Point Reyes National Seashore

National Park Service
U.S. Department of the Interior

Coastal Dune Restoration Vegetation Monitoring Report 2021











Lorraine Parsons, Michael Spaeth, and
Savannah Fuqua

March 2023

EXECUTIVE SUMMARY

Native dune habitat in Point Reyes National Seashore (Seashore) provides critical habitat for four federally listed and several additional rare and unique species of plants and animals and represents some of the largest expanses of rare native dune plant communities remaining in California. However, by the late 1990s, more than 60% (1,400 acres) of the park's roughly 2,200 acres of coastal dune, bluff, and scrub habitat has been invaded by European beachgrass (*Ammophila arenaria*) and iceplant (*Carpobrotus* spp.), which establish vast monocultures that crowd out native plant species, as well as threaten listed animal species that also depend on dune habitat such as Western snowy plover (*Charadrinus alexandrinus nivosus*) and Myrtle's silverspot butterfly (*Speyeria zerene myrtleae*). It is not exactly clear when these species arrived in the park, but it's believed that they were planted in the early to mid-1900's to stabilize dunes and protect adjacent ranches and roads from sand encroachment.

These impacts to native habitats spurred the Seashore to embark on an ambitious coastal dune restoration program starting in 2001. Three of the larger and more projects conducted include Abbotts Lagoon Coastal Dune Restoration Project (initiated in 2011), B Ranch North Restoration Project (initiated in 2013), and AT&T Coastal Dune Restoration Project (initiated in 2015). These projects relied on several different methods to remove the primary invasives (European beachgrass and iceplant), including manual removal, mechanical removal, and herbicide treatment. However, since the earliest project was conducted near the mouth of Abbotts Lagoon (Abbotts Triangle) in the early 2000's, the park has largely moved to herbicide treatment, because manual removal at Abbotts Triangle was not effective due to the extensive amount of retreatment needed, and mechanical removal at Abbotts Lagoon was too expensive and incurred too many indirect impacts to other native habitats and adjacent ranches through remobilization of unstabilized sands. To date, the Seashore has removed approximately 271 acres of non-native invasive plants from approximately 535 acres of coastal dunes.

With the exception of manual removal, both mechanical removal and herbicide treatment have been very effective in reducing abundance of primary invasives such as European beachgrass. Within one year following treatment, most beachgrass regrowth or new growth averaged less than 3- 5%. However, both of these disturbed environments proved vulnerable to invasion by a whole new suite of invasives, so-called "secondary invaders." Secondary invaders take advantage of decreases in cover of target species or primary invaders and increases in available space to gain a foothold and potentially preclude establishment of native species (Pearson et al. 2016). The suite of secondary invaders appears to vary depending on restoration approach and/or successional status (e.g., foredune, backdune). At Abbotts, European searocket (*Cakile maritima*) was relatively infrequent prior to and immediately after mechanical removal in 2011, but by summer 2016, its numbers had exploded within mechanically restored areas, literally blanketing in some instances newly established colonies of federally endangered Tidestrom's lupine (*Lupinus tidestromii*). "Sleeper weeds" such as European searocket often linger in this seemingly innocuous lag phase until conditions are optimal and then rapidly explode (Groves 1999), fueled in some instances by a change in biotic or abiotic resources or landscape disturbance such as restoration. Searocket continues to be a substantial maintenance issue at Abbotts, with an estimated 4,500 control hours invested to date. Secondary invaders have

encroached on all of the subsequent restoration areas, but lessons learned at Abbotts prompted park staff to pursue proactive measures to reduce abundance through vigilant manual removal and herbicide treatment, which has definitely paid off in terms of reducing resources invested in control.

Ultimately, what will define these dune restoration projects as being successful is not whether the park can eliminate both primary and now secondary invaders, but whether it can reestablish native dune ecosystem conditions, processes, and functions. In terms of convergence with the appropriate target native dune vegetation communities, the early monitoring results painted a pretty dismal picture. Both mechanical removal and herbicide treatment caused cover and richness of native dune plant species to plummet dramatically immediately following restoration. Mechanical removal instantaneously converted dense stands of beachgrass into vast expanses of unvegetated sand that ended up being largely nothing but bareground for seven (7) years or more, particularly in foredune areas. The only bright spot in terms of convergence with native dunes has been mechanical backdune areas, which in recent years have come to support native Dune Mat species. In contrast, herbicide treatment converted dense monocultural stands of live beachgrass into dense stands of dead biomass or necromass that, at least in backdunes, take more than seven (7) years to fully decompose, with decomposition generating large amounts of thatch and detritus that eliminate bareground or open space.

In some senses, the re-setting of the successional timeline was almost too extreme with mechanical removal, with soil conditions and dune processes either too primordial or too severe to support establishment of plants other than pioneer or nursery species such as Tidestrom's lupine. Conversely, in the case of herbicide treatment, restoration does not appear to have been extreme enough to provoke a successional reversion to native dune – except in foredune areas where sand overwash from adjacent beaches occurs. These successional trajectories have been strongly confounded by longer-term climatic conditions, with drought conditions persisting between 2012 and fall 2016, when many of these projects were initiated, and again between 2020 and 2021. Dry winters are often accompanied by high spring winds (Baye 2008), which further undermines native plant vegetation establishment. While easing of drought conditions in 2016 promoted colonization of native dune plants in both mechanical and herbicide treatment restoration areas at Abbotts, it also encouraged a strong surge in cover of secondary invaders and other non-native species.

Many past dune restoration projects have tacitly assumed that weed eradication alone would be sufficient to restore natural ecosystems and associated functions. However, restoration projects that rely entirely on invasives removal can be fraught with unexpected complications (Suding et al. 2004, Corbin and D'Antonio 2012, Konlechner et al. 2015). Past invasive plant control projects conducted in grasslands, wetlands, forest, and shrubland habitats have found that, while most have been successful at reducing invasive plant abundance, they were less successful in re-establishing natives (Reid et al. 2009, Kettenring and Adams 2011). For many of these projects, long-term retreatment has been necessary to prevent primary invaders from re-establishing and new or secondary invaders from becoming established (Reid et al. 2009, Kettenring and Adams 2011). Similar issues have plagued previous dune restoration projects, with post-restoration studies citing lower native plant cover/diversity (Zarnetske et al. 2010,

Konlechner at al. 2015, Pickart et al. 2021), increased abundance of secondary invaders (Novoa et al. 2013, Konlechner et al. 2015), and need for frequent re-treatment to prevent re-invasion (Novoa et al. 2013, Hesp and Hilton 2013, Darke et al. 2016).

Impacts of invasives may be more pervasive than simple displacement of native species (Novoa et al. 2013, Konlechner et al. 2015). Once established, invasives can alter soil chemistry and microbia, as well as insect-plant relationships (Bartomeus et al. 2008, Gornish et al. 2020; Pellegrini et al. 2021). Dune invasives such as iceplant and bush lupine (*Lupinus arboreus*) have well-documented effects on soil chemistry (Conser and Connor 2009, Novoa et al. 2013, Hetherington and Wilson 2019) and microbia (de la Peña et al. 2010, Badalamenti et al. 2016). Indirect effects of beachgrass invasion have not been as well-studied, but in northern California systems, invasion appears to affect both soil chemistry and microbia (Parsons et al. 2020, Parsons and Becker 2021), as well as natural foredune structure through aggressive sand accretion (Wiedemann and Pickart 1996).

Even if invasives are successfully removed and excluded, this may not be enough to fully restore dune ecosystems due to "legacy" effects of these invasives on both abiotic and biotic conditions of soils, dune structure, and other biological mutualisms of dune ecosystems still not fully understood (Suding et al. 2004, Suding and Hobbs 2009, Yelenik and Levine 2010, Yelenik and D'Antonio 2013). A number of restoration projects have shown that removal of areas long invaded by iceplant does not necessarily result in immediate establishment by natives, which researchers have surmised is due to any number of factors, including allelopathic effects of iceplant litter; changes in soil pH, organic matter, nutrient levels, and water-holding capacity; and adverse changes in soil biota (Andreu et al. 2010, Novoa et al. 2012, Novoa et al. 2014, Winsemius et al. 2015). In many cases, these "restored" areas end up being colonized primarily by weedy, opportunistic non-native species (Andreu et al. 2010, Hamingson and Voeller 2011, Novoa et al. 2014).

Legacy effects of European beachgrass are less well-understood, but it has been shown to alter structure of foredunes due to excessive sand accretion (Wiedemann and Pickart 1996). Research recently conducted at PRNS within the Abbotts-AT&T study areas has shown that beachgrass invasion alters both soil microbia and chemistry and that some effects persist even after restoration (Winsemius et al. 2015, Parsons et al. 2020, Parsons and Becker 2021). Even mechanical removal, which should have seemingly upended the potential for legacy effects, was not immune to problems, as treatment also affected soil microbia and chemistry (Winsemius et al. 2015, Parsons et al. 2020, Parsons and Becker 2021). Of 15 soil chemistry variables exhibiting "treatment" effects – changes associated with restoration approach, not invasion legacy -- 13 occurred exclusively in mechanical restoration areas (Parsons and Becker 2021). A soil inoculum study conducted at PRNS after mechanical removal found that, while germination of several native plants in "flipped" soils was comparable to that in native dune soils, aboveground biomass was considerably reduced, and it concluded that parasitic microbes in flipped soils could be potentially responsible (Winsemius et al. 2015). This microbial issue may have resulted more from treatment rather than a lingering invasion effect.

Another potential physical legacy effect associated with European beachgrass is the persistent standing dead beachgrass/litter, which may slow, if not possibly preclude, efforts to re-establish

native vegetation in backdunes. Standing dead biomass and thick thatch may potentially suppress plant germination and/or establishment (Facelli and Pickett 1991), including of target native dune plants. Dynamics of decomposition seemingly differ in herbicide-treated foredunes due to the fact that sand mobilized off the beach overwashes treated stands, burying standing dead biomass and litter, as well as soils with potential legacy and treatment effects. Invasives litter in PRNS dunes appears to even facilitate other weeds: Dead iceplant mats often support New Zealand spinach.

Results of a recent microbial research studies conducted jointly by the Seashore, University of Arizona, and University of California, Davis, may shed some light on this phenomenon. Heavily invaded sites were characterized by a higher abundance of cellulolytic bacteria involved in decomposition of cellulose in plants (Parsons et al. 2020). Following herbicide treatment, cellulolytic bacteria abundance dropped sharply despite the fact that a very large volume of now dead beachgrass material still persisted (Parsons et al. 2020). A follow-up study found that abundance of wood saprophytic fungi is also suppressed in beachgrass-invaded backdunes relative to native Dune Scrub, with fungal recovery not evident in herbicide-treated areas even 8 years following treatment (Yang et al. 2022). Saprophytic fungi initiate decomposition of cellulose, lignin, and lignocellulose and are considered the primary engine of the decomposition process (Francioli et al. 2021). Abundance of cellulolytic bacteria did appear to potentially rebound by Year 8 (B. Yang, University of Arizona, *pers. comm*.). These results mirror trends in levels of enzymes associated with breakdown of plant chemicals such as hemicellulose (xylanase) and cellulose (cellobiose and β-1,4 glucosidase), which also suggest recovery by Year 4 or Year 8, respectively (Yang et al. 2022). Structure equation modeling pointed to changes in enzyme activity being driven by changes in soil chemistry, which in turn was influenced by effects of habitat type (native, invaded, restored) and detritus on soil microbia (Yang et al. 2022). In this situation, legacy effects (saprophytic fungi) are being compounded by reversal of legacy effects (cellulolytic bacteria) to thwart timely decomposition of beachgrass. The park is currently partnering with University of Arizona on an adaptive restoration experiment to determine whether use of whole soil inoculum from intact native dune (Dune Scrub) habitats can jumpstart development of healthier microbial/decomposer communities in restored backdunes, thereby accelerating beachgrass decomposition and improving restoration success.

In addition to biota, soil chemistry may play some role in delayed decomposition, as well, as results of soil sampling suggest that invasion impacted 74% of 19 variables assessed, although there was a significant interaction in many cases with successional status (earlier vs later; Parsons and Becker 2021; Appendix G). Almost 60% of invasion effects persisted after restoration, with legacy effects prevalent in herbicide-treated backdunes where sand deposition from adjacent beaches could not mitigate effects as it could in herbicide-treated foredunes (Parsons and Becker 2021). Soil chemistry may decelerate decomposition of beachgrass due to the limited N available to enable microbial breakdown of the high C:N-ratio (70.8:1) litter, but microbial factors probably play a more important role (Parsons and Becker 2021).

Another potential repercussion of beachgrass invasion on native dune habitats is disruption of mycorrhizal networks within native soils, although invasives can also potentially gain a competitive edge by introducing arbuscular mycorrhizal fungi (AMF) where it did not exist

previously. In 2020, the Seashore sampled roots of beachgrass within AT&T South: Colonization rates were high ($\bar{X}$ = 79.3%) and greatly exceeded those previously documented for European beachgrass in both native and introduced ranges (23% to 51%; Tadych and Blaskowski 1999, Kowalchuk et al. 2002, David et al. 2016, Parsons et al. 2022; Appendix H). Native plants were not sampled as part of this study, but soil microbial analyses from 2017 and 2020 found that native Dune Mat and Dune Scrub at AT&T/North Beach had very low abundances of AMF, while moderately to heavily invaded dunes had very high abundances of three taxa (Parsons et al. 2022). Conversely, at BRN, Paraglomeromycetes was elevated within native late-successional Dune Mat habitat while reduced in heavily invaded beachgrass areas, which supported a higher abundance of a less common taxa, Archaeosporomycetes (Parsons et al. 2022). Herbicide treatment rapidly reversed AMF changes at both sites, with abundance becoming comparable to that within native dunes within two years after treatment (Parsons et al. 2022). These preliminary results suggest that beachgrass may be interacting extensively with mycorrhizal fungi and that its mode of interaction may vary spatially, which could have important consequences for native plant species in both invaded and uninvaded habitats, as strong AMF associations for beachgrass could give beachgrass a strong competitive advantage over non-mycorrhizal or weakly mycorrhizal native plants (Parsons et al. 2022). A more intensive study of mycorrhizal status of native and invasive dune plants at Point Reyes and other Pacific coast dune systems is currently underway at University of Arizona.

Post-treatment corrective measures explored to date such as direct seeding and post-herbicide treatment mowing have not necessarily reversed convergence issues in mechanical removal and herbicide treatment areas. Even if lack of native species propagules appeared to be a problem -- which, in this case, it does not -- active revegetation of at least mechanical removal areas relies on protection of plantings or seeding from high winds and appropriate nutrient/soil biota conditions for success. In terms of accelerating decomposition of dead beachgrass stands in herbicide-treatment areas, mowing and scraping did increase beachgrass decomposition rates and bareground cover, but did not increase native species cover and, in the case of dozing, actually decreased native species richness (Ender and Parsons 2017). Measures such as mowing, scraping, or even prescribed burning may introduce an element of disturbance that could actually disrupt, rather than accelerate, conversion to more native habitats.

Based on recent vegetation monitoring results, there are encouraging signs, however, that native vegetation establishment is beginning to occur in the older mechanical removal and herbicide treatment projects dating back to between 2011 and 2014. Multivariate and univariate analyses indicate that cover, richness, and diversity-related variables and species assemblages in Abbotts herbicide-treated backdune areas were finally comparable with target native habitat, Dune Scrub, after nine (9) years, with the exception of native species richness. Multivariate analyses confirmed that mechanical restoration areas had not yet converged with native Dune Mat, although cover of non-native species and detritus and non-native species richness showed comparability, as did mechanical backdune plots in terms of species assemblages. Vigilant re-treatment of primary and secondary invaders will be key, ultimately, to success of these projects. As Pickart and others have pointed out, re-treatment may not be a short-term obligation, but a long-term commitment, as new invaders brought by water, wind, or human will continue to try and establish in this naturally disturbance-prone environment.

One of the redeeming factors of the Abbotts project during those early post-restoration years was that, despite issues with recruitment of native plants and invasion by new weeds, many of the restored areas immediately experienced a dramatic increase in use by federally listed species such as Tidestrom's lupine, western snowy plover, and beach layia (*Layia carnosa*, FE). As noted earlier, one of the very first species to colonize mechanically restored areas at Abbotts was Tidestrom's lupine, and its numbers within mechanical removal areas have exploded to the point that the restored population is now probably larger than the adjacent historic or natural population. Not only did mechanical removal immediately generate suitable conditions for lupine establishment, but it immediately eliminated cover for native deer mice (*Peromyscus maniculatus*) that consumed large numbers of lupine seed in the historic population, threatening viability of this and other populations. One of the most imperiled lupine populations occurs at B Ranch North, where predation rates were extremely high, and plant numbers, extremely low (WU, unpub. data). Beachgrass surrounding the native Dune Mat supporting lupine was sprayed in 2014 to eliminate mice habitat, but restoration did not realize the same benefits for this population in terms of reducing predation and increasing population size, as it has for Abbotts. Predation rates have dropped from an average of 70% pre-restoration to 44% in 2021, compared to rates consistently below 10% at Abbotts (WU, unpub. data). Mice may be continuing to utilize dead beachgrass stands due to the persistence of decaying standing dead biomass that serves as cover from predators and rhizomes that may act as nesting material. The Seashore is currently partnering with U.S. Geological Survey researchers studying mice densities in different dune habitats, including treated beachgrass stands.

While beach layia did not colonize mechanical removal areas at Abbotts immediately, over time, abundance of this annual species has also skyrocketed in recent years, with the population currently estimated in the millions. Western snowy plover also began rapidly using the mechanically restored foredunes for nesting and even moved uncharacteristically at one point into backdune areas, but nest attempts dropped considerably after a peak in 2013 due probably to increased predation of nests and chicks by ravens due to low vegetation colonization in the foredunes that could provide cover. Whether slow increases in native plant colonization at Abbotts translates into increased suitability of habitat for successful plover nesting and fledging remains to be seen. Additional adaptive restoration measures may be needed, such as nest enclosures (already ongoing) and/or predator control.

For future coastal dune restoration projects, the Seashore may want to prioritize restoration sparsely invaded native Dune Mat and Dune Scrub, where invasion and legacy effects have not had time to develop. In addition, for moderately and heavily invaded beachgrass areas where invasion and legacy effects are probable, herbicide treatment efforts could be focused on foredune and mid-dune areas, as sand overwash from adjacent beaches accelerates conversion of invaded areas to native dune habitat by burying stand dead biomass and soils impacted by microbial and chemical legacy effects. While mechanical removal can also eliminate non-natives and reverse physical legacy effects of European beachgrass in foredune areas, the cost in terms of impacts to adjacent native habitats and ranchlands from sand remobilization is too high, and the approach itself is too costly to be feasible for larger future restoration efforts. Mechanical removal, too, appears to induce treatment effects in both soil chemistry and

microbia that may hamper efforts to restore native dunes.

Success of creating native dune habitats using herbicide in heavily vegetated backdunes is more questionable, however, jeopardized potentially by physical, chemical, and biological legacy effects of beachgrass, as well as minimal deposition of new sand, both of which may favor establishment of weedy, non-native annual species over natives. We do not have enough information yet to predict how persistent these decomposition-legacy effects will be, or how much of a challenge they will pose to successful restoration of dune habitats, but restoration project managers may need to find effective ways to tackle these legacy effects if restoration efforts are ultimately to be successful.

As noted earlier, the Seashore is currently conducting an experiment to evaluate adaptive restoration measures such as amendment with whole soil inoculum that could ameliorate some of these microbial legacy effects in herbicide-treated backdune and improve speed and success of these restoration efforts, particularly in maximizing establishment of natives and minimizing establishment of new weeds or secondary invaders. The Seashore is also currently exploring the possibility of conducting prescribed burning or flame torching in herbicide-treated backdunes to reduce volume of necromass in backdunes constrained by low decomposer abundances.

While the Seashore originally envisioned re-treatment as a short- to medium-term obligation, it is likely that long-term maintenance will be required to ensure that benefits of restoration are not lost over time. Managers who conducted one of the most successful dune restoration projects along the West Coast in Humboldt fully acknowledge that ongoing invasives removal is essential to continued project success. Ultimately, this management obligation may extend not only to vigilant re-treatment of weeds, but management of predators that decrease restored habitats' potential to support federally listed and other rare species.

INTRODUCTION

Native dune habitat in Point Reyes National Seashore (Seashore) provides critical habitat for four federally listed and several additional rare and unique species of plants and animals and represents some of the largest expanses of rare native dune plant communities remaining in California. However, more than 60% (1,400 acres) of the park's roughly 2,200 acres of coastal dune, bluff, and scrub habitat has been invaded by European beachgrass (*Ammophila arenaria*) and iceplant (*Carpobrotus* spp.), which establish vast monocultures that crowd out native plant species (Figure 1).

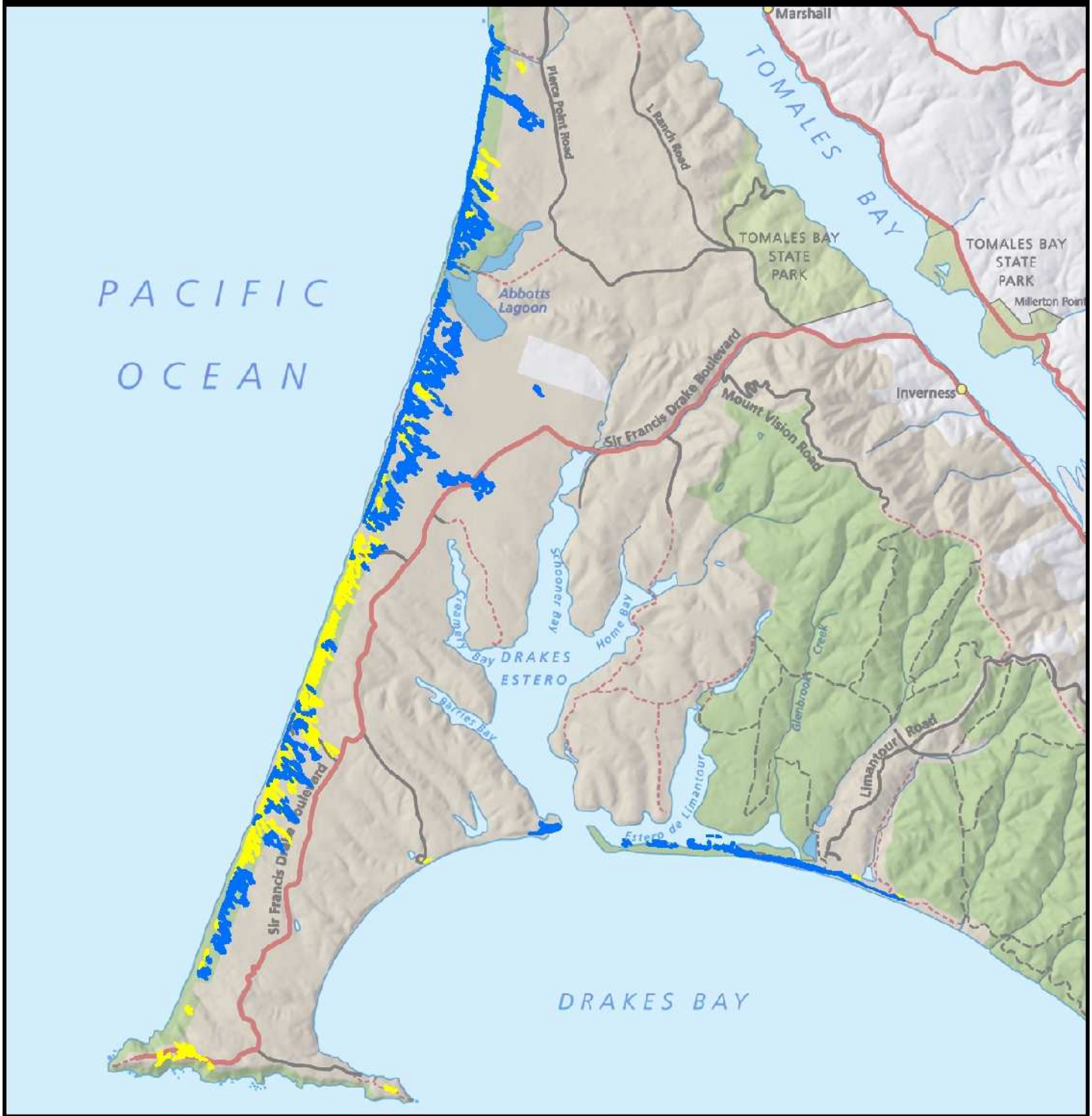
The continued encroachment of these species not only resulted ultimately in dense monocultures that supported fewer native dune plant species, but it had potential repercussions on other species as well, including the federally endangered Myrtle's silverspot butterfly (*Speyeria zerene myrtleae*) that feeds on the nectar on many dune plants and the federally threatened Western snowy plover (*Charadrinus alexandrinus nivosus*) that nests in unvegetated shorelines along the foredunes. Beachgrass can creep out into foreshore areas, and it also provides cover for potential predators of eggs, chicks, and adults. In addition, beachgrass indirectly impacted endangered dune plants such as Tidestrom's lupine (*Lupinus tidestromii*) by supporting much higher densities of native deer mice (*Peromyscus maniculatus*) that have been shown to eat as much as 82% of this species' seed, thereby threatening long-term viability of many of the Seashore's lupine populations (Dangremond et al. 2010).

These impacts have spurred the Seashore to embark on an ambitious coastal dune restoration program since 2000. The primary objectives related to dune restoration at the Seashore include:

- Remove non-native, invasive plant species from dune habitat where they interfere with natural physical processes such as sand movement and hydrology.
- Remove non-native, invasive plant species from dunes to create conditions under which native plant and wildlife species can flourish.
- Minimize potential for non-native species reinvasion of restored habitat.
- Increase potential coastal dune habitat for target threatened and endangered species affected by non-native, invasive plant species. The U.S. Fish and Wildlife Service (USFWS) Recovery Plan for endangered dune plant species calls for expanding population numbers and ranges through restoration and protection of dune habitats, with restoration to be focused on an "aggressive program to control non-native invasive plant species."
- Work with park ranchers to minimize impacts to beef and dairy cattle ranching operations on adjacent lands.

Monitoring of the effectiveness of restoration efforts was not necessarily identified as an objective, but several of the projects have attempted to monitor how successful projects have been in achieving at least some of these objectives. This report summarizes preliminary restoration results for vegetation-associated variables for three projects conducted by the Seashore as of 2021 – Abbotts Lagoon, which was conducted starting in 2011; B Ranch North, which was initiated in 2013, and AT&T, which was initiated in 2015.

Coastal Dune Restoration Environmental Assessment
Point Reyes National Seashore



National Park Service
Point Reyes National Seashore
Marin County, California

0 1 2 Miles

N

Figure 1. Beachgrass and Iceplant

- European Beachgrass
- Iceplant

S:\GIS\projects1\Dune_restoration\Dune_EA_2012\Project-based_EA_Figures\1_Beachgrass and Iceplant.mxd

The intent of the AT&T project is to link with the Abbotts Lagoon project to the north to create almost 400 acres of unfragmented, restored coastal dune habitat.

BACKGROUND ON COASTAL DUNE ECOSYSTEMS

Dune Processes

Coastal dunes are dynamic ecosystems formed by the combined action of wind and wave. Offshore sandbars and sediment deposited at the mouths of rivers are the most important sources of material for dune building. This sediment is carried by longshore currents down the coast and is deposited on the beach by wave action (CERES 2013). Dune formation begins when wind or so-called Aeolian transport blows dry sand particles landward from the beach (CERES 2013). Objects such as plants and logs can act as "drifts" that disrupt the wind flow and sediment transport, causing mounding of sand. Over time, the wind erodes sand particles from the windward side of these drifts or mounds and deposits them on the leeward side. Gradually, this process results in the dunes migrating inland, with more sand accumulating in these moving hills over time.

In some cases, dunes are formed as spits or long, narrow accumulations of sand. Spits establish from in-coming waves approaching the shoreline at an angle, generating a longshore current: this current transports sand that deposits and forms a bar in front of a bay or lagoon (Old Dominion University 2013). Limantour Spit is an example of a spit type of dune: waves refract around Point Reyes Headland, which leads to an angled wave approach along this section of coastline (Old Dominion University 2013).

As with many ecosystems, dunes often undergo periods of cyclic stabilization and rejuvenation (Pickart and Sawyer 1998). Rejuvenation events are the result of changes in relative sea level, which, in turn, have been attributed, at least in the past, to tectonic activity, including tsunamis (Vick 1988, Pacific Watershed Associates 1991, Clarke and Carver 1992, Komar and Shih 1993 *in* Pickart and Sawyer 1998). Both uplift and subsidence can trigger reactivation of dunes, with the former potentially building or expanding dunes through increased sediment supply, while the latter can destroy dunes through increased wave action or limit the expansion of new dunes (Pickart and Sawyer 1998).

During and in between these major dune-forming and dune-destruction periods, dune morphology continues to be shaped by other factors, including wave action and offshore winds. Offshore winds create "blowouts" or northwest-southeast trending swales or low areas that are parallel with the prevailing northwesterly winds. When dunes are sparsely vegetated, the strength of these winds is sufficient to mobilize sands and cause movement or creep of dunes inland over time. New foredunes and associated parabolic dunes are then created from new sediments supplied by the ocean.

In addition to tectonic activity, another factor that can stabilize or rejuvenate dune systems is climate change. Climate change can affect dune systems through a myriad of direct and indirect effects, including changes in temperature, wind, precipitation, freshwater hydrology, sediment supply and transport, sea level rise, and ocean circulation. Coastal environments are considered among the most vulnerable to changes from climate change, including direct changes (e.g.,

changes of temperature and precipitation) and indirect changes (e.g., sea level rise, wind and water circulation, increasing storm events). As climate change study is a relatively young science, the exact magnitude and extent– and even the direction – of these changes on the northern California coast is still a matter of active debate.

Dune Structure

Coastal dune fields form characteristic patterns. Most dune fields consist of two or three sets of parallel dunes, with the most recently formed foredunes nearest the beach, and the older dunes farthest inland; the inland dunes may by as much as 18,000 years old (CERES 2013). A common pattern in some California dune systems is a series of parallel ridges perpendicular to the prevailing winds, called "transverse ridges" (CERES 2013, Pickart and Sawyer 1998). These dunes occurred in Pismo and Santa Maria further south, as well as historically in San Francisco and Dillon Beach (P. Baye, *pers. comm*.).

The other common type of dune system in California is the parabolic dune, which consists of a series of U-shaped dunes with the concave side facing the prevailing wind direction (CERES 2013, Pickart and Sawyer 1998). Most of the California parabolic dunes are attenuated, narrow parabolic or U-shaped dune fields, also called “tongue” dunes (Cooper 1958), with minimal or no dune slack or swale development except in a few cases (Point Reyes, Monterey, Humboldt, and Morro Bay sandspit; P. Baye, *pers. comm*.). At Point Reyes, most of the dune mass occurs in an attenuated lobe rather than on the flanking ridges of the “U,” perhaps because these dunes are climbing up over a marine terrace with an erosion-resistant soil and vegetation community (P. Baye, *pers. comm*.).

In these systems, the primary foredune is a ridge of sand that forms parallel with the coast above the mean high tide line (Pickart and Sawyer 1998). It is buffeted by onshore winds and is vegetated by plants that are tolerant of sand burial (Pickart and Sawyer 1998). Under historical conditions (e.g., prior to invasion by non-native species), the foredune typically was a low-elevation, hummocky, non-continuous feature sparsely vegetated by species such as native American dunegrass (*Elymus mollis*), which allowed periodic blowouts of sand that leave valleys or openings within the foredune ridge (Pickart and Sawyer 1998). The wind pushes sand inland through these blowouts or valleys, forming crests and valleys or U-shaped dunes perpendicular to the coast.

The longitudinal ridges and valleys behind the foredune are also sparsely vegetated and move slowly inland by the forces of wind and slumping along the lee face as slopes exceed the angle of repose. In northern California, both parabolic and transverse dunes exist behind the foredune complex (Pickart and Sawyer 1998). Parabolic or U-shaped dunes move inland, sometimes merging into a large sand plain or sand sheet devoid of vegetation (Pickart and Sawyer 1998). As this highly mobile, unstabilized sheet moves, a large deflation plain or low elevation areas forms on which dune swale or dune slack wetlands can occur, fostered by a seasonal or perennial groundwater table exposed by sand loss (Pickart and Sawyer 1998). Wetland vegetation grows and stabilizes the dune swales. In some California dune systems, smaller transverse dunes or dunes oriented with crests perpendicular to the prevailing wind can develop on the open sand surfaces of so-called moving dunes, which include parabolic dunes

and sand sheets (Pickart and Sawyer 1998).

Both European beachgrass and iceplant have greatly altered the natural morphology of coastal dunes in many areas along the Pacific coast of North America, although European beachgrass more so. With colonization by European beachgrass, the primary foredune topography has changed to a steeply sloped, continuous ridge without blowouts, and the orientation of the ridges and troughs in areas behind the foredune complex are parallel rather than perpendicular to the coast (Cooper 1936; Barbour and Johnson 1988; Pickart and Sawyer 1998). Based on topographic analysis of dunes at Bodega, areas vegetated by European beachgrass can be 7- to 26 feet higher than unvegetated dunes (Cesmat et al. 2012). Rapidly accreting European beachgrass-dominated foredunes have an internal structure of interbedded unconsolidated sand (mostly horizontal or gently sloping beds) formed by pulses of rapid sand accretion (burial episodes) and shallow layers of persistent, dense beachgrass roots, rhizomes, and shoots (Baye 2008). European beachgrass roots and rhizomes concentrate in the upper 8- to 12 inches due to light exposure, but with frequent burial events, young, viable buds formed from rhizome breakage can be distributed throughout the foredune horizon (Baye 2008). This network of rhizomes, combined with the dense aboveground biomass, acts to traps sand and stabilizes the once active foredune, which prevents large-scale sand movement or fresh supplies of sand to dune areas inland of the primary foredune.

A second series of European beachgrass-dominated dunes occurs in the landward portion of the dune system, typically called the backdunes. Backdunes generally refer to dunes cut off from sand supply from the beach, and they are either stabilized or are internally remobilizing sand from blowouts or other mobile dunes inland from the beach (*p*. Baye, *pers. comm*.). At Point Reyes, most of the backdunes are heads or flanks of formerly migrating U-dune lobes that stabilized decades ago (Baye 2008). They are in early stages of Dune Scrub succession, as indicated by high frequency of coyote brush, wild cucumber, and mock heather (Baye 2008). Leading edges of U-dunes often stabilize by lateral, superficial colonization of European beachgrass, as the rate of dune migration inland slows. Because of the lower frequency of sand burial events in backdunes, rhizomes of European beachgrass do not extend as deeply down in the dune horizon in the backdunes as they do in the foredunes (Baye 2008).

At AT&T, just south of Abbott's Lagoon, both European beachgrass-dominated foredunes and backdunes exist, although the distance between foredune and backdunes is more compressed than at Abbott's, where foredunes and backdunes are separated by a large basin or deflation plain. Within the backdunes at AT&T, the ridges run in a perpendicular alignment to the beach, allowing for formation of moist meadow-drainage complexes between backdune ridges. A somewhat similar dune morphology exists at North Beach, although the size of foredune and backdune features is muted relative particularly to the northern portions of AT&T.

The dune systems at B Ranch, A Ranch, and Davis Property are different from those on the northern portions of the Great Beach. At B Ranch, there is a moderately steep drop from the dune "terrace" to the beach, with the oceanward portion of the dunes comprised of low-elevation European beachgrass-dominated foredunes. Behind the foredunes, the dunes slope gently upward in a series of concave basins bordered by marginal ridges to the elevated European beachgrass-dominated backdunes directly adjacent to the pasture. These basins at B

Ranch support a more native dune-open sand vegetation community with native plants and patches of iceplant, with ridges either comprised of native dune-open sand or colonized by European beachgrass or iceplant. At the Davis Property, the dune "terrace" is actually perched on a cliff.

Sand Movement

Presumably one of the primary differences between natural or native dunes and dunes heavily colonized by European beachgrass is the rate of sand movement, as species such as beachgrass and iceplant were planted to stabilize dunes and prevent encroachment into adjacent lands. In Humboldt, research found that parabolic dunes migrated on average 4.7 feet/year between 1939 and 1988, while some other parabolic dunes and transverse dunes moved as much as 15 to 21 feet/year (Wiedemann 1984; Pacific Watershed Associates 1991). Sand movement rates would be naturally expected to be much lower in invaded dune, as these species were plant to stabilize these systems. European beachgrass-dominated dunes at Bodega were estimated to accumulate on average as much as 5,232 cubic yards (CY) per year (Cesmat et al. 2012). In England, where European beachgrass is native, rates of inland movement of the dunes varied between 5-10 feet/year on the more stable dune sections and up to 22 feet/year on the most mobile dune sections (Ranwell 1958). The study at Bodega showed that, during peak wind conditions, sand transport was 450 times higher in unvegetated areas than in vegetated ones (Cesmat et al. 2012).

Some sediment transport monitoring has occurred in restored dunes. A series of plots at Little River State Beach in Humboldt County where excavation had been conducted to remove European beachgrass experienced sand movement at a rate of 3.9 cubic feet for every 10 square feet per year (Transou et al. 2007). During restoration, dunes moved, on average, 2.5 feet per month in a southeast or inland-trending direction, although the rate of inland dune movement may have been retarded by sand moving over existing European beachgrass stands (Vaughan and Fiori 2007). In the earlier restoration efforts at Abbott's Lagoon conducted by the Seashore between 2001 and 2005, sand movement was also assessed, and, while sand height did change, most of this change appeared to involve redistribution of sand within the treated area rather than actual movement of sand (Peterson et al. 2003).

Monitoring of the recent Abbott's mechanical removal project at Abbott's Lagoon showed that acreage of open sand areas, specifically on the inland perimeter of the dune system, increased from 2011 to 2013 (Johnson 2013b.). By spring 2013, approximately 10.1 acres of grassland, scrub, and wetland had been buried by newly remobilized sand (Johnson 2013b), which is equivalent to about 3% of the entire 300-acre restoration project area. Of the 10.1 acres, 7.3 acres involved inland "creep" of dunes, with the remainder being internal to the dune system (Johnson 2013b).

While the restored dunes visibly looked different in 2012 and 2013 relative to immediate post-construction conditions, this difference was not evident from topographic monitoring that was conducted in 2012 (NPS, unpub. data). Real-Time Kinematic GPS was used to survey longitudinal and latitudinal cross-sections of the Phase I project area and then compared to pre-restoration elevations estimated from LIDAR (2009) and immediate post-restoration elevations

that were also collected using RTK GPS (2011). Some sample cross-sections show a considerable reduction in dune elevations immediately after restoration, with little change between 2011 and 2012: The specifications called for no change in post-construction elevations, so either elevations were lowered, or, more likely, LIDAR data is not directly comparable to the RTK GPS data. Sands also had appeared to bury some of the adjacent native dune areas, as observed from "mounding" or burial of the many existing native dune plants, but, again, RTK GPS results suggest little immediate post-restoration change in elevations in these areas.

Dune movement is not isolated to natural and restored dune systems. While European beachgrass and iceplant were planted to stop movement of the dunes, the dunes have continued to migrate inland over the past 60- 70 years despite rapid expansion of these species. Dune expansion rates during the period 1943-2007 have varied from 16% at G Ranch to 59% at AT&T, with B Ranch estimated at 27%, resulting in average annual dune expansion rates during this period of 0.25% to 0.91% (Johnson 2013b). Analysis of historical aerial imagery at 20-year intervals between 1943 and 2007 suggests that this expansion occurred fairly continuously over approximately 65 years rather than in disjunct stages (Johnson 2013b). Coastal dune represents varying percentages of the total leased lands for different ranches, ranging from as low as 2% at G Ranch – currently, most of the dune system is outside the lease boundary at this ranch – to 24% at AT&T, with B Ranch estimated at 22% (Johnson 2013b).

Based on review of historic aerial imagery, the proportion of barren, sandy areas within the pasture adjacent to the restored dunes at Abbott's actually appears lower in 2013 – even with sand remobilization – than in 1963, around the time that the Seashore was established (Johnson 2013b). In general, sand movement since restoration at Abbott's in 2011 has tended to follow established historical sand deposition paths as evidenced by the fact that most of the ungrazeable areas buried were pre-existing dune "fingers" that extended into the pasture, which were already very sandy and dominated by dune and scrub type species. In the 1960s, two decades after European beachgrass was planted at Abbott's, these fingers are very distinguishable on aerial imagery as barren, sandy areas (Johnson 2013b). Over the next 30- to 40 years, these fingers remained within the pasture, but became more vegetated by dune and Dune Scrub plant species, as can be seen in 2010 aerial imagery (Johnson 2013b).

While there has been an inland creep from the perimeter of the Phase I mechanical removal area, this creep has largely been burying areas that have been repeatedly buried by sand in the past, with some very minor (<1.0 acre) exceptions. Inland sand migration rates were quite high during the first few years after Phase I implementation, averaging around 4 acres/year, but in 2014-2015, they dropped considerably to approximately 0.8 acre/year (NPS, preliminary unpub. data). Some of the movement during the first two years post-restoration may have been influenced by the two successive dry winters and the fact dry winters allow for reduced establishment by stabilizing vegetation and are accompanied by higher winds that discourage vegetation establishment (Baye 2008). Lower migration rates in 2014-2015 may be due to a reduction in maximum wind speed during at least 2014 (NPS, preliminary unpub. data). In the following two years (2015-2016 and 2016-2017), inland sand migration rates rose again, more than doubling to approximately 2.0 additional acres each of those years, but the wet winter also promoted re-colonization of some of these depositional areas, with the extent of colonized

areas jumping from 0.32 acre in 2015 to 4.98 acres om 2017 or approximately 29% of the 16.9 acres affected by sand remobilization (NPS, unpub. data).

In addition to sand movement being affected by weather-related factors, invasive species removal method also appears to influence sand migration rates. In the same year that mechanical removal was performed at Abbott's Lagoon, some of the backdune and foredune areas immediately adjacent to these areas were treated with herbicide. Although European beachgrass was almost completely killed during the initial treatment effort (>99%), the dead aboveground biomass and presumably the rhizomes did not decompose rapidly. This delayed decomposition may have reduced the potential for sands in these areas to remobilize, as they are being held in place by the dead rhizomes. No migration of dune had occurred inland of these herbicide-treated areas by 2013 (Johnson 2013b) or in subsequent years (2014-2017; NPS, unpub. data). In addition, monitoring of dune areas at AT&T in 2017 showed no inland migration of dune areas one year after treatment (NPS, unpub. data).

Dune Soils

Movement of sand not only affects dune topography but soil profiles and development. Much of the Seashore's coastal dune areas are mapped as the "Dune land" soil mapping unit, which is defined as consisting of hummocks, mounds, and hills of loose sand blown from nearby beaches (SCS 1985). Some areas are active and shifting, and other areas are partly stabilized by coastal sagebrush and grass (SCS 1985). As befitting its origins, Dune land "soils" exhibit no profile development (SCS 1985). Some small, low basins are surrounded by dunes that are wet most of the year: These areas are similar to Aquepts, seeped, but they commonly are sand or loamy sand throughout (SCS 1985).

Dune soils evolve as sands move inland. The landward edge of dunes is defined by unconsolidated sand that may have started to develop a soil profile, but does not show the red color of advanced chemical weathering characteristic of older sand deposits (Wiedemann 1984). Adjacent to the dunes are coastal marine terraces with paleodune soils, many of which have been mapped as Sirdrak Sand: Sirdrak Sand represents deposits of latest Pleistocene to Holocene-era Aeolian or wind-driven dune sand deposition approximately 30,000 years before present (Knudsen et al. 2000; Baye 2008).

Centuries of grassland establishment has led to establishment of a mollisol over most of these former dune sands. Mollisols develop through significant accumulation of humus or organic matter in the surface horizon, or uppermost layer, of soils: This organic matter always almost derives from grass vegetation. Mollisols have deep, high organic matter, nutrient-enriched surface soil (A horizon), typically between 60-80 cm thick, that is known as a mollic epipedon. Over time, these grassland-dominated terraces have been subject to natural dune transgression or migration, probably from specific episodes of climate-driven dune instability (Baye 2008). These are evident in the occurrence of the previously discussed dune "fingers" extending some distance sometimes into pastures or even in the presence of dune "islands" some distance from the beach, such as occurs at F Ranch.

Coastal dune sands tend to be nutrient limited, especially for the essential nutrients nitrogen (N), phosphorus (P), and potassium (K^+, Barbour et al. 1985). Unlike terrestrial ecosystems

where weathering of soils and rocks generates some of the K^+, magnesium (Mg^{2+}), calcium (Ca^{2+}), and P needed by plants, sandy soils in dunes are already highly weathered and mostly unable to supply these macronutrients (Etherington 1967; Art et al. 1974; Smith et al. 1985). Most inputs of N, oxygen (O), and sulfates (SO_4^{2-}), as well as base cations such as K^+, Mg^{2+}, Ca^{2+}, and sodium (Na^+), into coastal dunes come from meteorological sources such as rain, salt spray, and fog (Etherington 1967; Clayton et al. 1972; van der Valk 1974; Jacob et al. 1985; Holton et al. 1991). Due to the very low abundance of clay, sandy soils also do not have a strong capacity for binding cations that enter the system from elsewhere and are prone to considerable leaching (van der Valk 1974; Art et al. 1974). Organic matter (OM) provides nearly all the cation exchange – or nutrient binding – capacity (CEC) in the coarse-textured sands (Etherington 1967; Art et al. 1974), and OM adsorption is strongly dependent on pH, with CEC typically lower at lower pHs (Smith et al. 1985).

While soil nitrogen levels are low, N content within plants are actually similar to crop plants on fertile soils (Holton et al. 1991). Dune plants obtain N from episodic inputs of detritus (Ranwell 1972, Holton 1980), nitrogen fixation by symbiotic and associative bacteria (Wahab and Wareing 1980, Holton 1980, Robertson 1982, Rose 1988), and photosynthetic use (Barbour et al. 1985). A 1991 study by Holton and colleagues at a Point Reyes dune system characterized the total N pool at 348 lbs/acre, of which 78% was soil organic; 4% was soil inorganic; and 18% was tied up in plant biomass. Nitrogen contributions to this system principally came from fog condensation, bulk precipitation, and symbiotic nitrogen fixation, in exponentially decreasing order of importance (Holton et al. 1991). At Point Reyes, N inputs from fog were almost 3 times higher (4.2 kg N ha^{-1} yr^{-1}) than that from rainfall (Holton et al. 1991). Levels of N in Humboldt area foredunes ranged from 4.9 ppm for ammonium (NH^{4+}) and 5.9 ppm for nitrates (NO^{3-}), whereas P levels were triple those levels (17.7 ppm; Nature Conservancy, unpub. data *in* Pickart and Sawyer 1998). Point Reyes' dunes exhibited a similar pattern, although levels were lower: 0.7- to 1.8 ppm for NH^{4+}, 0.4- 0.8 ppm for NO^{3-}, and 4.8- to 6.4 ppm for P (Parsons and Becker 2021).

Based on the Point Reyes study, nodulated legumes, including beach pea, provided only minor N inputs to dune systems and only at a very localized level (Holton et al. 1991). However, other members of the pea family have been demonstrated to have much more effect on soil N levels. Bush lupine (*Lupinus arboreus*) -- a species that is native to California, but possibly not Marin County – is a tremendous nitrogen-fixer that derives 60% to 70% of its total N from atmospheric sources (Bentley and Johnson 1991). Nitrogen-enriched litter accumulates under the plants, contributing as much as 72- 185 kg of N per hectare per year to some systems (Gadgil 1971, Palaniappan et al. 1979, Skeffington and Bradshaw 1980, Maron and Jeffries, unpub. data *in* Maron and Connors 1996). With leaf drop or lupine death, high levels of exchangeable NH^{4+} and NO^{3-} become available to plant colonists, particularly weedy introduced grasses and forbs (Maron and Connors 1996). Once established, these weedy annual exotics create a labile litter that completely turns over organic matter and nitrogen, favoring persistence of weedy annuals (Zink et al. 1995).

Iceplant also effects soil nutrient status, but in a slightly differ manner. Iceplant-invaded soils appear to have higher OM content and lower pH than uninvaded areas (D'Antonio and Mahall 1991, de la Peña et al. 2010, Santoro et al. 2011, Winsemius et al. 2015) and, in some areas,

organic carbon (Vilà et al. 2006): This may be partially due to the much higher amounts of detritus produced by this species (Vilà et al. 2006). Iceplant leaves have high contents of tannin and antibacterial compounds (van der Watt & Pretorius 2001), which may reduce litter decomposition rates of detritus (Vilà et al. 2006). These compounds may subsequently leach into the soils and acidify them, inhibiting nitrification (conversion of ammonium to nitrate) and increasing leaching of Ca^{2+} and Mg^{2+} (D'Antonio and Haubensak 1998, D'Antonio and Mahall, unpub. *in* Conser and Connor 2009). Magnesium levels in dune soils at the Seashore were found to be lower in iceplant-invaded areas than in native dune (Winsemius et al. 2015). Soil acidification occurs naturally with dune succession, but iceplant invasion speeds up this process (D'Antonio and Mahall, unpub. *in* Conser and Connor 2009). Changes in soil nutrient content with iceplant encroachment ultimately appeared more variable, with some studies showing an increase in total N (Santoro et al. 2011), some showing a decrease (D'Antonio and Mahall, unpub. data), and some showing no change at all (Vilà et al. 2006). Chemical legacy effects can later lead to further changes in the soil microbial communities that may reinforce the competitive advantage for invasive species. A study in Spain documented that microbial communities changed substantially with invasion, which they attributed to iceplant creating higher levels of microbial carbon sources that resulted in an increase in total microbial biomass, a decrease in the bacteria/fungi ratio, and a shift in microbial species composition (Badalamenti et al. 2016).

Iceplant also appears to compete with native plant species for water (D'Antonio and Haubensak 1998). It could disrupt osmotic levels in the soil in a similar fashion to another member of the iceplant family, *Mesembryanthemum crystallinum*, which retains salts in its tissues that are then released into the soils after plant and leaf senescence (Vivrette 1973, Vivrette and Muller 1977). These salt infusions create an osmotic imbalance in dune soils that draw water out of neighboring plants, resulting in desiccation and death. Higher Na^{+} levels – salts are sodium chloride (NaCl) – have been found in soils invaded by iceplant (D'Antonio and Mahall, unpub. data *in* Conser and Connor 2009), although some studies have found lower levels of Na^{+} in iceplant-invaded soils (Winsemius et al. 2015). Removal of iceplant from the vicinity of two dune shrub species, mock heather and Menzies' goldenbush (*Isocoma menziesii*), resulted in higher pre-dawn water pressure potentials in shrubs compared to shrubs still surrounded by iceplant, suggesting that iceplant was utilizing water that would have otherwise been available to shrubs (D'Antonio and Mahall 1991). The greenhouse study conducted by Conser and Connor (2009) found no differences in soil moisture content between iceplant-invaded soils and native dune soils, but this may point more to a lack of long-term changes in soil properties that could negatively affect soil moisture-holding potential in soils rather than differences in field moisture conditions. Ultimately, even if iceplant does not compete for water, it appears to fare better under drier conditions, with iceplant in germinating in lower moisture soils than native dune plants in Spain (Novoa et al. 2012).

The effect of European beachgrass on soil nutrient pools and water availability does not appear as well studied of that of iceplant or bush lupine. Soil chemistry sampling conducted for two years at Point Reyes in 2019 and 2020 pointed to beachgrass invasion impacting 74% of the 19 variables assessed, although there was a significant interaction in many cases with successional status (earlier vs later; Parsons and Becker 2021; Appendix G). Other than $NH4^{+}$ and PO_4, almost

every organic (OM, TKN, TOC) and inorganic (NO_3^-, SO_4^{2-}, K^+, Mg^{2+}, Na^+) source of macronutrients and exchangeable cations (H^+, K^+, Mg^{2+}, Ca^{2+}) appeared altered by invasion, along with pH and CEC (Parsons and Becker 2021). Almost 60% of invasion effects persisted after restoration, with legacy effects prevalent in herbicide-treated backdunes where sand deposition from adjacent beaches could not mitigate effects as it could in herbicide-treated foredunes (Parsons and Becker 2021). Legacy effects were documented for pH, CEC, NO_3^-, SO_4^{2-}, Mg^{2+}, Na^+, Exc-H^+, and Exc-Mg^{2+} (Parsons and Becker 2021). Mechanical removal -- or inversion of invaded surface soils with less-contaminated subsoils -- resulted in fewer legacy effects, but more treatment effects, primarily in backdunes (Parsons and Becker 2021).

Sand transport and nutrients are not only the factors affected by non-native plant invasion. Despite its seemingly barren appearance, dune soils play host a number of bacterial, fungal, and pathogen species that appear to at least partially drive the dynamics of invasion and succession within dune systems. In its home range, European beachgrass acts more as a foredune pioneer species that thrives under influxes of windblown sand, with vigor of stands declining over time (Hope-Simpson and Jeffries 1966, van der Putten al. 1989). European beachgrass stabilizes sand, but stabilization may encourage microorganisms that infect beachgrass roots and lead to the decreased vigor and aboveground biomass production evident in backdune communities (van der Putten al. 1988, de Rooij-van der Goes 1995). Within European dune systems, soil-borne pathogens, primarily endoparasitic nematodes (*Heterodera arenaria* and *Pratylenchus* spp.) and fungi, build up over time as sand features stabilize (van der Stoel et al. 2002), leading to eventual replacement of beachgrass by other, later successional species that are more tolerant of these pathogens (van der Putten et al. 1993).

In areas outside Europe, however, European beachgrass does not function strictly as a foredune pioneer, but often rapidly spreads to establish a persistent monoculture in both foredunes and backdunes. Some researchers have surmised that this may result from a release from beachgrass's natural soil enemies in its home range. For example, in South Africa, the absence of this negative soil feedback loop appears to be part of the mechanism that allows European beachgrass to expand and persist unchecked (Knevel et al. 2004). Sampling of soils within European beachgrass's introduced range suggested that invasives are not necessarily escaping from all soil enemies (e.g., generalist nematode taxa), but only from specific ones such as specialist root-feeding nematodes (van der Putten et al. 2005).

Beckstead and Parker (2003) documented only partial escape in California dune soils, with pathogenic nematodes absent, but fungal species similar to those of beachgrass' native regions (Netherlands) present. Early growth of European beachgrass in unsterilized California dune soils was depressed by pathogens at very similar levels to those seen in corresponding studies from The Netherlands (van der Putten and Peters 1997), and in some cases, fresh sand from California showed even more of suppressive effect (Beckstead and Parker 2003). Studies of soils from other portions of beachgrass's invaded ranges in the United States showed that, while root knot nematodes found in beachgrass's native range were not present, sedentary, endoparasitic cyst nematodes were (van der Putten et al. 2005).

Similarly, while relative production of European beachgrass was higher in South African dune soils than in European ones, a comparison of production rates from sterilized and unsterilized

South African dune soils showed that local pathogens were inhibiting beachgrass growth to some degree (Knevel et al. 2004). A later study (Eppinga et al. 2006) argued on the basis of computer modelling incorporating plant competition and plant-soil community interactions that, rather than being released from its enemies, European beachgrass in California may accumulate local pathogens that eventually exclude native dune species. A similar dynamic may occur within the invaded ranges for iceplant. In some European systems, iceplant invasion is believed to be related more to the fact that iceplant may not be susceptible to existing soil pathogens: inoculation with rhizosphere soil from the invaded range in the Mediterranean region did not have a significant effect on plant biomass, while soils from the native range reduced iceplant biomass by 32% (van Grunsven et al. 2009).

Non-native invasives that are well-behaved within their home range may spread, then, either because their natural soil enemies are absent or that they are better able to tolerate accumulation of local pathogens than native neighbors. However, some researchers have argued introduced species are not always successful in invading native communities, because strong biotic interactions with native species can hinder their establishment and spread. A study on South African dunes found that one species, *Sporobolus virginicus*, might actually contribute to soil feedback resistance against European beachgrass and concluded that not only plant species diversity, but the type of plant species present, might be important in determining the potential for biotic resistance (Knevel et al. 2004). In a meta-analysis of 52 native–exotic pairwise feedback comparisons in 22 studies, researchers concluded that soil biotic resistance is somewhat frequency dependent: Soils conditioned by native species could provide resistance when frequency of the invader is low, even if the invader exhibits a positive plant-soil feedback, but this resistance may be adversely impacted by disturbance events or that enable invaders to spread quickly and establish high numbers in native ecosystems, particularly in situations in which non-native species have a dispersal or competitive advantage over native ones (Suding et al. 2013). Experiments have suggested that soil biotic community in at least some Mediterranean dune systems – where iceplant is not native – may initially resist the invader, but that, over time, the resistance to invasion breaks down (Vilà et al. 2006, de la Peña et al. 2010). High levels of a symbiotic fungal species, chytrids, were found in soils that had been invaded for some time by iceplant: This species may be detrimental to other native dune species, but not iceplant (de la Peña et al. 2010).

A 2017 study conducted jointly by the Seashore and researchers at University of Arizona and University of California, Davis, evaluated differences in soil microbial communities in coastal dunes with varying invasion intensity (sparsely invaded native Dune Mat and areas moderately or heavily invaded by beachgrass and iceplant) and different restoration approaches (herbicide, mechanical excavation) at Point Reyes (Parsons et al. 2020; Appendix E). Overall, the study found evidence of a strong link between bacterial and fungal soil communities and the presence of invasives and restoration approach (Parsons et al. 2020). Heavily invaded sites were characterized by a lower abundance of putatively identified nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs and a higher abundance of cellulolytic bacteria and a class of arbuscular mycorrhizal fungi (Archaeosporomycetes; Parsons et al. 2020). All these soil microbial changes imply shifts in carbon and nitrogen cycling with invasion success (Parsons et al. 2020). While the observed increase in cellulolytic bacteria is expected due to the

higher plant biomass in highly invaded soils and increased volume of plant material to be broken down through cellulolysis (Kourtev et al. 2002; Badalamenti et al. 2016), reduced nitrification might be due to bacteria competing with invasive plant roots for available ammonium (Kaye and Hart 1997) and/or due to acidification (Nicol et al. 2008). The decrease in taxa associated with fermentative processes together with the increase in taxa with cellulolytic potential might indicate that carbon is being processed differently in heavily invaded sites, with higher turnover of carbon and nutrients to support invasive plant growth (Parsons et al. 2020).

Interestingly, Halobacteria, a class of halophilic archaea that are extremely salt tolerant, showed the highest abundance in herbicide-treated sites while literally being absent from the rest of them (Parsons et al. 2020), pointing to herbicide changing salinity or some degradation by-product enhancing halophilic microbe activity (Le Borgne et al. 2008). Along these lines, glyphosate and imazapyr are often used in their isopropylamine salt rather than acid forms, and it has been shown that halophilic bacteria can break down glyphosate by utilizing carbon released after the organism breaks down the carbon-phosphorous bond (Sharifi et al. 2015).

Changes in soil microbiota did not fully dissipate following removal of invasives using herbicide, with exception of reductions in cellulolytic bacteria and Archaeosporomycetes abundance (Parsons et al. 2020). Mechanical restoration effectively removed both invasives and soil legacy effects by inverting or "flipping" rhizome-contaminated surface soils with soils from below, but as results from Winsemius et al. (2015) suggested, flipping of soils may have inadvertently induced other adverse effects on soils that precluded or at least greatly slowed re-establishment of native dune plants (Parsons et al. 2020).

As noted earlier, legumes or members of the pea family have strong associations with bacteria in their root systems that help them fix nitrogen. Dune plants form relationships with arbuscular mycorrhizal fungi (AMF) near roots that enhance P uptake, resistance to pathogens (Borowicz 2001, de la Peña et al. 2006), salt tolerance (Tsang and Maun 1999), and sand dune stabilization through aggregation of sand grains (Koske and Polson 1984). Strong nutrient limitation accentuates the importance of forming mycorrhizal associations for many dune species across the world (Koske and Halvorson 1981, Ernst et al. 1984, Logan et al. 1989, Stürmer and Bellei 1994, Beena et al. 2000, Rodríguez-Echeverríaa et al. 2007). AMF accounted for 30% of the total soil microbial biomass in Swedish coastal dunes (Olsson and Wilhelmsson 2000). In the U.S., AMF associations have been documented for most coastal dune species, including those in other northern California systems, with percent root colonization ranging from 5- to 90% (Koske and Polson 1984, Rose 1988, Pickart et al. 1990). AMF colonization is typically dramatically higher in foredunes, which are closer to the ocean, or in recently "fixed" or stabilized dunes than in older stabilized dunes, which are further from the beach and typically have more developed soils and later successional vegetation communities (Koske and Polson 1984, Rose 1988, Roy-Bolduc et al. 2015).

AMF colonization rates in native dune plant species at Point Reyes have not been explored in-depth yet, but the park is planning to collaborate with university researchers on a future study to more carefully evaluate mycorrhizal status of both native and invasive dune plants. Microbial results from DNA analyses conducted as part of the 2017 and a subsequent 2020 studies at Point Reyes suggest that, in northern sites such as AT&T, North Beach, and Abbotts,

native Dune Mat may have much lower general soil AMF abundance compared to heavily beachgrass-invaded areas possibly due in large part to naturally lower percent vegetation cover (22- 38%) and higher percent bareground cover (Parsons et al. 2022; Appendix H). Mycorrhiza would be expected to be strongly associated with the roots or rhizospheres of vegetation species, so sampling in largely open sand areas may underrepresent mycorrhizal communities.

In contrast, at southern sites such as B Ranch North, soil abundances of certain AMF taxa appeared comparatively higher in native Dune Mat (Paraglomeomycetes), with abundance of these taxa sharply reduced in heavily beachgrass-invaded areas, while abundance of another taxa, Archaeosporomycetes, increased (Parsons et al. 2022). Archaeosporomycetes abundance also jumped in beachgrass-invaded areas at AT&T and North Beach (Parsons et al. 2022). Interestingly, moderately invaded areas at B Ranch North dominated by iceplant with only spare beachgrass establishment did not support elevated Archaeosporomycetes levels (Parsons et al. 2022).

Differences in soil AMF abundance among different native Dune Mat areas may relate to successional status – B Ranch North supports a late-successional Dune Mat vegetation community characterized by higher percent vegetation with plants that are more evenly or uniformly distributed in space while that of the northern sites is more primary to mid-successional with patchier plant establishment (Parsons et al. 2022). A caveat to use of soil microbial data in understanding mycorrhizal status is that abundances or composition of species in the soil may be not wholly or even partially reflective of root colonization rates or of the AMF species colonizing roots (Parsons et al. 2022). The ongoing study will evaluate percent root colonization rates of native and non-native dune plants, along with identifying mycorrhizal species colonizing roots.

Within its home range, European beachgrass is characterized as facultatively mycorrhizal, with root colonization rates averaging 23- 51% (Giovanetti and Nicholson 1983, Tadych and Blaszkowski 1999, Kowalchuk et al. 2002, de la Peña et al. 2006, Rodríguez-Echeverría et al. 2007). Much higher rates of AMF colonization appear to occur in foredunes (76%) than backdunes (26%; Kowalchuk et al. 2002), with AMF potentially critical to beachgrass establishment (Read 1989). In addition to frequent sand accretion in foredunes providing a nematode-free substrate, higher AMF infection rates may also protect beachgrass against root-feeding nematodes (de la Peña et al. 2006).

Mycorrhizal status of European beachgrass within invaded habitats is less well-documented. In a 2012 study that evaluated AMF in European beachgrass and two other native (American dunegrass/*Elymus mollis*) and non-native (American beachgrass/*Ammophila breviliguta*) species in Oregon dunes, the incidence of AMF in the two non-native beachgrasses was high (European, 46.8%; American, 41.5%), but almost half that found in the native American dunegrass (74.5%), and AMF infection rates showed no relationship with biomass of either native or non-native species (David et al. 2016). In Chilean dunes, AMF colonized non-native European beachgrass in both foredunes and secondary dunes (i.e., fixed dunes behind foredunes), with colonization rates higher in the latter contrary to trends documented in European systems (Gonzales et al. 1995). Some limited sampling conducted at Point Reyes found that mycorrhizal colonization of beachgrass roots at AT&T-South/North Beach averaged 79.3 ±1.3% (SD), ranging narrowly

between 78.0% in the backdunes to 80.7 ± 7.0% in the mid-dunes (Parsons et al. 2022; Appendix H). Root colonization results for backdunes need to be interpreted cautiously, as the two other backdune root samples were not evaluated (Parsons et al. 2022). Slightly higher variability in AMF root colonization rates appeared to exist between sampling transects, with average colonization rates ranging from 70.5 ± 5.0% (ATT-S-2) to 87.0 ± 9.9% (Parsons et al. 2022).

Three major AMF taxa were detected in soil samples collected in 2017 and 2020, *Glomeromycetes, Paraglomeromycetes,* and *Archaeosporomycetes*, as well as the following genera: *Acaulospora, Archaeospora*, *Claroideoglomus*, *Diversispora*, *Glomus*, *Paraglomus*, *Rhizophagus*, and *Scutellospora* (Parsons et al. 2022-Appendix H). All three major taxa were present in moderately to heavily invaded dunes at AT&T/North Beach in 2017, with Glomeromycetes and Paraglomeromycetes being significantly elevated relative to native Dune Mat, where AMF was not detected (Parsons et al. 2022). Differences in Archaeosporomycetes abundance among dunes at AT&T and North Beach of varying invasion status was only weakly elevated and weakly significant (p=0.09; Parsons et al. 2022). At B Ranch North, Archaeosporomycetes showed higher abundance in heavily invaded dune soils compared to those from sparsely or moderately invaded habitats, possibly because the latter was dominated by iceplant, not beachgrass (Parsons et al. 2022). In contrast, as noted earlier, Paraglomeromycetes appeared to be more abundant in sparsely invaded late successional native Dune Mat soils than in moderately to heavily invaded habitats (Parsons et al. 2022).

In 2020, soil sampling efforts focused specifically on backdune habitats of varying invasion status at Abbotts, AT&T, and North Beach, specifically heavily beachgrass-invaded, herbicide-treated, and native Dune Scrub (Parsons et al. 2022). In 2020, soil abundance of Paraglomeromycetes did not differ between backdune habitats, but numbers of Glomeromycetes did, with invaded backdunes having seemingly higher abundance of Glomeromycetes than uninvaded native Dune Scrub (Parsons et al. 2022). Beachgrass-dominated backdune soils also appeared to have higher numbers Archaeosporomycetes, although differences were not statistically significant (Parsons et al. 2022). In both sampling years (2017/2020), herbicide-treated dunes appeared to quickly revert to pre-invasion levels of Glomeromycetes and Archaeosporomycetes after treatment, with herbicide treated habitats being statistically equivalent to uninvaded either native Dune Mat or Dune Scrub habitats (Parsons et al. 2022).

Invasive plants can either arrive with AMF already present in intact root systems, or they may be mycorrhizal generalists that can take advantage of AMF species already present in the new home (Pringle et al. 2009). Several studies have shown that invaders, once established, can increase abundance of AMF or influence abundance of particular AMF species that facilitates growth of the invader, but suppresses that of native plant species (Niu et al. 2007, Inderjit and van der Putten 2010, Zhang et al. 2010). While most of the evidence supporting this feedback mechanism comes from systems other than dunes, one study did show that germination of European beachgrass in iceplant-invaded soils resulted in lower aboveground biomass than in non-iceplant-invaded soils, which may relate to suppression of beneficial mycorrhizal interactions by non-mycorrhizal iceplant (de la Peña et al. 2010). Results from the 2017 soil sampling at B Ranch North suggest that invasion by beachgrass may be associated with a shift in

composition of mycorrhizal taxa, with abundance of Paraglomeromycetes decreasing and that of Archaeosporomycetes increasing in heavily beachgrass invaded areas: This needs to be confirmed in future studies (Parsons et al. 2022-Appendix H). Invasives can also target symbiotic mutualistic organisms such as nitrogen-fixing bacteria associated with the root systems of legumes. Studies identified increased pathogenicity and possibly reduced symbiosis as negatively influencing vigor of the late successional nitrogen-fixing dune shrub species, *Hippophaë rhamnoides* (Oremus and Otten 1981).

Whether soils in currently invaded areas supported different microbial communities prior to invasion than current "native dune refugia" that increased their vulnerability to invasion or whether active planting and spread of invasives broke down initial biotic resistance and subsequently changed soil biota is unknown (Parsons et al. 2020). The fact that islands of remnant native dune have persisted at Point Reyes for decades despite being surrounded by a sea of beachgrass points to some possible role of soil biota in resisting invasives when native communities are intact and not overwhelmed by anthropogenic or natural disturbance events as postulated by Suding et al. (2013).

Dune Vegetation

In general, species composition in dune vegetation along the Pacific Coast of North America does not vary greatly relative to latitude (Pickart and Sawyer 1998). The most significant ecological boundary for dunes on the Pacific coast occurs at 37 degrees North, where foredunes shift from being herb-dominated in the south and grass-dominated in the north (Barbour et al. 1976). Species composition, as well as species richness, appears more strongly correlated with distance from the shoreline than geographic location (Barbour 1978, 1992) or physical dune forms (Parker 1974).

Dunes are a harsh environment for plants. In general, constant cycles of sand burial make available critically limiting nutrients that normally would not be available, given the lack of organic matter in the soil, as well as the very poor nutrient-binding characteristics of sand (Pickart 1997). Specifically, it appears that P, N, and K^+ are most limiting in sand dunes and appear to have the ability to restrict and/or bolster growth habits of native dune plants, as well as non-native species such as European beachgrass (Willis 1965).

The type of communities and assemblage of species within communities can be strongly driven by physical processes and structure within dunes. Initially, scientists felt that vegetation communities within dunes were determined by salt spray, but in later years, more have come to think that communities are shaped by species' ability to tolerate sand burial (Maun and Perumal 2002). Another study found that species composition in dunes was driven by exposure to wind (Lortie and Cushman 2007), which in itself may be indirectly related to the degree of sand movement and burial.

Very few coastal plant species have the ability to survive burial of greater than 1-2 feet at a given time. Non-native European beachgrass has a distinct advantage over many native dune plants in that it thrives in areas of increased sand burial and is adapted to survive single burial events of up to 3 feet (Ranwell 1958, Huiskies 1979). Only a few other plant species at the Seashore have shown resilience and vigor in response to sand burial of greater than 1-2 feet at

a single time – salt rush (*Juncus lbreweri*), Pacific wildrye (*Elymus pacificus*), and Tidestrom's lupine are surprisingly capable of surviving and growing through such large-scale sand deposition. Species that are not adapted to sand burial through physiological adaptation mechanisms such as adventitious roots (European beachgrass) or rapid vegetative growth rates cannot survive these burial events. In this way, sand burial has the ability to "reset" the structure of a plant community to favor burial-tolerant species.

Representative Communities

American Dunegrass: This plant community is mostly restricted to the foredune area of a coastal dune system. Prior to development and the introduction of non-native species, native American dunegrass was common along the U.S. west coast from Monterey northward. Currently it can be found only at Point Reyes (near Abbott's Lagoon) and the Humboldt Bay dunes (Pickart and Sawyer 1998). This community is ranked by the Nature Conservancy as critically imperiled, which means five or fewer global occurrences of fewer than 2,000 acres remain. Vegetation in the native dunegrass community is sparse (25%-75% cover) and is dominated by American dunegrass. Other associated species include beach bursage (*Ambrosia chamissonis*), Douglas's bluegrass (*Poa douglasii*), beach morning glory (*Calystegia soldanella*), yellow sand-verbena (*Abronia latifolia*), and beach evening primrose (*Camissoniopsis cheiranthifolia*).

Dune Mat: Dune mat communities are comprised of mat-forming vegetation that is often variable and sparse. Species include beach bursage, dune sagebrush (*Artemisia pycnocephala*), coast buckwheat (*Eriogonum latifolium*), Douglas's bluegrass (*Poa douglasii*), yellow sand-verbena, beach pea (*Lathyrus littoralis*), beach strawberry (*Fragaria chiloensis*) and beach evening primrose. Dune mat habitat is considered rare and has been severely impacted by European beachgrass (Pickart and Sawyer 1998). Many federal, state and locally listed species are restricted to Dune Mat communities, including the federally endangered beach layia (*Layia carnosa*; FE) and Tidestrom's lupine, as well as northern curly-leaved monardella (*Monardella sinuata* ssp. *nigrescens*; California Native Plant Society (CNPS) List 1B.2-Proposed) and dune gilia (*Gilia capitata* ssp. *chamissonis*; CNPS List 1B.1). In 2012, bluff wallflower (*Erysimum concinnum*) was also listed (CNPS List 1B.2): The Seashore reputedly has one of the largest remaining populations of this species. At Limantour, a few patches of pink sand-verbena (*Abronia umbellata* var. *breviflora*; CNPS List 1B.1) also occur along the beachward side of the dunes where European beachgrass and iceplant grow intermixed.

Native dune vegetation communities occur throughout the Seashore's dunes, but they are often isolated remnants of once large communities that are now fragmented by expanding monocultures of European beachgrass and iceplant. At the three general areas prioritized for near-term restoration, approximately 25 acres of Dune Mat have been mapped at B Ranch, with 8.5 acres mapped at AT&T. Dune Mat vegetation also exists at North Beach and Davis Property, with a very small amount in the northern portion of A Ranch. No Dune Mat has been mapped at Limantour specifically, although pockets of Dune Mat vegetation may exist within invaded areas.

Species within these Dune Mat communities seem to differ slightly depending on age or

successional status of the dunes. Younger dunes with less differentiated soils (primary successional) support beach bursage, beach pea, beach morning glory, yellow sand-verbena, Tidestrom's lupine, and non-native species such as European searocket (*Cakile maritima*). Slightly more mature dune systems support vast numbers of Douglas's bluegrass, as well as beach evening primrose, although numbers of the latter are also often very high in stabilized dunes (mid-successional). As dune systems become even more stabilized – perhaps because they are surrounded by European beachgrass or iceplant stands that demobilize dunes or because they are on the inland edge of the inland-migrating dune systems – the composition of dominant species shifts again slightly towards species such as coast buckwheat, dune sagebrush, sea thrift (*Armeria maritima*), gumplant (*Grindelia stricta*), sand mat (*Cardionema ramosissimum*), and woolly lotus (*Acmispon heermanii*; late-successional). A few species appear to occur across a range of successional stages, such as seaside daisy (*Erigeron glaucus*; mid- to late successional), beach layia (primary to mid-successional), and beach evening primrose (mid- to late successional).

Dune Scrub: In the inland portions of active dune fields, a more shrub-dominated vegetation community can occur. Dune scrub once established on stabilized dunes stretching from Bodega Head to Los Angeles, but, by the 1970s, only Point Reyes-Dillon Beach and Morro Bay-Purisima Point still supported sizeable Dune Scrub areas.

Most of the Dune Scrub establishes in the more stabilized backdune areas. A study from 1979 defined at least four (4) predominant Dune Scrub communities. On stabilized dunes, bush lupine was dominant, with yarrow (*Achillea millefolium*), ripgut brome (*Bromus diandrus*), and sheep sorrel (*Rumex acetosella*) also present (Holton and Johnson 1979). A second association also occurred on stabilized slopes on inland sites and included bush lupine, mock heather (*Ericameria ericoides*), and coyote brush (*Baccharis pilularis*), with beach bursage and curly-leaved monardella as understory species (Holton and Johnson 1979). This tends to be a drier, more open type of site than in the first association (Holton and Johnson 1979). Deep, recently reworked sands on the top of ridges supports a community dominated by chamisso lupine (*Lupinus chamissonis*) and mock heather, with bush lupine much more infrequent (Holton and Johnson 1979). Even more exposed locations tend to be established only by chamisso lupine (Holton and Johnson 1979).

Intermediate between Dune Scrub and Dune Mat are two associations that are colonized by more herbs and sub-shrubs than the other associations. Bush lupine and lizard tail (*Eriophyllum staechadifolium*) occur on exposed slopes nearest the ocean (Holton and Johnson 1979). Even less stable sands play host to a mix of lizard tail and beach bursage, with Douglas's bluegrass and dune sagebrush also high in cover, particularly on semi-stable dunes immediately behind the foredunes (Holton and Johnson 1979). Bush lupine showed strong association with the highest nitrogen and organic matter content and smallest particle size of any of the scrub species (Holton and Johnson 1979). In terms of distance from the ocean, mock heather was restricted more to inland sites, while lizard tail occurred at more seaward locations: Dune sagebrush showed no relationship with distance to ocean, possibly because it colonizes recently disturbed sites, but is eventually replaced by other dune shrub species (Holton and Johnson 1979).

In general, most of these Dune Scrub areas have now been invaded by European beachgrass or iceplant, with Dune Scrub existing principally as “pockets” of native shrubs within dense European beachgrass stands or strongly intermixed within European beachgrass stands. Currently, most of these Dune Scrub pockets are dominated by coyote brush and mock heather, although other species may occur, including poison oak (*Toxicodendron diversilobum*), California blackberry (*Rubus ursinus*), bush lupine, bracken fern (*Pteridium aquilinum*), wild cucumber (*Marah fabaceus*), miner’s lettuce (*Claytonia perfoliata*), and fairy mist (*Pterostegia drymarioides*). Some limited Dune Scrub exists at AT&T/North Beach, B Ranch/A Ranch/Davis Property, Abbotts Lagoon, and at Limantour, although its extent has not been conclusively mapped.

Dune Slack Ponds or Swale Wetlands: In addition to rare foredune communities, many dune systems also include “dune swale” or “dune slack” wetlands. Coastal interdunal wetlands are common components of larger active and stabilized coastal dune fields, ranging from Coos Bay, Oregon, and south to San Luis Obispo County, California. They can be referred to as "slack dune ponds" when associated with larger and deeper water or "coastal dune swales" when water is shallow. In general, most of the dune swales and slacks within the Seashore tend to be large meadow or seasonal wetland features in low basins behind active foredunes or narrow drainages between dune ridges that are oriented perpendicular to the shoreline. Seasonal or even perennial ponding occurs when either sand supply is disrupted or sand is blown out, leaving certain areas closer to the groundwater table, or when dune movement interrupts and even “dams” surface water flow.

While obviously wetter than the adjacent dune communities, these seasonally wet areas may not be wet enough to be regulated as wetlands by regulatory agencies such as the U.S. Army Corps of Engineers (USACE). In some cases, only the lowest elevation portions of these distinct areas are wet enough to qualify as regulated wetlands. Most of these areas would be regulated by the California Coastal Commission (CCC) as species are typically listed as at least facultatively hydrophytic or occurring in wetlands at least 50% of the time, and the CCC only requires one parameter to be met to qualify as a wetland subject to oversight under the Coastal Act.

The vegetation at these sites typically falls into the sedge series and is dominated by a mix of sedge and rush species. Slough sedge (*Carex obnupta*), dune sedge (*Carex pansa*), and salt rush are the dominant species of the seasonally wet sedge series. Associated species include Pacific silverweed (*Potentilla anserina*), springbank clover, (*Trifolium wormskioldii*), marsh baccharis (*Baccharis douglasii*), beach starwort (*Stellaria littoralis*; CNPS List 4), and California blackberry. A few wetlands even support sparse stands of arroyo willow (*Salix lasiolepis*; Baye 2008). Cover commonly approaches 100% (Pickart and Sawyer 1998). Dune swale wetlands with a mix of meadow and riparian species represent an unusual plant association that is uncommon in central California coast dunes, although none of the component species are themselves that rare (Baye 2008). They appear to be intermediate between typical dune swale wetlands and freshwater fens, which are characterized by freshwater marsh and wetland scrub vegetation in low-flow systems that form on acidic sandy substrates (Baye 2008). One of these features occurs at Abbotts Dunes.

European beachgrass-dominated Foredunes and Backdunes: European beachgrass is a native perennial grass of Europe and North Africa introduced to California in the late 1800s to help stabilize blowing sand dunes. It is now recognized as an invasive species in North America, Australia, and New Zealand (Johnson 1982, Heyligers 1985, Wiedemann 1987). Throughout its range, beachgrass grows in sand dunes and adjacent coastal habitat (i.e., wetlands, grasslands, etc.). However, it grows most vigorously on open foredunes comprised of mobile and semi-fixed sand, where it tolerates extremes of substrate instability, water drainage, nutrients, organic matter content, and soil texture (Huiskes 1979). Beachgrass can actively grow in soil pH varying from 4.5 - 9.0 and soil temperatures varying from 10 - 40°C, although it appears less tolerant of higher salt concentrations, preferring levels between 1.0 and 1.5% (Slobodochikoff and Doyen 1977; Pickart 1997). In Britain, beachgrass tends to occur in two communities – mobile dune, where it is the dominant species, and semi-fixed dunes, where sand is less mobile, and European beachgrass becomes less competitive (British National Vegetation Classification System).

Because of this extreme adaptability, European beachgrass became a popular choice for stabilizing unstabilized dunes in the United States. European beachgrass was first planted on the west coast of North America at Golden Gate Park in San Francisco in the late 1800s (Lamson-Scribner 1895, Lamb 1898). The species was heralded as a desirable sand stabilizer and was eventually embraced by U. S. Soil Conservation Service and other agencies (Pickart 1997). Along the Pacific Coast, European beachgrass has spread to inhabit areas from Santa Barbara County, California, in the south to Canada in the north. Initially, beachgrass invasion appeared more severe in areas north of San Francisco more than in areas south, but, in recent years, beachgrass has been apparently rapidly expanding in some central and southern California coastal dune systems, including Guadalupe-Nipomo Dunes and Vandenberg Air Force Base (Chestnut 1997).

While European beachgrass was present in San Francisco as early as the late 1800s, there is no reliable information as to when this species arrived in the Seashore, which is approximately 30- to 40 miles northwest of San Francisco Bay. Anecdotal information suggests that ranchers and other landowners probably planted both European beachgrass and iceplant to stabilize the dunes: The Seashore did not acquire most of these lands until 1963 or later. Historical aerial photographs from the 1940s show scattered pockets of European beachgrass at Abbotts Lagoon and other beach areas such as the dunes near B Ranch. Since then, this species has rapidly spread along the Great Beach and at Limantour Beach. Beachgrass tends to be more prevalent than iceplant along some of the northern sections of the Great Beach, stretching from North Beach northward to north of Abbotts Lagoon up to Kehoe, and at Limantour Beach. While European beachgrass is present south of North Beach, extending to the dunes near the Lighthouse, iceplant often either co-dominates certain areas or is actually even more dominant. The reason for this geographic pattern in extent of particular invasive species is unknown, but may be related to what species was originally planted in particular dune areas by landowners prior to park ownership and to the structure of the dune system present.

European beachgrass predominantly spreads vegetatively through rhizomes or belowground root structures. Past research has documented that, as plants are buried by blowing sand, their shoots can grow up to 3.3 feet per year (Ranwell 1972, Barbour et al. 1985), however, other

studies have documented rates as high as 6.6 feet in six months (Bossard et al. 2000). If broken off, these rhizomes can re-establish plants from even the smallest rhizome fragment (<2 inches). Based on recent excavations in dunes at the Seashore, the depth of rhizomes can differ between foredune and backdune systems: In the foredune, rhizomes can extend anywhere from 6 to 12 feet, while in the backdune, rhizomes tend to be shallower, averaging between 3 and 6 feet. This agrees well depths from other studies, which showed a range between 3.3 feet (Salisbury 1952) to as much as 15 feet (Buchenau 1889).

The differences in backdune and foredune rooting depth may relate to a phenomenon known as the "Ammophila problem" (Marshall 1965) in which a lack of fresh sand accumulation results in reduced vigor of European beachgrass: Plants produce less roots (Wallén 1980, Gray 1985), which may potentially relate to the fact that roots of European beachgrass in stabilized dunes are infected by microorganisms that reduce formation of new tillers (van der Putten et al. 1988 *in* Pickart and Sawyer 1998). Fresh sand accumulation also provides nutrients (Pickart 1997) and inputs of arbuscular mycorrhizal fungi (symbiotic organisms that improve water uptake and nutrient acquisition) and prevents build-up of pathogens (van der Putten et al. 1988 *in* Pickart and Sawyer 1998). Apparently, fragments can even withstand prolonged seawater immersion, with viable fragments surviving in cold marine waters for as long as eight (8) tidal cycles (Baye 1990), although the plant itself can tolerate lower salt concentrations in dune soils than native plants (1.5%; Huiskes 1979).

Seed of European beachgrass is viable, but establishment by seedling is not common as vegetative establishment (Pickart and Sawyer 1998, Huiskes 1977) and may be more likely to occur only under certain climatic conditions or in certain types of microhabitat. A study in Great Britain found that seed only germinated in places where sand was comparatively damp, specifically at the base of slopes where direct rainwater concentrations and leaching was highest (Huiskes 1977). Most critically, seedlings of beachgrass require some form of retained moisture, decreased sand burial, and an overlay of damp, clay-like soil (Huiskes 1977).

Once established, European beachgrass tends to dominate in both foredune and backdune areas at Point Reyes, although community structure differs slightly. In foredune areas, cover of beachgrass tends to be dense, but other plant species that can establish and persist in "pockets" of open sand within the beachgrass stands include beach bursage, beach pea, sea rocket, yellow sand-verbena, beach morning glory, and Douglas's bluegrass. This community occurs extensively in the northern portion of the Great Beach, near AT&T/North Beach, and in certain areas of the southern portion of the Great Beach such as B Ranch/A Ranch/Davis, as well as along Limantour Spit. European beachgrass-dominated backdunes are also characterized by high densities of European beachgrass, but occur further inland than 160 feet from the beachfront. In some backdune areas, the vegetation cover includes a sizeable percentage of native shrubs -- coyote brush, mock-heather, and bush lupine – and herbs. This community occurs in the central and northern portions of the Great Beach, including at AT&T, and more sporadically further south such as at B Ranch, A Ranch, and Davis Property.

Iceplant-dominated Communities: Iceplant, a succulent, prostrate member of the Aizoaceae family, was introduced to California in the early 20th century to stabilize dunes adjacent to railroad tracks and was later planted along highways and on coastal sand dunes (Albert 1995a).

Its popularity as a planting along California's highways and freeways has led this species (*Carpobrotus edulis*) to be also called, "freeway iceplant." At one point, the California Department of Transportation was maintaining almost 6,000 acres of this species along roadways (Schmalzer and Hinkle 1987). *C. edulis* comes from South Africa, but the origins of another member of this family, *Carpobrotus chilensis*, is disputed (Albert 1995a). *C. chilensis* is not considered invasive, but hybrids of *C. edulis* and *C. chilensis* are (Albert 1995a). Unless otherwise noted, when this report refers to iceplant, it is denoting *C. edulis*.

Iceplant invades a number of coastal plant communities in California, including grasslands, dunes, and coastal scrub (D'Antonio 1993). Over time, in these habitats, this species forms dense, clonal mats up to 20 in thick that may overtop even native shrubs (D'Antonio and Mahall 1991). This succulent spreads both vegetatively and by seed. In one study, recruitment of seedlings accounted for only 1% to 2% of the change in cover measured over four years, suggesting that clonal growth often represents the primary means of expansion (D'Antonio 1993), although this is not always the case (de la Peña et al. 2010). Germination of iceplant seeds shows no effect from fire, but is enhanced by passage through animals' digestive system, with animals being important dispersers of *C. edulis* seeds (D'Antonio 1993). Seeds can remain viable in soils for at least two years, and the species is both self-fertile and self-compatible, meaning that it can successfully fertilize itself (D'Antonio 1990, Suehs et al. 2004). In terms of vegetative reproduction, a single individual of iceplant, specifically *C. edulis*, can form dense, circular mats over 33 feet wide and up to 20 inches deep (D'Antonio and Mahall 1991). This species can spread radially at rates high as 3.3 feet per year by growing from multiple axes and rooting where nodes can contact the soil (Wisura and Glen 1993).

While iceplant can be sensitive to water stress (MacDonald et al. 1984), this species can employ a special type of mechanism called Crassulacean Acid Metabolism (CAM) that increases its ability to tolerate water-limited, nitrogen-limited, and saline environments (Schmalzer and Hinkle 1987). Other abiotic and biotic factors that affect iceplant viability include frost sensitivity (MacDonald et al. 1984), scale insects introduced to California from the species' native South Africa (Tassan et al. 1982, MacDonald et al. 1984, Washburn and Frankie 1985), and hybridization (Albert 1995b, Albert et al. 1997). In one study, only 25% of the plants retained the original parental genotype, leading researchers to conclude that the overall species complex in California appears characteristic of a so-called hybrid swarm (Albert et al. 1997).

Iceplant can obviously directly affect other plant species by displacing them and even overgrowing them, which reduces biomass, life span, and reproductive output of adjacent plants (Pickart and Sawyer 1998). Iceplant reduces native species diversity (Vilà et al. 2006, Carboni et al. 2010), the fitness of neighboring native plants (D'Antonio and Mahall 1991), and the pollination network in invaded communities (Bartomeus et al. 2008). As discussed previously, it also has indirect effects, as well, by lowering pH, reducing nitrogen content and available water, increasing organic matter and carbon, and even changing the soil biota (higher levels of chytrid fungi and pathogens; lower levels of mycorrhiza (D'Antonio and Mahall 1991, Vilà et al. 2006, Conser and Connor 2009, de la Peña et al. 2010, Santoro et al. 2011). The species' dense fibrous root system, which is concentrated in the upper 25 inches of the soil, may enable it to compete more effectively for water with native plant species (D'Antonio and

Mahall 1991).

These direct and indirect effects work differently in different environments to promote iceplant establishment: In one study, iceplant established more readily in dunes than in coastal scrub due to lower abundance of brush rabbits and, therefore, reduced herbivory intensity (D'Antonio 1993). In grasslands, already established plants precluded establishment of iceplant seedlings, with disturbance and creation of gaps in the canopy cover required for successful seedling recruitment (D'Antonio 1993).

In dunes, resistance to invasion by iceplant in portions of its non-historic range may be mediated by soil biota (Vilà et al. 2006, de la Peña et al. 2010), with the resistance eventually broken down over time by the prolific seed rain (>1,000 seeds per fruit; de la Peña et al. 2010). As noted above, expansion by iceplant changes the soil by negatively affecting water availability for other plants, reducing soil pH, and altering nutrient and potentially soil biota dynamics (higher levels of chytrid fungi and pathogens; lower levels of mycorrhiza; D'Antonio and Mahall 1991, Vilà et al. 2006, Conser and Connor 2009, de la Peña et al. 2010, Santoro et al. 2011). These soil changes may cause iceplant to switch from sexual to asexual reproduction modes (de la Peña et al. 2010). In other regions, iceplant invasion appears to be successful, because the species is not affected by pathogenic species that are normally present in its native dune soils (Reinhart et al. 2005, van Grunseven et al. 2009 *in* de la Peña et al. 2010).

Over time, iceplant can develop into extensive carpets or mats that exclude almost all other species, but prior to this coalescence of patches, other species can often subsist within "pockets" of open, sandy areas within iceplant stands. The presence of iceplant appears to drive the successional status of native dune "pockets" towards a more mature, late successional community supporting species such as coast buckwheat, dune sagebrush, sea thrift, sand mat, and woolly lotus. A few species appear to occur across a range of successional stages, including beach evening primrose (moderately to highly stabilized), which can be quite common in iceplant-dominated dunes. Iceplant becomes more dominant between North Beach and South Beach and further south near B Ranch, A Ranch, Davis Property, and at the Lighthouse. Most of the iceplant at Limantour Spit occurs intermixed with European beachgrass, although there are approximately 9 acres of pure iceplant patches.

Secondary Invaders: Another threat to successful re-establishment of native dune habitats comes from so-called "secondary invaders." Secondary invaders are classified as species that are not the primary removal target, but that take advantage of decreases in cover of the primary target species and increases in available space to gain a foothold and potentially oust native species. Secondary invasion has been documented as issues for many invasives removal-related restoration projects (Pearson et al. 2016).

At Abbotts Lagoon, the most abundant secondary invaders in mechanically restored and native dune areas were European searocket (*Cakile maritima*), along with New Zealand spinach (*Tetragonia tetragonoides*), European black nightshade (*Solanum nigrum*), and iceplant (*Carpobrotus* spp.). These species have also encroached on other dune restoration areas, including at B Ranch North and AT&T, but the Seashore has actively countered these threats through both manual removal and herbicide treatment. A different suite of secondary species impacted herbicide-treated backdune areas, including ripgut brome (*Bromus diandrus*), fescue

brome (*Festuca bromoides*), common groundsel (*Senecio sylvestris*), and sow thistle (*Sonchus* spp.). Bush lupine is also problematic in backdunes, particularly at Abbotts.

Spread of secondary invaders appears strongly tied to several factors, including available space freed up by eradication of primary invaders, landscape disturbance associated with restoration (both mechanical removal and herbicide treatment), short-and long-term climatic cycles, and potentially increases in soil nitrogen associated with breakdown of dead beachgrass and colonization by the disturbance-adapted, nitrogen-fixing bush lupine. For example, at Abbotts, two secondary invaders are often found in close association with either live or dead bush lupine (New Zealand spinach and European black nightshade), suggesting that these new weeds are benefitting from nitrogen supplementation of soils by lupine.

Of these secondary invaders, European searocket and bush lupine currently appear to pose the most threat. In terms of sheer acreage, European beachgrass and iceplant are definitely the "primary" invaders at the Seashore, but European searocket and bush lupine have definitely made inroads at some of the dune systems, Abbotts Lagoon in particular. By summer 2016, European searocket and these other species had established in relatively moderate to dense abundance in a large percentage of the mechanically restored areas at Abbotts Lagoon, as well as in native dunes that have been affected by restoration by inland movement of destabilized sands. In certain instances, European searocket was actually blanketing some of the extensive Tidestrom's lupine colonies that had established in the mechanically restored areas.

European searocket reproduces by seed, which is distributed by ship ballasts, sand transport, tidal movement, and human activity (Cal-IPC 2012). In comparison to native dune plants, it produces more seed, disperses greater distances, and tolerates more disturbances (Cal-IPC 2012). It has a greatly extended flowering season that lasts almost year-found, at least in Point Reyes. Few sources of information could be found on seedbank longevity, but California Invasive Plant Council's Plant Assessment Form characterized the seedbank as relatively short-lived in foredune environments (at least 2 years; Knapp and DiTomaso 2005). However, "if *C. maritima* can survive into a second or even a third year of reproduction, it can produce an 18-fold difference in seed output" (Knapp and DiTomaso 2005). Seed production is increased during colonization, with plants allocating more resources to seed production than plant survival, and the species has high fecundity, with seed set often being 90% (Knapp and DiTomaso 2005). Ironically, native deer mice – which have impacted Tidestrom's lupine populations in the Seashore – also acts as a predator of European searocket seeds, potentially removing as much as 95% of the seed in some instances and, thereby, resulting in only 20% survivorship (Knapp and DiTomaso 2005). However, burial of seeds by moving sands can help them escape mice predation (Knapp and DiTomaso 2005).

Laboratory experiments demonstrate that the plant may have allelopathic effects, but these effects have not been observed in field experiments (Cal-IPC 2012). Disturbance rather than allelopathy appears to be how European searocket and some of the other secondary invaders gain a foothold in restored dunes. Secondary invaders such as European searocket and New Zealand spinach often subsist in relatively low abundance until a disturbance event provides them an opportunity to dramatically expand in numbers. In a sense, they are "sleeper weeds" that often exhibit a "lag phase" between establishment and large-scale expansion, waiting until

conditions become right for exponential growth (Groves 1999, Hui and Richardson 2017, Crooks 2005).

At Abbotts, the disturbance trigger for searocket population expansion was, undoubtedly, the restoration project, either directly through restoration activities or indirectly through deposition of remobilized sands. Not only does restoration remove potential competitors (European beachgrass and iceplant), but both mechanical removal and herbicide treatment created create open space that is more subject to wind and wave disturbance. Searocket also thrives on Camp Pendleton's beaches and dunes, which are continually disturbed by the military conducting tank maneuvers (B. Klink, GSRC, *pers. comm*.). In fall 2015, contractors removed 10 tons of European searocket plant material from the 7-mile weed treatment area at Camp Pendleton (Klink, *pers. comm*.).

Other Invasive Species: Bush lupine is native to California, but its historic range apparently incorporated only central and southern California. In 1902, Davy reported that bush lupine did not occur north of Point Reyes, while the Jepson Manual (1993) listed the historic range as Sonoma County south to Ventura. Botanical experts still question whether this species is native to Marin County (Pickart and Sawyer 1998, Baye 2008). There are some indications that bush lupine was planted with European beachgrass for stabilization and reclamation of coastal dunes after 1870 (McLaren 1924) and was introduced to North Coast dunes where it is highly invasive (Baye 2008).

As with other coastal areas in Sonoma County, bush lupine establishes in both grasslands and dunes (Pickart and Sawyer 1998). Without grazing, numbers of this species can increase dramatically in the park's coastal grasslands. In dunes at the Seashore, it can co-occur with European beachgrass in backdune areas, but it appears much less prevalent in these invaded communities than coyote brush and mock heather. At Tomales Dunes, it dominated the Dune Scrub community, however, and, after initial restoration at Abbotts, it established well in open sand conditions, as well as backdunes, within the park's dunes. In addition to altering soils as discussed earlier and, therefore, potentially altering vegetation communities, this species is also known to hybridize with other native lupine species such as manycolored lupine (*Lupinus variicolor*) and seashore lupine (*Lupinus littoralis*), so it could threaten viability of other lupine species within the dunes, including Tidestrom's lupine and chamisso lupine (Baye 2008).

COASTAL DUNE RESTORATION EFFORTS ALONG PACIFIC COAST

Land management agencies up and down the Pacific Coast have been experimenting with approaches for coastal dune restoration for decades. Some of the most well-known projects are in Humboldt, which focused primarily on manual removal using shovels or other hand-held tools. Early results from a Humboldt dune restoration project suggested this method showed considerable promise: the project successfully eliminated beachgrass, had relatively low re-treatment needs in the second year, and increased native plant cover from 2.7% to 38%, although it required re-treatment every week to every two (2) to four (4) weeks for approximately nine (9) months (Pickart and Sawyer 1998). The success of this project prompted larger projects to be conducted at Humboldt in 1992 and 1997, which also proved successful in terms of eliminating beachgrass and encouraging native species to colonize (Pickart and Sawyer 1998). Retreatment by hand required at least eight (8) visits during the first year and seven (7) visits the second year, followed by additional treatment during a third year (Pickart and Sawyer 1998).

A few agencies have used mechanical removal for treatment of European beachgrass. The U.S. Forest Service (USFS) first used mechanical removal at the Oregon Dunes National Recreation Area (Pickart and Sawyer 1998). Several projects conducted at this area between 1995 and 1996 excavated European beachgrass and its rhizomes to a depth of 3.3 feet and then "capped" these sands with another 3.3 feet of sand (U.S. Forest Service 1995 a,b *in* Pickart and Sawyer 1998). However, apparently, the depth of excavation and burial was inconsistent, and moderate to substantial resprouting occurred the next spring (Pickart and Sawyer 1998). Another technique used by the Bureau of Land Management (BLM) at Coos Bay Shorelands involved use of a ripper to sever rhizomes and break up soil compaction, followed by hand removal of re-sprouts. While this appeared successful, the program managers had previously applied saltwater to the beachgrass plants, which, while not successful, may have weakened them and affected treatment results (Pickart and Sawyer 1998).

