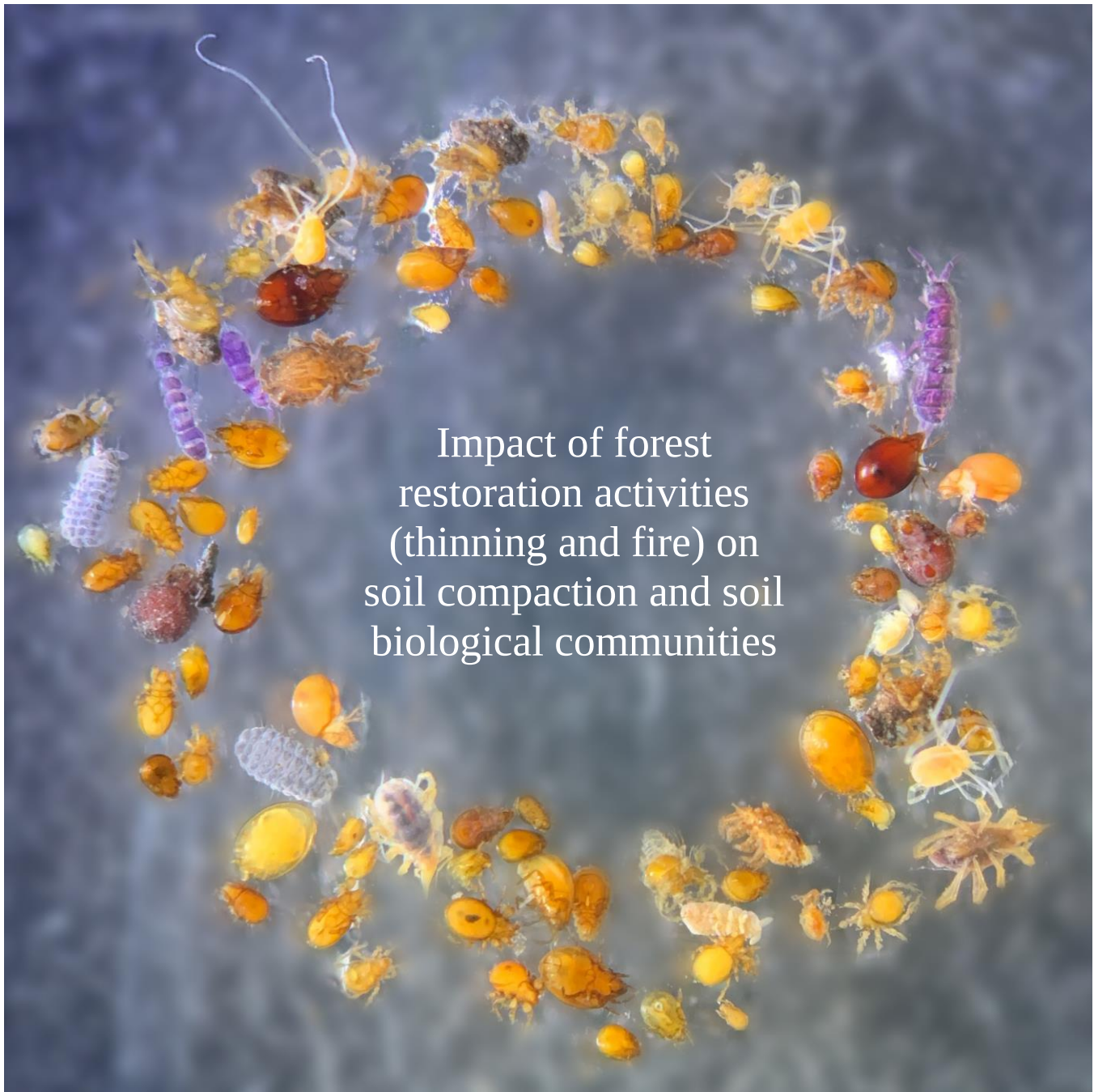




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Overview

Objectives

1. Understand the impacts of forest management activities on soil structure, function, and biota.
2. Add soil taxa to the All Taxa Biodiversity Inventory (ATBI).
3. Determine the potential of fire mosses as a Burned Area Emergency Response (BAER) restoration treatment.

Activities

Objective 1:

Study 1: We have completed an observational study of soil faunal and fungal responses to thinning and prescribed fire at Banco North.

Study 2: We have completed a manipulative study of impacts to nematodes and soil physical properties of increasing soil mechanical disturbance from logging machinery.

Study 3: We also completed sampling and characterization of fungal communities for a study of fuel loading effects on soil biota. Study 3 is continuing under our new cooperative agreement.

Study 4: In addition, leveraged support from the Joint Fire Science Program to complete a field mesocosm experiment investigating the relationship of soil faunal complexity to provision of ecological functions in thinned/burned and untreated management units at Banco North.

Objective 2: Our contribution to the ATBI is ongoing under our new cooperative agreement. We have identified nematode and microarthropod taxa collected from Banco North, Redondo Peak, and Seco 5. To contextualize soil biotic communities within the range of variation present at the preserve, we have also collected and analyzed samples and field data at South Mountain and within the old-growth, ponderosa-pine-dominated History Grove.