In recent decades, the USFS has at Oregon Dunes used a combination of mechanical techniques, including "scalp and bury," which removes 2.5 feet of the upper European beachgrass sand layer and buries it in pits approximately 6 to 8 feet in depth, or a scalp-and-push technique, which scalps vegetation to a 6- to 8 feet depth and then pushes materials onto beach for redistribution by wind and tidal action (USFS 2010). Many of these areas have been re-treated several times apparently (USFS 2010), which underlines the issue that European beachgrass does recolonize following this type of mechanical treatment.

In 2004-2005, California Department of Parks and Recreation (CDPR) conducted a pilot project experiment on mechanical removal at Little River State Beach in California. The project compared three different approaches to mechanical removal, including one that primarily involved excavators (Excavator), one that involved excavation by D8 or D850 bulldozers to a depth of 3 feet and burial of beachgrass with a 2-foot cap similar to the "scalp and bury" technique at Oregon Dunes (Dozer-Grade), and one that involved bulldozers with a rake attachment capable of pulling up rhizomes from 2 feet deep (Dozer-Rake; Transou et al. 2007). Re-treatment effort was 30 percent lower in the Dozer-Grade areas than in the other two treatments (Transou et al. 2007). Treatment results, combined with cost, led CDPR to conclude

that mechanical removal was the most effective beachgrass removal method (Transou et al. 2007). The success of this pilot project encouraged development of an 80-acre dune restoration project at Little River that used mechanical grading to remove beachgrass.

A number of restoration efforts along the Pacific Coast have used chemical control to treat European beachgrass. Chemical control originally involved almost exclusively use of glyphosate (Pickart and Sawyer 1998). Indeed, some early studies suggested that glyphosate might not have been very effective for removal unless applied at higher concentration rates (10%; Aptekar, *in prep*. *in* Pickart and Sawyer 1998). In Monterey, a 7% solution of glyphosate was successfully used to treat 10.4 acres at Sunset State Beach (Hyland and Holloran 2005). Initial experiments with a grass-specific product, Fusillade®, did not adequately control the invasive, so, later, the project area was first burned to eliminate thatch and stimulate regrowth before herbicide was applied (Hyland and Holloran 2005). Subsequent herbicide treatments occurred at 6 and 12 weeks later, with a total of five (5) follow-up treatments required to achieve 100% control (Hyland and Holloran 2005). By the sixth treatment, total herbicide used had dropped from 100 gallons to 2.5 gallons (Hyland and Holloran 2005).

Over time, many agencies have switched to using imazapyr with glyphosate for treatment, which appears to be more effective than just glyphosate and to require less retreatment. Importantly, lower concentrations of these chemicals can be used apparently without sacrificing efficacy: Glyphosate concentrations have dropped from 10% in those early projects to 1% in more recent restoration efforts (Pickart and Sawyer 1998), whereas imazapyr concentrations have remained fairly constant (1-1.5%). In addition, as noted earlier, some agencies have experimented with use of pre-treatment measures such as prescribed burning or mowing to remove beachgrass thatch and stimulate re-growth and improve uptake of herbicide (Hyland and Holloran 2005). Some agencies have reported that use of this particular herbicide combination has controlled almost 80- to 90% of the European beachgrass present with as little as one (1) to three (3) treatments.

Based on information presented at a North American Pacific coastal dune restoration symposium in January 2016, most land managers are using a combination of manual removal, mechanical removal, and herbicide treatment to eradicate invasives, with prescribed burning often used as a pre-treatment method for herbicide. Most of these projects relied on chemical treatment using some combination of imazapyr and glyphosate as the primary tool for initial treatment, but sometimes used mechanical and manual for treatment in certain areas or for re-treatment.

COASTAL DUNE RESTORATION AT POINT REYES NATIONAL SEASHORE

Since 2001, the Seashore has removed approximately 271 acres of non-native invasive plants from approximately 535 acres of coastal dunes.

Restoration at Abbotts Lagoon

Because of concerns about impacts of invasive plants on native dune habitat and threatened and endangered species, the Seashore began a concerted dune and coastal bluff restoration

process in 2001. Early on, park staff worked on removal of European beachgrass from the mouth of Abbotts Lagoon, and, starting in 2002, iceplant was removed from 100 acres of rocky coastal bluff near the Lighthouse. During the first few years of restoration at Abbotts, removal efforts focused on manually digging up European beachgrass, which has deep rhizomes that can extend anywhere from 3 to 12 feet or more (Figure 2). Because it was difficult to dig deeper than 1.5- to 3 feet manually, the beachgrass rapidly regrew. In 2007, one park staff member estimated that 20% of the treated area re-grew within as little as six months, and some areas required as many 15- 20 repeat treatments before control appeared to be achieved (Charles 2007, Peterson 2004).

These issues encouraged the Seashore to explore other approaches for removing at least European beachgrass. In 2004-2005, a 20-acre mechanical project was conducted in the same vicinity as the hand removal ones, but with more successful results: It appeared to be more practical – and perhaps more effective -- than hand removal ones, particularly for larger areas. Excavators were able to dig deeper and then bury and "cap" the rhizome-contaminated materials with clean sand. Because of this success, the park submitted a proposal to the federal government to fund a much more ambitious restoration project that would mechanically remove 132 net acres in an approximately 300 gross acre project area just south of Abbotts Lagoon (Figure 3).

The first phase of the Abbotts Lagoon Coastal Dune Restoration Project moved forward in early 2011. Unfortunately, bids for the project came back extremely high, so the project area was reduced to 80 net acres in an approximately 190-acre gross project area. The park focused the Phase I restoration efforts around the approximately 95-acre native dune area that was, at this point, completely surrounded by extensive stands of European beachgrass in both the foredunes and backdunes (Figure 3): The native dune supported the park's largest population of Tidestrom's lupine, as well as large populations of beach layia, curly-leaved monardella, and other rare plants. Hand removal of European beachgrass and iceplant was performed in the native dune in native dune and sensitive habitats such as wetlands and areas adjacent to wetlands.

While mechanical removal again appeared, at least initially, to be highly successful in eradicating European beachgrass similar to the earlier project, it was also highly expensive ($25,000 to $30,000/acre) due to the depth of excavation required (from 6- to 9 feet) to bury rhizome and biomass-contaminated material with a sufficient "cap" of relatively clean or less rhizome-contaminated sand (3 feet), so that buried materials could not re-sprout readily.

The Seashore wanted to build upon these seemingly very ecologically successful dune restoration efforts by expanding restoration southward, but it realized that it needed to shift its removal strategy to more cost-effective ones, such as those utilized by restoration projects conducted in some other California coastal state parks. Many of these state parks have used a combination of fire and herbicide to successfully treat these invasive species. As discussed earlier, the mixture of glyphosate with imazapyr has been particularly successful in treating almost 80- to 90% of the European beachgrass with as little as one to three treatments (William Maslach, CDPR, *pers. comm*.; Tim Hyland, CDPR, *pers. comm*., Brendan O'Neill, CDPR, *pers. comm*.).

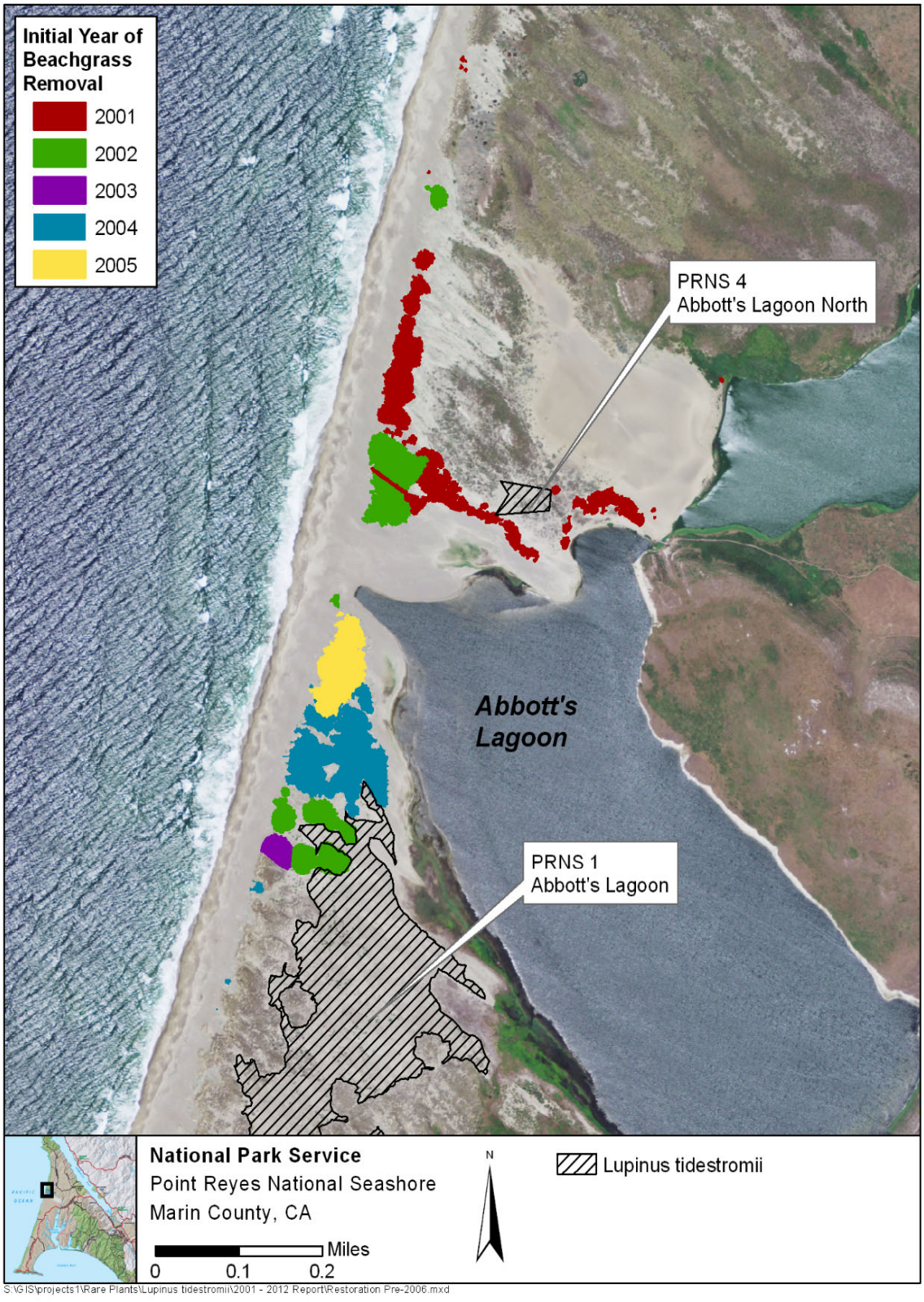


Figure 2. Location of restoration between 2001 and 2005 at Abbotts Lagoon.

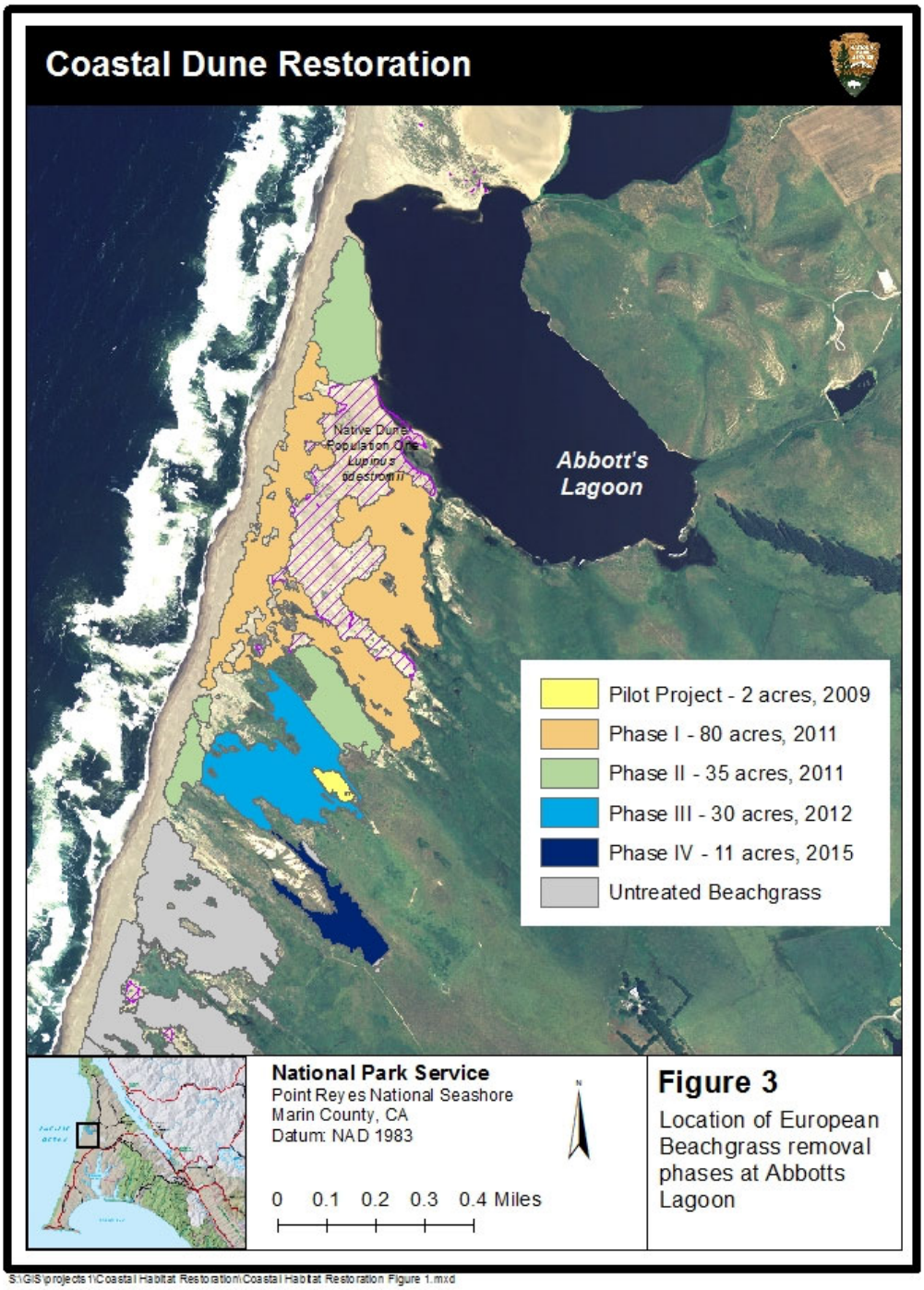


Figure 3. Location of initial restoration efforts between 2011 and 2015 at Abbotts Lagoon

To help develop the best methods for cost-effectively treating European beachgrass in the park, the Seashore initiated Phase II, which treated another approximately 31 acres using an experimental approach (Figure 3). The 31 acres included a portion of the area near the mouth of Abbotts Lagoon treated between 2001 and 2005 in which beachgrass had re-established due to insufficient re-treatment after 2005. Because various state park units have used different concentrations of glyphosate to successfully treat European beachgrass, two different concentrations of glyphosate (2% and 7%) were used with a uniform imazapyr concentration (1%), with treatments assigned randomly to plots in backdune and foredune stands to determine whether effectiveness varied based on physical location or age of stand. This pilot project was implemented in fall 2011, and the Seashore's early monitoring efforts showed that more than 95% of the beachgrass appeared to have been eradicated with just one treatment and that there appeared to be no differences due to glyphosate concentration. In addition, as part of this project, the dead aboveground biomass was physically removed from half of at least the southernmost Phase II plots in fall 2012 to determine whether biomass removal through mowing or shallow bulldozing improved and/or sped up colonization by native dune plant species: European beachgrass decomposes very slowly. Approximately 5.95 acres of backdune area were either mowed using a skid-steer loader with a rotary mower attachment or scraped with a bulldozer, while approximately 2.95 acres of foredune were mowed. Other methods used by state parks for reducing beachgrass biomass – and improving herbicide efficacy – such as controlled burning were not included as part of this experimental project due to the fact that more recent restoration efforts showed that treatment could be just as effective without burning (B. Maslach, *pers. comm*.), and burns can be difficult to conduct in the Point Reyes area due to highly variable climatic conditions.

Restoration efforts continued south of Abbotts Lagoon in the original project area in fall 2012 with herbicide treatment of another 30 acres of European beachgrass, with mechanical removal used in 25-foot buffers adjacent to ranchlands managed as organic pasture and hand removal used in wetlands (Figure 3). Based on the results from the Phase II project, which showed no noticeable differences between use of 2% versus 1% glyphosate, the Seashore elected to use a lower concentration of glyphosate (2%, AquaMaster®, currently marketed as Round-Up Custom®), as well as 1.5% imazapyr (Habitat®), 1% non-ionic vegetable oil surfactant (Competitor®), and 1.5% blue dye. Follow-up treatment of Phases I-III continued between 2012 and 2019.

The final phase of the Abbotts Lagoon project, comprised of 11 acres on Ammophila Mountain (Phase IV), was sprayed in 2015, completing at least one round of initial treatment of all European beachgrass (and iceplant) within the initial 255-acre Abbotts Lagoon Project Area.

Starting in 2018, staff also chemically treated some of the secondary invaders such as European searocket and New Zealand spinach. Between fall 2016 and summer 2020, 4,188 control hours were spent manually removing secondary invader species from Abbotts and the northern portion of AT&T, with more than 1,640 hours coming from limited staff resources. In comparison, chemical treatment hours – which largely focused on searocket -- totaled 96 in 2018, 103 in 2019, and 60 in 2020, although treatment efforts were circumscribed by issues associated with COVID-19 and a wildfire within another portion of the park.

In addition, in 2019 and 2020, a concerted effort to manually thin bush lupine from Phase II and III portions of the Project Area using weed wrenches, loppers, and pruning shears took place. Following two years of concerted manual efforts, most of the shrubs left were large, adult ones that could not be removed using weed wrenches. In 2021, after completing the necessary environmental compliance, the park moved forward with a low-volume drizzle approach (20% Round-Up Pro®; 30% Competitor®; 1% blue dye) and sprayed using a contractor crew approximately 14.1 net acres of bush lupine over 28.3 acres of restored backdune and Dune Scrub interface areas. Control hours more than doubled in 2021 due to easing of COVID-19 restrictions and initiation of chemical treatment for bush lupine.

Chemical re-treatment of most primary and secondary invaders has been conducted every year since initial treatment, with the exception of Ammophila Mountain (Phase IV), which is treated every other year.

Restoration at B Ranch North

In fall 2013, the park also initiated restoration efforts at B Ranch near the B Ranch North population, where Tidestrom's lupine numbers have decreased dramatically over the last decade. Due to this rapid decline, the Seashore requested funding from the USFWS to conduct an emergency project to halt the expansion of iceplant and remove plants that were directly displacing lupine from its habitat.

The first phase of the project involved hand removal of approximately 1.5 net acres of iceplant in 15.1 gross acres located in the B Ranch North population (Figure 4). Iceplant removal was not conducted at B Ranch South, even though it was included in compliance efforts. Follow-up treatment of iceplant removal at B Ranch North occurred in 2014, 2015, and 2016, with more opportunistic re-treatment between 2017 and 2021.

In September 2014, the Seashore conducted an initial treatment using herbicide of 14.9 net acres of European beachgrass in a 20.9 gross-acre project on the perimeter of the B Ranch North population (Figure 4). PRNS focused treatment efforts in a horseshoe-shaped project area around the Tidestrom's lupine, avoiding areas marked as "downwind" beachgrass areas (Figure 4). Herbicide treatment involved application of 2% glyphosate (Round-Up Custom®, formerly marketed as AquaMaster®), 1.0% imazapyr (Habitat®), 1.0% non-ionic vegetable oil surfactant (Competitor®), and 1.0% blue dye. Removal of European beachgrass along the perimeter was intended to reduce seed predation pressure, as previously discussed studies by Washington University in St. Louis, Missouri, have demonstrated that seed predation is reduced dramatically at least 75 m from the nearest European beachgrass stand. Follow-up treatment was conducted annually between 2015 and 2021, with another half-acre (0.53) of initial treatment performed at the Project Area's most northwestern boundary in 2019. Control hours have dropped from 58.5 in 2018 to 14.5 in 2021.

Restoration at ATT

In fall 2015, the park initiated restoration efforts at a third site, AT&T, which is directly south of the Abbotts Lagoon Coastal Dune Restoration Project Area. The project not only benefits federally listed species such as Tidestrom's lupine, beach layia, western snowy plover, and

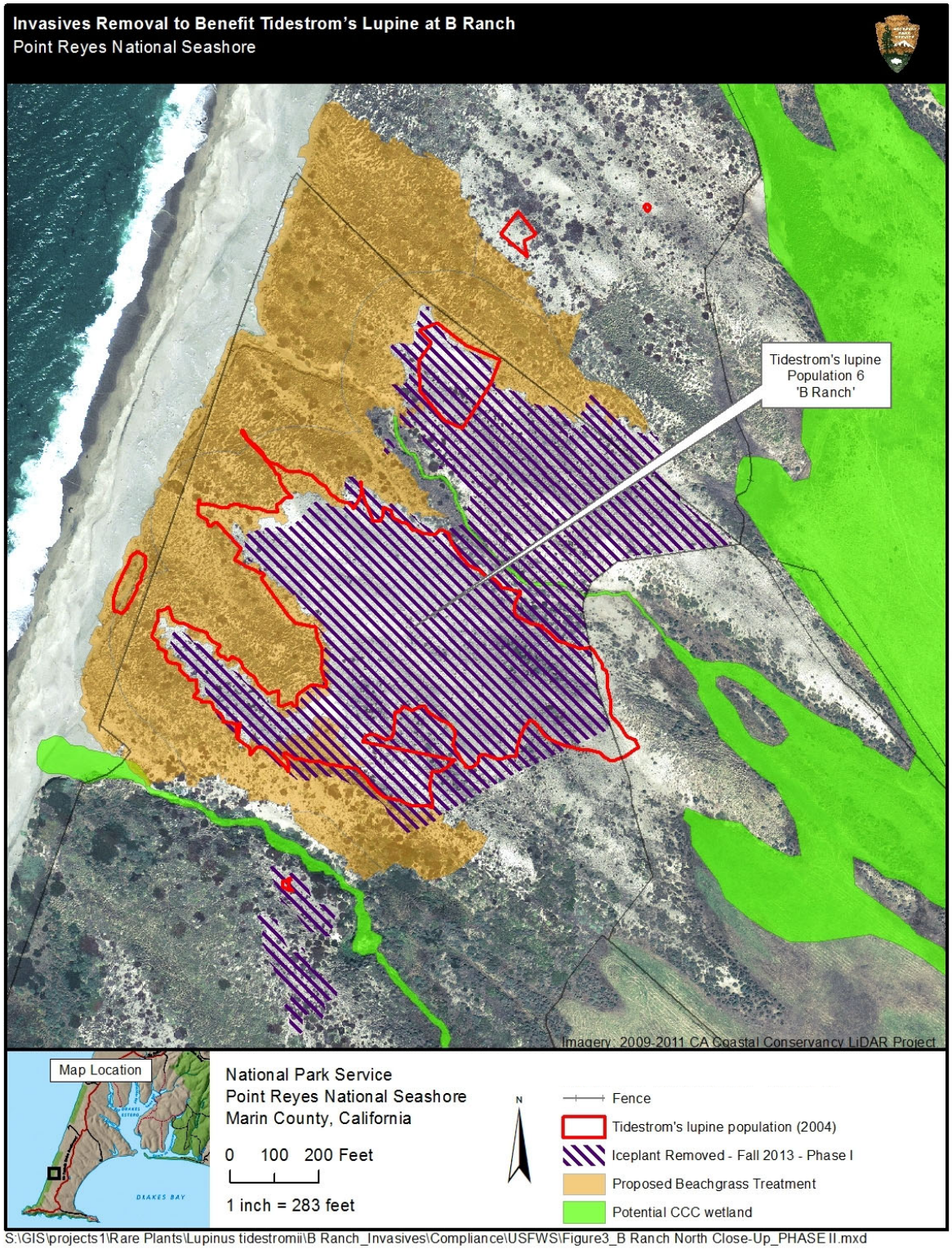


Figure 4. Location of initial restoration efforts at B Ranch North between 2013 and 2014

Myrtle's silverspot butterfly, but it expands the restoration envelope southward from Abbotts Lagoon, potentially creating almost 400 acres of unfragmented restored coastal dune habitat. The Seashore received funding from the National Park Service (Park Service), with additional funding raised by its non-profit organization, Point Reyes National Seashore Association (PRNSA) from National Fish and Wildlife Foundation for this project, which expanded treatment areas southward.

The first phase of the project in 2015 involved mechanical removal of approximately 2.4 acres of European beachgrass along a dune swale wetland within the AT&T project area where herbicide treatment could not occur (Figure 5). Another 13.5 gross acres of European beachgrass in the AT&T foredunes was mowed using a front-loader with a mower attachment to reduce the amount of biomass that would need to be treated the subsequent year (Figure 5).

In August-September 2016, contractors hired by the Seashore conducted an initial treatment using herbicide of 37.7 net acres of European beachgrass in a 68.0 gross-acre project focused on AT&T North or the north side dune swale wetland that roughly divides the project area in two (Figure 5). As with other projects, herbicide treatment involved application of 2% glyphosate (Round-Up Custom®, formerly marketed as AquaMaster®), 1.0% imazapyr (Habitat®), 1.0% non-ionic vegetable oil surfactant (Competitor®), and 1.0% blue dye. In 2016 and 2017, 4.79 net acres of dense iceplant in AT&T North was also treated with herbicide.

Initial treatment of European beachgrass areas south of the dune swale wetland occurred in summer 2017 and 2018, along with re-treatment of sprayed areas in AT&TNorth. AT&T South Phase I in 2017 treated 15.75 net acres of European beachgrass intermixed with iceplant and 0.3 net acres of standalone iceplant (Figure 5). Removal of beachgrass and iceplant was expanded further southward in 2018 with implementation of Phase II, which involved 16.6 acres of European beachgrass/iceplant treatment and 0.2 acres of scattered iceplant treatment (Figure 5). Most of the funding for AT&T South initial treatment and re-treatment came from National Fish and Wildlife Foundation through the grant awarded to PRNSA, with Park Service funding paying for AT&T North and a small portion of AT&T South (3.25 acres). In 2019, a small area in AT&T North just north of the Alopecurus Swale (but outside the buffer/fence) was initially treated (0.1 acre). By 2019, total acreage for area restored at AT&T North and AT&T South using both sources of funding was 74.7 acres of non-invasives in a 133.7-gross-acre coastal dune area.

Re-treatment has been conducted annually. Control hours have dropped from 110.7 in 2019 to 62.75 in 2021.

Restoration at Davis Property

Starting in fall 2016, the park and PRNSA began collaborating with volunteers to conduct manual removal of dense iceplant from Tidestrom's lupine population areas at Davis. A number of different volunteer groups have come out to assist at Davis, including Sierra Club service trips, Novato High School Yosemite Club, and a Boy Scout troop from Sonoma County. During this period, volunteers made significant inroads in pushing back the areal extent of iceplant from Dune Mat areas supporting Tidestrom's lupine, as well as beach layia and curly-leaved monardella. Iceplant was left on site, but pulled iceplant was piled to increase the footprint of

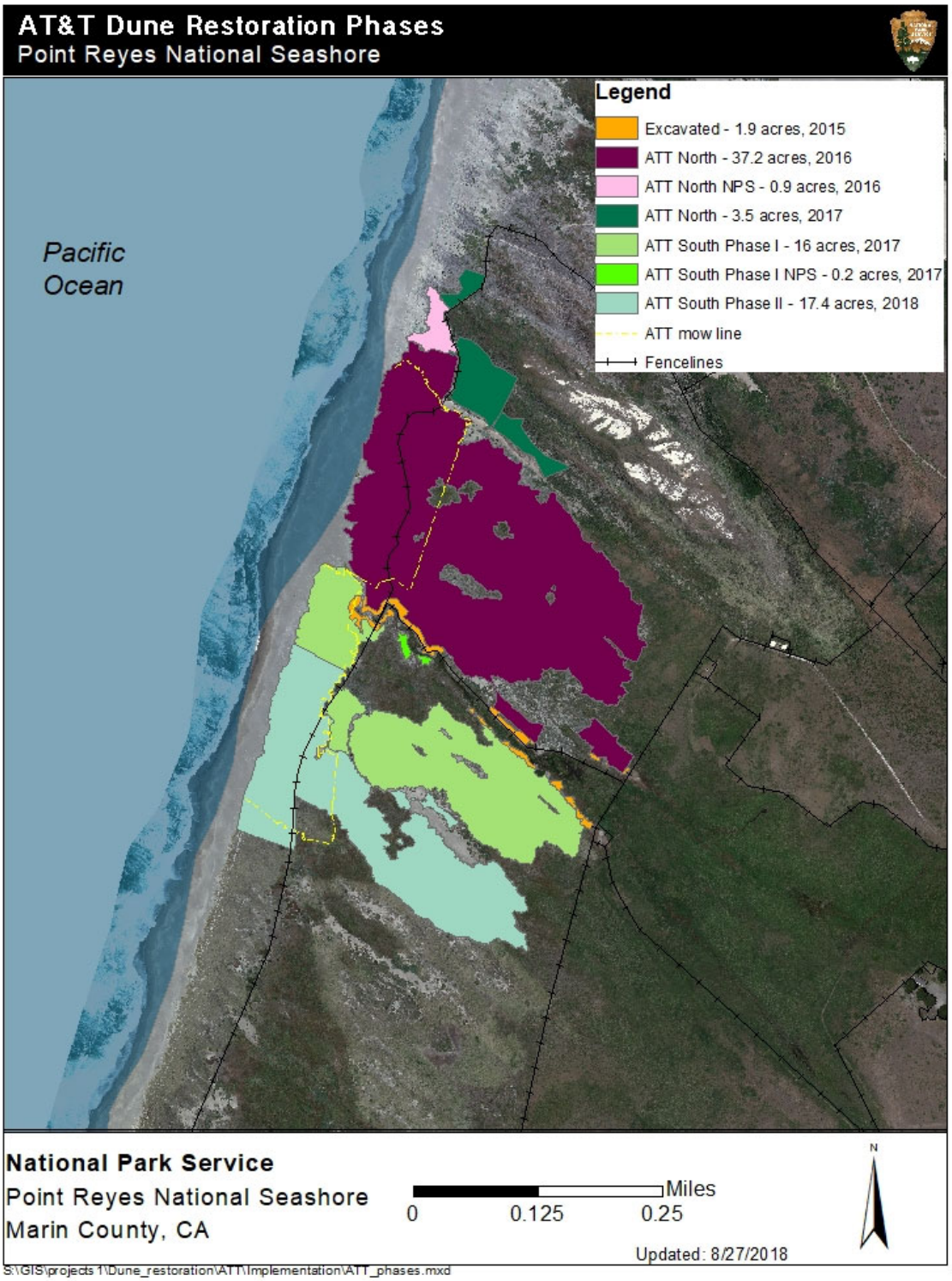


Figure 5. Location of restoration efforts at AT&T Coastal Dune Restoration Project Area (2015-2018)

non-invaded areas. By early 2020, 543 control hours had been expended manually removing iceplant from 2.3 acres, some of which was re-treatment of previously treated areas. COVID-19 restrictions greatly compromised volunteer efforts in the remainder of 2020 and 2021, but park staff did conduct one day of manual removal of iceplant in fall 2021. Park staff also chemically treated sparse European beachgrass patches in 2020-2021.

MONITORING SUCCESS OF RESTORATION EFFORTS

A key, but often unperformed, component of restoration projects is monitoring the success of restoration efforts. Funding is often not available to perform monitoring other than effectiveness monitoring aimed at determining whether re-treatment is necessary. Equally important, however, to the future of these restoration efforts is determining how successful the restoration project has been in changing and, hopefully, improving physical and biological conditions and ecological health of park resources.

At the Seashore, most of the monitoring associated with dune restoration has involved assessment of vegetation cover, number or areal extent of endangered plants, and Western snowy plover nesting attempts. In addition, some projects have attempted to evaluate changes in sand movement topography related to removal of invasive plant species, most of which alter natural physical processes and conditions in coastal dunes.

Early Monitoring Efforts at Abbotts Lagoon

Vegetation and sand movement monitoring was performed as part of the 2001-2005 Abbotts Lagoon project. Twenty-two 10X10-m macroplots were installed in a number of the areas treated both manually and mechanically (15) and in native dune areas (7) to assess changes in vegetation cover, European beachgrass stem density, and bareground (Peterson et al. 2003). The number of beach layia was counted, and the perimeter of the Tidestrom's lupine areas was mapped (Peterson et al. 2003). This monitoring continued after the restoration project was completed by researchers at Washington University until 2008, when monitoring efforts were discontinued. Sand movement was monitored in at least the first two years by measuring the height of sand along 1 m tall posts marking a corner of each permanent vegetation plot.

While results were never formally analyzed, some preliminary analyses suggested that percent cover of native vegetation in treated areas had reached equivalency with native dune areas by three years post-restoration, but that native species richness and evenness was considerably lower (Kroiss, unpub. data). Sand moved as part of restoration efforts, but, in general, this movement appeared more related to redistribution of sand within the restoration area, creating more natural topography and contours (Peterson et al. 2003).

Monitoring Efforts at Abbotts Lagoon

In 2009, the Seashore initiated a pre- and post-restoration monitoring program for the Abbotts Lagoon Coastal Dune Restoration Project. The monitoring program ultimately incorporated vegetation monitoring of both dunes and adjacent grassland, rare plant censuses and sampling, and inland dune movement or "creep." This report specifically focuses on dune vegetation monitoring.

Early on, staff decided against using the same vegetation monitoring protocol as was used for the earlier Abbotts Lagoon (and the Paleodunes) restoration projects. Subsampling larger macro-plots ultimately reduces the power of vegetation monitoring efforts as all the subsamples must be averaged for each macroplot, ultimately decreasing sample size while increasing sampling effort.

Staff reviewed the literature on monitoring for other coastal dune restoration projects throughout the world, but these projects have not been as extensively monitored as other types of vegetation systems. One approach used by several studies involved establishment of line transects perpendicular to the coastline, but this was considered too logistically complicated given the very steep and undulating pre-restoration terrain at Abbotts.

Similar to many other restoration projects, the monitoring program relies on a modified BACI sampling approach (Stewart-Oaten et al. 1986, Underwood 1991). "BACI" refers to monitoring of an "impact" (I) area both "before" (B) and "after" (A) an activity is implemented, with concurrent monitoring of "control" (C) areas. Based on this sampling design, we have been evaluating the Project Area (Abbotts Lagoon Project Area) before and after restoration was implemented and have been using native Dune Mat communities within the Project Area boundaries to better discern the effects of the restoration "impact" relative to natural variability.

Vegetation monitoring at Abbotts is being conducted using a different protocol based on establishment of 2X1 m plots throughout the Project Area, including both Phase I (mechanical removal) and Phase II/III (Herbicide Removal). The Project Area was divided into four sampling areas for the purpose of plot selection. Areas with European beachgrass were classified as "Invaded," and areas still supporting native dune vegetation were classified as "Native Dune" or "Reference." The Native/Reference and Non-Native/Treatment areas were then further divided into Foredune and Backdune strata for the purpose of random stratification. (Statistical analyses incorporated distance to the ocean as a covariate.) Within each sampling area, individual sampling plots were selected by placing a grid over a map of the Project Area. Each 50m by 50m grid square housed a single, computer-generated, random sampling plot location. A subset of the plot locations was randomly selected by hand within each sampling area. There are now 62 2X1-m monitoring plots in the 131-net-acre Project Area (Appendix A-1).

Selected UTMs were located in the field using a Trimble GeoXH handheld GPS unit with beacon. Plots were oriented in a north-south direction centered on the GPS coordinate. Marking consists of two pieces of rebar – one inside the southwest corner of the 2X1-meter sampling quadrat plot, and one inside the northeast corner – each with a copper tag indicating plot name and corner. Two (2) photos are taken of each plot – one directly of the plot and one including background landscape.

Cover of vegetation and other functional groups is assessed using a 2X1-m PVC quadrat separated into 10 X 10 cm grids that allows for estimation of cover using the point-intercept method, resulting in a maximum of 171 sampling points. The quadrat is positioned near the height of vegetation such that the grid plane is parallel to the general slope of the ground surface. A notepad is used to mark down the plot number, and then the quadrat and notepad are photographed from the south end. At each sampling point, plant species present (both alive

and dead) are recorded, along with functional groups such as bareground and detritus. The presence of plant species at each sampling point is only recorded once, even if there are multiple point-intercept "hits" at that point. Standing dead biomass is considered a dead plant, but if the biomass falls to the ground as thatch or breaks down further into smaller organic matter, it is recorded as "thatch" or "detritus," respectively. Monitoring is conducted between May and July to capture both annual and perennial species. If spring annuals are present that have senesced already, park staff make every effort to conduct "forensic" plant identification. Once cover assessment is finished, all species rooted within or crossing into the plot that were not detected during point-intercept sampling are recorded, and the number of individuals for rare plant species is counted.

For Native Dune areas, the full suite of 171 points is "read," but after the first few years, the number of sampling points within Invaded Dunes was reduced by half, because of the length of time it was taking to conduct the monitoring effort: Preliminary data analyses indicated that reducing the number of points did not alter data quality.

The 2X1 monitoring effort started in 2010, although some plots were added later in 2011 or 2012. A large percentage of the plots was installed prior to restoration, although a few Mechanical plots were added subsequently. The Seashore has been assessing Mechanical, Herbicide, and Native Dune plots almost every year since installation. Full monitoring was conducted in 2017 and 2019, but, in 2018, only herbicide-treatment plots were monitored. In 2020-2021, all of the Herbicide and Mechanical plots were monitored, with a portion of the Native Dune plots also monitored in 2020.

As noted earlier, in 2015, the park initiated restoration of the final area designated for invasives removal under the original Abbotts Lagoon plan – Ammophila Mountain. Vegetation in this area had not been incorporated into the 2X1 m monitoring effort being conducted elsewhere in the Project Area, so, for this 13-gross-acre area, photopoints were established within the treatment area and were assessed annually until 2019. Photographs were not taken in either 2020 or 2021.

Sand movement inland from Abbotts Lagoon has been assessed by walking the eastern edge of the dunes where it borders grassland/pasture. Sand burial was mapped using a hand-held GPS where there was 50% or greater cover by open, barren sand or a combination of sand and widely spaced Dune Mat- or Dune Scrub-characteristic vegetation compared to grasslands or wetlands (i.e., where there was <50% cover by grassland or wetland vegetation, with a relatively visible organic component on the surface). Starting in 2014, surveys focused specifically on inland sand migration into adjacent non-dune habitats and included mapping of the dune edge not only adjacent to mechanical removal areas, but adjacent to herbicide treatment areas, as well. In 2015, park staff started to notice that some "sand sheets" or large areas where sand had blown into former grassland or wetland areas were being colonized by vegetation: as vegetation could help to stabilize these sand sheets or "fingers," they were also mapped when vegetation cover exceeded 20%. Sand movement at Abbotts has been mapped through 2017; no monitoring was conducted between 2018 and 2021 due to lack of staff resources. These results are discussed under Chapter 1 in "Dune Processes."

2021 marks the 10th anniversary of the Mechanical phases of the Abbotts project, along with

the initial phases of the Herbicide treatment. Monitoring frequency of Herbicide and Mechanical plots will be reduced in future years to every other year starting in 2022 for at least six years, with a final monitoring proposed for Year 20 at 2031.

Recent Monitoring Efforts at Abbotts Triangle

After monitoring efforts were developed and implemented at Abbotts Lagoon, the Seashore recognized that additional treatment would be necessary within the 2001-2005 Project Area at Abbotts Lagoon if the park wanted to create an expanse of unfragmented, restored coastal dune that stretched from the mouth of Abbotts Lagoon to Ammophila Mountain or the boundary with AT&T. Treatment at Abbotts Triangle – as this area became known – involved exclusively herbicide treatment. Park staff wanted to monitor vegetation community changes and success in eradicating European beachgrass, but, for reasons discussed earlier, did not want to use the macroplot methodology originally established to assess success of the earlier project.

However, park staff also had concerns about the applicability of the 2X1-m quadrat method for Dune Mat or sparsely vegetated native dune communities. The point intercept method is one of the least biased and most objective measures of cover. However, species with low cover values such as Dune Mat species are often not sampled efficiently because points rarely intersect the species of interest. Also, detecting small changes, especially at low cover values, is problematic. Finally, the commonly used method of lowering a pin into the vegetation is subject to the bias with which the observer angels and moves the pin. The pin method may also overestimate cover – especially for narrow-leaved species – due to the diameter of the pin (Elzinga et al. 1998).

For these reasons, park staff experimented with a different vegetation monitoring method for this area. This method involves using sampling vegetation species frequency at uniformly spaced distances along uniformly spaced “line” transects. Frequency measurements can be more sensitive to small changes at low cover values than other types of sampling. This makes frequency sampling good for measuring changes in small, sparsely distributed species, as well as for detecting the recruitment of new species, including undesirable invaders (Elzinga et al. 1998).

Transect locations are established in GIS, and for sampling, a GPS unit is used to walk along each transect. These transect locations are temporary, have no markers, and are re-randomized each year. Transects run from northeast to southwest (314°), in the direction of prevailing winds, across the entire sampling area. The transect angle is meant to encompass any gradient that may occur due to wind and proximity to ocean. Transect locations are designed so that there is adequate interspersion between points along all transects (e.g., roughly 200 points within each sampling area) and between transects (with transects being located not more than twice or less than half of distance between sampling points positioned along the transect).

For this method, at each sampling point, both cover and frequency are assessed. Cover estimates are obtained using the point intercept method. Frequency sampling is conducted using nested quadrats of four sizes (10 cm, 50 cm, 100 cm, 200 cm). For frequency sampling, each quadrat is considered an individual sampling unit, assuming good interspersion. Sampling is scheduled to take place after germination of all species has occurred, but before annuals,

such as beach layia, have senesced and lost root-hold. The presence of any piece of vegetation that is attached to a rooted plant is considered present in the plot. For species that may not have fully leafed-out for the year, presence of leafless branches (possibly last-year's growth) is considered presence of that species in the plot.

At Abbotts Triangle, pre-restoration monitoring in summer 2011 involved sampling of 25 transects that had 15 m between transects and 20 m between quadrats along each transect for a total of 223 quadrats and 1,137 points. Frequency and cover monitoring took place between 2011 and 2015, except for 2014. The number of transects and quadrats/points was reduced slightly after 2011, but still averaged around 200.

In 2017, Park Service staff refined its monitoring methodology again. While adept at capturing species diversity within dunes, the frequency-transect method can be very time-consuming, particularly when repeated annually within a number of restoration areas. Also, the resulting data can be more cumbersome to analyze, as frequency of species cannot be easily collapsed into native/non-native or successional guilds as can be done with vegetation cover data. In 2017, park staff experimented with a new approach, transect-cover, that had been used already at B Ranch North. A similar grid of GIS transects is overlaid on the restoration area, but rather than collecting cover and frequency at each point, only vegetation cover is assessed. To improve the ability of this approach to discern changes in vegetation cover, the number of cover "points" per GPS transect line was increased relative to the frequency transects: every 15 feet versus every 60 feet. At each cover point, all plant species intercepted by the pointed toe of the boot were recorded, along with bareground and detritus. The sampling "point" or area on the toe of the boot was estimated to be approximately 2.5 cm wide. As it seemed successful, this approach was repeated in 2019 and 2021, 10 years following project implementation.

2021 also marks the 10th anniversary of the Abbotts Triangle project. Monitoring frequency at Abbotts Triangle will be reduced in future years to approximately every third year starting in 2023 for at least six years, with a final monitoring proposed for Year 20 at 2031.

Monitoring Efforts at B Ranch North

In conjunction with restoration efforts at B Ranch North, park staff developed a vegetation monitoring program to estimate percent cover and frequency of European beachgrass and iceplant and other plant species within treatment areas and, thereby, assess the effectiveness of restoration in eradicating these invasives and creating more habitat for native dune and rare plant species. Separate sampling areas were established for European beachgrass and iceplant treatment areas. In addition, due to rancher concerns about sand movement, park staff are also attempting to assess qualitatively change in vertical elevations within the Project Area and, thereby, infer how much sand movement may have occurred since restoration was implemented.

As B Ranch North contains a substantial amount of Dune Mat habitat and relatively moderate terrain conditions, the Seashore implemented frequency transects at this site, very similar to those developed for Abbotts Triangle. Sampling of transects first occurred for the iceplant area in 2013, which represents pre-restoration conditions. Sampling for the iceplant treatment area took place again in the summers between 2014 and 2016. Starting in 2017, the sampling

approached switched to the transect-cover approach described under Abbotts Triangle monitoring above. No monitoring was conducted in 2018, but it was in 2019, six years following project implementation. No monitoring has been conducted since then. Photopoints have been established within iceplant removal areas to track success of this project component in eliminating iceplant from within Population #6, but these have not been revisited in recent years (Appendix A-2).

Sampling for the European beachgrass area was initiated in 2014 and repeated in fall 2015, one year following initial treatment. For the first two years, the Seashore utilized the transect-frequency approach described earlier. In late summer 2016 (August), park staff moved to the transect-cover approach described under Abbotts Triangle monitoring above. To keep transect lengths reasonably approximate, the northern group of transects was divided into East and West. Transect-cover monitoring was repeated in 2017, 2019, 2020, and 2021, with 2021 representing the seventh year following implementation. The year 2021 also marks the 10th anniversary of the Abbotts Triangle project. Monitoring frequency at B Ranch North will be reduced in future years to approximately every third year starting in 2023 for at least six years, with a final monitoring proposed for Year 20 at 2034.

To monitor potential inland movement of sand at B Ranch North in response to dune restoration efforts, park staff installed transects of rebar posts throughout the restoration area. Sand movement transects are 100 m apart and are aligned in the direction of the prevailing wind, approximately 315 degrees, in the areas iceplant and beachgrass treatment areas. The transects stretch from the westernmost edge of the beachgrass to the westernmost portions of the pasture areas. Seven (7) transects were installed in 2013, and sampling points along the transects are designated by letters, with "A" closest to the ocean. Sampling points with dune areas are rebar posts with yellow caps, while those in grazed areas were rebar that had white PVC pipes installed on top. Measurements are taken to the nearest 1/8th inch: measurements can easily vary by ½" depending on technique and side measured, even when the post is relatively straight and undisturbed. During monitoring, lengths of all "sides" of the rebar or PVC pipe are measured, and an average distance to ground is calculated. Following measurements, photographs are taken standing 2 m back from the post in the oceanward direction along the entire transect. Monitoring has these rebar posts have not been conducted in the past few years, because rebar posts either knocked down by cows (in areas open to cattle) or blown sideways by winds, greatly complicating measurements of sand deposition and any meaningful interpretation of results. A better system would need to be developed to more accurately determine inland movement of sands from dune restoration efforts.

Monitoring Efforts at AT&T

Restoration efforts at AT&T North – located directly south of the Abbotts Lagoon Project Area – started in 2015.

Based on past experiences with 2X1 m plots and frequency/cover transects, park staff elected to go with 2X1 m plots at AT&T, using sample size analyses derived from results at Abbotts Lagoon to determine how many plots might be needed to adequately characterize vegetation communities. Pre-restoration surveys indicated that most of the native vegetation stands at

AT&T were primarily Dune Scrub with slightly varying species composition, as well as some Moist Meadow communities composed of rush (*Juncus*) spp.: Native Dune Mat only occurred in a few areas in the Project Area, and most of these had been invaded to some degree by iceplant, and, to a lesser extent, beachgrass. Based on results from Abbotts, the 2X1 m plots appear to function effectively in shrub- or large grass-dominated stands, but are less effective in more barren dune communities characterized by sparse vegetation and large expanses of sand. Also, the AT&T area is quite hilly, and the transect method is ill-suited to steep or sharply undulating topography, because it is difficult to maintain uniform distances between sampling points along the transects.

Separate sampling areas were established in AT&T for European beachgrass/iceplant treatment areas and native Dune Scrub communities. Pre-restoration plots were established the summer prior to treatment for each of the three phases (AT&T North/2016; AT&T South Phase I/2017; and AT&T South Phase II/2018). Using a random stratified sampling scheme within these two communities, 18 2X1 m plots were established within Dune Scrub at AT&T North, and 20 plots were installed in European beachgrass/iceplant areas (Appendix A-3). Sampling at AT&T North in 2016 established pre-restoration conditions in both invaded European beachgrass communities and in adjacent stands of relatively intact native Dune Scrub, although a few plots were installed in areas that were mechanically restored along the Alopecurus Swale in 2015. Due to the low number of plots, these were not included in the pre-restoration data analysis. Similar to the vegetation monitoring protocol for Abbotts Lagoon, the plots were monumented with rebar in the opposite corners and marked with flagging tape: locations were recording using handheld GPS with beacon (Trimble GeoXH). As part of the monitoring, two (2) photos are taken of each plot – one directly of the plot and one including background landscape.

An additional 14 plots were set up in invaded areas in AT&T South Phase I in 2017, along with four (4) plots in native Dune Scrub (Appendix A-4). An additional six (6) plots were established in 2018 in AT&T South Phase II in foredunes (3) and backdunes (3).

The AT&T South Phase I area included lightly invaded Dune Mat community that supports rare plant species such as Tidestrom's lupine and beach layia. This area was actually monitored starting in 2018 using the GPS-transect method discussed earlier for Abbotts Triangle and B Ranch North, with monitoring repeated in 2019.

In addition, 11 photopoints have been established within European beachgrass removal areas at AT&T North at high-elevation locations that provide a panoramic perspective of the restoration project, as well as eight (8) locations in AT&T South Phase I (Appendix A-5).

In 2017, the eastern or inland sand edge along AT&T was GPSed using the same technique as described earlier for Abbotts Lagoon. These results are discussed under Chapter 1 in "Dune Processes." This monitoring has not been conducted since then.

Monitoring of AT&T North and South plots have been conducted annually since plot establishment except for native Dune Scrub plots, which were monitored in 2016, 2017, and 2020-2021, and mechanical plots, which were last monitored in 2018. Due to staffing constraints related to COVID-19 and the 2020 wildfire, monitoring of Herbicide plots at both AT&T North and South were spread across two years (2020, 2021). Staffing constraints in 2020-

2021 also impacted photomonitoring, which has not been conducted since 2019.

Statistical Analysis

For 2X1-m plot vegetation, data analysis focused on comparison of cover, species richness, and diversity variables using parametric or non-parametric univariate or multivariate statistical testing, as well as species composition using multivariate ordination approaches. The analyses generally evaluated differences between 1) pre-restoration invaded areas (pre-treatment) vs. native dune reference areas (either Dune Mat or Dune Scrub); 2) pre-restoration invaded areas (pre-treatment) vs. immediate post-restoration invaded areas (post-treatment); and 3) post-restoration invaded areas (post-treatment) vs. reference areas. Dependent variables included percent cover of native species, non-native species, primary invaders (European beachgrass/iceplant), dead primary invaders, detritus, bareground, secondary invaders, as well as non-cover data such as native and non-native species richness, Shannon Diversity Index, Pielou species evenness index, and rare plant density. We also calculated percent cover of species separated into different successional types for native dune vegetation communities such as primary, mid- and late-successional native Dune Mat and Dune Scrub.

Data were evaluated to determine whether they fit assumptions of parametric testing such as homogeneity of variance and normal distribution of residuals. If assumptions were met, data were analyzed using a General Linear Model (GLM) in Minitab® (v. 17) or R (R Core Team 2021, version 4.1.2). If data did not meet assumptions, we transformed the data or employed bestNormalize (Peterson 2022) package in GLM in R, or we used a non-parametric statistical technique such as Mood Median test (Minitab®) or Permutation testing (lmPerm 2.1.0; Wheeler and Torchiano 2016) in R. If independent group variables were significant, a post-hoc Tukey test was performed to evaluate group differences. Post-hoc pairwise differences were assessed with pairwiseAdonis (Martinez 2020) or Tukey HSD, with the emmeans package (Lenth et al. 2022) used for GLM analyses. Cover and diversity data were also analyzed in R using Permanova (adonis, vegan package; Oksanen et al. 2022) with betadisper function to evaluate homogeneity of group dispersion. The park also evaluated changes in species composition among treatment types (treatment vs. control/reference) using multivariate analysis procedures such as Non-metric multidimensional scaling (NMS), Multiple Response Permutation Procedure (MRPP), and Indicator Species Analysis in either PcOrd® (Statistical Package v. 6) or R.

Summary statistics are presented with means or averages and standard errors (± S.E.). Some rare plant population data is presented with estimated total number of plants with a 90% confidence interval (C.I.). In this document, data from GIS-based line transects is presented simply as means or averages.

RESULTS/DISCUSSION

Pre-Restoration Conditions

Abbotts Lagoon: Prior to restoration, most of the restoration areas south of Abbotts supported largely non-native plant species, with non-native cover averaging 81.9 (± 4.1)% and 98.1 (± 4.2)% for mechanical removal and herbicide-treated areas, respectively (Figure 6; Table 1). These areas both differed significantly from the "reference" native Dune Mat areas, which supported only 4.8 (± 4.3)% non-native cover, almost all of which was European sea-rocket (GLM, df=2, F=116.4, p<0.0001; Figure 6; Table 1). The number of non-native species in these native dune areas was extremely low, averaging only 0.13 (± 0.07) species, significantly less than invaded dunes (GLM, df=2, F=101.0, p<0.0001, all adj. p<0.0001; log-transformed; Figure 7; Table 1).



Figure 8: Aerial view of Abbotts Lagoon Project Area in 2002 prior to any restoration. Source: californiacoastline.org

It is unclear why these native dune areas, which, in many cases, were bordered on all sides by European beachgrass, did not also become invaded (Figure 8). Historical aerial photographs taken since the 1940s show European beachgrass cover slowly increasing over the decades in the foredunes and backdunes areas adjacent to Abbotts Lagoon. Ultimately, build-up of the foredunes and backdunes due to European beachgrass stabilization led to native dunes being almost like a basin or valley in between the high-elevation beachgrass-dominated hills. Iceplant occurred in isolated patches throughout the native dunes, but these patches had not coalesced to create the dense, carpet-like mats that they had in other portions of the Seashore further south toward the Lighthouse. The opportunity for invasion was certainly there, as bareground accounted for 54.5 (± 5.4)% of the cover in native dune areas (Figure 6; Table 1). As discussed under the Background section and in a recent study at the Seashore (Appendix E), it is possible that some type of soil biota acted to protect these areas from invasion, making conditions inhospitable for large-scale colonization or expansion of invasive species and, thereby, increasing resistance of the seemingly vulnerable sparsely vegetated dunes.

Within invaded areas at Abbotts, European beachgrass accounted for most of the non-native cover, with mean values ranging from 78.5 (± 4.2)% in herbicide-treated areas to 79.5 (± 3.9)% in mechanical removal areas (lmPerm, df=2, p<0.0001; Figure 6; Table 1). In mechanical removal areas, European beachgrass represented almost the only non-native species, as reflected by the fact that non-native species richness averaged only 1.8 (± 0.2) species/2m^2 (Figure 7; Table 1). While beachgrass was also dominant in herbicide-treated areas, other non-

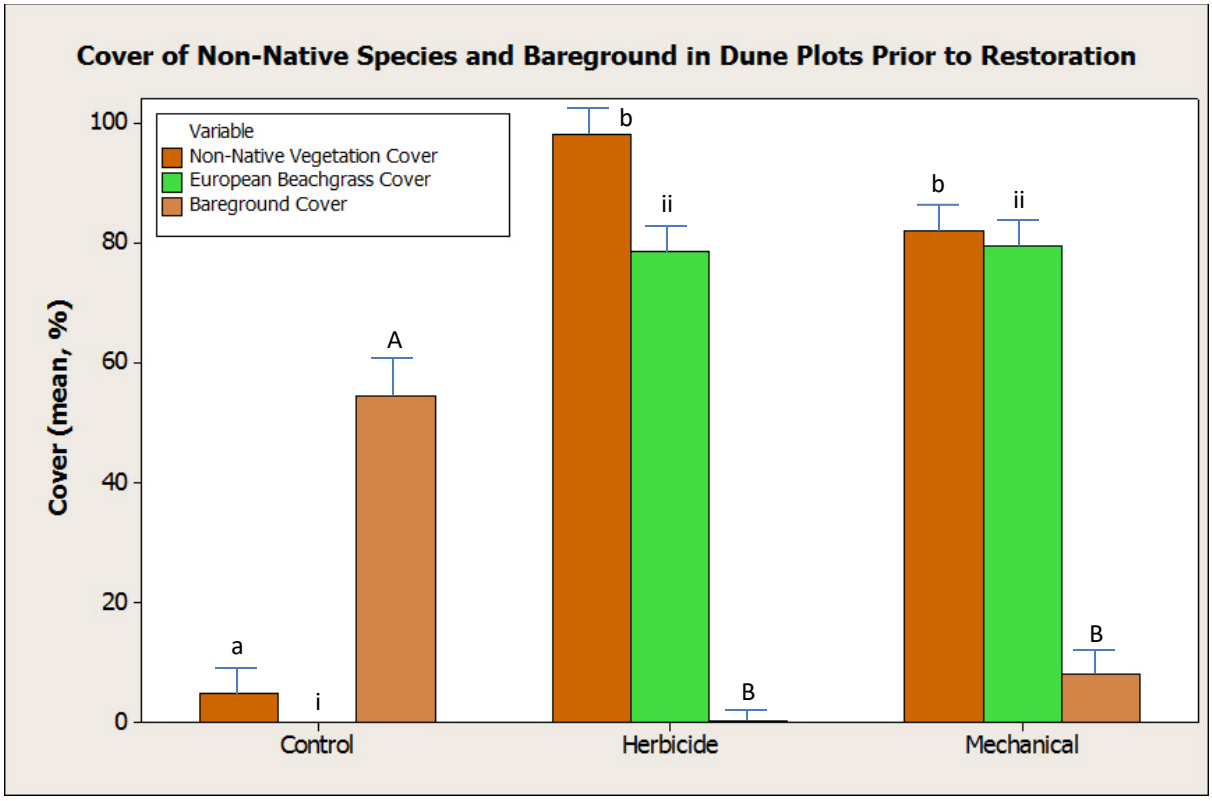


Figure 6. Mean percent cover of non-native species and bareground in pre-restoration plots at Abbotts Lagoon. Bars indicate standard error (S.E.) of the mean. Different letters/numerals indicate statistical differences between means.

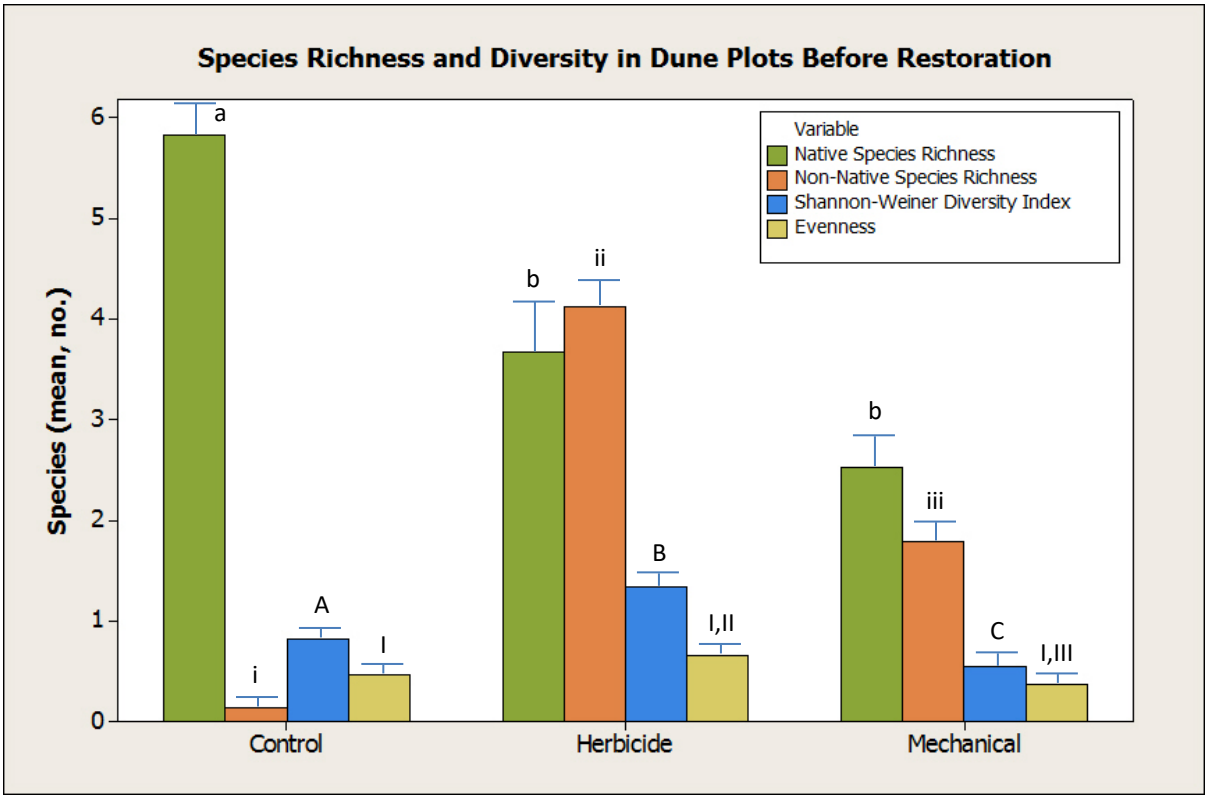


Figure 7. Mean percent cover of non-native species and bareground in pre-restoration plots at Abbotts Lagoon. Bars indicate standard error (S.E.) of the mean. Different letters/numbers indicate statistical differences between means.

native species occurred, as well, leading to significantly higher means for non-native species richness (4.1 ± 0.3 species/2m^2; GLM, adj. p<0.0001; log-transformed; Figure 7; Table 1).

Interestingly, despite the prevalence of non-native plants, native plants did occur in high numbers within all monitored areas, not just within the native Dune Mat plots. In fact, native plant species cover was higher in herbicide-treated areas (72.9 ± 1.5%) than in mechanical removal areas (31.0 ± 7.9%; GLM, df=2, F=3.89, p=0.027; adj. p=0.02; log-transformed) and, seemingly, native dune areas (39.9 ± 5.1%), although this difference was not statistically significant (p>0.18; Figure 9; Table 1). Native species richness was statistically equivalent between herbicide-treated (3.7 ± 0.4 species/2m^2) and mechanical (2.5 ± 0.3 species/2m^2) areas (GLM, df=3, F=19.6, p<0.0001; adj. p=0.83; Figure 7; Table 1). This equivalence accounts for the fact that native species richness increased with distance from the ocean (p=0.005) and that a much higher percentage of the herbicide treatment areas were located in the backdunes. These numbers suggest that a few native plant species accounted for considerable native plant cover in herbicide treatment areas prior to restoration such as coyote brush and mock heather. Within restoration areas, most of the native plant species probably established in open or bareground patches within beachgrass stands -- particularly in mechanical removal areas where bareground averaged 8.1 (± 2.9)% cover -- or as an understory to the taller beachgrass plants – particularly in herbicide-treated areas where bareground averaged only 0.3 (± 0.2)% (LM Permutation, p<0.0001; Figure 6; Table 1).

Not surprisingly, the number of native plant species was highest in the native Dune Mat areas (5.8 ± 0.6 species; GLM, all adj. p<0.002; Figure 7; Table 1). However, in terms of the Shannon-Weiner Diversity Index, herbicide treatment areas supported a higher plant diversity (1.3 ± 0.1) than either mechanical treatment (0.6 ± 0.1) or native dune areas (0.8 ± 0.1), probably due to the higher number of both native and non-native species (GLM, df=2, F=8.89, p<0.0001; all adj. p<0.04; Figure 7; Table 1). Evenness also appeared to differ, at least between mechanical and herbicide-treated areas (lmPerm, p=0.05), with the latter having a more balanced distribution of species and cover (0.65 ± 0.03) than the former (0.36 ± 0.06), probably due to the fact that non-beachgrass species within mechanical removal areas were patchier in distribution, while those in the herbicide-treated areas were interspersed within the beachgrass canopy (Figure 7; Table 1). Native Dune Mat plots fell intermediate in terms of evenness between herbicide-treated and mechanical removal areas – 0.46 (± 0.05); Figure 7; Table 1).

Based on species cover, most of the native plants in restoration areas were species more characteristic of native Dune Scrub (herbicide-treated areas) or Dune Scrub/late-successional dune communities (mechanical areas), while those in the native dune plots represented species considered more typical of mid- to primary successional Dune Mat communities (Chi-Square = 2372, df = 6, p< 0.0001; Figure 9). Decades of establishment by European beachgrass undoubtedly altered the sand structure, increasing organic matter and nutrient content, which improved abiotic conditions for plants that would typically not be adapted to the harsh dune environment. As discussed earlier, research on bush lupine, a species native to California that occurred historically only in central and southern portions of the state, has shown that this species creates high levels of exchangeable ammonium and nitrate with leaf drop or shrub death that then becomes available to weedy introduced grasses and forbs (Maron and Connors 1996). Once established, these weedy annual exotics created a labile litter layer that

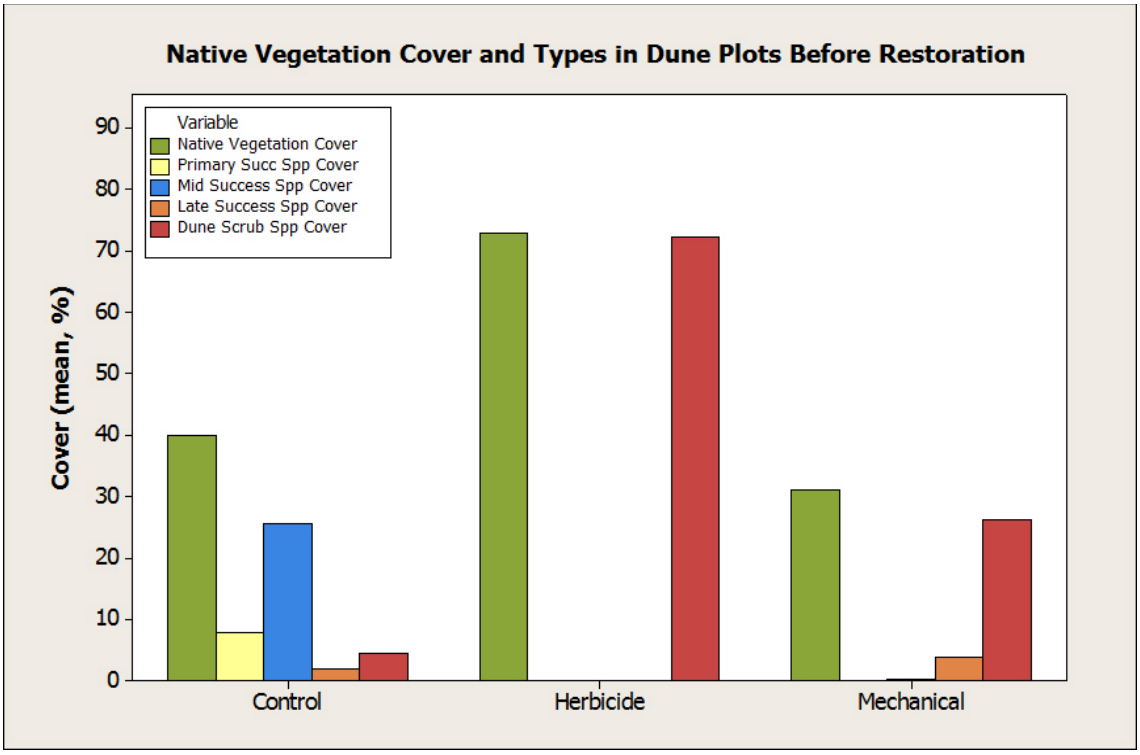


Figure 9. Mean percent cover of native dune species grouped by successional status in different treatment and control areas prior to restoration at Abbotts Lagoon.

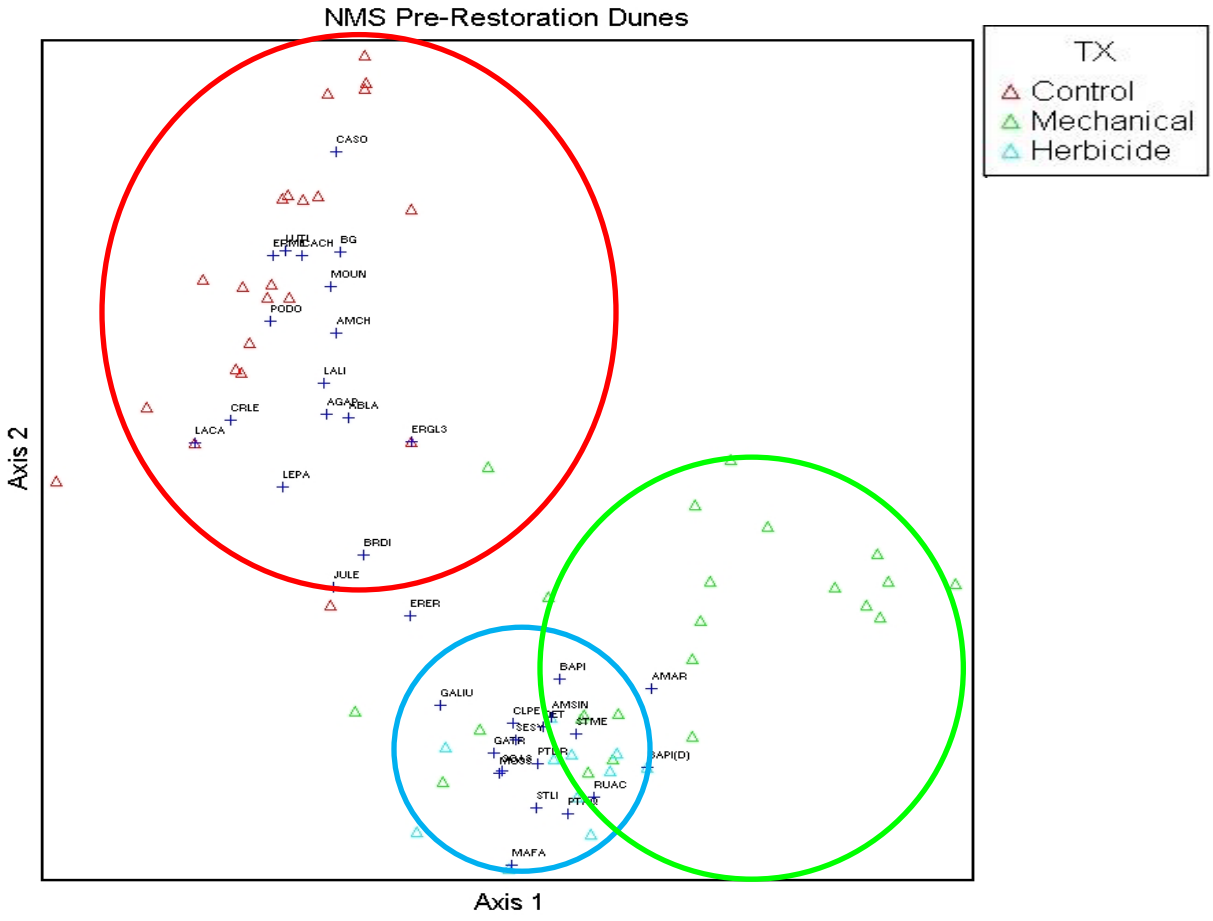


Figure 10. Non-metric multidimensional scaling multivariate analysis of Abbotts Lagoon pre-restoration vegetation communities

completely turns over organic matter and nitrogen, favoring persistence of weedy annuals (Zink et al. 1995). While no similar studies have been conducted on beachgrass facilitating invasion by non-natives, it seems possible that a similar phenomenon occurs in beachgrass-dominated dunes, although it may not be as strong a facilitator.

In retrospect, then, it is difficult at Abbotts to compare change between treatment methods, because, inadvertently, due to construction and funding logistics, the treatment approach was unevenly allocated among dune areas that were dissimilar even prior to restoration, despite sharing the common characteristic of being labeled, "invaded." Foredune and backdune areas near the lagoon that were treated mechanically were characterized by a more monotypic European beachgrass community with scattered pockets of bareground that provided habitat for a few other native and non-native plant species, most of which were characteristic of either Dune Scrub or, to a lesser extent, late successional plant associations. In contrast, herbicide treatment areas, which were largely located in the southern, more interior backdunes area (although some foredune areas were also treated), supported a denser and more diverse European beachgrass community that had a considerable number of other native and non-native plant species intermixed within the canopy, all of which would be considered Dune Scrub such as coyote brush and mock heather.

As noted earlier, in between these invaded treatment areas occurred a relatively pristine native Dune Mat community characterized almost exclusively by large expanses of open sand and a diverse mix of native mid- and primary successional plant species, although late successional and Dune Scrub species were more prevalent in portions of the native dune further from the ocean. By 2011, scattered iceplant and, to a lesser extent, beachgrass plants had established, but had not gained sufficient foothold to rapidly expand throughout this seemingly very vulnerable area. Multivariate analyses using Non-Metric Multidimensional Scaling (NMS) clearly show the strong distinction between native dune and restoration areas along Axis 2, while Axis 1 points to perhaps a less dramatic difference between restoration areas that would be primarily treated using either mechanical or herbicide approaches (Figure 10).

B Ranch North: Little information of comparable detail exists on other dune systems in the Seashore. The Seashore has conducted some pre-restoration monitoring efforts in conjunction with some of its other dune restoration projects, including B Ranch North, Abbotts Triangle, and AT&T, although monitoring methods were not always equivalent to those used at Abbotts. These sites do not support anywhere near as extensive a native dune or Dune Mat community as can be found at Abbotts Lagoon, even if some of these areas would have evolved into Dune Mat had they not been invaded.

At B Ranch North, dunes invaded by European beachgrass and, to a lesser extent, iceplant ringed a large Dune Mat area that also supports one of the Seashore's Tidestrom's lupine populations, as does the one at Abbotts (Figure 11). The large native dune area at B Ranch North, however, had been invaded by iceplant, although cover of this species was relatively sparse compared to other invaded dune areas: In 2-m square quadrats, frequency of iceplant within the B Ranch North Dune Mat area reached 61.9% of quadrats prior to restoration (2013), with occurrences typically composed of small to moderate-sized patches of iceplant scattered throughout the Tidestrom's lupine population. In contrast, frequency of European beachgrass



Figure 11: Aerial view of B Ranch North native Dune Mat area surrounded by European beachgrass (Source: californiacoastline.org)

in the native dune area was only 12.5%. There were very few individuals of European beachgrass: most of the European beachgrass colonization came from encroachment along the perimeter.

As with Abbotts, the relatively uninvaded nature of this Dune Mat area is particularly surprising, considering that it is surrounded on at least three sides by European beachgrass (Figure 11). Again, some type of soil biota may have enabled this isolated native dune area to resist invasion, particularly from European beachgrass, although, left unchecked, the scattered iceplant patches may have eventually coalesced and taken over this area. While not firmly entrenched in the Dune Mat area, iceplant, in fact, may have already been beginning to subtly alter successional dynamics of the dunes through its well-documented ability to change soil conditions, tipping the successional dynamic toward later successional communities. Most of the Dune Mat area supported mid- to late-successional type native dune species (combined 70% frequency), while Tidestrom's lupine appears to prosper best under more primary successional conditions (Pardini et al. 2015): primary successional species occurred in the Dune Mat area with only approximately 14% frequency.

The surrounding European beachgrass areas – which are largely foredunes with some mid-dune and backdune features running perpendicular to the shoreline – were characterized by moderate cover of European beachgrass (41%), with a considerable amount of bareground (22%) and, to a lesser degree, iceplant (8%). European beachgrass occurred in 95.6% of the 2-m frequency quadrats. Twenty-five (25) other, largely native species did co-exist with beachgrass and iceplant, with most of the species being either late successional (20% frequency) or Dune Scrub (13% frequency) ones that had colonized open bareground patches or established underneath beachgrass. Primary successional species occurred at frequency rate of less than 4%: occurrence of these species in heavily invaded dunes may directly relate to the fact that most of the treatment area occurred in foredunes directly adjacent to the beach.

Abbotts Triangle: At the Abbotts Triangle foredunes -- located north of the larger, more recent Abbotts project and just south of the mouth of Abbotts Lagoon (Figure 12) -- European beachgrass cover was considerably lower (30%) than other invaded areas in the Seashore, probably because this area had been treated (and re-treated) between 2001 and 2005, but not retreated subsequently. By 2011, beachgrass had reinvaded much of the area: the frequency of European beachgrass prior to re-treatment in 2011 was 73.4% of the 2-m frequency quadrats. However, there was a considerable amount of bareground remaining (54%), and native plants

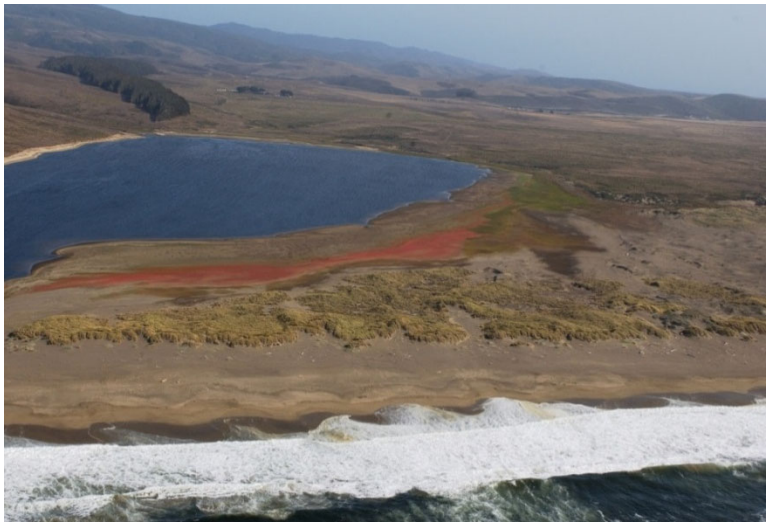


Figure 12: Aerial view of Abbotts Triangle in 2002 prior to any restoration. Source: californiacoastline.org

present appeared to be tilted more heavily toward primary successional species (21% frequency) and mid-successional (11%), probably because this area represents an active foredune area sandwiched between the ocean and Abbotts Lagoon.

AT&T North: Unlike the other restoration project areas, AT&T North primarily supported invaded dunes and pockets of native or lightly invaded Dune Scrub and, to a much lesser extent, Moist Meadow habitat: there was very small patches of remnant native Dune Mat in this project area. Average percent native and non-native cover, native species richness, and Shannon diversity indices all differed significantly between the invaded and the reference or native Dune Scrub plots (GLM, df=1, all F>14.7, all p<0.001; Table 2a). Similar to other dune systems being restored at the Seashore, average percent non-native cover averaged 83.1 (± 5.5)% in invaded plots, with European beachgrass accounting for 82.4% of the non-native species in invaded areas. In contrast, non-native cover in reference or Dune Scrub plots only averaged 17.3 (± 5.1)% (Table 2a). Despite differences in percent non-native cover, non-native species richness did not differ significantly between study areas, with medians being 2.5 in Dune Scrub and 2.0 in invaded plots (Mood Median, df=1, Chi-Square=2.1, p=0.15; Table 2a).

Percent native cover ranged from an average of 120.9 (± 10.1)% in native Dune Scrub to 55.3 (± 9.1)% in European beachgrass stands (GLM, df=1, F=21.87, p<0.0001; Table 2a). Average native species richness in Dune Scrub stands was also more than double that of beachgrass plots – 9.9 (± 0.7) versus 4.3 (± 0.5), respectively (GLM, df=1, F=44.87, p<0.0001; Table 2a). Species abundance was relatively evenly distributed within both study areas (GLM, df=1, F=2.6, p=0.12), but, due probably to higher number of natives in reference plots, species diversity in general was higher in Dune Scrub areas than beachgrass ones (GLM, df=1, F=14.7, p=0.001), with indices averaging 1.6 (± 0.1) and 1.0 (± 0.1), respectively (Table 2a).

Based on NMS results, plots that had already been restored mechanically showed strong differences from the Dune Scrub and Invaded plots (NMS, Final Stress=11.3, R^2=0.75, Figure 13). Almost all separation occurred along the first axis (R^2=0.57, GLM, df=2, F=177.5, p<0.0001), with mechanical plots on the left and Dune Scrub and invaded plots on the right: Dune Scrub and invaded plots also grouped pretty distinctly based on treatment type (Figure 13). European beachgrass represented the one species considered an indicator for invaded plots (Indicator Species Analysis, p=0.0002), while chamisso lupine, yarrow, maritime brome (*Bromus sitchensis* var. *maritimus*), ripgut brome, miner's lettuce, sand mat, and seaside fiddleneck (*Amsinckia*

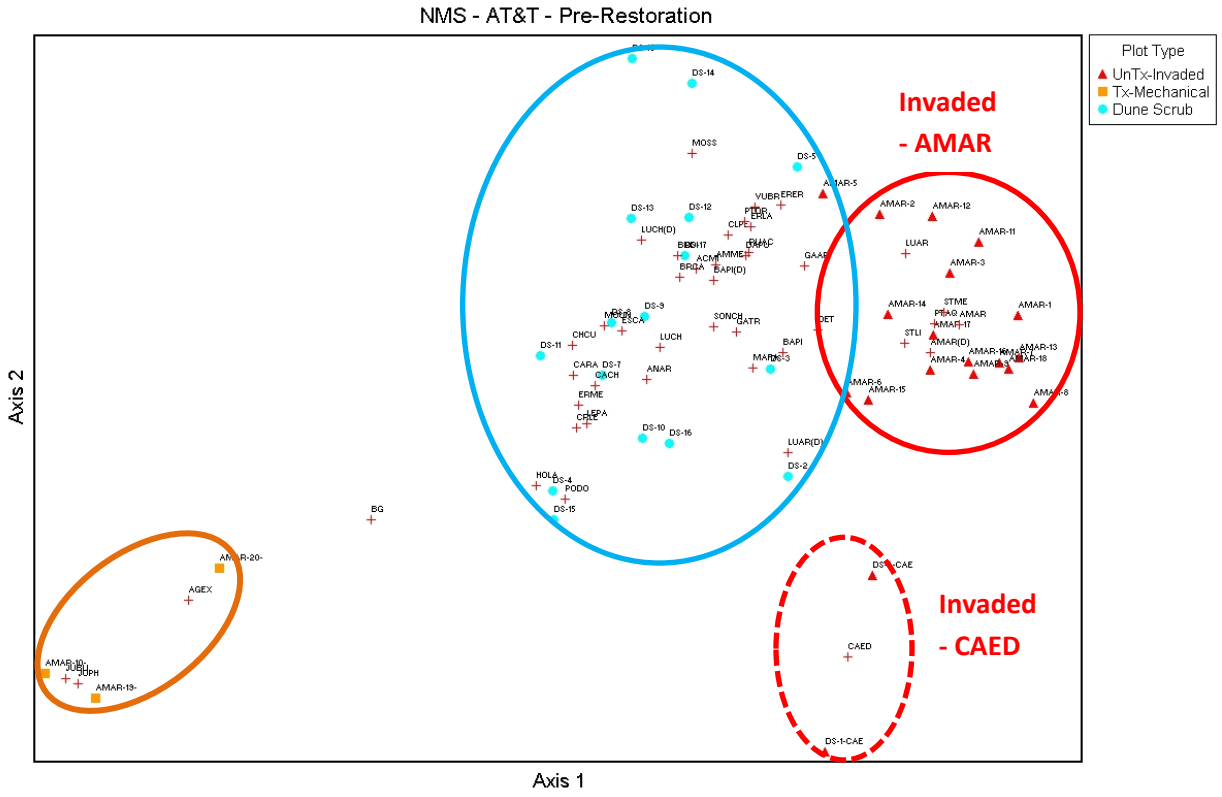


Figure 13. Non-metric multidimensional scaling multivariate analysis of AT&T North reference communities (Dune Scrub), invaded European beachgrass (AMAR) and iceplant (CAED) communities prior to treatment in 2016, as well as invaded communities following mechanical treatment in 2015.

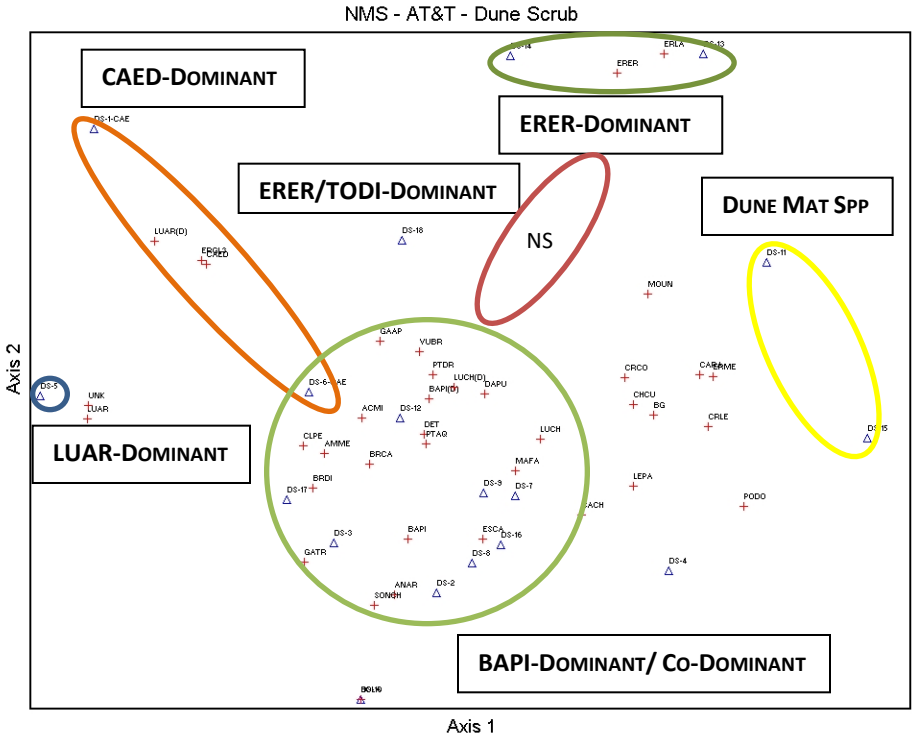


Figure 14. Non-metric multidimensional scaling multivariate analysis of AT&T North reference communities (Dune Scrub) in 2016, with communities dominated or co-dominated by particular shrub or herb species such as coyotebrush (BAPI), bush lupine (LUAR), mock heather (ERER), iceplant (CAED), poison oak (TODI), iceplant (CAED), or dune mat species. ERER/TODI community was not directly sampled (NS), but position estimated from species dominance.

spectabilis var. *spectabilis*) all distinguished Dune Scrub from other plots (all $p<0.05$). The mechanical plots differed strongly from the other types of plots such as Dune Scrub and invaded plots in terms of assemblages or groups of species largely because they primarily supported a number of seasonal wetland species, probably due to their proximity to the Alopecurus Swale, as well having more bareground or open sand. Very little bareground existed in either the Dune Scrub (10.1%) or the invaded (1.1%) plots (Table 2a).

The natural evolutionary trajectory for most of the areas at AT&T, with the possible exception of the foredunes, would probably be native Dune Scrub. From a geologic standpoint, Dune Scrub may represent another step in the evolutionary pathway between primary successional dune and terrestrial vegetation communities such coastal prairie, much of which at the Seashore is formed on historic dune soils. It could also represent a separate evolutionary pathway for higher elevation areas along the coast. Unlike its neighbor to the north (Abbotts), which has more low-lying dune areas, most of AT&T fall towards the higher end of the dune elevation spectrum, so, therefore, it is likely that most of AT&T would naturally have been Dune Scrub if these areas had not been invaded by European beachgrass and iceplant.

Dune Scrub could be considered a variant of Coastal Scrub, although it tends to support more dune plant species. While NMS results would potentially suggest that Dune Scrub stands at AT&T North were a relatively homogenous vegetation community, distinct differences between stands were apparent in terms of shrub and understory herb/forb composition. Most of the stands were characterized by having coyote brush as a dominant or co-dominant shrub species with a number of common understory species, while a very few plots were dominated either by mock heather/coast buckwheat or bush lupine (Figure 14; NMS, Final Stress=9.6, R^2=0.68). Common scrub-type understory species included maritime brome, scarlet pimpernel (*Lysimachia arvensis*), miner's lettuce, California poppy (*Eschscholzia californica*), chamisso lupine, and, to a lesser extent, woolly-headed spineflower (*Chorizanthe cuspidata* var. *villosa*; CNPS List 1B.2; Indicator Species Analysis, all $p<0.10$). At least one type of stand supported a dense thicket of mock heather and poison oak (*Toxicodendron diversilobum*), with coyote brush and wild cucumber as sub-dominants. (Cover of this plot was not directly sampled, but estimated visually). A few plots leaned slightly more toward Dune Mat in terms of species composition, supporting sand mat, coast cryptantha (*Cryptantha leioicarpa*), curly-leaved monardella, bluff wallflower, Douglas's bluegrass, Pacific wildrye, and beach evening primrose (Figure 14). Two plots were largely dominated by iceplant, with seaside daisy and dead bush lupine also present (Figure 14).

To some degree, cover of dominant species within Dune Scrub could vary interannually. AT&T North Dune Scrub plots were monitored in 2016 and 2017, and 2017 plots supported higher abundances of weedy species such as ripgut brome and common chickweed (*Stellaria media*). The 2017 monitoring season occurred after a very wet winter in 2016-2017 that terminated an extended drought. This is reflected in striking variation in non-native plant cover in Dune Scrub plots between years, with non-native cover jumping from 17.3% in 2016 to 67.1 (± 16.7)% in 2017 (GLM, df=2, F=7.72, p=0.009; Table 2a). Non-native species richness, however, remained roughly equivalent between 2016 (2.6 ± 0.4 spp/2m^2) and 2017 (3.1 ± 0.6 spp/2m^2; GLM, F=0.4, p=0.47; Table 2a), suggesting that a few non-native species rapidly expanded in cover. Wet winters often prompt an explosion in weedy, non-native species that take advantage of the

boom in abiotic resources such as water and nitrogen to spread quickly (Hobbs and Mooney 1991; Dukes and Mooney 1999; Milchunas and Lauenroth 1995; White et al. 1997 *in* Alpert et al. 2000).

The rare plant community is generally different from what would be found in Dune Mat vegetation communities. Rare plant species within Native Dune Scrub at AT&T North generally included woolly-headed spineflower, beach starwort, curly-leaved monardella, and bluff wallflower. At least two of these species co-occur in mid to late successional Dune Mat communities (curly-leaved monardella, wallflower), and woolly-headed spineflower can be found in late successional dune habitats, as well. Density of rare plants within native AT&T North Dune Scrub plots varied tremendously between years, again probably because of the difference in precipitation totals and the rapid response of many annual rare plants to changes in rainfall volume. Studies of other rare coastal annuals have found climatic variables to be an important determinant of plant establishment success (Parsons and Whelchel 2000, Fox et al. 2006, Levine et al. 2008). In 2016, density averaged 12.4 (± 7.20) plts/2m^2, but numbers jumped in 2017 (52.3 ± 32.4 plts/2m^2), largely because of high numbers with certain plots (Table 2a).

AT&T South: While topography in AT&T South is a little less extreme than in AT&T North, which has mountain-like dune features and highly undulating terrain, vegetation-related characteristics of these areas remained pretty similar. Percent cover of European beachgrass was significantly higher in the treatment group (83.6 ± 8.3%) than in the reference or native Dune Scrub areas in AT&T South (0 ± 0%; t=-8.05, df=7.00, p<0.0001; tangent transformed; Figure 15; Enders and Parsons 2018). Iceplant existed at low cover in both reference (0.6 ± 0.6%) and treatment plots (3.6 ± 3.6%; Figure 15). There was no significant difference in percent cover of iceplant between the two treatment groups (t=-0.83, df=7.35, p=0.433).

Native Dune Scrub communities in AT&T South supported high levels of both native (120.9 ± 33.1%) and non-native (95.1 ± 36.5%) plant cover with very little bareground (2.0 ± 2.0%) and relatively little rare plant cover (7.0 ± 4.3%; Figure 16). The high abundance of non-native species in AT&T South native Dune Scrub plots would seemingly differentiate these communities to some degree from those in AT&T North, which had low non-native species cover, at least in 2016 (17.3%). AT&T North plots also supported seemingly a lower number of non-native species (2.6 (± 0.4) species (spp)/ 2m^2) than AT&T South (4.5 (± 0.7)spp/2m^2). Not surprisingly, characteristic species differed between Dune Scrub plots in AT&T North and South. Based on multivariate analyses, AT&T South Dune Scrub plots were differentiated based on species such as ripgut brome, salt rush, beach starwort and, to a lesser extent, beach evening primrose and sheep sorrel (Indicator Species Analysis, all p<0.10). No federally listed and CNPS List 1B rare plants were detected in AT&T South Dune Scrub plots, although beach starwort (CNPS List 4) was present (Table 2b).

Prior to restoration, the herbicide treatment area supported less native cover (43.5±20.7%; t=2.07, df=10, p=0.065), but not significantly different levels of non-native cover (97.7±9.4%; t=-0.07, df=3.40, p=0.949), bareground (0.3±0.3%; t=0.85, df=3.12, p=0.456), or rare plant cover (4.2±3.6%) to Dune Scrub plots (t=0.49, df=7.21, p=0.636; Figure 16).

Total species richness, native species richness, and non-native species richness were all

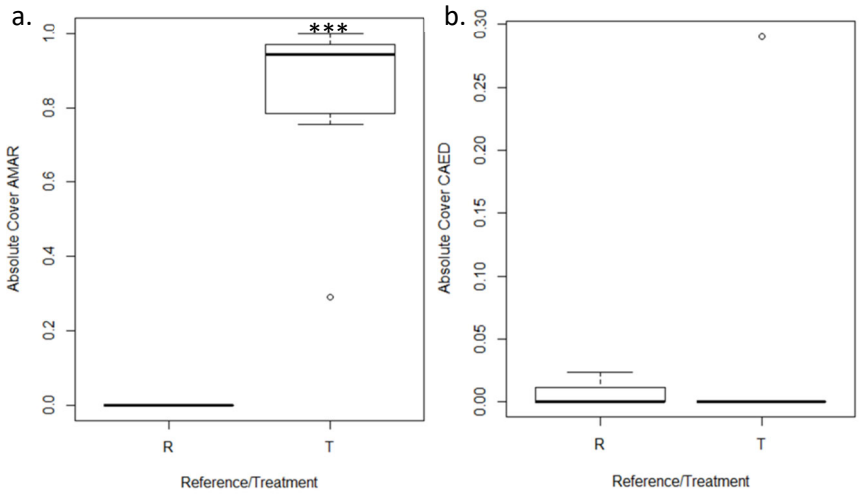


Figure 15. Box and whisker plots of AMAR (a) and CAED (b) cover in reference (R) and treatment (T) plots representing the median, interquartile range, data extremes, and outliers of the data. Asterisks above boxes indicate significant differences between means: '***' p<.0001; '**' p<.01; '*' p<0.05; '.' p<0.1.

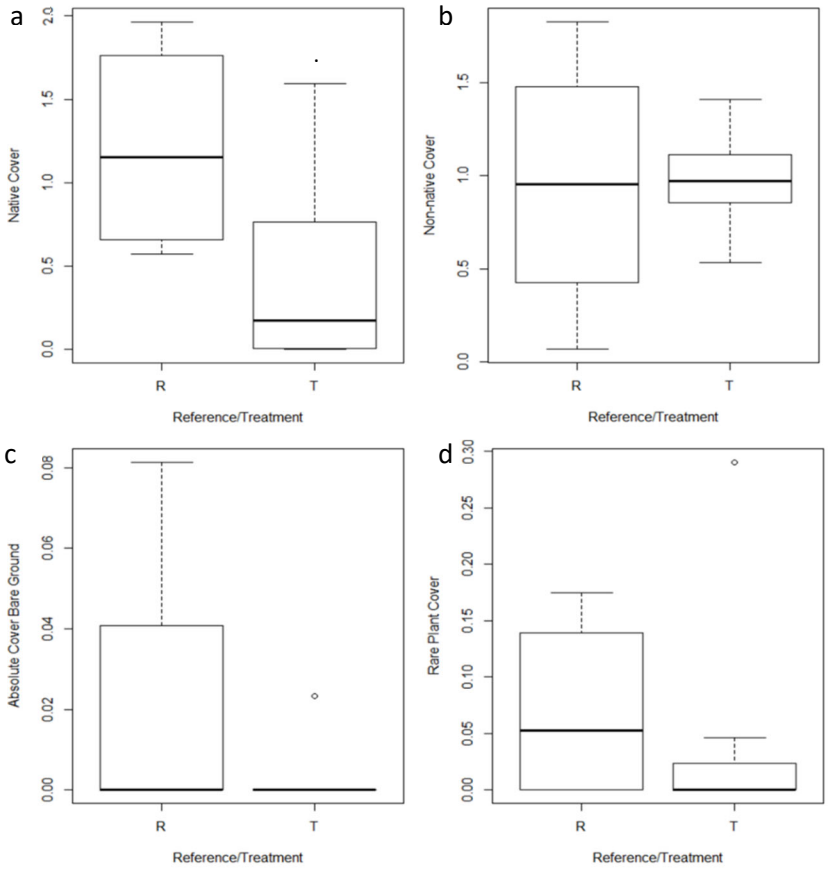


Figure 16. Box and whisker plots of native (a), non-native (b), bare ground (c) and rare plant (c) cover in reference (R) and treatment (T) plots representing the median, interquartile range, data extremes, and outliers of the data. Asterisks above boxes indicate significant differences between means: '***' p<.0001; '**' p<.01; '*' p<0.05; '.' p<0.1.

significantly higher in the native Dune Scrub plots than in the treatment plots (total, *t*=4.82, df=10, *p*=0.0001; native, *t*=3.95, df=10, *p*=0.003; non-native, *t*=2.62, df=10, *p*=0.026). Total species richness in reference plots averaged 11.8 (± 1.0) species, while treatment plots contained an average of 4.8 (± 0.9) species. Breaking down species richness into native and non-native richness, reference plots contained an average of 7.3 (± 1.0) native species and 4.5 ± 0.6 (S.E.) non-native species where treatment plots supported only 3.6 (± 0.7) native species and 2.1 (± 0.5) non-native species on average.

The two Study Areas were also significantly different in diversity and evenness. The reference plots supported a significantly higher Shannon-Weiner Diversity Index (1.9 ± 0.1) compared to the invaded community (0.7 ± 0.2; *t*=5.35, df=8.45, *p*=0.001). Likely driven by the high percent cover of European beachgrass in treatment plots, the invaded community also had significantly lower evenness (0.5 ± 0.1) than the native Dune Scrub one (0.8 ± 0.0; *t*=2.51, df=7.61, *p*=0.038).

The multivariate NMS analysis of differences in species composition between Study Areas shows separation between the reference and treatment plots along the first NMS axis (*t*=-6.23, df=7.32, *p*=0.0004), but no significant distinction between groups along the second axis (*t*=-0.98, df=10, *p*=0.349). The invaded plots aligned along the upper end of the first NMS axis correspond with the two target invasive species, European beachgrass and iceplant, and also a native species, bracken fern. The reference plots cluster on the lower end of the NMS Axis 1 characterized by species such as native beach starwort, common bedstraw (*Galium aparine*), and non-native species such as ripgut brome and bush lupine.

Davis Property: Formal pre-restoration monitoring was not conducted at Davis Property prior to restoration, but park staff had characterized vegetation conditions starting in 2004 with anecdotal observations reported in monitoring plans for Tidestrom's lupine and beach layia. In general, Davis Property is dominated by dense iceplant cover, with occasional patches and stands of European beachgrass. Dense iceplant generally surrounds a few isolated areas of native Dune Mat that are moderately invaded by iceplant and beachgrass. Dune sagebrush and open sand (40%) represented the dominant cover categories, along with iceplant. Sub-dominant native plant species consisted of seaside daisy, woolly lotus, coast buckwheat, gumplant, and coyote brush, which are representative of late-successional dune communities. Tidestrom's lupine and beach layia principally occurred in the westernmost portion of the native Dune Mat area being restored.

Post Restoration Conditions

Changes in Control or Reference Areas

Abbotts Lagoon: In theory, reference or control areas are ones that are not affected by the restoration action. However, as noted earlier, the native dune areas at Abbotts Lagoon were surrounded on almost all sides by invaded dunes, so the restoration did, inevitably, affect "control" areas. The almost instantaneous removal of European beachgrass, particularly on the foredune side, resulted in large-scale sand remobilization that buried portions of the native dune, with burial more evident in areas immediately to the foredunes and adjacent to distinct "lobes" or ridges of actively moving sand. In many instances, burial of both common and rare

plant species was quite evident.

However, based on data from plots, there were almost no statistically significant changes in most of the cover or diversity-related variables between pre-restoration (2010) and eight (8) years post-restoration. In 2019, the only variable to differ significantly was detritus (lmPerm, df=1, p=0.0014; Table 1). Percent cover of detritus jumped in 2019, averaging 16.7 (± 4.1) compared to 4.9 (± 0.01) in 2010 (Table 1). All parameters remained unchanged relative to pre-restoration conditions until 2015, towards the tail end of a four-year drought. In 2015, non-native cover did show a change relative to pre-restoration conditions, albeit a weakly significant one from a statistical standpoint (lmPerm, df=1, p=0.06; Table 1). However, there were no differences between pre- and post-restoration in non-native cover or richness the following year in 2016, despite the fact that the winter of 2015-2016 was the first wet one since the mechanical restoration project was implemented in 2011, so potentially dramatic shifts in vegetation cover might have been expected with the sharp increase in abiotic resources. It's possible that there was a bit of a lag factor, as there was a statistically significant change in 2017 with non-native species richness increasing slightly, climbing from 0.13 (± 0.07) species (spp)/2m^2 in 2010 to 0.39 spp/2m^2 in 2017 (lmPerm, df=1, p=0.04; Table 1). Increases appeared to be largely due to occasional, sparse establishment of plots by European searocket. In 2019, non-native species richness, which dropped to an average of 0.26 (± 0.09) species/2m^2, was back to being statistically equivalent to 2010 even though average richness was seemingly double that of 2010 (paired t-test, t= -1.14, df=22, p=0.27; Table 1).

These seemingly positive results, however, may ultimately be ascribed more to issues associated with our vegetation monitoring method than any kind of inherent invulnerability of native dune communities to invasives encroachment, restoration-related disturbance, or extreme climatic variability (drought/heavy rains). Our vegetation monitoring may not have accurately captured change due to the extreme amount of bareground and patchy nature of vegetation in native Dune Mat communities. Post-hoc analyses conducted on 2015 data from the reference or native Dune Mat plots suggested that power of these pre- and post-restoration comparisons may have been relatively weak, with power ranging from 0.42 to 0.65 where α=0.10, with low number of replicates (n=23) and high variability between plots potentially obscuring changes. This conclusion seems to be borne out by the fact that, despite data suggesting that no change had occurred in control plots in 2016, park staff had by this time noticed an alarmingly explosion in the abundance of European searocket and, to a lesser extent, other invasive, non-native species such as New Zealand spinach, iceplant, and European beachgrass in fall 2016, which probably resulted from higher rainfall levels during the preceding winter. Certain areas supported almost 75% cover of European searocket, with native Dune Mat areas closest to the mechanically restored foredunes appearing to be more vulnerable (Figure 17). These areas are more subject to frequent disturbance from moving foredune sands and high winds than more inland areas.

Of biggest concern was that monitoring conducted in June 2016 only documented four (4) plots with European searocket, all of which had cover totals below 2%: this includes mechanically restored areas, as well. The number of reference plots with searocket climbed to nine (9) in 2017, however, in general, estimated cover of non-native species remained low in 2017, as well. This may be at least partially due to the fact that the Seashore and PRNSA mounted an

extensive searocket removal effort starting in late summer 2016, with more than 4,188 hours of manual removal of secondary invaders by staff and volunteers invested through summer 2020. In 2019, the number of plots with European searocket dropped back down to four (4), which may at least partially attest to success of these extensive manual removal efforts, but may also be attributed to the inability of our monitoring method to adequately capture percent cover of both native and non-native species with a patchy distribution.



Figure 17: European searocket (*Cakile maritima*) in native Dune Mat at Abbotts Lagoon

Despite being buffeted by deposition of remobilized sands and encroachment of secondary invaders, successional communities in native Dune Mat communities appeared to remain relatively stable immediately following restoration. By 2016, five years after restoration, the percentage of species assemblages reflecting primary successional habitats had not changed literally at all, accounting for 7.8% both pre- and post-restoration. However, mid-successional habitats had seemingly dropped slightly from 25.5% in 2010 to 19.4% in 2016. Changes in late successional and Dune Scrub communities were quite small, averaging almost less than a percentage point difference. By 2019, this trend had shifted slightly, with primary (12.3%) and mid-successional (27.1%) dune species most strongly characterizing cover in native Dune Mat areas. These species included yellow sand-verbena, beach evening primrose, beach morning glory, bluff wallflower, curly-leaved monardella, Douglas' bluegrass, as well as bareground (Indicator Species Analysis, all $p<0.10$).

A key factor in many of these communities is the amount of bareground, as native Dune Mat is characterized by high amounts of bareground, and many rare plant species tend to cluster in areas with either higher or lower amounts of bareground or open space. Cover of bareground increased immediately after restoration, climbing from 54.5 (± 5.4)% in 2010 before restoration to 66.0% in 2013 (paired t-test, t=-2.40, df=22, p=0.025), and then dropping back down to roughly 57% in 2015 and 2017 and to 50.8 (± 6.0)% in 2019, which was statistically equivalent to pre-restoration conditions (Paired t-test, t=0.60, df=22, p=0.554; Table 1). The immediate post-restoration expansion in bareground, which undoubtedly resulted from sand remobilization, may have accounted for some of the temporary loss of species associated with mid-successional Dune Mat vegetation communities. As the volume of sand remobilized has dwindled over the years, bareground cover has dropped back down, as well, and this has been accompanied by increases in native plant cover and mid-successional dune species.

Increases in the amount of available unvegetated space often benefit rare plant species, and rare plant density did appear to increase over the years. In 2010, the vegetation monitoring plots supported an average of 2.4 (± 1.1) FE plants/2m^2, but, by 2015, average density had

climbed to 3.0 rare plants/2m^{2,} and, by 2017, that average had seemingly jumped to 18.0 FE plants/2m^2, although changes in median density were statistically insignificant (Mood Median test, Chi-Square=0.79, df=1, *p*=0.38), which suggests that density increases were largely focused in a few plots (Table 1). Endangered plant density averaged 5.5 (± 2.4) FE plants/2m^2 in 2019 (Table 1), more than double that present in pre-restoration plots, although differences were not statistically different from pre-restoration densities (lmPerm, df=1, *p*=0.55).

Given the predilection of searocket for disturbance, it is perhaps not surprising that European searocket also appears to respond positively to sand disturbance and quickly colonizes available open space (bareground), which may place in direct competition with other primary successional species such as Tidestrom's lupine (Figure 18). European searocket is often found co-occurring with Tidestrom's lupine, probably taking advantage of the nitrogen-fixing and microhabitat-building characteristics of this ecosystem pioneer.



Figure 18. European searocket establishing on newly deposited sands mobilized from adjacent restoration areas

As rare plant density numbers suggest, the existing population of Tidestrom's lupine in the native dune area has thrived since the restoration and has seemingly countered any of the initial adverse effects that remobilization of sands from adjacent mechanical removal areas and subsequent wind-driven re-deposition into lupine areas had on plant survival and reproduction. Indeed, it would appear that Tidestrom's lupine responds extremely well to disturbance activities, quickly re-establishing in newly scoured -- or deposited -- sands. Established populations may even be rejuvenated, to some degree, by moving sand. While data variability is too high to make any definitive conclusions, population numbers at Abbotts Lagoon appeared to mirror trends in bareground cover in native Dune Mat areas. Pre-restoration abundance in 2011 was estimated at 183,726 (± 24,290; 80% Confidence Interval (CI)). By 2015, lupine numbers had seemingly jumped, totaling an estimated 283,378 (± 65,534) in 2015. In recent years, estimated lupine numbers have been lower, but still in keeping with estimated pre-restoration abundance, totaling 186,756 (± 35,797) in 2017; 159,458 (± 24,250) in 2019; and 155,315 (± 23,710) in 2021 (NPS, unpub. data). With influxes of sand from adjacent restored areas reduced, the lupine population in the native Dune Mat area may be reverting back to a more stable equilibrium in terms of numbers that more closely parallels that prior to restoration, because the population is no longer being "boosted" by sand influxes that create more primary successional conditions.

In addition to improving habitat quality for Tidestrom's lupine, the restoration also appeared to succeed in one of its other objectives – reducing European beachgrass habitat for native deer mice that consume Tidestrom's lupine seed and, thereby, reduce reproductive success. Predation pressure plummeted almost immediately after the project was completed, most

likely due to the instantaneous removal of 80 acres of beachgrass and other vegetation that directly bordered the existing Tidestrom's lupine population, as well as removal of a sizeable proportion of the local mice population. Prior to implementation, the mean percentage of racemes or flowering stalks of the Abbott's Lagoon Tidestrom's lupine population that suffered predation ranged from 44.8% to 93.9% across a six-year observation period (Pardini and Knight 2013; Figure 19). Predation rates were significantly lower in the first two full years following the restoration (7.8% in 2012 and 5.1% in 2013 (Pardini et al. 2018; Figure 19). Since then, predation rates have ranged from 2.3% (2019) to 9.4% (2014; Pardini et al. 2018, WU, unpub. data; Figure 19). It should be pointed out there was a dip in predation pressure in 2009-2010 (44.8 – 57.6%) before restoration relative to pre-2009 rates (66.5% to 93.9%), therefore, not all of the decline in predation rates may not have been necessarily related to restoration activities.

Also, predation rates in 2019 were reduced in all lupine populations relative to the range of predation rates experienced in the years immediately prior (WU, unpub. data). Potential reduction in the numbers of deer mice associated with mechanical removal of European beachgrass – and, ultimately, mice --however, may ultimately also benefit continued spread of European searocket, the seeds of which are apparently also vulnerable to mouse predation (Knapp and DiTomaso 2005).

Beach layia, the other federally endangered species, has not been monitored as long as lupine, but its numbers grew dramatically after restoration. In 2016, the first year of monitoring, abundance was estimated at 186,343 (± 56,870); In 2017, these estimated numbers jumped to 1,811,168, although confidence intervals were very large (± 516,867). Park staff adjusted the monitoring approach for beach layia in subsequent years. In 2019, estimated layia abundance totaled 1,366,102, with reduced, but still large, confidence intervals (± 356,893). Interpreting population change for either beach layia or Tidestrom's lupine using these quantitative estimates must be conducted with extreme caution, given these very large confidence intervals. Due to the extremely patchy nature of both rare plant species, it is difficult to sufficiently narrow those confidence intervals to derive better estimates of population size, even with adjustments in the monitoring methods.

Changes in Mechanical Treatment Areas

Abbotts Lagoon

Restoration activities at Abbotts dramatically changed successional patterns of invaded dunes and seemingly in different ways. In mechanical removal areas, non-native and European beachgrass cover dropped dramatically following restoration, but, then, so did cover of all species (Table 1). Non-native plant cover plummeted from 81.9 (± 4.1)% pre- restoration to 0% immediately after restoration and remained at 0% until 2017, when non-native cover climbed slightly to 0.5% (Appendix B-1, Table 1). It climbed again in 2019 to 1.4 (± 0.8)%, but that was still significantly reduced compared to pre-restoration conditions (lmPerm, df=2, p<0.001, Tukey HSD, p<0.001). By 2021, non-native cover had dropped to 0.05% probably due to continued re-treatment over the 10 years since implementation. Non-native species richness dropped from 1.8 species (± 0.2) species/2m^2 pre-restoration to to 0.4 (± 0.2) spp/2m^2 in 2019

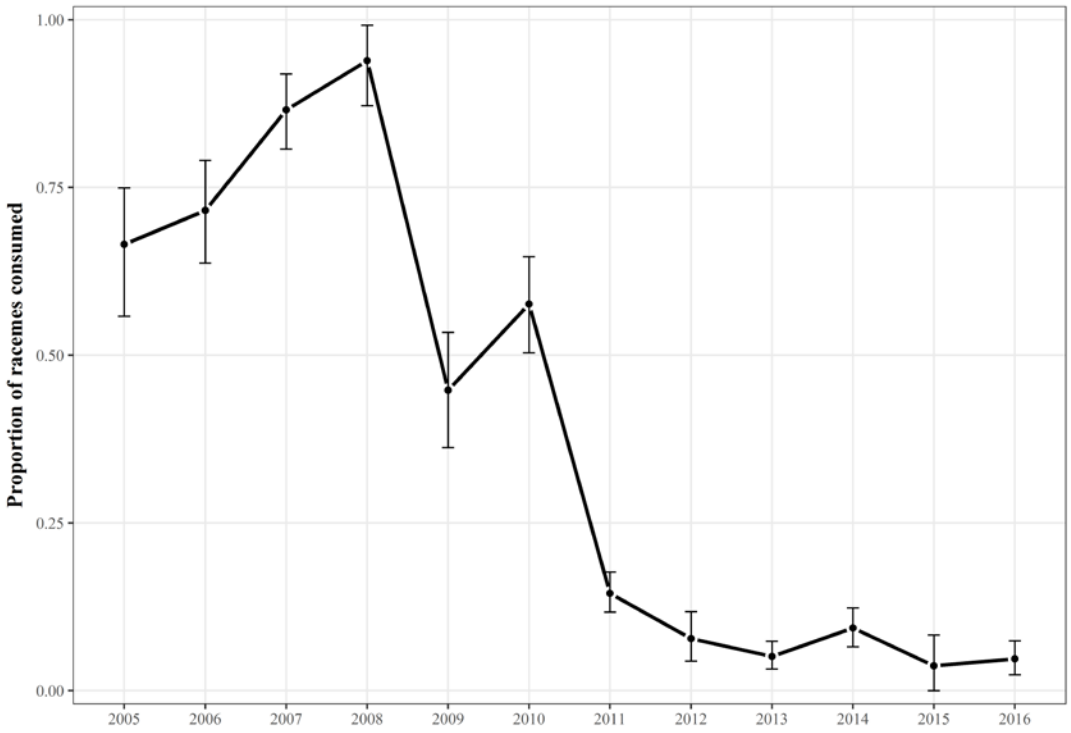


Figure 19. Proportion of racemes consumed of Tidestrom's lupine plants at Abbotts Lagoon Population #1 before restoration (2005 – 2010) and after restoration (2011-2016). Graph courtesy of Washington University.

(lmPerm, df=2, p<0.001) and even lower to 0.04 (± 0.2) spp/2m^2 in 2021 (Table 1).

With the loss of all this non-native plant cover came a dramatic increase in the amount of bareground. The mean percent cover of bareground jumped from 8.2 (± 2.9)% pre-restoration to 98.9% in 2013, two years after restoration, and has remained considerably high since then. In 2017, bareground cover still averaged 87.7%, but it dropped to 73.6 (± 0.1)% in 2019 and then climbed back up to 79.9 (± 5.8)% in 2021 (Appendix B-1, Table 1).



Figure 20. Oblique aerial photograph of Phase I Abbotts immediately after restoration in 2009 (Photographer: Robert Campbell).

However, the dramatic increase in bareground did not immediately translate into more opportunities for native plants, which also dropped dramatically directly after restoration. Immediate post-construction aerial photography shows large expanses of open sand with relatively little vegetation cover except for the remnant native dune areas (Figure 20). A full three years after restoration, percent native cover averaged 0.7% in 2014 compared to 31.0 (± 8.0)% during pre-restoration, when native plants were relegated to open areas between beachgrass stands or growing under the taller beachgrass canopy (Appendix B-1). Between 2015 and 2016, native plant cover climbed to between an average of 2 and 3%, but still remained pretty low. However, in 2017, native plant cover jumped to 13.1%, which, while still much lower than pre-restoration conditions (GLM, F=3.17, df=1, p=0.08), was a definitive improvement that was also evident on-site (Appendix B-1, Table 1; L. Parsons, *pers. obs*.). In 2019, average native plant cover climbed again to 20.0 (± 7.5)%, although this was still reduced relative to native cover prior to restoration (GLM, F=3.81, df=2, p=0.027; Appendix B-1, Table 1). Native plant cover dropped again in 2021 to 17.5% (± 7.5)% (Appendix B-1, Table 1).

In keeping with the drop in native plant cover immediately after mechanically flipping the dunes, native species richness also plummeted from 2.5 (± 0.3) species (spp)/2m^2 pre-restoration to 0.04 spp/2m^2 two years after restoration (Appendix B-1, Table 1). By 2015 and 2016, the number of native species had climbed incrementally to average around 0.5 species (Table 1). In 2017, richness almost doubled to 0.9 spp/2m^2 (Appendix B-1, Table 1). In 2019, native species richness climbed again to 1.8 (± 0.4) spp/2m^2, which was statistically equivalent

for the first time with pre-restoration conditions (GLM, F=19.26, df=2, p<0.0001; Tukey p>0.05, Table 1). However, by 2021, 10 years after restoration was implemented, it had slipped to 1.5 (± 0.4) spp/2m^2 (Appendix B-1, Table 1).

The Shannon Diversity Index strongly reflects the abrupt change in vegetation conditions, with indices declining from 0.55 (± 0.10) pre-restoration to between 0.03 and 0.07 through 2016. However, the Diversity Index more than tripled to 0.18 in 2017 and almost doubled from that in 2019 (0.30 (± 0.10), becoming at least statistically equivalent with pre-restoration levels (GLM, F=7.56, df=2, p=0.001, Tukey, p>0.05; Table 1). It dropped slightly in 2021 (0.25 ± 0.09). Evenness showed a similar pattern, dropping from 0.36 (± 0.06) in 2010 to between 0.03 and 0.07 during 2014-2016 and then rising in subsequent years. In 2017, evenness almost doubled to 0.12, and then it almost doubled again in 2019 to 0.20 (± 0.06), which, again, was statistically equivalent to pre-restoration levels (GLM, F=8.19, df=2, p=0.001, Tukey, p>0.05). In 2021, it slipped marginally to 0.18 (± 0.06).

The failure for native dune vegetation to re-colonize mechanically restored areas for many years immediately after restoration may have been due to many factors, including drought conditions, low soil nutrients, low soil biota, or even presence of some parasitic microbe in "flipped" soils. A study conducted by a University of California, Berkeley (UCB), student interning at the park in 2013 looked at plant establishment relative to several different soil conditions – native dune soils, mechanically treated or "flipped" soils, and iceplant-dominated soils with duff remaining and duff largely removed (Winsemius et al. 2015). Two rare plant species – beach layia (FE) and curly-leaved monardella (CNPS List 1B.2) – showed the highest germination rates in native dune soils (57.5- 72.5%), followed by flipped soils (30- 52.5%), with no germination in either of the two iceplant soils (Winsemius et al. 2015). However, flipped soils had the lowest average biomass per species – more than 100% less than native dune soils -- which led Winsemius to conclude that it was possible that flipped soils play host to some type of parasitic microbe that inhibits germination and, to a greater degree, plant growth (Winsemius et al. 2015).

A recent collaborative research effort between the Seashore and researchers at University of Arizona and University of California, Davis (UCD), supported the premise mechanically flipped soils have very different bacterial species assemblages and higher fungal species richness and diversity than do herbicide-treated soils (Parsons et al. 2020; Appendix E). Soil analyses conducted in 2019-2020 also suggested that soil chemistry of mechanical removal areas is more similar to the native Dune Mat than any of the other restored, unrestored, or native dune communities, with these areas having higher pH and exchangeable Na^{2+}, Mg^{2+}, and K^{+} and lower OM, exchangeable hydrogen (H^{+}), CEC, N, P, and other macronutrients (Figure 21; Parsons and Becker 2021; Appendix G). Mechanical foredunes were differentiated seemingly from mechanical backdunes and native Dune Mat areas by having lower levels of exchangeable Ca^{2+} (Parsons and Becker 2021; Figure 21). Beachgrass invasion impacted 74% of the 19 soil chemistry variables analyzed, with 60% of these persisting following restoration as "legacy effects," primarily in herbicide-treated backdunes (Parsons and Becker 2021). Mechanical removal resulted in fewer legacy effects, but more treatment effects, again primarily in backdunes (Parsons and Becker 2021).

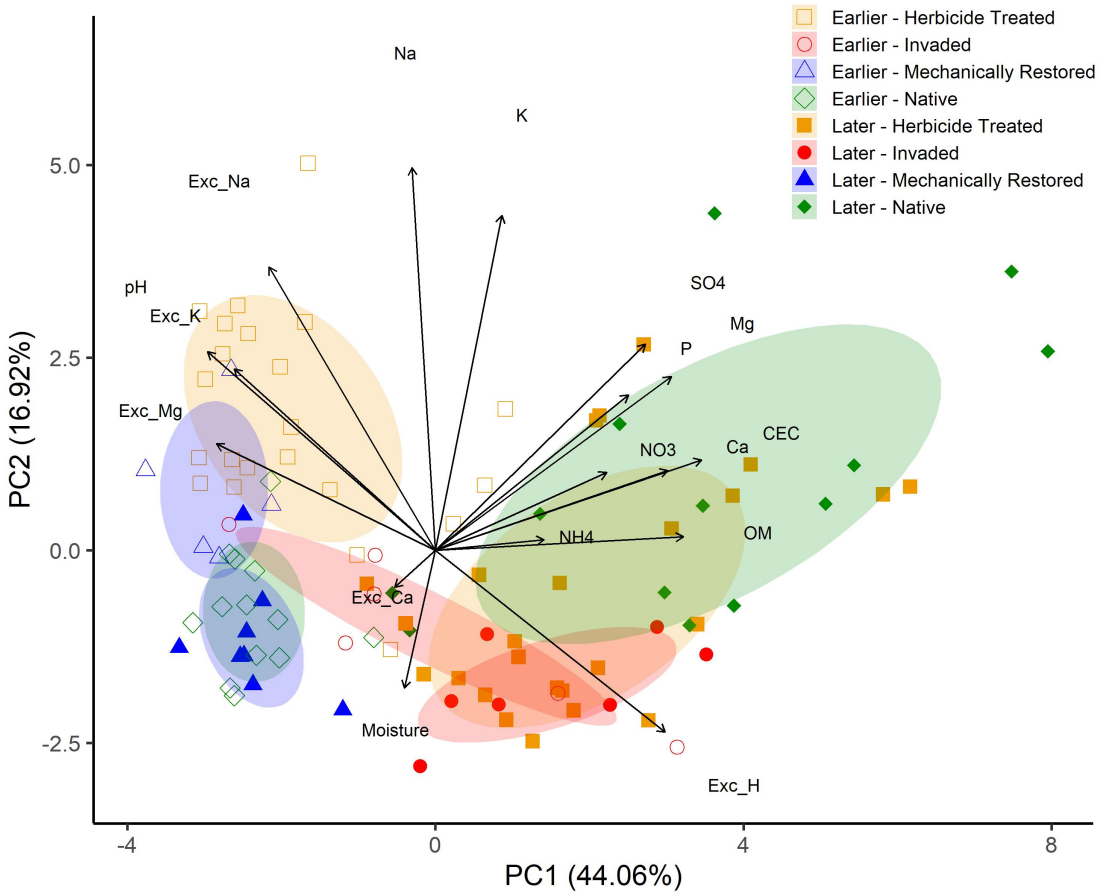


Figure 21. Results of Principal Components Analysis of soil data results from native Dune Mat ("Earlier-Native"), native Dune Scrub ("Later-Native"), Unrestored invaded foredunes ("Earlier-Invaded") and backdunes ("Later-Invaded"), Mechanically restored foredunes ("Earlier - Mechanically Restored") and backdunes ("Later-Mechanically Restored"), and Herbicide-treated foredunes ("Earlier-Herbicide Treated") and backdunes ("Later-Herbicide Treated") habitats at Abbotts and AT&T.

Another factor that may have played a role in limiting vegetation establishment in mechanical treatment areas were the very dry winters and springs between 2011 and 2015 immediately after project implementation. During these years, California was subjected to some very dry winters, with rainfall in many cases 6 inches below normal. While rainfall totals for 2012 and 2013 appeared average, both years were considered much drier than normal from a statewide perspective. Conditions became even drier in 2014 and 2015. By summer 2015, California was considered to be in the fourth – and seemingly driest – year of a four-year drought. While rainfall totals seemingly increased in 2015 relative to 2014, most of this increase came from torrential rains in December 2014, and the late winter/early spring was one of the driest periods on record, with the state of California declaring a Drought State of Emergency in January 2015.

Dry winters are often accompanied by high spring winds in coastal dune systems (Baye 2008). These winds compound the prohibitive lack of winter moisture by destabilizing dune systems through large-scale sand movement, where no vegetation currently exists to bind sands in-place, and by increasing moisture loss of both sand and the few hardy plants able to colonize to evaporation/evapotranspiration. Maximum wind speed exceeded 30 mph anywhere between 3.6% (Water Year (WY) 2013) and 7.9% (WY 2011) of the time during and immediately after restoration. Conditions calmed considerably in 2014, with winds exceeding 30 mph only 1.6% (WY 2014), only to pick up again somewhat in 2015 (WY 2015; 3.0%).

The effects of these strong spring winds were quite evident. Sand now free of stabilizing root systems began to move *en masse* across the restored landscape, burying portions of wetland and native dune features within the dune system. It also continued to move inland, extending in discrete, finger-like projections into the adjacent coastal grassland and wetlands. As discussed earlier in this document, an analysis of historic aerial imagery showed that, even with expansion of European beachgrass in the adjacent dunes after being planted probably in the 1940s, sand and dunes continued to encroach episodically into the adjacent grassland and that the spatial pattern of inland migration was identical to that occurring since 2011, with the prevailing northwesterly winds creating long, narrow ridge-like features aligned parallel with the wind direction (Johnson et al. 2013b). This large-scale movement of sand following dune restoration in 2011 essentially flattened the foredune and backdune features – in fact, so much so that the so-called native dune "basin" became seemingly higher in elevation than the surrounding restored areas. While no topographic cross-section surveys have been conducted of the Abbotts area since the year following restoration (2012), in 2020, a UCB student evaluated topographic changes in Abbotts using LIDAR and found that elevations within the mechanical areas had decreased in elevation by -0.19m compared to -0.04m in adjacent herbicide-treated areas (Hinson 2021).

Ironically, one of the few species that flourished in this highly disturbed environment was Tidestrom's lupine. A study conducted by Pardini and Knight in 2012 in the existing dune habitat at Abbotts showed that Tidestrom's lupine (and beach layia) were more frequently found in early successional microhabitats within the site, which are characterized high amounts of bareground (84.4%; Pardini et al. 2015). In addition, early successional habitats were more likely to contain newly recruited individuals or seedlings of Tidestrom's lupine (Pardini et al. 2015). However, within existing non-restored habitats, early successional habitats only

accounted for 15% of the 145 plots in the existing native dune area (Pardini et al. 2015). The remaining 85% of plots were either mid-successional (60% bareground) or late successional (18.8% bareground), which were characterized by much higher vegetation cover (Pardini et al. 2015). Interestingly, these results are in marked contrast to earlier hypotheses on habitat preferences of Tidestrom's lupine: The USFWS Recovery Plan characterized Tidestrom's lupine as a later successional species that was intolerant of sand burial (USFWS 1998).

Restoration converted the restoration area from heavily invaded Dune Scrub and late successional plant communities to one dominated by primary successional plants and, of course, extensive amounts of bareground. By flipping the dunes, mechanical removal essentially re-started the successional "clock," creating a very large, unfragmented expanse of primary (or even pre-primary) successional habitat. This primordial state appeared to dominate until possibly 2015, when data showed a small increase in the percentage of primary (2%), if not mid- (0.8%), and late successional (0%) dune vegetation communities (Appendix B-1). This evolutionary dynamic shifted slightly in 2017, with both primary and mid-successional species climbing to 6.9% and 2.4%, respectively (Appendix B-1). This trend continued in 2019, with primary increasing to 8.7% and mid-successional to 9.4%, respectively, but decreased again in 2021 to 6.8% and 8.7%, respectively (Appendix B-1).

Tidestrom's lupine responded to restoration efforts almost immediately once construction was completed. Thousands of Tidestrom's lupine germinated even in fall 2011 (Johnson et al. 2012). These plants persisted through a relatively dry winter and scouring winds in the spring, and, by February 2012, an estimated 15,884 plants had established in 15.8 of the 80 acres that were restored (Johnson et al. 2012). At least 25% of these new plants flowered and set seed, setting the stage for potentially even more plants to establish in the second year after restoration (Johnson et al. 2012). In 2013, lupine numbers rose to an estimated 20,552 individuals (NPS, unpub. data). While pre-restoration topsoils ended up almost 3 to 5 feet or more below the new soil surface -- which represented dune sands that had probably been buried for decades -- Tidestrom's lupine seed apparently persisted even in these deeper dune soils, as the almost immediate germination following restoration suggests that germinants must come from buried seed bank, not from blowing or movement of seed from adjacent native dune areas. Mechanical removal and movement of sands may have acted to scarify lupine seeds, promoting germination.

Despite the drought, numbers of Tidestrom's lupine continued to increase in the newly restored Abbotts Lagoon area. By 2014, Tidestrom's lupine populations had tripled to 74,111, with lupine establishing in most portions of the mechanically restored dunes and in several of the areas treated with herbicide where mechanical removal was performed nearby in wetland buffers (NPS, unpub. data). In 2015, use of sampling rather than census methods estimated the Tidestrom's lupine population in the restored dunes at 124,264 (± 31,706; NPS, unpub. data). These numbers have continued to climb almost every year, with abundance estimated at 118,186 in 2016; 297,637 in 2017; 575,450 in 2018; 443,605 in 2019; and 509,764 (± 86,736) in 2021 (NPS, unpub. data). In some areas, Tidestrom's lupine became so dense that it has created almost a "carpet," with little bareground between plants, and this vegetative mat appeared to be naturally stabilizing some of these mechanically restored dune areas (Figure 22).

Another rare species also characterized as favoring early successional habitats is beach layia. Interestingly, beach layia did not respond as strongly to this surfeit of primary successional dune, at least immediately after restoration. In the early years, the plant was only observed on the margins of the mechanical removal area. However, in 2014, 902 plants were counted within the restoration area. Those estimated numbers rose in 2015 to 7,797 and continued to rise exponentially in subsequent years, with abundance estimated at 54,526 in 2016; 886,278 in 2017; 4,795,310 in 2018; and 7,313,813 (± 3,332,388) in 2019. Beach layia has not been monitored in mechanical restoration areas in recent years due to staffing limitations associated with COVID and early senescence of plants in 2021 due to drought.



Figure 22. Tidestrom's lupine (*Lupinus tidestromii*) creates dense carpets within mechanically restored backdunes at Abbotts.

Based on observations on its occurrence, curly-leaved monardella might be categorized more as a mid- to late-successional species: Certainly, there did not appear to be strong overlap of curly-leaved monardella in mechanical restoration areas with either Tidestrom's lupine or beach layia. In 2015, only 44 individuals of curly-leaved monardella were counted in the Tidestrom's lupine transects. Subsequently, those numbers rose to 19,336 in 2016; 20,804 in 2017; and 16,975 in 2018. (Curly-leaved monardella was not monitored in mechanically restored areas in 2019 or 2021). As both beach layia and curly-leaved monardella are annuals, they may be better primed to take advantage of sudden changes in climatic conditions. The success of many rare annual species is closely tied to climate patterns (Higgins et al. 2000, Levine and Rees 2004, Levine et al. 2008). The failure of curly-leaved monardella to leverage additional rainfall in post-2015 years into as much population growth as beach layia may be due more to the fact that much of the mechanically restored area at Abbotts may still not provide optimal habitat for this mid- to late-successional species.

Tidestrom's lupine and beach layia were not necessarily the only species that took advantage of these early successional habitats. Beach evening-primrose and, less frequently, beach pea, Douglas' bluegrass, bluff wallflower, and chamisso lupine often established in the backdunes either behind or on top of mounds created by Tidestrom's lupine plants (Figure 23). Tidestrom's lupine, beach pea, and bush lupine – all early colonizers -- are members of the pea family, which are nitrogen-fixing and can, therefore, act as pioneering species to increase nitrogen resource within soils for other non-nitrogen-fixing species. Lupine is an ecosystem engineer, building mounds through sand accretion that appear to create hospitable micro-environments or micro-sites for other less hardy species. These mounds may shelter plants from high coastal winds: most of the non-lupine plant species appear to establish in the leeward side of mounds (L. Parsons, NPS, *pers. obs*.).

As data suggests, some of the early trends in vegetation establishment – or lack of vegetation establishment – started to reverse in 2016, probably due, in large part, to wetter winter conditions in 2015-2016 and subsequent years. Most of this change occurred in backdune areas, while foredune areas remained largely unvegetated. While this change only started being reflected in the 2X1 m plot data in 2017, vegetation staff observed an increase in colonization of mechanically restored areas by both native and non-native species earlier than that (Figure 23). Unfortunately, as with the reference or native Dune Mat areas, the most abundant colonizer following end of the drought appeared to be European searocket, which spread rapidly in the more heavily wind-disturbed areas such as the foredunes and so-called "Sand Alley," a linear feature oriented perpendicular to the shoreline where winds and sand movement are often highest. Searocket individuals also favored establishment in or around lupine mounds, often becoming so dense that they completely blanketed the lupine plants, although, in general, the smothered lupines did not appear to die (Figure 24). Searocket abundance decreased in the mechanically restored backdunes just south of Abbotts Lagoon where Tidestrom's lupine abundance is actually highest, so this would suggest that disturbance is the key factor to establishment of searocket, with presence of nutrient-enhanced lupine mounds being more of an ancillary or opportunistic factor enhancing searocket viability.



Figure 23. Diversity of native dune plant species in mechanically restored areas at Abbotts Lagoon in 2017.



Figure 24. European searocket establishing on Tidestrom's lupine mound.

Since 2019, California is again experiencing moderate to severe drought conditions. In Marin County, rainfall has averaged roughly 60% of normal in 2020 and less than 50% in 2021 (Marin Municipal Water District 2022). Drought may account for some of the decreases in native plant cover, native plant species richness, Shannon Diversity Index, and evenness and increases in bareground observed in 2021 relative to 2019. In addition, at least some of the reversal may potentially be attributed to a rogue group of beef heifers that roamed the dunes for several months in 2020 and consumed a considerable amount of vegetation in foredune areas,

including mounds dominated by sand verbena and beach morning-glory (L. Parsons, *pers. obs*.).

Convergence with Native Dune Communities: Ultimately, the most important determinant of restoration success for restored areas is not necessarily the amount of divergence from pre-restoration conditions, but the amount of convergence with native Dune Mat communities, which, given their topography and/or geographic location, is what these areas would most likely evolve into over time. Until 2017, the prospects for convergence of mechanically restored areas with native Dune Mat appeared bleak, given the protracted reluctance of native plant species to colonize the vast open sand expanses.

However, more recently, the mechanical restoration component appeared to be making a dramatic shift in terms of its evolutionary trajectory, making comparisons with native Dune Mat communities more reasonable. Over time, species assemblages in mechanical removal areas have incrementally moved closer towards that of native Dune Mat areas, but displayed no overlap in multivariate analyses (NMS). In 2021, mechanical plots showed for the first time overlap with native plots, although most remained somewhat distinct from them (NMS, Final Stress=0.06, Bray-Curtis, 3D; Figure 25a). Mechanical plots that did not overlap with native Dune Mat were largely characterized by higher bareground cover and corresponded to plots in foredune areas that are still struggling in terms of vegetation establishment. Axis 1 primarily highlighted differences between the herbicide-treated backdune and other plots, as well as convergence of mechanical removal/herbicide-treated foredune plots with Dune Mat (ANOSIM, p=0.0001).

When soil chemistry data is incorporated into multivariate analyses using a subset of the plots sampled during the 2020 study (Parsons and Becker 2021; Appendix G), results largely mimicked those of the Principal Components Analysis (PCA) performed in that study (Figure 21), suggesting that soils may be a critical factor driving community composition (Figure 25b). Higher pH and exchangeable cations such as Mg^{2+} and Na^{2+} closely aligned with mechanical removal and native Dune Mat sites, as well as herbicide-treated foredunes (Figure 25b; NMS, Final Stress = 9.67, Sorenson, 250 iter., 2D, R^2=0.876). Axis 2, which separated mechanical/native dune plots from herbicide-treated backdunes, accounted for most of the variation in the data (R^2=0.469) and was the only statistically significant component (GLM, F=5.67, df=3, p=0.006).

Based on Indicator Species Analysis, mechanical restoration areas continued to be differentiated from other study areas based on bareground cover (p<0.05), with Dune Mat characterized by higher abundances of beach morning glory, curly-leaved monardella, and Douglas' blue grass (all p<0.05).

By 2019, mechanical restoration showed for the first time no significant differences with reference or Dune Mat communities for a number of univariate variables, including non-native cover (lmPerm, df=2, p<0.0001, Tukey HSD, p=0.94); European beachgrass cover (lmPerm, df=2, p<0.001; Tukey HSD, p=0.99); non-native species richness (lmPerm, df=2, p<0.001; Tukey HSD, p=0.31), and detritus (lmPerm, df=2, p<0.001; Tukey HSD, p=0.29; Table 1). These monitoring results – and visual observations – suggested that mechanically restored areas were coming closer to converging with native Dune Mat communities.

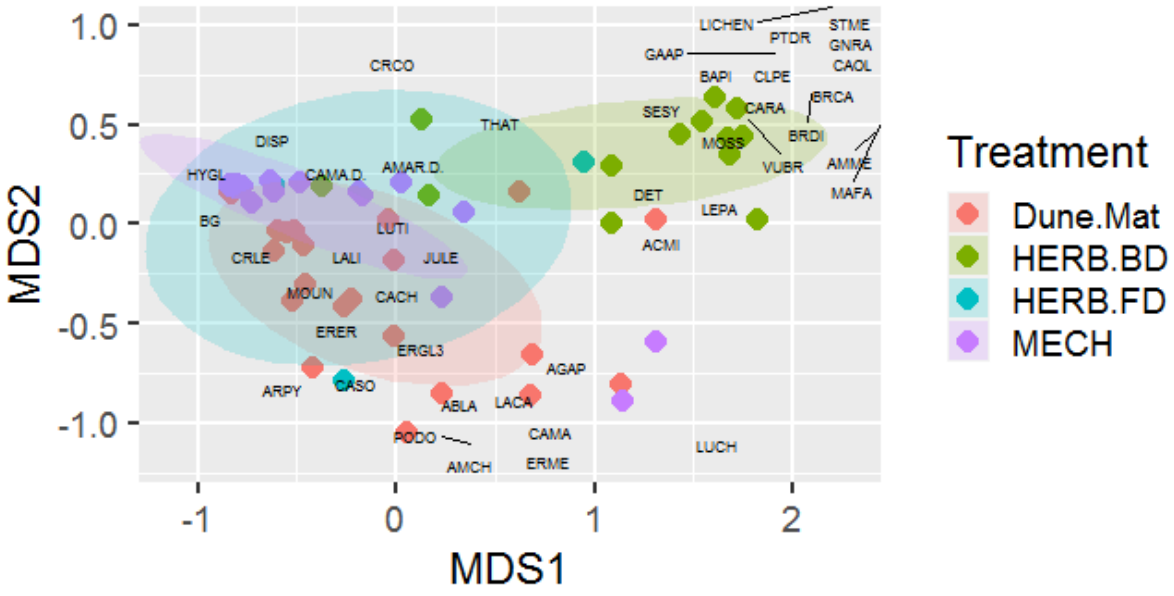


Figure 25a. Non-metric multidimensional scaling multivariate analysis of Abbotts Lagoon restoration areas in 2021, with native Dune Mat ("Dune.Mat"), mechanically restored areas ("MECH"), and herbicide-treated areas separated into foredune ("HERB.FD") and backdune ("HERB.BD"). Mechanically restored areas, particularly foredunes, and herbicide-treated foredunes would be most likely to evolve into native Dune Mat.

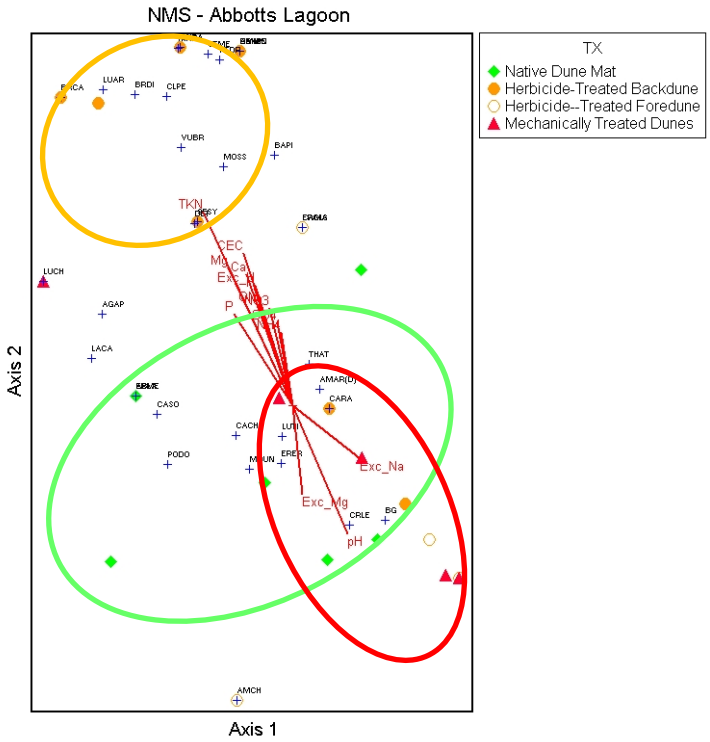


Figure 25b. Non-metric multidimensional scaling multivariate analysis of Abbotts Lagoon restoration areas in 2020 in a subsample of plots where soil sampling was conducted. Mechanically restored areas, particularly foredunes, and herbicide-treated foredunes would be most likely to evolve into native Dune Mat.

By 2021, however, not much further progress had been made toward convergence with native Dune Mat. Based on multivariate analyses, mechanical removal areas continued to differ significantly from native Dune Mat ones (Permanova, F=6.34, df=1, p=0.002, R^2=0.12; betadisper, F=1.61, p=0.21). Apart from non-native species cover (lmPerm, df=4, p=0.41), detritus cover (lmPerm, df=4, p=0.004; Tukey HSD, adj. p=0.18), and non-native species richness (Tukey HSD, adj. p=0.97), mechanical plots in Year 10 showed no statistical equivalence with native dune plots.

As noted earlier, support of rare species is typically considered a higher-level ecological function that evolves with time, but, in this instance, colonization by Tidestrom's lupine and, to a lesser extent, beach layia was almost immediate. In our situation, Tidestrom's lupine appears to be priming primordial dune areas for later colonization by other native dune plants, whereas, in other situations, it is often the reverse. In fact, colonization by Tidestrom's lupine appears to be accelerating successional aging of these once almost primordial successional communities, probably due to high nitrogen inputs from this nitrogen-fixing species. This may account for the dramatic increase in mid-successional communities between 2017 and 2019 (L. Parsons, *pers. obs*). Unfortunately, this could encourage incursion by secondary invader species currently more characteristic of backdune herbicide treatment areas such as sow thistle, as has been observed in areas first colonized by lupine (L. Parsons, *pers. obs*.).

Unlike iceplant, which, as discussed earlier, has a very well-documented effect on invaded dunes by reducing soil pH and available water and increasing OM, N, C and Na^+ levels (D'Antonio and Mahall 1991, Conser and Connor 2009, Novoa et al. 2014), the chemical legacy effects of beachgrass have not been well-studied. As was discussed earlier, to assess changes in chemistry associated with invasion and restoration, in 2019 and 2020, Seashore staff conducted some soil sampling of restored and unrestored dune habitats within the park's northern coastal dune systems (Parsons and Becker 2021; Appendix G). Results of multivariate analyses using Principal Components Analysis (PCA) showed some strong spatial separation between two groups: 1) herbicide-treated backdunes, unrestored invaded dunes, and native Dune Scrub areas and 2) native Dune Mat, mechanically restored dunes, and herbicide-treated foredunes (Parsons and Becker 2021; Figure 21).

Soil pH, TKN, OM, and some of the macronutrients (P, Ca^{2+}, Mg^{2+}) appeared to have the largest influence on separation of these two groups (Parsons and Becker 2021; Appendix G; Figure 21). Mechanical foredunes and herbicide-treated foredunes are characterized by higher pH and abundance of exchangeable macronutrients such as K^+, Mg^{2+}, and Na^{2+}, with strong overlapping of later successional habitats such as mechanically restored backdunes and native Dune Mat being slightly offset from the earlier successional ones by higher levels of exchangeable Ca^{2+} (Parsons and Becker 2021; Figure 21). These PCA results strongly mirror trends of those from NMS analyses discussed earlier. Mechanically restored areas involved "horizon flipping" or turnover of heavily invaded beachgrass soils with subsurface soils, so these soils would assumably be released from some of the chemical legacies of beachgrass invasion, as apparent in the soil chemistry results (Parsons and Becker 2021). However, instead of having to contend with lingering legacy effects of beachgrass invasion, restoration using horizon flipping created "treatment effects" in which the evolutionary trajectories of soil chemistry in foredunes and even more so in backdunes markedly diverged from those of target successional communities

(Parsons and Becker 2021). Almost 87% of treatment effects occurred in mechanical restoration areas (Parsons and Becker 2021). In addition, based on results of microbial studies (Winsemius 2015; Parsons et al. 2020), horizon flipping may have also led to treatment effects on soil microbia potentially unrelated to changes imparted by beachgrass invasion., These soil chemistry and microbial anomalies may explain why mechanical restoration areas have yet to fully converge with native Dune Mat habitats at Abbotts.

Changes in Large-Scale Manual Treatment Areas

B Ranch North

Immediately following removal of sparse iceplant patches in the native Dune Mat area at B Ranch North that supports a Tidestrom's lupine population, the frequency of iceplant dropped from 61.9 in 2013 to approximately 17.3 between 2013 in 2015 (Chi-Square, $p<0.0001$). Correspondingly, the frequency of dead iceplant also increased during this period from 0.6 to 13.3 (Chi-Square, $p<0.0001$). While these transects did not necessarily incorporate most of the European beachgrass treatment areas, there must have been some overlap, as the cover of this invasive grass declined significantly, as well, from a frequency of 20.8 to 11.0 (Chi-Square, $p=0.02$). In 2016, park staff noticed a resurgence in the abundance of iceplant, primarily seedlings, probably due to the wetter winter conditions, with frequency increasing to 37.9, which was still a significant decrease compared to pre-restoration conditions (Chi-Square, $p<0.0001$). The scattered stands of beachgrass within this sampling area, however, continued to decrease in frequency to 2.3 (Chi-Square, $p<0.0001$) despite changes in climatic conditions, possibly because of annual re-treatment efforts.

In 2017, the park decided to shift monitoring efforts to focus more on cover than frequency, because frequency results were hard to interpret. Results from GPS transect monitoring in 2017 was compared with cover results from earlier monitoring efforts, which included both frequency and cover, although the number of sampling points for cover was considerably less in prior years (2013-2016). This monitoring approach was repeated again in 2019, but limited staffing resources precluded another round of monitoring in 2020 or 2021. The graphic in Appendix B-2 shows five years of post-restoration cover data for the once lightly invaded Dune Mat area.

Results from 2017 and 2019 would suggest much lower primary invasives cover (iceplant), as well as lower cover of bareground and detritus relative to earlier years with a strong increase in native plant species cover, but results may be strongly influenced by the much higher number of sampling points. In 2017, there were 517 points compared to 168-174 between 2013 and 2016 (Appendix B-2). Based on cover data, primary invader cover (iceplant and European beachgrass) dropped from 10% in 2013 to 4.2% in 2014, with cover in subsequent years essentially zero (Appendix B-2), although scattered iceplant plants or patches continue to be present in 2021 (L. Parsons, *pers. obs*.). Cover of detritus, which includes both litter from natural senescence as well as that generated by invasives removal, rose from 11.3% in 2013 to a high of 28.0% in 2015, slipping slightly in following years with cover estimated at 24.1% in 2019.

Bareground cover remained roughly equivalent between pre-and post-restoration between 2013 (51.3%) and post-restoration (41.2% in 2014 to 47.0% in 2015) until 2017, when it dropped to 19.4%, similar to what was reported in 2019 (20.1%). This may either reflect a real change in the amount of unvegetated area or an artificial change related to the change in monitoring approach, as discussed earlier. A very similar trend was documented for cover of native plants, with percent cover hovering between 21.4% (pre-restoration) and 25.6% (2015) until 2017, when it seemingly jumped to 45.0%, and then later jumped again to 81.6% in 2019. (While estimates of percent cover in 2017 seem reasonable, those from 2019 appear to be unnaturally high: this may be a result of sampling error, as some of the staff conducting this monitoring were new.) Most of the increase in native plant species cover in 2017 and 2019 appears to come from species considered characteristic of mid- and late-successional communities, while those representative of primary successional communities remained low in abundance (Appendix B-2).

Expansion of native plant species in areas formerly invaded by iceplant isn't necessarily a foregone conclusion. Even if iceplant is fully removed, native dune vegetation communities may not be able to be fully restored due to changes in soil chemistry. As was discussed earlier in this document, iceplant not only alters soil properties (D'Antonio and Mahall 1991, de la Peña et al. 2010, Santoro 2011, Vilà et al. 2006, Novoa et al. 2012), but water availability (D'Antonio and Haubensak 1998). Iceplant leaves have been found to have high levels of tannin and antibacterial compounds (van der Watt and Pretorius 2001) that reduce litter decomposition rates (Vilà et al. 2006) and leach into soils, acidifying them and thereby reducing nitrate, calcium, and magnesium availability (D'Antonio and Haubensak 1998, D'Antonio and Mahall, unpub. *in* Conser and Connor 2009). Soil acidification occurs naturally with dune succession, but iceplant invasion speeds up this process (D'Antonio and Mahall, unpub. *in* Conser and Connor 2009).

These soil changes may preclude re-establishment of certain types of native dune vegetation communities, such as primary or mid-successional ones, and ultimately favor late-successional ones or even Dune Scrub. Studies conducted in Spain suggested that success of iceplant removal efforts could be compromised by lingering legacy effects (Novoa et al. 2012). While some legacy effects reverted within a year to pre-restoration conditions, others did not, with establishment of diverse native vegetation communities constrained in the interim by competition from opportunistic ruderal species (Novoa et al. 2014). In another study of restored dunes in Spain, removal of iceplant caused a significant increase in species richness, particularly of annual plants, but not necessarily in native species cover or diversity (Andreu et al. 2010). Similar preliminary results were found with an iceplant removal project near the Seashore's Lighthouse initially conducted in 2008, with manual removal seemingly increasing percent cover of non-native forbs and grasses (Hamingson and Voeller 2011). As was shown in Winsemius's 2013 study, even when iceplant duff was removed from the soil surface, neither beach layia nor curly-leaved monardella germinated in the former iceplant soils, although the greenhouse component indicated higher growth in soils without duff. One factor that may bode well for this site is that manual removal was conducted before iceplant had completely taken over the area and potentially irreversibly changed the soil biotic and abiotic conditions.

In terms of native dune plant species, three (3) native dune species appeared to immediately

respond to restoration. At least one late successional species, woolly lotus, increased in abundance, climbing in frequency from 11.9 in 2013 to 36.2 in 2016 (Chi-Square, $p<0.0001$). Beach evening primrose, a mid- to late-successional species, also climbed slightly in terms of frequency, increasing from 76.8 in 2013 to 96.1 in 2016. Both beach layia and curly-leaved monardella -- two rare dune plant species -- dropped in frequency the first year after restoration (2014; all $p < 0.006$), but appeared to rebound somewhat in Year 2 (2015). In 2016, beach layia frequency jumped considerably to 23.6, a significant increase from its pre-restoration abundance (Chi-Square, $p=0.02$).

One of the primary objectives of this project focused on improving habitat conditions for a very imperiled population of Tidestrom's lupine. Improving habitat conditions not only relied on removing iceplant from within the population itself in 2013, but removing moderate to dense European beachgrass stands on the perimeter of the population starting in 2014, as these stands could provide habitat for deer mice consuming lupine seed. Based on population sampling estimates from 2001-2011, plant numbers within Population 6 declined precipitously over those 10 years. Plant numbers dropped from an estimated high of 12,050 in 2004 to less than 2,000 plants between 2011 and 2013.

Around the time that restoration was implemented, the park switched from transect-based sampling to censuses due to the sharp reduction in population size. Immediately following iceplant removal, numbers climbed slightly to an estimated 2,186 in 2014 due partly to seedling recruitment. However, numbers plummeted to 706 plants in 2015 and slid even lower to 583 in 2016 despite a wetter winter. As numbers were low in many lupine populations in 2015, the precipitous decline in 2015 appeared linked more to severe spring drought conditions and related biotic impacts such as increased herbivory on remaining plants than to any direct or indirect impact from restoration. As was discussed in relation to the Abbotts Lagoon population, it may also be that the benefits of increased rainfall and more abiotic resources simply take longer to materialize in terms of boosting population numbers and viability for perennial plant species than annual plant species. Estimated plant numbers did double to 1,086 in 2017 (WU, unpub. data), remaining roughly equivalent in 2018 (1,146) before climbing moderately in 2019 (1,492) and 2021 (1,364; NPS, unpub. data). Some of the annual variation in numbers for this short-lived perennial species must be ascribed to sampling error as different people are typically conducting the counts each year, with some consistency built in through oversight by park or WU staff. However, this cannot account for all the variation. This population more so than many others appears heavily impacted by a combination of factors, including potentially drought stress, herbivory, low recruitment, genetic issues, and, perhaps most importantly, flower/seed/raceme predation by mice, rabbits, and insects.

Another indirect measure of overall project success involves changes in predation rates on Tidestrom's lupine seed. While iceplant may not seemingly provide much cover for mice, the larger project also included treatment of European beachgrass surrounding the Tidestrom's lupine population, which may have supported high numbers of this native rodent. Prior to 2014, Washington University researchers found that the proportion of racemes consumed at B Ranch North averaged 72.1% (WU, unpub. data). Since 2014, predation rates have dropped moderately, averaging 43.8% (WU, unpub. data). However, the decrease in the average predation rate does not result from a consistent reduction in predation following restoration,

but from some very large and sharp non-cyclical swings in predation rates between years (WU, unpub. data). For example, predation remained relatively low in 2018 (33.2%) and dropped even further in 2019 (7.9%), jumped sharply in 2020 (74.3%) and then plummeted to 17.7% in 2021 (WU, unpub. data). There may be some link between climate-influenced resource availability, mice carrying capacity, and foraging pressure.



Figure 26. Deer mouse *(Peromyscus maniculatus)* caught scaling side of Tidestrom's lupine enclosure at B Ranch North

As even the most effective treatment efforts would take time to yield results in terms of decreasing mice densities, park staff attempted some emergency management interim measures such as caging plants. Cages did reduce predation rates, but did not eliminate predation (Parsons and Ryan 2015, NPS, unpub. data). Mice were able to scale cages despite remedial measures park staff took to thwart predators, including placing smooth metal flashing around cage tops and covering tops with mesh netting (Parsons and Ryan 2015, NPS, unpub. data; Figure 26). Despite the fact that caging was not wholly successful in excluding mice, predation rates were much higher on control plants with no protection, averaging from 35% in 2016 to 68% in 2015 (Parsons and Ryan 2015; NPS, unpub. data). In contrast, predation rates on caged plants averaged between 9% (2014) and 30% (2015).

Based then on predation rates and population numbers, removal of European beachgrass (and iceplant) did not appear to have immediate or even intermediate-term beneficial impacts on the Tidestrom's lupine populations at B Ranch North. European beachgrass treatment was initiated in 2014, so changes in mice densities may only occur after beachgrass plants decompose, which can take five years or more. However, by 2020, most of the beachgrass immediately surrounding the population had decomposed (Appendix C-3).

It is also possible that mice have simply found alternate habitat in native shrubs or other persistent vegetation or that they remained, at least initially in the dense stands of standing dead beachgrass biomass. European beachgrass decomposes very slowly (>5 years), as does its rhizomes: persistence of the rhizomes even with eventual decomposition of aboveground biomass may maintain suitable nesting habitat for deer mice. Pitts and Barbour (1979) speculated that deer mice preferred European beachgrass and rush (*Juncus* spp.) for nesting habitat because of the surficially dense rooting patterns of these species and the fact that the dense aboveground cover kept the soil substrate cooler and more moist than native Dune Mat habitat. The park is currently collaborating with the U.S. Geological Survey on a study of deer mice abundance in different types of dune habitats surrounding Tidestrom's lupine populations that represent a range of population viability (decreasing, stable, increasing) and predation rate (low, high) conditions.

Large enough declines in rodent numbers to benefit Tidestrom's lupine reproductive success may not transpire unless there are drastic alterations in the landscape (such as the mechanical removal at Abbotts that left little to no vegetation and probably little to no mice either) or some type of predator management. As noted earlier, this population appears impacted by a number of factors: Even if mice populations decline, the Tidestrom's lupine population may still not be able to recover. Population Viability analyses conducted on 2008-2009 data by WU showed that, even if seed predation was removed, increases in population growth rate did not appear sufficient to ensure that the population would become stable.

These modeling results could potentially point to genetic issues limiting population viability. The park is currently collaborating Washington University and the Helmholtz Centre for Environmental Research at University of Halle in Germany to conduct analyses on genetic structure of Tidestrom's lupine populations within the Seashore. Preliminary results indicate that there is strong differentiation between Point Reyes' populations, with populations split into three groups: the northern group (Abbotts Lagoon, AT&T); a central group (North Beach and adjacent populations); and southern group (B Ranch North and South, Davis; A. Workman and W. Dietz, Helmholtz Center, *pers. comm*.). Tidestrom's lupine populations at all three southern sites have been struggling in terms of plant abundance, with B Ranch South having dwindled to as few as three (3) individuals. Inbreeding and low genetic variability do not seem to be a major threat to Tidestrom's lupine, but there is limited gene flow between park populations (A. Workman and W. Dietz, Helmholtz Center, *pers. comm*.). The Seashore may need to consider adaptive management measures involving translocation of seed or plants from more successful central or northern populations.

Davis Property

Formal monitoring has not been conducted at Davis Property either prior to or following the start of manual removal efforts in 2016, but, anecdotally, the cover of bareground appears to have increased by 30%, climbing from 40% to 70%. As noted earlier, dense iceplant was removed and placed in centralized piles. Interestingly, many of the beach layia and other native dune plant species found were growing in thick some of the thick duff left by removed iceplant, which not only would seemingly hinder emergence of beach layia and other seedlings due to reduced light and available bareground, but also because the duff has been shown to potentially alter soil chemistry and have allelopathic properties.

Davis Property supports populations of both Tidestrom's lupine (Population #3) and beach layia (Population #7). Since lupine monitoring began in 1983, numbers for this population had been very low, never exceeding 207 plants. Starting in 2003, abundance increased to between 579 and 982 plants for several years, but by 2007, numbers dropped back down to no more than 203 plants for five years. There was another brief period between 2012 and 2014 where plant numbers grew, with total population size ranging from 227 to 319. However, between 2015 and 2018, numbers dropped to pre-2012 levels (74 to 112), with non-reproductive adults accounting for most of the plants in 2015 and 2018. In 2019 and 2020, numbers rebounded slightly to 171 and 163 plants, respectively, and then dropped to 125 in 2021 (WU, unpub. data).

While volunteer efforts appear to have greatly benefited habitat quality, they do not appear to have translated directly into dramatic increases in lupine population size. This is in stark contrast to beach layia, numbers of which jumped 1,352% between 2018 (371) and 2020 (5,385; NPS, unpub. data). While beach layia abundance dropped in 2021, it was still much higher (1,099) than most previous years (NPS, unpub. data). Unlike beach layia, Tidestrom's lupine appears to be almost more impacted by indirect impacts of invasion than direct ones, which means that simply removal of primary invaders such as iceplant and beachgrass from its habitat may not be enough to jumpstart faltering populations or that it may take longer to realize any benefits from habitat improvement.

Washington University has characterized this Tidestrom's lupine population as one of the highest risk ones (E. Pardini, WU, *pers. comm*.). A preliminary population viability analysis for this population for the period 2008-2009 could not even be fully completed, because fertility was 0 during one of the years (E. Pardini, *pers. comm*.). Since 2010, seed predation rates have ranged from 20.7% (2018) to 86.4% (2019), averaging 52.2% (WU, unpub. data). In populations that are declining, if modeling populations with no seed predation does not improve projected population growth rates to stable or increasing, then other factors may be contributing to population decline (E. Pardini, *pers. comm*.). Certainly, the windy and exposed conditions at Davis may exacerbate impacts of drought during dry years and impact population health (E. Pardini, *pers. comm*.). However, as discussed above under B Ranch North, lupine at Davis may also be compromised by genetic issues related to poor gene flow between populations, particularly the more successful central and northern populations (A. Workman and W. Dietz, Helmholtz Center, *pers. comm*.).

In addition to poor reproductive success, encroachment from iceplant is perhaps the biggest threat to Tidestrom's lupine at this site. It is unknown whether iceplant, which is the primary habitat within and surrounding the population, provides good habitat for native deer mice as do European beachgrass and rush stands (Pitts and Barbour 1979), but it would be safe to assume that it does, given the predation history of this population and lack of sizeable stands of other habitats nearby.

Changes in Herbicide Treatment Areas

Abbotts Lagoon

Herbicide treatment resulted in a somewhat different restored environment at the Abbotts Lagoon Project Area than those restored mechanically. Die-back of European beachgrass was every bit as dramatic in backdune treated areas as in mechanical ones. Percent cover of live beachgrass in restored Abbotts backdunes dropped to 0.1% immediately after restoration in 2013, which represented more than a 100% drop from pre-restoration beachgrass invasion levels (90.2 ± 3.2%; Appendices B-3 & C-1; Table 1). These levels have remained extremely low since then, ranging from 0.1 to 1.0% and averaging 0.0 (± 0.0)% in 2019, a significant difference from percent cover of beachgrass prior to restoration (lmPerm, df=2, $p<0.001$, Tukey HSD, $p<0.001$; Appendices B-3 & C-1; Table 1). Beachgrass cover continued to remain relatively non-existent in 2021, nine (9) years after treatment (Appendices B-3 & C-1; Table 1).

While herbicide application was as effective as mechanical in almost immediately eliminating beachgrass after treatment, one key difference between the two restoration approaches was that herbicide treatment left large amounts of standing, dead biomass that did not decompose quickly (Figure 27). One (1) to two (2) years after treatment (2013), standing, dead biomass of beachgrass represented 62.2% of the cover in herbicide treatment plots (Appendix C-1). In 2015, three (3) to four (4) years after treatment, standing, dead biomass still accounted for 22.7% of the plot cover (Appendix C-1). However, by 2017, five (5)- to six (6) years post-treatment, standing dead beachgrass only averaged 4.7% and, by 2019, eight (8) years after restoration, dead cover had finally dwindled to 1.5% (± 0.7)%, significantly lower than pre-restoration cover (lmPerm, df=2, p=0.0076, Tukey HSD, p=0.03, Appendix C-1). By 2021, necromass cover had slipped further to 1.25% (Appendix C-1). Based on our data, the so-called "half-life" of beachgrass – or the time that it takes for half of the initial live beachgrass cover to decompose – would be about 2.5- 3 years at Abbotts and other restoration areas (Appendix C-1), which is surprising given that conditions along the coast are optimal for physical litter decomposition due to year-round temperate climate, high winds, high air moisture content, and strong solar radiation cycles. Physical and chemical degradation, including photodegradation, often plays an important role in breakdown of dead plants in more arid ecosystems such as deserts (Facelli and Pickett 1991). In addition, European beachgrass is not the only species that appears to be decomposing slowly: dead shrubs such as bush lupine also persist for years after being incidentally targeted by chemical spray (beachgrass or iceplant can occur intermixed with this and other shrub species) or being cut manually.



Figure 27. Undecomposed European beachgrass at Abbotts Lagoon Project Area five years after treatment. Recently treated individuals are yellower in color.

Once beachgrass starts to decompose, the stalks often fall to the ground, leaving a thick thatch on the soil surface (Figure 28). Over time, this thatch further breaks down into smaller and finer pieces of dead organic material or "detritus." Until recently, monitoring efforts combined both the larger thatch and smaller, organic material into the detritus category. Because plants are continually dying and decomposing, even without treatment, detritus levels in thickly vegetated stands can be quite high: they averaged 97.4 (± 0.7)% prior to restoration in herbicide treatment plots (Appendices B-3 & C-1; Table 1). Based on data collected, since initial treatment, detritus cover peaked in 2012 (97.2%) and has been declining ever since, averaging



Figure 28. Thatch layer of undecomposed European beachgrass.

53.0% in 2019, which is significantly different than pre-restoration (lmPerm, df=2, p<0.001; Tukey HSD, p<0.0001; Appendices B-3 & C-1; Table 1). In 2021, detritus cover actually increased to 71.6 (± 7.4)% (Appendices B-3 & C-1; Table 1). In contrast, cover of thicker litter or thatch steadily decreased from 9% in 2019 to 1% in 2021: Thatch was not separated from detritus in some of the earlier monitoring years (Appendices B-3 & C-1; Table 1).

What this means is that, while live European beachgrass was largely gone, the eradication of beachgrass did not immediately translate into new areas for native dune species to colonize because of the persistence of standing dead biomass, thatch, and detritus. In stark contrast to mechanical removal, herbicide treatment did not dramatically increase the amount of bareground or open space. Prior to restoration, bareground in backdunes was at a premium, averaging only 0.2 (± 0.2)% (Appendices B-3 & C-1; Table 1). Over the first few years after restoration, the percent cover of bareground slowly climbed to between 6.5 and 10.8% (2013-2019; Appendices B-3 & C-1; Table 1). While it seemingly doubled between Year 3 (7.8%) and Year 9 (16.6%), this change was not significant (GLM, β=0.38, SE=0.38, t=1.0, p=0.32). Buildup of undecomposed standing and litter biomass has both direct and indirect effects on establishment of native dune plant species. The necromass prevents light from reaching the soil surface and triggering germination and plant growth (Facelli and Pickett 1991). Depending on the density of dead culms or thickness of the litter, it may also act as a physical barrier that impedes target plant establishment (Facelli and Pickett 1991). While potential allelopathic characteristics of litter have received a lot of attention, Facilli and Picket (1991) argued that these effects are likely to be temporary at most.

In 2012, the park experimented with some post-treatment measures (mowing (“mow”) and scraping with a bulldozer (“dozer”)) in certain herbicide treatment areas to try and accelerate decomposition of European beachgrass. By 2016, there were visible differences between control areas (herbicide treatment, but no post-treatment) and post-treatment areas. Both post-herbicide treatments met one of the treatment goals – reduced dead European beachgrass cover – with the dozer treatment being the more extreme of the two treatments, resulting in the lowest dead European beachgrass cover, non-native species cover, and the highest amount of bareground (Ender and Parsons 2016; Appendix D). However, the dozer treatment also yielded the lowest species richness, evenness, and species diversity (Ender and Parsons 2016; Appendix D). The mow treatment also reduced dead standing European beachgrass, but was more similar to the control in terms of having higher non-native species cover, species richness, evenness, and diversity than doze plots (Ender and Parsons 2016; Appendix D). Native species cover did not differ among the three treatments (Ender and Parsons 2016; Appendix D).

Cover of both native and non-native species decreased significantly after restoration, even

though native and other non-native species were avoided as much as possible during herbicide application (Appendix B-3; Table 1). Non-native cover (European beachgrass plus other non-natives) dropped from 137.5 (±9.6)% pre-restoration to 12.8% in 2013, increasing somewhat over the next few years to 19.2% in 2016. The largest jump in non-native cover occurred in 2017 (58.3%) and 2019 (61.5%) of cover in the plots, which was still considerably lower than pre-restoration levels (GLM, F=45.27, df=2, $p<0.0001$; Tukey HSD, $p<0.05$; Appendix B-3). This sudden increase in non-native species other than beachgrass may be attributable to several factors, including a high rainfall year in 2015-2016 combined with more decomposition of standing dead beachgrass and thatch, which created more open space available for non-native colonization. European searocket – which quickly moved in on mechanical removal and native Dune Mat areas in 2016 – was not recorded in any of the herbicide-treated plots in 2017, however, backdune areas did seem to support increasing numbers over the years of weedy, non-native, annual species such as common groundsel, ripgut brome, fescue brome, and sow thistle (*Sonchus* sp.), as well as perennial species such as iceplant, European black nightshade, New Zealand spinach, and bush lupine (Figure 29).



Figure 29. Secondary invaders such as common groundsel (*Senecio sylvestris*) and fescue brome (*Festuca bromoides*) establishing in backdune treatment areas.

Bush lupine abundance in particular soared during recent years in treated backdunes at Abbotts (Figure 30). The park had been trying for years to thin bush lupine manually, but eventually most of the lupine remaining were very large individuals that could not be removed with weed wrenches. Previous efforts to cut larger shrubs at the base and notch the stump to discourage regrowth also proved largely unsuccessful, as many of the cut shrubs started regrowing below the notch. Abundance of larger bush lupine climbed precipitously in the last 5 years, which was extremely concerning because, as other published studies and anecdotal observations by staff have shown, this nitrogen-enriched species can convert restored areas to weedy habitats through decomposition of high N litter that promotes establishment by nitrophilous non-native annuals such as ripgut brome and to perennial secondary invaders such as New Zealand spinach, iceplant, and European black nightshade, which often occur either directly underneath and adjacent to living and decadent shrubs. In 2021, the park switched to low-volume drizzle spraying to reduce lupine spread in backdune areas. Follow-up treatment will continue in future years.

Cover of secondary species accounted 56.5% of the 61.5% cover of non-natives in 2019 (Appendices B-3 & C-1). By 2021, however, non-native cover had dropped to 15.9 (±4.6)%, with cover of secondary species also greatly reduced (10.8 ± 3.3%). Again, climatic conditions appear to wield the greatest influence over cover of secondary invaders, many of which are annuals, as

2021 was the second year in a two-year drought. Abundance of common groundsel, in particular, appeared to decrease in recent years relative to initial years after treatment.



Figure 30. Dense mature bush lupine (*Lupinus arboreus*) in northern portion of treated backdunes at Abbotts ("Lupine Alley")

This explosion in the abundance of secondary species within restored backdunes is supported by non-native species richness data. The number of non-native species dropped relative to pre-restoration conditions initially, averaging between 2.0 and 2.9 species (spp)/2m^2, but then climbed back up to in 3.9 spp/2m^2 in 2017 and 3.2 (± 0.6) spp/2m^2 in 2019 which was statistically equivalent to pre-restoration conditions (Lm-Perm, df=2, p=0.12; Table 1). By 2021, non-native species richness had dropped back down to 2.2 (± 0.4) spp/2m^2. Secondary invader (GLM, Tukey HSD, β=1.26, SE=0.29, t=4.4, adj. p=0.0009) and non-native cover (GLM, Tukey HSD, β=1.29, SE=0.33, t=3.9, adj. p=0.0.004) also declined by Year 9 relative to Year 3. This was probably a result of the onset of drought conditions in 2020.

The same initial decrease in vegetation cover occurred with native species. Immediately after restoration, native species cover plummeted by more than 50% to 26.1% compared to pre-restoration levels of 70.8 (± 12.4)% (Appendix B-3; Table 1). Native species cover has slowly climbed over the years, starting largely in 2015 (30.6%) and then increasing to 41.6 % in 2017 and 64.1 (± 14.3)% in 2019 (Appendix B-3; Table 1). By 2021, native cover had jumped to 95.5 (± 17.4)%, which was significant relative to cover in Year 3 (GLM, β=0.65, SE=0.18, t=3.6, p=0.001; Fig. 3C). Native species richness also dropped immediately after restoration, but it rebounded more quickly, reaching statistical equivalence with pre-restoration conditions in 2015 (Appendix B-3; Table 1). The number of native species rose from a low of 1.9 spp/2m^2 in 2014 to 3.0 spp/2m^2 in 2015 and then jumped to 5.2- to 5.5 spp/2m^2 between 2017 and 2021, with the latter being statistically significant relative to Year 3 (GLM, β =0.80, SE=0.33, t=2.4, p=0.01; Appendix B-3; Table 1).

Diversity indices also initially dropped after restoration, but, by 2017, they were equivalent between restoration periods, with Shannon-Weiner values averaging 1.42 (± 0.08) pre-restoration to 1.27 in 2017 and 1.29 (± 0.11) in 2019 (GLM, df=2, F=2.37, p=0.11; Tukey HSD, p>0.05; Table 1). Diversity indices remained relatively stable between 2019 and 2021 even with the drought, with the latter averaging 1.24 (± 0.13), significantly different from Year 3 (GLM, β=0.89, SE=0.36, t=2.5, p=0.05). Evenness values had already reached statistical equivalence earlier (2016). Evenness averaged 0.68 (± 0.02) prior to treatment and 0.62 (± 0.04) in 2019, approximately seven (7)- to eight (8) years following treatment (GLM, df=2, F=0.48, p=0.62). It slipped very slightly in 2021 to 0.61 (± 0.06).

This initial decline in native and other non-native plant cover and richness and diversity indices

in herbicide-treated plots could be ascribed to adverse impacts on non-target species either directly from being sprayed due to proximity to or being intermixed with target plant species or indirectly from absorption of released chemicals from decaying rhizomes. Treatment with imazapyr in certain types of vegetation communities has sometimes led to issues with adverse impacts on non-target plant species. Treatment of perennial pepperweed (*Lepidium latifolium*) in seasonal wetlands in the upper margins of San Francisco Bay resulted in "severe" and "persistent" non-target effects, with species richness and percent cover of non-target plant species significantly reduced even two years after treatment (Whitcraft and Grewell 2011). However, other dune projects that used imazapyr for control of European beachgrass have not encountered adverse impacts on non-target plant establishment or survival. In Mendocino County, no evident lasting effects were observed either with respect to germination or establishment of native dune plants, including rare plants (CDPR 2012). A restoration project at Año Nuevo State Reserve near Monterey that used imazapyr resulted in colonization "by a diverse suite of natives in a relatively short time" (T. Hyland, CDPR, unpub. data).

While the vegetation monitoring data summarized above would suggest that treatment activities had a large initial negative impact on non-target vegetation, visual observations immediately after initial treatment in 2011 or 2012 indicated otherwise. A number of species established in the first year post-treatment, including miner's lettuce, wild cucumber, common groundsel, galium (*Galium aparine* or *trifidum*), fairy mist, coast cryptantha, bitter-cress (*Cardamine oligosperma*), tarweed (*Hemizonia* sp.), seaside fiddleneck, fescue brome, sheep sorrel, and California poppy (Johnson 2013a). Additional species included sand mat, beach morning glory, beach evening primrose, and yarrow. Some individuals of beach layia, Tidestrom's lupine, and curly-leaved monardella were also observed (Figure 31). These results are somewhat similar to what Hyland and Holloran (2005) found after treatment of Monterey dunes, with initial colonization by native annual plant species, early successional woody shrubs such as bush lupine, and European searocket. These examples would suggest that herbicide treatment is not having "severe" or "persistent" effects on either the soils or emerging vegetation in this particular dune environment.



Figure 31. Beach layia (*Layia carnosa*) growing in treated European beachgrass

Tidestrom's lupine and beach layia were less frequent colonizers in herbicide treatment areas due probably due to the lack of open space or bareground for colonizing. In addition, herbicide treatment generally resulted in less early successional habitats with a few exceptions. Where mechanical removal had been performed in herbicide treatment buffer areas, Tidestrom's lupine again readily recruited without seeding being even necessary. Tidestrom's lupine also moved into more open, bare sand areas within herbicide treatment areas where scraping and, to a lesser degree, mowing had been performed in 2012 to accelerate decomposition of European beachgrass or where sand overwash from the beach occurred (Abbotts Lagoon

foredune treatment areas). Many of these more open areas in the backdunes received infusions of sand from the mechanical removal area, secondary access roads nearby, or the beach. Beach layia and curly-leaf monardella established in the early successional areas, but they were also present in herbicide treated areas with more late successional vegetation communities, particularly in small, unvegetated areas between dense stands of standing dead beachgrass. Similar to the mechanical restoration areas, Tidestrom's lupine typically colonized these larger, open sand areas first, followed a few years later by beach layia and curly-leaved monardella. Numbers of these species were combined with mechanical restoration results and presented earlier.

Multivariate statistical analysis continues to depict the backdune herbicide treatment areas as distinctly separate from both mechanical removal and control (native Dune Mat) areas, as well as herbicide-treated foredune areas (Figure 25a; NMS, Axis 1, R^2 =0.543, GLM, df=3, F=27.43, p<0.001). Foredune herbicide treatment plots aligned more with mechanical ones (Figure 25a). Herbicide-treated backdune plots are characterized by higher levels of OM, CEC, nitrogen and other macronutrients, and exchangeable H^+ and decreased pH and other exchangeable macronutrients such as Mg^{2+} and Na^{2+} (Figures 21 & 25b).

Convergence with Native Dune Communities: In general, after an initial post-treatment decline, most of the native plant species present in plots continue to be strongly representative of Dune Scrub vegetation communities, with cover of Dune Scrub-associated species climbing steadily over the years, averaging: 57.5% in 2019 and 92.7% in 2021 relative to 65.0 (± 12.2)% prior to restoration (Appendix B-3). As almost all of the Abbotts herbicide-treated plots are in the backdunes, this is not surprising, as the backdunes are more likely to evolve into a Dune Scrub rather than Dune Mat vegetation communities due to distance from the ocean and the infrequency of sand deposition events that would encourage colonization of more primary successional species. Less sand movement makes it more unlikely that early (or even mid-) successional dune species will establish in these areas (Zhang and Maun 1990, Maun 2004). At Abbotts, 2021 represented the first year that cover of plant species characteristic of Dune Scrub become almost comparable to that of reference communities (108.7 ± 12.0%). Native Dune Scrub, in general, also supports a higher percentage cover of late successional dune plant species (3.9 ± 1.6%) than either Abbotts post-restoration (1.1 ± 0.9%; 2021) or pre-restoration (0.0 ± 0.0%) backdune areas.

Dune Scrub exists on the western perimeter of the herbicide treatment area, but it is not intact due to invasion by secondary invaders such as iceplant, New Zealand spinach, and European black nightshade, so its value for determining whether restoration areas are heading on a trajectory toward eventual succession into Dune Scrub is diminished. However, the AT&T North Project Area supports a number of relatively intact Dune Scrub stands. Figure 32a shows a NMS analysis between Abbotts restored backdunes and AT&T Dune Scrub stands, as well as some other backdune areas restored using herbicide treatment at AT&T North and South. The results suggest that the Abbotts backdunes treated in 2012 finally showed some overlap with native Dune Scrub in 2021 (NMS, Final Stress=12.10, Bray-Curtis, 3D; Figure 32a). Axis 1 provided most of the separation between Abbotts treated backdunes/Dune Scrub and treated backdunes at AT&T (ANOSIM, p=0.0001; Figure 32a). Some Abbotts plots diverged considerably from Dune Scrub communities: These were typically characterized by more bareground and were closer to

foredunes spatially (Fig. 32a).

Fitting of soil chemistry variables from 2020 to a reduced subset of vegetation community data from that same year suggested that Abbotts backdunes and native Dune Scrub plots were differentiated from younger treated areas by increased TKN and exchangeable Ca^{2+} (NMS, Final Stress=11.96, 2D, 50 iter., R^2=0.874; Figure 32b). Axis 2 accounted for most of the variation (59.7%), but was not statistically significant (lmPerm, df=3, *p*=0.24). While accounting for less variation (R^2=0.277), Axis 1 also displayed clear division between older treated areas/Dune Scrub and younger treated areas (GLM, df=3, F=10.45; *p*<0.0001; Figure 32b). These and previous NMS results suggest that considerable time (8- 9 years) is needed for herbicide-treated backdunes to naturally evolve to a point where there is convergence, from a species perspective, with intact native Dune Scrub.

Other multivariate analyses based on percent cover and species diversity support the conclusion that, in general, herbicide-treated backdune areas remain dissimilar to native Dune Scrub (Permanova, F=4.53, df=3, *p*=0.001; R^2=0.25, betadisper=0.88), except for the oldest treated areas at Abbotts (pairwise adj. *p*=0.62). There was also some indication that Abbotts-Year 7 might have been converging with target communities (Permanova, pairwise adj. *p*=0.11). All other year/site (i.e., AT&T) combinations showed continued divergence from native vegetation conditions (all adj. $p < 0.03$) and a varying amount of overlap with other backdune herbicide treatment year/site combinations (all adj. *p*>0.05).

Species assemblages in native Dune Scrub areas differentiated from post-restoration communities based on higher percent cover of characteristic Dune Scrub species such as chamisso lupine and coast buckwheat (Indicator Species Analysis, all *p*<0.05). Abbotts treated backdunes were characterized by higher cover of bush lupine and ripgut brome than recently treated backdunes at AT&T, which supported more dead beachgrass, dead coyotebrush, thatch, and fescue brome (Indicator Species Analysis, all *p*<0.01).



Figure 33. Maritime brome (*Bromus sitchensis* var. *maritimus*) in backdune treatment areas

Herbicide-treated backdunes at Abbotts were differentiated from other Abbotts restored and unrestored areas by higher abundances of seaside fiddleneck, coyote brush, miner's lettuce, galium, wild cucumber, fairy mist, and moss, as well as secondary invaders (ripgut brome, fescue brome, common chickweed, bush lupine, and thatch (Indicator Species Analysis, all *p*<0.05). While statistical analyses did not detect this trend, park staff observed considerable expansion in abundance of maritime brome and little California melica (*Melica imperfecta*) within post-treatment backdune plots starting in 2019 (Figure 33) and in the fern species, California polypody (*Polypodium californicum*), in 2021 (Figure 34b with lichen). For the first time in 2021, backdune areas at Abbotts were not differentiated from other Abbotts restored areas by

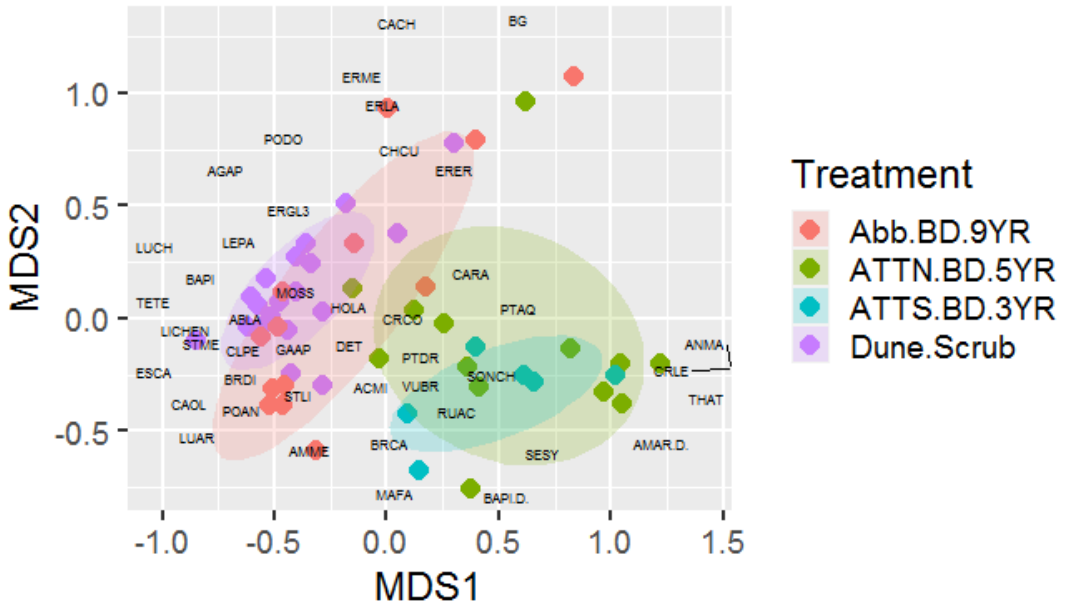


Figure 32a. Non-metric multidimensional scaling multivariate analysis of AT&T North Dune Scrub reference plots ("Dune.Scrub") and Abbotts Lagoon ("Abb.BD.9YR"; treated 2012), AT&T North ("ATTN.BD.5YR"; treated 2016), and AT&T South ("ATTS.BD.3YR"; treated 2018) backdune restoration areas in 2021. Backdune restoration areas would most likely evolve over time into Dune Scrub communities.

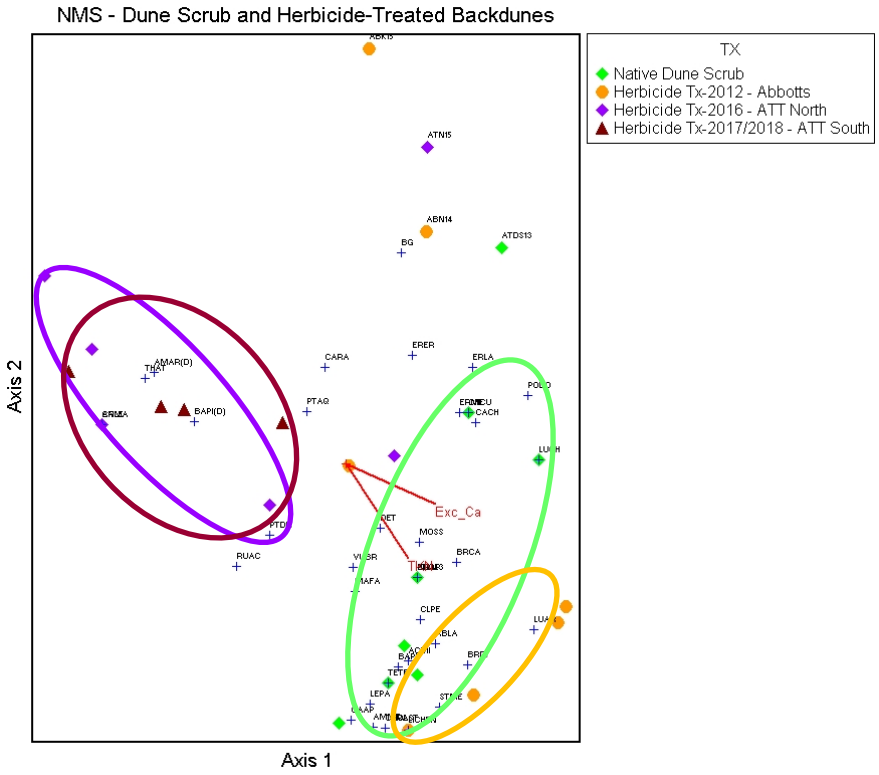


Figure 32b. Non-metric multidimensional scaling multivariate analysis of AT&T North Dune Scrub reference plots and Abbotts, AT&T North, and AT&T South backdune restoration areas in 2020 in a subsample of plots where soil sampling was conducted. Backdune restoration areas would most likely evolve over time into Dune Scrub communities.

increased abundance of dead beachgrass.



Figure 34. (a; top) Moss in native Dune Scrub at AT&T North. (b; bottom) Pixie-cup lichen and California polypody (*Polypodium californica*) in herbicide-treated backdunes.

One interesting finding in 2021 was the notable abundance of moss within herbicide-treated backdune areas at Abbotts. In general, native Dune Scrub communities typically support a much higher percentage cover of moss than any of the other dune habitats, including restored backdunes (L. Parsons, NPS, *pers. obs*.; Figure 34a). Moss cover in Dune Scrub is comprised of two species, including *Scleropodiun cespitans* and *Vinealobryum vinea* (Ken Kellman, *pers. comm*.). In contrast, lichen (pixie cup; *Cladonia chlorophaea* group; Shelly Benson, *pers. comm*.) appears more dominant in most of the restored backdunes (L. Parsons, *pers. obs*.; Figure 34b). Interestingly, in 2021, moss represented 2.6% cover in native Dune Scrub plots at AT&T North, while it actually accounted for 3.5% in Abbotts treated backdunes, compared to 0.01% and 0.04% in younger treated backdunes in AT&T North and South, respectively. Despite what has been observed by park staff, documented lichen cover within plots remained lower than 0.1% in all restored areas, however. Mosses, lichens, cyanobacteria, and algae are all considered components of the Biological Soil Crusts (BSCs). BSCs are known as "ecosystem-engineers" that have important, multifunctional ecological roles in primary production, nutrient and hydrological cycles, and stabilization of soils, especially in more arid systems (Schulz et al. 2016). Cyanobacteria appear to predominate more in primary successional dune habitats, while mosses and possibly lichen are more common in "climax" communities (Danin et al. 1989, Zellman et al. 2016).

As noted earlier, multivariate analyses point to older treated dunes at Abbotts finally beginning to converge with native Dune Scrub in terms of percent cover and diversity-based measures. By Year 9, older backdune herbicide treatment areas at Abbotts showed no significant differences from Dune Scrub for almost all measures of cover, richness, and diversity (GLM, Tukey HSD, all β >1.02, all SE<0.38, all t>-3.3, all adj. p>0.31). Native species cover and non-native species richness at Abbotts reached equivalence with target habitats by Year 7 (GLM, Tukey HSD, all β >-1.17, all SE<0.34, all t>-3.6, all adj. p>0.19), while Shannon diversity and dead beachgrass cover achieved equivalence even earlier (Year 5; GLM, Tukey HSD, all β >0.43, all SE<0.34, all t>-1.4, all adj. p>0.63). In 2021, no statistically significant differences were detected between the

target habitat and any treatment area, including the younger AT&T areas, for cover of non-native species, secondary invaders, bareground, and detritus and for non-native species richness (GLM, Tukey HSD, all β >-0.85. all SE<0.40, all t>-2.1, all adj. p>0.40). Only native species richness continued to differ between all treatment areas and Dune Scrub in 2021 (GLM, Tukey HSD, all β >-2.1, all SE<0.40, all t>-5.2, all adj. p<0.03), with restored sites supporting equivalent numbers of native species (GLM, Tukey HSD, all β >0.07, all SE<0.42, all t>0.2, all adj. p>0.16).

As discussed earlier, soil pH, TKN, OM, and some of the macronutrients (P, Ca^{2+}, Mg^{2+}) appeared to have the largest influence on separation herbicide-treated backdunes, invaded backdunes, and native Dune Scrub from foredune, native Dune Mat, and mechanical removal areas (Parsons and Becker 2021; Appendix G; Figure 21). Average pH, which accounted for a significant portion of the variability between groups (Adj. R^2=69%), varied dramatically, ranging from 6.6 to 7.3 for native Dune Mat, mechanically restored dunes, and herbicide-treated foredunes and from 5.3 to 5.9 for Dune Scrub, unrestored invaded dunes, and herbicide-treated backdunes (Parsons and Becker 2021). Herbicide-treated backdunes grouped very separately from herbicide-treated low-elevation foredunes, probably because the latter is subject to sand overwash from the adjacent beach. This sand overwash is not only burying the dead beachgrass clumps and thatch, but the formerly invaded soils, the chemistry of which has been shown to change as a result of beachgrass invasion. Periodic sand influx promotes a new sand substrate free of legacy effects and more like that found in native dune communities. This is reflected in the close proximity of these areas to native dune and mechanically restored dunes in multivariate space (Parsons and Becker 2021; Figure 21).

Within the PCA graph, herbicide-treated backdunes are positioned between the unrestored invaded dunes and native Dune Scrub, which, as explained earlier, is likely to be the community that these formerly invaded areas would evolve into if conditions permitted (Figure 21; Parsons and Becker 2021; Appendix G). Unrestored invaded dunes and herbicide-treated backdunes appeared to be differentiated from Dune Scrub by having higher soil moisture, lower OM, and higher NH^{4+} than the latter (Parsons and Becker 2021; Figure 21). Herbicide-treated backdunes were seemingly separated from unrestored invaded dunes by higher TKN, $NO3^-$, and P, although all these variables were still lower than levels reported in Dune Scrub (Parsons and Becker 2021; Figure 21). Average NH^{4+} levels ranged from 3.0 to 4.1 ppm in unrestored invaded, herbicide-treated backdune areas and native Dune Scrub at AT&T North, while all other areas had averages less than 3.0 ppm (Parsons and Becker 2021). Nitrates, on the other hand, were highest in Dune Scrub ($\bar{X}$=4.5 ppm) and herbicide-treated backdunes ($\bar{X}$=3.2 ppm), while averaging below 1.6 ppm in all other areas (Parsons and Becker 2021). Similarly, Dune Scrub had the highest average OM (1.3%), with herbicide-treated backdunes and unrestored invaded areas having the second highest (0.7 – 1.2%) and all other areas averaging below 0.5% (Parsons and Becker 2021).

The soil chemistry results point to unrestored invaded, herbicide-treated backdunes, and Dune Scrub at AT&T North as having significantly higher concentrations of NH^{4+} than the other areas (Parsons and Becker 2021; Appendix G). One of the recent microbial studies conducted at Point Reyes indicated that abundance of nitrifying bacteria was lowest in heavily invaded beachgrass stands and that restoration by chemical treatment did not alleviate this issue (Parsons et al.

2020). Nitrification can be affected by environmental factors such as salinity, temperature, oxygen, and pH. The fact that Dune Scrub areas had as low a pH as the unrestored invaded and herbicide-treated backdune areas -- but had among the highest nitrate levels -- would suggest that pH is not a constraining factor for nitrification in these dune soils. It is unclear, then, why, with such elevated NH^{4+} levels in these restored backdune areas, that nitrifying bacteria abundance remains low. Both plants and microbes can generally assimilate both forms of N, although certain species may preferentially use one form over another. Following restoration, NO^{3-} concentrations actually increased in treated backdunes relative to invaded backdunes, becoming more comparable with levels in native Dune Scrub (Parsons and Becker 2021), which suggests that any reduced abundance in nitrifying bacteria is not constraining N production or that NO^{3-} is being supplemented by other sources such as N-fixing bush lupine.

Abbotts Triangle

Dramatic declines in European beachgrass were also documented at herbicide-treated foredunes at Abbotts Triangle, with the frequency of beachgrass dropping from 73.4% prior to restoration in 2011 to 2.0% four years later in 2015 (Chi-Square, $p<0.0001$; Appendix C-2). Similar to B Ranch North, the Seashore switched monitoring methods in 2017 from frequency to cover using GPS line transects. Cover data from 2017 and 2019 were compared with more limited cover data from previous years. Immediately after treatment, cover of primary invasives such as European beachgrass dropped from 30.0% in 2011 to between 0.6 and 1.2% during the period 2012 – 2015 (Appendices B-4 & C-2). In 2017, cover of primary invasives fell to 0.2% cover and was 0% in 2019 and 2021 (Appendices B-4 & C-2).

In stark contrast to the higher-elevation herbicide-treated backdunes a little further south in the Abbotts Lagoon Project Area, standing dead biomass of European beachgrass appeared to rapidly decompose at Abbotts Triangle, except for areas on the most leeward side of the foredune system adjacent to the lagoon itself. However, despite this seemingly rapid beachgrass decomposition, the half-life of beachgrass at Abbotts Triangle – or the amount of time it takes for standing dead biomass to decompose to approximately half or less of the pre-restoration live standing crop – still averaged 2.5- to 3 years, which is in keeping with timeline of most of the other dune restoration projects (Appendix C-2). This section of Great Beach receives considerable sand inputs from wave-generated deposition, and considerable sand influx overwashed the Abbotts Triangle Project Area, burying much of the standing dead biomass, particularly on the windward portions of the low-elevation



Figure 35. Sand overwash in treatment area in foredunes at Abbotts Triangle just south of the lagoon

foredune ridge (Figure 35). Sand overwash also buried any detritus generated, rendering it a relatively minor component of this system, with detritus peaking at 17.6% cover in 2015 and dropping to 4.4% cover in 2021 (Appendices B-4 & C-2). Another factor that varied considerably from the herbicide-treated backdunes was bareground. The percent cover of bareground in the Abbotts Triangle was estimated at 54.0% in 2011, and it remained relatively stable until climbing to approximately 60% in 2017-2019 and then jumping to 72% in 2021, possibly due to drought conditions in the preceding years (Appendices B-4 & C-2).

In the first year following treatment, native dune plant species such as beach evening primrose and beach morning glory dropped significantly in frequency (Chi-Square, all $p<0.003$), with estimated native cover that year being 10.2% or roughly equivalent to pre-restoration conditions (9.5%; Appendices B-4 & C-2). Over the years, native cover has slowly, but steadily, grown, with the biggest increase coming in 2015 (21.6%; Appendices B-4 & C-2). Native cover remained stable until 2019, when it jumped to 33.8%, but drought conditions appear to reverse these gains, with native cover averaging 22.2% in 2021 (Appendices B-4 & C-2). Most of this increase appeared to come from species characterized as primary and, to a lesser extent, mid-successional species (Appendix B-4). The total number of native plant species jumped from four (4) species in 2011 (pre-restoration) to six (6) in 2012-2013, 12 in 2015, and 18 in 2019, slipping slightly in 2021 to 16 (Appendix B-4). Unfortunately, with increases in native plant cover and richness also came increases in cover of secondary invader species such as such as European searocket and common groundsel, with cover climbing from 1.5% in 2015 to 5.0% in 2019 (Appendices B-4 & C-2). Drought conditions again probably influenced the sharp reduction in secondary invader cover in 2021 to 0.4% (Appendices B-4 & C-2).

Tidestrom's lupine and, to a lesser extent, beach layia had been documented in the Abbotts Triangle area prior to the 2011 herbicide treatment. Following initial mechanical removal efforts at Abbotts Triangle in 2003-2004, park staff found 182 Tidestrom's lupine and 18 beach layia seedlings (Rodgers 2006). Most of these rare plant establishment efforts were later threatened, however, by re-growth of European beachgrass between 2005 and 2011. In monitoring efforts in subsequent years, abundance of these species in Abbotts Triangle was not differentiated relative to the adjacent historic population in the native Dune Mat area at Abbotts Lagoon, with some of the northernmost transects directly bordering the leeward portions of the Abbotts Triangle area. Since restoration in 2011, no species-specific sampling or census has been attempted, but data generated by the GPS line transects suggests that cover of rare plant species may have increased somewhat, climbing from 0% or nearly 0% in 2011-2012 to 3.0% in 2019 and 1.7% in 2021, with most of the rare plants being beach layia, Tidestrom's lupine, and curly-leaved monardella (Appendix B-4).