Objective 3:

Study 5: We have conducted a field experiment to evaluate the restoration potential of fire mosses (*Ceratodon purpureus*, *Funaria hygrometrica*, and *Bryum argenteum*) as a Burned Area Emergency Response tool.

Value added work: **A.** We completed an experiment to quantify the effect of storage time on nematode extraction. **B.** We also developed a protocol for high-efficiency extraction of nematodes from large soil subsamples by centrifugation with industrial-grade colloidal silica.

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Study 1. Banco North observational study

Collembolans and nematodes, but not mites, are resilient to restoration of a *Pinus ponderosa* forest with thinning, mastication, and prescribed fire

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This study is ready to submit to *Trees, Forests and People*

Abstract

Thinning, mastication, and prescribed fire are restoration treatments frequently employed in unnaturally dense second-growth *Pinus ponderosa* forests of the Western United States. Although a goal of these treatments is to restore ecosystem structure and function, little information is available regarding treatment effects on soil micro- and mesofauna, which comprise the overwhelming majority of metazoan forest inhabitants and occupy key positions in soil food webs. We quantified nematodes, mites, and collembolans in soil and litter habitats within untreated control, thinned (comprising thinning and masticating wood), and burned (comprising thinning and masticating, followed by broadcast burn) in *P. ponderosa* forest management units at Valles Caldera National Preserve in New Mexico, USA. We linked patterns in animal abundance to resource and habitat characteristics, hypothesizing that resources and available habitat for many taxa would increase with thinning and decrease with burning. Two years after thinning, densities of collembolans and nematodes in the thinned unit were higher than in the untreated control unit, but one-year post-fire, their densities in the burned unit were similar to those of the untreated control unit. Mite abundance, however, was not elevated in the thinned unit and was lower in the burned unit. Although faunal communities were highly heterogeneous, a significant proportion of the variance in faunal abundances was explained by easily and inexpensively measured habitat and resource characteristics: bulk density, SOM, pH, grass cover, and litter cover and depth. These findings demonstrate the abiotic and biotic factors that structure faunal habitats so that forest managers have a more complete understanding of the impacts of forest restoration treatments.

Introduction

Restoring frequent-fire-adapted *Pinus ponderosa* (ponderosa pine) forests is a high priority for forest managers in the western United States. Overharvesting, overgrazing, and fire suppression following Euro-American settlement have created dense stands of slow-growing, small-diameter trees with heavy litter accumulation and reduced understory vegetation, which are increasingly vulnerable to high-severity fire as the climate warms and dries (Allen et al., 2002). Mechanized thinning and prescribed or managed burning are restoration techniques employed to recreate historic stand structure, reduce the risk of stand-replacing wildfire, promote biological diversity, and improve ecosystem functions eroded by disruption of historic disturbance regimes (Covington et al., 1997; Reynolds et al., 2013).

While considerable research has tracked responses of aboveground organisms to these treatments, those of belowground biota have received little attention. Impacts on soil and litter dwelling micro- and mesofauna, including nematodes, mites, and collembolans, remain particularly understudied in xeric, fire-adapted forests of the Western United States. (An exception is Camann et al. (2012, 2008) who have reported a reduction in mite abundances following prescribed burning of a *P. ponderosa*/*P. jeffreyi* forest in the Pacific Northwest, with

signs of continuing decline in oribatid mites two years post-fire.) Furthermore, the effects of mastication (an increasingly common fuels treatment) on these numerically dominant metazoa have not been investigated. Since nematode and microarthropod detritivores, microbivores, root herbivores, and predators influence microbial communities (Crowther and A'Bear, 2012), carbon and nutrient cycling (Gan and Wickings, 2020), and pathogen populations (Sabatini and Innocenti, 2001), understanding how they are affected by forest treatments is crucial for evaluating restoration practices.

Both mechanized thinning and reintroduction of long-absent fire are likely to result in some immediate mortality of soil micro- and mesofauna. Animals may be crushed by heavy logging equipment. Litter-dwelling fauna are the most likely to experience lethal temperatures during burning, while those below the soil surface may or may not succumb depending on fire behavior, soil moisture, and small-scale fuels characteristics. Although heat transfer attenuates dramatically with depth, significant mortality of soil mites, collembolans, and nematodes may occur at temperatures well below the oft-cited “biological effects threshold” of 60°C (Malmström, 2008; Pingree and Kobziar, 2019), depending on heating duration, soil moisture, and an animal's cuticular structure. Given dramatic variation in soil micro- and mesofauna generation times (from days to years) and reproductive output (from single offspring to hundreds), post-treatment community changes due to mortality alone could potentially persist for years.