B Ranch North

The frequency of European beachgrass decreased dramatically after the first year of restoration, dropping from 95.6% frequency in 2014 to 4.1% frequency in 2015 (Chi-Square, $p<0.0001$). The frequency of iceplant within beachgrass stands also dropped significantly from 64.6- to 20.7%. As noted earlier, park staff switched monitoring methods of beachgrass-treated areas at B Ranch North in 2016. While it is not possible to directly assess changes between years due to the change in approach, more limited cover data was collected pre- and

immediate post-restoration. Cover of beachgrass averaged 50.9% in 2014 prior to treatment, but dropped to 3.4% in 2016 and 0.5% in 2017 and has remained low since then, averaging 0.4% in 2021 (Appendices B-5 & C-3).



Figure 36. Treated European beachgrass stands with intermixed iceplant at B Ranch North with untreated iceplant in the background.

As with Abbotts' backdune areas, most of the sprayed areas remained an extensive monoculture of standing, dead European beachgrass biomass the first few years, although, visually, plant decomposition appeared to occur more quickly at B Ranch North than at Abbotts (Figure 36). Standing dead biomass cover peaked at 37% the year immediately after restoration, dropping to 34.1% in 2016, and approximately 27.0% - in 2018 and 2019 (Appendices B-5 & C-3). Treated areas at B Ranch North are exposed to a harsher marine environment characterized by high winds and frequent fog than many of the herbicide-treated areas at Abbotts and AT&T, which would be perhaps expected to accelerate breakdown of dead plants. Dead beachgrass showed visible signs of shredding or tearing apart of aboveground stems that was not generally apparent for senescing plants at Abbotts or AT&T (Figure 37). However, despite these harsher climatic influences, the half-life of European beachgrass in terms of the amount of time that is required for standing dead biomass cover to be equivalent to 50% or less of the pre-restoration live beachgrass cover still appeared to be 2.5- 3 years, although decomposition appears to have stalled somewhat in subsequent years (Appendix C-3). While a large portion of the Project Area is "foredune" or directly adjacent to the beach, the dunes are situated on an elevated coastal marine terrace, which decreases, but does not necessarily eliminate, sand overwash, although most of the sand overwash is limited to the foredunes directly adjacent to the beach. In 2021, cover of standing dead beachgrass plummeted to 10.6%, which appeared related to a large influx of sand overwash during the preceding winter (Appendices B-5 & C-3; Figure 38).



Figure 37. European beachgrass shredded by exposure to the elements at B Ranch North

Detritus jumped from 10.3% pre-restoration to 30.3% in 2015 – probably because of

breakdown of standing dead beachgrass -- and then dropped to between 14.2 and 18.5% in the period 2016-2018 (Appendices B-5 & C-3). Between 2019 and 2020, detritus cover actually jumped back up to approximately 31%: this may be because of increases in breakdown of dead plants or may reflect simply differences in staff interpretation of detritus during monitoring (Appendices B-5 & C-3). By 2021, detritus cover had dropped to 23%, while cover of "thatch" – larger pieces of beachgrass litter (Figure 27) – had decreased from 10.4% in 2020 to 8.3% in 2021 (Appendices B-5 & C-3). The reduction in detritus and thatch may result from continued breakdown of litter or from the large influx of sand or a potentially a combination of both. Not surprisingly, bareground cover skyrocketed to 39.1% in 2021, after initially dropping immediately after restoration, hovering between 9.0% and 13.0% cover in 2015-2017, and then climbing to between 19.0 and 21.7% cover in 2018-2019 and 26.7% in 2020 (Appendices B-5 & C-3).



Figure 38. Winter storms caused large sand influx into herbicide-treated foredunes.

As with the other restoration projects, cover of native plant species also declined immediately after restoration. The only species that appeared to possibly increase in frequency immediately following treatment was coast cryptantha (Chi-Square, p=0.08). Based on visual observations by park staff, woolly lotus also appeared to be a frequent colonizer in the first year post-restoration, although data seemingly showed no difference in frequency (Chi-Square, p>0.1). In terms of cover, native vegetation dropped from 8.0% pre-restoration to 4.0% in 2015, but quickly jumped to 16.0% in 2016, 27.1% in 2017, and 36.8% in 2019 (Appendices B-5 & C-3). In keeping with trends observed at other restoration areas, native plant cover decreased during the recent drought, falling to 34.5% in 2020 and 23.1% in 2021, with the steep reduction in 2021 possibly also due to sand overwash (Appendices B-5 & C-3). Total number of native plant species or total native species richness was more than halved immediately after restoration, dropping from five (5) species to only two (2) in 2015, but has climbed dramatically in subsequent years, increasing to 19 in 2016, 25 in 2018, and 36 in 2019 (Appendix B-5). Some of this may be an artifact of the change in monitoring approach, but it does appear to reflect an actual increase in the number of native colonizers, at least until 2020 and 2021 when native species richness dropped back down to 28 and 25 species, respectively (Appendix B-5). Relative to pre-restoration conditions, most of the native species that have colonized within treated areas are mid- to late successional or Dune Scrub in nature (Appendix B-5), with primary successional species accounting for the lowest cover, which may be tied to the fact that foredune areas are on elevated terraces and not typically subject to a lot of beach overwash except for areas immediately adjacent to the terrace edge, at least until 2021 (Figure 38). In addition to late successional and Dune Scrub-associated species, former beachgrass areas at B Ranch North are also being colonized by coastal bluff species such as bluff lettuce (*Dudleya farinosa;* Figure 39).



Figure 39. Bluff lettuce (*Dudleya farinosa*) growing in European beachgrass thatch

Similar to other restoration projects, elimination of the primary invasive (European beachgrass) has opened the door to new invasives. Secondary invaders have climbed from 0.5% cover in 2015 to 4.0% cover in 2016, 12.2% in 2017, and 7.8% in 2019 (Appendix B-5). During drought years in 2020-2021, secondary invader cover dropped below 0.9% (Appendix B-5). The biggest re-invasion threat at B Ranch North has been iceplant, which existed within beachgrass stands at low frequency prior to treatment. Iceplant stands have seemingly showed more resistance to treatment efforts than have those near Abbotts and AT&T (Figure 40). As the treatment method is the same, it may be that the foggy conditions at night and early mornings increase condensation on the waxy surface of iceplant leaves to the point that herbicide treatment is not allowed to fully absorb, even with use of surfactants. While not as common as at Abbotts, European searocket has also made inroads, particularly closer to the foredunes and beach area. Other secondary invaders at B Ranch North include New Zealand spinach, sow thistle, and common groundsel.

Most of the rare plant monitoring at B Ranch North has focused on the lightly invaded Dune Mat areas that support existing populations of Tidestrom's lupine, beach layia, and other species. Some rare plant species such as beach layia have been observed in the beachgrass treatment areas, but they have not been censused. Rare plant cover averaged 0% between 2014 and 2017, 1.0% in 2019, 0.5% in 2020, and 0% in 2021 (Appendix B-5).



Figure 40. Treated iceplant at B Ranch North still partially alive following year.

AT&T North

Removal of primary invaders such as European beachgrass and, to a lesser extent, iceplant was very successful at AT&T North, which was implemented primarily in 2016, although some mechanical removal of beachgrass was performed along a wetland swale in 2015. As discussed earlier, prior to restoration, AT&T North largely supported beachgrass, coyote brush, and detritus, as determined from multivariate analysis (Indicator Species Analysis; $p<0.05$). Cover of live beachgrass dropped from an average of 77.7 (± 5.5)% in 2016 before restoration to 1.9% in 2017 and 0.0 (± 0.6)% in 2019 (lmPerm, df=4, $p<0.0001$) and 2020/2021 (Appendices B-6 & C-4; Table 2a). It should be noted that data presented represents only the herbicide treatment plots, although three plots were also established in mechanical removal areas: the low number of

plots made statistical comparisons infeasible. However, based on review of the data, regrowth in mechanical removal areas was also very low. In addition, while discussion of AT&T North and South and Abbotts are separated in this report, statistical analyses were combined and include both AT&T North and South and Abbotts, with treatment nested within these areas, because initial restoration implementation in Abbotts occurred first in 2011-2013, followed by AT&T North in 2016 and AT&T South in 2017-2018. In terms of convergence with native dune habitats, analyses are specifically focused on convergence of backdune areas with Dune Scrub. More than 75% of the Project Area is backdune, with foredune areas being slightly elevated relative to the adjacent beach.



Figure 41. Treated European beachgrass stands at AT&T North two years after treatment.

As was evident at Abbotts, standing dead beachgrass biomass presented more of an issue in the backdunes at AT&T North than the foredunes. Sand overwash in foredunes does occur, but the volume of sand available from wave deposition appears to be much smaller than that at Abbotts Triangle or in the herbicide-treated foredunes in Abbotts. Standing dead biomass of beachgrass in backdunes jumped dramatically from 2.9 (± 1.1)% prior to treatment to 79.3% in 2017, dropping to 39.5% in 2019 and 30.5 (± 7.3)% in 2021, which was not significantly different from Year 3 (GLM, Tukey HSD, β=0.34, SE=0.26, t=1.3; adj. p=0.89; Appendices B-6 and C-4). Figure 41 illustrates how treated beachgrass persists as large standing dead biomass even several years after treatment. Four to five years after implementation, half-life for decomposition of European beachgrass had just been reached, with slightly less than half (30.5 (± 7.3)%) of the pre-restoration beachgrass cover (77.1%) remaining as standing dead biomass (Appendices B-6 & C-4).

In backdunes, thatch or thick litter from beachgrass accounted for 28.6% cover in Year 5, compared to 75.8% cover for detritus or both fine and coarse litter, which was only a slight drop from detritus cover recorded in previous years (Appendices B-6 & C-4). Herbicide treatment also did not appreciably increase the percent cover of bareground relative to pre-restoration conditions, with cover climbing slowly from 1- 2% in 2016-2018 to 6.4 (± 3.8)% in 2020/2021, even though foredune areas with more sand overwash are incorporated (Appendices B-6 & C-4; Table 2a). The minimal amount of change in bareground cover can largely be attributed to the fact that most of the treated beachgrass or iceplant still remains as standing dead biomass and has not had time to break down into thatch or detritus, which would then decompose in turn and create more bareground or open space for new plant colonization.

As discussed earlier in this document, dieback of primary invaders can set the stage for invasion of other non-native species or so-called secondary invaders. Absolute cover of primary invaders and other non-native species at AT&T North plummeted from 83.1 (± 5.5)% in 2016 (pre-

restoration) to 7.4% in 2017, the first year after treatment (Appendices B-6 & C-4; Table 2a). However, average non-native cover rose abruptly over the next few years, peaking at 36.0% in 2019, only to fall to 21.2 (± 5.7)% in 2020/2021 (Appendix B-6, Table 2a). Much of this sharp variation in weed cover is probably strongly linked to climatic conditions during these years, with wetter winters (2017-2019) promoting proliferation of non-native species, particularly annuals, relative to drier winters or drought periods (2020-2021).

Potential increases in non-native cover appear attributable more to expansion in cover of a few species than establishment of many new non-native species following treatment, as non-native species richness remained roughly equivalent pre- ($\bar{X}$ = 2.1 ± 0.3 species (spp)/2m^2) and post-restoration ($\bar{X}$ = 2.9- 3.2 ± 0.4 spp/2m^2), although differences were statistically significant, at least in 2019 (GLM, F=4.67; p=0.002; Tukey HSD, p<0.05; Table 2a). Native species richness contracted slightly in 2020/2021 (5.3 spp (± 0.8)/2m^2; Appendix B-6; Table 2a). Year 1 post-restoration plots were characterized primarily by dead beachgrass and coyotebrush, as well as large amounts of detritus (Indicator Species Analysis; all p<0.05). By Year 4-5 post-restoration, dead beachgrass, thatch, brome fescue, and fairy mist contributed significantly to plant cover in 2020/2021 (Indicator Species Analysis; all p<0.05). Earlier years were characterized by non-natives such as common chickweed, common groundsel, and sheep sorrel, all of which were probably promoted by heavier winter rains. Sow thistle, another non-native, more commonly colonized foredune treatment areas for the first few years after restoration, but it was also present in the backdune areas (Indicator Species Analysis; all p<0.10). Many of these species are characterized as secondary invaders. At AT&T North, secondary invader abundance climbed from 3.6% in 2017 to 11.0% in 2018 and 26.4% in 2019, only to fall to 14.0 (± 4.5)% in 2020/2021, which, again, is probably due to two years of drought (Appendices B-6 & C-4; Table 2a).

In addition to non-native species cover, cover of native species also appeared to significantly decrease after restoration, dropping from 55.3 (±9.1)% in 2016 to 32.6- 33.9% in 2017-2018. While herbicide treatment involves use of backpack sprayers with wands and nozzles to try and avoid native species as much as possible, native species often grow amidst beachgrass and iceplant, so indirect effects on the native community are unavoidable. However, the objective is to minimize those indirect effects and leave as much native species as possible to allow for future expansion and regeneration of native species into treated areas. By 2019, however, any treatment-associated indirect impacts on native species appeared to have eased, as native cover climbed to 59.5(± 11.9)%, which was statistically equivalent to pre-restoration conditions (GLM, df=2, F=9.58; p<0.0001; Tukey HSD, p>0.05; Appendices B-6 & C-4; Table 2a). In 2020/2021, native cover dropped slightly to 49.3(± 10.0)%, but still remained relatively high considering two years of severe drought (Appendices B-6 & C-4; Table 2a).

Native species richness also dropped slightly immediately after restoration, averaging 4.3 (± 0.5) spp/2m^2 in 2016 and 3.2 spp/2m^2 in 2018. However, in 2019, species richness climbed to 5.8 (± 0.4) spp/2m^2, which was statistically equivalent for the first time to pre-restoration conditions (GLM, df=4, F=21.08, p<0.0001; Tukey HSD, p>0.05; Appendix B-6; Table 2a). Native species richness contracted slightly in 2020/2021 (5.3 spp (± 0.8)/2m^2; Appendix B-6; Table 2a). Notable native species that have recently colonized AT&T North include pearly everlasting (*Anaphalis margaritacea*), California polypody, lizard tail, and beach knotweed (*Polygonum*



Figure 42. Pearly everlasting (*Anaphalis margaritacea*) growing in dead beachgrass at AT&T North.

paronychia; Figures 42 & Figure 34b). In contrast, the Shannon Diversity Index remained at least statistically equivalent before and after treatment, averaging 0.95 in 2016 compared to 0.80- 0.84 in 2017-2018 and 1.18- 1.21 in 2019-2021 (Table 2a). Species evenness also stayed fairly equivalent between restoration periods, averaging 0.52 (± 0.05) in 2016 and 0.54 (± 0.8) in 2019 (GLM, df=4, F=3.97, p=0.006; Tukey HSD, p>0.05) and 0.57 (± 0.05) in 2020/2021 (Table 2a).

No rare plants were found in treatment plots in any of the monitoring years, but due to the persistent standing dead biomass of beachgrass and lack of bareground, this is not an unexpected result. As discussed earlier, the AT&T Project Area does support sizeable populations of several federally endangered plant species such as Tidestrom's lupine, beach layia, and Sonoma alopecurus. Sonoma alopecurus is a freshwater marsh species that occurs in the Alopecurus Swale in between AT&T North and AT&T South. As discussed earlier in this document, Tidestrom's lupine and beach layia tend to preferentially colonize in areas with more bareground or open sand, with Tidestrom's lupine being very disturbance-tolerant and stablishing in primary to mid-successional habitats, and beach layia preferring slightly more stabilized mid-successional habitats. Most of the existing primary to mid-successional dune habitats occur in AT&T South, which is where most of the established Tidestrom's lupine and beach layia populations are located.

At AT&T North, primary to mid-successional dune habitats exist now largely as pockets of open sand areas within former beachgrass stands. While these open sand pockets have not supported federally listed rare plants to date, there have been sporadic colonies of these species establishing in the UTV secondary access roads created (or enhanced) to implement the 2016 project. One "hotspot" for both species is the UTV road on the northwestern corner of AT&T North. In 2016, this area supported 45 Tidestrom's lupine adults and 10 seedlings, as well as beach layia. Other colonies have established within bareground or open areas in less dense native Dune Scrub, specifically on the south side of AT&T North near the Alopecurus Swale. This area had 12 Tidestrom's lupine adults and 3 seedlings in 2016. In 2019, 30 Tidestrom's lupine and 1,537 beach layia were counted in AT&T North on UTV roads and in the open sandy areas adjacent to native Dune Scrub. No census of rare plants in AT&T North was conducted in 2017-2018 or 2020-2021. Over time, rare plant habitat can be expected to increase, as foredune areas transition to more primary successional communities characterized by large expanses of open sand contributed from beach overwash. Indeed, a Tidestrom's lupine plant was sighted for the first time just last year in the treated foredunes where lupine had not been previously documented (L. Parsons, *pers. obs*.).

AT&T South

AT&T South was treated initially in 2017 (Phase I) and 2018 (Phase II). AT&T South areas are discussed separately from AT&T North in this report and presented by treatment year in Table

2b, but were statistically analyzed jointly with AT&T North and Abbotts. Due to staffing constraints related to COVID, only Phase II plots were monitored in 2021. Summary and other statistical results pertinent to AT&T South Phase II are relayed below. The graphs prepared for AT&T South also focuses on Phase II (2018), with 2021 characterizing post-treatment Year 3 data (Appendices B-7 & C-5).

Average percent cover of primary invaders such as European beachgrass treated as part of Phase II in 2018 dropped substantially from 79.1 (± 6.9)% pre-restoration to 4.0% after the first year (Table 2b). Regrowth of beachgrass appears to be consistently higher in Phase II than Phase I areas for some reason. Similarly, non-native cover as a whole, which included other non-native species such as ripgut brome, common velvetgrass (*Holcus lanatus*), and fescue brome, decreased significantly in Phase II areas from an average of 95.2% (± 12.6)% in 2018 to 7.8% immediately after treatment (2019; Table 2b). Overall, by 2021, post-treatment non-native cover in AT&T South Phase II areas averaged 2.5 (± 1.9)%, with drought potentially partially suppressing new weed establishment (Appendices B-6 & C-5; Table 2b).

Dead beachgrass dropped from 77.3% in Year 1 to 38.9 (± 7.3)% in AT&T South Phase II in 2021 (Table 2b). AT&T South is structured comparably to AT&T North, with extensive backdunes and a narrow foredune ridge elevated slightly relative to the adjacent beach. This substantially influences values of variables related to decomposition. Based on this data, AT&T South Phase II has reached after three years the half-life for beachgrass decomposition (i.e., the point at which standing dead beachgrass cover is equivalent to 50% of the pre-restoration live beachgrass cover; Appendix C-5). Conversely, percent cover of detritus in Phase II areas continued to climb to 80.7 (± 20.8)% in 2021 (Table 2b), as did percent cover of thatch (22.3%; Appendix C-5). Bareground also increased substantially in Phase II areas from 0.8% pre-restoration to 4.8% in 2019 and 12.0 (± 7.3)% in 2021 (Appendix C-5; Table 2b).

Similar to AT&T North and other Point Reyes dune restoration projects, native plant cover in AT&T South Phase II areas appeared to decrease the first year after treatment (6.6%) relative to pre-restoration levels (35.1%), but rebounded somewhat by Year 3 to average 21.1 (± 10.2)% (Table 2b). Similarly, native species richness dropped from 2.9 (± 0.6) species (spp)/$2m^2$ pre-restoration to 1.1 spp/$2m^2$ immediately after restoration, but climbed slightly in Year 3 to 1.7 (± 0.4) spp/$2m^2$ (Table 2b). The same trend was seen for non-native species richness, with species/$2m^2$ plots averaging 1.7(± 0.3) in 2018, 1.1 in 2019, and 1.3 (± 0.3) in 2021 (Table 2b). Wild cucumber, dead beachgrass, and dead coyotebrush were the most common plants or types of cover found in AT&T South invaded plots after treatment (Indicator Species Analysis, all $p<0.03$). Both species evenness and diversity dropped by almost 50% in Phase II areas immediately after treatment (Table 2b). By Year 3 or 2021, diversity and evenness averaged 0.58 (± 0.20) and 0.41 (± 0.12), respectively, which pointed to at least some recovery following treatment (Table 2b).

A sizeable, lightly invaded native Dune Mat area occurs at the boundary of Phase I and Phase II areas in AT&T South. The southern portion of AT&T supports the third largest population in the park of Tidestrom's lupine (Population #8) and one of the largest remaining populations of beach layia (Population #5). The center of these populations is the 0.37-acre native Dune Mat area that had been lightly invaded by iceplant (~10- 15% gross iceplant cover) with beachgrass

steadily creeping in along the perimeters. A small portion of the iceplant was treated in 2017, with most being sprayed in 2018. Surrounding beachgrass stands were treated in 2017 (north side) and 2018 (south side). Even the limited treatment conducted in 2017 appeared to have dropped cover of the primary invader, iceplant, to 4.3% in 2018, with further treatment decreasing iceplant extent to 0.9% in 2019 (Appendix B-8). By Year 2 following treatment, native plant cover and native species richness appeared to have increased considerably within the Dune Mat area (Appendix B-8), but it is difficult to determine whether this change is in response to restoration or in response to a wet winter, which could promote higher germination rates of certain species, particularly annuals. Additionally, it may reflect differences in interpretation by staff monitoring area. Monitoring of this area has not been conducted since 2019 due to lack of staffing resources.

Rare plant monitoring was not specifically conducted in beachgrass-invaded areas of Phase I or Phase II, but Washington University has monitored the established Tidestrom's lupine population (#8) for more than a decade. Interestingly, the Tidestrom's lupine population at AT&T had remained relatively small (234 – 413 individuals) until 2012, when it began to rapidly grow, more than doubling in numbers by 2014 (1,013) and peaking in 2019 (2,491), with the 2020 population still estimated at a relatively high 2,340 plants despite drought conditions (WU, unpub. data). In 2021, which was even drier, plant numbers dropped to 1,841 (WU, unpub. data). Despite drought effects, generally, these numbers represent almost a 500% increase since 2011, and very little of this increase would seemingly be attributable to restoration, as no invasives removal in AT&T South occurred until 2017.

A Population Viability Analysis conducted by Washington University on lupine data collected between 2005 and 2008 suggested that, based on reproduction rates, this population might be the only population with a stable or slightly increasing population growth rate and that removal of seed predation pressure by deer mice would be sufficient to induce a strongly positive population growth rate (>>1; Dangremond et al. 2010). However, data collected one year later (2008-2009) showed more equivocal results (WU, unpub. data). Seed predation rates have dropped moderately since 2012, with rates averaging 46% and 31%, respectively, during the periods prior to 2012 and afterwards (WU, unpub. data). Therefore, population growth could be a result of reduced predation. In 2021, predation rates averaged 12.3% (WU, unpub. data). The only other lupine population growing in population size – except for Abbotts, where restoration was performed – is located just south of AT&T (Population 9), and it has shown very similar trends in population growth, although predation rates are generally a little higher ($\bar{X}$ since 2015=38.9%, WU, unpub. data). The fact that these populations are growing despite continued moderate to heavy predation suggests that these populations are perhaps benefitting from some localized factor or common factors that have yet to be identified. Recent genetic studies have separated the Seashore's lupine populations into northern (Abbotts, AT&T), central (Population 9, North Beach), and southern populations (A. Workman and W. Dietz, Helmholtz Center, *pers. comm*.). It is possible that genetic differences are influencing population viability and how robust populations are to stressors such as seed predation.

It may not be a coincidence, then, that the only unrestored population of beach layia that has been stable since monitoring was established in 2004 occurs at AT&T South. Park staff has conducted intermittent monitoring of beach layia in the park at selected fixed census plot

locations since 2004. Layia estimates at Population #5 totaled 4,331 in 2004 and 4,216 in 2018, but dropped to 3,710 in 2019, only to skyrocket to 28,961 in 2020 (NPS, unpub. data). Numbers totaled 6,429 in 2021, which again was a strong drought year (NPS, unpub. data). Beach layia is not affected predation of seeds by mice (or seemingly any other mammals), so any benefits realized from the recent restoration would revolve more around an increase in available habitat once the abundance of iceplant and adjacent beachgrass declines, and cover of bareground expands as a result of eventual decomposition of dead invasives standing biomass and detritus. It is still too soon to see if invasives removal of iceplant and surrounding beachgrass will provide additional long-term benefits to these endangered species, however, it was very promising that, in 2019, an additional 231 beach layia were found in restored former beachgrass areas in AT&T South outside established population boundaries for this species.

Convergence with Native Dune Communities: Similar to backdune herbicide treatment areas at Abbotts, most of AT&T – both North and South – consists of backdunes that are topographically elevated and would likely evolve into Dune Scrub rather than Dune Mat vegetation communities. Foredune areas directly adjacent to the beach do have the potential to transition into Dune Mat, but these represent probably less than 25% of the entire Project Area.

As was discussed earlier for Abbotts herbicide-treated backdune plots, multivariate analyses of AT&T North and South backdune plots showed that more recently treated backdunes at AT&T differed substantially in species composition from those of older treated plots at Abbotts and in native Dune Scrub areas, with moderate overlap shown between widely dispersed points representing AT&T North and South plots (Figure 32a). Fitting of soil chemistry variables from 2020 to a reduced subset of this data suggested that Abbotts backdunes and native Dune Scrub were differentiated from more recently treated backdune areas at AT&T North and South by increased TKN and exchangeable Ca^{2+} (Figure 32b).

In terms of univariate analyses of percent cover and diversity variables, in 2021, AT&T herbicide-treated backdune areas had seemingly reached statistical equivalency with Dune Scrub or reference plots for cover of non-natives, secondary invaders, bareground, and detritus and for non-native species richness (GLM, Tukey HSD, all β >-0.85. all SE<0.40, all t>-2.1, all adj. p>0.40). At AT&T North, the Shannon index and non-native species cover in backdune areas more quickly converged with that of Dune Scrub than that at Abbotts, becoming equivalent by Year 3 (GLM, all β >-0.47, all SE<0.34, all t>-1.4, all adj. p>0.18). Analyzing these variables using multivariate Permanova (F=4.53, df=3, p=0.001, R^2=0.22; betadisper F=0.22; p=0.88), AT&T North and South continue to remain statistically equivalent to each other (adj. p=0.14) and dissimilar to native Dune Scrub (all adj. p<0.006), although AT&T North may be at least beginning to converge more with Abbotts (adj. p>0.10).

These and previous NMS results suggest that considerable time (8- 9 years) is needed for herbicide-treated backdunes to naturally evolve to a point where there is convergence, from a species perspective, with intact native Dune Scrub.

SUMMARY DISCUSSION ON CHANGES IN RESTORATION AREAS

The objective of dune restoration generally is to eradicate non-native, invasive species that are altering native dune ecosystems and threatening habitat quality and functions. The visualized endpoint for this restoration process is not only a system free of non-native, invasive plants, but a system that looks -- and functions -- like native dune systems. In many instances, invasives have either eliminated any resemblance to native dunes in either species or function, or they have forced these systems to age prematurely, transitioning them into later evolutionary phases or successional stages that don't support the same type of special status plants and wildlife that younger successional communities do.

In a sense, determining whether our restoration efforts have been successful is really a two-pronged question, starting with the question of whether we can successfully eliminate non-native, invasive species from the park's coastal dunes, particularly given how widespread European beachgrass and iceplant have become since being introduced sometime probably in the early to mid-1900's. As the "Invasion Curve" diagram suggests (Figure 43), the longer that an invasive species has been present, and the more pervasive it is in the landscape, the much harder it is to eradicate or contain. With more than 60% of coastal bluff, dune, and scrub habitat usurped by these invasives by the late 1990's, it seemed unlikely that the park would ever eradicate these species, although projects with far more daunting challenges such as the Invasive Spartina Project, which has largely eradicated widespread non-native or hybrid cordgrass (*Spartina* spp.) from San Francisco and surrounding estuaries, have pretty much done exactly that. Given the considerable logistical hurdles in eliminating beachgrass and iceplant from Point Reyes, the park chose to focus its initial restoration efforts on critical dune areas that support a number of special status plant and wildlife species.

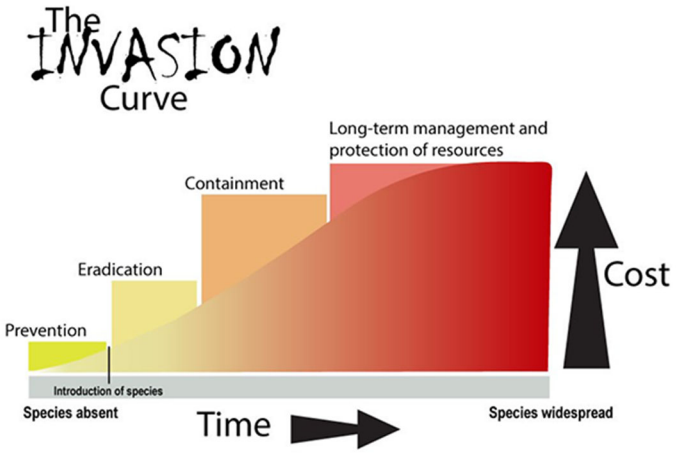


Figure 43. Species invasion curve showing feasibility/difficulty of removing invasives with increased time and abundance since invasion. *The invasion curve is adapted from Invasive Plants and Animals Policy Framework, State of Victoria, Department of Primary Industries, 2010. NPS Graphic/S. Sparhawk*.

Even with setting of these more modest goals for restoration, the potential for success early on seemed in doubt, as initial restoration efforts at Abbotts Triangle near the mouth of Abbotts Lagoon using strictly manual removal seemed almost a Sisyphean endeavor, with endless re-treatment rewarded only with endless re-growth. However, once the park switched to

mechanical removal and herbicide treatment, the success of eradication efforts improved dramatically: both methods reduced abundance of primary invaders such as European beachgrass and iceplant to less than 2- 5% within the first year after treatment, with cover continuing to decrease in subsequent years with minimal primary invader re-treatment.

However, the park's challenges were not over yet in terms of invasive species. While the battle against the primary invaders such as European beachgrass and iceplant within restoration areas appeared largely won, a new battle ensued against a much larger suite of secondary invader species, many of whom subsisted at low levels of abundance prior to restoration. These "sleeper weeds" wait until conditions are optimal and then rapidly explode out of this "lag phase" into a period of active growth, fueled by landscape disturbance or a change in biotic or abiotic resources. One of the most prevalent secondary invaders at Abbotts, European searocket, was only infrequently observed in Abbotts mechanical areas immediately following restoration in 2012. The park considered targeting it for removal, but, after consulting with online resources such as the California Invasive Plant Council threat rating ("Limited") and other biologists, dismissed it as inconsequential. Searocket persisted in relatively low and almost unnoticeable abundance at Abbotts through 2015, but, in 2016, searocket numbers exploded, expanding exponentially to the point that searocket plants were actually smothering newly established Tidestrom's lupine in some portions of the mechanically restored dunes. A similar issue arose with rapid spread of bush lupine in backdunes at Abbotts, with this N-fixing shrub potentially promoting other nitrophilous weeds such as ripgut brome, New Zealand spinach, European black nightshade, and iceplant.

This seemingly instantaneous explosion in numbers is why several invasives experts have argued that all weeds should be considered "guilty before being proven innocent" in terms of removal prioritization (Simberloff 2006, Edelaar and Tella 2012). This is by no means a unique problem to this project: as was discussed earlier in this report, a recent meta-analysis of research on invasives removal projects pointed to them often being very successful at eliminating the target species, but later these successes were significantly compromised – and sometimes even overshadowed -- by invasion of new, secondary invader species (Pearson et al. 2016). Unlike primary invaders, which were dominated by a few species that established dense monocultures, the suite of secondary invaders is more diverse and differs seemingly depending on successional status (foredunes, backdunes) and restoration approach (mechanical, herbicide). Foredune and mechanical secondary invaders are characterized by species such as European searocket, iceplant, and, to a lesser extent, New Zealand spinach and European black nightshade. Herbicide-treated backdunes support more terrestrial species such as ripgut brome, fescue brome, common groundsel, and bush lupine, along with New Zealand spinach and European black nightshade.

Despite more than approximately 4,500 control hours, the park continues to struggle with control of searocket at Abbotts due to a persistent seed bank that rapidly built following its explosion in 2016. Ultimately, problems with entrenched secondary invaders such as European searocket in Abbotts mechanical and foredune areas may prove insurmountable due to the extensive, and perhaps unsustainable, amount of long-term re-treatment required due to the delayed control response at this site. Certainly, the lessons learned at Abbotts have informed subsequent restoration efforts at AT&T and B Ranch North, with the park jumping on secondary

invaders before they could proliferate. Ultimately, what has become apparent is that vigilant re-treatment is critical to success of coastal dune restoration projects, and some level of long-term maintenance is inevitable.

So, it is apparent that European beachgrass and iceplant can be successfully eliminated even if they have been long established, although vigilant re-treatment is necessary, and re-treatment efforts are complicated by re-invasion by secondary invaders. However, the more difficult question to answer is whether our restoration efforts have been successful in creating native dune ecosystems. To date, the results of our restoration efforts have underscored the maxim that restoration is an inexact science – or a science its practitioners do not understand well enough yet. In some senses, mechanical removal almost proved too extreme in terms of re-setting the successional clock, while herbicide treatment and manual removal have not been extreme enough.

With mechanical removal, literally all non-native – and native – species were eliminated almost instantaneously, leaving vast expanses of open sand that have remained largely unvegetated for 10 years due to the vagaries of drought and high winds (Table 3). Soil conditions and dune processes proved too primordial except for the hardiest of pioneering species such as Tidestrom's lupine. Only mechanical backdune areas have begun to transform into a semblance of native Dune Mat in recent years, aided by Tidestrom's lupine enhancing soil nitrogen and creating mounds that provided safe sites for mid-successional natives to establish partially sheltered from the wind.

In stark contrast to the barren expanses of sand that typified mechanical treatment, herbicide treatment has generated little to no bareground, largely because dense stands of standing dead European beachgrass biomass are seemingly impervious to decomposition. The half-life for decomposing beachgrass biomass -- or the time elapsed for more than 50% of the pre-restoration standing biomass to be broken down – is roughly 2.5- 3 years or more in a coastal environment that seemingly should be conductive to rapid turnover of dead plant material due to wind, moisture, and temperate temperatures (Table 3). At the oldest backdune herbicide treatment site, Abbotts, it took nearly seven (7) to eight (8) years to reach almost complete breakdown of standing dead beachgrass biomass (2%), and necromass still persists even by Year 9- 10 (Table 3). Issues with decomposition are not just limited to beachgrass, as dead coyotebrush, bush lupine, and mock heather biomass also persists, but beachgrass accounts for the vast majority of the necromass present. Between the dead beachgrass and thick thatch and detritus, there has been little opportunity for open space or bareground to develop that could induce colonization of native plant species (Table 3). The only exception was in herbicide-treated foredune areas such as Abbotts Triangle where sand overwash occurred, blanketing the stands of dead beachgrass within a few years with new sand that provided substrate for natives (and secondary invaders) to more quickly gain hold (Table 3).

These restoration issues have confounded convergence of restored areas at Abbotts with the appropriate native dune community. Nine to 10 years post-restoration, only herbicide-treated backdunes showed signs of becoming similar to native Dune Scrub in terms of vegetation-related cover, richness, and diversity variables and species assemblages (Table 4). While cover and richness of native plants did climb starting 8 years post-treatment in mechanical areas,

foredunes, in particular, continue to be plagued with low native and plant cover (Tables 3 & 4). Similar trends are being observed at other dune restoration areas in the Seashore, although the time elapsed since treatment and recovery for native plants has seemingly been much shorter than at Abbotts, averaging 2 years at B Ranch North (implemented in 2014) and 3 years at AT&T North (implemented in 2016; Table 3). The only herbicide treatment project conducted entirely in foredunes (Abbotts Triangle) began recovering almost immediately in Year 1, probably due to the considerable influx of new sand that created more open space for dune plant colonization (Table 3). The influence of climate in shaping evolution of these restored systems cannot be ignored. Immediately following implementation of the Abbotts project in 2011, California entered into an extended drought, which are often accompanied by high spring winds in coastal dune systems (Baye 2008) that can destabilize newly denuded dunes and increase moisture loss in soils. Dune systems showing more rapid development in terms of native species cover and richness were restored later when the drought eased in late 2015.

Many past dune restoration projects have tacitly assumed that weed eradication alone would be sufficient to restore natural ecosystems and associated functions. However, restoration projects that rely entirely on invasives removal can be fraught with unexpected complications (Suding et al. 2004, Corbin and D'Antonio 2012, Konlechner et al. 2015). Past invasive plant control projects conducted in grasslands, wetlands, forest, and shrubland habitats have found that, while most have been successful at reducing invasive plant abundance, they were less successful in re-establishing natives (Reid et al. 2009, Kettenring and Adams 2011). For many of these projects, long-term retreatment has been necessary to prevent primary invaders from re-establishing and new or secondary invaders from becoming established (Reid et al. 2009, Kettenring and Adams 2011), some of which may pose more risk to ecosystem health than primary ones (Pearson et al. 2016). Similar issues have plagued previous dune restoration projects, with post-restoration studies citing lower native plant cover/diversity (Zarnetske et al. 2010, Konlechner at al. 2015, Pickart et al. 2021); increased abundance of secondary invaders (Novoa et al. 2013, Konlechner et al. 2015); and need for frequent re-treatment to prevent re-invasion (Novoa et al. 2013, Hesp and Hilton 2013, Darke et al. 2016).

Impacts of invasives may be more pervasive than simple displacement of native plant species (Novoa et al. 2013, Konlechner et al. 2015). Once established, invasives can alter soil chemistry and microbia, as well as insect-plant relationships (Bartomeus et al. 2008, Gornish et al. 2020, Pellegrini et al. 2021). Dune invasives such as iceplant and bush lupine have well-documented effects on soil chemistry (Conser and Connor 2009, Novoa et al. 2013, Hetherington and Wilson 2019) and microbia (de la Peña et al. 2010, Badalamenti et al. 2016). Indirect effects of beachgrass invasion have not been as well-studied, but in northern California systems, invasion appears to affect both soil chemistry and microbia (Parsons et al. 2020-Appendix E; Parsons and Becker 2021-Appendix G).

Some of these invader-mediated impacts persist long after invasives are removed, creating so-called legacy effects (Cuddington 2011, Corbin and D'Antonio 2012, Holmes et al. 2020). Removal of invasive Harding grass (*Phalaris aquatica*) from California sagebrush and orange wattle (*Acacia saligna*) from South African shrublands created changes in soil microbia or chemistry, respectively, that persisted for at least 7 to 10 years (Nsikani et al. 2017, Pickett et

al. 2018). A meta-analysis evaluating persistence of legacy effects for 42 studies involving restoration of agricultural old fields found that soil conditions and soil invertebrate richness and abundance had largely not converged with those of reference ecosystems even 50 years following restoration (Parkhurst et al. 2022). Thus, invasives removal may not be sufficient to reverse these broader legacy effects that may hinder or even preclude successful ecosystem restoration (Reid et al. 2009, Corbin and D'Antonio 2012, Holmes et al. 2020). Persistent legacy effects for soil chemistry and microbia have been reported in dune soils where iceplant, bush lupine, and beachgrass have been removed (Novoa et al. 2013, Hetherington and Wilson 2019).

As noted above, research recently conducted at PRNS within the Abbotts-AT&T study areas has shown that beachgrass invasion alters both soil microbia and chemistry and that some effects persist even after restoration (Winsemius et al. 2015, Parsons et al. 2020, Parsons and Becker 2021). Mechanical removal should have seemingly upended the potential for legacy effects associated with invasives as soil horizons were "flipped." However, mechanical areas were not immune to problems, as restoration approach or treatment also altered soil microbia and chemistry (Winsemius et al. 2015, Parsons et al. 2020, Parsons and Becker 2021). Of 15 soil chemistry variables exhibiting "treatment" effects – changes associated with restoration approach, not invasion legacy -- 13 occurred exclusively in mechanical restoration areas (Parsons and Becker 2021). A soil inoculum study conducted at PRNS after mechanical removal found that, while germination of several native plants in "flipped" soils was comparable to that in native dune soils, aboveground biomass was considerably reduced, and it concluded that parasitic microbes in flipped soils could be potentially responsible (Winsemius et al. 2015). Again, this might represent more of a treatment effect than a legacy effect imparted by past beachgrass invasion.

Soil microbial and chemistry changes following beachgrass invasion and treatment could also be slowing decomposition within backdunes. This persistent necromass phenomenon following herbicide treatment has not been well-documented within the literature, even though it occurs in other beachgrass removal projects in northern and central California (e.g., Manchester, Bodega, Año Nuevo; Terra Fuller, CDPR, *pers. comm*; Chris Heintzelman, CDPR, *pers. comm*; T. Hyland, CDPR, *pers. comm.*). Findings of other studies generally point to comparatively speedier decomposition of invasives relative to natives, with a meta-analysis of 94 studies conducted in various habitats showing that invasion increased litter decomposition rates by 117% (Liao et al. 2008). This meta-analysis did not incorporate studies in which invasives herbicide treatment was performed, but for those that did, decomposition of dead aboveground biomass did not appear delayed (Reynolds et al. 2017; Robichaud et al. 2021), although dead belowground material of herbicide-treated *Spartina foliosa* X *alterniflora* (invasive cordgrass) lingered for at least 6 years (Reynolds et al. 2017).

Litter decomposition rates would be expected to be strongly influenced by both plant and soil chemistry. Species having higher C:N ratios (> 25:1) typically decompose more slowly than those with lower ratios (<20:1; Brady and Weil 1999). Naturally senescing litter of invasive common reed (*Phragmites australis*) in East Coast marshes had C:N ratios almost twice as high (63:1) as those of a native cordgrass (*Spartina patens*; 35:1) and took more than twice as long to decompose (>9 years and 4 years, respectively; Windham 2001). Mean C:N ratio of untreated plants in the AT&T study area ranged from 17.8:1 for bush lupine (non-woody foliar) to 70.8:1

for beachgrass and 76.6:1 for coyotebrush (Parsons et al. 2020; Appendix F). Recalcitrant compounds such as lignin, other phenols, and silicon can also influence decomposition rates (Godoy et al. 2010): Concentrations for beachgrass at PRNS fell towards the lower end of the range generally reported for grasses, while lignocellulose or secondary cell wall structure was on the higher end (Parsons et al. 2020; Appendix F). A preliminary analysis of percentages of labile and recalcitrant compounds in beachgrass litter of varying ages since initial herbicide treatment at PRNS (< 1 year – 8 years) suggested that breakdown of more labile compounds such as N and hemicellulose occurred between Years 1 and 3, while that of more recalcitrant compounds did not begin until Year 3 (cellulose and lignocellulose) or even by Year 8 (lignin; Parsons 2020; Appendix F). A litterbag study conducted at PRNS found that herbicide-treated beachgrass litter had similar decomposition rates to untreated litter, at least during the first year (Fuqua et al. 2021), indicating that treatment does not seemingly alter litter quality.

Decomposition may also be stymied by low nutrients within ecosystems (Kueffer et al. 2008). As discussed earlier, coastal dune sands are strongly nutrient limited, especially for N, phosphorous (P), and, to a lesser extent, potassium (K^+; Willis & Yemm 1961, Barbour et al. 1985). Microbes must scavenge N within soils to enable breakdown of litter with higher C:N ratios, which in N-poor soils leads to immobilization rather than mineralization of N in litter and subsequent delays in decomposition (Brady and Weil 1999). One of the apparent microbial legacy effects in herbicide-treated backdunes was continued low abundance of nitrifying bacteria needed for conversion of ammonium (NH^{4+}) to nitrates (NO^{3-}; Parsons et al. 2020). However, both NH^{4+} and NO^{3-} levels were relatively high in herbicide-treated backdune soils, the latter of which is somewhat inconsistent with reduced abundance of nitrifying bacteria (Parsons and Becker 2021). It is possible that NO^{3-} is being supplemented from other sources, such as N-fixation by bush lupine. Given this, it seems unlikely that the decomposer community is being limited by N availability, even with the low amount of N available in the decomposing beachgrass material. However, in some ways, if N availability is limiting decomposition to some extent, this would help to temper the massive influx of nutrients to soils that would otherwise be expected from more immediate breakdown of such a large volume of dead biomass (Parsons and Becker 2021).

Ultimately, decomposition of beachgrass may be governed by an interaction between microbia and chemistry. Within our study areas at PRNS, abundance of wood saprophytic fungi was suppressed in beachgrass-invaded backdunes relative to native Dune Scrub, with fungal recovery not evident in herbicide-treated areas even after 8 years (Yang et al. 2022). Saprophytic fungi initiate decomposition of cellulose, lignin, and lignocellulose and are considered the primary engine of the decomposition process (Francioli et al. 2021). Abundance of cellulose-decomposing cellulolytic bacteria, which was high in heavily invaded dunes, also dropped immediately after herbicide treatment (Parsons et al. 2020a), but appeared to potentially rebound by Year 8 (B. Yang, University of Arizona, *pers. comm*.). These results mirror trends in levels of extracellular enzymes associated with breakdown of hemicellulose (xylanase) and cellulose (cellobiose and β-1,4 glucosidase), which also suggest recovery by Year 4 or Year 8, respectively (Yang et al. 2022). Structure equation modeling showed that differences in enzyme activity were driven by changes in soil chemistry, which in turn was shaped by effects of habitat type (native, invaded, restored) and associated detrital/thatch volume on soil

microbia (Yang et al. 2022). Modeling also substantiated a direct link between habitat type and soil chemistry (Yang et al. 2022). The response of these decomposers to restoration illustrates the complexity involved in legacy effects, with beachgrass removal reversing some invasion effects (cellulolytic bacteria) and perpetuating others (saprophytic fungi). While reversing microbial legacy effects of invaders is certainly desirable, reduced abundance of cellulolytic bacteria and saprophytic fungi while extensive necromass is still present may be strongly hindering restoration efforts, at least in the short- and intermediate-term.

The reason for this dramatic decline in cellulolytic bacteria following restoration is not known. Abundance of cellulolytic bacteria may have dropped due to dramatic reduction in live beachgrass material following herbicide treatment, or it may have been affected by the herbicide treatment itself. Herbicide treatment has the potential to affect soil biota through translocation by plants of herbicides into the soil through roots and rhizomes, infiltration of herbicides into the soil due to overspray, or run-off of chemicals from sprayed plants into the soils. Microorganisms, like higher plants, use the shikimate pathway to produce aromatic amino acids, so as glyphosate inhibits this pathway, it is potentially toxic to microorganisms (Issa 1999 *in* SERA 2011). While most studies conducted focused on different herbicides than were used in these projects, in general, the effect of herbicides on cellulolytic bacteria and fungi appears to depend on the chemical used, the rate of application, number of repeat treatments, and soil type, with microbial communities either inhibited or even stimulated by herbicides (Ismail and Yap 1994, Ismail and Wong 1994, Ismail et al. 1995, Pampulha et al. 2007, Schellenberger et al. 2012, Nguyen et al. 2018), but effects usually persist only for a certain period, with duration dependent on soil substrate type and sorption rates for individual chemicals (Ismail and Wong 1994, AMEC Geomatrix 2009). A few studies found some impairment of cellulose decomposition with glyphosate and imazapyr, the two chemicals used in dune restoration, however, most of these varied significantly depending on soil type and not always in the same way (Ismail et al. 1995, Nguyen et al. 2018, Ismail and Wong 1994, AMEC Geomatrix 2009). Even inhibitory effects have been characterized as relatively short-term due to binding or sorption of herbicide with soil or microbial breakdown of herbicide (AMEC Geomatrix 2009, Haney et al. 2017). Glyphosate treatment has been associated with reduced plant decomposition for black oats (Damin et al. 2012) and broad beans, but with increased decomposition for wheat biomass (Abdel-Mallek et al. 1994). Ultimately, changes in decomposition rates may have more to do with changes of the carbon-nitrogen (C:N) ratio within the plant detritus generated by glyphosate-induced senescence than herbicides themselves (Nguyen et al. 2018). Certainly, results of recent studies suggest that cellulolytic bacteria and enzymes associated with decomposition are temporarily impacted after herbicide application, but rebound by Year 8 or even Year 4 (Yang et al. 2022).

In a sense, this delayed decomposition, at least in herbicide-treated backdunes, represents perhaps yet another physical legacy effect of European beachgrass, with standing dead biomass impeding colonization of native plants. While belowground effects are most commonly documented, invasive species can also exert effects by physically changing ecosystems (Cuddington 2011). Beachgrass had been previously documented to alter the structure of foredunes in California systems (Wiedemann and Pickart 1996). A meta-analysis of 35 studies evaluating effect of litter on plant community dynamics found that positive effects (e.g.,

moisture retention) were outweighed by reduced species richness and germination (Xiong and Nilsson 1999). In the restored backdune areas, non-native plant species may benefit more than natives from the lingering microbial and physical legacy effects of beachgrass. Studies in iceplant-invaded systems have shown that legacy effects tend to favor non-native, weedy species over native ones (Andreu et al. 2010; Hamingson and Voeller 2011, Novoa et al. 2012, Novoa et al. 2014, Winsemius et al. 2015). Invasives iceplant litter in the Seashore's dunes appears to even facilitate other weeds: Dead iceplant mats often support New Zealand spinach.

Herbicide-treated foredunes can capitalize on frequent sand overwash from adjacent beaches to neutralize some of the consequences of at least legacy effects. Sand overwash is not only burying the dead beachgrass clumps and thatch, but the formerly invaded soils, creating soil conditions more like the native dune communities. However, even with this sand subsidy, foredunes are also not totally immune to legacy effects, specifically soil chemistry ones, as can be seen by the fact that herbicide-treated foredunes are offset from native Dune Mat in multivariate space (Figure 32a), which appears largely due to pH and higher levels of exchangeable K^+ and levels of Na^+ (Parsons and Becker 2021; Appendix G). Herbicide-treated foredunes were not specifically evaluated as part of the microbial study, so we cannot determine if there are any persistent microbial legacy effects in these areas.

Another invasion-related effect on microbia that appeared to readily dissipate following herbicide treatment was elevated abundances of a taxa of arbuscular mycorrhiza fungi (AMF), Archaeosporomycetes, strongly associated with heavily invaded European beachgrass dunes (Parsons et al. 2020; Appendix E). Archaeosporomycetes were more abundant in heavily invaded untreated sites than in herbicide-treated ones (Parsons et al. 2020), reinforcing the idea that invasive plants such as European beachgrass may establish or alter symbiotic relationships to enhance its success. AMF colonization rates of European beachgrass roots from AT&T South were high ($\overline{X}$ = 79.3%) and substantially exceeded those previously documented for beachgrass in both native and introduced ranges (23% to 51%; Tadych and Blaskowski 1999, Kowalchuk et al. 2002, David et al. 2016, Parsons et al. 2022; Appendix H). Microbial sampling conducted in 2017 and 2020 at Point Reyes suggest that, in northern sites such as AT&T, North Beach, and Abbotts, native Dune Mat and Dune Scrub soils may have very low AMF abundance compared to heavily beachgrass-invaded soils, which had elevated abundance of three taxa, including Archaeosporomycetes (Parsons et al. 2022). Within native Dune Mat, low AMF levels may correspond with naturally lower percent vegetation cover (22- 38%) and higher percent bareground cover (Parsons et al. 2022). Mycorrhiza would be expected to be strongly associated with the roots or rhizospheres of vegetation species, so sampling in largely open sand areas may underrepresent mycorrhizal communities. In contrast, at southern sites such as B Ranch North, soil abundances of certain AMF taxa appeared comparatively higher in native Dune Mat (Paraglomeomycetes), with abundance of these taxa sharply reduced in heavily beachgrass-invaded areas, while abundance of another taxa, Archaeosporomycetes, increased sharply in beachgrass-invaded dunes (Parsons et al. 2022). Differences in soil AMF abundance among different native Dune Mat areas may relate to successional status – B Ranch North supports a late-successional Dune Mat vegetation community characterized by higher percent vegetation with plants that are more evenly or uniformly distributed in space while that of the

northern sites is more primary to mid-successional with patchier plant establishment (Parsons et al. 2022).

These preliminary results suggest that beachgrass is interacting extensively with mycorrhizal fungi and that its mode of interaction may vary spatially, which could have important consequences for native plant species in both invaded and uninvaded habitats (Parsons et al. 2022; Appendix H). In northern habitats where native dune plants might be non- or weakly mycorrhizal, strong AMF associations for beachgrass could give beachgrass a strong competitive advantage (Parsons et al. 2022). In southern habitats, invasion of beachgrass may be accompanied by shifts in AMF taxa that may benefit beachgrass and hinder native plants. The park is currently collaborating with researchers at University of Arizona on a study to better investigate mycorrhizal status of both native and invasive dune plants and to determine how AMF relationships may be influencing invasibility of coastal dune habitats and the potential for successful restoration.

On the basis of some restoration project results, many researchers have argued that ecosystem restoration cannot be accomplished without taking additional steps to remediate some of the physical, chemical, and biological legacy effects. Habitat restoration cannot just rely on removing invasives, but may need to include "a perturbation that mitigates biogeochemical and physical effects" caused by invasives (Suding et al. 2004). These perturbations could include soil amelioration, topsoil removal, iceplant duff/litter removal, inoculation with fungal or bacterial taxa, mowing/removal of biomass, overseeding by native plant species, carbon addition, or selective removal of non-native species or species with strong biogeochemical feedbacks (Suding et al. 2004, Novoa et al. 2013, Corbin and D'Antonio 2012).

The park is currently collaborating with University of Arizona on a microbial inoculum experiment utilizing soils from native Dune Scrub to determine whether inoculum could jumpstart decomposition in herbicide-treated backdunes. Information from this study will be leveraged to develop a pilot adaptive restoration project that would experiment with different ways to accelerate beachgrass decomposition, including potentially prescribed burning/flame torching. Developing remedial measures for mechanically restored foredunes poses more of a challenge. Early on, the park attempted to conduct an active revegetation pilot project employing seeding/rhizome divisions, but plots were blown out immediately by high winds except for some direct transplants of American dunegrass in some portions of the foredunes. Time may be only the feasible prescription for the mechanical foredunes.

It is difficult to determine *a priori* whether these remedial measures are integral to restoration success or will they simply hasten the recovery process. As of now, previously implemented restoration projects hold some promise for re-creating systems that will eventually resemble native dunes, at least in terms of native plant species composition. However, it may be that full convergence with native dune communities in terms of ecological processes and functions cannot be achieved without some type of remediation. Typically, higher level ecosystem functions such as support of listed species would be expected to transpire after the habitat had reached equivalence with reference habitat condition. However, that has certainly not been the case at Abbotts. Tidestrom's lupine rapidly colonized the mechanical treatment area immediately after restoration, creating one of the largest sub-populations in the park. While



Figure 43. Myrtle's silverspot butterfly on curlyleaf monardella in restored area

slower to capitalize these new areas, beach layia also exploded in abundance both in mechanical and herbicide treatment dunes. Curly-leaved monardella, which is a nectar species for the federally endangered Myrtle's silverspot butterfly, has also slowly creeped into former beachgrass areas eliminated by herbicide treatment and, to a lesser extent, mechanical treatment (Figure 43). Western snowy plover also moved into mechanically restored dunes almost immediately after restoration was implemented, with some post-restoration years marked by a high number of nest attempts (Figure 44). Nesting and fledging success has been constrained considerably, though, by predation of nests and chicks by ravens and other predators due to the lack of vegetation cover in mechanical foredunes (David Press, NPS, *pers. comm*.).



Figure 44. Snowy plover eggs in Tidestrom's lupine plant

Given all this – threats from new weeds, legacy effects left by primary invaders even after removal, and climatic variation and uncertainties associated with climate change -- the reality is that restoration is not a one-year or even a five-year prospect. It is likely to be an ongoing, long-term maintenance responsibility and an iterative learning process that may require some tinkering or remedial measures to ultimately achieve restoration success. Adaptive restoration/management may include actions not only aimed at improving basic indicators of success such as accelerating native species re-colonization rates and boosting overall native species cover and diversity, but those aimed at improving higher level functions such as federally listed species support. For example, improvement of plover nesting and fledging success in restored dunes may require some management of predators to improve plover nesting and fledging success, even if cover of native dune plant species continues to increase.

Regardless, key to success of these efforts will be vigilant re-treatment of these areas to eliminate resurgence of primary invaders and keep out new secondary invaders. Similar to many other disturbance-prone ecosystems such as wetlands, disturbance from wind and wave exposure definitely drives the threat of perpetual non-native re-invasion. One of the most successful dune restoration projects along the West Coast – Lanphere/Ma-le'l in Humboldt County – openly acknowledges that ongoing invasives removal maintenance is required to maintain the success of this project. In southern California, invasives removal and predator control are considered long-term obligations of many mitigation and restoration projects, probably due to the high and consistent level of disturbance in such an urbanized environment. This, of course, does not conform to the seemingly widely held belief that restoration projects

are not long-term obligations and should adhere to some type of ecological “Field of Dreams” standard (“Build it, (walk away), and they (listed species) will come….”). However, managers need to recognize that building habitats that effective restoration – including projects that incorporated listed species recovery -- will need some form of routine maintenance and perhaps even adaptive restoration into the future to continue to be successful, as the processes and conditions that forged these habitats over millennia have been greatly altered by both natural and anthropogenic influences and will continue to be so as coastal environments adapt to climate change and other long-term impacts.

TABLE 3. Evolutionary trajectories in vegetation successional trends at Point Reyes coastal dune restoration projects. The first column summarize time elapsed until 50% of the original European beachgrass cover was no longer present as standing dead biomass. The second and third columns reference time elapsed until native vegetation appeared to start rebounding or until secondary invaders became more prevalent. Columns 4-6 provide additional indicators related to beachgrass eradication as of 2021.

Site/ Treatment Type	Beachgrass Half-Life (Yrs)	Native Veg Recovery (Yrs)	Secondary Invaders (Yrs)	Dead Beachgrass – 2020/21 (% Cover)	Detritus/ Thatch – 2020/21 (% Cover)	Bareground – 2020/21 (% Cover)
Abbotts/ Mechanical *Foredune-Backdune* (2011)	<<1	8	5	0%	13%	80%
Abbotts/ Herbicide *Backdune* (2011-2012)	2.5- 3	4	4	1.3%	70%	17%
Abbotts Triangle/ Herbicide *Foredune* (2011)	2.5- 3	1	3	0.3%	4%	72%
B Ranch North/ Herbicide *Foredune/Backdune* (2014)	~2.5- 3	2	2	11%	23%	39%
AT&T North/ Herbicide *Backdune/Foredune* (2016)	~4-5	3	3	35%	46%	6%
AT&T South/ Herbicide *Backdune/Foredune* (2017-2018)	~3	1-2	1-2	39%	59%	12%

TABLE 4. Convergence of key vegetation variables in restored areas with those either of pre-restoration (PRE) conditions or conditions in native reference habitats (REF). NSppR refers to native species richness; NNSppR refers to non-native species richness.

Site - Treatment Type (Treatment Yr)	**Native Cover**		**Non-Native Cover**		**BG**		**Detritus**		**NSppR**		**NNSppR**		**Shannon**		**Evenness**	
	PRE	*REF*	*PRE*	*REF*	*PRE*	*REF*	*PRE*	*REF*	*PRE*	*REF*	*PRE*	*REF*	*PRE*	*REF*	*PRE*	*REF*
Abbotts - ***Mechanical*** (2011)				**8 yrs** (2019)				**8 yrs** (2019)	**8 yrs** (2019)			**8 yrs** (2019)	**8 yrs** (2019)		**8 yrs** (2019)	
Abbotts - ***Herbicide*** (2011-2012)	**7- 8 yrs** (2019)	**7 yrs** (2019)		**9 yrs** (2021)		**9 yrs** (2021)		**9 yrs** (2021)	**3- 4 yrs** (2015)		**5- 6 yrs** (2017)	**7 yrs** (2019)	**5- 6 yrs** (2017)	**5 yrs** (2017)	**4- 5 yrs** (2016)	**9 yrs** (2021)
AT&T North - ***Herbicide*** (2016)	**3 yrs** (2019)			**3 yrs** (2019)	**3 yrs** (2019)	**5 yrs** (2021)	**3 yrs** (2019)	**5 yrs** (2021)	**3 yrs** (2019)			**5 yrs** (2021)	**3 yrs** (2019)	**3 yrs** (2019)	**3 yrs** (2019)	
AT&T South - ***Herbicide*** (2017-2018)	**1- 2 yrs** (2019)			**3 yrs** (2021)	**1- 2 yrs** (2019)	**3 yrs** (2021)	**1- 2 yrs** (2019)	**3 yrs** (2021)	**1- 2 yrs** (2019)		**1- 2 yrs** (2019)	**3 yrs** (2021)	**1- 2 yrs** (2019)		**1- 2 yrs** (2019)	

CURRENT RECOMMENDATIONS FOR COASTAL DUNE RESTORATION IN THE SEASHORE

- Large-scale restoration projects that involve non-native invasive plant removal need to be designed with potential legacy effects in mind, even if they are not fully understood for that project area or system. Weed removal alone may be insufficient to achieve restoration objectives. Additional concurrent or post-project remediation measures may be required for successful restoration. Project managers need to acknowledge the threat of secondary invasion by new or novel weeds and be prepared to quickly respond to this threat early on before the rapid expansion phase. Otherwise, managers may find themselves having to spend years, if not decades, trying to dig themselves out of the retreatment "black hole" that many of these secondary (and primary) weeds create.
- Ultimately, restoration efforts may benefit from more careful selection of which areas to restore. Planning should perhaps prioritize removing non-natives from relatively intact systems or on moderately invaded areas or on recently invaded areas where legacy effects may not have had sufficient time to develop following invasion. This approach maximizes the amount of habitat that can be more quickly restored for a considerably reduced staff and financial investment.
- Other high priority restoration options would include areas where legacy effects can be countered more easily such as herbicide treatment of foredunes where sand overwash from adjacent beaches blankets standing dead beachgrass and thatch and provides a new substrate free from the soil microbial and chemistry legacy effects imparted by beachgrass (Parsons et al. 2020, Parsons and Becker 2021). These areas also have the potential to support multiple listed and rare species, such as western snowy plover, Tidestrom's lupine, and, to a lesser extent, beach layia, curly-leaved monardella, bluff wallflower, and Myrtle's silverspot butterfly. When possible, restored areas should be discrete units of invasive plants to limit potential for re-invasion by adjacent stands of primary invader species. While this approach does not alter the landscape as dramatically as mechanical treatment, and therefore may not directly promote as extensive colonization by listed plant species, these species should colonize over time as the habitat transitions from invasive plant stands to native dunes.
- Continued restoration of backdune areas in the Seashore appears more high risk at this point, at least until we can better determine how to optimize beachgrass decomposition and conversion to native vegetation communities. The Seashore will continue to work with university researchers to better understand what factors are limiting success in existing projects and to develop measures to improve success. The next challenge will be to determine what approach or measures might need to be taken to avoid these issues prior to restoration.
- While mechanical treatment undeniably has yield huge benefits for listed species at Abbotts, the indirect impacts to adjacent native habitats and ranchlands makes use of this method highly problematic. Any use of mechanical restoration in the future should be reduced in scale and carefully planned to avoid large-scale remobilization of decades of accumulated sands. Mechanical restoration could be used in mid-dune areas, for example,

in conjunction with herbicide treatment of foredunes: Decomposing European beachgrass and associated rhizomes would minimize wind-associated erosion of foredunes and transport of those sands inland, while allowing sand overwash to rebuild native dune communities on top of decomposing biomass. In the meantime, mechanical restoration would rejuvenate dune habitats where sand deposition rates are much lower and less likely to effect large-scale habitat conversion. Mechanical removal should NOT be used adjacent to wetlands, organic pastures, or existing native dune habitat. The Seashore had originally proposed to use mechanical removal in herbicide-treatment buffers, but the risk to these habitats and listed species using them is too great.

- Manual removal is most appropriate for initial treatment of sparse iceplant and re-treatment of European beachgrass in mechanically restored areas and within buffers. Manual removal is ineffective for initial treatment of European beachgrass due to the extensive depth of the rhizome system, unless the project is simpler from a logistical standpoint (e.g., easy to access) and well-supported by volunteers or funding for contractors.
- Both external and internal iceplant removal projects have clearly documented that, regardless of method, removal of iceplant may not be enough, particularly in highly invaded areas due to legacy effects on soil chemistry and biota. Removal of duff may improve "restorability" of these areas, but, ultimately, until measures can be found to counter these legacy effects, the park may want to direct its limited restoration resources to lightly to perhaps moderately invaded iceplant areas or areas that have only been invaded for a short while.
- Project managers should prioritize removal of secondary invaders that act as facilitators for other invaders. Secondary or primary invaders that are nitrogen-fixing are prime targets due to their tendency to attract nitrophilous species. Within dune systems, this may include nitrogen-fixing bush lupine, which promotes colonization of primary and secondary invaders such as iceplant and New Zealand spinach.
- Retreatment is absolutely critical to success of these type of restoration projects and will need to be acknowledged as a long-term investment of resources.

SUMMARY DISCUSSION ON CHANGES IN MONITORING APPROACHES

Monitoring of dune vegetation communities is challenging due to the wide disparity in percent cover of bareground relative to plants and large diversity in species present. Dune mat communities are characterized by large expanses of bare sand, colonized seemingly intermittently by a wide variety of small- to medium-sized herbs along with the occasional shrubs. In contrast, Dune Scrub communities have less to sometimes no bareground and are largely colonized by medium- to large- sized shrubs, with herbs and grasses growing within the bare patches or underneath the shrub canopy.

The early Abbotts project emphasized monitoring of subplots within a larger macroplot, however, some of the statistical power for analyzing change was lost, because subplots technically represented replicates for the particular macroplot, not independent replicates. For

the later Abbotts project, the Seashore staff wanted to increase the number of replicates and, thereby, the power of our statistical analyses, so we switched to 2X1 m plots. However, methods such as the 2X1 m plots – which have been used fairly successfully in other vegetative communities – did not appear to capture the vegetative character of Dune Mat communities very well, overemphasizing bareground and considerably underemphasizing certain, less common plant species. Staff was concerned that changes in Dune Mat communities could occur without this type of monitoring approach being able to detect these changes. Certainly, the large-scale invasion by European searocket of mechanically restored and native dune areas completely escaped detection despite these monitoring efforts, which raises some definite red-flags regarding the adequacy of the current protocol, at least within certain types of dune ecosystems. Results from data collected within the 44-acre native dunes at Abbotts between 2010 and 2015 suggested that anywhere from 30 (bareground) to 121 (percent native cover) permanent quadrats would be needed to detect at least a 20% change between monitoring years. There are currently 23 plots.

To remedy this, a different type of monitoring approach was used for the Abbotts Triangle and B Ranch North areas. This method relied on "line" transects actually established using a GPS and systematically collecting point cover and species frequency data along these GPS transects, which were systematically replicated throughout the Project Areas. Based on results, this method appeared to reflect more accurately the diversity of species within Dune Mat areas, but frequency data does not lend itself as well as cover data to summary calculations of total percent cover of vegetation and bareground, as well as cover of species within different successional communities. In addition, this method is dependent on systematic interspersion of points/frequency measurements for statistical power. It is difficult to maintain the required interspersion when the terrain within dunes becomes much more steeply undulating, with large dune hills and steep slopes, because staff cannot adequately maintain consistent pacing between points. In 2016, park staff explored a variant of this in the European beachgrass treatment areas at B Ranch North, which systematically collected point-intercept vegetation cover across the entire treatment area using GIS-generated "transects" that were navigated in the field with a hand-held GPS. The long-term value of this approach is not yet known, but it is quicker than the frequency method and seems to provide more usable data. Currently, this technique is used at Abbotts Triangle, B Ranch North, and the sparsely invaded native Dune Mat areas at AT&T.

Ultimately, it may be that dune monitoring may require a combination of approaches. For very sparsely vegetated Dune Mat communities within less steeply undulating terrain, GPS line transects collecting cover data may continue to represent the best choice – at least until another method can be developed. However, for more densely vegetated communities such as invaded, Dune Scrub, or later successional native dune communities – particularly in more steep or undulating terrain – the 2X1 m plot may remain the better solution, as it's easier to navigate to randomized points, and it appears to capture both bareground and vegetative diversity quite well.

A sample size analysis for the 2X1 m plots established in the 50-acre herbicide treatment plots in the backdunes at Abbotts suggested that as few as 3 to 12 permanent plots would be needed to assess percent non-native cover, native cover, and some of the diversity indices with a 20%

chance of detecting change between years: more plots would be needed, however, to adequately assess the percent cover of bareground (23). The backdune areas at Abbotts are likely to transition to Dune Scrub, so the 2X1 m plot method should be able to accommodate this vegetative shift. In addition, almost all of the native dune vegetation communities in AT&T are some variant of Dune Scrub, which is also the likely native plant target community for the invaded dunes, which were treated starting in 2015. This, combined with the steep and highly rugged terrain, suggested that the 2X1 m plot might be a better option for monitoring in this area, so park staff opted for this monitoring method for all of AT&T, although some power analyses conducted in 2017 with results from the first year suggested that, at least at AT&T, more replicates or plots might be needed to maintain adequate statistical analysis power, ranging anywhere from 13 to 80 plots depending on the variable.

The optimal method – or methods – for monitoring vegetation in dunes may still yet to be developed. Seashore staff will keep exploring for ways to improving monitoring changes in these unique vegetation communities.

CITATIONS

Abdel-Mallek, A.Y., M.A. Abdel-Kader, A.M.A. Shonkeir. 1994. Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. Microbiological Research. 149: 69-73.

Albert, M., C. M. D'Antonio, and K. Schierenbeck. 1997. Hybridization and introgression in *Carpobrotus* spp.(Aizoaceae) in California. I. Morphological evidence. American Journal of Botany 84:896–896.

Albert, M. E. 1995a. Portrait of an invader II: the ecology and management of *Carpobrotus edulis*. CalEPPC News. Spring:4–6.

Albert, M. Ed. 1995b. Morphological variation and habitat associations within the *Carpobrotus* species complex in coastal California. University of California, Berkeley.

AMEC Geomatrix. 2009. Human Health and Ecological Effects Risk Assessment. Imazapyr. Washington State. Submitted to the Washington Department of Agriculture. Olympia, WA.

Andreu, J., E. Manzano-Piedras, I. Bartomeus, E.D. Dana, M. Vilà. 2010. Vegetation response after removal of the invasive *Carpobrotus* hybrid complex in Andalucía, Spain. Ecological Restoration. 28(4):440-448.

Art, H. W., F. H. Bormann, G. K. Voigt, and G. M. Woodwell. 1974. Barrier Island Forest Ecosystem: Role of Meteorologic Nutrient Inputs. Science 184:60–62.

Badalamenti E, V. Armando Laudicina, A. Novara, S. Pasta, and T. La Mantia. 2016. The impact of *Carpobrotus* cfr. *acinaciformis* (L.) L. Bolus on soil nutrients, microbial communities structure and native plant communities in Mediterranean ecosystems. Plant and Soil 409:19–34

Barbour, M. G. 1978. Salt Spray as a Microenvironmental Factor in the Distribution of Beach Plants at Point Reyes, California. Oecologia 32:213–224.

Barbour, M. G. 1992. Life at the leading edge: the beach plant syndrome. Coastal plant communities of Latin America: 291–307.

Barbour, M. G., T. M. deJong, and A. F. Johnson. 1976. Synecology of Beach Vegetation Along the Pacific Coast of the United States of America: A First Approximation. Journal of Biogeography 3:55–69.

Barbour, M. G., T. M. DeJong, and B. M. Pavlik. 1985. Marine beach and dune plant communities. Pages 296–322 *in* B. F. Chabot and H. A. Mooney, editors. Physiological Ecology of North American Plant Communities. Springer Netherlands.

Barbour, M. G., and A. F. Johnson. 1988. Beach and dune. Pages 223–262 *in* M. G. Barbour and A. A. Major, editors. Terrestrial Vegetation of California, 2nd edition. California Native Plant Society.

Bartomeus, I., J. Bosch, and M. Vilà. 2008. High Invasive Pollen Transfer, Yet Low Deposition on Native Stigmas in a *Carpobrotus*-invaded Community. Annals of Botany 102:417–424.

Bastow, J.L., E.L. Preisser, D.R. Strong. 2008. Wood decomposition following a perennial lupine die-off: A 3-year litterbag study. Ecosystems. 11: 442–453. DOI: 10.1007/s10021-008-9132-5

Baye, P. R. 1990. Comparative growth responses and population ecology of European and American beachgrasses (*Ammophila* spp.) in relation to sand accretion and salinity. Ph.D., The University of Western Ontario (Canada), Canada.

Baye, P. R. 2008, May 28. NEPA support for assessment of impacts to wetlands and sensitive plants affected by *Ammophila* control/removal in dunes west of southern Abbotts Lagoon, Point Reyes National Seashore. Memorandum.

Beckstead, J and IM Parker. 2003. Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? Ecology 84(12): 2824-2831.

Beena K.R., Raviraia N.S., Sridhar K.R. 1999. Seasonal variations of arbuscular mycorrhizal fungal association with *Ipomoea pes-caprae* of coastal sand dunes, Southern India. J. Environ. Biol. 21(4):341-347.

Bentley, B.L. and N.D. Johnson ND. 1991. Plants as food for herbivores: the roles of nitrogen fixation and carbon dioxide enrichment. *In*: Price PW, Lewinsohn TM, Fernandes GW, Benson WW (eds). Plant-animal interactions: evolutionary ecology in tropical and temperate regions. Wiley, New York, pp 257–272.

Borowicz, V.A., 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology. 82(11):3057-3068.

Bossard, C. C., J. M. Randall, and M. C. Hoshovsky. 2000. Invasive plants of California's wildlands. Edited by Carla C. Bossard, John M. Randall, and Marc C. Hoshovsky. University of California Press.

Brady, N.C. and R.R. Weil. 1999. The nature and properties of soils. 12th ed. New Jersey: Prentice-Hall. 881 p.

Buchenau, F. 1889. Über die Vegetationsverhältnisse des "Helms"(*Psamma arenaria* Röm. et Schultes) und der verwandten Dünengräser. Naturwissenschaftlicher Verein zu Bremen 10:397–412.

Busse, M.D., A.W. Ratcliff, C.J. Shestak, R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol. Biochem. 33: 1777-1789.

California Invasive Plant Council. 2012. Cal-IPC: *Cakile maritima*. http://www.cal-ipc.org/ip/management/plant_profiles/Cakile_maritima.php.

California Native Plant Society. 2013. Inventory of Rare and Endangered Plants (online edition, v8-01a). http://www.rareplants.cnps.org/.

Campbell, C. 2015. Draft - Monitoring Western snowy plovers at Point Reyes National Seashore, Marin County, California. 2015 Annual Report. Natural Resource Technical Report NPS/SFAN/NRTR.

Carboni, M., R. Santoro, and A. T. R. Acosta. 2010. Are some communities of the coastal dune zonation more susceptible to alien plant invasion? Journal of Plant Ecology 3:139–147.

CDPR. 2012. Dune Rehabilitation Project Initial Study/Mitigated Negative Declaration: Inglenook Fen - Ten Mile Dunes Natural Preserve, MacKerricher State Park: Appendix E – Integrated Pest Management Analysis. Draft, California Department of Parks and Recreation.

CERES. 2013. California's Coastal Dunes. http://ceres.ca.gov/ceres/calweb/coastal/dunes.html.

Cesmat, R., S. Werner, M.E. Smith, T. Riedel, R. Best, and S. Olyarnic. 2012. Quantifying the effects of European beachgrass on Aeolian sand transport over the last century: Bodega Marine Reserve, California. American Geophysical Union Fall Meeting. San Francisco, CA.

Charles, J. 2007, November 21. Non-native beach grass thwarts dune's drift. Point Reyes Light. Point Reyes Station, CA.

Chenot, J., L. Affre, R. Gros, L. Dubois, S. Malecki, A. Passetti, A. Aboucaya, and E. Buisson. 2018. Eradication of invasive Carpobrotus sp.: effects on soil and vegetation. Restoration Ecology, Wiley, 2018, 26 (1), pp.106-113. ff10.1111/rec.12538ff. ffhal-01795595f

Chestnut, J. 1997. The distribution of rare species and the distribution and trend of invasive weeds on the Mobil Coastal preserve, Guadalupe-Nipomo Dunes, California. Unpublished document. The Nature Conservancy, San Francisco, CA. In: Pickart and Sawyer. 1998. Ecology and Restoration of Northern California Coastal Dunes. California Native Plant Society.

Clarke, S.H., Jr., and Carver, G.A. 1992. Late Holocene tectonics and paleoseismicity, southern Cascadia subduction zone: Science, v. 255, p. 188-192.Clayton, J. L. 1972. Salt spray and mineral cycling in two California coastal ecosystems. Ecology:74–81.

Clayton J.L. 1972. Salt spray and mineral cycling in two California coastal ecosystems. Ecology 53(1):74-81

Conser, C., and E. F. Connor. 2009. Assessing the residual effects of *Carpobrotus* edulis invasion, implications for restoration. Biological Invasions 11:349–358.

Cooper, W. S. 1936. The strand and dune flora of the Pacific Coast of North America: a geographic study. University of California Press.

Corbin J.D., C.M. D'Antonio. 2012. Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Science and Management 5:117–124

Crooks, Jeff. 2005. Lag times and exotic species: The ecology and management of biological invasions in slow-motion. Ecoscience. 12. 316-329. 10.2980/i1195-6860-12-3-316.1.

D'Antonio, C. M. 1990. Seed Production and Dispersal in the Non-Native, Invasive Succulent *Carpobrotus edulis* (Aizoaceae) in Coastal Strand Communities of Central California. Journal of Applied Ecology 27:693–702.

Cuddington, K. 2011. Legacy effects: the persistent impact of ecological interactions. Biol Theory 6:203–210.

D'Antonio, C. M. 1993. Mechanisms Controlling Invasion of Coastal Plant Communities by the Alien Succulent *Carpobrotus Edulis*. Ecology 74:83–95.

D'Antonio, C. M., and K. Haubensak. 1998. Community and ecosystem impacts of introduced species. Fremontia 26(4):13–18.

D'Antonio, C. M., and B. E. Mahall. 1991. Root Profiles and Competition between the Invasive, Exotic Perennial, *Carpobrotus edulis*, and Two Native Shrub Species in California Coastal Scrub. American Journal of Botany 78:885–894.

Damin, V., P.C.O. Trivelin, T.d.G. Barbosa, S.J.P.d. Carvalho, M.F. Moraes. 2012. Mineralization and corn recovery of ^{15}Nitrogen from Black Oats residues treated with herbicides. Plant Nutrition 35: 1830-1842.

Dangremond, E. M., E. A. Pardini, and T. M. Knight. 2010. Apparent competition with an invasive plant hastens the extinction of an endangered lupine. Ecology 91:2261–2271.

Danin, A., Y. Bar-Or, I. Dor, and T. Yisraeli. 1989. The role of cyanobacteria in stabilization of sand dunes in southern Israel. *Ecologia Mediterranea*, *15*(1), pp.55-64.

Darke I.B., I.J. Walker, P.A. Hesp. 2016. Beach–dune sediment budgets and dune morphodynamics following coastal dune restoration, Wickaninnish Dunes, Canada. Earth Surface Processes and Landforms. 41(10):1370-85.

David, A.S., May G., Schmidt D., Seabloom, E.W. 2016. Beachgrass invasion in coastal dunes is mediated by soil microbes and lack of disturbance dependence. Ecosphere 7(11):e01527. 10.1002/ecs2.1527

de La Peña, E., S. R. Echeverría, W. H. Van Der Putten, H. Freitas, and M. Moens. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytologist 169:829–840

de La Peña, E., N. De Clercq, D. Bonte, S. Roiloa, S. Rodríguez-Echeverría, and H. Freitas. 2010. Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biological Invasions 12:3637–3648.

de Rooij-van der Goes, P.C.E.M. 1995. The role of plant-parasitic nematodes and soil-borne fungi in the decline of Ammophila arenaria (L.). The New Phytologist, Vol. 129, No. 4:661-669.

Dukes, J.S. and Mooney, H.A. 1999. Does global change increase the success of biological invaders? Trends in Ecology and Evolution, 14,135–139. *in* Alpert, P., E. Bone, and K. Holzapfel. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspectives in Plant Ecology, Evolution and Systematics. Vol. 3/1, pp. 52–66.

Edelaar, P. and J.L. Tella. 2012. Managing non-native species: don't wait until their impacts are proven. Ibis (2012), 154, 635–637.

Elzinga, C.L., D.W. Salzer, and J.W. Willoughby. 1998. Measuring and monitoring plant populations. BLM Technical Reference 1730-1. BLM/RS/ST-98/005+1730.

Ender, C. and L. Parsons. 2018. Pre-treatment Conditions at ATT South Phase I. Memorandum prepared for National Fish and Wildlife Foundation. March 2, 2018.

Eppinga, M. B., M. Rietkerk, S. C. Dekker, P. C. De Ruiter, and W. H. Van Der Putten. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114:168–176.

Ernst W.H.O., van Duin W.E., Oolbekking G.L. 1984. Vesicular arbuscular mycorrhiza in dune vegetation, Acta Bot. Neerl. 33:151–160.

Etherington J.R. 1967. Studies of nutrient cycling and productivity in oligotrophic ecosystems: I. Soil potassium and wind-blown sea-spray in a South Wales dune grassland. Journal of Ecology 55(3):743-752.

Facelli, J.M. and S.T.A. Pickett. 1991. Plant litter: Its dynamics and effects on plant community structure. The Botanical Review 57(1):1-32.

Francioli D, van Rijssel SQ, van Ruijven J, Termorshuizen AJ, Cotton TE, Dumbrell AJ, Raaijmakers JM, Weigelt A, Mommer L (2021) Plant functional group drives the community structure of saprophytic fungi in a grassland biodiversity experiment. Plant and Soil 461(1):91-105.

Fuqua, S. and L. Parsons. 2021. Effects of herbicide treatment on *Ammophila arenaria* decomposition. Poster. Ecological Society of America conference. August 2-6, 2021. Long Beach, CA.

Fuller, T. *pers. comm*. Senior Specialist Environmental Scientist. CA State Parks, Sonoma-Mendocino District. Series of personal communications dated April 2019 and July 1, 2022.
Gadgil, R. L. 1971. The nutritional role ofLupinus arboreus in coastal sand dune forestry. Plant and Soil 34:575–593.
Giovannetti M., T.H. Nicolson. 1983. Vesicular-arbuscular mycorrhizas in Italian sand dunes. Trans. Brit. Mycol. Soc. 80:552-557.
Gonzales B., R.B. Godoy, H.S. Figueroa. 1995. Seasonal dynamics of the vesicular-arbuscular mycorrhizal fungi in Chilean central-south sand dune ecosystems, Agricultura Tecnica 55:267–271.
Godoy, O., P. Castro-Díez, R.S.P. Van Logtestijn, J.H.C. Cornelissen, F. Valladares. 2010. Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: a broad phylogenetic comparison. Oecologia 162:781–790
Gornish E.S., K. Franklin, J. Rowe, A. Barberán. 2020. Buffelgrass invasion and glyphosate effects on desert soil microbiome communities. Biol Invasions 22:2587–2597
Gray, A. J. 1985. Adaptation in perennial coastal plants — with particular reference to heritable variation in *Puccinellia maritima* and *Ammophila arenaria*. Pages 179–188 *in* D. I. W. G. Beeftink, D. J. Rozema, and D. A. H. L. Huiskes, editors. Ecology of coastal vegetation. Springer Netherlands.
Grossbard, E. and G.I. Wingfield. 1978. Effects of paraquat, aminotriazole and glyphosate on cellulose decomposition. Weed Research. 18(6): https://doi.org/10.1111/j.1365-3180.1978.tb01172.x
Hamingson, E. H., and D. J. Voeller. 2011, March 14. A Case Study: Monitoring Iceplant Removal at Point Reyes National Seashore. The George Wright Society Conference on Parks, Protected Areas and Cultural Sites. New Orleans, LA.
Haney, R. L., S. A. Senseman, F. M. Hons, D. A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science. 48 (1):89-93. DOI:https://doi.org/10.1614/00431745(2000)048[0089:EOGOSM]2.0.CO;2
Hayman, D. S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Canadian Journal of Botany 61:944–963.
Heintzelman, C. Environmental Scientist California Department of Parks and Recreation, Sonoma Mendocino Coast District. Personal communication dated November 10, 2022.
Hesp P.A., M.J. Hilton. 2013. Restoration of foredunes and transgressive dunefields: case studies from New Zealand. Pages 67-92. In: Martínez LM, Gallego-Fernández JB, Hesp PA (eds) Restoration of coastal dunes. Springer, Berlin, Heidelberg.
Hetherington J.K., J.B. Wilson. 2019. Short-term soil nutrient and plant community responses to the eradication of a nitrogen fixing tree, *Lupinus arboreus*. Journal of Coastal Conservation 23:49–58
Heyligers, P. C. 1985. Impact of introduced grasses on foredunes in south-eastern Australia. *in* J. R. Dodson and M. Westoby, editors. Are Australian Ecosystems Different? Proceedings of a Symposium. Sydney, August 1984.
Higgins, S.I., Pickett, S.T.A., and Bond, W.J. 2000. Predicting extinction rates for plants: environmental stochasticity can save declining populations. Trends in Ecology and Evolution 15: 516-520.

Hinson, G.J. 2021. Understanding geomorphological changes of dunes after invasive plant species restorations. Undergraduate research project. University of California, Berkeley. Spring 2021.

Hobbs, R.J. and Mooney, H.A. 1991. Effects of rainfall variability and gopher disturbance on serpentine annuals grassland dynamics. Ecology, 72, 59–68. . *in* Alpert, P., E. Bone, and K. Holzapfel. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspectives in Plant Ecology, Evolution and Systematics. Vol. 3/1, pp. 52–66.

Holmes, P.M., K.J. Esler, M. Gaertner, S. Geerts, S.A. Hall, M.M. Nsikani, D.M. Richardson, S. Ruwanza. 2020. Biological invasions and ecological restoration in South Africa. Biological Invasions in South Africa. 665-700.

Holton, B. 1980. Some aspects of the nitrogen cycle in a northern Californian coastal dune-beach ecosystem, with emphasis on *Cakile maritima*. Dissertation Abstracts International, B 41:805.

Holton, B., and A. F. Johnson. 1979. Dune Scrub Communities and Their Correlation with Environmental Factors at Point Reyes National Seashore, California. Journal of Biogeography 6:317–328.

Holton Jr, B., M. G. Barbour, and S. N. Martens. 1991. Some aspects of the nitrogen cycle in a Californian strand ecosystem. Madroño 38:170–184.

Hope-Simpson, J.F. and R. L. Jefferies. 1966. Observations relating to vigour and debility in Marram Grass (*Ammophila arenaria* (L.). Journal of Ecology, Vol. 54, No. 1:271-274

Hui, C. and D.M. Richardson. 2017. Invasion Dynamics. Oxford University Press, New York, NY.

Huiskes, A. H. L. 1977. The Natural Establishment of *Ammophila arenaria* from Seed. Oikos 29:133–136.

Huiskes, A. H. L. 1979. *Ammophila Arenaria* (L.) Link (*Psamma Arenaria* (L.) Roem. et Schult.; *Calamgrostis Arenaria* (L.) Roth). Journal of Ecology 67:363–382.

Hyland, T., and P. Holloran. 2005. Controlling European beachgrass (*Ammophila arenaria*) using prescribed burns and herbicide. Page 29. Proceedings of the California Invasive Plant Council Symposium.

Hyland, T. 2022. Natural Resources Program Manager, California Department of Parks and Recreation, Santa Cruz District. Personal communications dated February 8, 2012, and November 8, 2022.

Inderjit, and W.H. van der Putten. 2010. Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology and Evolution Vol.25 No.9.

Irvine IC et al. (2013) Relationships between methylobacteria and glyphosate with native and invasive plant species: implications for restoration. Restoration Ecology 21:105–113

Ismail, B.S., H.P. Wong. 1994. Effects of herbicides on cellulolytic activity in peat soil. Microbios. 78(315):117-123.

Ismail, B.S. and M. Y. Yap. 1994. Effects of two acetanilide herbicides on microbial populations and their cellulolytic activities. Bulletin of Environmental Contamination and Toxicology. 52:61–68. https://doi.org/10.1007/BF00197358.

Ismail, B.S., A.J. Kader, O. Omar. 1995. Effects of glyphosate on cellulose decomposition in two soils. Folia Microbiologica. 40(5): 499–502.

Jacob D.J., Waldman T.J.M., Monger J.W., Hoffmann M.R. 1985. Chemical composition of fogwater collected along the California coast. Environ. Sci. Technol. 19:730-736
Jepson, W. L. 1993. The Jepson manual: higher plants of California. University of California Press.
Johnson, P. N. 1982. Naturalised plants in south-west South Island, New Zealand. New Zealand Journal of Botany 20:131–142.
Johnson, W. C. 2013a. Abbotts Lagoon Dune Restoration Project: Dune Movement Assessment. Point Reyes National Seashore, Point Reyes Station, CA.
Johnson, W. C. 2013b. Abbotts Lagoon Dune Restoration Project: Phase II and III Retreatment Assessment. Point Reyes National Seashore, Point Reyes Station, CA.
Johnson, W. C., S. L. Minnick, and L. Parsons. 2012. Tidestrom's lupine (*Lupinus tidestromii*) census at Abbott's Lagoon dunes and B Ranch – July 2012. Revised Nov. 5, 2012. Point Reyes National Seashore, Point Reyes Station, CA.
Kaye J.P. and S.C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology & Evolution 12:139–143
Kettenring K.M., C.R. Adams. 2011. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. Journal of Applied Ecology 48(4):970-9.
Knapp, J.J. and J.M. DiTomaso. 2005. Cal IPC Plant Assessment Form
Knevel, I. C., T. Lans, F. B. J. Menting, U. M. Hertling, and W. H. Van Der Putten. 2004. Release from Native Root Herbivores and Biotic Resistance by Soil Pathogens in a New Habitat Both Affect the Alien *Ammophila arenaria* in South Africa. Oecologia 141:502–510.
Knudsen, K. L., and C. M. Wentworth. 2000. Preliminary maps of Quaternary deposits and liquefaction susceptibility, nine-county San Francisco Bay Region, California: A digital database. US Geological Survey, Reston, VA, USA.
Konlechner T.M., J.M. Lord. 2015. Plant community response following the removal of the invasive *Lupinus arboreus* in a coastal dune system: Community response following *L. arboreus* removal. Rest. Ecol. 23(5): 607–614
Koske, R.E., Halvorson W.L. 1981. Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Canad. J. Bot*. 59: 1413–1422.
Koske, R. E., and W. R. Polson. 1984. Are VA Mycorrhizae Required for Sand Dune Stabilization? BioScience 34:420–424.
Kourtev P.S., J.G. Ehrenfeld, M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166
Kowalchuk GA et al. (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek 81:509–520
Kowalchuk G.A., De Souza F.A., Van Veen J.A. 2002. Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch coastal sand dunes. Molecular Ecology 11(3):571-81.
Kueffer C., G. Klingler, K. Zirfass, E. Schumacher, P.J. Edwards, S. Güsewell. 2008. Invasive trees show only weak potential to impact nutrient dynamics in phosphorus-poor tropical forests in the Seychelles. Functional Ecology 22:359–366
Lamb, F. H. 1898. Sand dune reclamation on the Pacific coast. The Forester 4:141–142.
Lamson-Scribner, F. 1895. Grasses as sand and soil binders. US Government Printing Office.

Le Borgne S., D. Paniagua, and R. Vazquez-Duhalt. 2008. Biodegradation of organic pollutants by halophilic bacteria and archaea. Journal of Molecular Microbiology and Biotechnology 15:74–92.

Lenth R., P. Buerkner P, I. Giné-Vázquez, M. Herve, M. Jung, J. Love, F. Miguez, H. Riebl, H. Singmann. 2022. Package "emmeans." Version 1.8.3. https://cran.r-project.org/package=emmeans

Levine, J. M. and M. Rees. 2004. Effects of temporal variability on rare plant persistence in annual systems. American Naturalist. 164: 350-363

Levine, J. M., A. K. McEachern, and C. Cowan. 2008. Rainfall effects on rare annual plants. Journal of Ecology. 96:795-806.

Liao C., R. Peng, Y. Luo, X. Zhou, X. Wu, C. Fang, J. Chen, B. Li. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706–714.

Logan V.S., Clark P.J., Allaway W.G. 1989. Mycorrhizas and root attributes of plants of coastal sand-dunes of New South Wales. Australian Journal of Plant Physiology 16(1): 141–146.

Lortie, C. J., and J. H. Cushman. 2007. Effects of a directional abiotic gradient on plant community dynamics and invasion in a coastal dune system. Journal of Ecology 95:468–481.

MacDonald, J. D., J. R. Hartman, and J. D. Shapiro. 1984. Pathogens of ice plant in California [*Carpobrotus* spp., *Pythium aphanidermatum*, *Phytophthora*, *Phomopsis* sp., *Verticillium dahliae*]. Plant Diseases 68:965–967.

Maron, J. L., and P. G. Connors. 1996. A Native Nitrogen-Fixing Shrub Facilitates Weed Invasion. Oecologia 105:302–312.

Maron J.L., R.L. Jefferies. 2001. Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11:1088–1100

Marshall, J. K. 1965. *Corynephorus Canescens* (L.) P. Beauv. as a Model for the *Ammophila* Problem. Journal of Ecology 53:447–463.

Martinez Arbizu, P. 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4

Maslach, W. Environmental Scientist. California Department of Parks and Recreation. Personal communication dated February 29, 2012.

Maun, M. A., and J. Perumal. 1999. Zonation of vegetation on lacustrine coastal dunes: effects of burial by sand. Ecology Letters 2:14–18.

Maun, M. A. 2004. Burial of plants as a selective force in sand dunes. Coastal Dunes: Ecology and Conservation. Springer-Verlag, Berlin pp. 119-135.

McLaren, J. 1924. Gardening in California: Landscape and flower. With illus. and planting plans. 3rd edition. AM Robertson, San Francisco, CA.

Milchunas, D.G. & Lauenroth, W.K. (1995) Inertia in plant community structure: state changes after cessation of nutrient-enrichment stress. Ecological Applications, 5, 452–458. *in* Alpert, P., E. Bone, and K. Holzapfel. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspectives in Plant Ecology, Evolution and Systematics. Vol. 3/1, pp. 52–66.

Nguyen, B.D. 2018. Impacts of glyphosate and its formulation on soil microbial function. Dissertation thesis. Southern Cross University, Australia.

Nicol G.W., S. Leininger, C. Schleper, and J.I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10:2966–2978.

Niu H., W. Liu, F. Wan, B. Liu. 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. Plant and Soil 294(1-2):73–85.

Novoa, A., L. González, L. Moravcová, and P. Pyšek. 2012. Effects of Soil Characteristics, Allelopathy and Frugivory on Establishment of the Invasive Plant *Carpobrotus edulis* and a Co-Occuring Native, *Malcolmia littorea*. PLoS ONE 7(12):e53166.

Novoa, A., L. González, L. Moravcová, and P. Pyšek. 2013. Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: Implications for restoration. Biological Conservation. 164:1–9.

Nsikani, M.M., A. Novoa, B.W. van Wiglen, J-H Keet, M. Gaertner. 2017. *Acacia saligna*'*s* soil legacy effects persist up to 10 years after clearing: Implications for ecological restoration. Austral Ecology 42(8):880-889.

Oksansen J., G.L. Simpson, F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner. 2022. Vegan: Community Ecology Package. Version 2.5-6. https://CRAN.R-project.org/package=vegan

Old Dominion University. 2013. Laboratory 15 – Coastal Processes, Landforms, Hazards, and Risks. http://www.ocean.odu.edu/~spars001/physical_geology/laboratory/laboratory_15/solutions_
laboratory_15.html. Accessed 2013.

Olsson P.A., Wilhelmsson P. 2000. The growth of external AM fungal mycelium in sand dunes and in experimental systems. Plant and Soil 226:161–169.

O'Neill, B. Environmental Scientist. California Department of Parks and Recreation. Personal communications dated 2012.

Oremus, P.A.I. and H. Otten. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoide*s L. ssp. *rhamnoide*s in soils from two successional stages of dune formation. Plant and Soil 63, 317-331 (1981).

Pacific Watershed Associates. 1991. Physical processes, geomorphology and management options for the coastal sand dunes of Humboldt Bay, Humboldt County, California. Unpublished document. Humboldt County Planning Department, Eureka, CA. In: Pickart and Sawyer. 1998. Ecology and Restoration of Northern California Coastal Dunes. California Native Plant Society.

Palaniappan, V. M., R. H. Marrs, and A. D. Bradshaw. 1979. The Effect of *Lupinus arboreus* on the Nitrogen Status of China Clay Wastes. Journal of Applied Ecology 16:825–831.

Pampulha, M.E., M. A. S. S. Ferreira and A. Oliveira. 2007. Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. Journal of Basic Microbiology. 47:325–331

Pardini, E. pers. comm. Professor, Washington University, St. Louis, MO. Personal communication dated November 21, 2012.

Pardini, E. A., and T. M. Knight. Unpublished data.