Changes to habitat quality and resource availability with restoration could also have profound effects on micro- and mesofauna assemblages. Because soil micro- and mesofauna are too small to physically shift soil particles, pore size and connectivity are critical parameters governing habitat availability (Erktan et al., 2020). Compaction from logging machinery and combustion of organic matter should each be expected to decrease accessible pore space for many soil animals. On the other hand, deposition of thinning residues (i.e., slash or masticated wood) ought to increase habitat for litter-dwelling fauna. This influx of organic matter, along with root necromass from cut trees, also provides a slow resource pulse for saprotrophic organisms (particularly fungi) and their consumers—at least until these residues are burned. Precipitous declines in microbial biomass, and especially of fungi, are well-documented after fire (Pressler et al., 2019). Primary and secondary consumers in the soil food web are also likely to respond to post-treatment shifts in understory plant diversity and abundance, which govern long-term carbon and nutrient inputs to the soil food web and shape microbial communities (Hättenschwiler et al., 2005). Finally, any changes in soil moisture due to altered canopy cover, ground cover, or water holding capacity should be of great consequence to soil fauna in xeric forests, and particularly to nematodes, which are aquatic organisms that depend on water films for movement. We examined responses of soil mites, collembolans, and nematodes to thinning with mastication alone or with prescribed fire in a *P. ponderosa* forest within Valles Caldera National Preserve, New Mexico. Ponderosa pine forests in the region historically experienced low-severity surface fires with decadal to subdecadal return intervals (Touchan et al., 1994). The Native Jemez people heavily influenced fire regimes from the pre-colonial era until the late 1600s, increasing ignitions and reducing fuel connectivity (Swetnam et al., 2016). Relative to the period following Jemez population collapse, fires were more frequent but smaller in spatial extent, and were effectively decoupled from interannual climate variation (Roos et al., 2021). Depopulation resulted in more widespread, less frequent, more intense (although still low-severity) fires driven primarily by climate variability (Swetnam et al., 2016). This period of “free range” fires persisted until the late 1800s, when intensive livestock grazing by Euro-American colonists eliminated fine fuels and inaugurated the current era of fire suppression (ibid.). Recruitment after clear-cut logging in the 1930s (Anschuetz and Merlan, 2007), and a federal policy of fire suppression, produced the thickets of stunted trees that are now common in ponderosa pine forests across much of the US Southwest.

Our study took place within three adjacent management units at different treatment stages. Our objective was to link patterns in post-treatment abundance of these three faunal groups with relevant habitat and resource characteristics. We hypothesized that resources and available habitat for soil micro- and mesofauna increase following thinning and mastication and decrease with subsequent prescribed fire. We thus posited that nematodes and microarthropods would be more abundant (relative to an untreated control unit) within a thinned

and masticated treatment unit due to an increase in masticated wood, soil organic matter, and herbaceous plants, and less abundant within an adjacent unit that had been thinned, masticated, and broadcast burned as a function of reduced litter cover and soil organic matter.

Methods

Study site and sample collection

Responses of nematodes and microarthropods to forest fuels treatments were assessed in three adjacent ponderosa pine-dominated management units at Valles Caldera National Preserve, New Mexico (**Fig. 1 A**). The study area was located on Banco Bonito, a rhyolite lava flow from ~68,000 years ago (Goff, 2009). Annual precipitation averaged 590 mm from 1981–2010, and the mean annual temperature for this period was 6.6 °C, with monthly mean temperatures ranging from -2.3 °C in January to 17 °C in July (PRISM, 2021). Soils in the study area are rhyolite-derived sandy loams to loamy sands belonging to the Totavi-Jemez-Rock Outcrop association (Hacker and Banet, 1987). Two management units—hereafter the “burned” (82 ha) and “thinned” (120 ha) units—were treated in 2012 and 2014, respectively, with mechanical thinning (and removal of harvested wood) followed by mastication of unmarketable timber. These treatments resulted in an average tree density of 60 trees ha⁻¹. The burned unit received a low-intensity broadcast burn in October of 2015 after thinning as described above. An adjacent portion of an untreated control unit (encompassing approximately 90 ha) served as a control retaining ~300 trees ha⁻¹.

In late August and early September of 2016, we selected eight areas within each unit with approximately matching slope and aspect, then randomly selected origin points for arrays of four quadrats as depicted in **Fig. 1B** and **1C**. In order to assess ground cover changes with restoration treatments, we measured ground cover and litter depth at each quadrat. A 0.5 m x 0.5 m gridded frame was used to estimate percentage cover by grasses, forbs, litter, mosses, lichens, scat, rock, wood, mushrooms, bare ground, and tree seedlings or exposed roots. Litter depth was measured as near as possible to the center of the quadrat. Overall slope and aspect were also recorded for each quadrat.

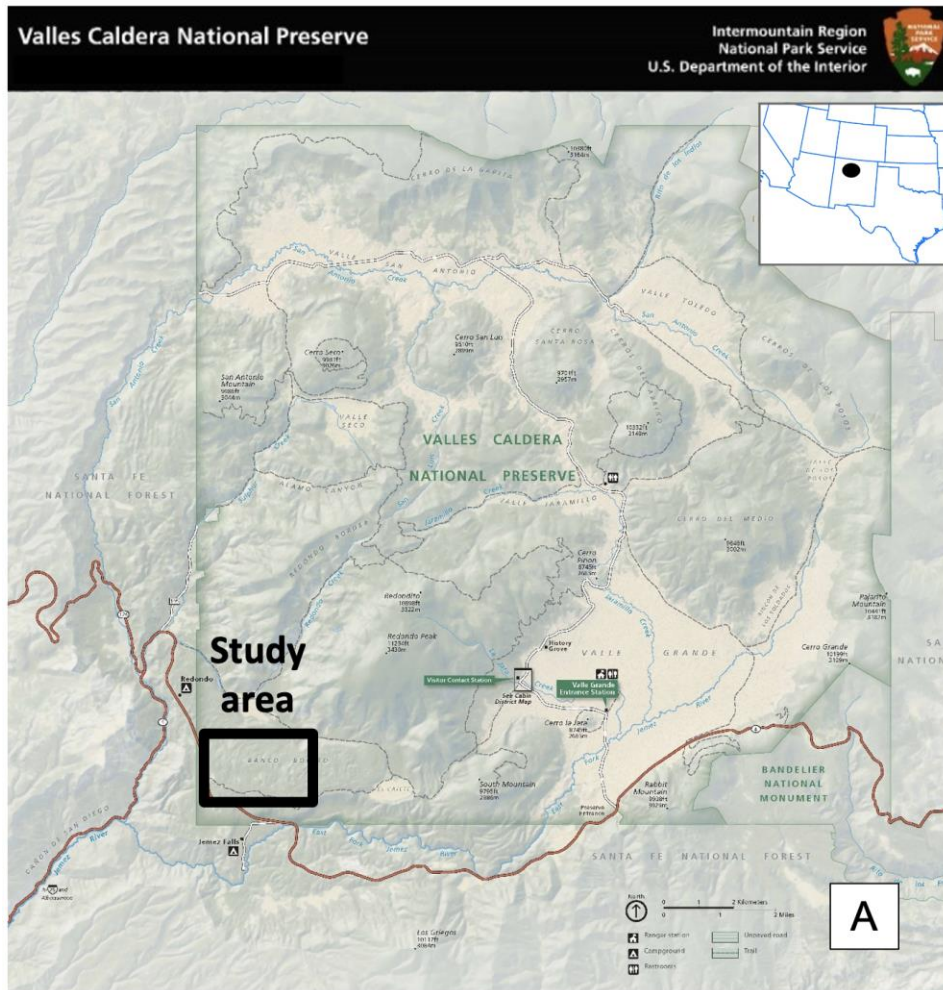


Fig. 1. A. Location of study area within Valles Caldera National Preserve and the US state of New Mexico.

One of the four quadrats in each array was randomly selected for collection of soil cores. Three 5.08 cm diameter soil cores were collected from the uppermost 10 cm of mineral soil and the O_a layer, if present. One core each was reserved for extraction of nematodes, microarthropods, and for abiotic analyses requiring dried soil, respectively. We used a 10.16 cm diameter circular corer to collect a litter sample (if litter cover was present) for extraction of microarthropods before sampling the underlying soil for these organisms. An additional smaller soil core (2.5 cm diameter, to a depth of 9.5 cm) was collected from each randomly selected quadrat for quantification of hyphal lengths. Microarthropod soil samples were transported intact in the PVC core samplers to reduce animal mortality. The other core samplers were rinsed with water and then cleaned with 90% isopropyl alcohol. Soil cores to be dried for abiotic analyses were stored in open air while in the field, then were dried at 47 °C upon return to Northern Arizona University. Nematode and microarthropod soil and litter cores were stored in coolers on ice, and soil samples for hyphae extraction were frozen on dry ice until arrival at NAU. Nematode and microarthropod core samples were then refrigerated at 4 °C until animals were extracted. Samples for hyphae extraction were stored at -20 °C.