Pardini, E. A., and T. M. Knight. 2013, February 20. Memo: Benefits of dune restoration at Abbotts Lagoon to two federally listed endangered species, Tidestrom's Lupine and Beach Layia.
Pardini, E.A., K.E. Vickstrom, T.M. Knight. 2015. Early successional microhabitats allow the persistence of endangered plants in coastal sand dunes. PLoS ONE 10(4): e0119567. doi:10.1371/journal.pone.0119567
Pardini, E.A., L.S. Parsons, V. Ștefan, T.M. Knight. 2018. GLMM BACI environmental impact analysis shows coastal dune restoration reduces seed predation on an endangered plant. Restoration Ecology. doi: 10.1111/rec.12678
Parker, J. 1974. Coastal dune systems between Mad River and Little River, Humboldt county, California. Humboldt State University.
Parkhurst, T., S.M. Prober, R.J. Hobbs, R.J. Standish. 2022. Global meta-analysis reveals incomplete recovery of soil conditions and invertebrate assemblages after ecological restoration in agricultural landscapes. Journal of Applied Ecology. 59(2):358-72.
Parsons, L. and A. Ryan. 2015. Implement Emergency Management Measures to Prevent Extinction of Highly Endangered Plant Species, PMIS 171131: Installation of Exclusion Caging to Improve Viability of Rapidly Declining Tidestrom's Lupine Population at B Ranch North. September 21, 2015. Grant, Final Report. National Park Service, Point Reyes National Seashore.
Parsons, L, J. Sayre, C. Ender, J.L.M. Rodrigues, A. Barberán. 2020. Soil microbial communities in restored and unrestored coastal dune ecosystems in California. Restoration Ecology. Published web location. https://doi.org/10.1111/rec.13101
Parsons, L.S., Becker, B.H. 2021. Invasion by *Ammophila arenaria* alters soil chemistry, leaving lasting legacy effects on restored coastal dunes in California. Invasive Plant Science and Management 14(2):75-91.
Parsons, L, S. Fuqua, B. Yang, A. Barberán. 2022. European beachgrass invasion and restoration on soil fungal communities in California coastal dunes. Unpublished memorandum.
Patten, K. 2003. Persistence and non-target impact of imazapyr associated with smooth cordgrass control in an estuary. Journal of Aquatic Plant Management 41:1–6.
Pearson, D. E., Ortega, Y. K., Runyon, J. B. & Butler, J. L. 2016. Secondary invasion: the bane of weed management. Biol. Conserv. 197, 8–17.
Pellegrini E., F. Boscutti, G. Alberti, V. Casolo, M. Contin, M. De Nobili. 2021. Stand age, degree of encroachment and soil characteristics modulate changes of C and N cycles in dry grassland soils invaded by the N2-fixing shrub *Amorpha fruticosa*. Science of The Total Environment. 792:148295.
Peterson, B. 2004. The Use of Heavy Machinery (Excavators) to Remove *Ammophila arenaria* (European beachgrass) from Native Sand Dunes at Point Reyes National Seashore. Pages 58–61 *in* C. Pirosko, editor. Proceedings of the California Invasive Plant Council Symposium. Vol. 8: 2004. California Invasive Plant Council.
Peterson, B., J. Bromberg, and K. Cooper. 2003. Coastal Dune Restoration PMIS #60235. Final Report, Point Reyes National Seashore, Point Reyes Station, CA.
Peterson R.A. 2022. bestNormalize: Normalizing Transformation Functions. Version 1.8.3. https://cran.r-project.org/package=bestNormalize

Pickart A, S.C. McCleneghan, B.T. Little. 1990. Vesicular-arbuscular mycorrhizae in the Dune Mat Community, Humboldt County, California. Report submitted to Menzies' Wallflower Research Program, Humboldt State University.

Pickart, A. J. 1997. Control of European beachgrass (*Ammophila arenaria*) on the west coast of the United States. Symposium of the California Exotic Pest Plant Council.(The Nature Conservacy Lanphere-Christensen Dunes Preserve: Arcata, CA).

Pickart, A. J., and J. O. Sawyer. 1998. Ecology and restoration of northern California coastal dunes. California Native Plant Society.

Pickart A.J., W.R. Maslach, L.S. Parsons, E.S. Jules, C.M. Reynolds, L.M. Goldsmith. 2021. Comparing restoration treatments and time intervals to determine the success of invasive species removal at three coastal dune sites in northern California, USA. Journal of Coastal Research 37(3):557-67.

Pickett, B., I.C. Irvine, E. Bullock, K. Arogyaswamy, E. Aronson. 2019. Legacy effects of invasive grass impact soil microbes and native shrub growth. Invasive Plant Science Management. 12:22–35.

Press, D. Natural Resources Program Lead. Point Reyes National Seashore. Personal communication (undated).

Pringle A., M.C. Rillig, M. Gardes, J. Klironomos. 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715

Ranwell, D. 1958. Movement of Vegetated Sand Dunes at Newborough Warren, Anglesey. Journal of Ecology 46:83–100.

Ranwell, D. S. 1972. Ecology of salt marshes and sand dunes. Chapman and Hall: London, England.

Read, D.J. 2011. Mycorrhizas and nutrient cycling in sand dune ecosystems. Proceedings of the Royal Society of Edinburgh. Section B: Biological Sciences Volume 96: Coastal Sand Dunes. Cambridge University Press.

Reid A.M., L. Morin, P.O. Downey, K. French, J.G. Virtue. 2009. Does invasive plant management aid the restoration of natural ecosystems? Biological Conservation 142(10):2342-9.

Reinhart, K. O., A. A. Royo, W. H. Van Der Putten, and K. Clay. 2005. Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. Journal of Ecology 93:890–898.

Reynolds, P.L., J. Glanz, S. Yang, C. Hann, J. Couture, E. Grosholz. 2017. Ghost of invasion past: legacy effects on community disassembly following eradication of an invasive ecosystem engineer. Ecosphere 8(3):e01711.

Robichaud, C.D. and R.C. Rooney. 2021, Effective suppression of established invasive *Phragmites australis* leads to secondary invasion in a coastal marsh. Invasive Plant Science and Management 14(1):9-19.

Robertson, G. P. 1982. Factors Regulated Nitrification in Primary and Secondary Succession. Ecology 63:1561–1573.

Rodgers, J. 2006. Tidestrom's Lupine (*Lupinus tidestromii*). USFWS Permit TE018180-2: Annual Report. Point Reyes National Seashore.

Rodríguez-Echeverría S., W.H.G. Hol, H. Freitas, W.R. Eason, R. Cook. 2007. Arbuscular mycorrhizal fungi of *Ammophila arenaria* (L.) Link: Spore abundance and root colonisation in six locations of the European coast. European Journal of Soil Biology 44(1):30–36.

Rose, S. 1988. Above and belowground community development in a marine sand dune ecosystem. Plant and Soil 109:215–226.

Rose, S. L. and C. T. Youngberg. 1981. Tripartite associations in snowbrush (*Ceanothus velutinus*): effect of vesicular-arbuscular mycorrhizae on growth, nodulation, and nitrogen fixation. Canadian Journal of Botany 59:34–39.

Roy-Bolduc A, T.H. Bell, S. Boudreau, M. Hijri. 2015. Comprehensive sampling of an isolated dune system demonstrates clear patterns in soil fungal communities across a successional gradient. Environmental Microbiology Reports 7(6): 839–848. doi:10.1111/1758-2229.12312

Salisbury, E. J. 1952. Downs & dunes. Bell.

Santoro, R., T. Jucker, M. L. Carranza, and A. T. R. Acosta. 2011. Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter? Community Ecology 12:234–240.

Schellenberger, S., H.L. Drake, and S. Kolb. 2012. Impairment of cellulose- and cellobiose-degrading soil bacteria by two acidic herbicides. FEMS Microbiology Letters, Volume 327, Issue 1, February 2012, Pages 60–65, https://doi.org/10.1111/j.1574-6968.2011.02460.x

Schmalzer, P., and C. Hinkle. 1987. Species biology and potential for controlling four exotic plants (*Ammophila arenaria*, *Carpobrotus edulis*, *Cortaderia jubata* and *Gasoul crystallinum*) on Vandenberg Air Force Base, California.

Schulz, K., T. Mikhailyuk, M. Dreßler, P. Leinweber, and U. Karsten. 2016. Biological soil crusts from coastal dunes at the Baltic Sea: cyanobacterial and algal biodiversity and related soil properties. Microbial Ecology. 71:178-193.

SERA. 2011. Glyphosate Human Health and Ecological Risk Assessment. Final Report for USDA/Forest Service. Prepared by Syracuse Environmental Research Associates, Manlius, NY. 313 pp. SERA TR-052-22-03b

Sharifi Y., A.A. Pourbabaei, A. Javadi, M.H. Abdolmohammadi, M. Saffari, A. Morovvat. 2015. Biodegradation of glyphosate herbicide by *Salinicoccus* spp isolated from Qom Hoze-Soltan Lake, Iran. Environmental Health Engineering and Management Journal 2:31–36

Skeffington, R. A., and A. D. Bradshaw. 1980. Nitrogen Fixation by Plants Grown on Reclaimed China Clay Waste. Journal of Applied Ecology 17:469–477.

Simberloff D. 2006. Risk assessments, blacklists, and white lists for introduced species: are predictions good enough to beuseful? Agric Resour Econ Rev 35:1–10

Slobodchikoff, C. N., and J. T. Doyen. 1977. Effects of *Ammophila arenaria* on sand dune arthropod communities. Ecology 58:1171–1175.

Smith S.M., Allen R.B., Daly B.K. 1985. Soil-vegetation relationships on a sequence of sand dunes, Tautuku Beach, South-east Otago, New Zealand. Journal of the Royal Society of New Zealand 15(3):295-312

Soil Conservation Service (SCS). 1985. Soil survey of Marin County California. United States Department of Agriculture, Soil Conservation Service. United States Department of Agriculture.

St. John, T. V. 1990. Mycorrhizal Inoculation of Container Stock for Restoration of Self-Sufficient Vegetation. Pages 103–112 *in* J. J. Berger, editor. Environmental Restoration: Science And Strategies for Restoring the Earth: selected papers from the Restoring the Earth Conference.

Island Press, Washington, D.C. *In*: Pickart, A. J., and J. O. Sawyer. 1998. Ecology and restoration of northern California coastal dunes. California Native Plant Society.
Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment: pseudoreplication in time? Ecology 67:929-940.
Stürmer SL, Bellei MM (1994) Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. Can. J. Bot. 72:359-363.
Suding, K.N., K.L. Gross, and G.R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution. 19(1):46-53.
Suding, K.N. and R.J. Hobbs. 2009. Models of ecosystem dynamics as frameworks for restoration ecology. In New Models for Ecosystem Dynamics.
Suding, K.N., W.S. Harpole, T. Fukami, A. Kulmatiski, A.S. MacDougall, C. Stein, and W.H. van der Putten. 2013. Consequences of plant–soil feedbacks in invasion. Journal of Ecology. 101: 298–308.
Tadych M., J. Blaszkowski. 1999. Growth responses of maritime sand dune plant species to arbuscular mycorrhizal fungi. Acta Mycologica 34(1):115-123. DOI: 10.5586/am.1999.010
Tassan, R., K. Hagen, and D. Cassidy. 1982. Imported natural enemies established against ice plant scales in California. California Agriculture 36:16–17.
Transou, A. N., P. R. Vaughan, and M. A. Forys. 2007. Little River State Beach European beachgrass (*Ammophila arenaria*) removal project - A pilot study. Unpublished report prepared for California Department of Parks and Recreation, North Coast Redwoods District, Eureka CA.
Tsang A., Maun M.A. 1999. Mycorrhizal fungi increase salt tolerance of *Strophostyles helvola* in coastal foredunes. Plant Ecology 144:159–166.
Underwood, A. J. 1991. Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. Australian Journal of Marine and Freshwater Research 42:569-587.
USFS. 1995a. Decision notice and finding of no significant impact: snowy plover/pink sand verbena habitat restoration. Unpublished document. US Forest Service, Suislaw National Forest, Oregon Dunes National Recreation Area, Reedsport, OR. In: Pickart and Sawyer. 1998. Ecology and Restoration of Northern California Coastal Dunes.
USFS. 1995b. Environmental assessment: snowy plover/pink sand verbena habitat restoration project. Unpublished document. US Forest Service, Suislaw National Forest, Oregon Dunes National Recreation Area, Reedsport, OR. In: Pickart and Sawyer. 1998. Ecology and Restoration of Northern California Coastal Dunes.
USFS. 2010. Solicitation/Contract/Order for Commercial Items: CCRD Dune Restoration European Beach Grass Removal Phase 2: Siuslaw National Forest: Central Coast R.D. U.S. Forest Service.
USFWS. 1998. Seven Coastal Plants and the Myrtle's Silverspot Butterfly Recovery Plan. U.S. Fish and Wildlife Service, Portland, OR.
Van der Putten, W. H., C. van Dijk, and S. R. Troelstra. 1988. Biotic Soil Factors Affecting the Growth and Development of *Ammophila arenaria*. Oecologia 76:313–320.

Van der Putten, W. H., J.T. Van der Werf-Klein Breteler, and C. van Dijk. 1989. Colonization of the root zone of *Ammophila arenaria* by harmful soil organisms. Plant and Soil 120: 213-223.

Van der Putten, W. H., C. Van Dijk, and B. a. M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56.

Van der Putten, W.H. and A.M. Peters. 1997. How Soil-Borne Pathogens May Affect Plant Competition. Ecology. 78(6): 1785-1795

Van der Putten, W.H., G.W. Yeates, H. Duyts, C. Schreck Reis, G. Karssen. 2005. Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. Biological Invasions. 7: 733–746. DOI 10.1007/s10530-004-1196-3

Van der Watt E., J.C. Pretorius. 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Journal of Ethnopharmacology 76:87–91

Van der Valk, A. G. 1974. Mineral Cycling in Coastal Foredune Plant Communities in Cape Hatteras National Seashore. Ecology 55:1349–1358.

Van der Stoel, C.D., W.H. van Der Putten, and H. Duyts. 2002. Development of a Negative Plant-Soil Feedback in the Expansion Zone of the Clonal Grass *Ammophila arenaria* Following Root Formation and Nematode Colonization. Journal of Ecology. 90(6): 978-988

Van Grunsven, R. H. A., F. Bos, B. S. Ripley, C. M. Suehs, and E. M. Veenendaal. 2009. Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean. South African Journal of Botany 75:172–175.

Vaughan, P. R., and R. A. Fiori. 2007. Pilot Project for Assessment of Sand Movement Following Vegetation Removal: Little River State Beach, Humboldt County, CA. Prepared for California State Parks, North Coast Redwoods District, Eureka CA.

Vilà, M., M. Tessier, C. M. Suehs, G. Brundu, L. Carta, A. Galanidis, P. Lambdon, M. Manca, F. Médail, and E. Moragues. 2006. Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. Journal of Biogeography 33(5):853–861.

Vivrette, N. J. 1973. Mechanism of invasion and dominance of coastal grassland by *Mesembryanthemum crystallinum* L. University of California, Santa Barbara.

Vivrette, N. J., and C. H. Muller. 1977. Mechanism of Invasion and Dominance of Coastal Grassland by *Mesembryanthemum crystallinum*. Ecological Monographs 47:301–318.

Wahab, A. A., and P. F. Wareing. 1980. Nitrogenase activity associated with the rhizosphere of *Ammophila arenaria* L. and effect of inoculation of seedlings with Azotobacter. New Phytologist 84:711–721.

Wallén, B. 1980. Changes in Structure and Function of *Ammophila* during Primary Succession. Oikos 34:227–238.

Washburn, J. O., and G. W. Frankie. 1985. Biological studies of iceplant scales, *Pulvinariella mesembryanthemi* and *Pulvinaria delottoi* (Homoptera: Coccidae), in California. Hilgardia-California Agricultural Experiment Station 53.

Wheeler B, M. Torchiano. 2016. Permutation tests for linear models: lmPerm. Version 2.1.0. URL https://github.com/mtorchiano/lmPerm

Whitcraft, C. R., and B. J. Grewell. 2011. Evaluation of perennial pepperweed (*Lepidium latifolium*) management in a seasonal wetland in the San Francisco Estuary prior to restoration of tidal hydrology. Wetlands Ecology and Management. Published online.

White, T.A., Campbell, B.D. & Kemp, P.D. 1997. Invasion of temperate grassland by a subtropical annual grass across an experimental matrix of water stress and disturbance. Journal of Vegetation Science, 8, 847–854. *in* Alpert, P., E. Bone, and K. Holzapfel. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspectives in Plant Ecology, Evolution and Systematics. Vol. 3/1, pp. 52–66.

Wiedemann, A. M. 1984. Ecology of Pacific Northwest Coastal Sand Dunes: A Community Profile. Evergreen State Coll., Olympia, WA (USA).

Wiedemann, A. M. 1987. The ecology of European beachgrass (*Ammophila arenaria* (L.) Link): a review of the literature. Technical Report 87-1-01, Oregon Department of Fish and Wildlife, Nongame Wildlife Program.

Wiedemann, A.M. and Pickart, A., 1996. The *Ammophila* problem on the Northwest coast of North America. Landscape and Urban Planning. 34(3-4):287-299.

Willis, A. J. 1965. The Influence of Mineral Nutrients on the Growth of *Ammophila Arenaria*. Journal of Ecology 53:735–745.

Willis, J.E. and E.W. Yemm. 1961. Braunton Burrows: mineral nutrient status of the dune soils. Journal of Ecology 49 (2):377-390

Windham, L. 2001. Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21:179–188

Winsemius S., Stein C., Parsons L., Minnick S., Ryan A., Suding K. 2015. Plant-soil interactions and implications for restoration of coastal sand dunes at Point Reyes National Seashore. Poster. California Native Plant Society Conference. San Jose, CA. January 13-17, 2015.

Wisura, W., and H. F. Glen. 1993. The South African species of *Carpobrotus* (*Mesembryanthema*: Aizoaceae). Contrib. Bolus Herb:76–107.

Wolfe, B. E., and J. N. Klironomos. 2005. Breaking New Ground: Soil Communities and Exotic Plant Invasion. BioScience 55:477–487.

Xiong S. and C. Nilsson. 1999. The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology 87:984–994

Yang, B., S.R. Fuqua, L. Parsons, A. Barberán. 2022. Managing the soil microbiome to overcome legacy effects of beachgrass invasion. Poster. Ecological Society of America conference. August 14-19, 2022. Montreal, Canada.

Yang, B. Doctoral candidate. University of Arizona. Series of personal communications dated August 2021.

Yelenik, S.G. and J.M. Levine. 2010. Processes limiting native shrub recovery in exotic grasslands after non-native herbivore removal. Rest. Ecol. 18(2):418-425.

Yelenik, S.G. and C.M. D'Antonio. 2013. Self-reinforcing impacts of plant invasions change over time. Nature. 503(28):517-521.

Zarnetske P.L., E.W. Seabloom, S.D. Hacker. 2010. Non-target effects of invasive species management: beachgrass, birds, and bulldozers in coastal dunes. Ecosphere 1(5):1-20.

Zellman, K.L. 2014. Changes in vegetation and biological soil crust communities on sand dunes stabilizing after a century of grazing on San Miguel Island, Channel Islands National Park, California. Monographs of the Western North American Naturalist. 225-245.

Zhang, J., and M. A. Maun. 1990. Sand burial effects on seed germination, seedling emergence and establishment of *Panicum virgatum*. Holarctic Ecology 13:56-61.

Zhang Q., R. Yang, J. Tang, H. Yang, S. Hu, X. Chenet. 2010. Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. PLoS ONE 5:e12380

Zink, T. A., M. F. Allen, B. Heindl-Tenhunen, and E. B. Allen. 1995. The effect of a disturbance corridor on an ecological reserve. Restoration ecology 3:304–310.

Table 1. Variables monitored at Abbotts Lagoon Coastal Dune Restoration Project (+/1 Standard Error (S.E.)). Certain years were not monitored (e.g., 2018).

	CONTROL							MECHANICAL									HERBICIDE									
	PRE	*Post-Yr 2*	*Post-Yr 3*	*Post-Yr 4*	*Post-Yr 5*	*Post-Yr 6*	*Post-Yr 8*	*PRE*	*Post-Yr 2*	*Post-Yr 3*	*Post-Yr 4*	*Post-Yr 5*	*Post-Yr 6*	*Post-Yr 8*	*Post-Yr 9*	*Post-Yr 10*	*PRE*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*	*Post-Tx*
Variable	**2010**	**2013**	**2014**	**2015**	**2016**	**2017**	**2019**	**2010**	**2013**	**2014**	**2015**	**2016**	**2017**	**2019**	**2020**	**2021**	**2010**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
Non-Native Cover (%)	4.8 (4.3)	0.0 (0.0)	0.4 (0.3)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)	2.2 (1.0)	81.9 (4.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)	1.4 (0.8)	1.0 (0.5)	0.04 (0.04)	137.5 (9.6)	19.7 (4.8)	12.8 (4.7)	13.6 (0.04)	19.2 (5.2)	58.3 (10.6)	36.4 (9.5)	61.5 (11.4)	22.4 (6.2)	15.9 (4.6)
European Beachgrass Cover (%)	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	79.5 (3.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	90.2 (3.2)	1.9 (1.1)	1.0 (0.7)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Native Cover (%)	39.9 (5.1)	30.7(5.3)	33.7 (6.1)	40.2 (5.7)	35.5 (4.9)	39.8 (5.8)	48.3 (6.8)	31.0 (7.9)	0.5 (0.5)	0.7 (0.5)	3.9 (2.3)	2.3 (1.6)	13.1 (6.3)	20.0 (7.5)	20.9 (9.2)	17.5 (6.0)	70.8 (12.4)	26.1(7.3)	27.5 (8.6)	30.6 (10.3)	36.1 (11.5)	41.6 (8.9)	34.9 (9.0)	64.1 (14.3)	54.4 (11.5)	95.5 (17.4)
Bareground (%)	54.5 (5.4)	66.0 (4.6)	53.9 (5.8)	57.7 (5.0)	61.6 (4.5)	57.8 (5.4)	50.8 (6.0)	8.2 (2.9)	98.9 (0.1)	98.1 (0.8)	87.9 (0.6)	97.4 (1.6)	87.7 (5.2)	73.6 (7.1)	79.6 (7.7)	79.9 (5.8)	0.2 (0.2)	7.8 (6.3)	6.5 (4.3)	7.8 (2.7)	15.1 (4.3)	11.3 (3.6)	10.2 (3.1)	10.8 (3.6)	10.5 (3.9)	16.6 (6.6)
Native Plant Richness	5.83 (0.55)	4.96 (0.59)	4.57 (0.56)	5.70 (0.68)	5.74 (0.68)	6.30 (0.66)	5.43 (0.63)	2.52 (0.31)	0.04 (0.04)	0.35 (0.14)	0.50 (0.19)	0.46 (0.15)	0.85 (0.25)	1.77 (0.42)	1.85 (0.51)	1.54 (0.37)	3.91 (0.37)	2.46 (0.48)	1.85 (0.36)	3.00 (0.45)	3.54 (0.60)	5.23 (0.71)	2.46 (0.27)	5.46 (0.61)	4.00 (0.62)	5.23 (0.66)
Non-Native Plant Richness	0.13 (0.07)	0.0 (0.0)	0.09 (0.06)	0.04 (0.04)	0.17 (0.08)	0.91 (0.38)	0.83 (0.40)	1.78 (0.23)	0.00 (0.00)	0.08 (0.05)	0.04 (0.04)	0.0 (0.0)	0.15(0.07)	0.39 (0.17)	0.40 (0.17)	0.04 (0.04)	4.27 (0.24)	2.77 (0.51)	2.15 (0.27)	2.00 (0.34)	2.85 (0.37)	3.85 (0.55)	2.08 (0.33)	3.15 (0.59)	2.25 (0.41)	2.15 (0.39)
Shannon-Weiner Diversity Index	0.82 (0.09)	0.71 (0.11)	0.74 (0.12)	0.74 (0.12)	0.87 (0.12)	0.93 (0.12)	0.85 (0.19)	0.55 (0.10)	0.00 (0.00)	0.03 (0.03)	0.07 (0.04)	0.03 (0.02)	0.14 (0.07)	0.30 (0.10)	0.37 (0.12)	0.25 (0.09)	1.42 (0.08)	0.95 (0.19)	0.88 (0.16)	0.80 (0.14)	1.04 (0.12)	1.27 (0.10)	0.70 (0.12)	1.29 (0.11)	0.85 (0.13)	1.24 (0.13)
Evenness	0.46 (0.05)	0.41(0.06)	0.43 (0.07)	0.40 (0.06)	0.47 (0.06)	0.52 (0.06)	0.56 (0.07)	0.36 (0.06)	0.00 (0.00)	0.03 (0.03)	0.07 (0.04)	0.04 (0.03)	0.12 (0.05)	0.20 (0.06)	0.26 (0.08)	0.18 (0.06)	0.68 (0.02)	0.52 (0.09)	0.64 (0.11)	0.51 (0.08)	0.58 (0.06)	0.60 (0.03)	0.46 (0.08)	0.62 (0.04)	0.46 (0.06)	0.61 (0.06)
FE Plant Density															41.3 (23.0)	6.2 (2.2)									0.08 (0.08)	0.08 (0.08)

Table 2a. Variables monitored at AT&T-North

	CONTROL (DUNE SCRUB)				HERBICIDE				
	PRE	*Post-Tx-Yr 1*	*Post-Tx-Yr 2/3*	*Post-Tx-Yr 4/5*	*PRE*	*Post-Tx-Yr 1*	*Post-Tx-Yr 2*	*Post-Tx-Yr 3*	*Post-Tx-Yr 4/5*
Variable	**2016**	**2017**	**2018/2019**	**2020/2021**	**2016**	**2017**	**2018**	**2019**	**2020/2021**
Primary Invaders (%)	0.0 (0.0)	0.0 (0.0)	NS	0.0 (0.0)	77.7 (5.5)	1.9 (0.9)	0.8 (0.5)	0.6 (0.6)	0.0 (0.0)
Primary Invaders (D; %)	0.0 (0.0)	0.0 (0.0)	NS	0.1 (0.1)	2.9 (1.1)	79.3 (5.9)	55.2 (7.8)	47.1 (7.8)	32.3 (7.7)
Non-Native Cover (%)	17.3 (5.1)	67.1 (16.7)	NS	23.9 (6.9)	83.1 (5.5)	7.4 (2.5)	16.1 (5.1)	36.0 (8.6)	21.2 (5.7)
Secondary Invaders (%)	9.0 (3.0)	44.6 (10.2)	NS	9.2 (3.2)	13.0 (6.3)	3.6 (2.0)	11.0 (3.3)	26.4 (6.8)	14.0 (4.5)
Detritus (%)	77.7 (5.3)	55.9 (6.5)	NS	79.6 (3.8)	88.5 (4.1)	77.6 (6.0)	89.1 (2.6)	74.7 (5.1)	74.6 (5.2)
Bareground (%)	10.1 (2.9)	7.5 (2.2)	NS	4.4 (1.9)	1.1 (0.9)	1.1 (1.0)	1.9 (1.2)	3.1 (2.0)	6.4 (3.8)
Native Cover (%)	120.9 (10.1)	128.9 (7.8)	NS	108.7 (10.8)	55.3 (9.1)	32.6 (8.3)	33.9 (8.5)	59.5 (11.9)	49.3 (10.0)
Native Species Richness	9.9 (0.7)	10.5 (0.7)	NS	8.5 (0.8)	4.3 (0.5)	3.9 (0.7)	3.2 (0.6)	5.8 (0.4)	4.6 (0.6)
Non-Native Species Richness	2.6 (0.4)	3.1 (0.6)	NS	2.2 (0.4)	2.1 (0.3)	2.1 (0.5)	2.1 (0.4)	3.2 (0.4)	2.9 (0.4)
Shannon-Weiner Diversity Index	1.61 (0.12)	1.82 (0.07)	NS	1.45 (0.10)	0.95 (0.12)	0.80 (0.15)	0.84 (0.16)	1.18 (0.11)	1.05 (0.14)
Evenness	0.64 (0.04)	0.70 (0.02)	NS	0.63 (0.03)	0.52 (0.05)	0.43 (0.07)	0.45 (0.08)	0.54 (0.04)	0.51 (0.05)
Rare Plant (#/2m^2)	12.4 (7.2)	52.3 (32.4)	NS	3.8 (3.0)	0	0	0	0	0

Table 2b. ***Variables monitored at AT&T-South - Phase I (2017)***

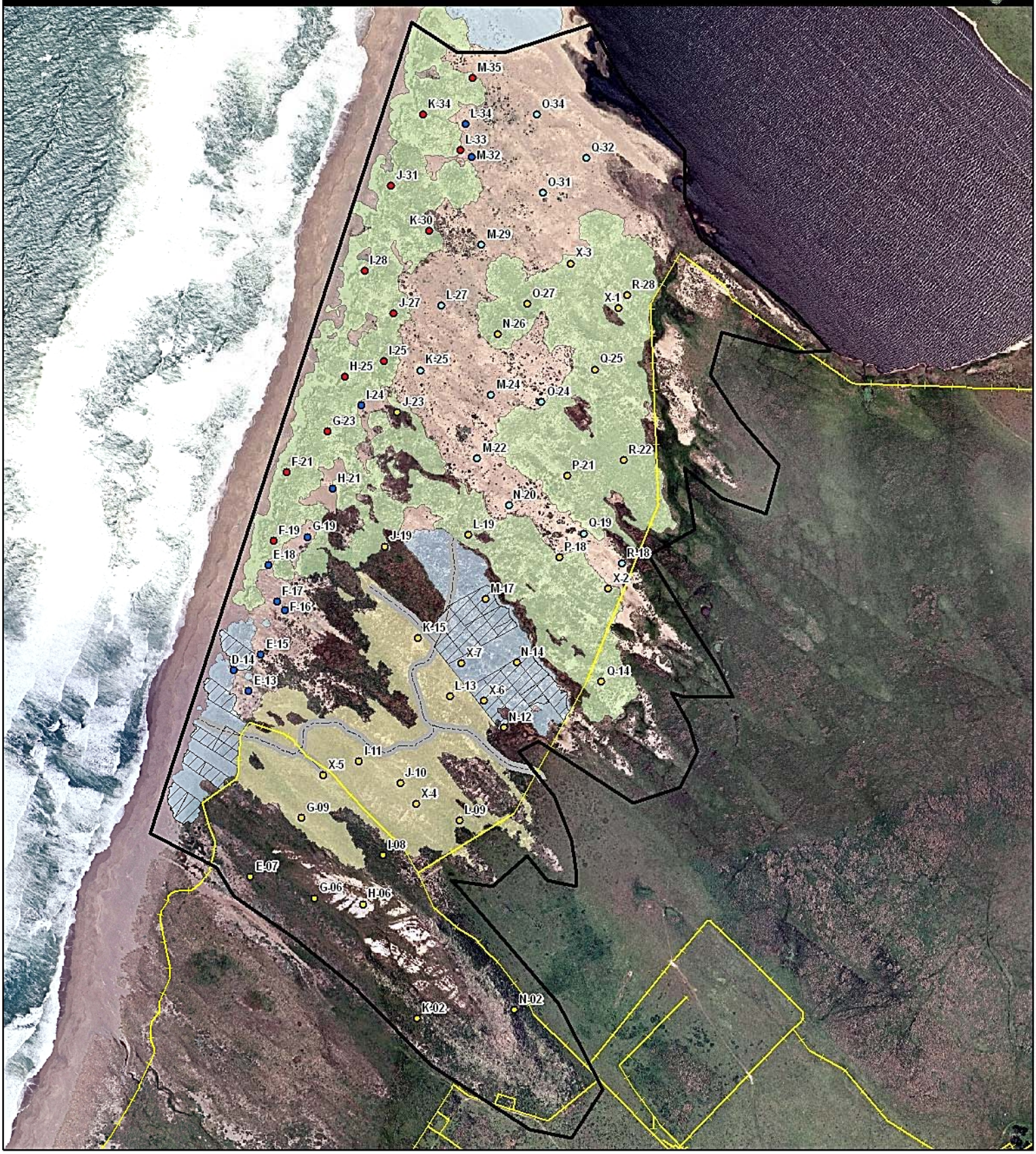
	CONTROL (DUNE SCRUB)			HERBICIDE			
	PRE	*Post-Tx-Yr 1*	*Post-Tx-Yr 2*	*PRE*	*Post-Tx-Yr 1*	*Post-Tx-Yr 2*	*Post-Tx-Yr 4*
Variable	**2017**	**2018**	**2019**	**2017**	**2018**	**2019**	**2021**
Primary Invaders (%)	0.6 (0.6)	NS	NS	83.6 (8.4)	2.8 (1.0)	0.5 (0.5)	0.0 (0.0)
Primary Invaders (D; %)	0.0 (0.0)	NS	NS	11.2 (6.1)	83.9 (9.7)	65.3 (13.2)	49.4 (17.3)
Non-Native Cover (%)	95.1 (36.5)	NS	NS	97.7 (9.4)	3.2 (0.9)	7.8 (5.9)	0.9 (0.9)
Detritus (%)	87.2 (12.6)	NS	NS	53.2 (13.2)	70.6 (9.0)	63.6 (12.8)	55.5 (17.1)
Bareground (%)	2.0 (2.0)	NS	NS	0.3 (0.3)	2.0 (1.6)	2.7 (2.1)	3.2 (2.1)
Secondary Invaders (%)	60.2 (23.5)	NS	NS	11.2 (7.7)	0.2 (0.2)	1.2 (0.7)	0.3 (0.3)
Native Cover (%)	121.2 (33.0)	NS	NS	43.5 (20.8)	15.0 (9.8)	43.7 (17.3)	58.7 (22.0)
Native Species Richness	7.3 (1.0)	NS	NS	2.6 (0.7)	1.0 (0.4)	3.1 (0.7)	3.3 (0.6)
Non-Native Species Richness	4.5 (0.7)	NS	NS	2.1 (0.6)	1.1 (0.3)	2.0 (0.8)	0.5 (0.5)
Shannon-Weiner Diversity Index	1.88 (0.07)	NS	NS	0.73 (0.20)	0.35 (0.16)	0.68 (0.19)	0.67 (0.29)
Evenness	0.76 (0.02)	NS	NS	0.48 (0.11)	0.41 (0.19)	0.39 (0.10)	0.57 (0.21)
Rare Plant (#/2m^2)	0 (0)	NS	NS	0 (0)	0 (0)	0 (0)	0 (0)

Variables monitored at AT&T South - Phase II (2018)

	HERBICIDE		
	PRE	*Post-Tx-Yr 1*	*Post-Tx-Yr 3*
Variable	**2018**	**2019**	**2021**
Primary Invaders (%)	79.1 (6.9)	4.0 (2.2)	0.3 (0.2)
Primary Invaders (D; %)	25.1 (14.4)	77.0 (7.3)	38.9 (7.3)
Non-Native Cover (%)	95.2 (12.6)	7.8 (4.0)	2.5 (1.9)
Detritus (%)	53.5 (13.3)	54.6 (9.8)	80.7 (20.8)
Bareground (%)	0.8 (0.6)	4.8 (2.6)	12.0 (7.3)
Secondary Invaders (%)	16.1 (10.6)	0.3 (0.3)	0.2 (0.2)
Native Cover (%)	35.1 (11.7)	6.6 (4.1)	21.1 (10.2)
Native Species Richness	2.9 (0.6)	1.1 (0.7)	1.7 (0.4)
Non-Native Species Richness	1.7 (0.3)	1.1 (0.3)	1.1 (0.3)
Shannon-Weiner Diversity Index	0.70 (0.22)	0.31 (0.18)	0.47 (0.10)
Evenness	0.44 (0.11)	0.25 (0.13)	0.51 (0.14)
Rare Plant (#/2m^2)	0 (0)	0 (0)	0 (0)

APPENDIX A. VEGETATION MONITORING AND PHOTOPOINT PLOTS AT ABBOTTS LAGOON COASTAL DUNE RESTORATION AREA, B RANCH NORTH, AND AT&T COASTAL DUNE RESTORATION PROJECT

Restore Critical Dune Habitat to Protect Threatened and Endangered Species
Vegetation Monitoring - All Plots





Point Reyes National Seashore
Marin County, CA

0 0.1 0.2 Miles

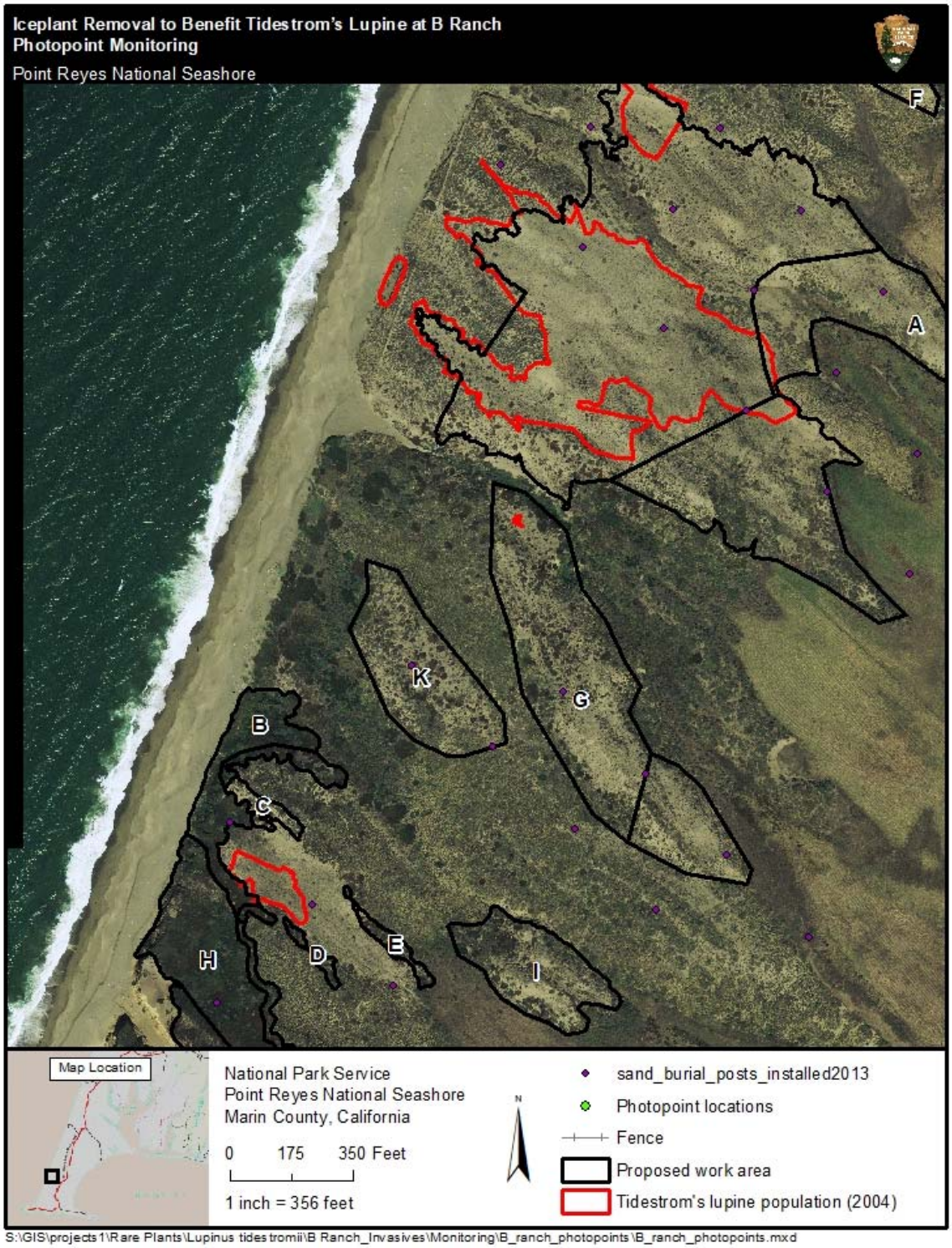
N

Legend

- Fence
- REFERENCE, BACKDUNE
- TREATMENT, FOREDUNE
- REFERENCE, FOREDUNE
- TREATMENT, BACKDUNE
- DELETED
- project area
- Mowed Fall 2012
- Access Road
- Phase I - mechanical 2011
- Phase II - herbicide 2011
- Phase III - herbicide 2012

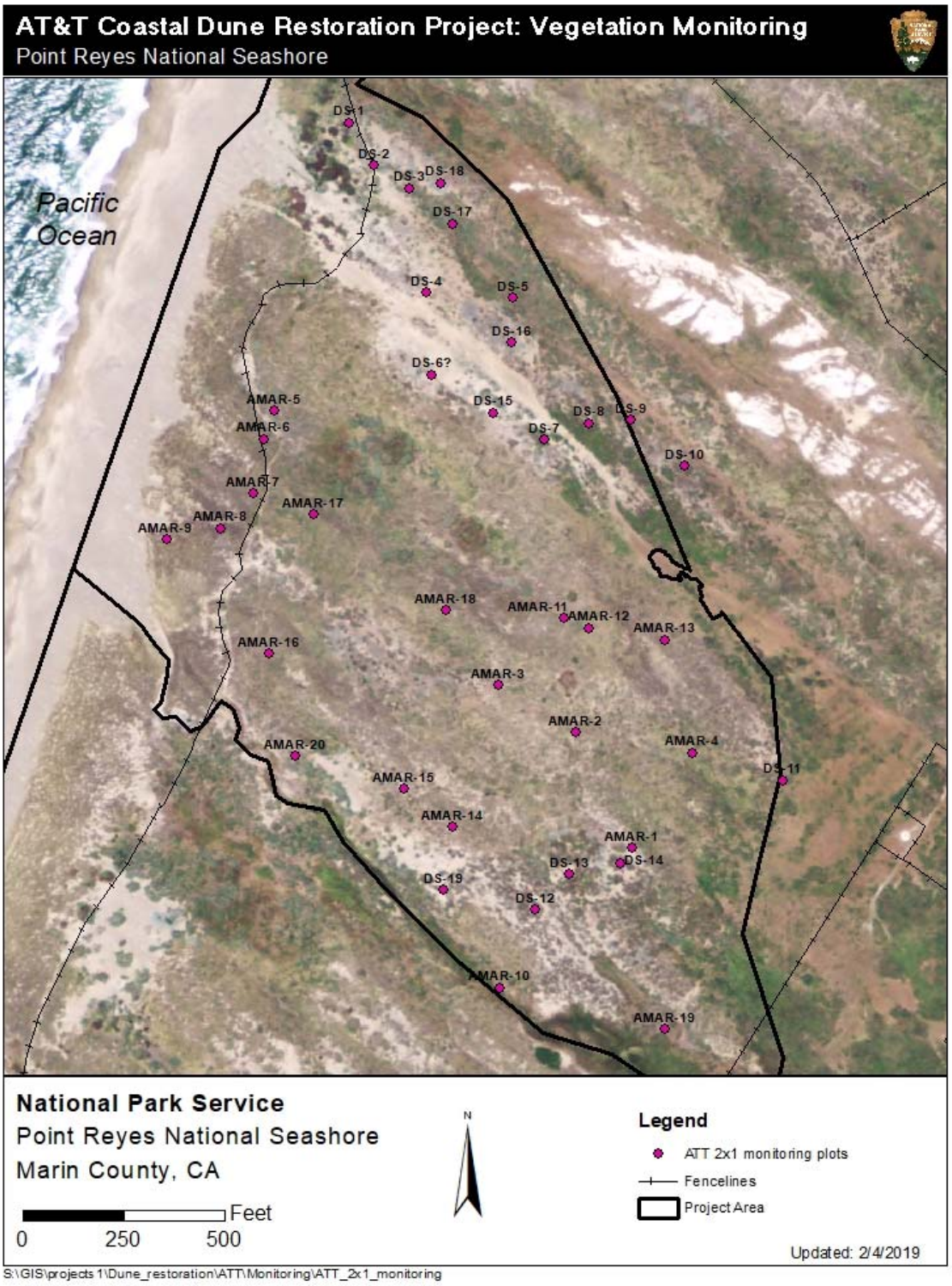
S:\GIS\projects\Dune_restoration\Vegetation Monitoring\2mx1m plots\Vegetation Monitoring Plots_8x11.mxd

Appendix A-2: B Ranch North Photopoints

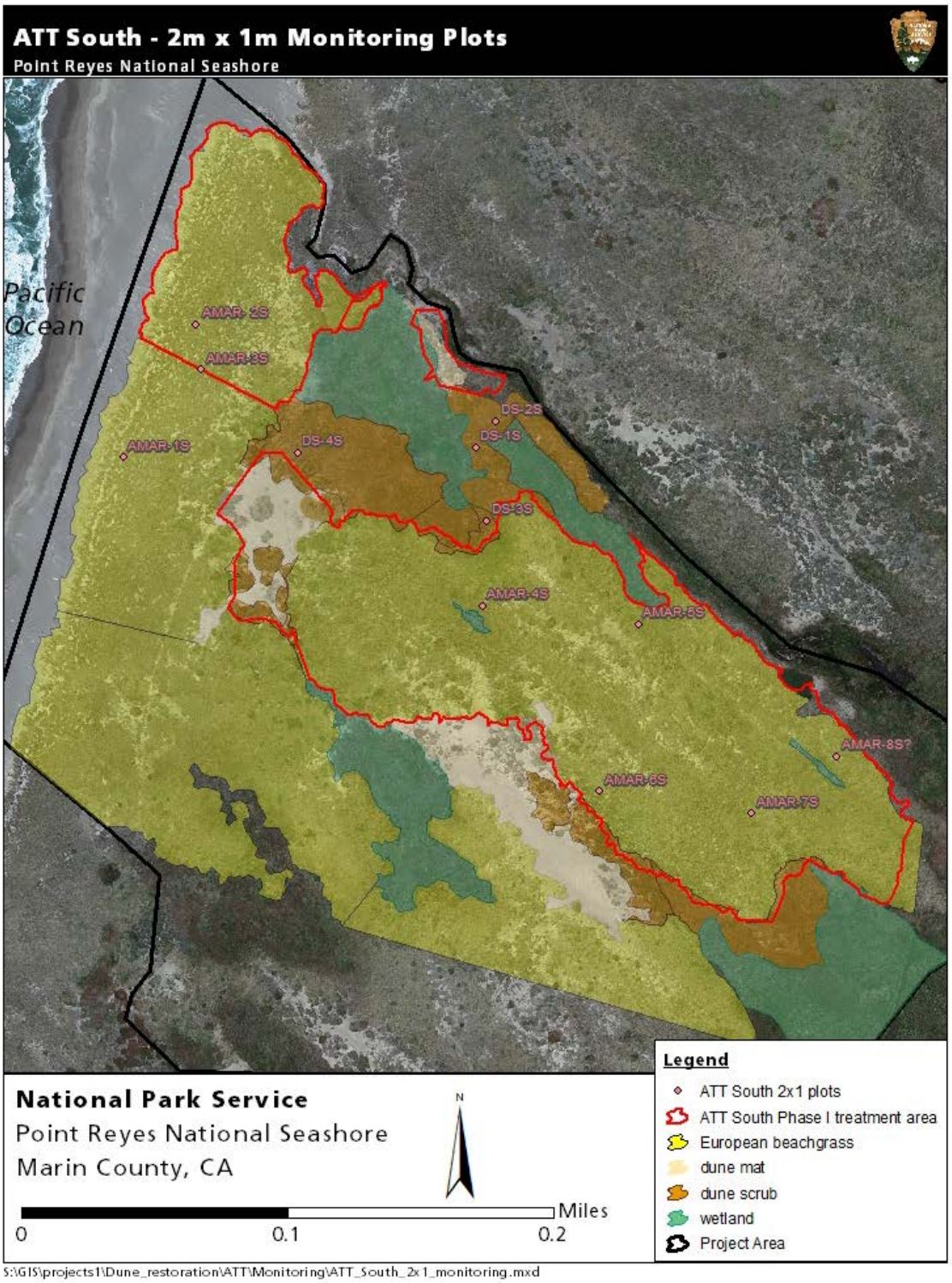


S:\GIS\projects1\Rare Plants\Lupinus tidestromii\B Ranch_Invasives\Monitoring\B_ranch_photopoints\B_ranch_photopoints.mxd

Appendix A-3: ATT North - 2X1 Vegetation Monitoring Plots



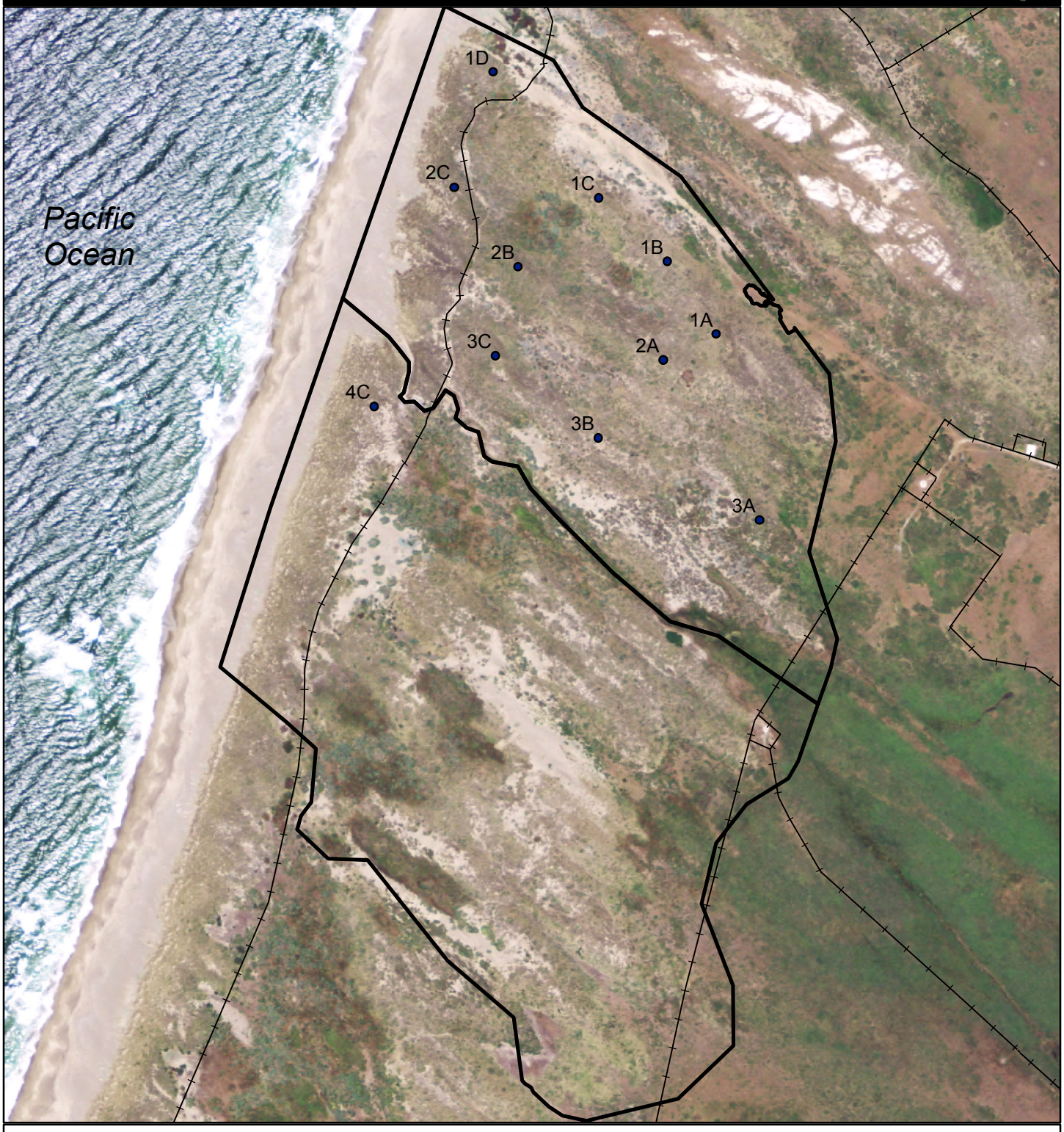
S:\GIS\projects 1\Dune_restoration\ATT\Monitoring\ATT_2x1_monitoring



Appendix A-4. ATT South Phase I permanent monitoring plot locations. “DS” or Dune Scrub plots were chosen in native dune scrub habitat as reference sites, and “AMAR” plots were chosen in European beachgrass/iceplant-invaded areas. Note that plot AMAR-1S is outside of the Phase I treatment area – consequently, this plot was removed from statistical analyses.

AT&T Coastal Dune Restoration Project: Photomonitoring

Point Reyes National Seashore



** Eight overlapping photos taken at each point, start facing north.

National Park Service
Point Reyes National Seashore
Marin County, CA

Miles
0 0.125 0.25

N

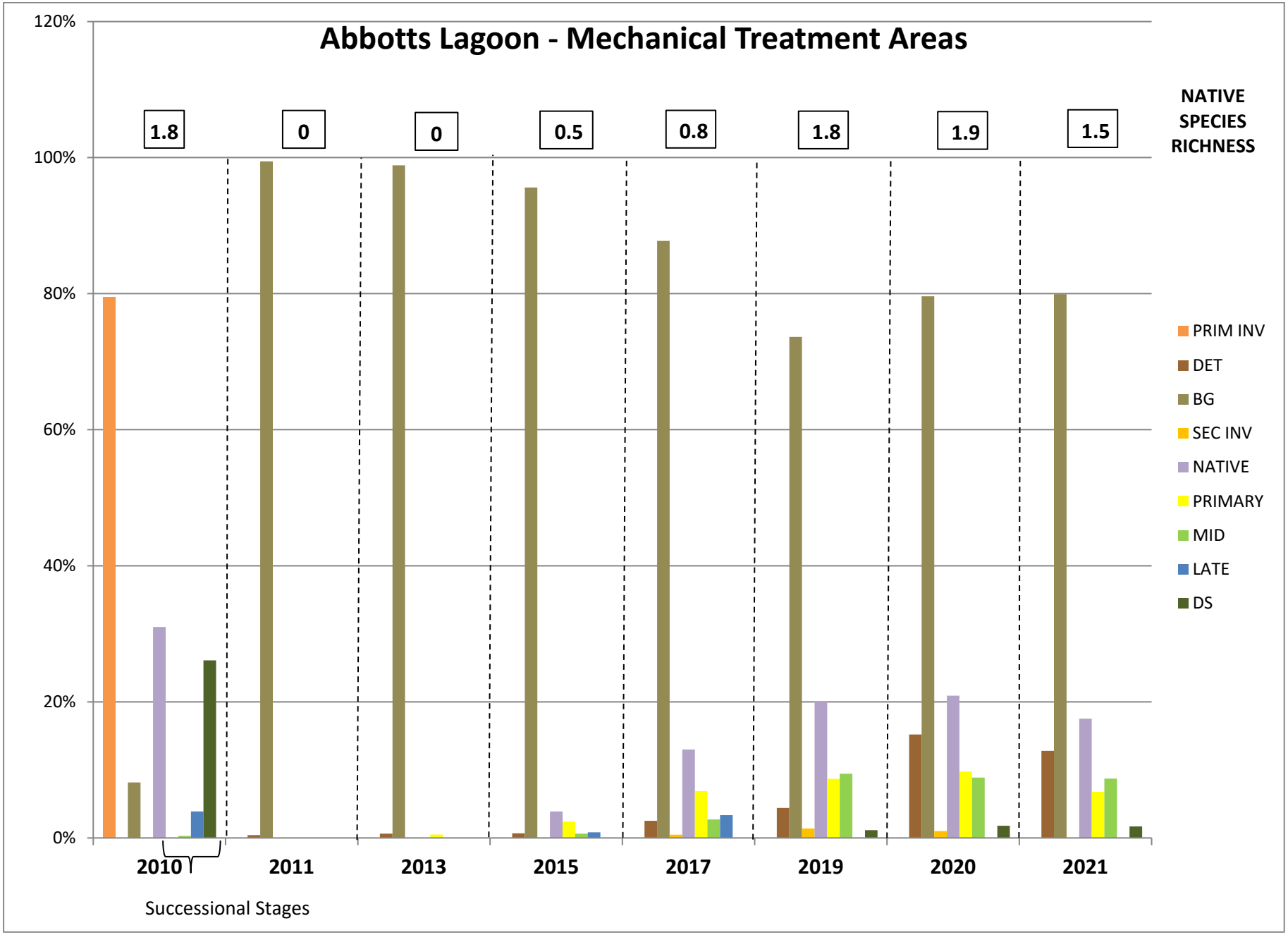
Legend

- ATT photopoints
- Fencelines
- ATT Project Area

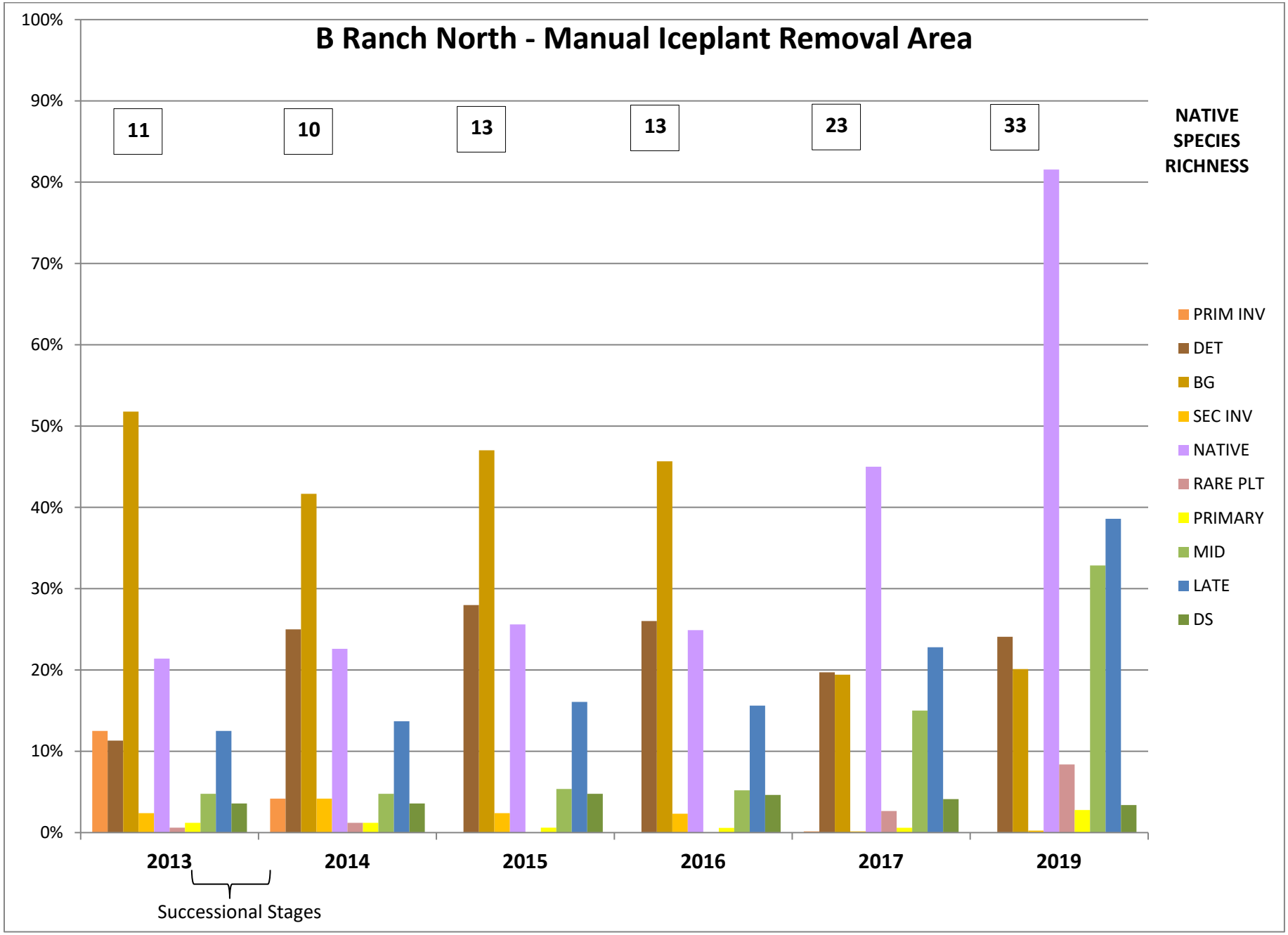
S:\GIS\projects1\Dune_restoration\ATT\Monitoring\ATT_photomonitoring.mxd

APPENDIX B. GRAPHS DISPLAYING VEGETATION COMMUNITY DATA FOR MULTIPLE DUNE RESTORATION PROJECTS AT POINT REYES NATIONAL SEASHORE

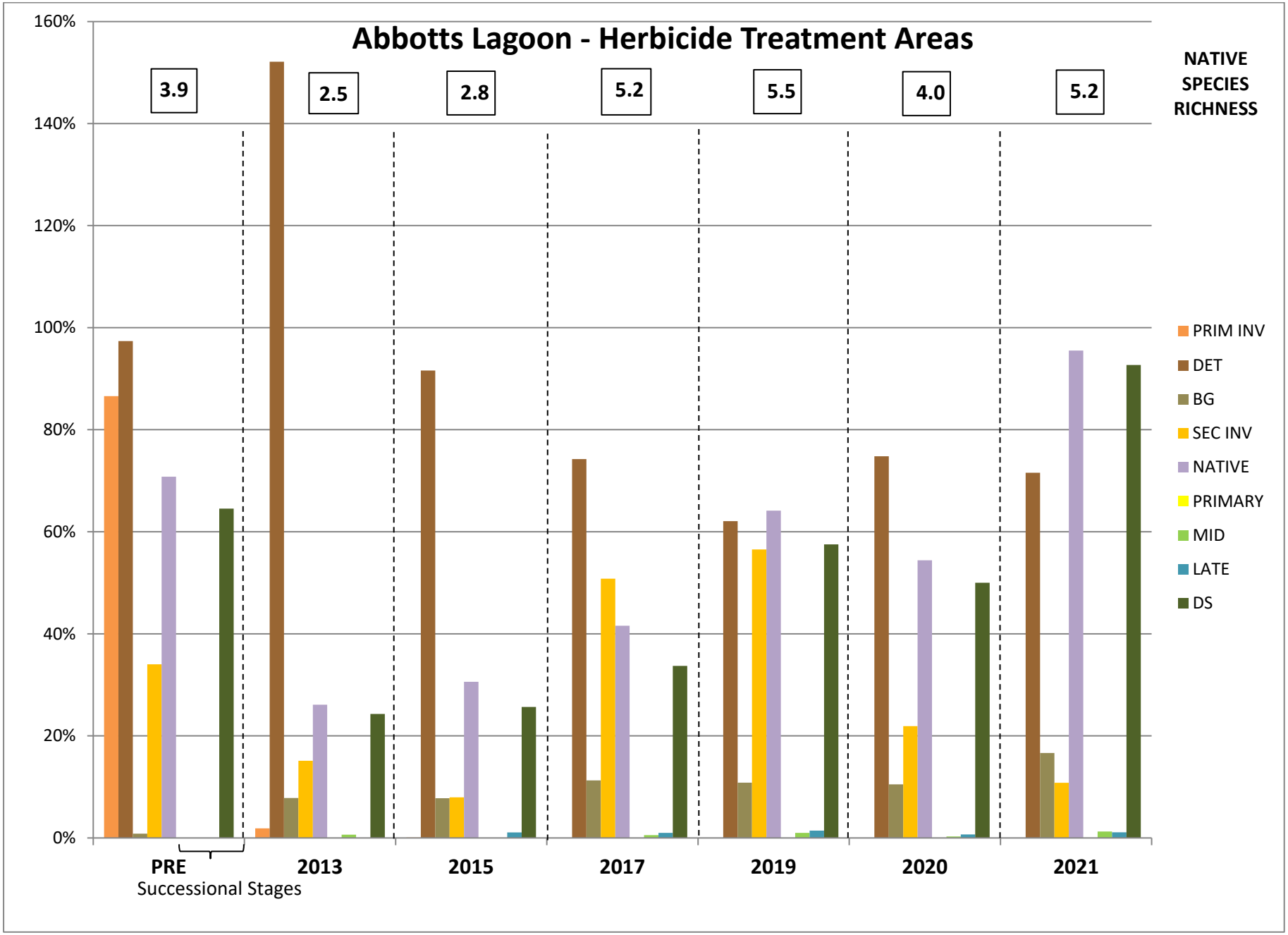
APPENDIX B-1


Abbotts Lagoon - Mechanical Treatment Areas
NATIVE SPECIES RICHNESS
1.8
0
0
0.5
0.8
1.8
1.9
1.5
120%
100%
80%
60%
40%
20%
0%
PRIM INV
DET
BG
SEC INV
NATIVE
PRIMARY
MID
LATE
DS
2010
2011
2013
2015
2017
2019
2020
2021
Successional Stages

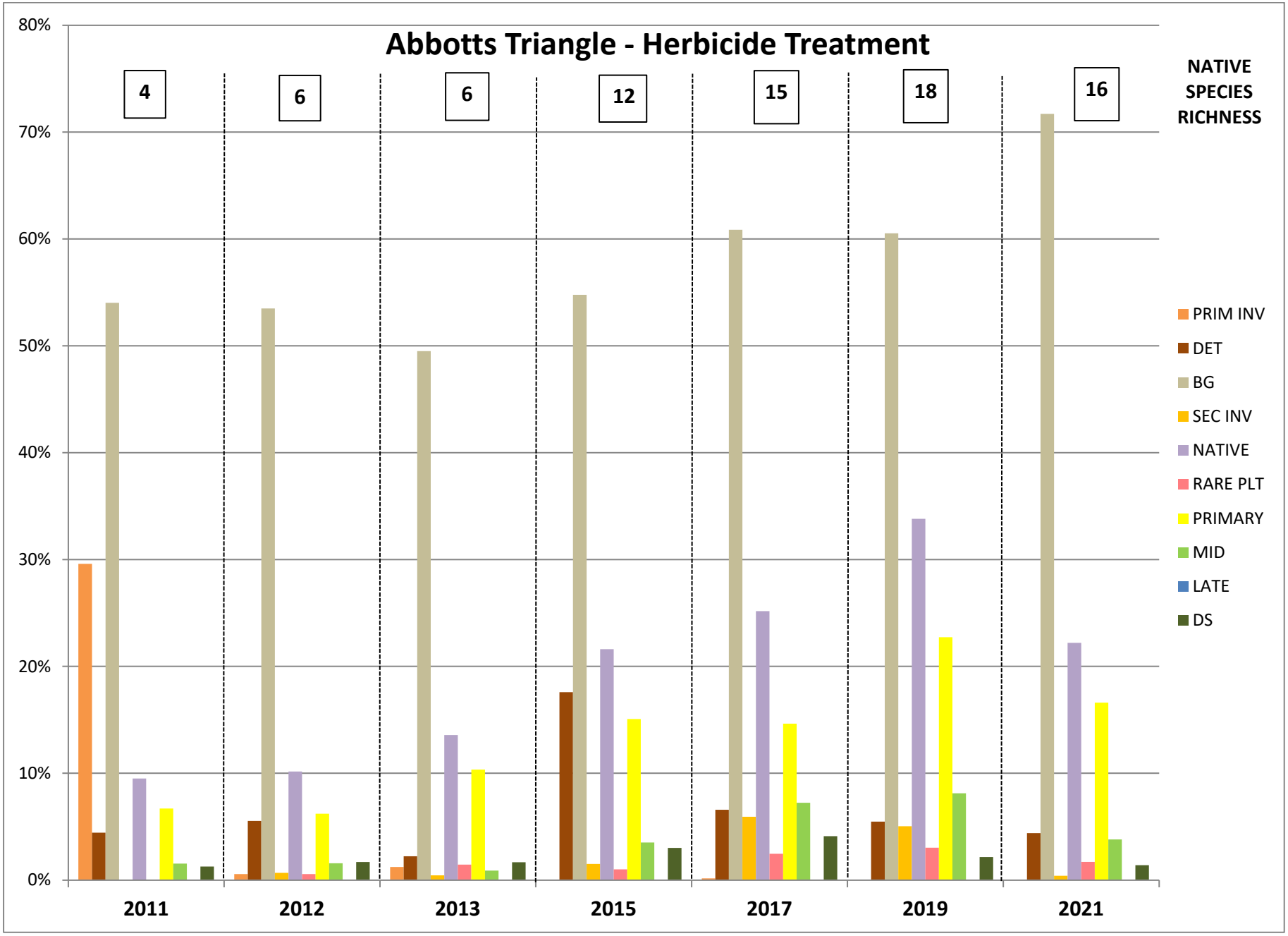
APPENDIX B-2


B Ranch North - Manual Iceplant Removal Area
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
11
10
13
13
23
33
NATIVE SPECIES RICHNESS
PRIM INV
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS
2013
2014
2015
2016
2017
2019
Successional Stages

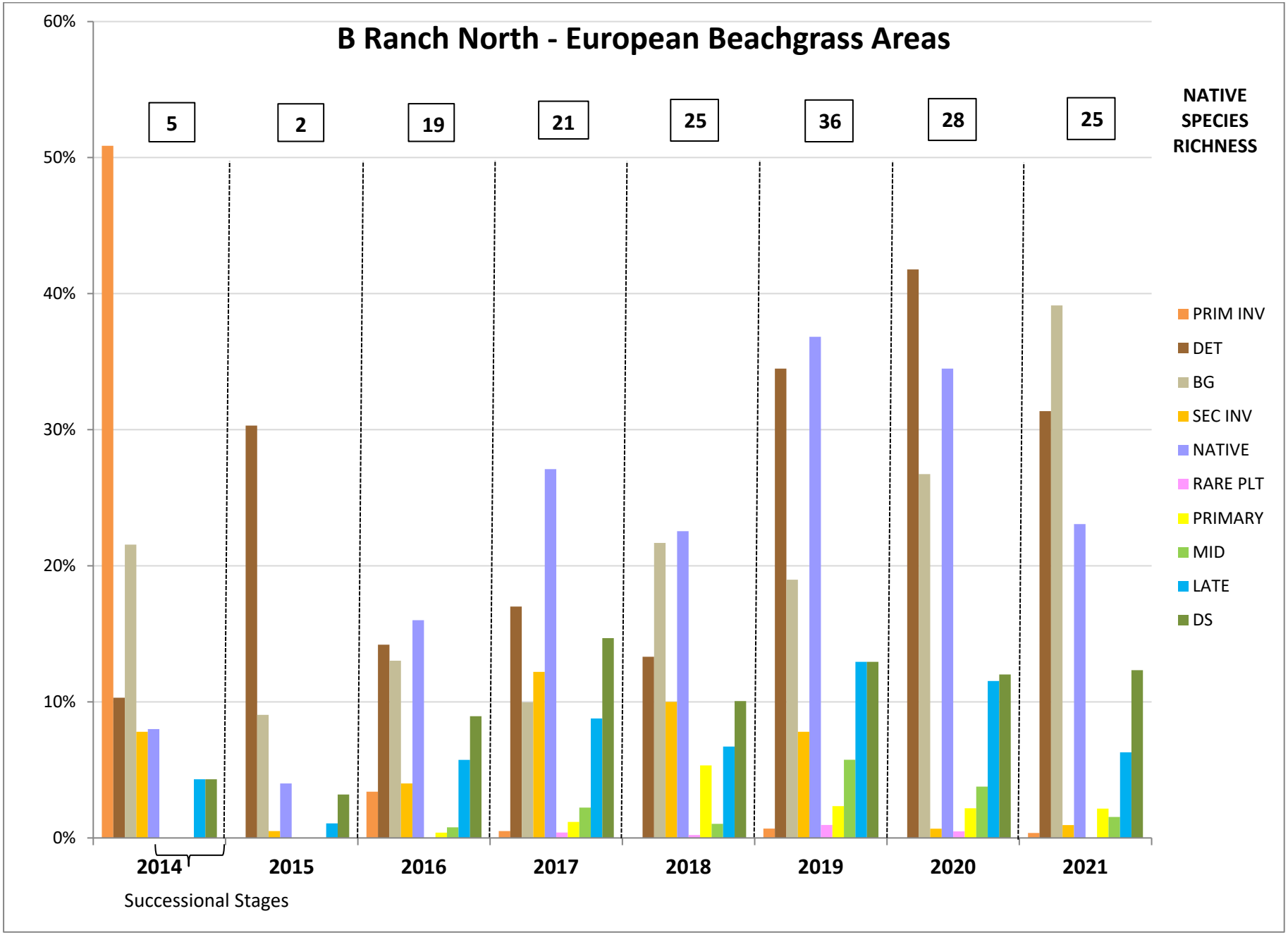
APPENDIX B-3


Abbotts Lagoon - Herbicide Treatment Areas
NATIVE SPECIES RICHNESS
3.9
2.5
2.8
5.2
5.5
4.0
5.2
160%
140%
120%
100%
80%
60%
40%
20%
0%
PRE
2013
2015
2017
2019
2020
2021
Successional Stages
PRIM INV
DET
BG
SEC INV
NATIVE
PRIMARY
MID
LATE
DS

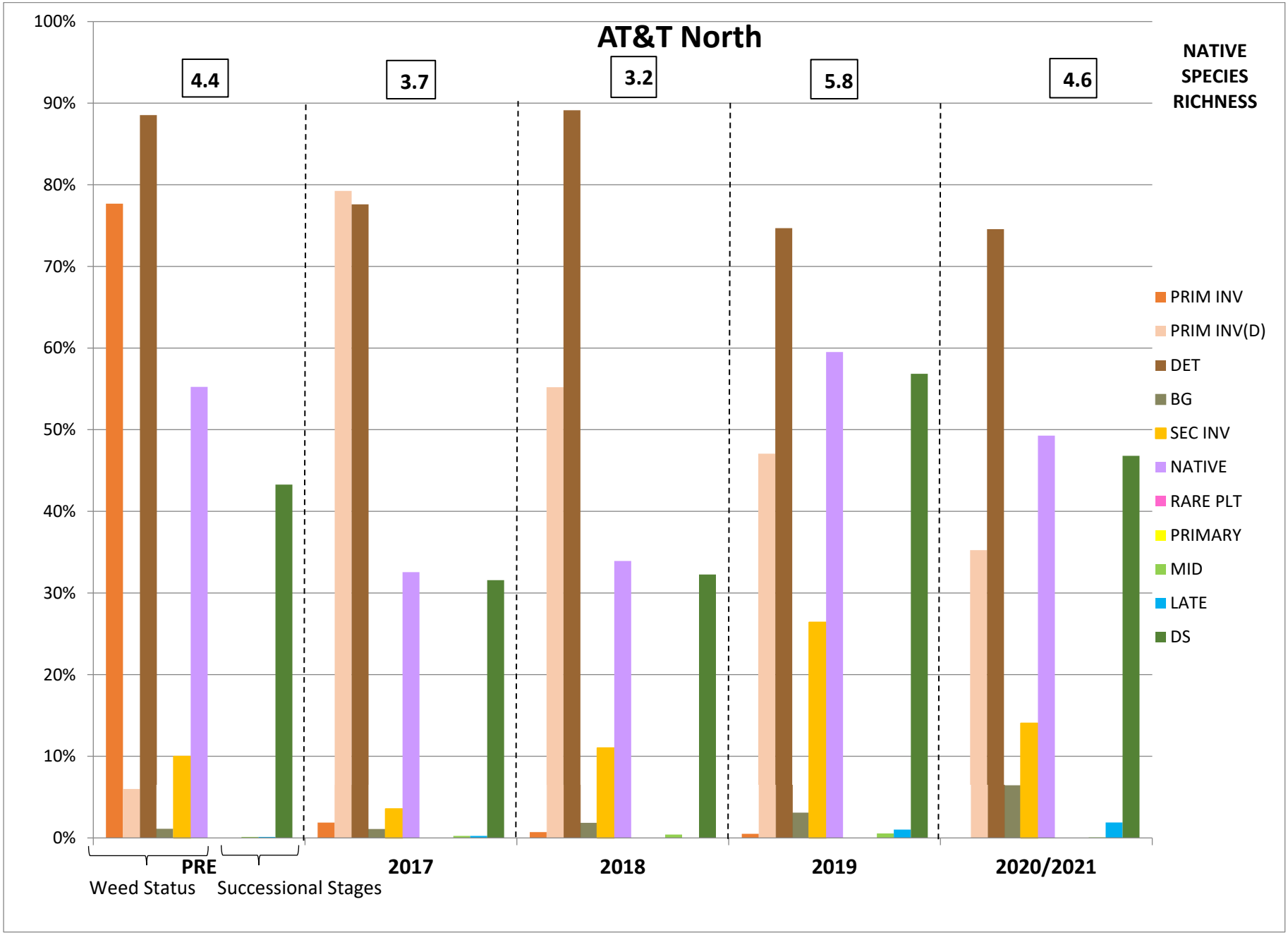
APPENDIX B-4


Abbotts Triangle - Herbicide Treatment
NATIVE SPECIES RICHNESS
4
6
6
12
15
18
16
80%
70%
60%
50%
40%
30%
20%
10%
0%
2011
2012
2013
2015
2017
2019
2021
PRIM INV
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS

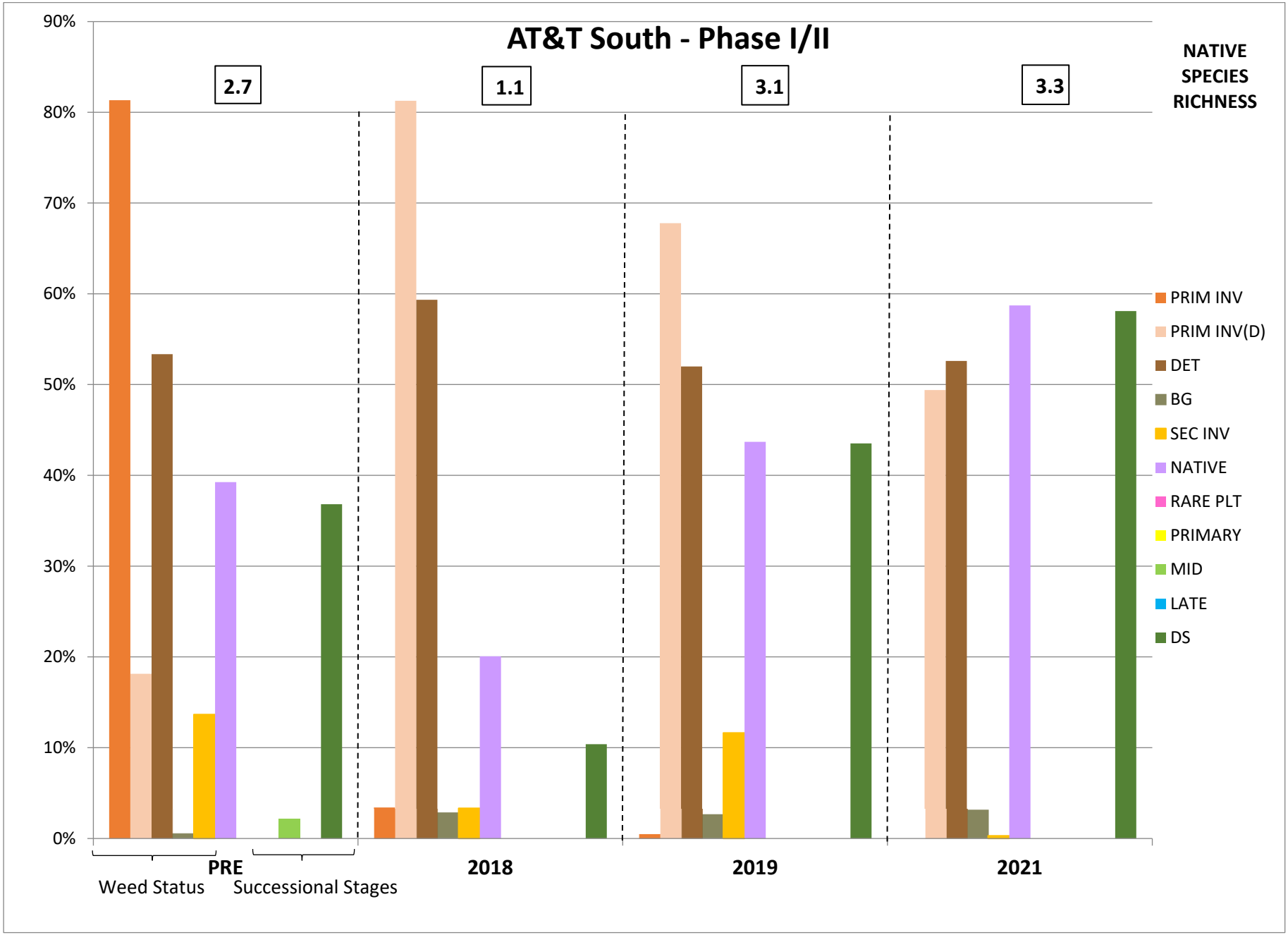
APPENDIX B-5


B Ranch North - European Beachgrass Areas
NATIVE SPECIES RICHNESS
5
2
19
21
25
36
28
25
60%
50%
40%
30%
20%
10%
0%
2014
2015
2016
2017
2018
2019
2020
2021
Successional Stages
PRIM INV
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS

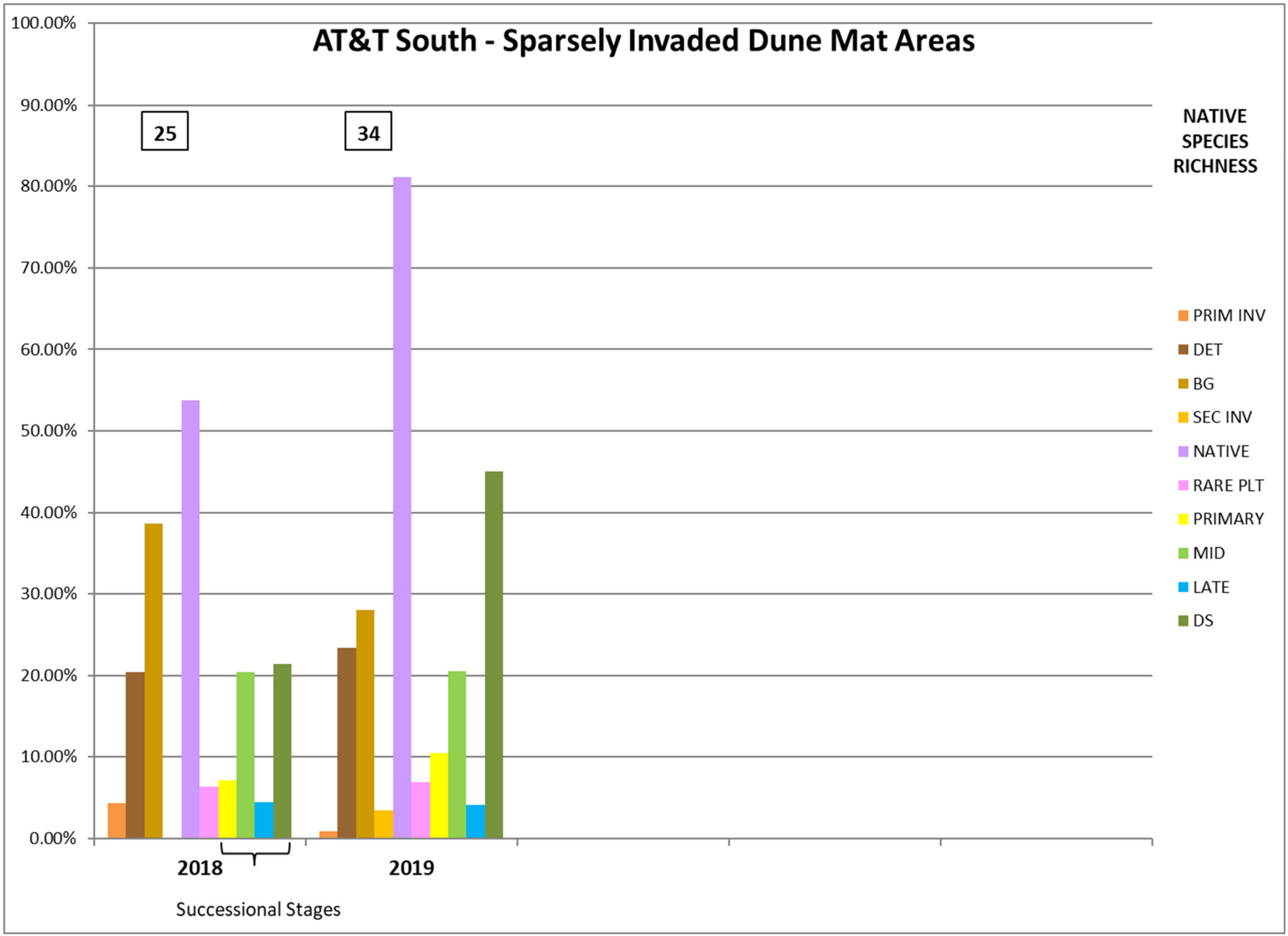
APPENDIX B-6


AT&T North
NATIVE SPECIES RICHNESS
4.4
3.7
3.2
5.8
4.6
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
PRIM INV
PRIM INV(D)
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS
PRE
2017
2018
2019
2020/2021
Weed Status
Successional Stages

APPENDIX B-7

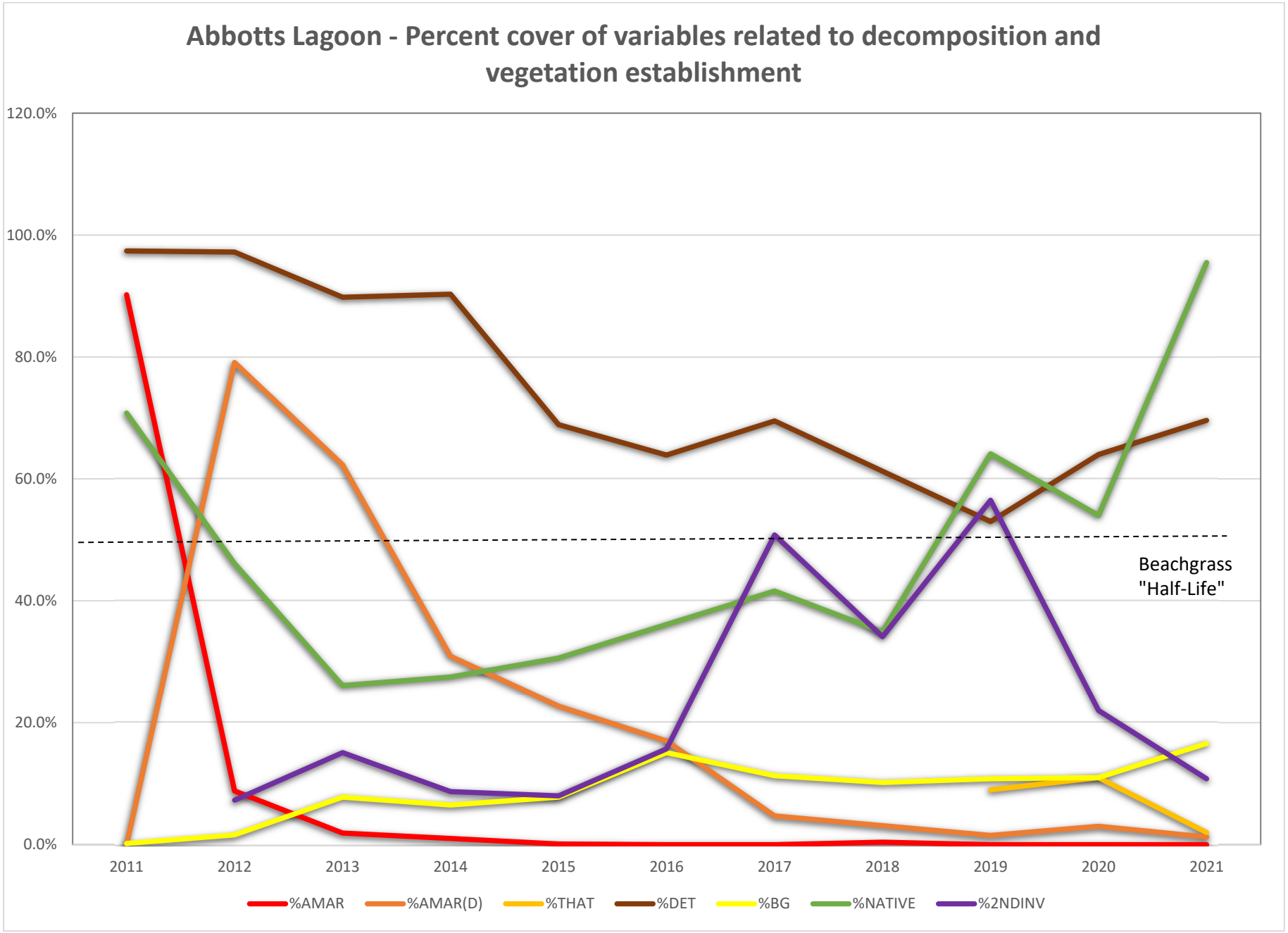

AT&T South - Phase I/II
NATIVE SPECIES RICHNESS
2.7
1.1
3.1
3.3
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
PRIM INV
PRIM INV(D)
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS
PRE
2018
2019
2021
Weed Status
Successional Stages

APPENDIX B-8

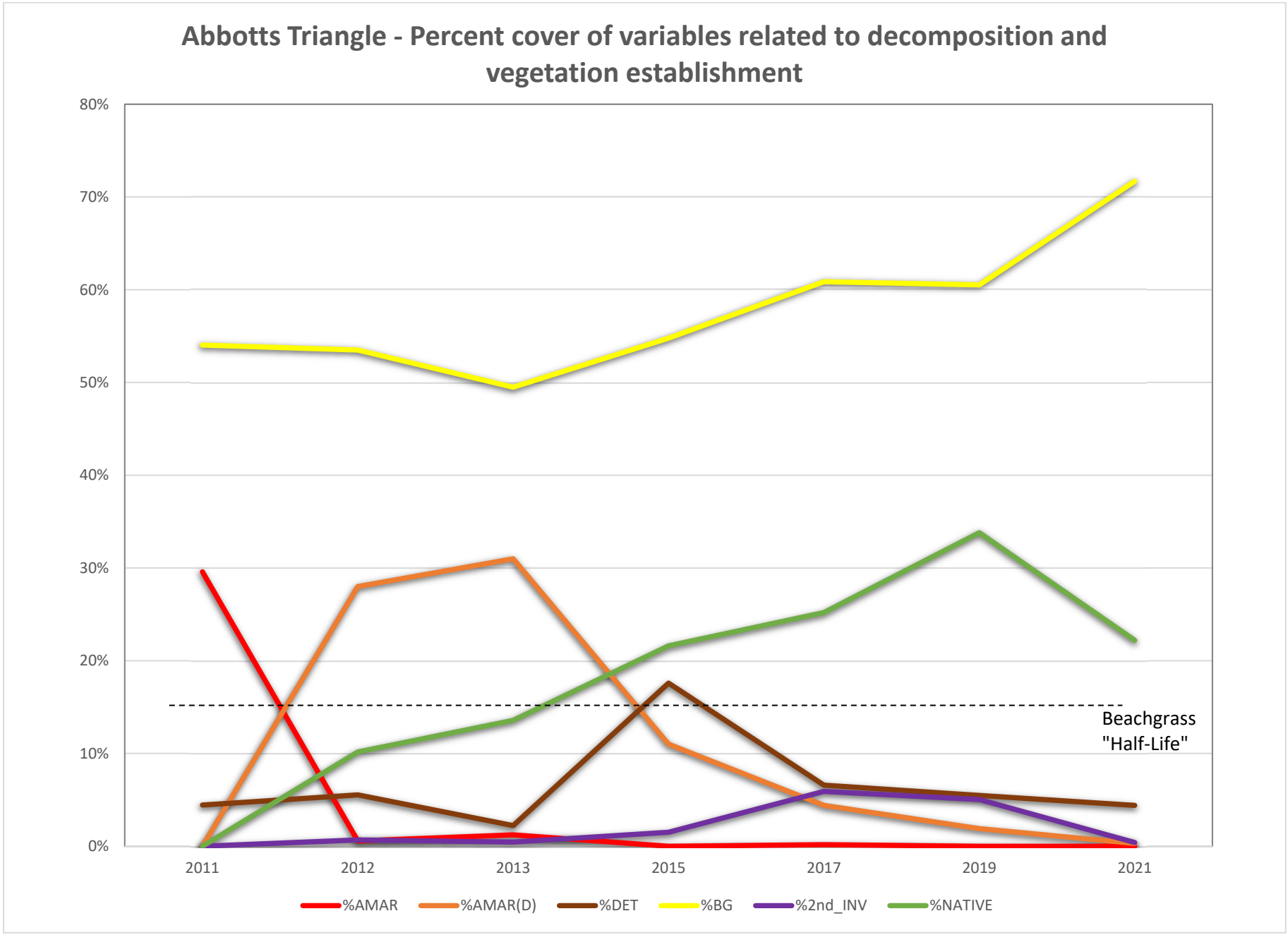

AT&T South - Sparsely Invaded Dune Mat Areas
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
25
34
2018
2019
Successional Stages
NATIVE SPECIES RICHNESS
PRIM INV
DET
BG
SEC INV
NATIVE
RARE PLT
PRIMARY
MID
LATE
DS

APPENDIX C. DECOMPOSITION-RELATED DYNAMICS OF EUROPEAN BEACHGRASS AT POINT REYES COASTAL DUNE RESTORATION PROJECT AREAS

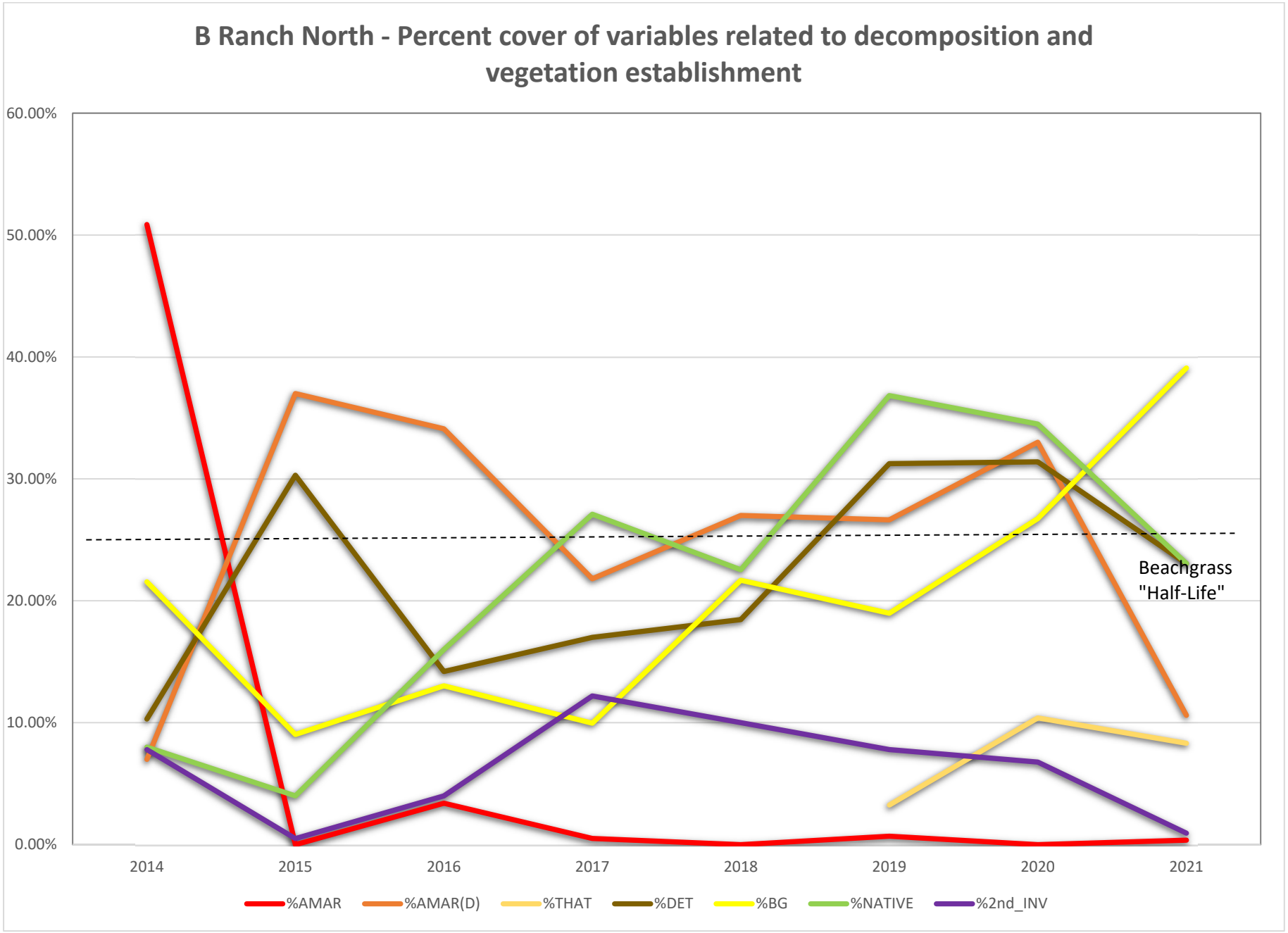
APPENDIX C-1


Abbotts Lagoon - Percent cover of variables related to decomposition and vegetation establishment
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
Beachgrass "Half-Life"
%AMAR
%AMAR(D)
%THAT
%DET
%BG
%NATIVE
%2NDINV

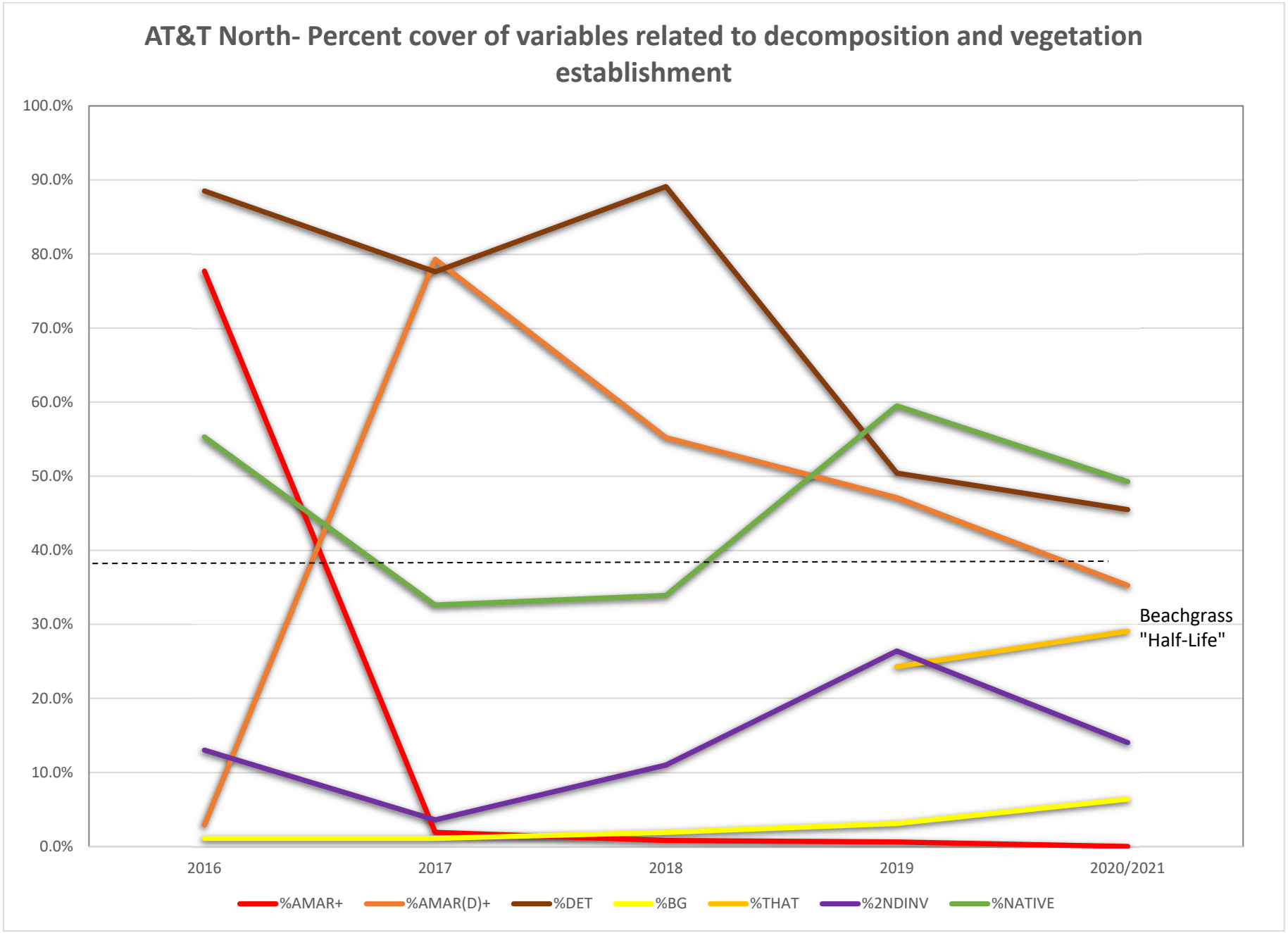
APPENDIX C-2


Abbotts Triangle - Percent cover of variables related to decomposition and vegetation establishment
80%
70%
60%
50%
40%
30%
20%
10%
0%
2011
2012
2013
2015
2017
2019
2021
Beachgrass "Half-Life"
%AMAR
%AMAR(D)
%DET
%BG
%2nd_INV
%NATIVE

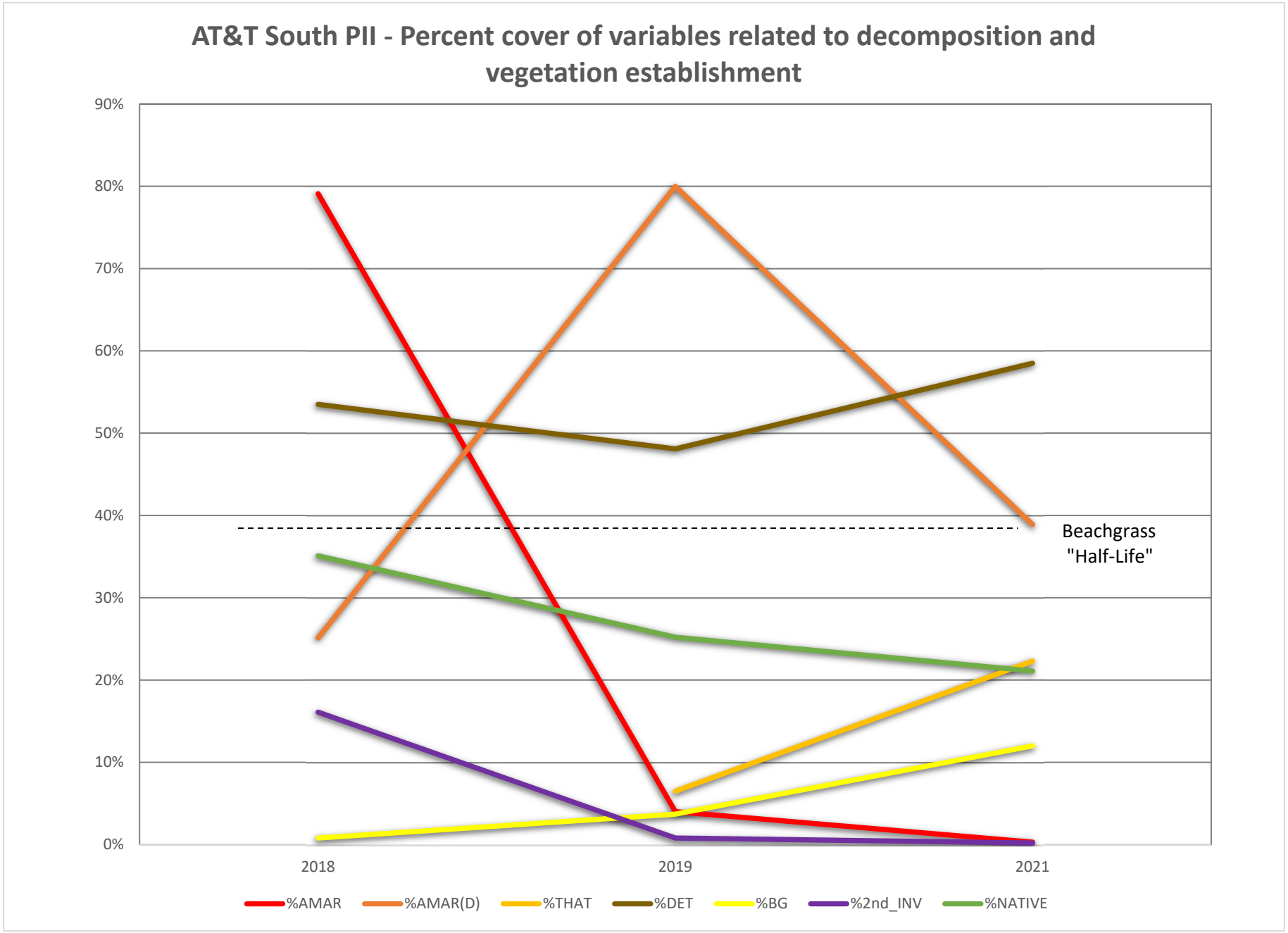
APPENDIX C-3


B Ranch North - Percent cover of variables related to decomposition and vegetation establishment
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
2014
2015
2016
2017
2018
2019
2020
2021
Beachgrass "Half-Life"
%AMAR
%AMAR(D)
%THAT
%DET
%BG
%NATIVE
%2nd_INV

APPENDIX C-4


AT&T North- Percent cover of variables related to decomposition and vegetation establishment
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
2016
2017
2018
2019
2020/2021
Beachgrass "Half-Life"
%AMAR+
%AMAR(D)+
%DET
%BG
%THAT
%2NDINV
%NATIVE

APPENDIX C-5


AT&T South PII - Percent cover of variables related to decomposition and vegetation establishment
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2018
2019
2021
Beachgrass "Half-Life"
%AMAR
%AMAR(D)
%THAT
%DET
%BG
%2nd_INV
%NATIVE

APPENDIX D. POST-HERBICIDE TREATMENT OF EUROPEAN BEACHGRASS AT ABBOTTS LAGOON

Post-herbicide treatment of European beachgrass (*Ammophila arenaria*) at Abbotts Lagoon

Cody Ender and Lorraine Parsons
Point Reyes National Seashore
Point Reyes, CA 94956

January 27, 2017

Background

In fall 2012, a portion of the Phase II herbicide treated area (treated the previous year in 2011) received additional post-herbicide treatment with the intent of speeding up decomposition of dead European beachgrass (*Ammophila arenaria*; AMAR) and improving colonization by native dune plant species. Lingering biomass above- and below-ground poses an obstacle to successful dune restoration by reducing bare ground characteristic of native dune communities, occupying space that could be colonized by native species, and preventing sand remobilization. On the other hand, standing dead biomass may help to prevent excessive sand movement into nearby pastures or low-lying swales and existing native dune habitats and may help prevent colonization by secondary invaders or other non-native species originally in much lower abundances prior to restoration. European beachgrass decomposes very slowly: dead aboveground biomass -- as well as rhizomes belowground -- have been found at the Abbotts Lagoon restoration area up to five years post herbicide treatment (Figure 1). This pattern of persistent dead biomass has also been observed by California Department of Parks and Recreation (CDPR) staff at Bodega (Chris Heintzelman, CDPR, *pers. comm.*).