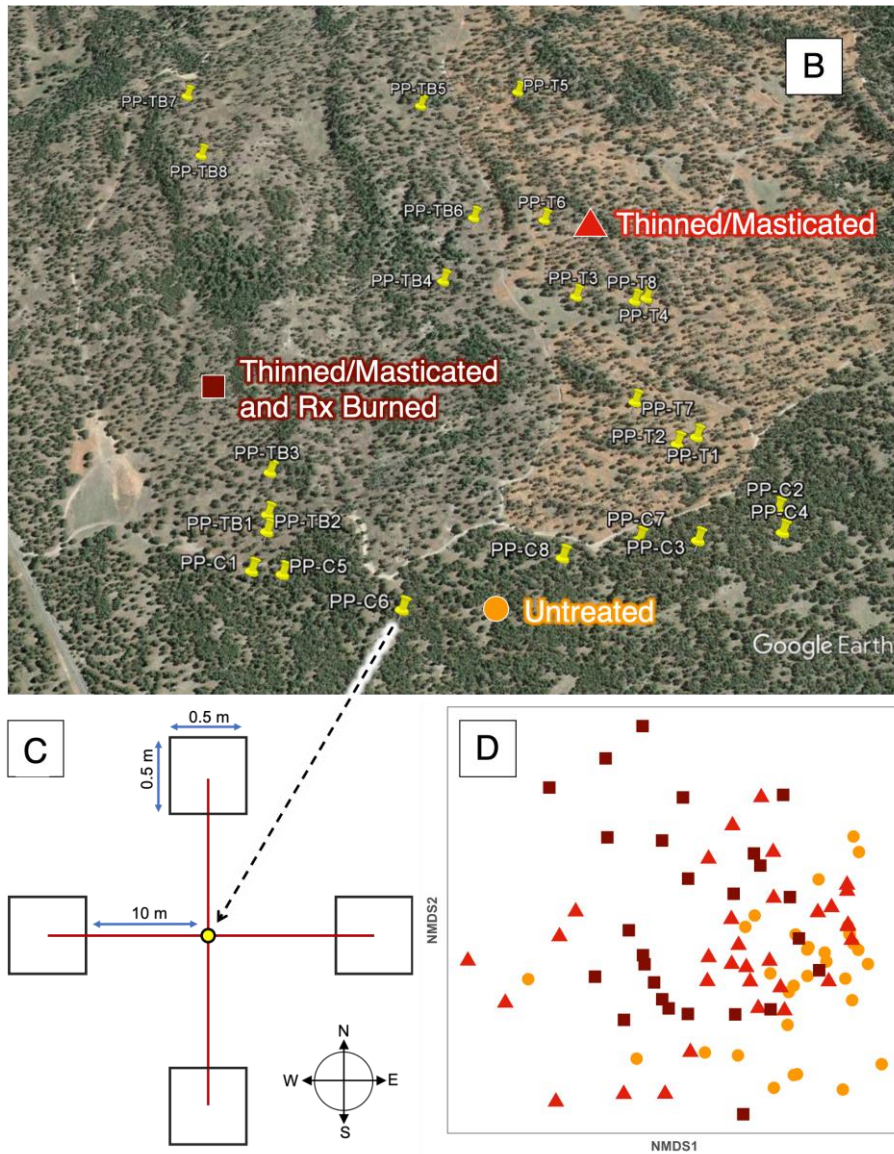


Fig. 1. B. Sampling locations in thinned, burned, and untreated management units within the study area at Banco Bonito, Valles Caldera National Preserve, New Mexico, USA. Each yellow pin represents the origin point of a quadrat array as shown in **C**. Ground cover and litter depth were measured at each quadrat, and soil and litter cores were collected from one randomly selected quadrat per array. **D**. Non-metric multidimensional scaling ordination of ground cover in quadrats, colored by treatment.

Soil biota extraction

Microarthropods were extracted from soil and litter within two weeks of collection using high-gradient Tullgren funnels (similar to Crossley and Blair, 1991). Soil or litter samples were spread on cheesecloth within funnels which emptied into scintillation vials holding tap water. Insulated chambers housing the collection vials were cooled with ice bottles to maintain a strong temperature gradient as light was gradually intensified over 4 days, followed by a 2-day period of maximum illumination by 15W bulbs. Two microarthropod soil and litter samples—one from the untreated control unit and one from the burned treatment unit—were excluded due to sample damage. Collected microarthropods were preserved in approximately 70% ethanol and refrigerated. Microarthropods were counted using a dissecting microscope at 20–63x magnification and sorted to mites, collembolans and other fauna (e.g., ants, macroarthropod larvae, and enchytraeids).

Nematodes were initially extracted from 30 g of soil using modified Baermann trays (“nematode rafts”: Gibson, 2016), without a decanting and sieving pre-extraction step, and preserved in DESS solution (Yoder et al., 2006). When samples were examined the following year, however, nematode abundances were improbably low (compared to abundances determined from preliminary sampling). Nematodes were thus re-extracted from archived refrigerated samples in random order by autumn of 2017 using decanting, sieving, and sucrose centrifugation (Ferris, 2012). Approximately 100 cc of soil (unless less than this amount remained from the previous extraction) was used for each extraction. Two damaged samples were excluded from each of the thinned and burned treatments. Nematodes were counted using a dissecting microscope at 20x–63x magnification.

Fungal hyphae were extracted from 4 g of soil according to Abbott et al. (1984) and classified as fine ($<5\ \mu\text{m}$) and coarse ($>5\ \mu\text{m}$) arbuscular mycorrhizal (AM) fungi, heavily melanized septate fungi, and other septate fungi using a compound microscope. Hyphae were examined under a compound microscope at 200x magnification and quantified using the gridline intersect method (Abbott et al., 1984).

Analysis of soil abiotic properties

Dried soil from the 203 cc abiotic core was sieved to 2 mm and the fine fractions were weighed to estimate bulk density. Most samples contained few particles $> 2\ \text{mm}$. Dried soil was subsampled using a microsplitter to ensure representative fractions for analyses. Soil organic matter (SOM) was measured by loss on ignition after five hours at $550\ ^\circ\text{C}$ (Heiri et al., 2001). Particle size distribution was analyzed at the Northern Arizona University Sedimentary Records of Environmental Change Laboratory with laser diffraction using a Beckman-Coulter LS-230 Particle Size Analyzer. We measured soil pH according to the method described in Grover et al. (2020). Available N and P were extracted using the methods of Keeney and Nelson (1982) and measured in the Colorado Plateau Analytical Laboratory. Nitrate and ammonium content was determined following Wendt (1999), and orthophosphate content was analyzed by the Olsen Method (Olsen et al., 1954).

Data analysis

All analyses were conducted in R (v.4.0.3; R Core Team, 2020). Where model assumptions of normality and homogeneity of variance could be met, we used ANOVA to detect treatment differences in soil abiotic properties and in densities of microarthropods, nematodes, and hyphae, and performed post-hoc comparisons with Tukey’s honestly significant difference test ($\alpha \leq 0.1$ to account for heterogeneity and small sample size). Data were log-transformed to meet assumptions of normality and homogeneity of variance. When assumptions for ANOVA could not be met, we used Kruskal-Wallis and performed post-hoc comparisons with pairwise Wilcoxon rank sum tests, using the Benjamini and Hochberg correction for multiple comparisons. *P*-values calculated with Wilcoxon rank sum tests are approximate where ties occurred.

Non-metric multidimensional scaling ordination (NMDS) was used to examine relationships among soil faunal communities, soil properties, hyphal densities, and ground cover (litter depth was omitted because of three missing values). Multi-response permutation procedure (MRPP) was used to test the significance of faunal community differences among treatments. This multivariate non-parametric technique tests for differences among *a priori* groups and provides the test statistic *A*, the chance-corrected proportion of between-sample distances explained by group identity. An *A* value of 0 indicates that within-group heterogeneity is equal to the chance expectation, an *A* value < 0 suggests more within-group heterogeneity than would be expected to occur by chance, and *A* will equal 1 if all samples within groups are identical, but groups are distinct. An *A* value > 0.1 is considered likely to represent ecologically meaningful group differences. The NMDS and MRPP analyses were based on total densities of nematodes, collembolans, mites, and other fauna (mainly ants, enchytraeids, and macroarthropod larvae). We produced NMDS plots and performed MRPP analyses with the package “vegan” (Oksanen et al., 2020) using Bray-Curtis dissimilarity distance values. Figs were created using the package “ggplot2” (Wickham, 2011), with the exception of correlation matrices included in the supplementary information, which were constructed with the package “corrplot” (Wei and Simko, 2017).

We used an automated stepwise model selection procedure based on $\Delta AIC_c < 2.00$ to explore the most important habitat and resource predictors for abundances of individual faunal groups in soil and (if applicable) litter habitats. Litter depth was excluded due to multiple missing observations, but values were imputed by using treatment averages for variables missing only one observation. After removing highly collinear predictor variables ($R^2 > 0.6$) and applying log transformations as needed to satisfy model assumptions of normality, linearity, and homogeneity of variance, we used the “step” function to search for the optimal model for each faunal group. We specified a lower scope limit of fauna ~ 1 and an upper limit of fauna $\sim \log.NO_3 + \log.PO_4 + \text{percent.Wood} + \text{percent.Litter} + \log.\text{percent.Grasses} + \log.\text{percent.Forbs} + \log.\text{percent.Bare} + \log.SOM + \text{BulkDensity} + \text{Clay} + \text{Fine.AM} + \text{Coarse.AM} + \text{Total.NonAM}$.

Results

Habitat and resource characteristics

Soil fauna resource and habitat characteristics within control, thinned, and burned management units are shown in **Table 1**. Soil organic matter in the thinned unit was 44.3% higher on average than in the control unit, and 58.6% higher than in the burned unit; this pattern was also evident, but weaker, for %C. The inverse trend was observed for bulk density, which was 34.5% higher on average within the burned unit than within the thinned unit, and intermediate within the control. Relative to the control unit, soils in the thinned unit were slightly more acidic and soils in the burned unit were marginally more alkaline. Phosphorus and nitrogen tended to be most available in the burned unit, intermediate in the thinned unit, and lowest in the untreated control unit, but variance in PO_4 , NH_4 , and NO_3 was large, especially within the burned unit. Soil texture differences were detected with the control unit having higher fine soil fraction compared to the two treatment units. Of the fungal hyphae categories quantified, only abundance of coarse AM hyphae differed by treatment: it was 76% higher on average in the thinned unit than in the untreated control unit, and intermediate in the burned unit.

Meso- and microfauna abundance

The abundance of micro- and mesofaunal groups differed across restoration treatments. Lower densities of soil and litter mites were observed within the burned unit than within the thinned and control units, which had similar soil mite abundances (**Fig. 2**). Soil and litter collembolans (**Fig. 2**) and soil nematodes (**Fig. 3**) showed similar patterns across treatment units, in that members of these three groups were most abundant in the thinned unit, while densities in control and burned units were similarly low. Treatment differences were stronger for soil collembolans and nematodes than for litter collembolans. Most samples did not contain individuals in the “other soil fauna” and “other litter fauna” categories, and abundances of these groups did not differ across treatments (**Supplemental Fig. 1**).

Habitat and resource predictors of soil faunal communities

Community separation of soil only, soil and litter, and microarthropod assemblages was evident for thinned vs burned treatments, and for burned vs control treatments, but not for the control vs. thinned units (**Table 2**). Across faunal group subsets, grass and litter cover, bare ground, and nitrate consistently correlated with community dissimilarity; ammonium was also strongly correlated in ordinations based on all fauna in all habitats, and on microarthropods alone, while fine sand and phosphate were correlated with soil fauna community differences (**Fig. 4**). Selected multiple regression models for nematodes and soil, litter, and total microarthropod densities are summarized in **Table 3**. In addition, nematodes were negatively correlated with pH ($r^2=0.41$, $P<0.01$ for the bivariate correlation), and litter mites were positively correlated with litter depth ($r^2=0.40$, $P<0.01$ for the bivariate correlation), two habitat variables that were excluded from the model selection procedure due to strong collinearity with SOM and missing values, respectively.