Figure 1. Standing dead European beachgrass (above) and dead rhizomes (below) in 2016.

In order to accelerate decomposition of European beachgrass, the Seashore experimented with mowing or scraping with a bulldozer one year after herbicide treatment. The mowing treatment (mow) removed vegetation approximately 4- 5 inches on average above ground level leaving much of the basal portion of plants and their root systems intact. The

scraping treatment (doze) removed vegetation just below ground level with minimal root disturbance. For comparison, some areas received no post-treatment (control). The post-herbicide treatments were meant to decrease standing biomass without substantial soil disturbance and loss of native species that occurred with mechanical removal. These three post-treatments (mow, doze, and control) were all located in the Phase II herbicide treatment area (Figure 2).

Other European beachgrass removal projects using herbicide have experimented with use of pre-treatment measures such as prescribed burning or mowing to remove beachgrass thatch, stimulate re-growth, and improve uptake of herbicide (Hyland and Holloran 2005). At Bodega dunes, staff and volunteers have actually pulled by hand individual dead European beachgrass clumps from the ground and stockpiled clumps in central locations to reduce standing dead biomass (C. Heintzelman, *pers. comm*.). However, we are not aware of other attempts at post-treatment measures to remove dead biomass.

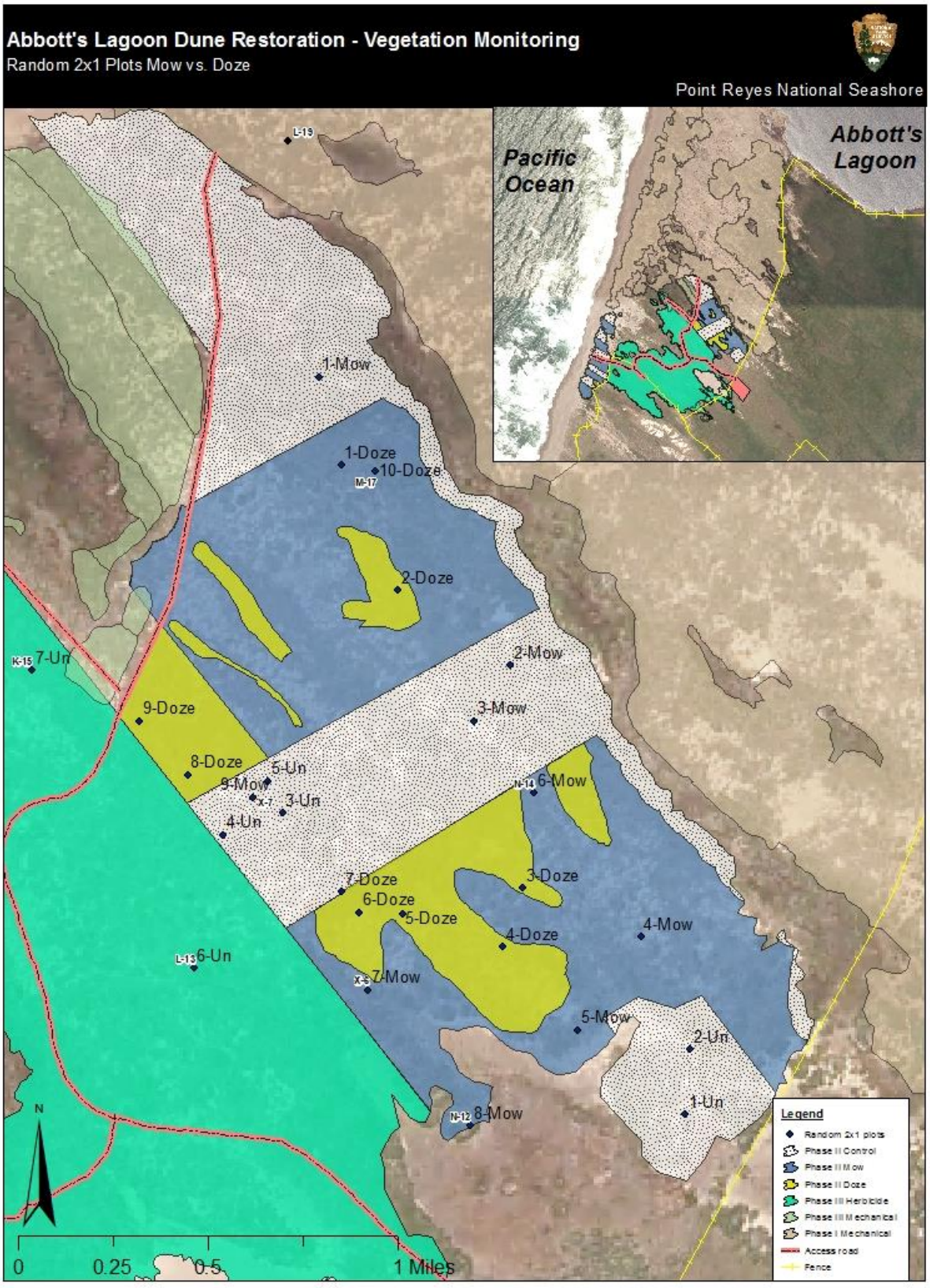
A study by Pearson et al. (2016) showed that, after control of the primary target invasive species, it is very common for those target species to be replaced by secondary invaders instead of native species. We wanted to encourage native species establishment without also acquiring more invasive plant species. We were especially concerned about bush lupine (*Lupinus arboreus*; LUAR) becoming dominant in restored areas because bush lupine fixes nitrogen in the soil, produces abundant nitrogen-rich leaf litter, and provides shade, all characteristics that could encourage weedy annuals (Zink et al. 1995, Maron and Connors 1996). Bush lupine became prolific enough in the mechanically restored dunes by 2016 that efforts were made to remove seedlings and adult plants. We hypothesized that the doze treatment -- the treatment with more soil disturbance and more similar to the mechanically treated dunes -- might result in increased bush lupine cover.

Methods

To compare post-herbicide treatments with no post-treatment, we monitored 26 randomly chosen and permanent plots for species composition and cover in the summer of 2016 (five years after herbicide treatment, four years after post-treatment). From a larger set of randomly generated points, we hand-selected a subset of points to monitor within each treatment type (Figure 2). Existing permanent 2x1 plots that landed in each of the treatment areas were also included in data analysis (M-17 in doze, N-12, N-14, X-6 and X-7 in mow and L-13 and K-15 in control). In total, we sampled 10 plots from the doze treatment, nine (9) plots from the mow treatment, and seven (7) plots that received no post-herbicide treatment.

Seashore staff used the same sampling methods as the permanent monitoring plots at Abbotts Lagoon, using a 2x1 m quadrat to quantify species cover using point-intercept sampling and identifying all species present in the plot (Parsons et al. 2016). All plots were located in restored dunes so we used the "reduced" sampling protocol, only reading 68 points on the quadrat instead of the full 171 points.

Because one of the main goals of post-herbicide treatment was to reduce standing dead European beachgrass, we analyzed overall dead European beachgrass cover (dead AMAR) as well as dead cover separated into three height classes (dead AMAR short, medium, and tall). We also analyzed bush lupine



S:\GIS\projects 1\Dune_restoration\Abbott's Lagoon Coastal Dune Restoration Project\Phase_II_Mow

Figure 2. Locations of post-herbicide treatments and subsequent 2x1 monitoring plots.

cover separately to see if any of the treatments had an effect on the colonization of this secondary invader. We grouped all other species (including European beachgrass and bush lupine) into native and non-native functional groups to test for differences in native cover, non-native cover, native species richness, and non-native species richness across the three treatment groups. Additionally, we tested for differences in overall species richness, Shannon-Weiner diversity, evenness, detritus, and bareground cover.

For all response variables except the dead AMAR height classes, we fitted generalized linear models (GLM) with post-herbicide treatment (mow, doze, or control) as an independent factor. All cover responses (native, non-native, dead AMAR, bush lupine, detritus, and bareground cover) were fitted using a binomial distribution. Species richness, native species richness, and non-native species richness were fitted using a Poisson distribution. Diversity and evenness were fitted using a Gaussian distribution. Whenever treatment had a significant effect, Tukey multiple comparisons tests were used to evaluate differences among the means. Contingency table analysis was used to assess changes in the proportion of dead European beachgrass in the short, medium, and tall height classes. We performed non-metric multidimensional scaling (NMDS) analysis on individual species cover in the 2x1 m plots and then tested for differences in species composition among the three post-herbicide treatments using plot ordination scores from NMDS as dependent factors (NMDS1 and NMDS2) and post-herbicide treatment as an independent factor.

Results

Both mow and doze post-herbicide treatments significantly reduced standing dead European beachgrass compared to control plots (mow, df=23, z=-9.53, p<0.0001; doze, df=23, z=-13.16, p<0.0001), from 35.5 ± 11.5 (S.E.)% in control plots to 5.1 ± 2.9% in doze and 13.0 ± 5.4% in mow plots (Figure 3a). The proportion of dead European beachgrass in each of the height categories (short, medium, or tall) varied

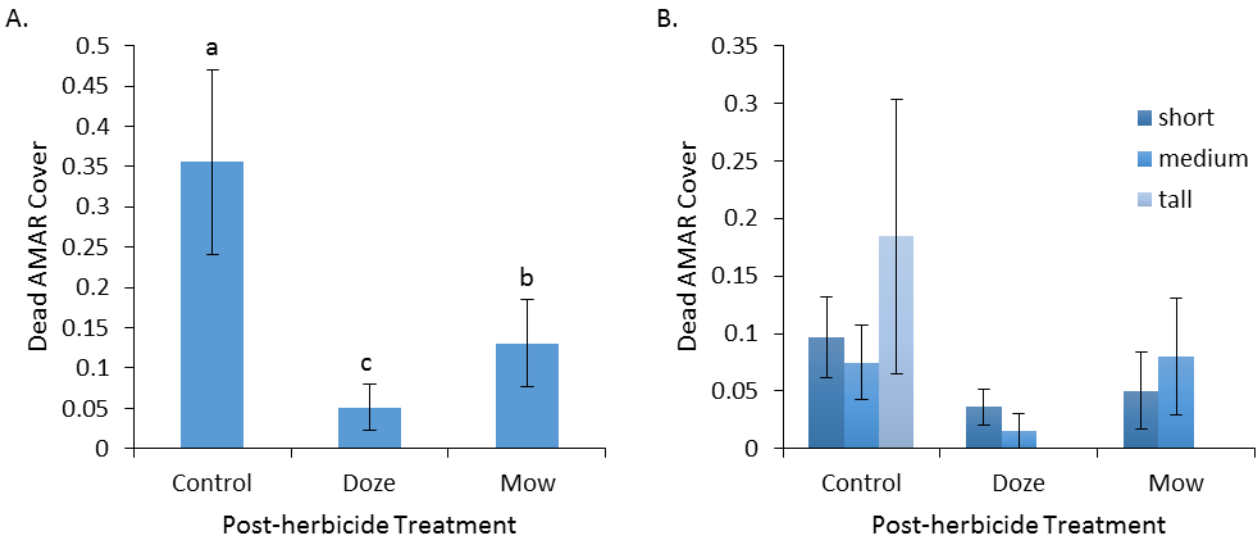


Figure 3. Mean (± S.E.) cover of all dead European beachgrass (a) and dead European beachgrass by size class (b) as a function of post-herbicide treatment (control, doze or mow). Letters above bars correspond to the results from Tukey multiple comparison tests.

significantly with post-herbicide treatment (X^2 = 15.49, df=4, p=0.004, Figure 3b).

Post-herbicide treatment had a significant effect on non-native species cover, with lowest cover in the doze treatment (3.5 ± 1.7%, df=23, z=-5.66, p<0.0001) and with no difference between the mow treatment (15.1 ± 6.6%) and the control (11.5 ± 2.6%; df=23, z=1.96, p=0.05; Figure 4a). Native cover was unaffected by post-herbicide treatment (control, 24.3 ± 8.9%; mow, 21.6 ± 10.7%, df=23, z=-1.17, p=0.24; doze, 22.8 ± 11.0%, df=23, z=-0.64, p=0.52; Figure 4b). Another focal species with invasive tendencies, bush lupine (LUAR), was only found in doze plots, yet we failed to find any significant effect of treatment on LUAR cover (mow, df=23, z=0, p=1; doze, df=23, z=0.01, p=0.99). One of the most visible effects of post-herbicide treatment was on bareground cover. Treatment had a significant effect on bareground cover, with the doze treatment increasing bareground from 11.8 ± 4.2% in control plots to 60.1 ± 6.4% in doze plots (df=23, z=16.79, p<0.0001). The mow treatment increased bareground to a

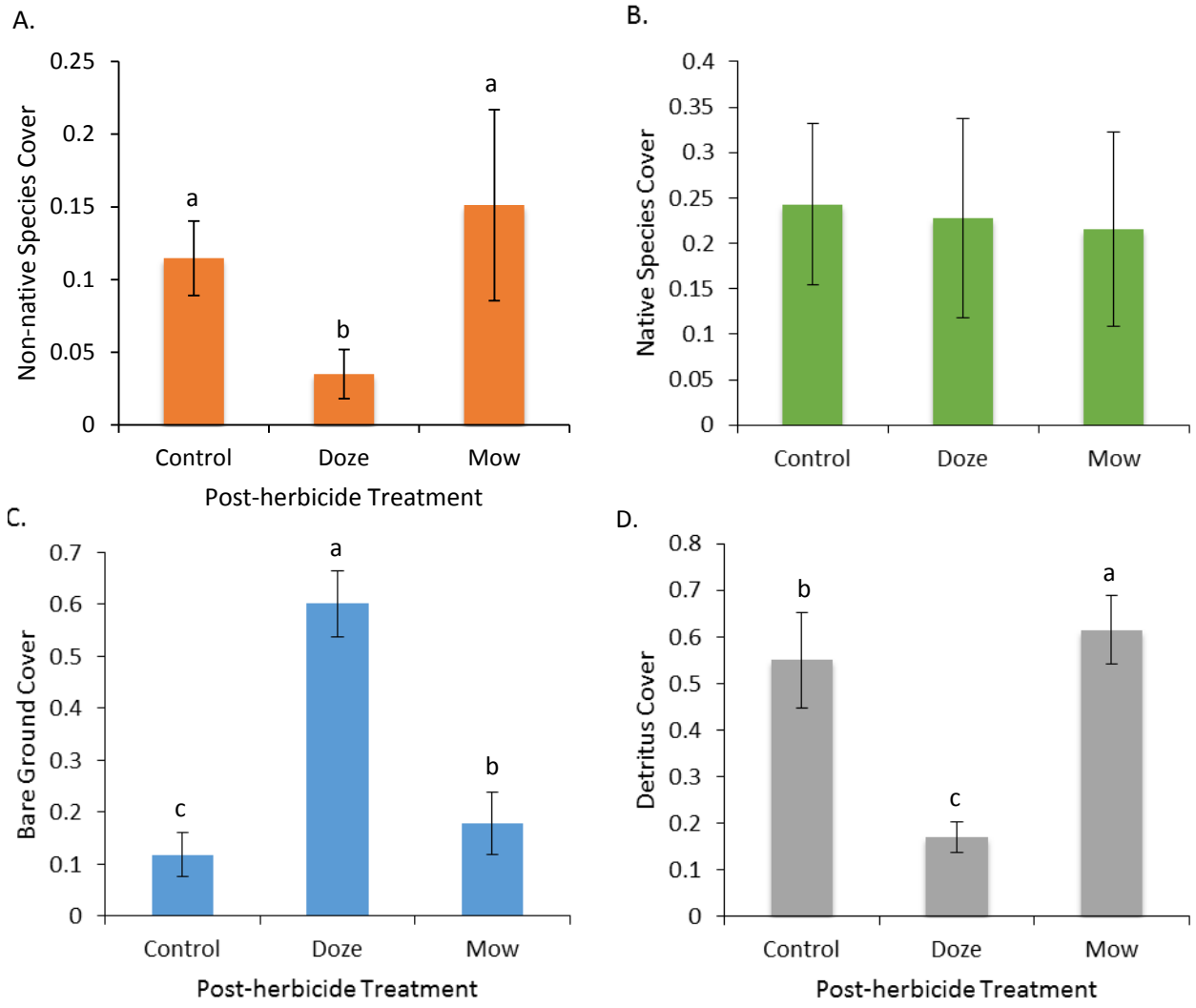


Figure 4. Mean (± S.E.) cover of non-native species (a), native species (b), bare ground (c), and detritus (d) as a function of post-herbicide treatment (control, doze or mow). Letters above bars correspond to the results from Tukey multiple comparison tests.

lesser degree with 17.8 ± 6.1% bareground cover in those plots (df=23, z=3.08, p=0.002; Figure 4c). Detritus was significantly increased compared to the control in mow plots (control, 55.0 ± 10.3%; mow 61.5 ± 7.3% df=23, z=2.43, p=0.02). Conversely, the doze treatment decreased detritus compared to the control (doze 17.0 ± 3.3%, df=23, z=-14.61, p<0.0001; Figure 4d).

Species richness varied significantly among post-herbicide treatments, with decreased richness in the doze treatment compared to the control (control, 6.0 ± 0.8; doze, 2.2 ± 0.6; df=23, z=-3.81, p=0.0002) but no significant difference between mow treated areas and the control (4.6 ± 0.4; df=23, z=-1.25, p=0.21; Figure 5a). Non-native species richness of doze plots was significantly lower than control plots (control, 2.7 ± 0.5; doze, 0.8 ± 0.2; df=23, z=-2.90, p=0.004), while non-native richness of mow plots was not significantly different from either the control or doze treatments (mow, 1.8 ± 0.3; df=23, z=-1.25, p=0.21; Figure 6a). Native species richness showed a similar pattern with significantly lower values in doze plots (control, 3.3 ± 0.8; doze, 1.4 ± 0.5; df=23, z=-2.52, p=0.01) and no significant difference in mow plots when compared to doze plots or the control (mow, 2.8 ± 0.5; df=23, z=-0.58, p=0.56; Figure

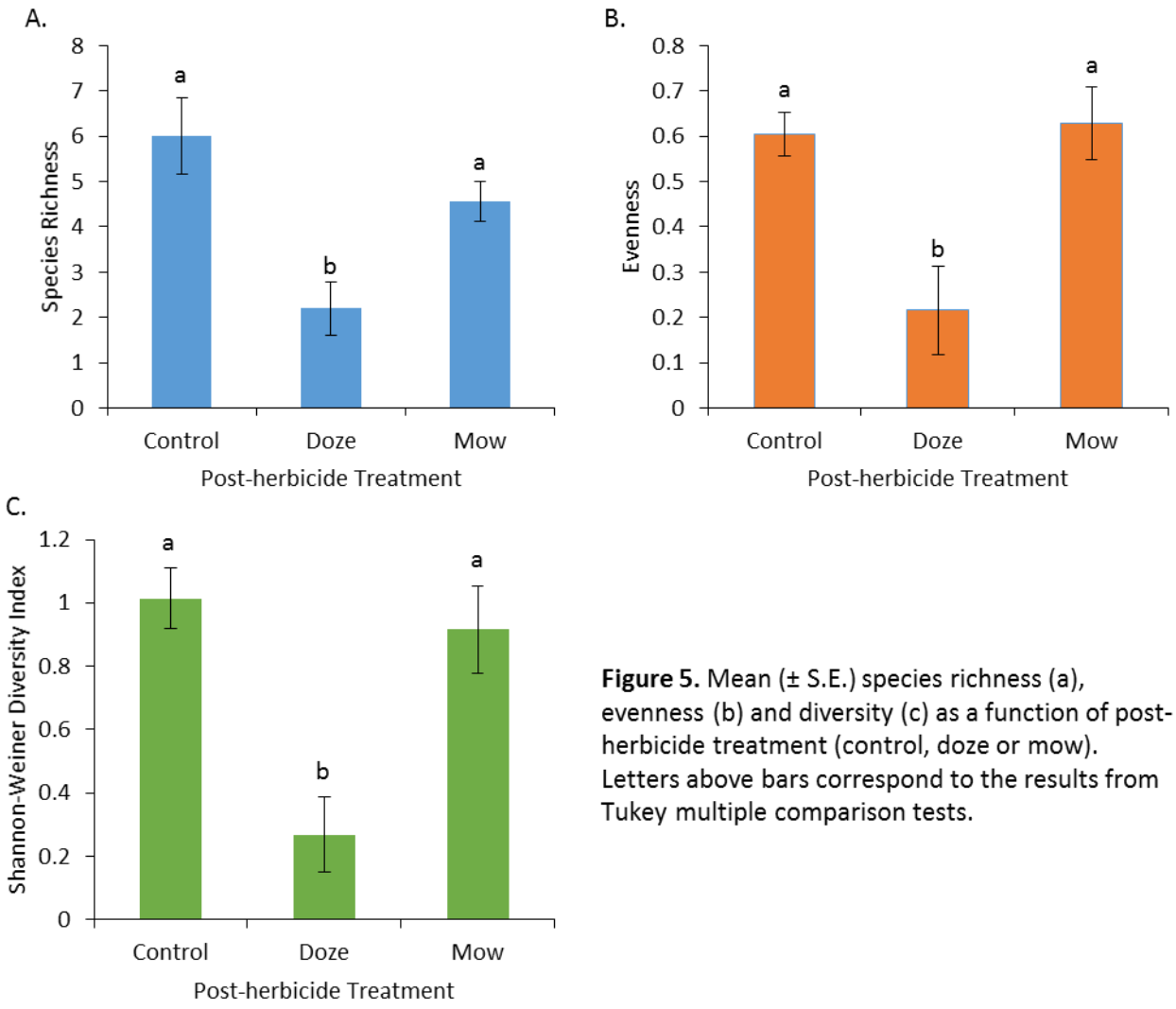


Figure 5. Mean (± S.E.) species richness (a), evenness (b) and diversity (c) as a function of post-herbicide treatment (control, doze or mow). Letters above bars correspond to the results from Tukey multiple comparison tests.

6b). Doze plots had significantly lower species evenness than control plots (control, 0.61 ± 0.05; doze, 0.22 ± 0.10; df=23, z=-3.18, p=0.004), but we failed to find any significant difference in mow plots with either doze or control plots (mow, 0.63 ± 0.08; df=23, z=0.19, p=0.85; Figure 5b). Similarly, we found significantly lower diversity (Shannon-Weiner diversity index) in doze plots compared to controls (control, 1.01 ± 0.09; doze, 0.27 ± 0.12; df=23, z=-4.17, p=0.0004), but failed to detect a difference in mow plots with either control or doze plots (mow, 0.92 ± 0.14; df=23, z=-0.54, p=0.60, Figure 5c).

We found significant differences in species composition among post-herbicide treatment groups along NMDS axis 1 ($F_{2,23}$=34.9, p<0.0001), but no significant difference along NMDS axis 2 ($F_{2,23}$=0.38, p=0.69; Figure 7). The NMDS analysis showed species overlap between mow and control plots and a separation along the first axis with doze plots (Figure 7). Doze plots clustered lower on the first NMDS axis along with bareground, rhizomes from dead European beachgrass, woolly lotus (*Lotus heermanii*), curly leaf monardella (*Monardella sinuata* ssp. *nigrescens*), bush lupine, and gumplant (*Grindelia stricta*). Control and mow plots clustered higher along the first NMDS axis along with detritus, ripgut grass (*Bromus diandrus*), dead European beachgrass, coyote brush (*Baccharis pilularis*), woodland groundsel (*Senecio sylvaticus*), fairy mist (*Pterostegia drymarioides*), miner's lettuce (*Claytonia perfoliata*), sheep sorrel (*Rumex acetocella*), bracken fern (*Pteridium aquilinum*), and sand mat (*Cardionema ramosissimum*).

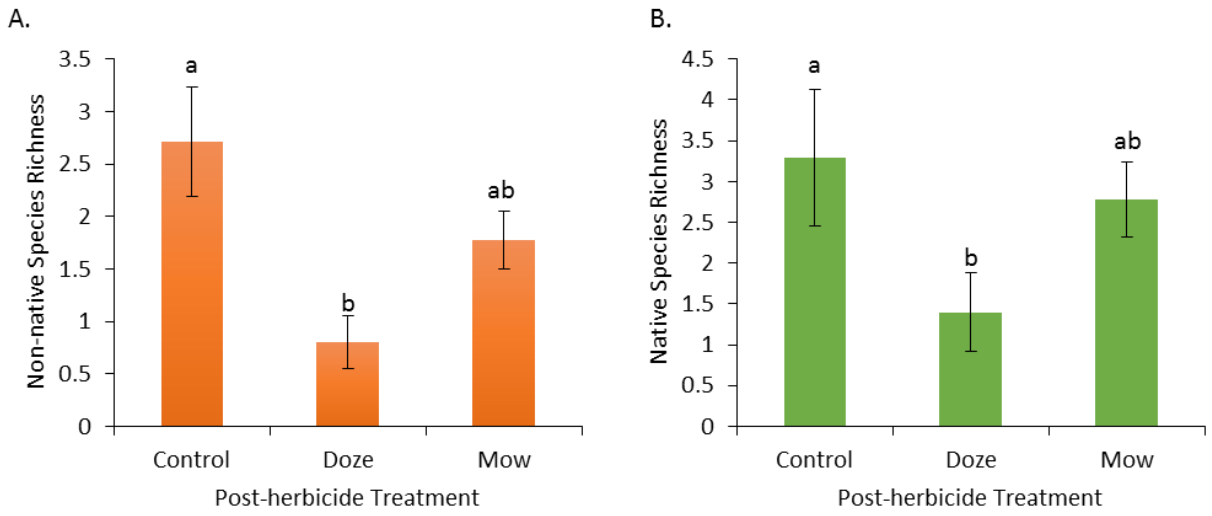


Figure 6. Mean (± S.E.) non-native species richness (A) and native species richness (B) as a function of post-herbicide treatment (control, doze or mow). Letters above bars correspond to the results from Tukey multiple comparison tests.

Conclusions

Overall, both post-herbicide treatments met one of the treatment goals -- reduced dead European beachgrass cover -- but have not yet shown an increase in native species establishment. The doze treatment was the more extreme of the two treatments resulting in the lowest dead European beachgrass cover, non-native species cover, and the highest amount of bareground, but also the lowest species richness, evenness, and diversity. The mow treatment also reduced dead standing European

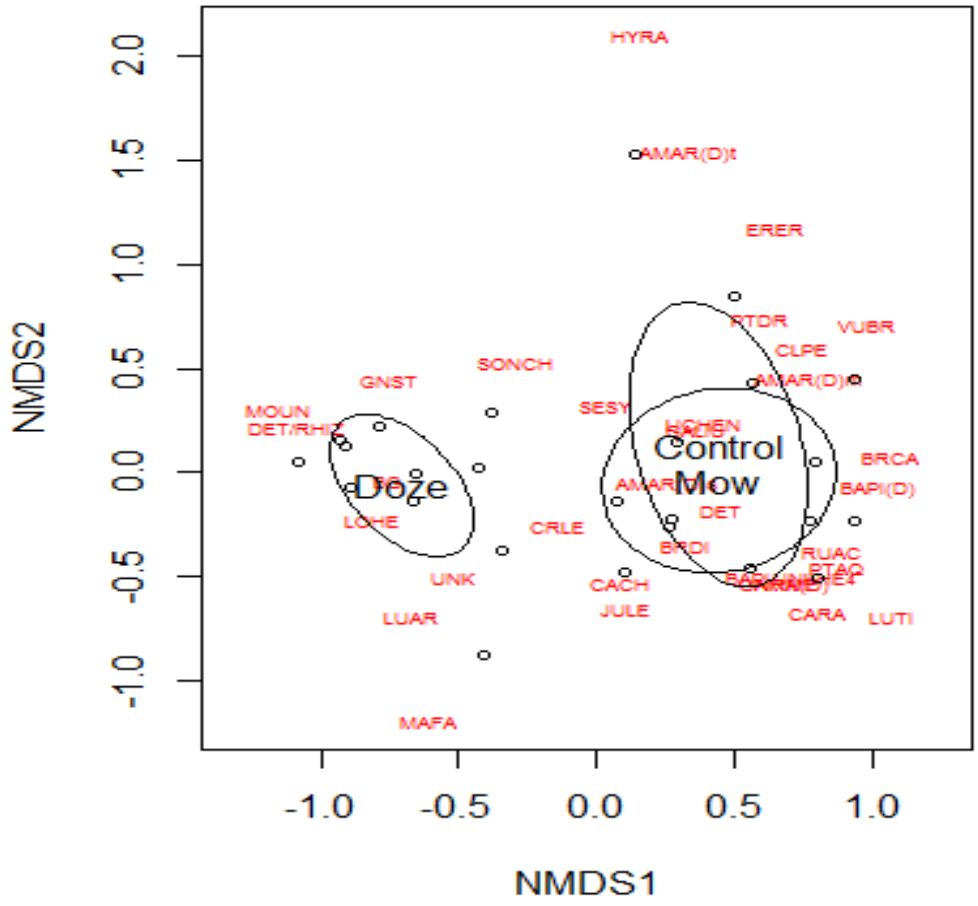


Figure 7. Non-metric multidimensional scaling multivariate analysis of plots from control, doze or mow post-herbicide treatments.

beachgrass, but was more similar to the control in terms of having higher non-native species cover, species richness, evenness, and diversity than doze plots. Native species cover did not differ among the three treatments.

While bush lupine definitely appears to be a more prominent species in the doze and, to a slightly lesser extent, mow treatments than control ones, our results were unable to discern any statistically significant difference, possibly because statistical power was too low. This issue underscores some of the difficulties with use of the 2X1 plots for evaluating vegetation changes in the restored dunes: due to the vagaries of random plot placement and heterogeneity in spatial patterns of dune species cover, the plots do not always capture the full complexity of what is going on vegetation-wise in the dunes. However, we have yet to find a time-efficient and yet biologically powerful vegetation monitoring method to effectively replace this one, as vegetation sampling in the dunes can be quite complicated due to extreme topographic variability in foredunes and backdunes.

Post-herbicide treatment reduced standing dead European beachgrass cover to 13% for the mow treatment and 5% for the doze treatment. Considering that the control with no post-treatment had an average cover of 35%, treatments were successful from the standpoint of reducing standing dead biomass. Of the remaining dead plants, there was a lower proportion of tall plants in mow and doze treated plots as well as a lower proportion of medium-sized plants in doze treated plots. Plots with the doze treatment also had a higher proportion of short dead European beachgrass. An overall decrease in the volume and height of standing dead biomass should increase light available for new plant establishment. Although standing aboveground biomass of dead AMAR was also reduced in the mow treatment, detritus was higher in these treatment areas than doze ones, which could reduce germination of both native and non-native species (Xiong and Nilsson 1999) or possibly tip the establishment success scales toward non-native species.

While our monitoring efforts did not evaluate belowground variables, post-herbicide treatment did not appear to affect belowground rhizomes, which continued to stabilize the soil. Less sand movement might make it less likely that early (or even mid-) successional dune species will establish in these areas (Zhang and Maun 1990, Maun 2004). Interestingly, NMDS results suggest that doze plots have supported colonization of mid- to late-successional dune species such as woolly lotus, curly leaf monardella, and gumplant, while the species assemblage in the mowed and control plots (coyote brush, fairy mist, bracken fern, miner's lettuce) are more characteristic of dune scrub communities, with the possible exception of sand mat, a species common in late-successional dune communities. As Phase II is largely a backdune area, somewhat removed from the shore and ocean- and wind-associated movement of sand, we have assumed that the evolutionary trajectory in terms for Phase II in terms of native vegetation communities would be a dune scrub assemblage, however, these results suggest that disturbance – natural or anthropogenic – could shift evolution on a different trajectory.

There was no increase in native species cover for either post-herbicide treatment, and the doze treatment actually had the opposite from the desired effect, decreasing native species richness (there was no change in native species richness in mow plots compared to control plots). Additional monitoring in the future may show a different story, but as of now, the post-herbicide treatments show no advantage compared to the control in terms of native species colonization.

In addition to decreasing standing dead European beachgrass and increasing native plant establishment, we wanted to avoid colonization by other exotic and/or invasive species. The more destructive doze treatment decreased both non-native species cover and richness, while the mow plots showed no significant difference compared to the control. Fewer non-native species in the doze plots can likely be attributed to the destruction caused by scraping that decreased cover and richness of all species. Bush lupine was found at very low cover only in doze plots. Although this finding suggests that the doze treatment might have increased bush lupine establishment, statistically, our results failed to show that post-herbicide treatments had any significant effects on bush lupine cover.

Implications for Future Management

Dead European beachgrass was reduced in both the mow and doze treatments compared to no post-herbicide treatment. However, removing standing dead biomass has not produced the desired ancillary effects of increasing native species. The mow treated plots were not different from control plots in non-native species cover or richness, native cover or richness, bareground cover, or overall species richness, evenness, or diversity. In this case, the results of post-herbicide treatment do not seem to warrant the cost of bringing heavy machinery to the dunes for treatment. The doze treatment did reduce non-native species cover and richness, but these results came with undesired effects like reducing native species richness and overall species richness, evenness, and diversity. From this point of view, the doze treatment also does not seem to be a viable option for post-herbicide treatment. It is important to keep in mind that over a longer period of time post-herbicide treatment might prove to be valuable in recruiting native dune species, and additional monitoring may be needed.

Finally, additional post-treatment efforts such as direct seeding or outplanting may be necessary to attain the desired native community in herbicide treated plots. Revegetation plants were established in winter 2015-2016 to set up seeding with coyotebrush and mock heather within different types of herbicide-treatment areas: formal monitoring was not conducted in 2016, but staff observations of plots indicated that, despite an increase in rainfall last winter, there was not abundant germination of sown seed (L. Parsons, NPS, *pers. obs*.). However, these seeds may germinate and flourish more in future years.

Literature Cited

Hyland, T., and P. Holloran. 2005. Controlling European beachgrass (Ammophila arenaria) using prescribed burns and herbicide. Page 29. Proceedings of the California Invasive Plant Council Symposium.

Maron, J. L., and P. G. Connors. 1996. A Native Nitrogen-Fixing Shrub Facilitates Weed Invasion. Oecologia 105:302–312.

Maun, M. A. 2004. Burial of plants as a selective force in sand dunes. Coastal Dunes: Ecology and Conservation. Springer-Verlag, Berlin pp. 119-135.

Parsons, L., S. Minnick, and A. Ryan. 2016. Coastal Dune Restoration Vegetation Monitoring Report. Point Reyes National Seashore, Point Reyes Station, CA.

Pearson, D. E., Y. K. Ortega, J. B. Runyon, and J. L. Butler. 2016. Secondary invasion: The bane of weed management. Biological Conservation 197:8-17.

Xiong, S., and C. Nilsson. 1999. The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology 87:984-994.

Zhang, J., and M. A. Maun. 1990. Sand burial effects on seed germination, seedling emergence and establishment of *Panicum virgatum*. Holarctic Ecology 13:56-61.

APPENDIX E. PAPER PUBLISHED BY PARSONS ET AL. (2020): "*SOIL MICROBIAL COMMUNITIES IN RESTORED AND UNRESTORED COASTAL DUNE ECOSYSTEMS IN CALIFORNIA*"

RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Soil microbial communities in restored and unrestored coastal dune ecosystems in California

Lorraine S. Parsons[1,2], Jordan Sayre[3], Cody Ender[1], Jorge L. M. Rodrigues[3], Albert Barberán[4]

Most restoration projects involving invasive plant eradication tend to focus on plant removal with little consideration given to how these invasives change soil microbial communities. However, soil microorganisms can determine invasibility of habitats and, in turn, be altered by invasives once established, potentially inhibiting native plant establishment. We studied soil microbial communities in coastal dunes with varying invasion intensity and different restoration approaches (herbicide, mechanical excavation) at Point Reyes National Seashore. Overall, we found evidence of a strong link between bacterial and fungal soil communities and the presence of invasives and restoration approach. Heavily invaded sites were characterized by a lower abundance of putatively identified nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs and a higher abundance of cellulolytic bacteria and a class of arbuscular mycorrhizal fungi (Archaeosporomycetes). Changes in soil microbiota did not fully dissipate following removal of invasives using herbicide, with exception of reductions in cellulolytic bacteria and Archaeosporomycetes abundance. Mechanical restoration effectively removed both invasives and soil legacy effects by inverting or "flipping" rhizome-contaminated surface soils with soils from below and may have inadvertently induced other adverse effects on soils that impeded reestablishment of native dune plants. Land managers should consider additional measures to counteract lingering legacy effects and/or focus restoration efforts in areas where legacy effects are less pronounced.

Key words: dune, herbicide, invasive plant, microbial, restoration, soil

Implications for Practice

- Even when invasive plant species are successfully removed, soil microbial legacy effects may persist that could derail efforts to reestablish functioning, intact, and resilient native dune ecosystems.
- Legacy effects may impede efforts to reestablish native dune plant species and/or promote either re-invasion by primary target species or invasion by new weeds or "secondary" invaders better adapted to changed soil conditions.
- Project managers may need to consider additional restoration measures (e.g. iceplant duff removal, soil amelioration) to ensure the success of restoration efforts or focus restoration efforts on low-risk, high-yield activities such as invasives removal in sparsely to moderately invaded areas or in foredune areas, where sand overwash can counteract beachgrass legacy effects.

Introduction

Invasive plants pose one of the largest threats to coastal ecosystems, along with development and climate change (Pickart & Sawyer 1998). Two of the most problematic invasive species within coastal dunes are European beachgrass (*Ammophila arenaria*) and iceplant (*Carpobrotus* spp.), which are native to northern Europe and South Africa, respectively, but have been introduced to southern Europe, South Africa, Australia/New Zealand, and North America (Weber 2003; van der Putten et al. 2005). While the spread of invasive plants in some coastal ecosystems often builds slowly over time due to accidental introductions, these two species were in many instances deliberately planted to stabilize coastal areas and protect roads, railroads, and other infrastructure or land uses. This gives these invasives a tremendous jump-start in wholescale conversion of previously diverse native ecosystems into monotypic, dense stands of European beachgrass and iceplant (Wiedemann & Pickart 1996, 2008). These invasive plants not only displace, outcompete, and directly reduce native plant species diversity, but can induce indirect effects on native plant communities by altering soils and insect-plant relationships and providing habitat for predators of both plants and animals (Barbour et al. 1976; Vilà et al. 2006; Conser & Connor 2009). Through these

Author contributions: LSP, AB developed project concept, developed sampling scheme, conducted field sampling, wrote and edited the manuscript; JS, CE conducted field sampling; AB, JS ran molecular microbiome analyses; AB performed data analysis; JLMR, JS edited the manuscript.

[1]Point Reyes National Seashore, Point Reyes Station, CA 94956, U.S.A.
[2]Address correspondence to L. S. Parsons, email Lorraine_Parsons@nps.gov
[3]Department of Land, Air and Water Resources, University of California, Davis, CA 95616, U.S.A.
[4]Department of Environmental Science, University of Arizona, Tucson, AZ 85721, U.S.A.

© 2020 Society for Ecological Restoration
doi: 10.1111/rec.13101

direct and indirect impacts, they also threaten the wildlife and rare plants that depend on intact ecosystems for persistence (Dangremond et al. 2010; Muir & Colwell 2010).

Plant–soil feedbacks are important for aboveground community composition (Wardle et al. 2004; van der Heijden et al. 2008). In particular, soil microbial communities might facilitate or inhibit the establishment and success of invasive plant species, as well as native plant ones (Wolfe & Klironomos 2005; Inderjit & van der Putten 2010; Kardol & Wardle 2010). Among the ecosystems where plant–soil feedbacks have been of interest are coastal dunes. Many studies have focused on understanding factors enabling certain dune species such as European beachgrass and iceplant to be more invasive in terms of prevalence and persistence in their introduced ranges than in their native ranges. In general, results from these studies suggest that factors potentially differ depending on geographic region. For example, in some areas, European beachgrass and iceplant appear to flourish when they are either completely or partially released from their natural enemies that inhibit expansion and persistence of these species in their "home" range (Beckstead & Parker 2003; Knevel et al. 2004; Van Grunsven et al. 2009). Another California study argued that, rather than being wholly or partially released from its enemies, European beachgrass accumulates local pathogens and that it can tolerate higher pathogen levels than native plants, giving beachgrass a competitive advantage (Eppinga et al. 2006). Invasives may also be able to spread because they disrupt native species' relationships with symbiotic mutualists or because they are able to form stronger symbiotic relationships than native species (Pringle et al. 2009; Inderjit & van der Putten 2010). For example, germination of European beachgrass in iceplant-invaded soils resulted in lower aboveground biomass of beachgrass than in non-iceplant-invaded soils, which may relate to suppression of beneficial mycorrhizal interactions by iceplant (de la Peña et al. 2010).

However, it has been argued that introduced species are not always successful in invading native communities, because strong interaction between soil biota and native species can hinder their establishment and spread (Knevel et al. 2004; Suding et al. 2013). Along these lines, a study on South African dunes found that one species, *Sporobolus virginicus*, might actually contribute to soil feedback resistance against European beachgrass and concluded that not only plant species diversity, but also plant community composition, might be important in determining the potential for biotic resistance (Knevel et al. 2004). In a meta-analysis of native–exotic feedback comparisons, researchers concluded that soils conditioned by native species can resist invaders when invader frequency is low, even if the invader exerts a positive plant–soil feedback, but that this resistance can be negated by disturbance or other events that enable invaders to spread quickly or establish in high numbers, particularly when non-native species have dispersal or competitive advantages over native ones (Suding et al. 2013). Experiments suggested that the soil community in some Mediterranean dune systems may initially resist iceplant, but the resistance breaks down over time, with an increase in abundance of a symbiotic fungal species (chytrids) eventually being detrimental to native plant species (Vilà et al. 2006; de la Peña et al. 2010).

Once established within dune systems, invasive non-native species have developed a number of mechanisms that enable them to rapidly spread and displace native counterparts. These mechanisms include physical, chemical, and biological "legacy" effects. European beachgrass leaves a lasting physical legacy on foredune systems by capturing sands and creating larger, taller foredune systems than would have existed naturally (Wiedemann & Pickart 1996; Corbin & D'Antonio 2012). Iceplant exerts a very well-documented physiochemical influence on invaded dunes by reducing soil pH and available water and increasing organic matter, nitrogen, carbon, and sodium levels (D'Antonio & Mahall 1991; Conser & Connor 2009; Novoa et al. 2014). Iceplant leaves have high levels of tannin and antibacterial compounds (van der Watt & Pretorius 2001) that may reduce litter decomposition rates (Vilà et al. 2006) and acidify soils, thereby reducing availability of nitrate, calcium, and magnesium (Conser & Connor 2009). Physiochemical legacy effects can later lead to further changes in the soil microbial communities that may reinforce the competitive advantage for invasive species. A study in Spain documented that soil microbial communities changed substantially with invasion, which they attributed to iceplant creating higher levels of microbial carbon sources that resulted in an increase in total microbial biomass, a decrease in the bacteria/fungi ratio, and a shift in microbial species composition (Badalamenti et al. 2016). Once invaded, these persistent legacy effects can have tremendous ramifications on dunes and the potential for restoring them in the future.

In this study, we assessed soil microbial communities by sequencing environmental DNA (Breed et al. 2019) in a large-scale coastal dune restoration program that Point Reyes National Seashore (PRNS; California, U.S.A.) has been conducting since 2001 to eliminate European beachgrass and iceplant. These species were believed to have been planted in PRNS in the early to mid-1900s by ranchers and other residents trying to prevent coastal dune sands from migrating into adjacent pastures and ranch facilities and on to roads. By 2009, more than 60% of the park's roughly 890 ha of coastal dune, bluff, and scrub habitat was estimated to be dominated by European beachgrass and iceplant, with beachgrass being more prevalent in areas where most of the restoration has been conducted to date (National Park Service 2009).

We hypothesize that differences in soil microbiota exist between areas invaded by European beachgrass and uninvaded areas, and these changes may promote persistence and expansion of invaders while suppressing that of native plant species. We also hypothesize that the approach used for removing European beachgrass (mechanical removal, herbicide treatment) may have differential effects on soil microbial communities, which may thereby affect the potential for re-establishing a native flora, as well as the potential for being re-invaded by either beachgrass or other weeds. There has been little study about the effect of mechanical removal on soil microbial communities, but research has suggested that herbicides might alter soil biota

due to persistence of chemicals in soils and the fact that herbicides such as glyphosate can affect microbial, as well as plant, metabolic pathways (Issa 1999; Durkin 2011).

The availability in PRNS of areas with varying invasion intensity, as well as of restored and unrestored invaded habitats, offers a valuable opportunity to evaluate impacts of invasion and restoration approach on soil microbial communities in coastal dune ecosystems. Understanding these mechanisms would enable land managers to optimize site selection and approaches for habitat restoration.

Methods

Study Area

PRNS is a unit of the National Park Service and is located approximately 48 km north of San Francisco, California, U.S.A. (Fig. 1A). The climate is Mediterranean, with hot/dry summers and cool/wet winters. Coastal areas in California are generally cooler and have less intra-annual variability in temperature than inland regions, with fog and winds being important climatic influences. Annual temperature is estimated to average 12.1°C, and precipitation is estimated to average 45–52 cm per year. Soil types in dune areas consist of hummocks/mounds and hills of loose sand blown from nearby beaches with no profile development (Soil Conservation Service 1985). Coastal dune sands tend to be nutrient limited, especially for the essential nutrients nitrogen, phosphorus, and potassium (Barbour et al. 1985). PRNS's dunes provide habitat for up to 11 species listed by the U.S. Fish and Wildlife Service as endangered or threatened.

Between 2001 and 2014, approximately 200 gross hectares of coastal dunes have been restored using a combination of manual removal (e.g. hand pulling or small hand tools), mechanical removal, and chemical treatment. Mechanical removal involves use of excavators and bulldozers to invert or "flip" the surface 1–1.5 m of rhizome-contaminated soils with less-contaminated soils 2–3 m below the soil surface. Initial chemical treatment and subsequent re-treatment are performed annually in the late summer with herbicide backpacks and directed spraying using a single nozzle wand of a mixture of 1.5% imazapyr, 2% glyphosate, a modified vegetable oil surfactant, and blue dye onto target invasive plants.

The park's restoration efforts have focused on invaded areas that would have the most benefit for critical listed species. While invasive plants often account for more than 90% of the vegetation in these targeted restoration areas, some retain vestiges of native dune mat or dune scrub habitat. These "islands" of native dune mat habitat have been surrounded by dense invasive stands for decades, yet retain the ability to resist encroachment despite patchy colonization by iceplant. Native dune refugia often support sizeable rare plant populations, as well as a diverse native plant community, and range in size from 1 to 20.8 ha.

Selection of Sites for Soil Sampling

The sampled areas included Abbotts Lagoon (122°57′33.46″W, 38°6′36.272″N), AT&T/North Beach (122°57′51.737″W, 38°5′47.147″N), and B Ranch North (123°0′0.289″W, 38°1′12.915″N; Fig. 1A). Soil sampling in native dune and unrestored invaded areas occurred along a continuum of invasion, from lightly invaded (native dune "islands" with <5% cover of invasives, primarily iceplant) to moderately invaded (approximately 25–50% cover of European beachgrass, iceplant, or secondary invaders such as European searocket/*Cakile maritima*) to heavily invaded (>75% cover of invasives, primarily beachgrass) (Fig. 1B–D).

We also sampled areas restored by either herbicide treatment or mechanical removal, ensuring equal sampling effort between foredunes and backdunes, as backdunes represent older, more stabilized dunes with a different vegetation community (Miller 2015) that could, therefore, have different soil chemistry and belowground biota. Chemical treatment of European beachgrass and, to a lesser degree, iceplant has been performed at all three areas, but mechanical removal was only conducted at Abbotts Lagoon in 2011 due to high cost per acre and indirect impacts from sand remobilization on adjacent native habitats and ranchlands. Due to these issues, PRNS switched restoration approach primarily to herbicide treatment following that project, with initial herbicide treatment occurring in 2011–2014 (Abbotts), 2014 (B Ranch North), and 2016 (AT&T/North Beach). Soil sampling represented a range in terms of restoration age: mechanical removal (6 years) and herbicide treatment (1–6 years).

Soil Sampling Methodology

In general, two to three subsampling areas were selected within each of these sampling categories (invasion intensity/restoration approach) at all areas (Table 1). At each soil sampling location, four surface soil cores (approximately 7 cm depth) were collected in the peak growing season (26 April, 2017) that varied in proximity to plants, although areas directly beneath plants with dense roots were avoided. Composite soil samples were homogenized, sieved to 4 mm to remove roots and stones, placed in plastic bags, and frozen in the field until samples could be transported on ice at −20°C to the laboratory for storage at −80°C until analysis.

Molecular Analyses

We used DNA metabarcoding coupled with high-throughput sequencing to describe the soil microbial communities based on taxonomic markers. Genomic DNA was extracted using the QIAGEN DNeasy PowerLyzer PowerSoil kit. For bacteria and archaea, we sequenced the V4 hypervariable region of the 16S rRNA, using the 515-F (GTGCCAGCMGCCGCG GTAA) and 806-R (GGACTACHVGGGTWTCTAAT) primer pair, and for fungi, we sequenced the first internal transcribed spacer (ITS1) region of the rRNA operon, using the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primer pair (Barberán et al. 2015). Primers included Illumina adapters and unique error-correcting 12-bp barcodes. PCR products were quantified and pooled in equimolar concentrations for sequencing on an Illumina MiSeq at the University of Arizona (Tucson, AZ, U.S.A.).

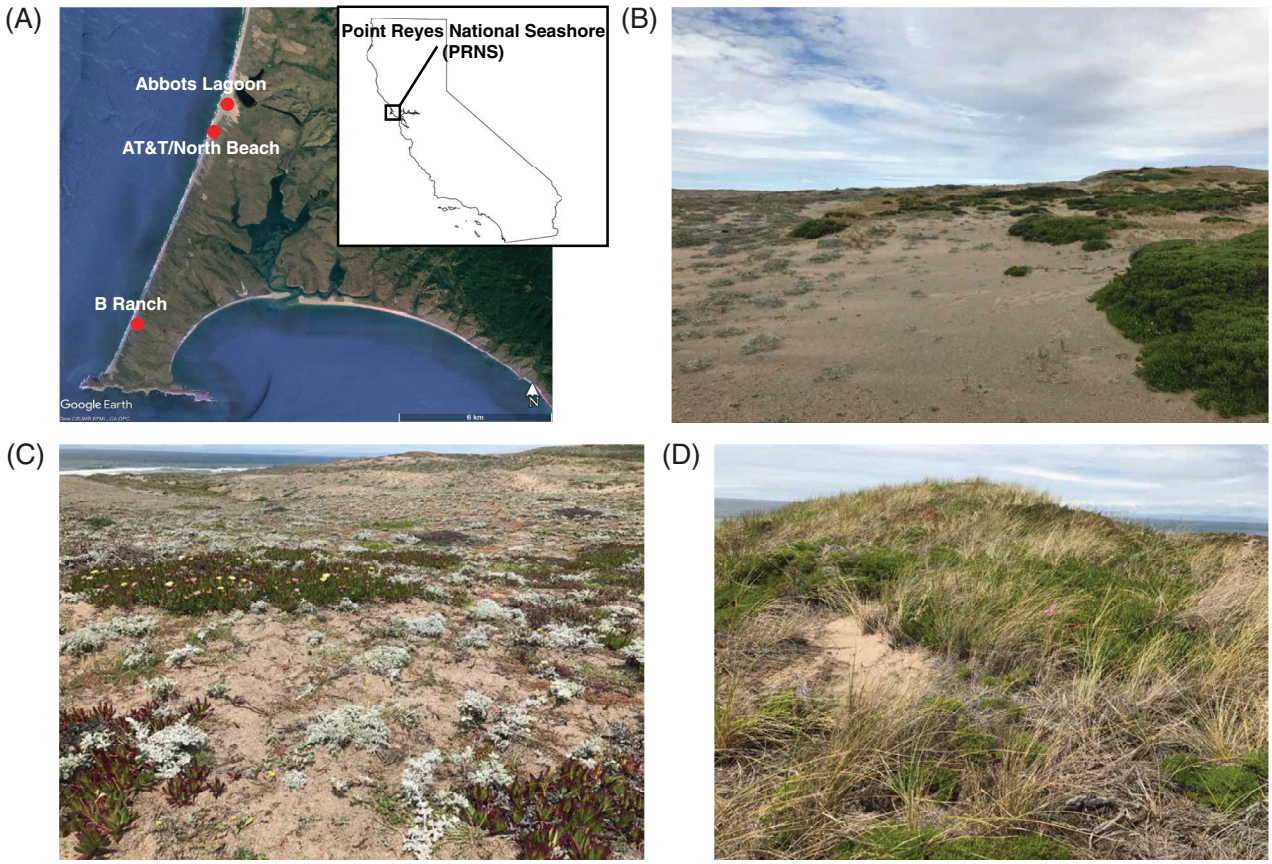


Figure 1. Map of the study site in Point Reyes National Seashore (PRNS), California (A). Pictures showing examples of lightly invaded dunes (B), moderately invaded dunes (C), and heavily invaded dunes (D).

Table 1. Number of soil samples analyzed from restored and unrestored dunes in Point Reyes National Seashore. NA = not available (i.e. mechanical restoration not performed in all areas). NS = present, but not sampled.

Invasion Intensity/Treatment Approach	*Foredune vs. Backdune (Where Applicable)*	*Abbotts Lagoon*	*AT&T/ North Beach*	*B Ranch North*
Lightly invaded native dune mat	Foredune	3	2	3
	Backdune			
Moderately invaded native dune mat	Foredune	3	3	3
	Backdune			
Heavily invaded dunes—untreated	Foredune	NA	1	NS
	Backdune		2	3
Heavily invaded dunes—restored using herbicide	Foredune	3	3	3
	Backdune	3	3	3
Heavily invaded dunes—restored mechanically	Foredune	2	NA	NA
	Backdune	2		

Sequence Processing

After demultiplexing, raw reads were subject to quality filtering, dereplication, chimera detection, and merging of paired-end reads using dada2 (Callahan et al. 2016). The dada2 algorithm uses a parametric error model to correct and group sequences into unique phylotypes (Callahan et al. 2016). The ITS region is highly variable in length, and thus the first step prior to sequence processing was the removal of primers using cutadapt (Martin 2011). Unique sequence phylotypes were assigned taxonomic affiliation using the Ribosomal Database Project naïve Bayesian classifier (Wang et al. 2007) trained on the 16S rRNA SILVA database (Quast et al. 2012) or the ITS UNITE database (Abarenkov et al. 2010) for bacteria and fungi, respectively. Sequences unclassified at the domain level or classified as mitochondria, chloroplasts, or Eukaryota were removed. We removed potential contaminants by discarding phylotypes present in the control blanks. We normalized phylotype counts using a cumulative-sum scaling approach (Paulson et al. 2013).

Finally, we inferred putative functions using FAPROTAX (Louca et al. 2016) and FUNGuild (Nguyen et al. 2016) for bacteria and fungi, respectively. Although each DNA metabarcoding step can potentially introduce artifacts and biases, we sequenced gene regions shown to provide accurate taxonomic information; we employed broadly used primers with few biases against specific taxa; we filtered out sequencing errors and chimeras using astringent thresholds; we used up-to-date taxonomic reference databases, and we removed potential contaminants through sequencing negative controls (Zinger et al. 2019). Sample information, phylotype abundance tables, and representative sequences are publicly available in FigShare (https://doi.org/10.6084/m9.figshare.9036740.v1).

Statistical Analyses

We used analysis of variance (invasion levels nested within sites) to assess the effect of the different treatments on microbial richness and taxonomic or inferred functional differences. We represented microbial community similarity patterns by non-metric multidimensional scaling using Bray-Curtis distance metric. We used permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) to assess the effect of the different treatments on microbial community similarity patterns. We corrected for multiple testing using false discovery rate (FDR) correction (Benjamini & Hochberg 1995). All statistical analyses were implemented in the R environment (www.r-project.org) using the *vegan* package (vegan.r-forge.r-project.org).

Results

The total number of unique phylotypes was 28,295 for bacteria across 41 soil samples and 8,249 for fungi across 39 soil samples. The average number of unique phylotypes per soil sample was 1,791 and 969 for bacteria and fungi, respectively. Overall, bacterial community composition in PRNS dune soils was dominated by the taxonomic classes Actinobacteria (19.5%), Alphaproteobacteria (19.1%), Thermoleophilia (10.3%), Bacteroidia (9.2%), Gammaproteobacteria (8.6%), Verrucomicrobiae (4.4%), Acidobacteria (4.3%), Deltaproteobacteria (4.1%), and Bacilli (3.5%). Fungal communities were dominated by Dothideomycetes (29.2%), Sordariomycetes (25.9%), Eurotiomycetes (17.9%), Agaricomycetes (9.2%), Leotiomycetes (6.9%), Tremellomycetes (4.3%), and Mortierellomycetes (1.5%).

Bacterial richness showed no significant differences among sites (Abbotts, AT&T/North Beach, B Ranch; analysis of variance [ANOVA], $df = 2$, $F = 0.06$, $p = 0.94$) or among invasion levels nested within sites (ANOVA, $df = 5$, $F = 0.54$, $p = 0.74$; Fig. 2A). Similar results were observed using Shannon's diversity index (ANOVA, $df = 2$, $F = 0.78$, $p = 0.48$ for sites; $df = 5$, $F = 0.83$, $p = 0.55$ for invasion nested within sites). Bacterial community similarity patterns did vary among sites (PERMANOVA, $df = 2$, $r^2 = 0.29$, $p < 0.001$) and by invasion levels nested within sites (PERMANOVA, $df = 5$, $r^2 = 0.38$, $p < 0.001$; Fig. 2B). Inferred methylotrophy, nitrification, denitrification, nitrate reduction, nitrogen respiration, chitinolysis, cellulolysis, dark oxidation of sulfur compounds,

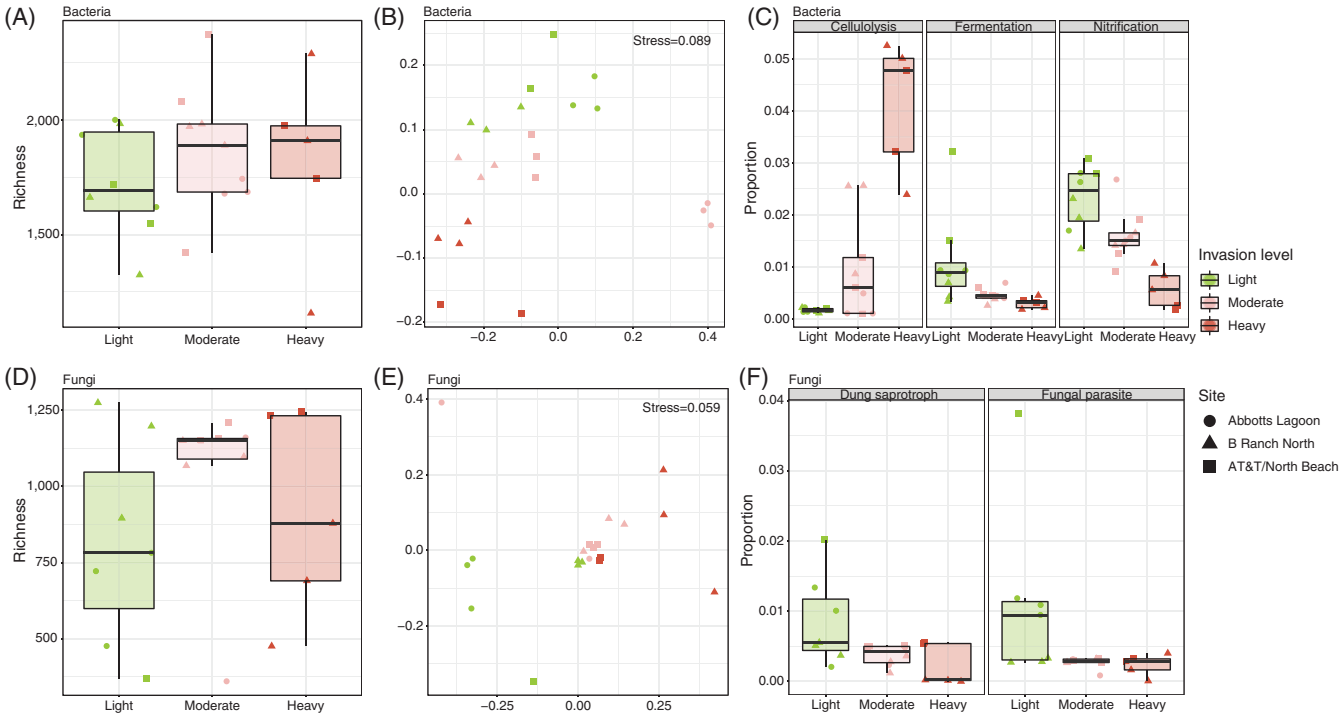


Figure 2. Observed richness (number of different phylotypes per sample) for bacterial and fungal soil communities (A, D). Similarity patterns for bacterial and fungal soil communities represented using non-metric multidimensional scaling (B, E). Significant differences ($p < 0.05$, nested ANOVA after false discovery rate correction) were found in the inferred abundance of bacterial metabolic functions and fungal functional guilds (C, F).

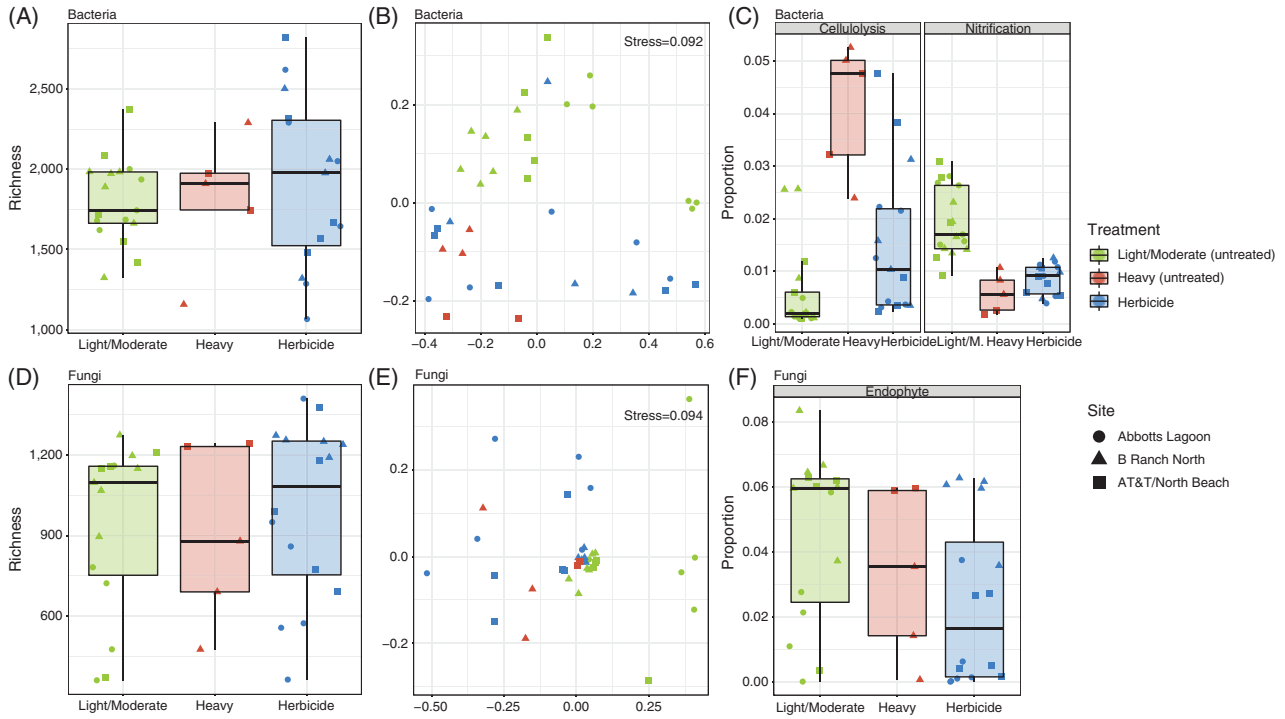


Figure 3. Observed richness (number of different phylotypes per sample) for bacterial and fungal soil communities (A, D). Similarity patterns for bacterial and fungal soil communities represented using non-metric multidimensional scaling (B, E). Significant differences ($p < 0.05$, nested ANOVA after false discovery rate correction) were found in the inferred abundance of bacterial metabolic functions and fungal functional guilds (C, F).

manganese oxidation, fermentation, and oxygenic photoautotrophy exhibited significant differences among invasion levels ($p < 0.05$ after FDR correction). The metabolic functions differing the most were cellulolysis, nitrification, and fermentation (Fig. 2C). Inferred cellulolysis showed increasing levels with increasing invasion intensity (in parallel to an increasing proportion of Alphaproteobacteria with increasing invasion; $p < 0.05$ after FDR correction), while nitrification and fermentation showed the opposite pattern (as did classes Nitrososphaeria, Nitrospira, Chloroflexia, Chthonomonadetes, and Gemmatimonadetes; $p < 0.05$ after FDR correction).

Fungal richness differed among sites (ANOVA, $df = 2$, $F = 4.22$, $p = 0.04$) and invasion levels nested within sites (ANOVA, $df = 5$, $F = 4.29$, $p = 0.02$; Fig. 2D). Similar results were observed using Shannon's diversity index (ANOVA, $df = 2$, $F = 5.72$, $p = 0.02$ for sites; $df = 5$, $F = 3.14$, $p = 0.04$ for invasion nested within sites). AT&T/North Beach samples and moderate/heavily invaded samples tended to have higher fungal diversity. Fungal community similarity patterns also varied among sites (PERMANOVA, $df = 2$, $r^2 = 0.23$, $p < 0.001$) and invasion levels nested within sites (PERMANOVA, $df = 5$, $r^2 = 0.41$, $p < 0.001$; Fig. 2E). Focusing on inferred fungal functional guilds, lightly invaded areas had significantly higher abundance of putative dung saprotrophs and fungal parasites than moderate and heavily invaded areas ($p < 0.05$ after FDR correction; Fig. 2F). This pattern was opposite to the one observed for Archaeosporomycetes ($p < 0.05$ after FDR correction).

Herbicide treatment did not affect bacterial richness, with no significant differences between formerly heavily invaded areas that had been herbicide-treated, heavily invaded untreated areas, and lightly to moderately invaded untreated areas (ANOVA, $df = 5$, $F = 0.14$, $p = 0.98$; Fig. 3A). Shannon's diversity index was also not significant (ANOVA, $df = 5$, $F = 0.43$, $p = 0.82$). Bacterial community patterns varied, though, between treatments nested within sites (PERMANOVA, $df = 5$, $r^2 = 0.24$, $p < 0.001$; Fig. 3B). Bacterial community composition of lightly to moderately invaded areas remained distinct from those of heavily invaded untreated and herbicide-treated sites, which were strongly overlapping (Fig. 3B). Inferred cellulolysis levels were the highest in heavily invaded untreated areas, while nitrification (as taxonomic classes Nitrososphaeria, Chloroflexia, and Entotheonellia; $p < 0.05$ after FDR correction) was highest in lightly to moderately invaded untreated samples ($p < 0.05$ after FDR correction; Fig. 3C). Halobacteria abundance was present and significantly highest in herbicide-treated samples and absent from all the untreated samples whether lightly, moderately, or heavily invaded ($p < 0.05$ after FDR correction).

Similarly, fungal species richness showed no significant differences among herbicide-treated areas, heavily invaded untreated areas, and lightly to moderately invaded untreated areas (ANOVA, $df = 5$, $F = 2.00$, $p = 0.11$; Fig. 3D). Shannon's diversity index was also not significant (ANOVA; $df = 5$, $F = 2.29$, $p = 0.07$). Fungal community composition varied significantly between treatments nested within sites (PERMANOVA, $df = 5$, $r^2 = 0.25$, $p < 0.001$). As with

bacterial communities, fungal composition showed separation between lightly/moderately invaded areas and herbicide-treated and heavily invaded untreated areas, which overlapped considerably (Fig. 3E). Fungal endophytes were significantly different across treatments with herbicide-treated samples showing the lowest relative abundance ($p < 0.05$ after FDR correction; Fig. 3F). At the taxonomic level, Archaeosporomycetes were significantly more abundant in heavily invaded untreated samples compared to both lightly to moderately invaded untreated and herbicide-treated samples ($p < 0.05$ after FDR correction).

Similar results were found when comparing heavily invaded areas that had been treated either by herbicide or through mechanical removal. Bacterial phylotype richness or diversity showed no significant differences between mechanically treated areas and herbicide-treated ones (ANOVA, $df = 2$, $F = 1.37$, $p = 0.32$ for richness; $df = 2$, $F = 2.69$, $p = 0.15$ for Shannon's diversity). Similarly, as with all of the other analyses, there were no observed significant differences based on dune location (foredune/backdune; ANOVA, $df = 1$, $F = 0.35$, $p = 0.58$ for richness; $df = 1$, $F = 0.63$, $p = 0.46$ for Shannon's diversity). Bacterial community composition, however, varied significantly among treatment approach nested within dune position (PERMANOVA, $df = 2$, $r^2 = 0.36$, $p = 0.013$) and dune position (PERMANOVA, $df = 1, r^2 = 0.19, p = 0.013$). Also, in contrast to bacterial results, there was a weakly significant difference in soil fungal phylotype richness/Shannon's diversity for treatment approach nested within dune position (ANOVA, $df = 2$, $F = 4.25$, $p = 0.08$ for richness; $df = 2$, $F = 6.92$, $p = 0.04$ for Shannon's diversity). There was also a stronger relationship between richness and dune position (foredune/backdune) for fungi (ANOVA, $df = 1$, $F = 9.23$, $p = 0.03$), with fungal richness generally higher for mechanical treatment and foredune areas, although dune position did not influence Shannon's diversity (ANOVA, $df = 1$, $F = 2.49$, $p = 0.18$). No significant differences were observed for fungal community composition as a function of restoration treatment or dune position (PERMANOVA, $df = 1, r^2 = 0.14, p = 0.127$ for dune position; $df = 2$, $r^2 = 0.34$, $p = 0.127$, for restoration treatment nested within dune position). In this case, we did not evaluate specific differences in taxonomic classes or inferred functions due to low sample size.

Discussion

Although plant–soil feedbacks might facilitate or inhibit the initial establishment and later success of invasive plant species (Wardle et al. 2004; Kardol & Wardle 2010), above-belowground interaction mechanisms and outcomes are complex, highly variable, and ecosystem-specific (Shannon et al. 2012; Carey et al. 2015; Bansal & Sheley 2016; Gornish et al. 2016). In this study, we assessed soil microbial communities using DNA metabarcoding in the presence of plant invaders and approaches for removing these invaders in coastal dunes. Overall, we found evidence of a strong link between soil microbial communities and the presence of invasives, invasion intensity, and restoration approach.

Our results show that soil microbial community composition of coastal dunes at PRNS varies dramatically with invasion intensity. Whether soils in currently invaded areas supported different microbial communities prior to invasion than current "native dune refugia" that increased their vulnerability to invasion or whether active planting and spread of invasives broke down initial biotic resistance and subsequently changed soil biota is unknown. The fact that islands of remnant native dune have persisted at PRNS for decades despite being surrounded by a sea of beachgrass points to some possible role of soil biota in resisting invasives when native communities are intact and not overwhelmed by anthropogenic or natural disturbance events as postulated by Suding et al. (2013). There are little data available showing how quickly biotic and abiotic conditions in native dune soils change once invaded, but, in Mediterranean island ecosystems, differences in soil chemistry and vegetation communities became apparent only 8 years after iceplant invasion (Badalamenti et al. 2016).

Heavily invaded sites were characterized by a lower abundance of nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs and a higher abundance of cellulolytic bacteria. All these soil microbial changes imply shifts in carbon and nitrogen cycling with invasion success. While the observed increase in cellulolytic bacteria is expected due to the higher plant biomass in highly invaded soils and increased volume of plant material to be broken down through cellulolysis (Kourtev et al. 2002; Badalamenti et al. 2016), reduced nitrification might be due to bacteria competing with invasive plant roots for available ammonium (Kaye & Hart 1997) and/or due to acidification (Nicol et al. 2008). The decrease in taxa associated with fermentative processes together with the increase in taxa with cellulolytic potential might indicate that carbon is being processed differently in heavily invaded sites. The increase in taxa with non-fermentative characteristics suggests an increased turnover of carbon and other nutrients to support invasive species growth.

Fungal guilds were also influenced by invasion levels. Although we did not observe significant changes in overall fungal plant pathogen or arbuscular mycorrhizal fungi (AMF) abundance, Archaeosporomycetes, a class of AMF, showed a significant increase with invasion levels. Several studies have explored the importance of AMF for native dune plants, with mycorrhizal associations enhancing phosphorus uptake, promoting sand aggradation, improving salt tolerance, and increasing nitrogen fixation in legumes (Koske & Polson 1984; Tsang & Maun 1999). In Dutch dune systems, AMF infection rates were positively associated with vigor of beachgrass stands (Kowalchuk et al. 2002), but not necessarily in areas outside its native range, with AMF infection rates showing no relationship with beachgrass biomass in Oregon dunes (David et al. 2016). Invasive plants can either arrive with AMF already present in intact root systems, or they may be mycorrhizal generalists that can take advantage of AMF species already present in their new home (Pringle et al. 2009; Vogelsang & Bever 2009; Lekberg et al. 2013; Gornish et al. 2016). Several studies have shown that invaders, once established, can increase abundance of AMF or influence abundance of particular AMF species that facilitates growth of the invader, but suppresses

that of native plant species (Pringle et al. 2009; Zhang et al. 2010). It is possible that widespread establishment of European beachgrass in Point Reyes selectively promoted expansion of Archaeosporomycetes, which may have been naturally present, but at low levels, and that higher abundance of this particular AMF had a positive feedback on beachgrass while having a negative feedback on native dune plant species.

As other studies have shown, simply removing invasive plants does not necessarily eliminate the legacy effects that these species have had on native ecosystems. Coastal scrub sites in southern California that had been restored 11 years previously with herbicide treatment of invasive Harding grass (*Phalaris aquatica*) still suffered from microbial legacy effects, with symbiotic nitrogen fixers found in plant root nodules, nitrate-reducing bacteria, and rhizophilic AMF being more abundant in post-invasive soil, while the nitrite-oxidizing bacteria, *Nitrospira*, was more abundant in uninvaded soil (Pickett et al. 2019). In our study, soil prokaryotic and fungal communities from heavily invaded untreated sites were almost indistinguishable from formerly heavily invaded sites that had been herbicide-treated anywhere from 1 to 6 years previously. With the exception of nearshore areas affected by beach sand overwash, herbicide treatment areas would be expected to have similar soil microbiota to heavily invaded untreated areas, as herbicide treatment is intended to only eradicate aboveground biomass and associated roots of target invasives. However, herbicide treatment has the potential to affect soil biota through translocation by plants of herbicides into the soil through roots and rhizomes, infiltration of herbicides into the soil due to overspray, or run-off of chemicals from sprayed plants into the soils. Some laboratory studies have shown impacts of glyphosate on soil microorganisms (Irvine et al. 2013), but field studies on glyphosate and imazapyr have been more equivocal, showing either no effect or even an enhancement of microorganisms (Busse et al. 2004; Lupwayi et al. 2004; Sederholm et al. 2018).

The strong similarity between soils from heavily invaded untreated and herbicide-treated sites would suggest that herbicide treatment did not in and of itself shift overall bacterial and fungal composition or cause any change in bacterial and fungal richness. The one exception might be that Halobacteria, a class of halophilic archaea that are extremely salt tolerant, showed the highest abundance in herbicide-treated sites while literally being absent from the rest of them, pointing to herbicide changing salinity or some degradation by-product enhancing halophilic microbe activity (Le Borgne et al. 2008). Along these lines, glyphosate and imazapyr are often used in their isopropylamine salt rather than acid forms, and it has been shown that halophilic bacteria can break down glyphosate by utilizing carbon released after the organism breaks down the carbon-phosphorus bond (Sharifi et al. 2015).

However, while herbicide may not have had a large overall direct or indirect impact on the soil community, it also did not release restored systems from some of the soil legacies left behind by beachgrass. For example, nitrifierswere as scarce in herbicide-treated sites as in heavily invaded untreated sites, suggesting legacy effects in nitrogen cycling. The decrease in nitrifiers with invasion disputes previous findings that showed how invasive plants can increase nitrification by over 50% (Hawkes et al. 2005; Liao et al. 2008). This persistent legacy may have implications for the success of restoration efforts in that, given the continued absence of nitrifiers in herbicide-treated areas, species that are capable of fixing nitrogen such as bush lupine may have a competitive advantage in colonizing these areas, which could thwart the native vegetation recovery process.

Some changes in soil microbiota did occur, however, after restoration. The abundance of cellulolytic bacteria in herbicide-treated sites dropped compared to heavily invaded untreated sites even though large volumes of undecomposed beachgrass material remained. Also, Archaeosporomycetes were more abundant in heavily invaded untreated sites than in herbicide-treated ones, reinforcing the idea that invasive plants such as European beachgrass may establish symbiotic relationships to enhance its success. By removing beachgrass, restoration may be able to reverse at least some of the changes in soil biota associated with invasion, which may have been precluding re-establishment by native species. In addition, loss of this AMF from herbicide-treated areas may shift the competitive advantage away from beachgrass in terms of re-invasion, making it easier to preclude re-establishment of this particular primary invasive.

In addition to microbial legacy effects, it is likely that restored areas must also contend with other types of legacy effects, as well. At PRNS, most of the herbicide-treatment areas (particularly in the backdunes where sand overwash from adjacent beaches is unlikely) may have also been impacted by the physical legacy effects associated with the long-lingering stands of dead European beachgrass (Parsons et al. 2019), with standing dead material decreasing space available for colonization and the slowly decomposing thick layer of "thatch" or litter material potentially suppressing plant germination and/or establishment (Xiong & Nilsson 1999). Recruitment rates of native dune plant species remained lower than those of weedy species or so-called secondary invaders, which include ripgut grass (*Bromus diandrus*), brome fescue (*Festuca bromoides*), common groundsel (*Senecio sylvestris*), and sow thistle (*Sonchus* spp.; Parsons et al. 2019). Only herbicide treatment in foredune areas has resulted in almost immediate colonization by native dune plants, with sand overwash burying the dense beachgrass stands (and soils) and providing a new substrate for colonization (Parsons et al. 2019).

In the restored backdune areas, these non-native plant species may benefit from the lingering microbial and physical legacy effects of beachgrass, as well as any potential changes that beachgrass may have had on soil organic matter, nutrient pools, and chemistry. It stands to reason that, given the very large volume of plant matter produced by dense beachgrass monocultures, this species could have a dramatic impact on organic matter content of dune soils. However, unlike iceplant, there appears to have been no studies published on how beachgrass changes soil chemistry and the persistence of these changes once beachgrass is removed. Studies on chemical legacy effects of other species suggest that changes can be persistent. After removal of iceplant from coastal dunes in Spain, soil pH, organic matter, ammonium, and nitrate/nitrite levels were still altered 12 months

after removal of iceplant from coastal dunes in Spain (Novoa et al. 2014). Similarly, areas mowed to remove bush lupine (*Lupinus arboreus*) showed no difference from still invaded ones in soil nitrogen levels 5 years after eradication (Maron & Jefferies 2001).

At Abbotts Lagoon, mechanical treatment conducted by "horizon flipping" almost instantaneously eliminated both beachgrass and seemingly any of its potential microbial, physical, or chemical legacy effects. As discussed earlier, this resulted in different soil microbial composition than herbicide-treated areas. However, while releasing restored areas from the legacy effects of beachgrass, this approach may have had its own repercussions. A soil inoculum study conducted 2 years after the mechanical restoration project was completed found that aboveground biomass of several native dune plant species grown in "flipped" soils was considerably reduced relative to that of plants grown in native dune soils and concluded that microorganisms in flipped soils could be detrimental to growth of germinated individuals (Winsemius et al. 2015). Vegetation monitoring results seemed to support this conclusion. Mechanical removal resulted in almost immediate eradication of primary invasives, but also left vast expanses of barren sand and little to no colonization by native dune plants, with cover ranging from 0.5 to 2.3% up through Year 5 post-restoration (Parsons et al. 2019). There was one notable exception: the federally endangered plant species Tidestrom's lupine (*Lupinus tidestromii*), a leguminous species capable of nitrogen fixation, quickly expanded throughout mechanical removal areas immediately after restoration (Parsons et al. 2019).

On the basis of some of these dune restoration project results, many researchers have argued that ecosystem restoration cannot be accomplished without taking additional steps to remediate some of the physical, chemical, and biological legacy effects. Habitat restoration cannot just rely on removing invasives, but may need to include "a perturbation that mitigates biogeochemical and physical effects" caused by invasives (Suding et al. 2004). These perturbations could include soil amelioration, topsoil removal, iceplant duff/litter removal, inoculation with fungal or bacterial taxa, mowing/removal of biomass, overseeding by native plant species, carbon addition, or selective removal of non-native species or species with strong biogeochemical feedbacks (Suding et al. 2004; Corbin & D'Antonio 2012; Novoa et al. 2014).

It remains to be seen whether intact, resilient native dune plant and soil microbial communities will eventually re-establish in these restored areas, with or without additional restoration measures. Not only has re-colonization by natives been very slow regardless of restoration approach, but both herbicide-treated and mechanically restored areas have been invaded by new weeds or secondary invaders that are taking advantage of the decrease in primary invader abundance and disturbance associated with restoration to gain a foothold in restored systems (Parsons et al. 2019). Ultimately, more information is needed on the persistence of legacy effects of invaders on coastal dune soils and how these effects might be most effectively countered to jumpstart dune restoration efforts and improve their success. Both aboveground and belowground interventions may be necessary to restore functional plant–soil interactions characteristic of a native ecosystem (Kardol & Wardle 2010). In all cases, vigilant, long-term re-retreatment will be critical to success of restoration efforts, as coastal ecosystems are disturbance-prone and therefore vulnerable to invasion and re-invasion events (Carboni et al. 2010). Until effective measures to remediate legacy effects are developed, land managers might best be served to focus restoration efforts on low-risk, high-yield restoration efforts such as removal of iceplant in lightly or very recently invaded areas or herbicide treatment of European beachgrass or iceplant in foredune areas where sand overwash creates a new substrate for native dune plant establishment unimpacted by lingering legacy effects. Future studies should delve more deeply into changes in soil chemistry, carbon-nitrogen cycling, and biota associated with beachgrass invasion and restoration of invaded areas, as well as evaluate potential remediation measures that might mitigate persistent legacy effects that could ultimately preclude native plant reestablishment.

LITERATURE CITED

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, et al. (2010) The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytologist 186:281–285

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46

Badalamenti E, Gristino L, Laudicina VA, Novara A, Pasta S, La Mantia T (2016) The impact of *Carpobrotus* cfr. *acinaciformis* (L.) L. bolus on soil nutrients, microbial communities structure and native plant communities in Mediterranean ecosystems. Plant and Soil 409:19–34

Bansal S, Sheley RL (2016) Annual grass invasion in sagebrush steppe: the relative importance of climate, soil properties and biotic interactions. Oecologia 181:543–557

Barberán A, McGuire KL, Wolf JA, Jones FA, Wright SJ, Turner BL, et al. (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. Ecology Letters 18:1397–1405

Barbour MG, de Jong TM, Johnson AF (1976) Synecology of beach vegetation along the Pacific coast of the United States of America: a first approximation. Journal of Biogeography 3:55–69

Barbour MG, De Jong TM, Pavlik BM (1985) Marine beach and dune plant communities. Pages 296–322. In: Physiological ecology of North American plant communities. Springer Netherlands, Dordrecht

Beckstead J, Parker IM (2003) Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? Ecology 84:2824–2831

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B 57:289–300

Breed MF, Harrison PA, Blyth C, Byrne M, Gaget V, Gellie NJC, et al. (2019) The potential of genomics for restoring ecosystems and biodiversity. Nature Review Genetics 20:615–628

Busse MD, Fiddler GO, Ratcliff AW (2004) Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. Water, Air, & Soil Pollution 152:23–34

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583

Carboni M, Santoro R, Acosta ATR (2010) Are some communities of the coastal dune zonation more susceptible to alien plant invasion? Journal of Plant Ecology 3:139–147

Carey CJ, Beman JM, Eviner VT, Malmstrom CM, Hart SC (2015) Soil microbial community structure is unaltered by plant invasion, vegetation clipping, and nitrogen fertilization in experimental semi-arid grasslands. Frontiers in Microbiology 6:466

Conser C, Connor EF (2009) Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. Biological Invasions 11:349–358

Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Science and Management 5:117–124

Dangremond EM, Pardini EA, Knight TM (2010) Apparent competition with an invasive plant hastens the extinction of an endangered lupine. Ecology 91:2261–2271

D'Antonio CM, Mahall BE (1991) Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. American Journal of Botany 78: 885–894

David AS, Seabloom EW, May G (2016) Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. Microbial Ecology 71:912–926

Durkin P (2011) Glyphosate human health and ecological risk assessment. Final report. Submitted to USDA/Forest Service, Southern Region, Atlanta, Georgia. Prepared by Syracuse Environmental Services, Manlius, New York. https://www.fs.fed.us/foresthealth/pesticide/pdfs/Glyphosate_SERA_TR-052-22-03b.pdf

Eppinga MB, Rietkerk M, Dekker S, De Ruiter P, van der Putten WH (2006) Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114:168–176

Gornish ES, Fierer N, Barberán A (2016) Associations between an invasive plant (*Taeniatherum caput-medusae*, medusahead) and soil microbial communities. PLoS One 11:e0163930

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8:976–985

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310

Inderjit, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology & Evolution 25:512–519. https://www.sciencedirect.com/science/article/abs/pii/S016953471000145X

Irvine IC, Witter MS, Brigham CA, Martiny JBH, et al. (2013) Relationships between methylobacteria and glyphosate with native and invasive plant species: implications for restoration. Restoration Ecology 21:105–113

Issa AA (1999) Interference of glyphosate with the shikimate pathway by cyanobacteria in chemostat culture. Microbios 100:47–55

Kardol P, Wardle DA (2010) How understanding aboveground–belowground linkages can assist restoration ecology. Trends in Ecology & Evolution 25:670–679

Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends in Ecology & Evolution 12:139–143

Knevel IC, Lans T, Menting FBJ, Hertling UM, van der Putten WH (2004) Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. Oecologia 141:502–510

Koske RE, Polson WR (1984) Are VA Mycorrhizae required for sand dune stabilization? Bioscience 34:420–424

Kourtev PS, Ehrenfeld JG, Häggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166

Kowalchuk GA, De Souza FA, Van Veen JA (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81:509–520

Le Borgne S, Paniagua D, Vazquez-Duhalt R (2008) Biodegradation of organic pollutants by halophilic bacteria and archaea. Journal of Molecular Microbiology and Biotechnology 15:74–92

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW (2013) Severe plant invasions can increase mycorrhizal fungal abundance and diversity. The ISME Journal 7:1424–1433

Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706–714

Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272–1277

Lupwayi NZ, Harker KN, Clayton GW, Turkington TK, Rice WA, O'Donovan JT (2004) Soil microbial biomass and diversity after herbicide application. Canadian Journal of Plant Science 84:677–685

Maron JL, Jefferies RL (2001) Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11:1088–1100

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

Miller TE (2015) Effects of disturbance on vegetation by sand accretion and erosion across coastal dune habitats on a barrier island. AoB Plants 7:plv003

Muir JJ, Colwell MA (2010) Snowy plovers select open habitats for courtship scrapes and nests. The Condor 112:507–510

National Park Service (2009) *Abbotts Lagoon area dune restoration plan environmental assessment*. https://www.nps.gov/pore/learn/management/upload/planning_dunerestoration_ea_090209.pdf

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menkea J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248

Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10:2966–2978

Novoa A, Rodríguez R, Richardson D, González L (2014) Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br. Biological Invasions 16:429–443

Parsons L, Ender C, Ryan A (2019) Coastal dune restoration vegetation monitoring report. Point Reyes National Seashore, Point Reyes Station, California. https://irma.nps.gov/DataStore/Reference/Profile/2265431

Paulson JN, Stine OC, Bravo HC, Pop M (2013) Differential abundance analysis for microbial marker-gene surveys. Nature Methods 10:1200–1202

de la Peña E, de Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H (2010) Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biological Invasions 12:3637–3648

Pickart AJ, Sawyer JO (1998) Ecology and restoration of Northern California coastal dunes. California Native Plant Society, Sacramento

Pickett B, Irvine IC, Bullock E, Arogyaswamy K, Aronson E (2019) Legacy effects of invasive grass impact soil microbes and native shrub growth. Invasive Plant Science and Management 12:22–35

Pringle A, Rillig MC, Gardes M, Klironomos J (2009) Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715

van der Putten WH, Yeates GW, Duyts H, Schreck Reis C, Karssen G (2005) Invasive plants and their escape from root herbivory: a worldwide comparison of the root feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. Biological Invasions 7:733–746

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590–D596

Sederholm MR, Schmitz BW, Barberán A, Pepper IL (2018) Effects of metam sodium fumigation on the abundance, activity, and diversity of soil bacterial communities. Applied Soil Ecology 124:27–33

Shannon S, Flory SL, Reynolds H (2012) Competitive context alters plant–soil feedback in an experimental woodland community. Oecologia 169:235–243

Sharifi Y, Pourbabaei AA, Javadi A, Abdolmohammadi MH, Saffari M, Morovvati A (2015) Biodegradation of glyphosate herbicide by *Salinicoccus* spp

isolated from Qom Hoze-Soltan Lake, Iran. Environmental Health Engineering and Management Journal 2:31–36

Soil Conservation Service (1985) *Soil survey of Marin County, California*. https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/california/marinCA1985/marinCA1985.pdf

Suding KN, Gross KL, Houseman GR (2004) Alternative states and positive feedbacks in restoration ecology. Trends in Ecology & Evolution 19: 46–53

Suding KN, Harpole WS, Fukami T, Kulmatiski A, MacDougall AS, Stein C, van der Putten WH (2013) Consequences of plant-soil feedbacks in invasion. Journal of Ecology 101:298–308

Tsang A, Maun MA (1999) Mycorrhizal fungi increase salt tolerance of *Strophostyles helvola* in coastal foredunes. Plant Ecology 144: 159–166

Van Grunsven RHA, Bos F, Ripley BS, Suehs CM, Veenendaal EM (2009) Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean. South African Journal of Botany 75:172–175

Vilà M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, et al. (2006) Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. Journal of Biogeography 33:853–861

Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90:399–407

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73: 5261–5267

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633

van der Watt E, Pretorius JC (2001) Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Journal of Ethnopharmacology 76:87–91

Weber E (2003) Invasive plant species of the world: a reference guide to environmental weeds. CAB International Publishing, Wallingford, United Kingdom

Wiedemann AM, Pickart A (1996) The *Ammophila* problem on the northwest coast of North America. Landscape and Urban Planning 34: 287–299

Wiedemann AM, Pickart AJ (2008) Temperate zone coastal dunes. Pages 53–65. In: Martinez M, Psuty N (eds) Coastal dunes: ecology and conservation. Springer, Heidelberg, Germany

Winsemius S, Stein C, Parsons L, Minnick S, Ryan A, Suding K (2015) Plant-soil interactions and implications for restoration of coastal sand dunes at Point Reyes National Seashore. Poster. California Native Plant Society Conference. San Jose, California

Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. Bioscience 55:477–487

Xiong S, Nilsson C (1999) The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology 87:984–994

Zhang Q, Yang R, Tang J, Yang H, Hu S, Chen X (2010) Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. PLoS One 5:e12380

Zinger L, Bonin A, Alsos IG, Bálint M, Bik H, Boyer F, et al. (2019) DNA metabarcoding—need for robust experimental designs to draw sound ecological conclusions. Molecular Ecology 28:1857–1862

Guest Coordinating Editor: Martin Breed

Received: 3 August, 2019; First decision: 20 September, 2019; Revised: 8 October, 2019; Accepted: 10 December, 2019

APPENDIX F. NPS UNPUBLISHED MEMO SUBMITTED TO POINT REYES NATIONAL SEASHORE ASSOCIATION: *"PRELIMINARY RESULTS OF PLANT AND SOIL CHEMISTRY ANALYSES FROM NATIVE, UNRESTORED INVADED, AND RESTORED COASTAL DUNE COMMUNITIES AT POINT REYES NATIONAL SEASHORE"*

PRELIMINARY RESULTS OF PLANT CHEMISTRY ANALYSES FROM NATIVE, UNRESTORED INVADED, AND RESTORED COASTAL DUNE COMMUNITIES AT POINT REYES NATIONAL SEASHORE

GRANT REPORT TO POINT REYES NATIONAL SEASHORE ASSOCIATION

LORRAINE PARSONS
VEGETATION/WETLAND ECOLOGIST
POINT REYES NATIONAL SEASHORE

APRIL 21, 2020

INTRODUCTION

Point Reyes National Seashore (Seashore) contains some of the highest quality remaining coastal dune habitat in the nation. Park legislation mandates protection of diminishing coastline and specifically calls for the preservation of declining coastal dune habitat. This habitat, however, has been seriously threatened by the encroachment of European beachgrass and iceplant since being planted in the early 1900s. As of the late 1990s, these species accounted for 60% of the Seashore's 2,200 acres of coastal bluff, dune, and scrub habitat. Concerned about the impact on native dune habitats and listed plant and wildlife species, the park embarked in 2001 on an ambitious program of coastal dune restoration. Since then, the park has removed 271 acres of invasive European beachgrass and iceplant (*Carpobrotus edulis*) from 525 acres of dune habitat at Abbotts Lagoon, AT&T, and B Ranch North.