Table 1. Litter depth, soil abiotic properties, and lengths of arbuscular mycorrhizal (AM) and septate hyphae (m g^{-1}). Groups with different letters are significantly different at $P < 0.1$ according to univariate ANOVA or Kruskal-Wallis (KW), after correction for multiple comparisons using Tukey's test of honestly significant differences for ANOVA or Benjamini Hochberg adjustment for KW.

	Test	<i>Control</i>				<i>Thinned</i>				<i>Burned</i>			
		n	Mean	SE		n	Mean	SE		n	Mean	SE	
Litter depth (cm)	KW	7	3.92	1.25	a	8	3.03	1.01	a	6	1.44	0.64	a
Bulk density (g/cm^3)	ANOVA	8	0.92	0.05	ab	8	0.77	0.04	b	7	1.02	0.07	a
pH	ANOVA	8	4.85	0.16	ab	8	4.56	0.22	b	8	5.25	0.23	a
% SOM	KW	8	6.00	0.77	ab	8	8.66	1.45	a	8	5.46	1.26	b
% C	KW	8	2.82	0.54	a	8	3.34	0.81	a	8	2.34	0.71	a
PO_4 $\mu\text{g/g}$	KW	7	3.44	0.69	a	8	3.73	0.75	a	8	6.43	1.57	a
NH_4 $\mu\text{g/g}$	KW	7	5.90	0.39	a	8	7.92	1.14	a	8	18.48	9.48	a
NO_3 $\mu\text{g/g}$	KW	7	0.21	0.02	a	8	0.33	0.10	a	8	0.59	0.21	a
% N	KW	8	0.12	0.02	a	8	0.13	0.02	a	8	0.11	0.04	a
% Clay	ANOVA	8	9.28	0.94	a	8	5.10	0.40	b	8	6.43	0.90	b
% Silt	ANOVA	8	43.71	4.18	a	8	25.78	2.24	b	8	32.06	4.31	b
% Very fine sand	ANOVA	8	19.60	1.39	a	8	12.50	0.89	b	8	16.08	1.71	ab
% Fine sand	ANOVA	8	14.32	2.02	b	8	20.17	1.56	a	8	19.33	1.86	ab
% Medium sand	ANOVA	8	7.67	2.28	b	8	18.91	1.61	a	8	13.92	2.78	ab
% Coarse sand	ANOVA	8	5.00	2.00	b	8	16.41	1.88	a	8	11.29	2.43	ab
Fine AM hyphae (m/g)	ANOVA	8	7.49	0.95	a	8	8.01	1.20	a	8	7.22	1.51	a
Coarse AM hyphae (m/g)	ANOVA	8	1.81	0.33	b	8	3.19	0.40	a	8	2.31	0.34	ab
Septate hyphae (m/g)	ANOVA	8	3.41	0.84	a	8	4.12	0.72	a	8	4.59	1.03	a