While re-treatment was anticipated during project development, park managers did not anticipate how strong climatic variation – years of drought followed by two years of moderate to heavy rainfall – would not only finally cause a resurgence in cover of native plant species, but also promote establishment of new weeds or secondary invaders such as European searocket (*Cakile maritima*), New Zealand spinach *(Tetragonia tetragonioides*), European black nightshade (*Solanum nigrum*), iceplant (*Carpobrotus edulis*), and bush lupine (*Lupinus arboreus*, the latter of which is native to California, but probably not to Marin County). This is in addition to non-native annual species such as ripgut brome (*Bromus diandrus*), fescue brome (*Festuca bromoides*), common groundsel (*Senecio sylvestris*), and sow thistle (*Sonchus* spp.). Spread of secondary invaders appears strongly tied to several factors, including available space freed up by eradication of primary invaders, landscape disturbance associated with restoration (both mechanical removal and herbicide treatment), short-and long-term climatic cycles, and potentially increases in soil nitrogen associated with breakdown of dead beachgrass and colonization by the disturbance-adapted, nitrogen-fixing bush lupine. For example, at Abbotts, two secondary invaders are often found in close association with either live or dead bush lupine (New Zealand spinach and European black nightshade), suggesting that these new weeds are benefitting from nitrogen supplementation of soils by lupine.

The Seashore has been working with researchers to identify potential interactions between soil chemistry and soil microbial and other "legacy effects" that might be negatively influencing

restoration by substantially delaying decomposition of beachgrass; limiting recruitment of native plant species; and encouraging invasion by secondary weeds or even re-invasion by primary ones. Results of a collaborative study by the Seashore with University of Arizona and University of California Davis suggest that invasion and the intensity of invasion changes the soil microbial community relative to native dune areas and that restoration alleviates some – but not all – of these changes (Parsons et al. 2019). Heavily invaded sites were characterized by a lower abundance of putatively identified nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs and a higher abundance of cellulolytic bacteria and a class of arbuscular mycorrhizal fungi (Archaeosporomycetes; Parsons et al. 2019). Changes in soil microbiota did not fully dissipate following removal of invasives using herbicide, with exception of reductions in cellulolytic bacteria and Archaeosporomycetes abundance (Parsons et al. 2019). Abundance of fermentative and nitrifying bacteria, the latter of which converts ammonium to nitrates, remained low in herbicide-treated dunes and similar to that of highly unrestored areas still invaded by European beachgrass, even in those herbicide-treated areas treated six (6) years ago (Parsons et al. 2019). Mechanically restored dunes had a very different microbial community than herbicide-treated dunes, which may have resulted from the fact that this restoration process involved deeply burying invaded beachgrass surface soils and covering with subsurface soils (Parsons et al. 2019).

Ironically, while there has been considerable research on the legacy effects of iceplant invasion on soil chemistry, there has been little to no research on legacy effects of European beachgrass, chemical or otherwise. Researchers in Humboldt have referenced the fact that European beachgrass artificially elevates dune height, which persists unless it is mechanically treated, and that this could be considered a "physical legacy" effect of taller foredune systems than would have existed naturally (Wiedemann and Pickart 1996). Another physical legacy effect that we have observed is that, since herbicide treatment efforts were started in 2011, European beachgrass stands treated chemically decompose very slowly, leaving huge monocultures of standing dead biomass for three (3) years or more. An analysis conducted showed that, on average, it takes 2.5 – 3 years for standing dead biomass cover to reach 50% of the initial pre-restoration cover of beachgrass (Parsons et al. 2020). Even after eight (8) years post-treatment, standing beachgrass clumps still remain plentiful, along with extensive thatch or thick litter from recently broken down plants (70% cover; Parsons et al. 2020). European beachgrass is not the only species that appears to be decomposing slowly: dead shrubs such as bush lupine also persist for years after being incidentally targeted by chemical spray (beachgrass or iceplant can occur intermixed with this and other shrub species) or being cut manually.

According to Facelli and Pickett (1991), dead plant organs of some herbaceous species and most perennial grasses remain standing upright for some time after plant death, with members of the Poaceae family having among slowest decomposition rate for herbaceous species (Cornelissen 1996). In a study comparing litter decomposition dynamics in U.S. East Coast marshes between the native saltmeadow cordgrass (*Spartina patens*) and the non-native invasive, common reed (*Phragmites australis*), standing dead biomass accounted for 65% of the total litter pool for common reed, while litter represented the highest fraction for cordgrass (95%; Windham 2001). After 390 days, 79% of common reed litter remained versus 57% for cordgrass (Windham 2001). Culms of common reed may remain standing for four (4) years before entering surface detrital pool (Haslam 1972 *in* Windham 2001). Windham (2001) calculated that, to reach 10% of its

original ash-free dry weight, cordgrass would need four (4) years, while common reed would require nine (9) years.

Slower decomposition of invasives relative to native counterparts may be the exception more than the rule. Many studies have found that invasives decompose more quickly than natives, which may greatly alter ecosystem nutrient cycling through rapid turnover of nitrogen (N)-rich, labile litter that dramatically boosts mineralization rates and inorganic N pulses (Liao et al. 2008). A meta-analysis study on effects of invasive plants on soil chemistry and litter dynamics in forest, grassland, and wetland ecosystems that incorporated data from 94 experimental studies found that invasion increased litter decomposition rates by 117% (Liao et al. 2008). However, other researchers have countered that decomposition rates of invasives are not necessarily faster than that of natives or, even if they are, do not necessarily alter nutrient cycling (Kueffer et al. 2008, Jo et al. 2016, Prescott and Zukswert 2016). As with common reed, for some invasives, decomposition may actually proceed more slowly than natives', particularly when lignin content of invasives is comparatively higher or in nutrient-poor ecosystems (Kueffer et al. 2008, Godoy et al. 2010).

Buildup of undecomposed standing and litter biomass has both direct and indirect effects on establishment of native dune plant species. The necromass prevents light from reaching the soil surface and triggering germination and plant growth (Facelli and Pickett 1991). Depending on the density of dead culms or thickness of the litter, it may also act as a physical barrier that impedes target plant establishment (Facelli and Pickett 1991). While potential allelopathic characteristics of litter have received a lot of attention, Facilli and Picket (1991) argued that these effects are likely to be temporary at most. A meta-analysis of 35 studies on the effect of litter on plant community dynamics found that negative effects outweighed the potential positive (e.g., litter helps to retain soil moisture) and that species richness and germination were more affected by litter than plant biomass and establishment (Xiong and Nilsson 1999).

Decomposition rates of necromass can be highly influenced by external factors such as climate, latitude, and invertebrate and microbial communities, but they are also strongly driven by plant chemistry. Some plant species are more resistant to being broken down and, therefore, could naturally take longer to decompose. For example, species with a high carbon-nitrogen (C:N) ratio tend to decompose more slowly than those with lower ratios, with ratios between 20:1 to 30:1 often being considered optimal for microbial breakdown. N in plant materials with ratios lower than this tends to be mineralized, whereas nitrogen is immobilized in plant materials with higher ratios (Liu and Sun 2013). Many woody plant species, for example, have higher C:N ratios and, therefore, naturally decompose more slowly. For instance, forest-leaf litter has a C:N ratio of 58:1–88:1 (McGroddy et al. 2004).

Interestingly, no published information on C:N ratios or chemical composition of European beachgrass could be found in the scientific literature. For this reason, we elected to conduct a preliminary study on plant chemistry of European beachgrass at PRNS to determine if plant chemistry could be influencing decomposition rates. We sampled naturally recently senesced plant material, as well as undecomposed material litter from herbicide-treated plants of varying treatment ages (~1 year, 3 years, and 8 years).

MATERIALS AND METHODS

Seashore staff sampled in 2019 dead European beachgrass leaves or litter of differing treatment status/ages (naturally senesced -- dead <2 mos and herbicide-treated <1 yr ago; herbicide-treated ~3 yrs ago; herbicide-treated ~7-8 yrs ago). Naturally senesced material (n=4) was collected from several locations along the Seashore's Great Beach (AT&T, South Beach, and Davis), with AT&T and Davis representing the northern and southern ends. Herbicide-treated material was collected at AT&T South (<1 year since treatment; n=3); AT&T North (~3 years since treatment, n=3); and Abbotts Lagoon (~7- 8 years since treatment; n=3). For comparison purposes, the Seashore staff also sampled recently senesced bush lupine material (n=1); remnant bush lupine material from manual cutting 2 years prior (n=1); and plant material indirectly sprayed with herbicide more than 3 years prior (n=1).

Collected plant materials were sent to two different laboratories: University of California at Davis (UCD; Davis, CA) and Cumberland Valley Analytical Sciences (CVAS; Waynesboro, PA). We shipped senesced leaves to UCD for analysis of carbon (combustion coupled with thermal conductivity/IR detection; % dry matter (DM)), nitrogen (combustion coupled with thermal conductivity/IR detection; %DM), silicon (Si; ICP AES; %DM), and total phenol (Folin-Ciocalteu colorimetric assay; mg gallic acid equivalents per gram of sample (mg GAE g-1)). As costs of these analyses were prohibitive, older (3-year, 8-year) and bush lupine samples were not sent to UCD for analyses of carbon, nitrogen, lignin, and silicon, only naturally, recently senesced (n=4) and herbicide-treated <1 year ago for European beachgrass (n=3). However, we collected beachgrass material for total phenols from each of the four treatment groups in 2020 (n=1 for each of the four treatment groups).

A portion of the collected material was sent to CVAS for analysis of lignin (basic Near Infrared Analysis (NIR); % DM); acid-digestible fiber (ADF; NIR: % DM); and amylase neutral digestible fiber (aNDF; NIR; %DM). The bush lupine material sent to CVAS included both leaves and stems.

We calculated the C:N ratio of beachgrass by dividing % C by %N, with cellulose percentages of both beachgrass and lupine estimated by subtracting lignin from %ADF.

Beachgrass data were analyzed using either a linear method permutation procedure (lmPerm package; Wheeler and Torchiano 2016) or a non-parametric Wilcoxon ranked sums test in the statistical analysis program "R" version 4.0.2.

RESULTS AND DISCUSSION

C:N ratios of European beachgrass that had naturally senesced within the past two months ranged from 81.2:1 to 122.1:1, averaging 84.7:1 (± 26.4:1; SD), based on laboratory results from UCD (Table 1). The average C:N ratio of European beachgrass at the northern end of the Great Beach, AT&T, averaged 70.8:1 (± 14.6:1; SD). In comparison, bush lupine, a semi-woody shrub that occurs in both dunes and adjacent grasslands, averaged a C:N ratio of 47.74:1 at a coastal prairie site adjacent to dunes in Bodega, which is located just north of Point Reyes (Bastow et al. 2008). Non-native annual grasses occurring at this prairie site in Bodega had very similar C:N

ratios (47.36:1; Bastow et al. 2008). European beachgrass is perhaps more similar to common reed in that both are large, perennial grass species that establish dense stands. Common reed growing in East Coast marshes had C:N ratios that were almost twice as high (63:1) as those for the native cordgrass (35:1), a much smaller grass species (Windham 2001).

The C:N ratio for European beachgrass would suggest that decomposition might be delayed, because microbes would need to scavenge enough N from soils to bring C:N ratios of European beachgrass down to ratios more favorable for bacterial activity (20:1 to 30:1). Mineralization of annual grasses at Bodega dunes occurred within a year (Bastow et al. 2008), but soils in these dunes were comparatively N-enhanced due to high cover of N-fixing bush lupine. Even in these N-subsidized soils, mineralization of co-occurring bush lupine at Bodega, which had a similar C:N ratio (47.74:1), still took almost 3 years, probably due to the higher lignin content (14.7%; Bastow et al. 2008). While native cordgrass in East Coast marshes had higher rates of N incorporation, annual rates of N immobilization were almost 300% higher for the invasive common reed, probably because of the greater amount of recalcitrant litter, its slower deposition rate, and its ability to retain exogenous N from more than one year (Windham 2001, Windham and Ehrenfeld 2003). Immobilization typically results in a N depression period in soils, severely limiting plant uptake until mineralization starts to recur. However, simultaneous breakdown of buried organic matter in common reed marshes appeared to offset greater immobilization in litter, with 300% greater mineralization rates in common reed soils than cordgrass ones, leading to no dramatic changes in the net nitrogen budget (Windham and Ehrenfeld 2003). Both of these systems were likely higher in organic and inorganic N than those that don't support large numbers of N-fixing species or a large volume of buried organic matter due to oxygen limitations on decomposition. With nitrogen content of coastal dune soils typically very low (Willis and Yemm 1961, Holton and Johnson 1979, Barbour et al. 1985, Holton et al. 1991), immobilization of N in beachgrass plant litter would be expected to persist for some time and cause delays in decomposition, as has been documented in other nutrient-poor ecosystems (Kueffer et al. 2008, Godoy et al. 2010).

C:N ratios are not the only factor driving decomposition rates. Some plant species contain higher percentages of more recalcitrant compounds. Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starches, and some of the hemicellulases are decomposed first at rapid rate, followed by proteins at a somewhat slower rate, while insoluble compounds such as hemicellulose, cellulose, and phenols, which form a large portion of organic matter, are decomposed much more slowly (Khatoon et al. 2017). Phenols such as lignin is what makes woody plants "woody," but even non-woody plants such as grasses can contain lignin. In general, plant biomass averages 10%–30% of lignin (Khatoon et al. 2017), with grasses often at the lower end of that scale (Lozano 2015). Our recently senesced, non-herbicide-treated European beachgrass plant material averaged 8.8 (± 1.3; SD) lignin for PRNS (Table 1) and 9.7 (± 0.9; SD) for AT&T only, so that falls well within range of these other grasses. Recently senesced bush lupine from PRNS had lignin content totaling 14.1% DM, which agrees well with the reported lignin content of bush lupine at Bodega (14.7%; Bastow et al. 2008).

In addition to lignins, phenols can include other recalcitrant compounds such as tannins and other secondary metabolites protection against insects, herbivores and pathogens (biotic) and/or to ensure survival under abiotic stresses (Gebashe et al. 2020). In a study on total phenolic

content of South African medicinal grasses, Gebashe et al. (2020) found that total soluble phenolic content ranged from 4.2 to 30.9 mg GAE/g DW. Total phenolic content of our recently, naturally senesced beachgrass material was 2.0 mg GAE/g DM (Table 1).

Plants also assimilate silicon from their environment to produce silica, which plays many important roles for plants, including protection against herbivory and the stresses of saline environments. Cornelissen and Thompson (1997) attributed at least some of the slow decomposition rates in graminoids to high Silicon (Si) content. The percentage of Si in plants can range from 0.1% to as high as or even higher than 10% DM, with dryland grasses such as oats and ryes having values around 1% and the "wetland" grass such as paddy-grown rice having values of 5% or higher (Epstein 1994). Silicon in recently senesced beachgrass from the park ranged from 0.70 to 1.19% DM, averaging 0.91 (± 0.20; SD)% DM, based on UCD lab results (Table 1). Silicon content of plants at AT&T appeared slightly lower (0.80 ± 0.13; SD)% DM.

Other compounds that can affect decomposition rates are cellulose and hemicellulose. Average composition for dry matter (DM) of plants are 35–50% of cellulose and 20–35% of hemicellulose (Wyman 1996). Another study provided a slightly different range for grasses of 25–40% cellulose and 25–50% of hemicellulose (Wongwatanapaiboon et al. 2012). Our recently senesced beachgrass material falls solidly within these ranges: cellulose was estimated at 35.8 (±1.9 SD)% DM, and hemicellulose was estimated at 32.5 (± 3.1; SD)% DM, respectively (Table 1). Means for recently senesced plants at AT&T were slightly different, totaling 37.1 (±2.1 SD)% DM and 34.3 (± 2.8; SD)% DM for cellulose and hemicellulose, respectively. In comparison, recently senesced bush lupine at PRNS had higher cellulose, but seemingly lower hemicellulose, content than beachgrass, totaling 41.1 and 16.3% DM, respectively.

In grasses, hemicellulose and cellulose are combined into complex composites with other compounds such as polysaccharides, phenolics (monomers and polymers), and protein through a variety of chemical linkages to provide structural support, resist water loss, and protect against mechanical stress (Bhatia et al. 2017). Lignocellulosic biomass accounts for ~60%–80% of dry matter yields in grasses and is primarily composed of secondary cell walls comprised mainly of cellulose (~25%–55%), hemicellulose (~20%–50%), and lignin (~10%–35%; Marriott et al. 2015, Vogel 2008). The analyte, Neutral Detergent Fiber (aNDF or amylase-treated NDF), represents proportions of cell-wall fractions, as it determines forage value of grasses, with higher aNDF being less digestible. The aNDF of beachgrass at PRNS averaged 79.0 (±2.1 SD)% DM (Table 1).

Based on these laboratory results, with the exception of C:N, plant chemistry does not appear to entirely explain the issue with delays in decomposition of beachgrass within herbicide-treated stands. Yet results point to delays in microbially- and physically-mediated processing of dead material. Not even the favorable degradation conditions present at PRNS such as year-round temperate climate, high winds, high air moisture content, and strong solar radiation cycles that are associated with Point Reyes' heavily marine-influenced environment appear to be able to hasten decomposition. Physical and chemical degradation, including photodegradation, often plays an important role in breakdown of dead plants in more arid ecosystems such as deserts (Facelli and Pickett 1991).

Over time, the percentage of some of these more recalcitrant compounds would be expected to decline as physical factors and the microbial community slowly work at breaking them down. However, in many instances, the percentage of these compounds are actually increasing. Over time, dead plant material loses moisture. This is reflected in the percentage of dry matter increasing, at least negligibly, from 85.5 (± 2.4; S.D.) in recently, naturally senesced beachgrass material (Year 0) to 91.1 (± 1.7; S.D.) in beachgrass material treated by herbicide eight (8) years prior (Year 8; LmPerm, df=3, P=0.005, Tukey HSD P=0.01). Based on a comparison between recently senesced and 1-year-old herbicide-treated beachgrass material (Year 1), there was a slight loss in N (3%), but a slight increase in C (1%), with the resulting C:N ratio climbing 3% during the first year (Table 1). All of these changes were statistically insignificant (Wilcoxon rank sum test, all P>0.86). Small sample size undoubtedly compromised power of our analyses.

The percentage of hemicellulose, one of the more easily degradable carbohydrate compounds, remained roughly similar between Year 0 and beachgrass material treated with herbicide three (3) years prior (Year 3; 0% change), but seemingly dropped in Year 8 material (6%; Table 1; Figure 1), although that change was not statistically significant (LmPerm, df=3, P=0.76). Conversely, the percentage of cellulose, which resists breakdown more than hemicellulose, climbed from Year 0 through Year 3 (8%; (LmPerm, df=3, P=0.06; Tukey HSD, P=0.08), and then plateaued seemingly between Year 3 and Year 8 (6%; Tukey HSD, P=0.93; Table 1; Figure 1). Cell-wall fractions (aNDF) followed a very similar pattern to cellulose, increasing through Year 3 (6%) and then leveling off or falling slightly in Year 8 (4%; Table 1; Figure 1), although differences were not statistically significant (LmPerm, df=3, P=0.12). Again, power of our analyses were impacted by small sample sizes.

Some of the largest percentage increases were recorded with lignin and total phenols. Lignin climbed from 8.8 (± 1.3; SD)% DM for recently senesced beachgrass to 11.5% (± 0.3 DM; S.D.) for beachgrass herbicide treated eight (8) years ago, a 30% increase (LmPerm, df=3; P=0.02; Tukey HSD, P=0.03; Table 1; Figure 1). While we did not have replication for phenol data, phenols appeared to potentially increase even more steeply to 10% by Year 3 and 70% by Year 8 (Table 1). Similarly, bush lupine from PRNS appeared to show an increase in lignin content from 14.1% DM in Year 0 to 29.5% DM in Year 3.

Ultimately, plant chemistry results suggest that, in beachgrass at PRNS, breakdown of more labile compounds such as N or hemicellulose took place starting in Years 1-3, while less labile compounds such as cellulose and cell wall fractions didn't begin to decompose until sometime between Years 3 and 8. Breakdown of the most recalcitrant compounds, lignin and phenols, did not appear to have begun even by Year 8.

One interesting finding from the soil microbial study that might bear on this phenomenon is that, as noted earlier, cellulolytic bacteria dropped considerably after herbicide treatment was performed (Parsons et al. 2019). As their name implies, cellulolytic bacteria and fungi are responsible for breakdown of cellulose and hemicellulose. While we originally interpreted this change in cellulolytic as a positive development, as it improved the restored area's comparability with native dune areas and suggested that some of the legacy effects associated with invasion could be reversed, it may have actually hindered the decomposer community's ability to process such an extremely large volume of dead material. The reason for this dramatic decline in

cellulolytic bacteria is not known. Abundance of cellulolytic bacteria may have dropped due to dramatic reduction in live beachgrass material following herbicide treatment, or it may have been affected by the herbicide treatment itself.

The effects of herbicides on the microbial community and on decomposition of cellulose in particular has received considerable scientific attention, with studies coming to very different conclusions. Microorganisms, like higher plants, use the shikimate pathway to produce aromatic amino acids, so as glyphosate inhibits this pathway, it is potentially toxic to microorganisms (Cox 2002, Issa 1999 *in* SERA 2011). In general, the effect of herbicides on cellulolytic bacteria and fungi appears to depend on the chemical used, the rate of application, number of repeat treatments, and soil type, with microbial communities either inhibited or even stimulated by herbicides (Ismail and Yap 1994, Ismail and Wong 1994, Ismail et al. 1995, Busse et al. 2001, Pampulha et al. 2007, Schellenberger et al. 2012, Nguyen et al. 2018). Even inhibitory effects have been characterized as relatively short-term due to either binding or sorption of herbicide with soil or rapid microbial degradation of herbicide, as microbial organisms often use herbicide as a source of carbon (Ismail and Wong 1994, Haney et al. 2000, Busse et al. 2001, AMEC Geomatrix 2009, SERA 2011). While most studies conducted focused on different herbicides than were used in these projects (glyphosate and imazapyr), some laboratory studies have shown both inhibitory and stimulatory impacts of glyphosate on soil microorganisms (Grossbard and Wingfield 1978, Busse et al. 2001, Irvine et al. 2013).

However, field studies have been harder to interpret. One study found reduced cellulose decomposition in peat soils treated with glyphosate, while decomposition rates in sandy clay loam were actually higher (Ismail et al. 1995). Meanwhile, another study evaluating effects of glyphosate and one of its brand name products (Roundup CT), actually reported contradictory findings depending on the number of application treatments. A single treatment with glyphosate, but not Roundup CT, reduced decomposition rates for 27 days in clay-based Vertosols (Nguyen 2018). However, repeat treatments within a single season of both glyphosate and/or Roundup CT led to lower cellulose decomposition rates, as well as more change in bacterial community structure, in light-textured, low organic matter soils relative to clay and loam soils (Nguyen 2018). In this study, abundance of four (4) Acidobacteria members were reduced in light-textured soils: Acidobacteria species play a key role in soil biogeochemistry, particularly cellulose degradation (Nguyen 2018). Another study found that imazapyr, the other chemical used in our projects, inhibited cellulose degradation in peat soils (Ismail and Wong 1994), although these effects may level off over time as imazapyr binds to the soils (AMEC Geomatrix 2009). Longer term impacts of imazapyr on these functional microbial groups may occur in more alkaline soils or soils with low organic matter (AMEC Geomatrix 2009). Glyphosate treatment has been associated with reduced plant decomposition for crops such as black oats (Damien et al. 2012) and broad beans, but increased decomposition of wheat (Abdel-Mallek et al. 1994). Ultimately, Nguyen postulated that changes in decomposition rates may have more to do with changes of the C:N ratio within the plant detritus generated by glyphosate-induced senescence than necessarily with the use of herbicides themselves (Nguyen 2018).

The significant delay in decomposition of European beachgrass standing dead biomass and litter has not only hindered reestablishment of native dune plant species, but it may also be encouraging colonization by new or secondary invaders. It may be that the soil chemistry or the

soil microbial community is more conducive to some of these disturbance-adapted species, many of which flourish under a high nitrogen condition. As noted earlier, interestingly, we were unable to find any scientific papers that delved into chemical legacy effects of beachgrass, unlike iceplant, which has a very well-documented effect on invaded dunes by reducing soil pH and available water and increasing organic matter, nitrogen, carbon and sodium levels (D'Antonio and Mahall 1991, Conser and Connor 2009, Novoa et al. 2014).

Ultimately, the Seashore hopes to better understand the dynamics of these restored communities relative to native ones such as Dune Mat and Dune Scrub, so that effectiveness of restoration efforts can greatly be improved. The next steps are to better define soil chemistry of native, restored, and unrestored invaded areas throughout the park during the active growing season and to further evaluate differences in microbial communities – particularly decomposer communities – that might be limiting turnover of dead European beachgrass and perhaps even promoting establishment of other weeds or non-native plant species over that of native plant species. It would be invaluable to look at differences between decomposer and other components of the microbial communities between Herbicide-treated backdunes and its ecological target community, Dune Scrub, as this was not evaluated in the earlier study (Parsons et al. 2019). Unlike native Dune Mat communities, which are characterized by a rather sparse cover of annual and perennial herbs and forbs, native Dune Scrub communities are characterized by a dense cover of shrubs and subshrubs. It would stand to reason that Dune Scrub may play host to a thriving decomposer community capable of breaking down the woody and dense herbaceous detritus generated during normal plant turnover. It would also be good to continue tease apart differences between the Unrestored invaded communities and Herbicide-treated backdunes to better understand whether restoration using herbicide can overcome some of the microbial and chemical legacies imparted by years of invasion by beachgrass and other species such as iceplant.

Citations

Abdel-Mallek AY, MA Abdel-Kader, AMA Shonkeir. 1994. Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. Microbiological Research. 149: 69-73.

AMEC Geomatrix. 2009. Human Health and Ecological Effects Risk Assessment. Imazapyr. Washington State. Submitted to the Washington Department of Agriculture. Olympia, WA.

Bastow JL, EL Preisser, DR Strong. 2008. Wood decomposition following a perennial lupine die-off: A 3-year litterbag study. Ecosystems. 11: 442–453
DOI: 10.1007/s10021-008-9132-5

Bhatia R, JA Gallagher, JD Gomez, M Bosch. 2017. Genetic engineering of grass cell wall polysaccharides for biorefining. Plant Biotechnology Journal. https://doi.org/10.1111/pbi.12764

Busse MD, AW Ratcliff, CJ Shestak, RF Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol. Biochem. 33: 1777-1789.

Conser C, EF Connor. 2009. Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. Biological Invasions 11:349–358

Cornelissen JHC. 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. Journal of Ecology 84: 573-582

D'Antonio CM, BE Mahall. 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. American Journal of Botany 78:885–894

Damin V, PCO Trivelin, TdG Barbosa, SJPd Carvalho, MF Moraes. 2012. Mineralization and corn recovery of ^{15}Nitrogen from Black Oats residues treated with herbicides. Plant Nutrition 35: 1830-1842.

Epstein E. 1994. The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci. USA Vol. 91, pp. 11-17, January 1994.
Facelli, JM, STA Pickett. 1991. Plant litter: Its dynamics and effects on plant community structure. The Botanical Review 57(1):1-32. DOI: 10.1007/BF02858763

Godoy O, P Castro-Díez, RSP Van Logtestijn, JHC Cornelissen, F Valladares. 2010. Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: a broad phylogenetic comparison. Oecologia 162:781–790 https://doi.org/10.1007/s00442-009-1512-9

Grossbard E., GI Wingfield. 1978. Effects of paraquat, aminotriazole and glyphosate on cellulose decomposition. Weed Research. 18(6): https://doi.org/10.1111/j.1365-3180.1978.tb01172.x

Haney RL, SA Senseman, FM Hons, DA Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science. 48 (1):89-93. DOI:https://doi.org/10.1614/00431745(2000)048[0089:EOGOSM]2.0.CO;2

Haslam SM. 1972. Biological flora of the British Isles no. 128: *Phragmites communis* Trin. Journal of Ecology 60:585–610.

Haynes RJ. 1996. Nitrification. *In*: Haynes, R.J. (Ed.), Mineral Nitrogen in the Plant–Soil System. Academic Press, New York, USA, pp. 127–165

Irvine IC, MS Witter, CA Brigham, JBH Martiny. 2013. Relationships between Methylobacteria and Glyphosate with Native and Invasive Plant Species: Implications for Restoration. .

Ismail BS, HP Wong. 1994. Effects of herbicides on cellulolytic activity in peat soil. Microbios. 78(315):117-123.

Ismail BS, MY Yap. 1994. Effects of two acetanilide herbicides on microbial populations and their cellulolytic activities. Bulletin of Environmental Contamination and Toxicology. 52:61–68. https://doi.org/10.1007/BF00197358.

Ismail BS, AJ Kader, O Omar. 1995. Effects of glyphosate on cellulose decomposition in two soils. Folia Microbiologica. 40(5): 499–502.

Jo I, JD Fridley, DA Frank. 2016. More of the same? In situ leaf and root decomposition rates do not vary between 80 native and nonnative deciduous forest species. New Phytologist 209:115–122 doi: 10.1111/nph.13619

Khatoon H, P Solanki, M Narayan, L Tewari, JPN Rai. 2017. Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. International Journal of Chemical Studies. 5(6): 1648-1656

Kueffer C, G Klingler, K Zirfass, E Schumacher, PJ Edwards, S Güsewell. 2008. Invasive trees show only weak potential to impact nutrient dynamics in phosphorus-poor tropical forests in the Seychelles. Functional Ecology 22:359–366 doi: 10.1111/j.1365-2435.2007.01373.x

Liao C, R Peng, Y Luo, X Zhou, X Wu, C Fang, J Chen, B Li. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706–714

Liu C, X Sun. 2013. A Review of Ecological Stoichiometry: Basic Knowledge and Advances *in* Reference Module in Earth Systems and Environmental Sciences.

Lozano RR. 2015. Silica and lignin in grasses. *In*: Grass Nutrition. Edited by R. Ramirez Lozano. pp. 57-66. Palibrio Press, Bloomington, Indiana USA. ISBN: 978-1-5065-0808-5

Maier RM. 2009. Biogeochemical Cycling *in* Environmental Microbiology. Second Edition. 624 pp.

Marriott PE, LD Gómez, SJ McQueen-Mason. 2015. Unlocking the potential of lignocellulosic biomass through plant science. New Phytol. 209, 1366– 1381.

McGroddy, ME, T Daufresne, LO Hedin. 2004. Scaling of C:N:P stoichiometry in forests worldwide: Implications of terrestrial Redfield-type ratios. Ecology, 85(9), 2004, pp. 2390–2401.

Nguyen, BD. 2018. Impacts of glyphosate and its formulation on soil microbial function. Dissertation thesis. Southern Cross University, Australia.

Novoa AR, D Rodríguez, D Richardson, L González. 2014. Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br. Biological Invasions 16:429–443

Pampulha ME, MASS Ferreira, A Oliveira. 2007. Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. Journal of Basic Microbiology. 47:325–331

Parsons LS, J Sayre, C Ender, JL Mazza Rodrigues, A Barberán. 2019. Soil microbial communities in restored and unrestored coastal dune ecosystems in California. Restoration

Ecology. Published web location. https://doi.org/10.1111/rec.13101

Parsons LS, M Spaeth, C Ender. 2020. Coastal Dune Restoration Vegetation Monitoring Report. Point Reyes National Seashore. Point Reyes Station, CA, USA. 128 pp.

Pitts WD, MG Barbour. 1979. The microdistribution and feeding preferences of *Peromyscus maniculatus* in the strand at Point Reyes National Seashore, California. American Midland Naturalist. 101(1): 38-48

Prescott CE, JM Zukswert. 2016. Invasive plant species and litter decomposition: time to challenge assumptions New Phytologist 209:5–7

Robertson GP, PM Groffman. 2015. Nitrogen transformations. Pages 421-446 *in* E. A. Paul, editor. Soil microbiology, ecology and biochemistry. Fourth edition. Academic Press, Burlington, Massachusetts, USA.

Schellenberger S, HL Drake, S Kolb. 2012. Impairment of cellulose- and cellobiose-degrading soil bacteria by two acidic herbicides. FEMS Microbiology Letters, Volume 327, Issue 1, February 2012, Pages 60–65, https://doi.org/10.1111/j.1574-6968.2011.02460.x

SERA. 2011. Glyphosate Human Health and Ecological Risk Assessment. Final Report for USDA/Forest Service. Prepared by Syracuse Environmental Research Associates, Manlius, NY. 313 pp. SERA TR-052-22-03b

Vogel J. 2008. Unique aspects of the grass cell wall. Curr. Opin. Plant Biol. 11, 301– 307.

Wiedemann AM, A Pickart. 1996. The *Ammophila* problem on the Northwest coast of North America. Landscape and Urban Planning 34:287–299

Wheeler B, M Torchiano. 2016. Permutation tests for linear models for "R." Version 2.1.0. August 29, 2016. https://github.com/mtorchiano/lmPerm

Windham L. 2001. Comparison of biomass production and decomposition between Phragmites australis (common reed) and Spartina patens (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21:179–188 https://doi.org/10.1672/0277

Wongwatanapaiboon J, K Kangvansaichol, V Burapatana, R Inochanon, P Winayanuwattikun, T Yongvanich, W Chulalaksananukul. 2012. The Potential of Cellulosic Ethanol Production from Grasses in Thailand. Journal of Biomedicine and Biotechnology. Article ID 303748. doi:10.1155/2012/303748

Wyman CE. 1996. Handbook on Bioethanol Production and Utilization. Washington, D.C.: Taylor & Francis.

Xiong S, C Nilsson. 1999. The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology 87:984–994.

Table 1. Changes in plant chemistry variables for European beachgrass at PRNS at different senescence/treatment intervals (TX0=recently, naturally senesced; TX1=herbicide-treated <1 yr prior; TX3=herbicide-treated ~ 3 yrs prior; TX8=herbicide-treated ~7-8 yrs prior). Bold-faced % change indicates apparent increase; while italicized % change indicates apparent decrease.

Variable	**TX0**	**TX1**	*%Chg*	**TX3**	*%Chg*	**TX8**	*%Chg*
%DM	85.5	86.6	**1%**	92.0	**8%**	91.1	7%
%N	0.60	0.58	*-3%*	NS		NS	
%C	47.50	47.80	**1%**	NS		NS	
C:N	84.7	87.5	**3%**	NS		NS	
%Hemicell	34.3	34.0	*-1%*	34.1	0%	32.4	*-6%*
%Cellulose	35.8	37.5	**5%**	38.6	**8%**	37.9	*6%*
%CellWall(%aNDF)	79.0	82.2	**4%**	83.8	**6%**	81.8	*4%*
%Silicon	0.91	0.87	*-4%*	NS		NS	
%Lignin	8.8	10.4	**18%**	11.1	**26%**	11.5	**30%**
Total Phenols	2	2	0%	2.2	**10%**	3.4	**70%**

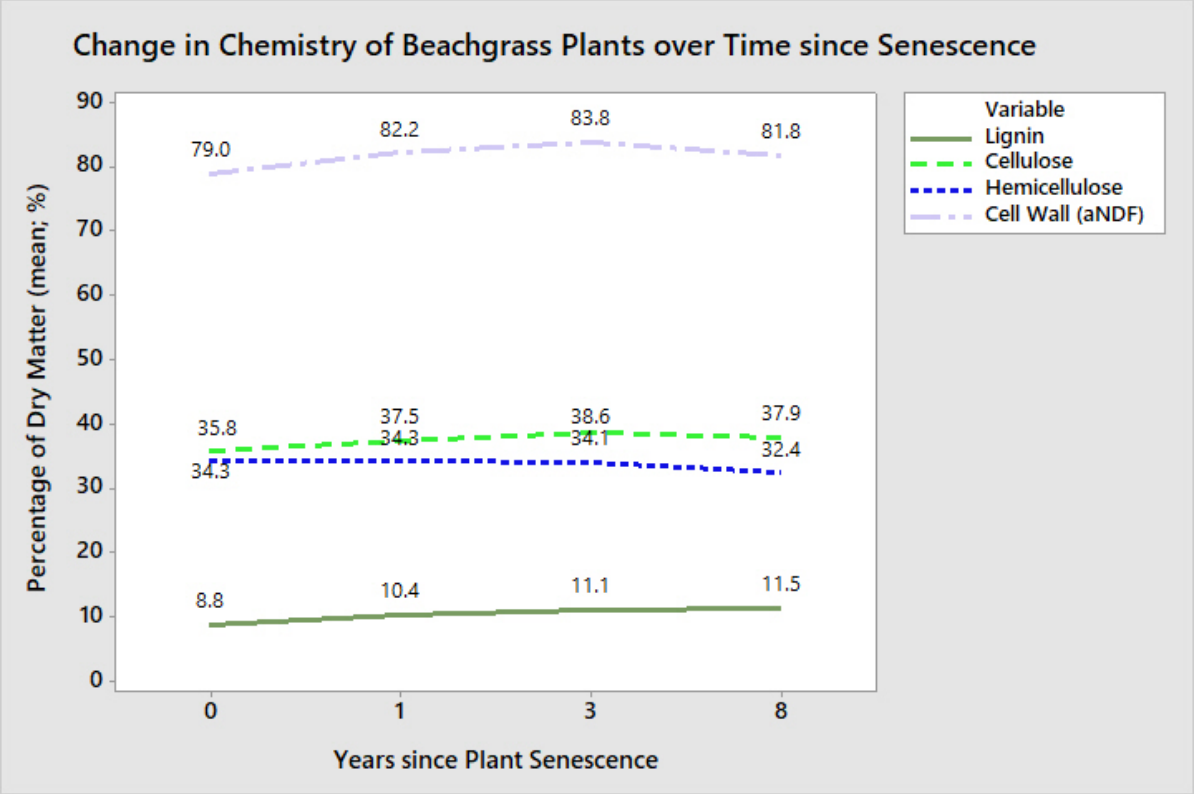


Figure 1. Change in key plant chemistry variables in senesced European beachgrass based on senescence or treatment age of material.

APPENDIX G. PAPER PUBLISHED BY PARSONS AND BECKER (2021): "*INVASION BY AMMOPHILA ARENARIA ALTERS SOIL CHEMISTRY, LEAVING LASTING LEGACY EFFECTS ON RESTORED COASTAL DUNES IN CALIFORNIA*"

Invasive Plant Science and Management

www.cambridge.org/inp

Invasion by *Ammophila arenaria* alters soil chemistry, leaving lasting legacy effects on restored coastal dunes in California

Lorraine S. Parsons[1] and Benjamin H. Becker[2]

[1]Vegetation/Wetland Ecologist, Point Reyes National Seashore, National Park Service, Point Reyes Station, CA, USA and [2]Science Adviser/Research Coordinator, Californian Cooperative Ecosystems Studies Unit, National Park Service, University of California Berkeley, Berkeley, CA, USA

Research Article

Cite this article: Parsons LS and Becker BH (2021) Invasion by *Ammophila arenaria* alters soil chemistry, leaving lasting legacy effects on restored coastal dunes in California. Invasive Plant Sci. Manag **14**: 75–91. doi: 10.1017/inp.2021.16

Received: 3 March 2021
Revised: 4 May 2021
Accepted: 12 May 2021
First published online: 17 May 2021

Associate Editor:
Edith Allen, University of California, Riverside

Keywords:
Delayed decomposition; herbicide; invasive plant; restoration; secondary invaders

Author for correspondence:
Lorraine Parsons, Point Reyes National Seashore, 1 Bear Valley Road, Point Reyes Station, CA 94956.
(Email: Lorraine_Parsons@nps.gov)

Abstract

Many restoration projects rely on invasive plant removal to restore ecosystems. However, success of restoration efforts relying on invasive removal can be jeopardized, because in addition to displacing native plants, invasives can also dramatically impact soils. Many studies have documented invasives' effects on soil chemistry and microbiota. While European beachgrass [*Ammophila arenaria* (L.) Link] is a worldwide invasive problem in coastal dunes outside northern Europe, little attention has been paid to effects of this species on soil chemistry following invasion, even though it establishes persistent, dense monocultures. In our study, we evaluated effects of *A. arenaria* invasion on soil chemistry of coastal dunes at Point Reyes National Seashore (PRNS); persistence of effects following removal by mechanical or herbicide treatment (legacy effects); and effects of treatment independent of invasion. Dune restoration efforts at PRNS have met with mixed success, especially in herbicide-treated backdunes, where decomposition of dead *A. arenaria* has been greatly delayed. Based on results, invasion impacted 74% of 19 variables assessed, although there was a significant interaction in many cases with successional status (earlier vs. later). Almost 60% of invasion effects persisted after restoration, with legacy effects prevalent in herbicide-treated backdunes where sand deposition from adjacent beaches could not mitigate effects as it could in herbicide-treated foredunes. Mechanical removal—or inversion of invaded surface soils with less-contaminated subsoils—resulted in fewer legacy effects, but more treatment effects, primarily in backdunes. Soil chemistry may decelerate decomposition of *A. arenaria* due to the limited nitrogen (N) available to enable microbial breakdown of the high carbon(C):N (70.8:1) material, but microbial factors probably play a more important role. Success of restoration at PRNS may not be fully realized until legacy effects are resolved through additional actions such as inoculation with healthy microbiomes or necromass reduction through controlled burning.

Introduction

Many habitat restoration projects that focus on removal of invasive plants end up foundering due to unexpected ecological complications (Corbin and D'Antonio 2012; Konlechner and Lord 2015; Suding et al. 2004). Either long-term maintenance is needed to eradicate the primary invaders targeted, or primary invaders are eliminated, only to be replaced by new weeds or secondary invaders (Pearson et al. 2016). Restoration is complicated by the fact that, while efforts are generally "aboveground centric," focusing on the highly visible displacement of native plant species by non-native ones, the effects of invasives are often not, effecting equally as pernicious effects on belowground conditions (Wolfe and Klironomos 2005). Invasives' impacts on soils can either be chemical or microbial or a combination of both (Corbin and D'Antonio 2012; Inderjit and van der Putten 2010), with changes in soil chemistry influencing microbial communities and vice versa (Badalamenti et al. 2016; Ehrenfeld 2004). Soil changes can exert a positive feedback effect on established invasives, enhancing their competitiveness at natives' expense (Corbin and D'Antonio 2012; Inderjit and van der Putten 2010; Reinhart and Callaway 2006), or invasives can have direct, negative feedbacks on natives by introducing or amplifying pathogens (de la Peña et al. 2010; Eppinga et al. 2006; Vilà et al. 2006), producing allelochemicals (Inderjit et al. 2008; Novoa et al. 2012; Vivrette and Muller 1977), or disrupting or altering symbiotic mutualistic relationships (Gornish et al. 2020; Lekberg et al. 2013; Pringle et al. 2009; Stinson et al. 2006).

Effects of invasion by nonnative plants on soil chemistry have been documented for a large number of plant types and habitats, including nonnative grasses in Hawaiian woodlands and California shrublands (Mack et al. 2001; Yelenik and D'Antonio 2013; Yelenik and Levine 2010); nonnative shrub and grass species in deciduous forests in the eastern United States (Ehrenfeld et al. 2001); and nonnative herbs in Chinese forests (Niu et al. 2007). While these

© U.S. National Park Service, 2021. This is a work of the US Government and is not subject to copyright protection within the United States. Published by Cambridge University Press on behalf of Weed Science Society of America.

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Management Implications

Undertaking restoration projects that focus on invasive plant removal without taking into consideration the broader ecological picture is a mistake. Natural ecosystems supporting predominantly native species are often characterized by a myriad of complex biotic and abiotic interactions, many of which can be substantially altered by invasion by nonnative plants. As results of many prior projects have shown, just removing invasive plants is not always enough to reverse aboveground and belowground changes effected by plant invasion. If habitat restoration projects are ultimately to be successful, resource managers need to determine what relationships may have been impacted before moving ahead with invasive removal and to include measures to remediate these invasion effects. Some adaptive restoration measures that have been discussed include soil amelioration, topsoil removal, dead biomass or litter removal, inoculation with fungal or bacterial taxa, overseeding with native plant seed, carbon addition, or selective removal of nonnative species or species with strong biogeochemical feedbacks. The restoration approach also needs to be carefully considered, as the method used to remove weeds influences legacy and treatment effects. Even if removal efforts should initially appear successful, restored systems continue to be vulnerable to invasions by new weeds or secondary invaders, so project planning should include contingency plans to address these issues as well. While the focus for many restoration projects is often on re-creating natural ecosystems that, once restored, will need no maintenance, some type of long-term maintenance will probably always be needed to control opportunistic secondary invaders that often flourish in systems disturbed by restoration activities and to potentially implement post-project "tweaks" that will maintain the project on a successful restoration trajectory. Until restoration approaches are refined to integrate measures that address both aboveground and belowground invasion effects, managers would be best served by focusing on restoring areas with fewer invasion effects or a lower potential for legacy effects or focusing on areas where these effects are more easily countered.

studies linked invasion to change in a wide range of soil chemistry variables, nonnative plant invasion appears to have particularly impacted nitrogen (N) dynamics within ecosystems (Ehrenfeld 2010; Liao et al. 2008), which may stem partly from many studies focusing on invasion of N-fixing species into habitats without a history of N-fixers (Corbin and D'Antonio 2011). A meta-analysis of effects of both N-fixing and non–N-fixing invasive plants on N and carbon (C) cycling in forest, grassland, and wetland ecosystems using data from 94 experimental studies and 19 soil variables found that soil ammonium (NH_4^+) and nitrate (NO_3^-) pools climbed by 30% and 17%, respectively, following invasion, while mineralization and nitrification rates jumped 52% and 53%, respectively (Liao et al. 2008). Conversely, invasion appeared to be accompanied by less dramatic changes in soil C pools (~7%; Liao et al. 2008).

Coastal dunes are one ecosystem in which soil chemistry changes following invasion have been well studied, at least for certain species. Iceplant [*Carpobrotus edulis* (L.) L. Bolus], a common invader in many of the world's dune systems, exerts a well-documented physiochemical influence on invaded areas by reducing soil pH and available water; increasing organic matter (OM); and in certain instances, increasing total N, NO_3^-, NH_4^+, phosphorus (P), organic C, and sodium (Na^+) levels (Badalamenti et al. 2016; Conser and Connor 2009; D'Antonio and Mahall, unpublished data *in* Conser and Connor 2009; D'Antonio and Mahall 1991; Novoa et al. 2013, 2014; Vilà et al. 2006). *Carpobrotus edulis* leaves have high levels of tannin and antibacterial compounds (Novoa et al. 2014; van der Watt and Pretorius 2001) that may reduce litter decomposition rates (Vilà et al. 2006) and acidify soils, thereby reducing nitrification rates and increasing leaching of calcium (Ca^{2+}) and Mg^{2+} (D'Antonio and Mahall, unpublished data *in* Conser and Connor 2009). All these soil chemistry changes create a positive feedback effect for *C. edulis*, favoring it over native dune plant species (Novoa and González 2014). N-fixing shrubs such as bush lupine (*Lupinus arboreus* Sims) have also proven to be an issue in New Zealand dunes and northern California prairies, almost doubling N soil concentrations and increasing mineralization rates within normally N-limited systems (Hetherington and Wilson 2019; Maron and Jefferies 1999; Pickart et al. 1998). Continued N deposition through *L. arboreus* death or leaf drop promotes habitat conversion by encouraging establishment of weedy annuals such as ripgut grass (*Bromus diandrus* Roth) (Maron and Connors 1996; Maron and Jefferies 1999).

Another common invader in coastal dune systems worldwide is European beachgrass [*Ammophila arenaria* (L.) Link], a C_3 grass native to northern Europe (van der Putten et al. 2005; Weber 2003). Many studies have evaluated how *A. arenaria* has been able to proliferate in introduced portions of its range, with most focusing on how differences in the soil microbial community between native and introduced ranges might enable this species to flourish, often at the expense of native plants and wildlife (Dangremond et al. 2010; Muir and Colwell 2010). Little, if any research, however, has focused on changes in soil chemistry following invasion by *A. arenaria*, even though, given its predilection to form dense monocultures in invaded portions of its range, this species would seemingly have a strong potential to exert an effect. A recent study at Point Reyes in northern California demonstrated that *A. arenaria* invasion is accompanied by distinct changes in the soil microbial community; specifically, lower abundances of putatively identified nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs and a higher abundances of cellulolytic bacteria and a class of arbuscular mycorrhizal fungi (Archaeosporomycetes; Parsons et al. 2020b). Reductions in nitrifiers and changes in the proportions of cellulolytic and fermentative bacteria would strongly point to the potential for changes in cycling of both N and C in *A. arenaria*–invaded dunes.

Changes in soil chemistry—and microbial communities—with invasion are concerning, because many of these invasion-associated soil changes do not dissipate with restoration, creating "legacy effects" that ultimately may hamper, or even preclude, the success of restoration efforts (Corbin and D'Antonio 2011; Reinhart and Callaway 2006; Yelenik and D'Antonio 2013). Nutrient concentrations and native plant cover at intermediate (13 mo) and long-term (5 yr) post-restoration intervals in New Zealand and California sites where *L. arboreus* was removed largely returned to levels similar to or even slightly higher, respectively, than those of untreated areas (Hetherington and Wilson 2019; Maron and Jefferies 2001). A number of *C. edulis* removal studies have found that, while some soil chemical legacy effects reverted within a year to pre-restoration conditions (salinity, Na^+, Mg^{2+}), others did not (pH, OM, NH_4^+, NO_3^-/NO_2^-), and other variables were potentially intermediate between uninvaded and invaded conditions (P, soil moisture; D'Antonio and Mahall, unpublished data *in* Conser and Connor 2009; Novoa et al. 2013; Santoro et al. 2011, 2012). These chemical legacy effects promote new invasion by ruderal nitrophilous species within *C. edulis* removal areas

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

(Novoa et al. 2013; Santoro et al. 2011, 2012), which reduces establishment of native dune species and overall species diversity (Badalamenti et al. 2016; Novoa et al. 2013).

Starting in 2001, Point Reyes National Seashore (PRNS), a unit of the U.S. National Park Service (NPS) located along the northern California coast, embarked on an extensive program of coastal dune restoration by removing *A. arenaria* and *C. edulis*. These species were likely planted in the early to mid-1900s by ranchers and other residents trying to prevent coastal dune sands from migrating into adjacent pastures, ranch facilities, and roads. By 2009, more than 60% of the park's roughly 890 ha of coastal dune, bluff, and scrub habitat was estimated to be dominated by *A. arenaria* and *C. edulis* (NPS 2009). Between 2001 and 2018, approximately 110 ha of primarily *A. arenaria* and, to a much lesser degree, *C. edulis* had been removed from 200 ha of coastal dunes using a combination of manual removal (i.e., hand pulling or small hand tools), mechanical removal (i.e., using heavy equipment), and chemical treatment.

These restoration efforts have met with mixed success, with mechanical removal areas remaining largely unvegetated for many years except for recruitment by a very rare plant species (Tidestrom's lupine [*Lupinus tidestromii* Greene]) and a disturbance-adapted weed (European searocket [*Cakile maritima* Scop.]). Herbicide treatment areas in the backdunes, which are older dune features further from the beach that typically support later successional vegetation communities, have largely been dominated by persistent standing dead *A. arenaria* and its thick litter ("thatch") for 5 to 8 yr posttreatment, essentially creating a physical legacy effect (Parsons et al. 2020b). With the exception of herbicide-treated foredunes (younger dune features directly adjacent to the beach), both types of restoration approaches at least initially failed to promote extensive or diverse recruitment by native dune plant species despite an abundant seedbank and were instead subject to invasion by new weeds or "secondary" invaders (Parsons et al. 2020a). In recent years, colonization by natives within mechanical removal areas has improved substantially, but issues with native recruitment and secondary invader establishment persist within herbicide-treated backdunes (Parsons et al. 2020a). As cover of primary invaders (*A. arenaria* and *C. edulis*) within restored areas remains low due to vigilant retreatment efforts, other factors appear to be constraining native dune habitat evolution and development. The recent microbial study at PRNS suggested that some of the changes in the microbial soil community brought about by invasion of *A. arenaria*, such as reduced abundances of nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs did not dissipate even 5 yr after restoration by herbicide treatment (Parsons et al. 2020b).

We hypothesize that long-term colonization by dense stands of *A. arenaria* changes the chemistry of dune soils at PRNS relative to uninvaded areas (invasion effects) and that some of these changes may persist following invasives' removal, creating legacy effects that may ultimately decelerate or even derail restoration efforts. In addition, physical legacy effects such as delayed decomposition of *A. arenaria* litter or thatch in herbicide-treated backdunes may at least partially stem from soil chemistry alterations. We further hypothesize that, even if chemical legacy effects do not persist, soils in restored areas may not necessarily revert to conditions more characteristic of comparable native dune habitats due to restoration itself having an effect that may differ depending on the approach used (treatment effect). The presence or intensity of invasion, legacy, and treatment effects may vary depending on secondary factors such as habitat successional stage; year or interannual variation in climatic conditions such as precipitation; distance from the ocean; or elevation. In this study, we conducted 2 yr of soil sampling within native dune (dune mat, dune scrub) and highly *A. arenaria*–invaded habitats, as well as former highly *A. arenaria*–invaded habitats that have been restored either mechanically or through herbicide treatment to evaluate OM, C, N, P, other macronutrients, pH, soil moisture, and exchangeable cations between habitats. We also assessed whether relationships existed between soil chemistry and vegetation-related measures of habitat evolution such as percent cover of native and nonnative plants, standing dead *A. arenaria* biomass, thatch, and bare ground.

Materials and Methods

Study Area

PRNS is located approximately 48 km north of San Francisco, CA, USA (Figure 1). Most of the lands at PRNS fall on a peninsula that juts out from California's coastline into the Pacific Ocean and is perched along the western edge of the San Andreas Fault. Dunes at PRNS are largely composed of a modern largely continuous foredune ridge established by current and past *A. arenaria*–dominated vegetation and a complex series of undulating backdunes and depressions associated with an older, mostly stabilized parabolic dune field, including narrow deflation plains (slacks). In northern portions of a property formerly owned by the telecommunications company AT&T (AT&T North/South; Figure 1), climbing dunes overlay a high-elevation sandstone feature that reaches 61 m at its highest point.

PRNS dunes represent a range of successional stages from early to midsuccessional communities such as foredunes and native dune mat, so-called yellow dunes, to later successional ones such as backdunes and native dune scrub. The latter two are often referred to as "gray" and "brown" dunes and are usually situated further inland (>300 m from the ocean) or, in the case of dune scrub, sometimes at higher elevations along the immediate coast (Parsons et al. 2020a). Both uninvaded foredunes and dune mat are sparsely vegetated, largely open sand habitats supporting herbaceous communities, including those in the following alliances: *Leymus mollis* (Trin.) Pilg.; *Abronia latifolia* Eschsch.–*Ambrosia chamissonis* (Less.) Greene; and *Eriophyllum stoechadifolium* Lag.–*Erigeron glaucus* Ker Gawl.–*Eriogonum latifolium* Sm. (Buck-Diaz et al. 2021). Dune scrub is a dense, shrub-dominated habitat represented by alliances such as *Lupinus chamissonis* Eschsch.–*Ericameria ericoides* (Less.) Jeps. (Buck-Diaz et al. 2021). A range of invasion conditions exist within PRNS dunes from sparsely to highly invaded, with the highly clonal nature of *A. arenaria* and *C. edulis* favoring eventual establishment of dense monocultures exceeding 98% cover (Parsons et al. 2020a). Some invaded systems retain vestiges of native dune mat or dune scrub that have either not been invaded or only very sparsely invaded by *C. edulis* and a few other nonnatives despite being, in some instances, almost completely surrounded by *A. arenaria* or *C. edulis* for decades (Parsons et al. 2020a). The largest native dune mat habitat in PRNS is found at Abbotts Lagoon (Abbotts; ~20.8 ha), although there are also smaller dune mat areas at AT&T (~1 ha). Native dune scrub is prevalent at both Abbotts and AT&T, although the most intact stands in terms of low number and percent cover of nonnatives occur at AT&T North.

PRNS dunes provide habitat for 11 species listed by the U.S. Fish and Wildlife Service as endangered or threatened. Therefore, dune restoration efforts have focused on areas that

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

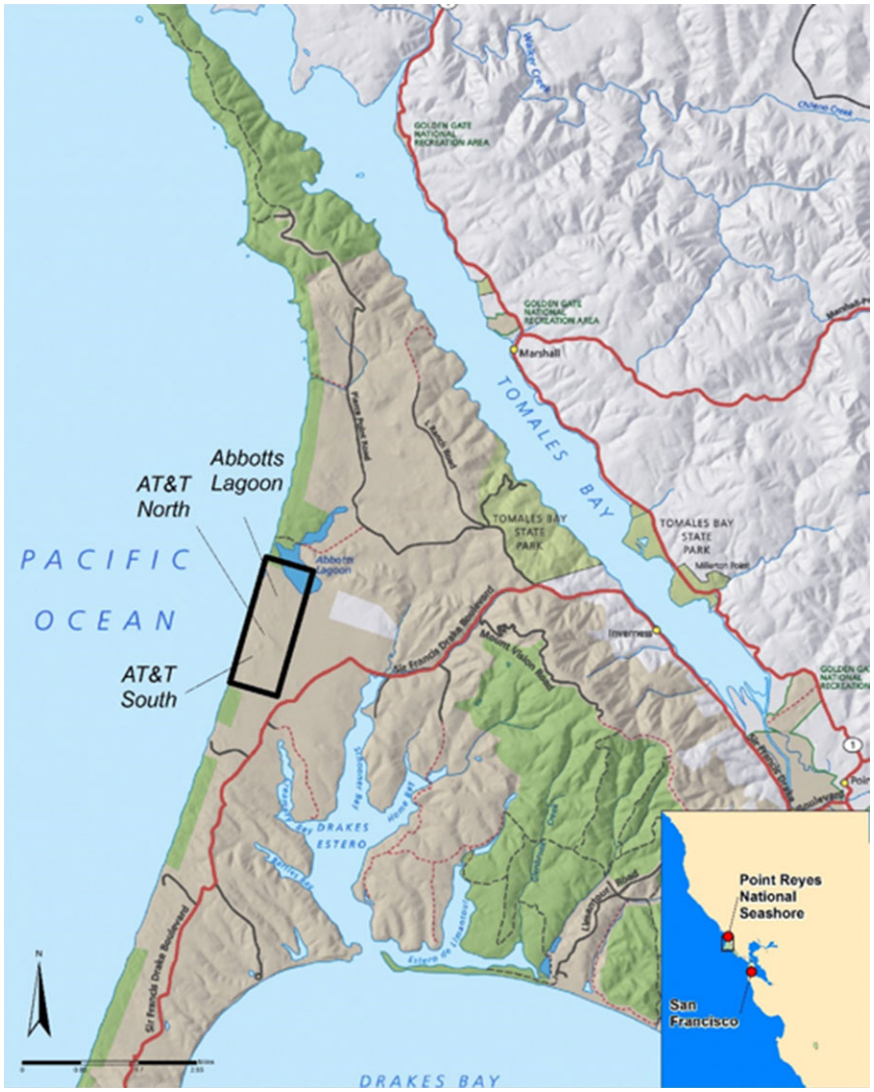


Figure 1. Map of the study areas in Point Reyes National Seashore (PRNS), showing three sites (Abbotts Lagoon, AT&T North, and AT&T South) and location of PRNS along the northern California coast.

should have the greatest benefit for critical rare species. To date, most of the park's restoration efforts have focused on removing *A. arenaria* using a combination of manual removal, mechanical removal, and chemical treatment. Mechanical removal involves use of excavators and bulldozers to invert or "flip" the surface 1 to 1.5 m of rhizome-contaminated soils with less-contaminated soils 2 to 3 m below the soil surface. Initial chemical treatment is performed using herbicide backpacks with a single nozzle wand and directed spraying of a mixture of 1% imazapyr (Habitat®, isopropylamine (IPA), 27.77% ai, SePRO, Carmel, IN, USA); 2% glyphosate (Roundup Custom®, IPA, 53.8%, Monsanto, St Louis, MO, USA); 1.5% of a modified vegetable oil surfactant (Competitor®, 98.0%, Wilbur-Ellis, Fresno, CA, USA); and 1% blue dye (various manufacturers) onto target invasive plants. PRNS elected—and received approval—to use aquatic-label herbicides (e.g., Habitat®) to minimize toxicity within sensitive dune habitats, even though they are not aquatic.

Soil types in PRNS dunes consist of hummock mounds and hills of loose sand with no profile development (Soil Conservation Service 1985). For this reason, coastal dune sands tend to be strongly nutrient limited, especially for the essential nutrients N and P and, to a lesser extent, K^+, while Ca^{2+} and Mg^{2+} levels are typically adequate for plant growth (Barbour et al. 1985; Koske and Polson 1984; Willis and Yemm 1961; Woodhouse and Hanes 1967). Nutrient cycles in California dunes have not been studied except at Point Reyes (Holton et al. 1991) and Humboldt Bay (Nature Conservancy, unpublished data *in* Pickart and Sawyer 1998; Rose 1988). Holton et al. (1991) found that total N in earlier successional habitats (foredune, native dune mat) at Point Reyes averaged only 0.004%. In contrast, total N in later successional habitats such as dune scrub ranged from 0.5% to 6.0%, while OM remained low (0.004% to 0.2%; Holton and Johnson 1979).

The climate in Point Reyes is Mediterranean, with hot/dry summers and cool/wet winters. Coastal areas in California are generally cooler and have less intra-annual variability in temperature than inland regions, with fog, winds, and salt spray being important climatic influences. Annual temperature is estimated to average 12.1 C, and precipitation near the project area is estimated to average 54 cm yr^{-1} (2006 to 2020; WRCC 2020). Total rainfall can vary dramatically between years, as is evident for total precipitation numbers for 2019 (89.5 cm) and 2020 (32 cm; WRCC 2020). Fog

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

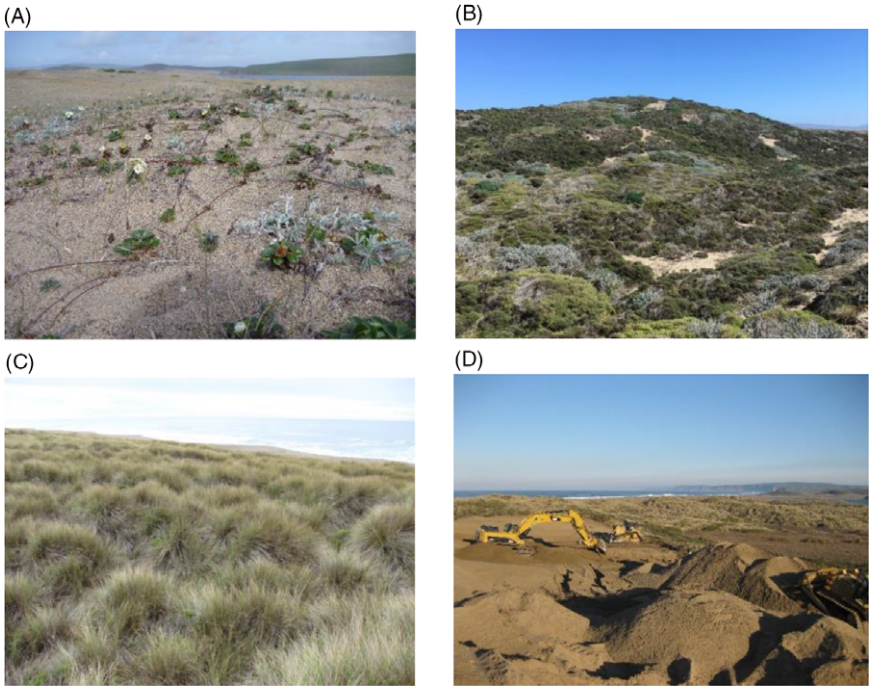


Figure 2. Representative photographs of sampled habitats, including (A) sparsely invaded native dune mat; (B) sparsely invaded native dune scrub; (C) highly invaded *Ammophila arenaria* monoculture; and (D) former highly invaded areas being restored mechanically.

blankets the Point Reyes coast about 30% of the time between June and October (Goodman 1982 *in* Ingraham and Matthews 1995), generating anywhere from 1.0 to 5.2 L site^{-1} yr^{-1} of additional moisture during what is otherwise California's driest period (June to October; Ingraham and Matthews 1995). Another climatic influence is windblown salt spray, which occurs during both winter and summer seasons (Clayton 1972). Salt spray studies conducted at Point Reyes estimated deposition values at coastal sites ranging from 3 to 18 mg (dm^2)$^{-1}$ d^{-1} (Barbour 1978; Holton and Johnson 1979).

Selection of Sites for Soil Sampling

Samples were collected at 51 sites in 2019 and 2020 at Abbotts Lagoon (38.110076°N, 122.959294°W) and AT&T (38.09643°N, 122.964371°W; Figure 1). The Abbotts area falls south of a large coastal lagoon, with AT&T directly bordering Abbotts to the south (Figure 1). To evaluate whether *A. arenaria* invasion changed soil chemistry relative to conditions in uninvaded dune habitat, we focused soil sampling in (1) uninvaded or sparsely invaded (<5% cover of invasives) native dune habitats (dune mat, dune scrub; Figure 2A and B) and (2) highly invaded *A. arenaria* (>90% cover) dunes (Figure 2C). We also assessed the potential for legacy or treatment effects by sampling formerly highly invaded *A. arenaria* dunes that had been restored either (3) mechanically (Figure 2D) or (4) using herbicide.

We used a random stratified sampling approach to either select sampling locations from existing 2 by 1 m vegetation monitoring plots or to create plots in invaded areas, as well in native dune mat, dune scrub, and herbicide-treated foredunes. We generally interspersed sampling within habitats among sites (Abbotts, AT&T North, AT&T South); however, this was not always possible (Table 1). Chemical treatment of *A. arenaria* has been performed at all three sites, but mechanical removal was only conducted at Abbotts in 2011 due to the high restoration cost per acre and indirect impacts from subsequent sand remobilization on adjacent native habitats and ranchlands (Table 1). Subsequently, PRNS switched its restoration approach primarily to herbicide treatment, with treatment projects occurring in a spatially sequential manner starting in the north with Abbotts (2011 to 2015) and then moving south to AT&T North (2016) and AT&T South (2017 to 2018; Table 1; Figure 1). Most of the remaining heavily invaded *A. arenaria* areas primarily occur at the very southern portion of AT&T, south of current restoration efforts, although a few isolated stands of backdune *A. arenaria*–invaded areas do persist within restoration areas where nontreatment buffers to organic pastures were established (Table 1). Sampling of native dune mat and dune scrub was also more intensive at Abbotts and AT&T North, respectively, due to higher proportions of these habitats at those sites (Table 1).

Within areas of varying invasion status (native, invaded, mechanically restored, herbicide treated), we also ensured approximately equal sampling effort between earlier successional habitats (native dune mat, foredunes) and later successional habitats (backdunes, native dune scrub), as soil texture and chemistry would be expected to differ based on successional stage (Holton and Johnson 1979), and successional status may influence invasion, legacy, and treatment effects (Table 1). In 2020, a few sampling sites were dropped and new ones added to improve interspersion of habitats among sites and uniformity of sampling effort among successional habitats (Table 1).

Soil Sampling Methods

We conducted soil sampling in late May (20, 22, 29 to 30) in 2019 and early June (1 to 3) in 2020, the peak growing season for most dune vegetation communities. Within plots, surface soils were collected using a 30-cm slotted mini-soil probe to a depth of 30 cm, with multiple cores taken using a cross-diagonal sampling pattern. Sampling location varied in proximity to plants, although sampling avoided collection of dense roots. We separated soils into several

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Table 1. Number of soil samples analyzed from restored and unrestored dunes in Point Reyes National Seashore (PRNS).[a]

Invasion status/ treatment approach	Successional status (earlier vs. later)[b]	Abbotts Lagoon (78.3 ha)		AT&T North (15.5 ha)		AT&T South (18.2 ha)	
		2019	2020	2019	2020	2019	2020
Sparsely invaded native dune	Dune mat	5	6	—	—	NS	2
	Dune scrub	NS	2	6	6	NS	NS
Heavily invaded dunes—untreated	Foredune	—	—	—	—	3	3
	Backdune	—	—	NS	1	3	4
Heavily invaded dunes—restored mechanically	Foredune	2 (2011)	3	—	—	—	—
	Backdune	5 (2011)	3 (2011)	—	—	—	—
Heavily invaded dunes—restored using herbicide	Foredune	6 (2012)	4 (2012)	4 (2016)	3 (2016)	2 (2018)	3 (2018)
	Backdune	7 (2012)	5 (2012)	5 (2016)	3 (2016)	3 (2018)	3 (2018)

[a]For restored areas, initial treatment year is shown in parentheses below sample number. Dash connotes that specified habitat was not available at study site. NS, present, but not sampled.
[b] Successional status column shows sites in earlier successional habitats in darker shading and later successional habitats with lighter shading.

plastic bags marked for different analytical laboratories and transported samples in our backpacks in contractor bags with multiple ice packs until samples could be transferred to coolers in our vehicles.

Vegetation Sampling Methods

To evaluate whether soil chemistry appeared linked to changes in vegetation-related variables in herbicide-treated backdunes, we conducted vegetation monitoring using previously and newly established 2 by 1 m plots in 2020. We assessed cover of vegetation and other functional groups using a PVC quadrat separated into 10 by 10 cm grids for cover estimation by the point-intercept method, with a maximum of 171 sampling points. At each sampling point, plant species present (both alive and dead) were recorded once regardless of the number of "hits," along with functional groups such as bare ground, thatch (intact dead stems >10 cm), and detritus (finer OM ≤10 cm). In addition to grouping species into native, nonnative, and primary and secondary invader species, we also grouped native dune species according to successional stage, as certain species appear strongly associated with different successional stages of dune habitat (early, mid, late, and dune scrub) related to their ability to tolerate disturbance from wind, wave, salt spray, and sand burial (Barbour et al. 1976; Hesp 1991). Native and nonnative species richness was calculated from the total number of native and nonnative plant species present in each plot regardless of whether "hit" by intercept sampling or not.

Laboratory Analyses—Soils

We used commercial laboratories for soils analyses. In 2019, soils were only sent to Western A&L Agricultural Laboratory (Western A&L, Modesto, CA, USA). In 2020, soils were shipped to both Western A&L and University of California, Davis, Analytical Laboratory (UC Davis, Davis, CA, USA). Soils for Western A&L were stored in coolers in a basement at PRNS until all sampling was completed during 2019 and 2020 and then shipped with ice packs. Samples for UC Davis were shipped priority overnight with ice packs after every sampling day for analysis of NH_4^+ (2.0 N KCL extract; ppm), NO_3^- (2.0 N KCL extract; ppm), total Kjeldahl nitrogen (TKN; wet method; %), PO_4^- (weak Bray; ppm), and total organic carbon (TOC; combustion; %).

The following additional analyses were performed using standard laboratory methods: OM (loss-on-ignition at 360 C; %); PO_4^- (Olsen; ppm); sulfate (SO_4^{2-}; 1.0 N ammonium acetate at pH 7; ppm); K^+ (saturated paste–inductively coupled plasma atomic emission spectrometry [ICP AES]; ppm); Mg^{2+} (saturated paste–ICP AES; ppm); Ca^{2+} (saturated paste–ICP AES; ppm); Na^+ (saturated paste–ICP AES; ppm); pH (saturated paste); soil moisture ((wet wt.–dry wt./dry wt.); %); and soluble salts (saturated paste–conductivity; mmhos cm^{-1}). Percent exchangeable (Exc) cations (K^+, Mg^{2+}, Ca^{2+}, Na^+, H^+) and CEC (meq 100 g^{-1}) were calculated using the procedure of 1.0 N ammonium acetate extraction at pH 7. Soluble salts were not assessed in 2020. In 2020, soil texture (Na-hexametaphosphate hydrometer; % sand/silt/clay) was assessed for 12 sampling sites. We calculated the C:N ratio of soils for June 2020 samples using the ratio of %TOC divided by %total N, which was calculated by adding %TKN to the very small percentage of inorganic N (NO_3^-, NH_4^+).

Statistical Analyses

Hypothesis Testing

Invasion effects were evaluated by comparing soil chemistry of sparsely invaded native dune mat or dune scrub sampling sites with that of highly invaded *A. arenaria* ones. For variables demonstrating an invasion effect, legacy effects were explored by comparing results of highly invaded *A. arenaria* sites with those of herbicide-treated and mechanically restored areas to determine whether restoration resulted in a change. As restoration may affect soil chemistry, even if invasion does not, variables without legacy effects that differed between restored and native dune areas following restoration were classified as treatment effects. Secondary factors that may indirectly influence invasion, legacy, and treatment effects were also evaluated. These included successional status ("earlier" successional or foredune/dune mat, "later" successional or backdune/dune scrub), year (2019, 2020), site (Abbotts, AT&T North, AT&T South), distance from the ocean, and elevation.

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Permutational Multivariate Analysis Of Variance

We used a permutational multivariate analysis of variance (PERMANOVA; Oksanen et al. 2019) to assess competing hypotheses explaining overall dune soil chemistry values. Sixteen candidate models consisting of additive and interaction terms of successional status, invasion status, year, site, distance from ocean, and elevation were ranked using a custom script to calculate Akaike's information criterion (AIC) for small sample sizes. Based on our analyses, the best model incorporated a direct effect of year and an interaction between invasion status and successional status.

Principal Components Analysis

Before analysis was conducted, three sample sites were removed using Mahalanobis distance to detect outliers in multivariate space (distances > 6.58). We then conducted unconstrained ordination on scaled soil chemistry variables from 2019 and 2020 using principal components analysis (PCA) with rotated axes (Oksanen et al. 2019). We linked the PCA to PERMANOVA results. Variables in PERMANOVA models with the lowest AIC were used to plot visual groupings on the PCA results. We used the scree plot technique to determine the number of principal components that should be retained for interpretation.

GLMM

We used separate generalized linear mixed models (GLMM; Bates et al. 2015) to explain patterns for each individual soil chemistry variable that showed strong factor loadings in PCA. (Soil moisture and soluble salts were not analyzed.) Based on AIC rankings in PERMANOVA, models were built with an interaction between invasion status and successional status, a direct effect of year, and both fixed and random intercepts for site. We did not assess competing models for each variable; rather, we followed the form of the best (lowest AIC) model found for the PERMANOVA using all the soil chemistry values. Traditional transformations of dependent variable distributions were unsuccessful in providing reasonable GLMM residual plots, and we therefore normalized data via ordered quantile normalization (Peterson 2019). We corrected for multiple testing using the false discovery rate method (Benjamini and Hochberg 1995).

Non-metric Multidimensional Scaling

To evaluate temporal changes in vegetation-related and soil chemistry variables in backdunes based on time since herbicide treatment, we conducted unconstrained ordination with non-metric multidimensional scaling (NMDS; Oksanen et al. 2019) on data from backdune areas where herbicide was applied in 2012, 2016, or 2018. We then fit environmental vectors selected based on permutation tests onto the NMDS to determine soil chemistry variables that best explained changes in vegetation-related variables over time.

All analyses were done in R v. 4.0.2 (R Core Team 2020).

Results and Discussion

Invasion Status

Invasion by *A. arenaria* exerted a dramatic effect on soil chemistry relative to that of sparsely invaded native dune habitats, influencing 74% of 19 variables assessed, with the exception of C:N, NH_4^+, PO_4^-, Ca^{2+}, and Exc-Na^+. Table 2 presents results of statistical analyses, while Table 3 summarizes which variables showed invasion effects (i.e., which variables in invaded habitats were not statistically equivalent with those in native ones) and, for variables exhibiting invasion effects, whether means were generally higher or lower than those in native habitats (up/down arrows) and whether differences were restricted to just one successional stage ("early" or "late"). Other than NH4$^+$ and PO_4, almost every organic (OM, TKN, TOC) and inorganic (NO_3^-, SO_4^{2-}, K^+, Mg^{2+}, Na^+) source of macronutrients and exchangeable cations (H^+, K^+, Mg^{2+}, Ca^{2+}) appeared altered by invasion, along with pH and CEC (Tables 2 and 3). Invasion effects were more pronounced in certain successional habitats for half of the variables (Tables 2 and 3). This is evident in Figure 3, which graphically displays standardized means for earlier and later successional stages in both native and invaded habitats (as well as restored habitats). Only TKN, NH_4+, C:N, PO_4^-, Exc-K^+, and Exc-Ca^{2+} variables showed no interaction between invasion and successional status (Tables 2 and 3). Supplementary Table 1 provides summary statistics for all soil chemistry variables analyzed for different invasion and successional status habitats in 2019 and 2020.

Multivariate ordination results point to successional status factors perhaps being more critical to soil chemistry than invasion status. On the PCA's first axis (PC1; 44.06% of variance), earlier successional habitats generally grouped on the left side of the biplot regardless of invasion or restoration status with a few exceptions (Figure 4). Mechanically restored backdune plots (later successional) overlapped strongly with earlier successional native dune mat plots, and invaded foredune plots fell in the center of the biplot, straddling earlier and later successional habitat groupings (Figure 4). Interpretation of PC2—which accounted for less than a third of the variance (16.92%) explained by PC1—was more complicated, but it largely represented the separation between restored and unrestored habitats, with sparsely invaded native dune mat (Figure 4, bottom left) grouping separately from mechanically restored and herbicide-treated foredunes (Figure 4, upper left). Sparsely invaded native dune scrub (Figure 4, upper right) grouped separately from invaded backdunes (Figure 4, bottom right), with herbicide-treated backdunes juxtaposed between them. Both PC2 and PC1 showed a statistically significant relationship between the interaction of invasion and successional status, as well as generally with invasion status, but only PC1 differed between sparsely and highly invaded habitats (Table 3).

Macronutrient dynamics within coastal dunes are a balancing act between geologic and climatic forces. Unlike terrestrial ecosystems where weathering of soils and rocks generates some of the K^+, Mg^{2+}, Ca^{2+}, and P needed by plants, sandy soils in dunes are already highly weathered and mostly unable to supply these macronutrients (Art et al. 1974; Etherington 1967; Smith et al. 1985). Most inputs of N, oxygen (O), and SO_4^{2-}, as well as base cations such as K^+, Mg^{2+}, Ca^{2+}, and Na^+, into coastal dunes come from meteorological sources such as rain, salt spray, and fog (Clayton 1972; Etherington 1967; Holton et al. 1991; Jacob et al. 1985; van der Valk 1974). Due to the very low abundance of clay, sandy soils also do not have a strong capacity for binding cations that enter the system from elsewhere and are prone to considerable leaching (Art et al. 1974; van der Valk 1974). OM provides virtually all the exchange surface capable of binding cations (Art et al. 1974; Etherington 1967), and OM adsorption is strongly dependent on pH, with CEC typically lower at lower pH values (Smith et al. 1985).

Our results corroborate the well-documented pH gradient between early and late successional coastal dune habitats (Figure 3A), with lower pH values (<5.0 to 6.5) prevalent in late

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Table 2. Statistical results for GLMM analyses.[a]

Covariate	pH	OM	TOC	C:N	TKN	NH_4^+	NO_3^-	PO_4^-	SO_4^{2-}	K^+	Mg^{2+}	Na^+	Ca^{2+}	CEC	Exc–H^+	Exc–K^+	Exc–Mg^{2+}	Exc–Ca^{2+}	Exc–Na^+
(Intercept)	0.44	**−0.49** *	**−0.53** *	−0.40	−0.30	0.21	**−0.54** *	0.19	**−1.01** ***	−0.27	−0.29	**−0.86** ***	**−0.66** **	**−0.85** **	−0.53	0.60	**1.11** ***	0.23	−0.08
InvStatus [Invaded]	**−1.17** **	**1.24** ***	**1.03** *	0.80	**0.87** *	0.60	0.27	−0.23	**1.05** **	0.02	0.66	**0.71** *	−0.23	**1.02** **	**1.29** ***	**−1.03** *	**−0.89** *	**−1.41** *	0.05
InvStatus [MechRest]	**0.95** **	−0.17	**−1.13** *	−1.12	**−0.85** *	−0.42	−0.17	**−1.37** ***	0.66	0.27	**−0.71** *	**1.44** ***	0.31	0.13	**−0.73** *	0.13	**−1.05** *	0.71	**1.04** **
InvStatus [HerbTx]	**0.50** *	0.30	0.10	0.03	−0.18	0.29	**0.77** **	−0.44	**1.06** ***	**1.08** ***	0.44	**1.73** ***	0.19	**0.66** **	−0.36	0.36	−0.34	−0.56	**0.97** ***
SuccStatus [Later]	−0.54	**1.76** ***	**1.55** ***	0.59	**1.50** ***	**0.73** **	**1.88** ***	**0.67** *	**1.62** ***	**1.22** ***	**1.33** ***	**0.94** ***	**2.04** ***	**1.88** ***	**0.65** *	**−0.91** *	**−1.71** ***	0.55	**−0.88** ***
Year [2020]	−0.22	**−0.62** ***			**−0.49** **	**−1.38** ***	**−0.65** ***	**−0.41** **	0.17	**−0.63** ***	**−0.35** *	**0.39** **	−0.17	−0.14	0.20	**−0.37** *	−0.19	−0.09	**0.47** ***
InvStatus [Invaded] * SuccStatus [Later]	0.26	**−1.33** ***	**−1.26** *	0.20	−0.99	−0.39	**−1.32** **	−0.14	**−1.22** *	**−1.31** *	**−1.11** *	**−1.42** **	−0.58	**−1.25** **	−0.36	0.36	0.61	0.30	−0.04
InvStatus [MechRest] * SuccStatus [Later]	−0.34	**−1.66** ***	−0.86	0.09	−0.60	−0.21	**−1.75** ***	0.19	**−2.32** ***	−1.21	**−1.19** *	**−3.04** ***	**−1.94** ***	**−2.16** ***	0.01	1.07	**2.29** ***	−0.48	−0.55
InvStatus [HerbTx] * SuccStatus [Later]	**−1.13** **	**−0.72** *	−0.52	0.54	−0.32	−0.17	**−1.19** ***	0.57	**−1.40** ***	**−1.74** ***	**−0.90** *	**−2.34** ***	**−1.01** **	**−0.99** **	**0.96** **	−0.43	0.37	−0.57	**−0.92** **
Random effects																			
σ^2	0.34	0.26	0.32	0.50	0.43	0.36	0.44	0.31	0.42	0.72	0.35	0.44	0.51	0.30	0.32	0.50	0.53	0.64	0.37
τ_{00}	0.09_{Site}	0.04_{Site}	0.03_{Site}	0.02_{Site}	0.08_{Site}	0.00_{Site}	0.01_{Site}	0.19_{Site}	0.07_{Site}	0.00_{Site}	0.21_{Site}	0.00_{Site}	0.00_{Site}	0.09_{Site}	0.08_{Site}	0.14_{Site}	0.00_{Site}	0.09_{Site}	0.00_{Site}
ICC	0.21	0.14	0.09	0.04	0.16		0.01	0.38	0.14		0.37			0.24	0.20	0.22		0.12	
N	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}	3_{Site}
Observations	99	99	48	48	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
Marginal R^2/ conditional R^2	0.576/ 0.667	0.668/ 0.714	0.640/ 0.672	0.495/ 0.517	0.533/ 0.606	0.645/NA	0.574/ 0.580	0.465/ 0.667	0.394/ 0.477	0.315/NA	0.404/ 0.625	0.569/NA	0.509/NA	0.569/ 0.671	0.592/ 0.674	0.430/ 0.554	0.494/NA	0.333/ 0.416	0.640 / NA

[a]Bold values represent significant *t*-test differences for slope estimates of variables relative to InvStatus [Native], SuccStatus [Early], Year [2019], and InvStatus [Native]*SuccStatus [Early]. Information on random effects related to site include variance (σ^2), random intercept variance (τ_{00}), and intraclass correlation coefficient (ICC). Marginal and conditional R^2 of the model are also presented. We corrected for multiple testing using false discovery rate (FDR) method (Benjamini and Hochberg 1995). Exc, exchangeable; OM, organic matter; TOC, total organic carbon; TKN, total Kjeldahl nitrogen.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Table 3. Effects of invasion by *Ammophila arenaria* and habitat restoration on soil variables from 2019 to 2020.[a]

	Invasion effect (invaded ≠ native) difference invaded relative to native	Legacy effect (restored = invaded)		Treatment effect (restored ≠ native) difference restored relative to native)		Interaction factor (InvStatus *SuccStatus)
Variable		Mechanical	Herbicide	Mechanical	Herbicide	
PC1	↑ *Early*	—	—	↓	—	Y
PC2	↓ *Late*	Y	Y	↑ *Early*	↑ *Early*	Y
pH	↓	—	Y *Late*	↑	↑ *Early*	Y
OM	↑ *Early*	—	—	↓ *Late*	—	Y
TOC	↑ *Early*	—	—	↓	—	Y
TKN	↑	—	—	↓	—	Y
C:N	—			—	—	—
NH_4^+	—			—	—	—
NO_3^-	↓ *Late*	Y	—	—	↑ *Early*	Y
PO_4^-	—			↓	—	Y
SO_4^{2-}	↑ *Early*	Y	Y	↓ *Late*	—	Y
K^+	↓ *Late*	—	—	—	↕	Y
Mg^{2+}	↑ *Early*	—	Y	↓	—	Y
Ca^{2+}	—			↓ *Late*	↓ *Late*	Y
Na^+	↕	—	Y *Late*	↕	↑ *Early*	Y
CEC	↑ *Early*	—	Y	↓ *Late*	—	Y
Exc-H^+	↑	—	Y *Late*	↓	—	Y
Exc-K^+	↓	—	—	—	—	—
Exc-Mg^{2+}	↓	Y *Early*	—	↑ *Late*	—	Y
Exc-Ca^{2+}	↓	—	—	—	—	—
Exc-Na^+	—			↑	↑ *Early*	Y

[a]Invasion effects are those in which there are significant differences between sparsely invaded native and highly invaded areas. Effects that persist following restoration of invaded areas are characterized as legacy effects. Variables without legacy effects that differ between restored and native areas following restoration are classified as treatment effects. Variables with significant statistical interaction between invasion and successional status are noted in the last column. Legacy and treatment effects are separated on the basis of restoration approach (mechanical removal, herbicide treatment). Arrows indicate direction of difference in specified habitat (invaded or restored) relative to native habitats (↑, higher; ↓, lower; ↕, both higher and lower depending on successional status). Italicized references to *Early* or *Late* refer to effects present only in specific type of successional status habitat (earlier, later). Exc, exchangeable; OM, organic matter; TOC, total organic carbon; TKN, total Kjeldahl nitrogen.

successional or "gray/brown" dunes (except for those deriving from calcareous geologic substrates such as limestone) and higher pH values (>6.5) common in early successional "yellow" dunes (Espejel 1992; Kooijman et al. 2020). Higher alkalinity of earlier successional soils may be reinforced by higher levels of calcium carbonate, which is often leached from later successional soils (Randall 1973; Smith et al. 1985), as well as higher cation influx due to proximity to the ocean. Within our study area, pH values ranged from 6.24 to 7.27 in native dune mat and mechanically restored foredunes ("yellow" dunes) to 5.10 to 5.90 in backdunes and native dune scrub ("gray" dunes; Figure 3A). The percentage of OM increased with successional age consistent with the fact that percent vegetation and shrub cover is higher in native dune scrub than in foredunes or native dune mat (Barbour et al. 1985; Kooijman et al. 2020; Figure 3B). Higher OM concentrations in later successional soils were associated with higher levels of organic nutrients such as TOC and TKN compared with those in earlier successional ones (Figure 3C and D). Interestingly, CEC, which is typically reduced in soils with lower pH, was much higher in later successional habitats with lower pH than that of earlier successional ones with higher pH (Figure 3L).

OM and TOC levels in highly invaded soils exceeded those in native dunes, but differences are particularly distinct between invaded foredunes and native dune mat (Table 3; Figure 3B and C). These differential invasion effects on foredunes may account for invaded foredunes being the only earlier successional habitat that did not group clearly on the left of PC1, but rather was shifted toward later successional habitats on the right (Figure 4), suggesting that invasion successionally ages foredunes. Accumulation of high amounts of OM increases CEC in highly invaded foredunes relative to that present in sparsely invaded ones (Figure 3L). However, OM decomposition generates acidic H^+ cations in both invaded foredunes and backdunes seemingly in excess of atmospheric contributions of base cations from fog, rainfall, and salt spray, leading to reduced pH, higher levels of acidic cations (Exc-H^+), and lower levels of exchangeable base cations such as K^+, Mg^{2+}, and Ca^{2+} (Table 3; Figure 3M–P). At lower pH values, acidic cations (H^+) preferentially bind to OM, displacing base cations such as K^+, Mg^{2+}, and Na^+ (Brady and Weil 1999; Figure 3M–O). Exchangeable base cations start becoming more important at a pH of 5 and increase considerably at pH 6, at which point Exc-H^+ (and Exc-Al^{3+}) concentrations taper off considerably (Brady and Weil 1999). Of the exchangeable base cations, only Exc-Na^+ did not appear affected by invasion (Tables 2 and 3).

While pH was moderately acidic in both invaded and native later successional habitats, NO_3^- levels remained much lower in the former than the latter (Tables 2 and 3; Figure 3F). Recent microbial studies at PRNS documented that invaded dunes supported significantly fewer nitrifying bacteria than sparsely invaded ones (Parsons et al. 2020b). Higher concentrations of NO_3^- in acidic later successional habitats (1.47 to 5.53 ppm) relative to circumneutral earlier successional ones (0.39 to 2.77 ppm) suggests that lower pH values are not constraining nitrification: even in low pH situations, nitrification can still proceed if enough Ca^{2+} and Mg^{2+} are present (Brady and Weil 1999). Anions such as NO_3^- and SO_4^{2-} would be expected to quickly leach out of sandy systems, as they do not bind to OM, but primarily to clay particles and oxides (Brady and Weil 1999). Dune soils at PRNS were uniformly low in clay (2%) and silt (3%; $n = 12$). Sulfate

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

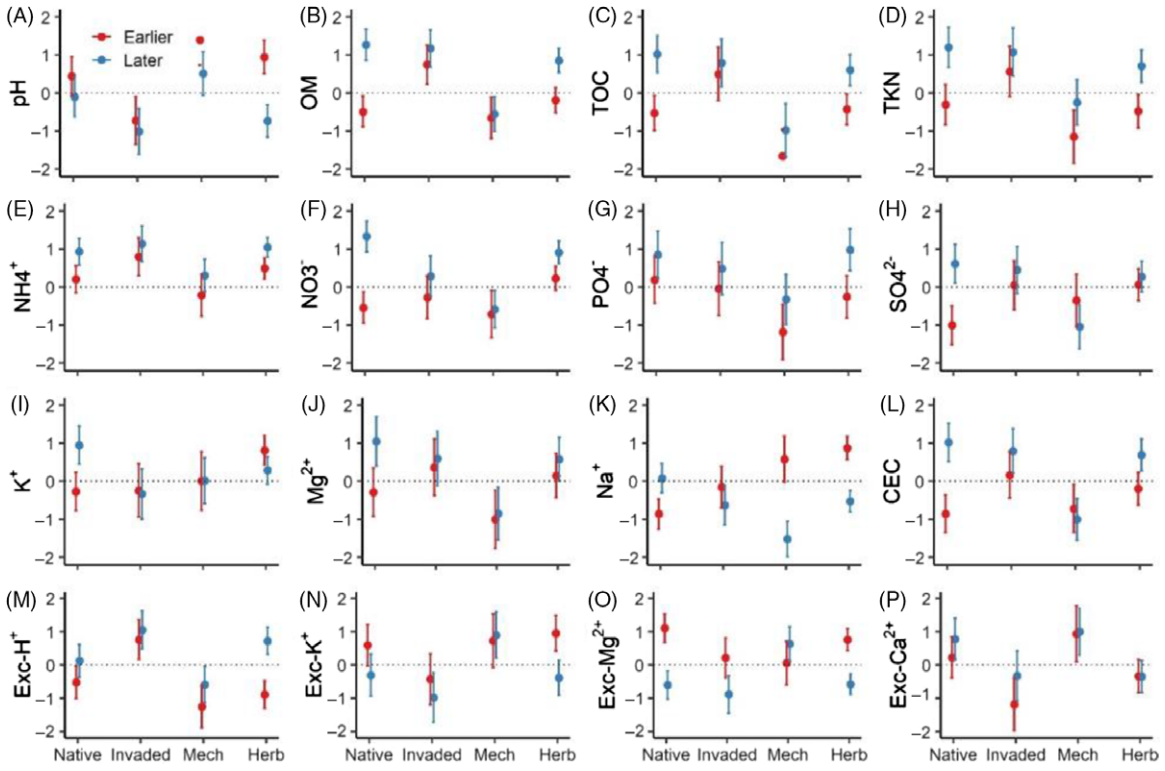


Figure 3. Individual GLMM effects plots for invasion status (*x* axis) by successional status (earlier/later) for selected soil chemistry variables. Plots present standardized means with 95% confidence intervals, with dashed line representing overall means for each variable standardized to 0.

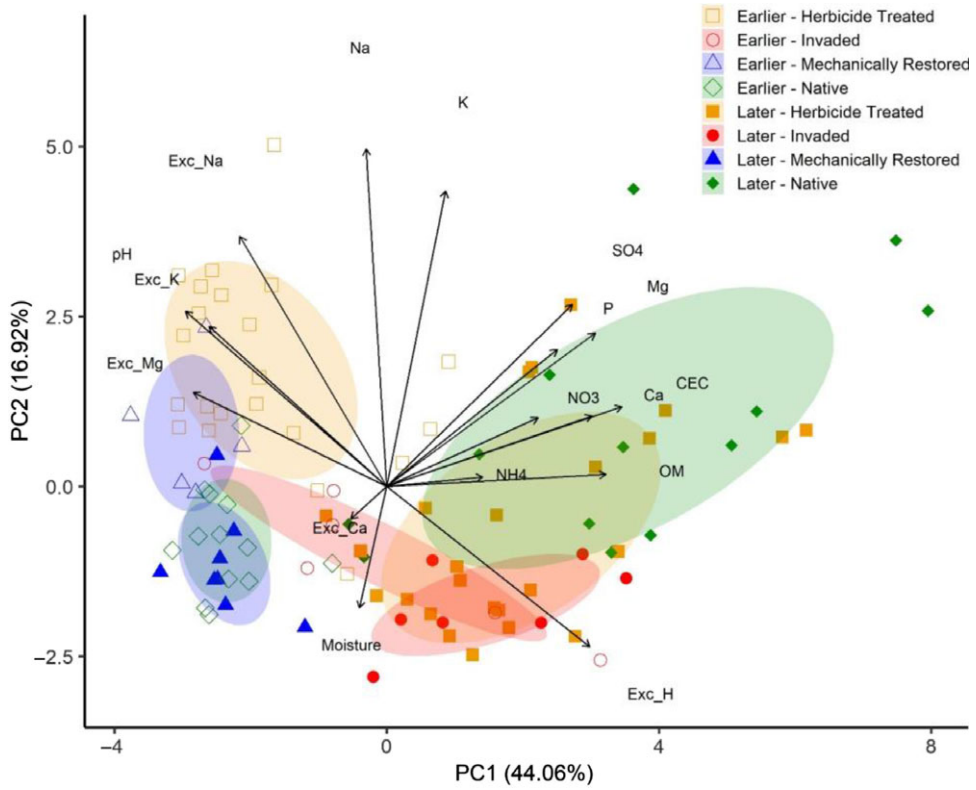


Figure 4. Principal components analysis (PCA) biplot depicting relationship of habitats of varying invasion status and successional stage and quantitative soil chemistry variables for 2019 and 2020. Scree plots indicate that two components captured most of the variance. The arrows point in the direction of maximum change of that variable, and their length is proportional to their correlation with the ordination axis and thus their relation to pattern of habitat variation. Ellipses represent 50% confidence intervals. Exc, exchangeable; OM, organic matter.

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

concentrations were higher in invaded foredunes than native earlier successional habitats, which may result from a combination of higher rates of production from OM decomposition and potentially lower rates of leaching than in other less densely vegetated sandy soils (Tables 2 and 3; Figure 3H).

P is typically a major limiting nutrient in dune and other terrestrial ecosystems, but as noted earlier, invasion did not appear to affect inorganic PO_4^-, despite it affecting related factors such as OM. While other studies have shown lower-pH dune habitats to have reduced concentrations of inorganic PO_4^- relative to higher-pH habitats (Kooijman et al. 2020), inorganic PO_4^- levels in later successional habitats at PRNS were substantially higher (7.3 to 25.7 ppm) than in circumneutral earlier successional ones (2.1 to 9.4 ppm; Figure 3G). In northern Europe, inorganic phosphate levels were lower in moderately acidic dunes than in higher pH calcareous ones, but levels of phosphates more weakly sorbed to OM and organic P—rather than to ferric oxides—increased, which rendered more PO_4^- available for plant uptake in lower-pH dunes (Kooijman et al. 2020).

While Exc-Mg^{2+} was lower in invaded soils, Mg^{2+} in soil solution was higher, at least in invaded foredunes (Tables 2 and 3; Figure 3J). As noted earlier, cations such as Mg^{2+} are probably largely supplied by meteorological sources. While appreciable differences in nutrient deposition would not be expected between early and late successional habitats with respect to fog or rainfall, a Point Reyes study found that sites 500 m inland received 20% to 50% of the salt spray volume received by seaward cliff-edge sites (Barbour 1978; Holton and Johnson 1979). Deposition rates of Ca^{2+}, Na^+, and Mg^{2+} from winter salt spray in central California coastal areas exceeded those of inland sites (2.2 km from coast) by as much as a factor of 4, while K^+ influx was roughly equivalent (Clayton 1972). Large amounts of these cations are taken up by plants (van der Valk 1974), with plant tissue concentrations roughly similar between inland and coastal sites despite differential deposition rates (Clayton 1972). Ionic patterns within soils reflect steady-state cycling of K^+, Mg^{2+}, and Ca^{2+} in California coastal systems, with root exudation and litterfall release increasing concentrations in surface soils, while sandy soils reduce concentrations at greater depths (1 to 2 m) through leaching (Clayton 1972). While deposition rates of cations may be higher in earlier successional habitats, denser, taller vegetation in later successional areas may better trap cations from meteorological inputs through foliar absorption and eventual turnover through root exudation or litterfall release, leading to higher levels of cations in these soils. Later successional native dunes in PRNS supported higher concentrations of K^+, Ca^{2+}, Mg^{2+}, and Na^+ in soils (Figure 3I–K).

Invaded foredunes may have deviated from this successional trend of higher base cations in later successional soils, because dense monocultures of *A. arenaria* in invaded foredunes enhance the potential for canopy interception, foliar absorption, and translocation of Mg^{2+} into roots or litter and eventually into soils. Soil Mg^{2+} levels in native dune scrub, which supports relatively dense stands of intermixed shrubs and forbs, appeared comparable to those of invaded backdunes (Figure 3J). Similar trends might have been expected with K^+, Ca^{2+}, and Na^+, but instead showed slightly different patterns. Invaded foredunes did have higher Na^+—if not K^+—concentrations than native dune mat, but invaded backdunes had much lower Na^+ and K^+ levels than native dune scrub (Tables 2 and ; Figure 3I–K). Ca^{2+} showed no effect with regard to invasion status (Tables 2 and 3).

Legacy Effects

Legacy effects were interpreted as invasion effects that did not dissipate following restoration, with soil conditions remaining comparable to those in earlier or later successional invaded habitats or both (Table 3). Restoration of formerly highly invaded *A. arenaria* habitats through either mechanical removal or herbicide treatment appeared to reverse only a few invasion effects. Of the 14 variables affected by *A. arenaria* invasion, roughly 60% (8) exhibited some type of legacy effect, with persistent differences in pH, CEC, NO_3^-, SO_4^{2-}, Mg^{2+}, Na^+, Exc-H^+, and Exc-Mg^{2+} (Tables 2 and 3). In half of the cases, differences persisted in only one type of successional habitat, even if conditions under invaded scenarios differed from native habitats for both types (Table 3).

Herbicide-treated areas were subject to 50% more chemical legacy effects than mechanical restoration ones, specifically for pH, SO_4^{2-}, Mg^{2+}, Na^+, CEC, and Exc-H^+, with effects split equally between both types of successional habitats or just later successional ones (Table 3; Figure 3). Legacy effects in soil chemistry following restoration are likely to be highly influenced by other invasion legacies, namely physical ones such as delayed decomposition of *A. arenaria* in herbicide-treated backdunes (Parsons et al. 2020b). At PRNS, approximately 50% of herbicide-treated *A. arenaria* in backdunes remained as undecomposed standing dead biomass 2 to 3 yr posttreatment (Figure 5A), with standing dead cover in the oldest site, Abbotts, not dropping to single-digit percentages until 6 yr after restoration (Parsons et al. 2020a; Figure 5B). Litter cover also stayed elevated after 6 yr (70%; Parsons et al. 2020a; Figure 5C), which may limit plant establishment by suppressing germination (Xiong and Nilsson 1999).

Some researchers have postulated that invasives can exert effects on soil chemistry by decomposing very quickly, which, for N-rich weeds, can create a pulse of inorganic N that attracts persistent nitrophilous weeds (Liao et al. 2008) such as those observed in the herbicide-treated backdunes (e.g., *B. diandrus*). Others have countered that decomposition rates of invasives are not necessarily faster than those of natives or, even if they are, do not necessarily influence nutrient cycling (Ehrenfeld 2003; Jo et al. 2016; Kueffer et al. 2008; Prescott and Zukswert 2016). In some instances, decomposition of invasives' litter may actually proceed more slowly than that of natives, particularly when lignin content of invasives is comparatively higher (Godoy et al. 2010; Windham 2001), with delayed decomposition often leading initially to immobilization and depletion of N from soils by microbes.

While no published information could be found on lignin content or other plant chemistry characteristics of *A. arenaria*, preliminary data from plant material collected in PRNS suggest that these factors cannot entirely account for the substantial decomposition delays (Parsons et al. 2020a). With the exception of C:N (70.8:1), values for cellulose, hemicellulose, lignin, total phenols, and silicon fell at the lower or, at most, intermediate range of those reported for other grass species (Parsons et al. 2020a). Annual grasses occurring in coastal prairie at Bodega, directly north of Point Reyes, had C:N ratios closer to 47.36:1, very similar to *L. arboreus* (47.74:1), with highest N mineralization occurring in Year 1 for grasses and Year 3 for *L. arboreus*, probably due to the higher lignin content of *L. arboreus* (14.7%; Bastow et al. 2008). A study on U.S. East Coast marshes found that both standing dead culms and litter of invasive common reed [*Phragmites australis* (Cav.) Trin. ex Steud.], which had a C:N ratio of 63:1, decomposed extremely slowly, with senescent material projected to take 9 yr to

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

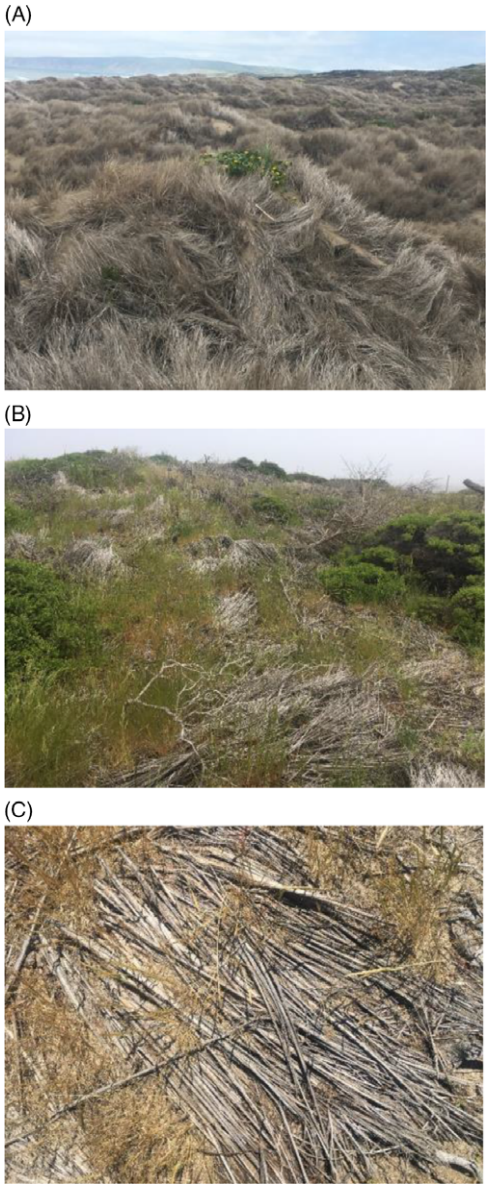


Figure 5. Representative photographs of (A) standing dead *Ammophila arenaria* biomass in 3-yr-old plot at AT&T South with sparse native colonization and *A. arenaria* regrowth; (B) remaining standing dead *A. arenaria* biomass in 7-yr-old herbicide-treated plot at Abbotts with increased colonization by native dune plants and secondary invaders such as nonnative annual grasses; and (C) *A. arenaria* "thatch" or large litter.

reach 10% of its original dry weight (Windham 2001). Based on our plant chemistry data, litter processing rates would be expected to decelerate, as microbes would need to scavenge N from soils to bring C:N ratios of *A. arenaria*—which otherwise contains low amounts of recalcitrant compounds—down to ratios more favorable for bacterial activity. Given that the N content of coastal dune soils is naturally low, at least moderate delays in decomposition should be anticipated due to N limitation, as has been documented in other nutrient-poor ecosystems (Godoy et al. 2010; Kueffer et al. 2008). Soil C:N ranged from 15.8 to 17.8 in herbicide-treated backdunes (Supplementary Table 1) compared with medians of 12:1 in most arable soils (Brady and Weil 1999), which supports the premise of reduced N and slower breakdown and accumulation of OM.

Soil chemistry—and physical—legacy effects appeared to be reversed much more quickly in herbicide-treated foredunes, probably due to frequent deposition of sand washed up onto adjacent beaches (Parsons et al. 2020a). Sand influxes thickly blanketed *A. arenaria* necromass within 4 yr (<10% standing dead cover, 18% litter; Parsons et al. 2020a), creating a new soil substrate largely freed from invasion effects (Table 3). Slow decomposition of buried *A. arenaria* litter may have continued, however, to fuel elevated SO_4^{2-} and Mg^{2+} concentrations in herbicide-treated foredunes and to maintain higher OM in soils that contributed to elevated CEC more consistent with invaded foredunes (Figure 3B, H, J, and L).

Without countering influences from frequent sand deposition, standing dead *A. arenaria* biomass and thatch in herbicide-treated backdunes are considerably more persistent, and slowly decomposing litter may have a more pervasive influence on soil chemistry. Gradual breakdown of persistent OM in herbicide-treated backdunes may continue to generate H^+ in excess of what could be countered by base ion influx, leading to higher levels of Exc-H^+ and continued reductions in pH (Table 3; Figure 3A and M). However, decomposition in herbicide-treated backdunes seemingly did not much alter N levels (TKN, NH_4^+) post-restoration (Table 3; Figure 3D and E). While recent microbial research at PRNS suggested that abundance of nitrifying bacteria remained low after herbicide treatment (Parsons et al. 2020b), NO_3^- concentrations actually increased in treated backdunes relative to invaded backdunes, becoming more comparable with levels in native dune scrub (Table 3; Figure 3F).

Based on NMS multivariate ordination of vegetation-related variables in herbicide-treated backdune plots, strong overlap existed between different treatment years (i.e., 2012, 2016, 2018), but plots with higher standing dead *A. arenaria* biomass and thatch cover characterized more recently treated plots (2018, some 2016; Figure 6). Standing dead biomass/thatch cover dropped in the oldest treated plots (2012), which were distinguished by higher cover of native species, including those representative of native dune scrub communities, as well as cover of nonnative and secondary invader species (Figure 6). Plots of intermediate treatment age had higher diversity of native and nonnative plant species, but lower total cover and possibly higher levels of detritus than older plots (Figure 6). Statistically significant higher levels of soil OM and N (TKN, NH_4^+) occurred in older plots, as shown in Figure 6 (permutation, $P < 0.05$), which may reflect eventual physical breakdown of standing biomass into surface litter and then into soil OM. It may also capture a shift in N cycling from immobilization—and possible depletion of N in more recently treated plots—to mineralization and increases in N in older treatment plot soils, as the C:N ratio of remaining litter slowly drops over time, and litter N is mobilized.

Fewer chemical ($n = 3$) and physical legacy effects persisted following mechanical removal (Table 3). By flipping surface soils with subsurface soils, mechanical removal in the foredunes exposed subsoils that may have been higher in SO_4^{2-} due to past leaching (Figure 3H). Inversion of topsoil and subsoil layers may also account for continued depletion relative to native habitats of NO_3^- in mechanically restored backdunes and of Exc-Mg^{2+} in mechanically restored foredunes (Figure 3F and O), possibly because most cations such as Mg^{2+} are leached out of subsoil layers, unlike Na^+ (Clayton 1972; Etherington 1967). As Figure 4 indicates, with the exception of Ca^{2+}, exchangeable cations such as Mg^{2+}, K^+, and Na^+ were generally higher in earlier successional habitats at PRNS despite the highest soil solution concentrations being in later successional soils (Figure 3N and O).

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

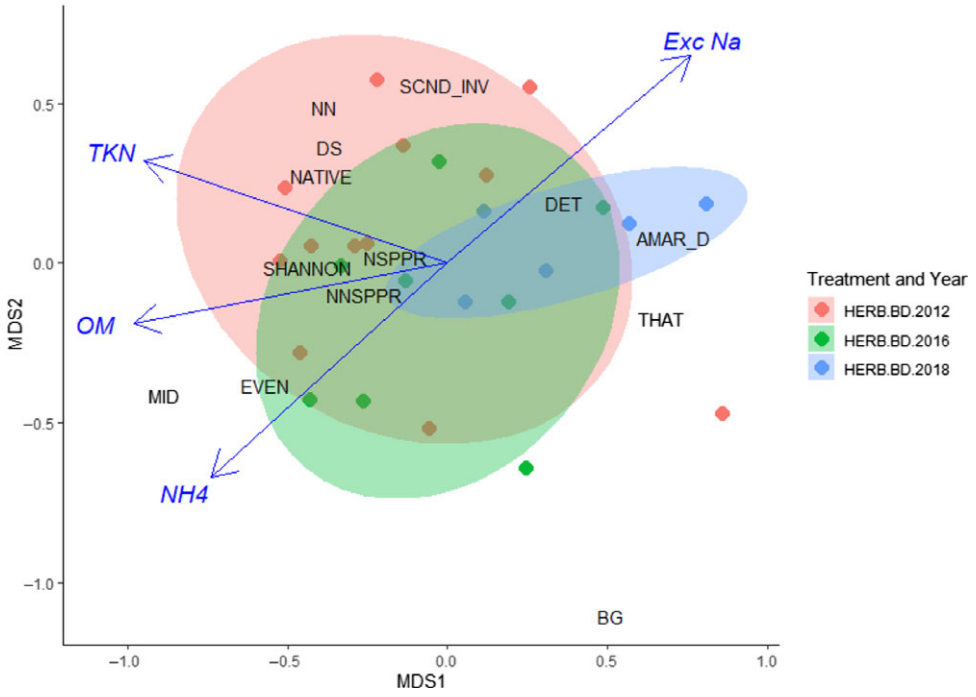


Figure 6. Non-metric multidimensional scaling (NMDS) biplot depicting relationship of vegetation-related community variables in herbicide-treated backdune areas of varying treatment age (2012, 2016, 2018) and statistically significant soil chemistry variables in 2019 and 2020. Statistically significant soil chemistry variables were identified through permutation tests. Ellipses represent 80% confidence intervals

Treatment Effects

Ultimately, the goal of restoration is to shift systems altered by invasion toward conditions—and processes—that more closely mimic those of target native habitats. This goal can certainly be derailed by lingering legacy effects of invasion, but restoration can change certain aspects of soil chemistry even if invasion did not. These treatment effects may, or may not, bring restored systems closer to re-creating desired conditions. At PRNS, native dune mat and dune scrub generally represent the target habitats for earlier and later successional areas, respectively.

Based on GLMM analyses (Table 3), both mechanically restored and herbicide-treated areas did not have soil chemistry similar to native habitats, regardless of time since restoration. Of the 19 soil chemistry variables assessed, 79% ($n = 15$) showed some evidence of a treatment effect in which soil conditions within restored areas not impacted by legacy effects were still dissimilar to those in native habitats (Table 3). While herbicide treatment areas have had to contend more with legacy effects, particularly in backdunes, mechanical restoration through "horizon flipping" has—not surprisingly—upended soil chemistry evolutionary trajectories in foredunes and even more so in backdunes relative to those of target successional communities. Almost 87% of treatment effects occurred in mechanical restoration areas. Our PCA ordination points to mechanically restored backdunes converging more with native dune mat than dune scrub, with PC1 and PC2 axes both showing strong overlap between these groups (Figure 4). Mechanically restored foredunes, meanwhile, are slightly offset from these two groups, positioning closer to herbicide-treated foredunes along PC2 (Figure 4). Mechanical removal essentially reset the successional clock, at least in the backdunes. It is possible that, over time, mechanically restored backdunes will evolve from a soil chemistry perspective, incrementally moving closer to conditions in native dune scrub communities, but the data are certainly not suggestive of any convergence at 8 to 9 yr post-restoration. In comparison, in herbicide-treated dunes, only 32% of variables evinced evidence of treatment effects, with most of these concentrated in foredunes (Table 3; Figure 3). Herbicide-treated foredunes had higher pH, NO_3^-, K^+, Na^+, and Exc-Na^+ than native dune mat, while herbicide-treated backdunes had lower K^+ and Ca^{2+} than native dune scrub (Table 3; Figure 3).

Year

Sampling year was identified as another important factor in the GLMM analysis, although it did not show any interaction with invasion or successional status. A number of variables showed a statistically significant relationship with sampling year, including OM, N (TKN, NH_4^+, NO_3^-), PO_4^-, K^+, Mg^{2+}, Na^+, and some exchangeable cations (K^+, Na^+; Table 2). In general, most variables exhibited higher concentrations in 2019 (OM, N, PO_4^-, K^+, Mg^{2+}, and Exc-K^+), while only Na^+ and Exc-Na^+ displayed higher levels in 2020.

The years 2019 and 2020 bracketed two extremes in California climatic variability, with 2019 having considerably above-average rainfall and 2020 having considerably below-average rainfall. Levels of organic and inorganic N and inorganic P (PO_4^-) seemingly flourished under the wetter climatic regime. Earlier research at PRNS indicated that rainfall could funnel as much as 1.6 kg N ha^{-1} yr^{-1} into coastal dune systems, with NH_4^+ contributions slightly higher (0.9 kg ha^{-1}) than NO_3^- (0.7 kg ha^{-1}; Holton et al. 1991). In addition to higher rainfall supplying more nutrients, higher soil moisture during wet years may also accelerate breakdown of OM, increasing organic N (TKN) and NH_4^+ and NO_3^- in years such as 2019. As discussed earlier, turnover of OM may play one of the largest roles in N and P cycling within coastal dunes.

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Holton et. al. (1991) estimated that, of the approximately 390 kg ha^{-1} total N budgeted for PRNS dunes, 78% of that was organic N in soils, with another 18% tied up in plant biomass, and the remaining 4% being inorganic.

Higher litter decomposition rates in wet years may also trigger pulses of K^+ and Mg^{2+} into soils, as K^+ concentrations within coyote brush (*Baccharis pilularis* DC.) sampled at both coastal and inland sites in central California were among the highest of any of cations, with Mg^{2+} third highest behind Ca^{2+} (Clayton 1972). During wet years, higher levels of K^+ and Mg^{2+} influx into soils from moisture-associated increases in decomposition would also be subsidized by increased deposition from rainfall and a higher number and intensity of storm-driven salt spray events (Clayton 1972; van der Valk 1974). While plant uptake of Na^+ is reduced compared with other cations, Clayton (1972) suggested that, in coastal California soils, it is less subject to leaching, at least at deeper depths. Less rainfall in 2020 may have resulted in less translocation of Na^+ to lower soil depths in PRNS dunes, while salt spray and fog deposition continued to maintain atmospheric inputs to the system.

Summer fog drip might be perceived as a boon for California's coastal systems during low-rainfall years such as 2020. At PRNS, N input from fog was almost 3 times higher (4.2 kg N ha^{-1} yr^{-1}) than that from rainfall (Holton et al. 1991). In contrast to trends for rainfall, concentrations of NO_3^- were much higher in fog than NH_4^+ (Holton et al. 1991; Jacob et al. 1985), resulting in contribution of approximately 1.4 kg ha^{-1} NO_3^- and 1.1 kg ha^{-1} NH_4^+ during the summer sampling period (Holton et al. 1991).

Another potential non–precipitation-related atmospheric source of N to coastal systems could be N fixation, which has been documented as a bigger contributor to N budgets than atmospheric deposition in Welsh dunes (Jones et al. 2008). N-fixing taxa in coastal dunes include not only nodulated legumes, but cyanobacteria in biological soil crusts (Jones et al. 2008) and *A. arenaria* and other dune grasses (*L. mollis*) that support N-fixing bacteria that are either endophytic (Oregon; Dalton et al. 2004) or free-living in the rhizosphere (e.g., Wales and northwest Europe; Abdel Wahab and Wareing 1980; Ruppel 1989). Based on Holton's work, N fixation contributed negligible N to PRNS dunes due to the relatively sparse abundance of N-fixing species such as lupines (*Lupinus* spp.) and beach pea [*Lathyrus littoralis* (Nutt.) Endl.], and there was no evidence of either N-fixing algae or endophytic or free-living bacteria in soil and roots of *A. arenaria* at PRNS (Holton et al. 1991). In systems such as those in Europe and Oregon, where *A. arenaria* is dominant and known to support N-fixing bacteria, N fixation could potentially represent a much larger contributor to the N budget than it may at PRNS.

Given that coastal dunes are heavily reliant on meteorological contributions from rain, fog, and salt spray for nutrient budgets, this system would seem particularly vulnerable to potential climate change effects. While models for Mediterranean regions generally predict a drier and warmer climate in the future, some models suggest that certain portions of northern and central California could become wetter (Maloney et al. 2014; Polade et al. 2017), with coastal areas turning windier, cooler, and either less or more foggy during warmer months (Lebassi et al. 2009; O'Brien 2011; Pozo Buil et al. 2021; Snyder et al. 2003; Sydeman et al. 2014). These disparities in modeling results create a high degree of uncertainty regarding future impacts of climate change on coastal dune systems. Regardless, some of these changes could alter and potentially exacerbate the impact that invasion of *A arenaria*, as well as *C. edulis*, have on soil chemistry.

In conclusion, invasion by *A. arenaria* strongly influences soil chemistry, altering conditions relative to native habitats for 74% (14) of the measured variables. Specifically, invasion appeared to successionally "age" invaded foredunes, edging them closer to later successional habitats in terms of chemistry. Almost 60% (8) of the invasion effects persisted following restoration, with legacy effects particularly prevalent in herbicide-treated backdunes where active sand deposition could not counter legacy effects of invasion as it did in herbicide-treated foredunes through burial of standing dead *A. arenaria* biomass, litter, and invasion-altered soils with new sand. Mechanical removal resulted in fewer legacy effects, but more treatment effects that left mechanically restored areas definitively dissimilar to native habitat counterparts. Only 5 of the 19 assessed variables in mechanically restored areas showed convergence with conditions in comparable native habitats. Removal of *A. arenaria* through inversion of surface and subsoil layers essentially restarted the successional timeline in mechanically restored backdunes, creating soil conditions more comparable with earlier successional native habitats. Soil chemistry appears to play only a minor role in delayed decomposition of *A. arenaria*, although litter breakdown may have been limited to some extent by low N in soils. Ultimately, N-mediated delays in microbial decomposition of the high C:N litter may have acted to ecologically "amortize," or incrementally stagger over time, the considerable impacts to nutrient cycling that would have otherwise been expected from more immediate breakdown of such a large volume of invasive biomass.

Invasive plant removal alone may not be sufficient to restore native ecosystems due to lingering physical, chemical, and biological legacy effects inherited from invaders (Corbin and D'Antonio 2012; Kardol and Wardle 2010; Suding et al. 2004). As we and others have observed, the type of legacy—and treatment—effects may be strongly linked to eradication technique, with effects differing depending on the type of invaded habitat being restored (Hetherington and Wilson 2019). At PRNS, herbicide treatment has yielded so far the best results for foredunes, and mechanical removal, perhaps the best for backdunes, but cost and indirect impacts associated with mechanical removal make future use of this method infeasible. Regardless of technique, however, additional restoration measures—or "interventions that mitigate biogeochemical and physical effects" such as soil amelioration or removal of biomass (Kardol and Wardle 2010; Suding et al. 2004; Wolfe and Klironomos 2005)—may be required to successfully restore PRNS dunes, particularly herbicide-treated backdunes. Ultimately, the primary constraint on decomposition within backdunes at PRNS is probably microbial, not chemical. Previous microbial research at PRNS has shown that the abundance of cellulolytic bacteria—or microbes involved in breakdown of cellulose in plant litter—dropped dramatically following restoration using herbicide treatment (Parsons et al. 2020b). Large reductions in celluloytic bacteria could stymie microbial efforts to process the extremely large volume of litter in treated *A. arenaria* monocultures, thereby helping to foster this physical legacy effect.

Future studies will attempt to further elucidate disconnects in the microbial decomposition process in herbicide-treated backdunes that may be hindering efforts to restore coastal dunes at PRNS. This information would then be used to design an adaptive restoration project. Decomposition could potentially be accelerated through introduction of whole-soil inoculum from native communities with higher expected decomposer abundance (e.g., dune scrub; Wolfe and Klironomos 2005.) Alternatively, demand

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

on decomposers could be reduced through pre- or post-restoration controlled burns. Until restoration approaches can be developed that integrate methods for resolving legacy effects, PRNS might best be advised to focus efforts on areas in which restoration yields more immediate benefits, such as herbicide treatment of foredune areas, where frequent sand deposition from adjacent beaches can almost instantaneously bury legacy effects. In the interim, vigilant, long-term retreatment of both primary and secondary invaders will be critical to restoration success.

Acknowledgments. We thank Michael Spaeth, Savannah Fuqua, and Jacob Longmeyer for assistance with field sampling. We thank P. Baye for information on dune morphology and J. Callaway for information on soils and for reviewing the draft article. Funding came from the U.S. National Park Service, as well as grants from nonprofit organizations (Point Reyes National Seashore Association, Point Reyes Station, CA, USA; Hanford Fund, Sonoma, CA, USA). Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government. No conflicts of interest have been declared.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2021.16

References

Abdel Wahab AM, Wareing PF (1980) Nitrogenase activity associated with the rhizosphere of *Ammophila arenaria* L. and effect of inoculation of seedlings with *Azotobacter*. New Phytol 84:711–721

Art HW, Bormann FH, Voigt GK, Woodwell GM (1974) Barrier Island forest ecosystem: role of meteorologic nutrient inputs. Science 184:60–62

Badalamenti E, Gristino L, Laudicina VA, Novara A, Pasta S, La Mantia T (2016) The impact of *Carpobrotus* cfr. *acinaciformis* (L.) L. Bolus on soil nutrients, microbial communities structure and native plant communities in Mediterranean ecosystems. Plant Soil 409:19–34

Barbour MG (1978) Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. Oecologia 32:213–224

Barbour MG, De Jong TM, Johnson AF (1976) Synecology of beach vegetation along the Pacific Coast of the United States of America: a first approximation. J Biogeogr 3:55–69

Barbour MG, De Jong TM, Pavlik BM (1985) Marine beach and dune plant communities. Pages 296–322 *in* Chabot BF, Mooney HA, eds. Physiological Ecology of North American Plant Communities. Dordrecht, Netherlands, Springer

Bastow JL, Preisser EL, Strong DR (2008) Wood decomposition following a perennial lupine die-off: a 3-year litterbag study. Ecosystems 11:442–453

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B 57:289–300

Brady NC, Weil RR (1999) The Nature and Properties of Soils. 12th ed. Englewood Cliffs, NJ: Prentice-Hall. 881 p

Buck-Diaz J, Sikes K, Evens JM (2021) Vegetation Classification of Alliances and Associations in Marin County, California. Draft. Sacramento: California Native Plant Society. 73 p

Clayton JL (1972) Salt spray and mineral cycling in two California coastal ecosystems. Ecology 53:74–81

Conser C, Connor EF (2009) Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. Biol Invasions 11:349–358

Corbin JD, D'Antonio CM (2011) Abundance and productivity mediate invader effects on nitrogen dynamics in a California grassland. Ecosphere 2:ar32:1–20

Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Sci Manag 5:117–124

Dalton DA, Kramer S, Azios N, Fusaro S, Cahill E, Kennedy C (2004) Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. FEMS Microbiol Ecol 49:469–479

Dangremond EM, Pardini EA, Knight TM (2010) Apparent competition with an invasive plant hastens the extinction of an endangered lupine. Ecology 91:2261–2271

D'Antonio CM, Mahall BE (1991) Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. Am J Bot 78:885–894

de la Peña E, de Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H (2010) Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biol Invasions 12:3637–3648

Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523

Ehrenfeld JG (2004) Implications of invasive species for belowground community and nutrient processes. Weed Technol 18:1232–1235

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol System 41:59–80

Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecol Appl 11:1287–1300

Eppinga MB, Rietkerk M, Dekker S, De Ruiter P, van der Putten WH (2006) Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114:168–176

Espejel I (1992) Coastal sand dune communities and soil relationships in the Yucatan Peninsula, Mexico. Pages 323–335 *in* Seeliger U, ed. Coastal Plant Communities of Latin America (Physiological Ecology). San Diego: Academic

Etherington JR (1967) Studies of nutrient cycling and productivity in oligotrophic ecosystems: I. Soil potassium and wind-blown sea-spray in a South Wales dune grassland. J Ecol 55:743–752

Godoy O, Castro-Díez P, Van Logtestijn RSP, Cornelissen JHC, Valladares F (2010) Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: a broad phylogenetic comparison. Oecologia 162:781–790

Goodman J (1982) Water Potential from Advected Fog. San Jose, CA: Department of Meteorology, San Jose State University Progress Report 1. 45 p

Gornish ES, Franklin K, Rowe J, Barberán (2020) Buffelgrass invasion and glyphosate effects on desert soil microbiome communities. Biol Invasions 22:2587–2597

Hesp PA (1991) Ecological processes and plant adaptations on coastal dunes. J Arid Environ 21:165–191

Hetherington JK, Wilson JB (2019) Short-term soil nutrient and plant community responses to the eradication of a nitrogen fixing tree, *Lupinus arboreus*. J Coastal Conserv 23:49–58

Holton B, Barbour MG, Martens SN (1991) Some aspects of the nitrogen cycle in a Californian strand ecosystem. Madroño 38:170–184

Holton B, Johnson AF (1979) Dune scrub communities and their correlation with environmental factors at Point Reyes National Seashore, California. J Biogeogr 6:317–328

Inderjit, Seastedt TR, Callaway RM, Pollock JL, Kaur J (2008) Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biol Invasions 10:875–890

Inderjit, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends Ecol Evol 25:512–519

Ingraham NL, Matthews RA (1995) The importance of fog-drip water to vegetation: Point Reyes Peninsula, California. J Hydrol 164:269–285

Jacob DJ, Waldman TJM, Monger JW, Hoffmann MR (1985) Chemical composition of fogwater collected along the California coast. Environ Sci Technol 19:730–736

Jo I, Fridley JD, Frank DA (2016) More of the same? In situ leaf and root decomposition rates do not vary between 80 native and nonnative deciduous forest species. New Phytol 209:115–122

Jones MLM, Sowerby A, Williams DL, Jones RE (2008) Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. Plant Soil 307:219–234

Kardol P, Wardle DA (2010) How understanding aboveground–belowground linkages can assist restoration ecology. Trends Ecol Evol 25:670–679

Konlechner TM, Lord JM (2015) Plant community response following the removal of the invasive *Lupinus arboreus* in a coastal dune system: community response following *L. arboreus* removal. Restor Ecol 23:607–614

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Kooijman A, Morriën E, Jagers op Akkerhuis G, Missong A, Bol R, Klumpp E, van Hall R, van Til M, Kalbitz K, Bloem J (2020) Resilience in coastal dune grasslands: pH and soil organic matter effects on P nutrition, plant strategies, and soil communities. Ecosphere 11:e03112

Koske RE, Polson WR (1984) Are VA mycorrhizae required for sand dune stabilization? BioScience 34:420–424

Kueffer C, Klingler G, Zirfass K, Schumacher E, Edwards PJ, Güsewell S (2008) Invasive trees show only weak potential to impact nutrient dynamics in phosphorus-poor tropical forests in the Seychelles. Funct Ecol 22:359–366

Lebassi B, González J, Fabris D, Maurer E, Miller N, Milesi C, Switzer P, Bornstein R (2009) Observed 1970–2005 cooling of summer daytime temperatures in coastal California. J Climate 22(13), 10.1175/2008JCLI2111.1

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW (2013) Severe plant invasions can increase mycorrhizal fungal abundance and diversity. ISME J 7:1424–1433

Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytol 177:706–714

Mack MC, D'Antonio CM, Ley RE (2001) Alteration of ecosystem nitrogen dynamics by exotic plants: a case study of C_4 grasses in Hawaii. Ecol Appl 11:1323–1335

Maloney E, Camargo SJ, Chang E, Colle B, Fu R, Geil KL, Hu Q, Jiang X, Johnson N, Karnauskas KB, Kinter J, Kirtman B, Kumar S, Langenbrunner B, Lombardo K, *et al.* (2014) North American climate in CMIP5 experiments: Part III: assessment of twenty-first-century projections. J Climate 27:2230–2270

Maron JL, Connors PG (1996) A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105:302–312

Maron JL, Jefferies RL (1999) Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80:443–454

Maron JL, Jefferies RL (2001) Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. Ecol Appl 11: 1088–1100

Muir JJ, Colwell MA (2010) Snowy plovers select open habitats for courtship scrapes and nests. The Condor 112:507–510

[NPS] National Park Service (2009) Abbotts Lagoon Area Dune Restoration Plan Environmental Assessment. Point Reyes Station, CA: Point Reyes National Seashore, National Park Service. 198 p

Niu H, Liu W, Wan F, Liu B (2007) An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. Plant Soil 294:73–85

Novoa A, González L (2014) *Impacts of Carpobrotus edulis* (L.) N.E.Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. PLoS ONE 9(9):e107557

Novoa A, González L, Moravcová L, Pyšek P (2012) Effects of soil characteristics, allelopathy and frugivory on establishment of the invasive plant *Carpobrotus edulis* and a co-occurring native, *Malcolmia littorea*. PLoS ONE 7(12):e53166

Novoa A, González L, Moravcová L, Pyšek P (2013) Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: implications for restoration. Biol Conserv 164:1–9

Novoa A, Rodríguez R, Richardson D, González L (2014) Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br. Biol Invasions 16:429–443

O'Brien TA (2011) The Recent Past and Possible Future Decline of California Coastal Fog. Ph.D thesis. Santa Cruz: University of California, Santa Cruz. 193 p

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GB, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R Package v. 2.5-6. https://CRAN.R-project.org/package=vegan. Accessed: January 16, 2020

Parsons L, Ender C, Ryan A (2020a) Coastal Dune Restoration Vegetation Monitoring Report. Point Reyes Station, CA: Point Reyes National Seashore, National Park Service. 128 p

Parsons, L, Sayre J, Ender C, Rodrigues JLM, Barberán A (2020b) Soil microbial communities in restored and unrestored coastal dune ecosystems in California. Restor Ecol 28(S4):S311–S321

Pearson DE, Ortega YK, Runyon JB, Butler JL (2016) Secondary invasion: the bane of weed management. Biol Conserv 197:8–17

Peterson RA (2019) Ordered quantile normalization: a semiparametric transformation built for the cross-validation era. J Appl Stat 47:2312–2327

Pickart AJ, Sawyer JO (1998) Ecology and Restoration of Northern California Coastal Dunes. Sacramento: California Native Plant Society. 152 p

Polade SD, Gershunov A, Cayan DR, Dettinger MD, Pierce DW (2017) Precipitation in a warming world: assessing projected hydro-climate changes in California and other Mediterranean climate regions. Sci Rep 7:10783

Pozo Buil M, Jacox MG, Fiechter J, Alexander MA, Bograd SJ, Curchitser EN, Edwards CA, Rykaczewski RR, Stock CA (2021) A dynamically downscaled ensemble of future projections for the California Current System. Front Mar Sci 8:612874

Prescott CE, Zukswert JM (2016) Invasive plant species and litter decomposition: time to challenge assumptions. New Phytol 209:5–7

Pringle A, Rillig MC, Gardes M, Klironomos J (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol Syst 40:699–715

R Core Team (2020) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org

Randall RE (1973) Calcium carbonate in dune soils: evidence for geomorphic change. Area 5:308–310

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytol 170:445–457

Rose SL (1988) Above and belowground community development in sand dunes. Plant Soil 109:215–226

Ruppel S (1989) Isolation and characterization of dinitrogen-fixing bacteria from the rhizosphere of *Triticum aestivum* and *Ammophila arenaria*. Dev Soil Sci 18:253–262

Santoro R, Jucker T, Carboni M, Acosta ATR (2012) Patterns of plant community assembly in invaded and non-invaded communities along a natural environmental gradient. J Veg Sci 23:483–494

Santoro R, Jucker T, Carranza ML, Acosta ATR (2011) Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter? Commun Ecol 12:234–240

Smith SM, Allen RB, Daly BK (1985) Soil-vegetation relationships on a sequence of sand dunes, Tautuku Beach, South-East Otago, New Zealand. J R Soc NZ 15:295–312

Snyder MA, Sloan LC, Diffenbaugh NS, Bell JL (2003) Future climate change and upwelling in the California Current. Geophys Res Lett 30:1823

Soil Conservation Service (1985) Soil Survey of Marin County California. Washington, DC: Soil Conservation Service, U.S. Department of Agriculture. 229 p

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol 4(5):e140

Suding KN, Gross KL, Houseman GR (2004) Alternative states and positive feedbacks in restoration ecology. Trends Ecol Evol 19:46–53

Sydeman WJ, García-Reyes M, Schoeman DS, Rykaczewski RR, Thompson SA, Black BA, Bograd S (2014) Climate change and wind intensification in coastal upwelling ecosystems. Science 345:77–80

van der Putten WH, Yeates GW, Duyts H, Schreck Reis C, Karssen G (2005) Invasive plants and their escape from root herbivory: a worldwide comparison of the root feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. Biol Invasions 7:733–746

van der Valk AG (1974) Mineral cycling in coastal foredune plant communities in Cape Hatteras National Seashore. Ecology 55:1349–1358

van der Watt E, Pretorius JC (2001) Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J Ethnopharmacol 76:87–91

Vilà M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, Lambdon P, Manca M, Médail F, Moragues E, Traveset A, Troumbis AY, Hulme PE (2006) Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. J Biogeogr 33:853–861

Vivrette, NJ, Muller CH (1977) Mechanism of invasion and dominance of coastal grassland by *Mesembryanthemum crystallinum*. Ecol Monogr 47:301–318

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

Weber E (2003) Invasive Plant Species of the World: A Reference Guide to Environmental Weeds. Wallingford, UK: CAB International Publishing. 548 p

[WRCC] Western Regional Climate Center (2020) Climate Data from Point Reyes RCA CA Weather Station. https://wrcc.dri.edu/cgi-bin/rawMAIN.pl?nvprca. Accessed: December 15, 2020

Willis JE, Yemm EW (1961) Braunton Burrows: mineral nutrient status of the dune soils. J Ecol 49:377–390

Windham L (2001) Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21:179–188

Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. BioScience 55:477–487

Woodhouse WW, Hanes RE (1967) Dune Stabilization with Vegetation on the Outer Banks of North Carolina. Washington, DC: U.S. Army Coastal Engineering Research Center Tech Memo No. 22. 53 p

Xiong S, Nilsson C (1999) The effects of plant litter on vegetation: a meta-analysis. J Ecol 87:984–994

Yelenik SG, D'Antonio CM (2013) Self-reinforcing impacts of plant invasions change over time. Nature 503:517–521.

Yelenik SG, Levine JM (2010) Processes limiting native shrub recovery in exotic grasslands after nonnative herbivore removal. Restor Ecol 18: 418–425

Downloaded from https://www.cambridge.org/core. U.S. Fish & Wildlife Services, on 13 Jul 2021 at 22:07:21, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2021.16

APPENDIX H. UNPUBLISHED PAPER BY PARSONS ET AL. (2022): "*ROLE OF MYCORRHIZA IN EUROPEAN BEACHGRASS INVASION IN CALIFORNIA COASTAL DUNES*"

EFFECTS OF EUROPEAN BEACHGRASS INVASION AND RESTORATION ON SOIL FUNGAL COMMUNITIES IN CALIFORNIA COASTAL DUNES

LORRAINE PARSONS, BEN YANG, SAVANNAH FUQUA, AND ALBERT BARBERÁN

MARCH 22, 2022 DRAFT

Introduction

Invasive plants can drastically alter the structure of ecological communities, potentially leading to a disruption of important symbiotic associations (Reinhart and Callaway 2006; Pringle et al. 2009; Inderjit and van der Putten 2010). There is increasing evidence that introduction of invasive plants into ecosystems impacts the soil microbial community (Parsons et al 2020a), including the abundance and associations of arbuscular mycorrhizal fungi (AMF) (Zubek et al 2016). Understanding whether these changes in AMF communities persist after the invasive species is removed is not well understood and is critical for restoring native plants that heavily rely on mycorrhizal associations.

Invasive plants are often non-mycorrhizal or facultatively mycorrhizal (Reinhart and Callaway 2006; Pringle et al. 2009) and their introduction can disrupt intact AMF networks, adversely impacting native plant species that are either dependent on or fare better with AMF (Reinhart and Callaway 2006; Pringle et al. 2009; Inderjit and van der Putten 2010). For example, garlic mustard (*Alliaria petiolata*), a non-mycorrhizal European weed, suppresses growth of native tree seedlings in U.S. forests by suppressing AMF and ectomycorrhizal (EM) associations (Stinson et al. 2006). Conversely, mycorrhizal invasives can hijack AMF already present in native habitats and form stronger symbiotic relationships than AMF have with resident species (Marler et al. 1999; Pringle et al. 2009; Zhang et al. 2010; Lekberg et al. 2013). In some cases, invasives may even introduce non-native mycorrhizal species when mycotrophic weeds are actively transplanted into non-native ranges (Pringle et al. 2009).

The consequences of invasion for AMF networks are especially important in strongly nutrient limited systems, where AMF enhance inorganic phosphorous (P) and nitrogen (N) uptake and in some instances account for up to 70% of P obtained by plants (Koske and Polson 1984; Kooijman et al. 2020). Coastal dune systems tend to be strongly nutrient limited, especially for the essential nutrients N and P and, to a lesser extent, potassium (K^+; Willis and Yemm 1961; Woodhouse and Hanes 1966; Koske and Polson 1984). Strong nutrient limitation accentuates the importance of forming mycorrhizal associations for many dune species across the world (Koske and Halvorson 1981; Ernst et al. 1984; Logan et al. 1989; Stürmer and Bellei 1994; Beena et al. 2000; Rodríguez-Echeverríaa et al. 2007). AMF accounted for 30% of the total soil microbial biomass in Swedish coastal dunes (Olsson and Wilhelmsson 2000). In the U.S., AMF associations have been documented for most coastal dune species, including those in other northern California systems, with percent root colonization ranging from 5- to 90% (Koske and Polson 1984; Rose 1988; Pickart et al. 1990).

European beachgrass (*Ammophila arenaria*) has been introduced to many dune systems outside its native range in Europe, including the U.S. Pacific coast, where it has exerted tremendous direct and indirect adverse impacts on native ecosystems, including wholescale displacement of native plants and conversion to beachgrass monocultures (Barbour et al. 1976; Wiedemann and Pickart 1996; Vilà et al. 2006). Within its home range, European beachgrass is characterized as facultatively mycorrhizal, with root colonization rates averaging 23- 51% (Giovanetti and Nicholson 1983; Tadych and Blaszkowski 1999; Kowalchuk et al. 2002; de la Peña et al. 2006; Rodríguez-Echeverría et al. 2007). Much higher rates of AMF colonization appear to occur in foredunes (76%) than backdunes (26%; Kowalchuk et al. 2002), with AMF

potentially critical to beachgrass establishment (Read 1989). In European dune systems, higher AMF infection rates may also protect beachgrass against root-feeding nematodes (de la Peña et al. 2006).

Mycorrhizal status of European beachgrass within invaded habitats is less well-documented. In Chilean dunes, AMF colonized non-native European beachgrass in both foredunes and secondary dunes (i.e., fixed dunes behind foredunes), with colonization rates higher in the latter contrary to trends documented in European systems (Gonzalez et al. 1995). In a 2012 study conducted in Oregon dunes, AMF colonization of *Ammophila arenaria* roots was 46.8%, falling within the range of rates seen in its home range (David et al. 2016). Interestingly, the native plant *Elymus mollis* (American dunegrass) showed much higher rates of AMF colonization (74.5%, David et al. 2016), highlighting the potential for important consequences of beachgrass invasion on AMF communities in this system.

Point Reyes National Seashore (PRNS), a unit of the U.S. National Park Service (NPS), contains some of the highest quality remaining coastal dune habitat in the U.S. Foredune and backdune habitat at PRNS, however, has been seriously threatened by encroachment of European beachgrass and iceplant (*Carpobrotus* spp.) since being planted for dune stabilization in the early 1900s. Concerned about the impact on native dune habitats and rare plant and wildlife species, PRNS embarked in 2001 on an extensive restoration program, with 110 hectares of invasive European beachgrass and 11 hectares of iceplant removed from invaded dune habitat over the past 20 years.

We investigated soil fungal community composition of native, invaded, and restored coastal dune habitats in PRNS, with a particular focus on AMF communities. We hypothesized that (1) invasion by beachgrass would decrease AMF abundance and diversity and (2) these

changes would persist following restoration, creating a legacy effect that could impede future restoration efforts. To better understand the interactions between beachgrass and soil fungi in this system, we collected beachgrass roots from foredunes and backdunes in an invaded area in PRNS and determined percent fungal colonization rates.

Materials and Methods

Study site

PRNS is located approximately 48 km north of San Francisco, California, USA (Fig. 1). Dunes at PRNS are composted of a modern largely continuous foredune ridge established by current and past European beachgrass-dominated vegetation and a complex series of undulating backdunes and depressions associated with an older, mostly stabilized parabolic dune field.

The climate at Point Reyes is Mediterranean, with hot/dry summers and cool/wet winters. Coastal areas in California are generally cooler and have less intra-annual variability in temperature than inland regions, with fog, winds, and salt spray being important climatic influences and sources of nutrients to soils (Clayton et al. 1972; van der Valk 1974; Jacob et al. 1985; Holton et al. 1991; Ingraham and Matthews 1995). Annual temperature is estimated to average 12.1°C, and precipitation near the project area is estimated to average 54 cm per year (2006-2020; Western Regional Climate Center (WRCC), Pt Reyes RCA), although there can be dramatic variability between years in rainfall totals. Fog blankets the Point Reyes coast about 30% of the time between June and October (Goodman 1982 *in* Ingraham and Matthews 1995), generating anywhere from 1.0 to 5.2 L site^{-1} yr^{-1} of additional moisture during what is otherwise California's driest period (June-Oct; Ingraham and Matthews 1995).

PRNS dunes represent a range of successional stages from early to mid-successional communities such as foredunes and native Dune Mat, often characterized as "yellow dunes," to later successional ones such as backdunes ("grey" or "brown" dunes) and native Dune Scrub. Native Dune Mat often occurs closer to the ocean (Fig. 2A), while native Dune Scrub and backdunes are usually situated further inland (>300 m) from the coast (Fig. 2B; Parsons et al. 2020b). Both uninvaded foredunes and Dune Mat are sparsely vegetated, largely open sand habitats supporting herbaceous communities, including the following primary successional Dune Mat species, American dunegrass, sand verbena (*Abronia latifolia*), and beach bur-sage (*Ambrosia chamissonis*), as well as mid- to late successional ones such as seaside daisy (*Erigeron glaucus*), and coast buckwheat (*Eriogonum latifolium*; Buck-Diaz et al. 2021). Dune Scrub is a dense, shrub-dominated habitat characterized by species such as chamisso lupine (*Lupinus chamissonis*), mock heather (*Ericameria ericoides*; Buck-Diaz et al. 2021), which at PRNS is often intermixed with coyotebrush (*Baccharis pilularis*) and bush lupine (*Lupinus arboreus*).

A range of invasion conditions exist within PRNS dunes from sparsely to heavily invaded, with the highly clonal nature of European beachgrass and iceplant favoring eventual establishment of dense monocultures with cover often exceeding 98% (Fig. 2D; Parsons et al. 2020b). Some invaded systems retain vestiges of native Dune Mat or Dune Scrub that have either not been invaded or only very sparsely invaded by iceplant and a few other non-natives despite being surrounded by beachgrass or iceplant for decades (Parsons et al. 2020b; Fig. 2A). The largest native Dune Mat habitat in PRNS is found at Abbotts Lagoon (~20.8 hectares; "Abbotts"), although there are also smaller Dune Mat areas at AT&T (~1 hectare; "ATT") and B Ranch North (6.1 hectares; "BRN"; Fig. 1). Native Dune Scrub is prevalent at both Abbotts and

ATT, although the most intact stands in terms of low non-native cover occur at ATT North (Fig. 1).

To date, most of the park's restoration efforts have focused on removing European beachgrass and, to a lesser extent, iceplant using a combination of manual removal, mechanical removal, and chemical treatment, with the latter used more commonly in recent years. Initial chemical treatment of European beachgrass is performed using herbicide backpacks with a single nozzle wand and directed spraying of a mixture of 1% imazapyr (Habitat®, IPA, 27.77% ai, SePRO, Carmel, IN); 2% glyphosate (Roundup Custom®, IPA, 53.8%, Monsanto, St. Louis, MO); 1.5% of a modified vegetable oil surfactant (Competitor®, 98.0%, Wilbur-Ellis Co., Fresno, CA); and 1% blue dye (various manufacturers) onto target invasive plants. PRNS elected -- and received approval -- to use aquatic-label herbicides (e.g., Habitat®) to minimize toxicity within sensitive dune habitats, even though they are not aquatic.

Soil sampling – Microbial community analyses - 2017

In 2017, microbial communities in dune soils were sampled across a gradient of invasion intensity, from sparsely invaded (Dune Mat with <5% cover of invasives, primarily iceplant; Fig. 2A) to moderately invaded ((~25 to 50% cover of iceplant and European beachgrass; Fig. 2C) to heavily invaded (>75% cover of invasives, primarily beachgrass; Fig. 2D), along with foredune and backdune former beachgrass areas that had been restored using herbicide treatment (Fig. 2E-2F; Table 1). The sampled sites included ATT/North Beach ("ATT/NB"; 122°57'51.737"W 38°5'47.147"N) and BRN (123°0'0.289"W 38°1'12.915"N; Fig. 1). In general, two- to three subsites or plots were selected within each of these four sampling categories (three levels of invasion intensity + herbicide-treated) at the two sites (Table 1). At each subsite, four surface soil cores (~7 cm depth) were collected in the peak growing season (April 2017). Composite soil

samples were homogenized, sieved, placed in plastic bags, and frozen in the field until samples could be transported on ice at -20°C to the laboratory for storage at -80°C until analysis.

Soil sampling – Microbial community analyses - 2020

Follow-up research on microbial communities focused on sampling backdune habitats of varying invasion status at two sites: ATT/NB and another restored dune system directly to the north, Abbotts (122°57'33.46"W 38°6'36.272"N; Fig. 1). Because this sampling effort specifically targeted backdunes, we did not sample BRN, because there are much fewer hectares of beachgrass-invaded and herbicide-treated backdunes at this site. Habitats sampled included: heavily beachgrass-invaded backdunes; herbicide-treated backdunes of varying ages or time since initial treatment, and sparsely invaded native Dune Scrub (Fig. 2B; Table 1). At the northern restoration areas (ATT, Abbotts), treatment projects occurred in a spatially sequential manner starting in the north with Abbotts (2011-2015) and then moving south to ATT North (2016) and ATT South (2017-2018; Fig. 1, Table 1). Most of the remaining heavily invaded beachgrass areas primarily occur at the very southern portion of ATT/NB, south of current restoration efforts, although a few isolated stands of beachgrass-invaded backdunes persist within restoration areas where non-treatment buffers to organic pastures were established.

Thirty soil samples were collected from five subsites in heavily invaded backdunes (NB/ATT South); five subsites each in herbicide-treated areas initially treated in 2012 (Abbotts; “8-YR”) and 2016 (ATT North; “4-YR”); four subsites herbicide-treated in 2018 (ATT South; “2-YR”); and six subsites in native Dune Scrub (ATT North; Table 1). Within subsites or plots, surface soils were collected in September 2020 using a sterilized trowel to a depth of 10 cm, with multiple cores taken using a cross-diagonal sampling pattern. Sampling location varied in proximity to plants, although sampling avoided collection of dense roots. Composite soil

samples were homogenized, placed in plastic bags, and transported via backpacks in contractor bags with multiple icepacks until samples could be transferred to coolers in our vehicles. Once out of the field, samples were frozen until they could be shipped overnight using dry ice to University of Arizona (Tucson, AZ, USA), where they were stored at -80°C until analysis.

Molecular analyses – Microbial community sampling

We used high-throughput amplicon sequencing methods to describe the soil microbial communities based on taxonomic markers. Microbial DNA was first extracted using QIAGEN DNeasy PowerLyzer PowerSoil kits according to manufacturer instructions. We sequenced the first internal transcribed spacer (ITS1) region of the rRNA operon, using the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primer pair (Schoch et al. 2012). Primers included Illumina adapters and unique error-correcting 12-bp barcodes. PCR products were quantified and pooled in equimolar concentrations for sequencing on an Illumina MiSeq at the University of Arizona.

Sequence processing – Microbial community sampling

After demultiplexing using idemp (https://github.com/yhwu/idemp), raw reads were subject to quality filtering, dereplication, chimera detection, and merging of paired-end reads using dada2 (Callahan et al. 2016). Unique sequence phylotypes were assigned taxonomic affiliation using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang et al. 2007) trained on the ITS UNITE database (Abarenkov et al. 2010). We removed potential contaminants by discarding phylotypes present in the control blanks. Finally, we inferred putative functions using FUNGuild (Nguyen et al. 2016). Sample information, phylotype abundance tables, and representative sequences of 2017 data are publicly available in FigShare

(https://doi.org/10.6084/m9.figshare.9036740.v1). Sequence data of 2020 samples have been deposited in the NCBI Sequence Read Archive under BioProject accession code PRJNA795998.

Root and soil sampling – AMF root colonization and soil spore

On October 22, 2020, PRNS sampled relatively dense (>80% gross cover) European beachgrass stands at the very southern end of ATT South and the northern portion of NB (Fig. 1). Three transects were set up within these areas at an orientation perpendicular to the ocean/beach – two in ATT South and one in NB. Sampling locations were selected using a random stratified method to collect beachgrass from foredune, "mid-dune" (essentially halfway between foredunes and backdunes), and backdunes. Using a sterilized long-bladed shovel, holes were dug, and both root and soil samples were collected at each location. Roots collected for analysis were selected to represent both larger and smaller roots collected within the rhizophere of beachgrass plants. A total of nine root samples and nine soil samples were collected from foredunes (3), mid-dunes (3), and backdunes (3). The samples were placed in contractor bags with ice to keep them cool during transport and brought back to the office and later shipped to University of California, Riverside (UCR; Riverside, CA, USA), for analysis.

Laboratory analyses – AMF root colonization and soil spore

PRNS collaborated with the laboratory of Dr. Sydney Glassman at UCR on analyses of AMF and non-AMF root colonization and spore abundance. In December 2020, UCR performed analyses of root material from European beachgrass to 1) determine whether roots were colonized by AMF and non-AMF species and 2) determine the percentage of roots colonized by fungal taxa. The laboratory stained the beachgrass roots, mounted 10 pieces of stained root on microscope slides, and then, under 200x magnification, analyzed 10 cross sections per root for fungi. Due to time constraints, only seven of the nine root samples were evaluated, with

backdune locations along Transects 2 and 3 not being processed. However, all soil samples were analyzed. Spores were extracted from soil samples, mounted on slides, and evaluated under the microscope to determine density and number of morphotypes. Representative photographs were taken of the stained roots and spores.

Statistical analyses

Due to small sample size, we could not ensure assumptions for use of parametric statistical analysis techniques were met. Therefore, we used the non-parametric resampling method, permutation, with 5000 randomizations of the observed responses using the lmPerm package (Wheeler and Torchiano 2016) in the R environment (www.r-project.org). We analyzed the 2017 and 2020 microbial data separately, because the 2020 sampling effort was structured very differently than the 2017 one, with only backdune habitats assessed in 2020. For the 2017 analyses, both site (ATT/NB, BRN) and invasion intensity (sparsely invaded native Dune Mat and moderately invaded, heavily Invaded, and herbicide-treated dunes) represented fixed factors. The sample size was too small to incorporate an interaction term between these fixed factors, so invasion intensity was analyzed independently for each site. For the 2020 analysis, the fixed factor invasion status (sparsely invaded native Dune Scrub, heavily invaded backdunes, and herbicide-treated backdunes in 2012, 2014, and 2016) was nested within site (Abbotts, ATT North, ATT South). Tukey HSD post-hoc analyses were run in the lmPerm package. We corrected for multiple testing using false discovery rate (FDR) correction (Benjamini & Hochberg 1995). As the root colonization and soil spore study was considered exploratory, analysis is restricted to presentation of summary statistics.

Results

Microbial community analyses – 2017

Trends in AMF abundance varied by site. At BRN, we detected a non-significant trend of lower AMF abundance in moderately to heavily invaded dunes compared to sparsely invaded dunes ($p = 0.22$, Fig. 3A, Table 2). Conversely, at ATT/NB, AMF abundance was significantly higher in moderately to heavily invaded habitats ($p < 0.0007$; Table 2; Fig.3A) than in sparsely invaded native Dune Mat, where AMF were not detected ($\bar{X} = 0.00$). At ATT/NB, herbicide treatment appeared to reverse changes caused by invasion, with AMF abundance dropping relative to invaded areas (all $P < 0.00004$) and becoming statistically equivalent to native Dune Mat ($P=0.57$; Table 2; Fig. 3A).

We detected three major AMF taxa in our samples, *Glomeromycetes, Paraglomeromycetes,* and *Archaeosporomycetes*, as well as the following genera: *Acaulospora, Archaeospora*, *Claroideoglomus*, *Diversispora*, *Glomus*, *Paraglomus*, *Rhizophagus*, and *Scutellospora*. All three major taxa were present in moderately to heavily invaded dunes at ATT/NB, with Glomeromycetes ($P=0.0014$) and Paraglomeromycetes ($P=0.04$) being significantly elevated relative to native dunes (Table 2; Fig. 3C-D). Differences in Archaeosporomycetes abundance among dunes at ATT/NB of varying invasion status was only weakly significant ($P=0.09$; Fig. 3B). At BRN, abundances of Archaeosporomycetes and Paraglomeromycetes varied according to invasion status (all $p < 0.005$), whereas Glomeromycetes abundance did not significantly vary across habitats ($p = 0.26$). Archaeosporomycetes showed higher abundance in heavily invaded compared to sparsely or moderately invaded habitats (all $p < 0.0003$; Table 2; Fig. 3B). In contrast, Paraglomeromycetes was more abundant in sparsely invaded native Dune Mat than in moderately to heavily invaded habitats (all $P< 0.02$; Table 2; Fig. 3D).

Within herbicide-treated areas, restoration again reversed some of these changes, with abundance of Paraglomeromycetes increasing ($P=0.04$) and Archaeosporomycetes decreasing ($P<0.0001$) at BRN relative to heavily invaded habitats and becoming statistically equivalent to sparsely invaded dunes (all $P>0.18$; Table 2; Fig. 3B and 3D). At ATT/NB, abundance of Glomeromycetes and Paraglomeromycetes dropped relative to beachgrass-infested dunes following herbicide treatment (all $P<0.02$), with one exception (Paraglomeromycetes; Heavily invaded = Herbicide-treated; $P=0.44$; Fig. 3C-D). Following treatment, abundance of these taxa in herbicide-treated dunes became similar to that in sparsely invaded dunes (all> P.0.76; Table 2; Fig. 3C-D).

Presence of fungal endophytes also showed significant differences between habitats of varying invasion intensity at ATT/NB ($P=0.0038$), as well as possibly at BRN ($P=0.08$; Fig. 3E). As with AMF, trends in endophyte abundance at BRN and ATT/NB were strikingly different. At BRN, abundance of endophytes in sparsely to moderately invaded dunes appeared much higher than in heavily invaded dunes (Table 2), although only differences between moderately and heavily invaded habitats showed marginal significance ($P=0.08$; Fig. 3E). At ATT/NB, endophyte abundance remained highest in moderately to heavily invaded habitats and lowest in sparsely invaded (all $P<0.09$; Table 2; Fig. 3E). With restoration, endophyte abundance appeared to revert towards pre-invasion levels at both sites. At ATT/NB, numbers dropped dramatically within moderately to heavily invaded dunes following herbicide treatment (all $P\leq 0.01$) and became statistically equivalent to those in native Dune Mat ($P=0.84$; Table 2). At BRN, we detected no significant differences in endophyte abundance between habitats, with all herbicide-treated areas statistically equivalent to sparsely, moderately, and heavily invaded dunes

(all $P > 0.42$). Primary taxa identified during microbial analyses included putatively pathogenic genera (*Capronia* and *Leptodontidium*).

Microbial community analyses – 2020

In our follow-up study focused on backdune habitats at northern restoration sites, native Dune Scrub and herbicide-treated habitats all showed lower AMF abundances (all $p < 0.004$; Fig. 4A) compared to heavily invaded beachgrass areas ($p = 0.0038$; Table 3; Fig. 4A).

We detected the same dominant AMF taxa as well as specific AMF genera as in our 2017 study. Changes in dominant taxa were more difficult to tease apart in the 2020 study than in the 2017 one. Abundance of Paraglomeromycetes did not differ between backdune habitats ($P=0.87$; Fig. 4D), but numbers of Glomeromycetes did ($P=0.01$), with invaded backdunes having seemingly higher abundance of Glomeromycetes than uninvaded ones ($P=0.10$; Table 3; Fig. 4C). Beachgrass-dominated backdunes also appeared to have higher numbers of Archaeosporomycetes (Fig. 4B; Table 3), although differences were not statistically significant ($P=0.12$). As with the earlier study, herbicide-treated dunes appeared to quickly revert to pre-invasion levels of Glomeromycetes after treatment (Fig. 4C; Table 3), with herbicide treated habitats being statistically equivalent to uninvaded habitats ($p = 0.99$; Fig 4C).

Uninvaded native Dune Scrub appeared to have low abundance of other fungal endophytes (Fig. 4E) compared to invaded dunes (Fig 4E; Table 3). However, the only statistically significant difference detected was between 8-YR herbicide treatment and uninvaded dune scrub habitats ($p = 0.04$); differences between 8-YR herbicide and other restored dune habitats were marginally significant ($p < 0.08$; Table 3). Primary taxa identified included pathogenic, as well as non-pathogenic, genera, including *Capronia* and *Cadophora*.

Beachgrass root colonization and soil spore analyses – 2020

Mycorrhizal colonization of beachgrass roots averaged 79.3 ±1.3% (SD), ranging narrowly between 78.0% in the backdunes to 80.7 ± 7.0% in the mid-dunes (Table 4; Fig. 5). Root colonization results for backdunes need to be interpreted cautiously, as the two other backdune root samples were not evaluated. Slightly higher variability in AMF root colonization rates appeared to exist between sampling transects, with average colonization rates ranging from 70.5 ± 5.0% (ATT-S-2) to 87.0 ± 9.9% (NB-1; Table 5).

Non-AM endophytic fungi had also colonized beachgrass roots in these dune areas, although colonization rates were seemingly lower overall and more variable spatially (Tables 4-5; Fig. 5). Non-AMF colonization averaged 33 ± 16.3%, ranging from a low of 21 ± 14.1% in the foredunes to potentially a high of 52% in the backdunes (Table 4). Strong variability in non-AM fungi was also evident between sampling transects, with the NB-1 having the lowest colonization rate and ATT-S-2 having the highest colonization rate (Table 5).

Within soil samples collected in the root zone, there was also strong variability in the total count of AMF and non-AMF spores. Spore count averaged 2.16/g soil but ranged from 1.36 ± 0.63/g soil in the backdunes to 3.64 ± 4.85/g soil in the mid-dunes, largely based on a very high count in the ATT-S-2 mid-dune sampling site (Tables 4-5). Within sampling transects, spore count ranged considerably from 0.68 ± 0.11/g soil at NB-1 to 4.57 ± 4.08/g soil at ATT-S-2 (Table 5). Nine different morphotypes were identified using microscopy on spores. Both ATT-S-1 and NB-1 supported only two morphotypes that were moderately abundant. Six different morphotypes occurred in the ATT-S-2 transect.

Discussion

Our results suggest that invasion by *Ammophila arenaria* can alter soil fungal communities. The effects of invasion on AMF abundances varied by site, which may relate to the successional status of these native communities. Sparsely invaded Dune Mat at ATT/NB supports more primary to mid-successional species, while that at BRN supports late-successional Dune Mat species (Parsons et al. 2020b). AMF colonization rates of native dune species have not been evaluated at PRNS, but similar species occurring at dunes at Humboldt in northern California did not show great disparity between colonization rates for primary/mid (5- 19%) and late successional (12- 22%) Dune Mat species (Pickart et al. 1990), almost all of which also occur at PRNS. Differences may also involve spatial or geographic considerations, as BRN is considerably south of ATT/NB and has a different microclimate as well as probably geologic history. Future work is needed to uncover the underlying processes causing these geographic differences in beachgrass invasion effects.

While invasion reduced abundance of certain AMF taxa (Paraglomeromycetes) at BRN, it showed the opposite effect at ATT/NB, with higher abundance of both Paraglomeromycetes and, to a lesser extent, Glomeromycetes in moderately to heavily invaded dunes compared to sparsely invaded dunes. On the other hand, Archaeosporomycetes increased in heavily invaded areas compared to sparsely invaded dunes at both BRN and ATT/NB. Interestingly, unlike at ATT/NB, Archaeosporomycetes remained low in moderately invaded dunes at BRN. This difference could be due to a high presence of iceplant in moderately invaded dunes at BRN, as iceplant is considered non-mycorrhizal (Conser and Connor 2009, de la Peña et al. 2010) and has been documented to have substantial effects on both soil chemistry and microbia once established (Vilà et al. 2006; Conser & Connor 2009; D'Antonio and Mahall, unpub. data *in* Conser and Connor 2009; Novoa et al. 2013; Badalamenti et al. 2016).

We found that the effects of beachgrass on AMF communities did not persist after beachgrass was treated. Restoration using herbicide appeared to rapidly reverse AMF changes at both sites, with abundance becoming more comparable to that within native dunes at each site, sometimes within two years after treatment. Our restoration treatment has the potential to affect AMF communities both directly, through effects of herbicide chemicals on AMF, and indirectly, through changes in the aboveground plant community. Previous studies have found that application of glyphosate can reduce viability and amount of AMF spores in the soil, although it is unclear how long these effects last following application (Druille et al 2013, Zaller et al 2014). While our study does not allow us to directly determine which of these effects is driving changes in AMF abundance, reversion of AMF to abundances more similar to those in native dune habitats suggests that herbicide application potentially does not have lingering effects on mycorrhiza and that changes may be more reflective of changes in plant species composition with elimination of beachgrass.

Results from our 2020 follow-up study in northern backdune areas at PRNS were generally consistent with findings of the 2017 study for ATT/NB. AMF, particularly Glomeromycetes, was almost absent from native uninvaded Dune Scrub and herbicide-treated backdunes of varying times since treatment while being more abundant in heavily beachgrass-invaded backdunes. The lower abundance of AMF in native backdune habitats such as Dune Scrub are perhaps to be expected. AMF are very sensitive to pH, which is typically lower in older fixed ("grey" or "brown") backdunes (3.8 to 5.2; Kooijman et al. 2020). In addition, inorganic P such as $CaPO^{4-}$ -- the form of phosphate most likely uptaken by fungal mycelium (Frey-Klett et al. 2007) -- dissolves in acidic soils (Hinsinger 2001). Many species within northern European older dunes are non-mycorrhizal species that rely on other physiological

adaptations to enhance P uptake such as ericoid mycorrhizal associations or proteoid and cluster roots that exude carboxylates or enzymes to release P from organic sources (Lambers et al. 2008; Richardson et al. 2009; Cairney and Meharg 2003; Read 1989; Roy-Bolduc et al. 2015). Native Dune Scrub in PRNS had an average pH ranging from 5.5- 5.9 (Parsons and Becker 2021), which suggests that reduced abundance of mycorrhiza in Dune Scrub may be more reflective of the mycorrhizal status of species present than a pH constraint.

While AMF abundance in soil microbial communities showed considerable variation across sites and habitats at PRNS, AMF colonization of beachgrass roots was remarkably consistent across foredune, mid-dune, and backdune areas at both sites. Colonization rates were high ($\bar{X}$ = 79.3%) and greatly exceeded those previously documented for European beachgrass in both native and introduced ranges (23% to 51%; Tadych and Blaskowski 1999; Kowalchuk et al. 2002; David et al. 2016). While loss of some of our backdune samples complicates a direct comparison, the one backdune sample that was analyzed had high colonization rates (78%), which aligned very closely with rates found in our foredune and mid-dune areas and was much higher those found in European backdunes (20-32%; Kowalchuk et al. 2002). The high rates of colonization found in our study suggest that beachgrass is interacting extensively with mycorrhizal fungi in this system, which could have important consequences for the interactions between native plant species and AMF in invaded habitats.

We did not find strong successional gradients in AMF spore density, unlike studies at other northern California dunes, where spore densities were much higher in foredunes than other dune habitats (Rose 1988; Pickart et al. 1990). Interestingly, we found high variation in both spore abundance and diversity between sites, with the ATT-S-2 transect having higher total spore abundance and diversity of spore morphotypes than the other two transects.

In addition to mycorrhiza, beachgrass roots at Point Reyes also showed colonization by non-mycorrhizal fungi. While non-AMF species were not specifically identified, based on microbial soil analyses, fungal endophyte composition potentially represents a combination of primarily pathogenic, as well as some non-pathogenic, species. Average rates of non-AMF root colonization were generally much lower than mycorrhizal ones, ranging from 21% in foredunes to potentially as high as 52% in the backdunes. Beachgrass in later successional habitats within its native range is constrained by buildup of nematodes and pathogenic fungi, leading to decreased cover and reduced aboveground and belowground biomass (de Rooij-van der Goes 1995; van der Stoel et al. 2002). Previous studies have hypothesized that the reason beachgrass is able to colonize both foredunes and backdunes in its introduced range is due to a full or partial release from the natural soil enemies present in backdunes in its home range. For example, Beckstead and Parker (2003) documented partial escape in California dune soils, with pathogenic nematodes absent, but fungal species similar to those of beachgrass' native regions (Netherlands) present. In Oregon dunes, David et al. (2016) found that the abundance of nematodes in soils and roots of European beachgrass and other native and non-native dune grasses was generally low, and incidence of the pathogenic fungal endophyte species, *Microdochium bolleyi,* did not differ either between the three grasses, averaging 20- 25%.

Our study serves as an initial survey of the interaction between beachgrass and soil fungal communities, especially AMF, at PRNS. Our data do not allow us to draw any conclusions about the benefit that beachgrass receives from AMF associations, if any. However, our results provide interesting insights into the effect of beachgrass invasion on AMF communities. Interestingly, interactions between AMF and European beachgrass vary spatially, especially with regards to the native plant community. At BRN, where AMF is present within the native Dune Mat habitat,

invasion by beachgrass may reduce abundance of certain AMF taxa, while increasing abundance of a less common taxa, Arachaeosporomycetes. Given that many of the native Dune Mat species have been shown to be moderately to strongly mycorrhizal in other nearby systems (Pickart et al. 1990; David et al. 2016), this shift in AMF community composition may tip the competitive advantage to European beachgrass over natives at BRN, allowing it to establish monocultures once it gains a foothold. Similar patterns have been documented in other systems. For example, an invasive forb in China, goldenrod (*Solidago canadensis*), boosted abundance of one AMF species, while decreasing that of another more common one, enhancing its competitiveness over a native legume shrub (Zhang et al. 2010). Conversely, native habitats at ATT/NB are predominantly Dune Scrub, with some native Dune Mat. Dune Scrub supports later successional species that are typically less mycorrhizal in other dune systems (Read r1989; Kooijman et al. 2020). Native Dune Mat at ATT/NB had very low to no AMF abundance. In these northern sites, European beachgrass may be more mycorrhizal than the native species, leading to the observed increase in AMF abundance following invasion.

In many cases, changes in microbial community with invasion can persist long after the invader is gone, creating legacy effects that impede habitat restoration efforts (Corbin and D'Antonio 2012; Pickett et al. 2018). However, invasion effects that involve changes in relative abundance rather than complete elimination of AMF may allow for quicker recovery of native vegetation communities (Corbin and D'Antonio 2012; Wolfe and Klironomos 2005). Our data suggests that impacts of beachgrass on AMF status may potentially be reversible, with AMF abundance increasing in restored dunes at BRN and decreasing in restored habitats at ATT and Abbotts, even two years following restoration. Furthermore, AMF data from 2020 pointed to AMF abundance in older herbicide-treated areas (restored in 2012 and 2016) becoming more

equivalent to that in native Dune Scrub communities, which our restored backdunes are most likely to evolve into should we be able to successfully counter or reverse other physical, chemical, and biological legacy effects related to delayed necromass/litter decomposition and soil chemistry changes (Parsons et al. 2020a; Parsons and Becker 2021; Yang et al., *in prep*.).

Future studies should more extensively evaluate the mycorrhizal status of both European beachgrass and native dune plants in various successional stages (primary, mid, and late-successional Dune Mat and Dune Scrub) across a broader range of dune systems within PRNS. Also, where restoration appeared to cause a rebound in the native mycorrhizal taxa associated with sparsely invaded native Dune Mat such as at BRN, it would be good to determine if the same suite of genera or species of AMF is present, not just higher order taxa. It may be that, while abundance of particular mycorrhizal orders increases, abundance of specific genera or species strongly associated with late successional native Dune Mat do not, which could hinder full recovery of these systems. Should that be the case, more active adaptive restoration measures may be necessary such as directly inoculating either soils or container plants targeted for outplanting with the appropriate AMF taxa (Corbin and D'Antonio 2012; Pickett et al. 2018).

REFERENCES

Barbour MG, de Jong TM, Johnson AF (1976) Synecology of beach vegetation along the Pacific Coast of the United States of America: a first approximation. Journal of Biogeography 3:55–69

Beckstead J, Parker IM (2003) Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? Ecology 84:2824–2831

Beena KR, Raviraia NS, Sridhar KR (1999) Seasonal variations of arbuscular mycorrhizal fungal association with lpomoea pes-caprae of coastal sand dunes, Southern India. J. Environ. Biol. 21(4):341-347.

Buck-Diaz J, Sikes K, Evens JM (2021) Vegetation classification of alliances and associations in Marin County, California. Draft. California Native Plant Society.

Cairney JWG, Meharg AA (2003) Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. European Journal of Soil Science 54:735–740.

Clayton JL (1972) Salt spray and mineral cycling in two California coastal ecosystems. Ecology 53(1):74-81.

Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Science and Management 5:117–124

David, AS, May G, Schmidt D, Seabloom, EW (2016) Beachgrass invasion in coastal dunes is mediated by soil microbes and lack of disturbance dependence. Ecosphere 7(11):e01527. 10.1002/ecs2.1527

De Rooij-Van der Goes PCEM. 1995. The role of plant-parasitic nematodes and soil-borne fungi in the decline of *Ammophila arenaria* (L.) Link. The New Phytologist 129 (4): 661-669.

Druille, M., Omacini, M., Golluscio, R. A., & Cabello, M. N. (2013). Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Applied Soil Ecology*, *72*, 143-149.

Ernst WHO, van Duin WE, Oolbekking GL (1984) Vesicular arbuscular mycorrhiza in dune vegetation, Acta Bot. Neerl. 33:151–160.

Frey-Klett P, Garbaye J, Tarkka M (2007) Tansley review: the mycorrhiza helper bacteria revisited. New Phytologist 176:22–36.

Gonzalez B, Godoy RB, Figueroa HS (1995) Seasonal dynamics of the vesicular-arbuscular mycorrhizal fungi in Chilean central-south sand dune ecosystems, Agricultura Tecnica 55:267–271.

Goodman J (1982) Water potential from advected fog. Progress Report 1. Department of Meteorology, San Jose State University, San Jose, CA.

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237:173–195.

Holton B, Barbour MG, Martens SN (1991) Some aspects of the nitrogen cycle in a Californian strand ecosystem. Madroño 38:170–184.

Inderjit, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology & Evolution 25:512–519.

Ingraham NL, Matthews RA (1995) The importance of fog-drip water to vegetation: Point Reyes Peninsula, California. Journal of Hydrology 164:269-285.

Jacob DJ, Waldman TJM, Monger JW, Hoffmann MR (1985) Chemical composition of fogwater collected along the California coast. Environ. Sci. Technol. 19:730-736

Koske, RE, Halvorson WL (1981) Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Canad. J. Bot*. 59: 1413–1422.

Koske RE, Polson WR (1984) Are VA Mycorrhizae Required for Sand Dune Stabilization? BioScience 34:420–424.

Kowalchuk GA, De Souza FA, Van Veen JA (2002) Community analysis of arbuscular mycorrhizal fungi associated with Ammophila arenaria in Dutch coastal sand dunes. Molecular Ecology 11(3):571-81.
Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23:95–103.

Olsson PA, Wilhelmsson P (2000) The growth of external AM fungal mycelium in sand dunes and in experimental systems. Plant and Soil 226:161–169.

de la Peña, Rodriguez-Echeverria S, van der Putten WH, Freitas H, Moens M (2006) Mycorrhizal fungi control migratory endoparasitic nematodes in *Ammophila arenaria*, New Phytol. 169:829–840.

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW (2013) Severe plant invasions can increase mycorrhizal fungal abundance and diversity. The ISME Journal 7:1424–1433

Logan VS, Clark PJ, Allaway WG (1989) Mycorrhizas and root attributes of plants of coastal sand-dunes of New South Wales. Australian Journal of Plant Physiology 16(1): 141–146.

Marler MJ, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80:1180–1186.

Parsons, L, Sayre J., Ender C, Rodrigues JLM, Barberán A (2020a) Soil microbial communities in restored and unrestored coastal dune ecosystems in California. Restoration Ecology https://doi.org/10.1111/rec.13101

Parsons L, Ender C, Ryan A (2020b) Coastal Dune Restoration Vegetation Monitoring Report. Point Reyes National Seashore. Point Reyes Station, CA, USA. 128 pp. https://irma.nps.gov/DataStore/Reference/Profile/2284461

Parsons L, Becker B (2021). Invasion by *Ammophila arenaria* alters soil chemistry, leaving lasting legacy effects on restored coastal dunes in California. Invasive Plant Science and Management, 14(2), 75-91. doi:10.1017/inp.2021.16

Pickart A, McCleneghan SC, Little BT (1990). Vesicular-arbuscular mycorrhizae in the Dune Mat Community, Humboldt County, California. Report submitted to Menzies' Wallflower Research Program, Humboldt State University.

Pickett B, Maltz M, Aronson E (2018) Impacts of invasive plants on soil fungi and implications for restoration *in* Diversity and Ecology of Invasive Plants. Charan Sahu S, Kumar S, eds. IntechOpen. DOI: 10.5772/intechopen.82108

Pringle A, Rillig MC, Gardes M, Klironomos J (2009) Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715

van der Putten WH, van Dijk C, Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56.

Read DJ (1989) Mycorrhizas and nutrient cycling in sand dune ecosystems. Proceedings of the Royal Society of Edinburgh. Section B: Biological Sciences Volume 96: Coastal Sand Dunes. Cambridge University Press.

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytologist 170:445–457. doi: 10.1111/j.1469-8137.2006.01715.x

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimize access to soil phosphorus. Crop & Pasture Science 60:124–143.

Rose SL (1988) Above and belowground community development in sand dunes. Plant and Soil 109:215-226.

Roy-Bolduc A, Bell TH, Boudreau S, Hijri M (2015) Comprehensive sampling of an isolated dune system demonstrates clear patterns in soil fungal communities across a successional gradient. Environmental Microbiology Reports 7(6): 839–848. doi:10.1111/1758-2229.12312

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences. 109(16):6241-6246.

Soil Conservation Service (1985) Soil survey of Marin County California. California https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/california/marinCA1985/marinCA1985.pdf

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, et al. (2006) Invasive Plant Suppressesthe Growth of Native Tree Seedlings by Disrupting Belowground Mutualisms. PLoS Biol 4(5): e140.https://doi.org/10.1371/journal.pbio.0040

van der Stoel CD, van der Putten WH, Duyts H (2002) Development of a negative plant-soil feedback in the expansion zone of the clonal grass *Ammophila arenaria* following root formation and nematode colonization. Journal of Ecology 90:978–988.

Stürmer SL, Bellei MM (1994) Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. Can. J. Bot. 72:359-363.

Tadych M, Blaszkowski J (1999) Growth responses of maritime sand dune plant species to arbuscular mycorrhizal fungi. Acta Mycologica 34(1):115-123. DOI: 10.5586/am.1999.010

van der Valk AG (1974) Mineral cycling in coastal foredune plant communities in Cape Hatteras National Seashore. Ecology 55(6):1349-1358 http://www.jstor.com/stable/1935462

Vilà M et al. (2006) Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. Journal of Biogeography 33:853–861

Western Regional Climate Center (WRCC) (2020) Climate data from Point Reyes RCA CA weather station.

Wheeler B, Torchiano M (2016). Permutation tests for linear models: lmPerm. Version 2.1.0. URL https://github.com/mtorchiano/lmPerm

Wiedemann AM, Pickart A (1996) The *Ammophila* problem on the Northwest coast of North America. Landscape and Urban Planning 34:287–299

Willis JE, Yemm EW (1961) Braunton Burrows: mineral nutrient status of the dune soils. Journal of Ecology 49 (2):377-390 https://doi.org/10.2307/2257270

Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. BioScience 55:477–487

Woodhouse WW, Hanes RE (1966) Dune stabilization with vegetation on the Outer Banks of North Carolina. U.S. Army Coastal Engineering Research Center Tech Memo No. 22. August 1967.

Zaller, J. G., Heigl, F., Ruess, L., & Grabmaier, A. (2014). Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Scientific reports, 4(1), 1-8.

Zhang Q, Yang R, Tang J, Yang H, Hu S, Chen X (2010) Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. PLoS ONE 5(8): e12380. https://doi.org/10.1371/journal.pone.0012380

Zubek, S., Majewska, M.L., Błaszkowski, J. et al. Invasive plants affect arbuscular mycorrhizal fungi abundance and species richness as well as the performance of native plants grown in invaded soils. Biol Fertil Soils 52, 879–893 (2016).

Table 1. Number of soil samples analyzed from restored and unrestored dunes in Point Reyes National Seashore (PRNS). NA = Not available (e.g., heavily invaded dunes not present at all sites due to prior restoration efforts).

Invasion Intensity/Restoration Status	B Ranch North (19.4 ha)	AT&T/North Beach				Abbotts Lagoon (78.3 ha)
		North (15.5 ha)		**South/NB** (18.2 ha)		
	2017	2017	2020	2017	2020	2020
Native – *Sparsely Invaded*						
Dune Mat	3	NA	--	2	--	--
Dune Scrub	--	--	6	--	--	--
Invaded Dunes - *Untreated*						
Moderately Invaded	3	NA	--	3	--	--
Heavily Invaded	3	--	--	2	5	NA
Dunes Heavily Invaded by European Beachgrass – Restored Using Herbicide						
Restored - *2012*	--	--	--	--	--	5
Restored - *2014*	6	--	--	--	--	--
Restored - *2016*	--	6	5	--	--	--
Restored - *2018*	--	--	--	--	4	--

Site (2017)	BRN				ATT/NB			
	Sparsely Invaded (Dune Mat)	*Moderately Invaded*	*Heavily Invaded*	*Herbicide Treated*	*Sparsely Invaded (Dune Mat)*	*Moderately Invaded*	*Heavily Invaded*	*Herbicide Treated*
Microbial Guild or Taxa	**N=3**	**N=3**	**N=3**	**N=6**	**N=2**	**N=3**	**N=2**	**N=6**
	(All values x 10^{-2})				*(All values x 10^{-2})*			
AMF	0.37% (0.01)	0.14% (0.17)	0.23% (0.13)	0.23% (0.14)	0.00% (0.00)	0.35% (0.07)	0.36% (0.04)	0.05% (0.04)
Archaeosporomycetes	0.03% (0.02)	0.02% (0.01)	0.15% (0.02)	0.02% (0.02)	0.00% (0.00)	0.03% (0.02)	0.04% (0.01)	0.02% (0.02)
Glomeromycetes	0.23% (0.01)	0.10% (0.13)	0.08% (0.14)	0.15% (0.09)	0.00% (0.00)	0.23% (0.03)	0.24% (0.01)	0.02% (0.03)
Paraglomeromycetes	0.11% (0.02)	0.02% (0.03)	0.00% (0.00)	0.07% (0.04)	0.00% (0.00)	0.09% (0.04)	0.05% (0.07)	0.01% (0.02)
Fungal Endophytes	4.68% (0.17)	5.59% (2.35)	1.61% (0.02)	3.58% (1.92)	1.65% (1.82)	4.76% (0.17)	4.49% (0.05)	0.96% (1.11)

Table 2. Relative abundances (%) of mycorrhizae (AMF), specific mycorrhizal taxa, and non-mycorrhizal fungal endophytes in 2017 microbial community study. Data represent mean and standard deviation (SD) with units of x 10^{-2}.

Site (2020)	ATT/NB & Abbotts				
	Sparsely Invaded Dune Scrub	*Heavily Invaded Dune Scrub*	*Herbicide-Treated 2-YR (2018)*	*Herbicide-Treated 4-YR (2016)*	*Herbicide-Treated 8-YR (2012)*
Microbial Guild or Taxa	***N=6***	***N=5***	***N=4***	***N=5***	***N=5***
		(All values x 10^{-3})			
AMF	0.07% (0.11)	3.11% (2.43)	0.03% (0.06)	0.06% (0.13)	0.14% (0.09)
Archaeosporomycetes	0.00% (0.00)	0.98% (0.72)	0.03% (0.06)	0.06% (0.13)	0.03% (0.06)
Glomeromycetes	0.07% (0.11)	1.71% (2.27)	0.00% (0.00)	0.00% (0.00)	0.04% (0.09)
Paraglomeromycetes	0.00% (0.00)	0.03% (0.03)	0.00% (0.00)	0.00% (0.00)	0.03% (0.07)
Fungal Endophytes	1.68% (0.68)	3.51% (1.71)	1.46% (1.48)	1.83% (1.48)	4.29% (1.61)

Table 3. Relative abundances (%) of mycorrhizae (AMF), specific mycorrhizal taxa, and non-mycorrhizal fungal endophytes in 2020 microbial community study in backdune areas at ATT/NB and Abbotts. Data represent means and standard deviation (SD) with units of x 10^{-3}.

Successional Stage/ Dune Location	N	Root Colonization (%)		Soil Spores (#/g soil)
		AMF	Non-AMF	
Foredunes	3	79.3% (± 13.7)	21% (± 14.1)	1.48 (± 1.20)
Mid-Dunes	3	80.7% (± 7.0)	27.7% (± 20.3)	3.64 (± 4.85)
Backdunes	1	78.0%	52.0%	1.36 (± 0.63)
Average		*79.3% (± 1.3)*	*33.6% (± 16.3)*	*2.16 (± 0.65)*

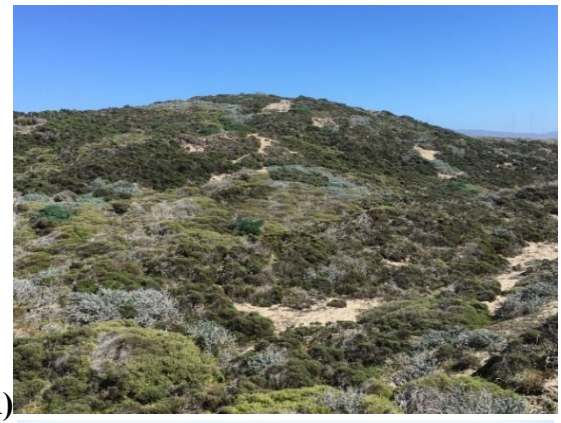
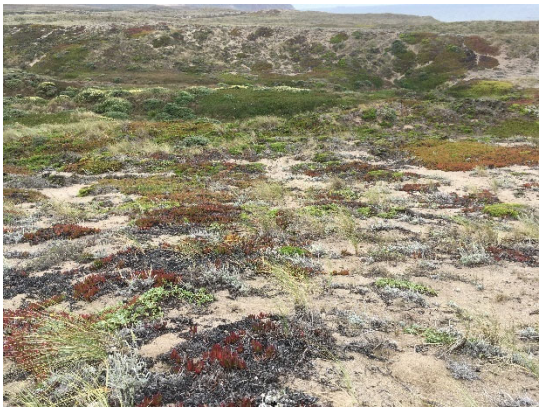
Table 4. Percent colonization of *Ammophila arenaria* roots by AMF and non-mycorrhizal fungi and fungal spore density (# spores/g soil) across three dune locations representing a gradient of successional stages.

Transect	Root Colonization (%)		Soil Spores (#/g soil)
	AMF	Non-AMF	
ATTS-1	81% (± 6.1)	26% (± 23.1)	1.23 (± 0.50)
ATTS-2	70.5% (± 5.0)	43.5% (± 10.6)	4.57 (± 4.08)
NB-1	87% (± 9.9)	16.5% (± 3.5)	0.68 (± 0.11)

Table 5. Percent colonization of *Ammophila arenaria* roots by AMF and non-mycorrhizal fungi and fungal spore density (# spores/g soil) in three dune sampling transects oriented perpendicular to the shoreline.



Figure 1. Location of Point Reyes National Seashore (PRNS) along northern California coast and sampling sites within PRNS.


(A)

(B)

(C)

(D)

(E)

(F)

Figure 2A-2E. (A) Sparsely invaded native Dune Mat; (B) Sparsely invaded native Dune Scrub; (C) Moderately invaded dunes with iceplant and sparse European beachgrass; (D) Heavily invaded dunes with European beachgrass; (E) Herbicide-treated dunes two years after restoration (2018); (F) Herbicide-treated dunes eight years after restoration (2012).

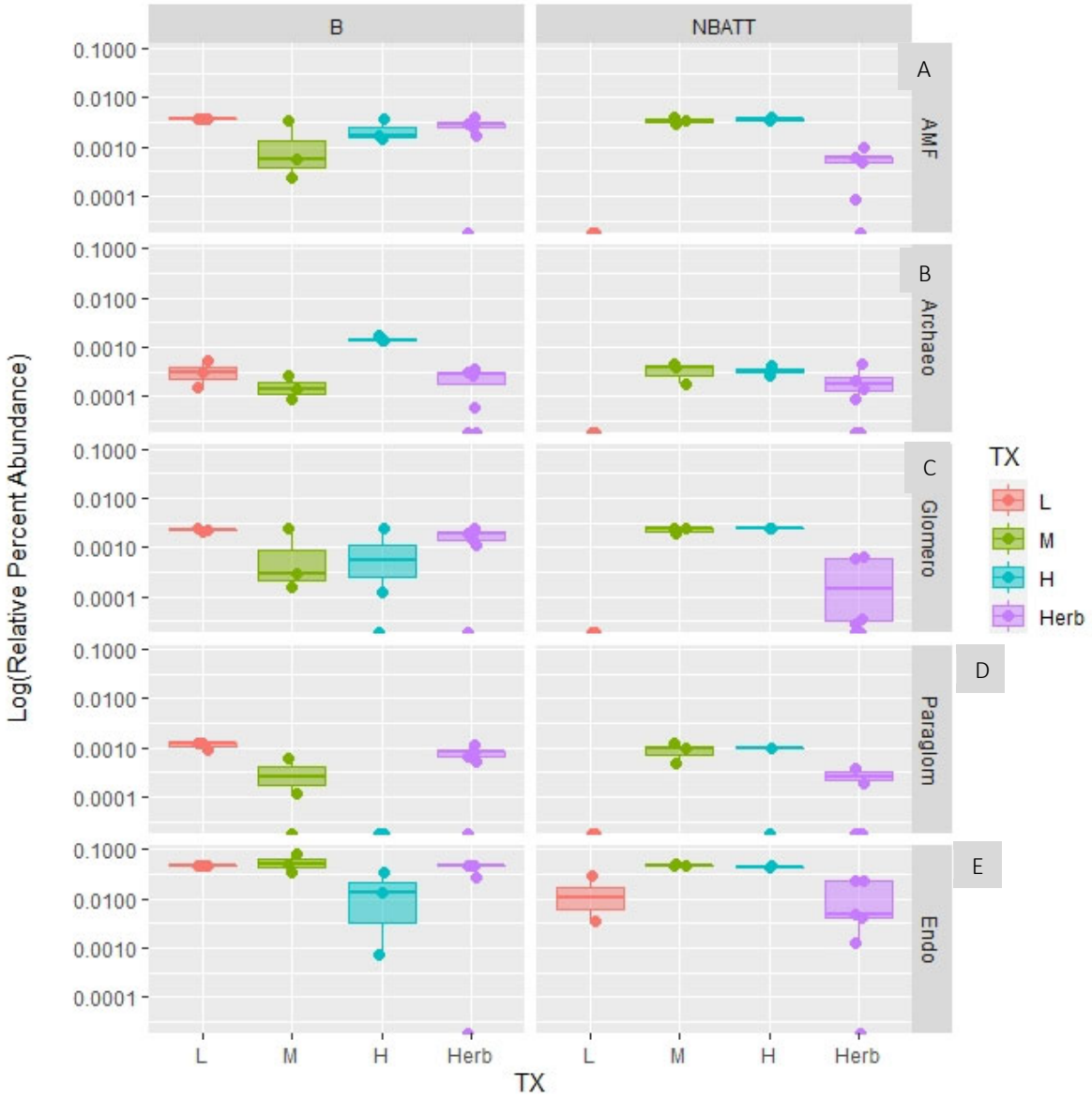


Figure 3A-3E. Box plots for 2017 microbial study of relative abundance in dune areas of varying invasion intensities (Low or sparsely invaded ("L"); Medium or moderately invaded ("M"); High or heavily invaded ("H'); Restored using herbicide treatment "Herb") for arbuscular mycorrhizal fungi (AMF; A); different taxa of mycorrhiza (Archaeosporomycetes, B; Glomeromycetes, C; Paraglomeromycetes, D); and non-AMF endophytic fungi (E).

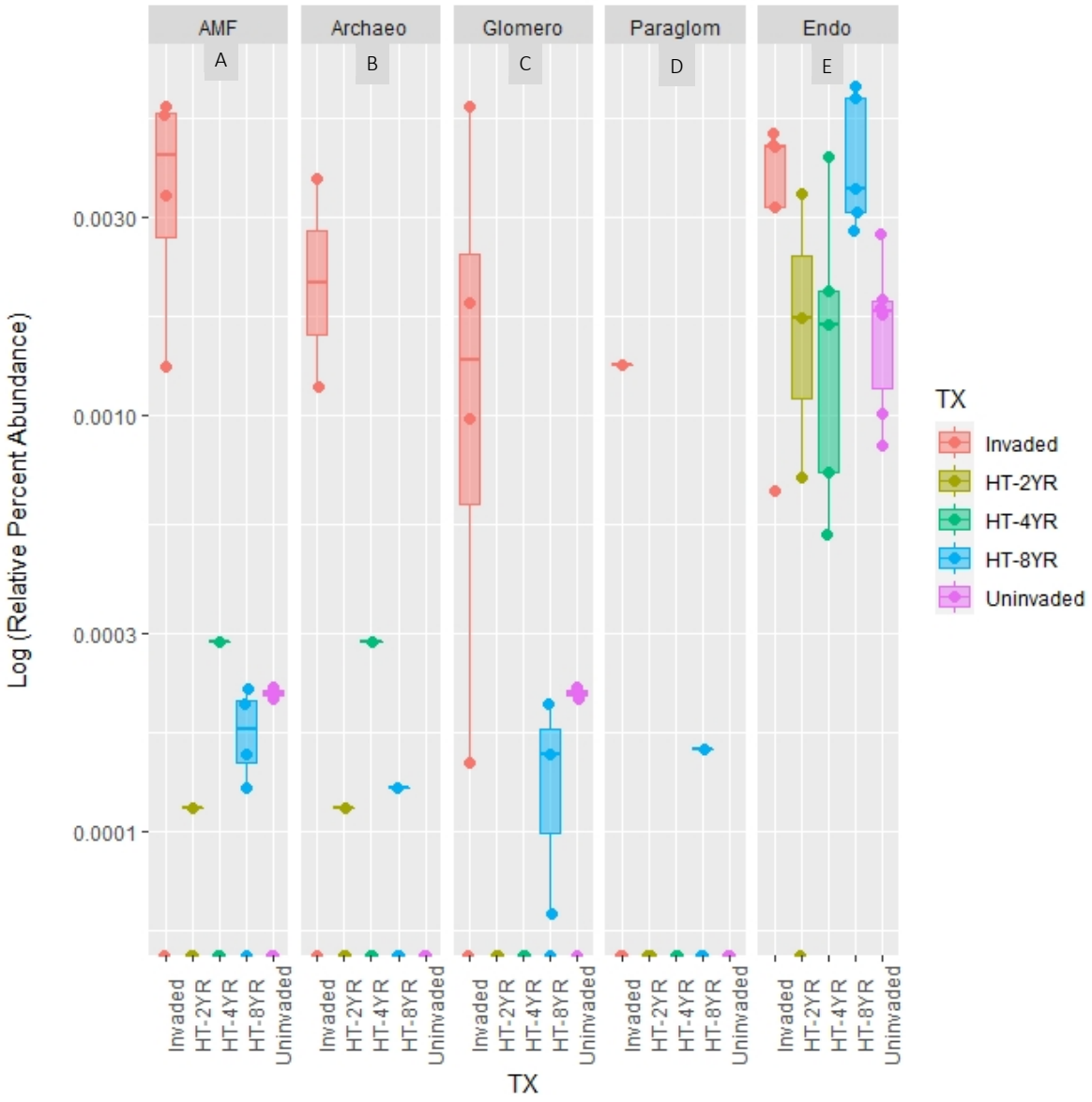


Figure 4A-4E. Box plots for 2020 microbial study of dune areas with varying restoration status -- Heavily invaded European beachgrass ("Invaded"); Herbicide treated two years ago (2018; "HT-2YR"); Herbicide-treated four years ago (2016; "HT-4YR"); Herbicide-treated eight years ago (2012; "HT-8YR"); and native Dune Scrub ("Uninvaded"). Relative abundance is shown for arbuscular mycorrhizal fungi (AMF; A); different taxa of mycorrhiza (Archaeosporomycetes, B; Glomeromycetes, C; Paraglomeromycetes, D); and non-AMF endophytic fungi (E).

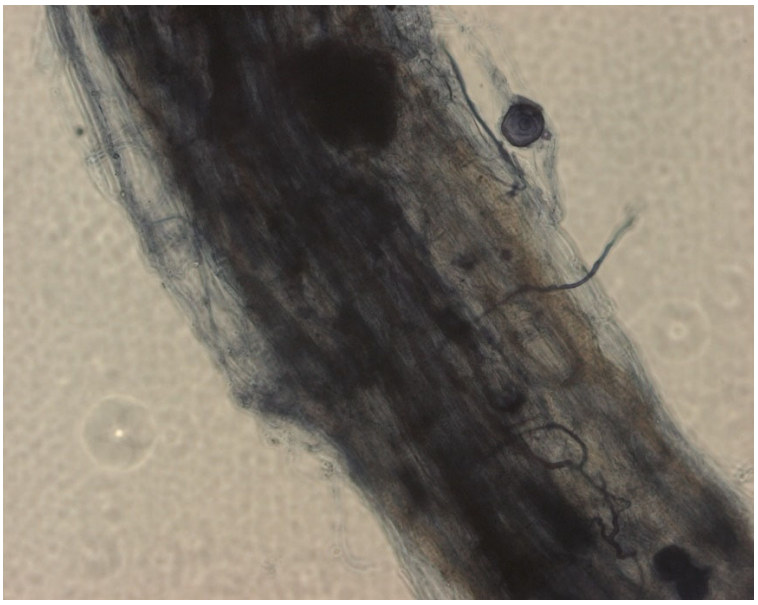


Figure 5. Roots of European beachgrass from ATT/NB under 200X magnification using a compound microscope. The blue, thick, aseptate hyphae are AMF, and the blue spore is also AMF. The brown spores and brown hyphae were classified as non-AMF fungi. Photo courtesy of Dr. Sydney Glassman.