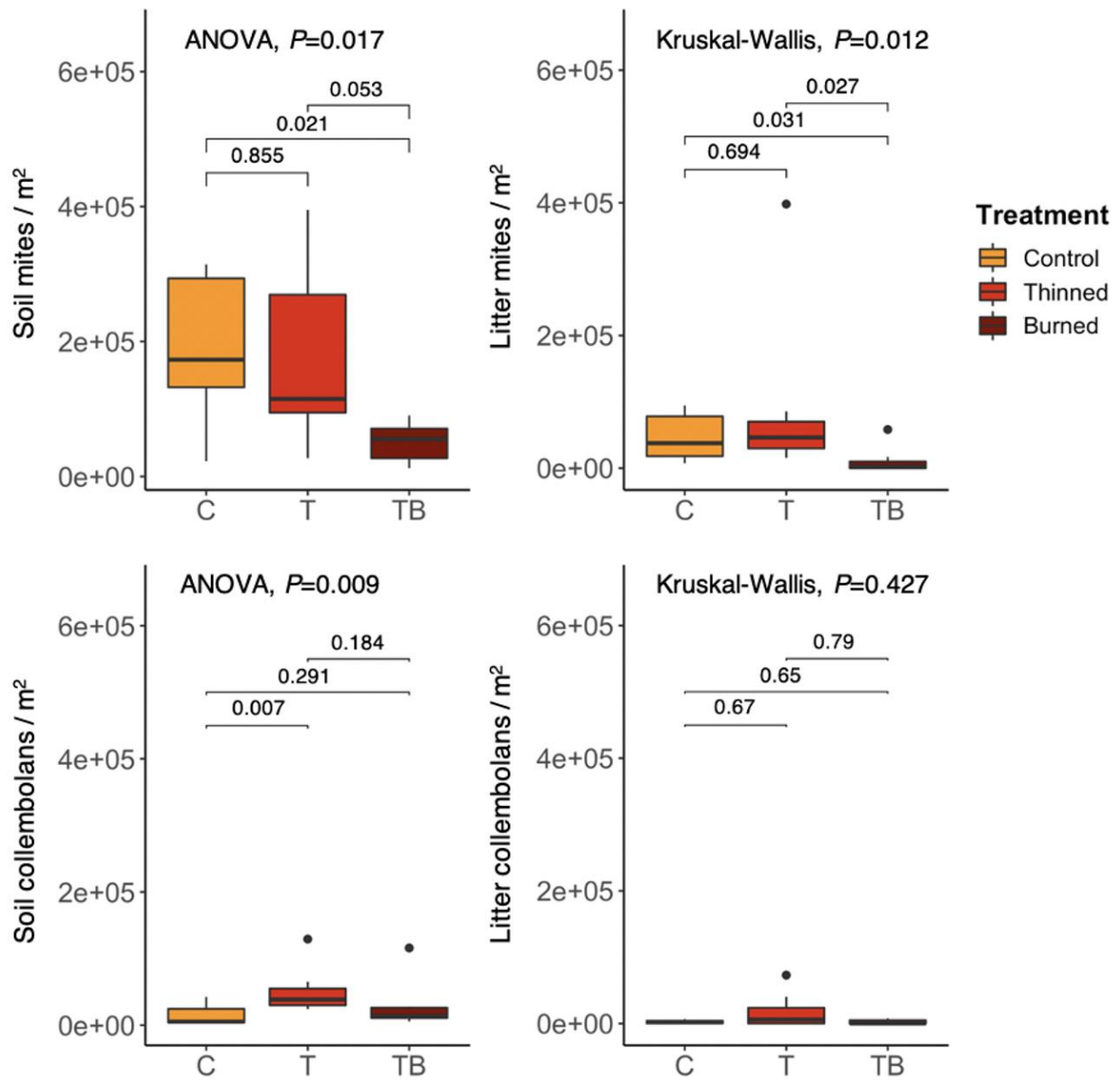


Fig. 2. Densities of mites and collembolans in soil and litter habitats within untreated control, thinned, and burned management units. The center line within each box represents the median for the treatment, and the lower and upper bounds of the box represent the first and third quartiles, respectively. The whiskers show any data points extending up to 1.5 times the interquartile range from the bounds of the box. Any dots are extreme values more than 1.5 times the interquartile range from the upper or lower box boundaries. Horizontal bars above the boxes show P -values for pairwise comparisons, adjusted for multiple comparisons by Tukey's test of honestly significant differences for ANOVA or Benjamini Hochberg adjustment for Kruskal-Wallis.

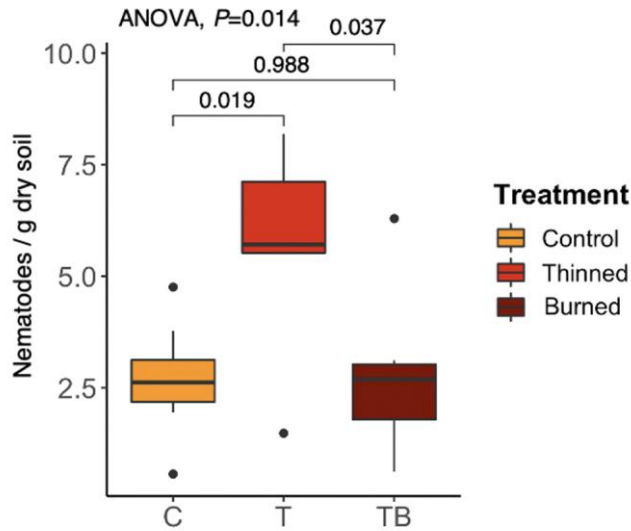


Fig. 3. Nematode abundance in untreated control, thinned, and burned treatment units. Horizontal bars show pairwise comparison P -values, adjusted for multiple comparisons by Tukey's test of honestly significant differences.

Table 2. Multi-response permutation procedure (MRPP) results for soil fauna only (mites, collembolans, nematodes, and others), soil and litter fauna, and microarthropods only, grouped by treatment. The test statistic A represents the chance-corrected proportion of between-sample distances explained by group identity. $A=0$ when within-group heterogeneity is equal to the chance expectation, $A < 0$ indicates more within-group heterogeneity than would be expected to occur by chance, and A will equal 1 if all samples are identical, but groups are distinct. An A value > 0.1 is considered likely to represent ecologically meaningful group differences.

Soil fauna				
	<i>Thinned</i>		<i>Burned</i>	
	<i>A</i>	<i>P</i> -value	<i>A</i>	<i>P</i> -value
<i>Control</i>	-0.005744	0.46	0.1624	0.023
<i>Thinned</i>			0.219	0.006
Soil and litter fauna				
	<i>Thinned</i>		<i>Burned</i>	
	<i>A</i>	<i>P</i> -value	<i>A</i>	<i>P</i> -value
<i>Control</i>	-0.01394	0.591	0.1197	0.014
<i>Thinned</i>			0.1641	0.008
Microarthropods only				
	<i>Thinned</i>		<i>Burned</i>	
	<i>A</i>	<i>P</i> -value	<i>A</i>	<i>P</i> -value
<i>Control</i>	-0.002202	0.448	0.112	0.007
<i>Thinned</i>			0.1012	0.004

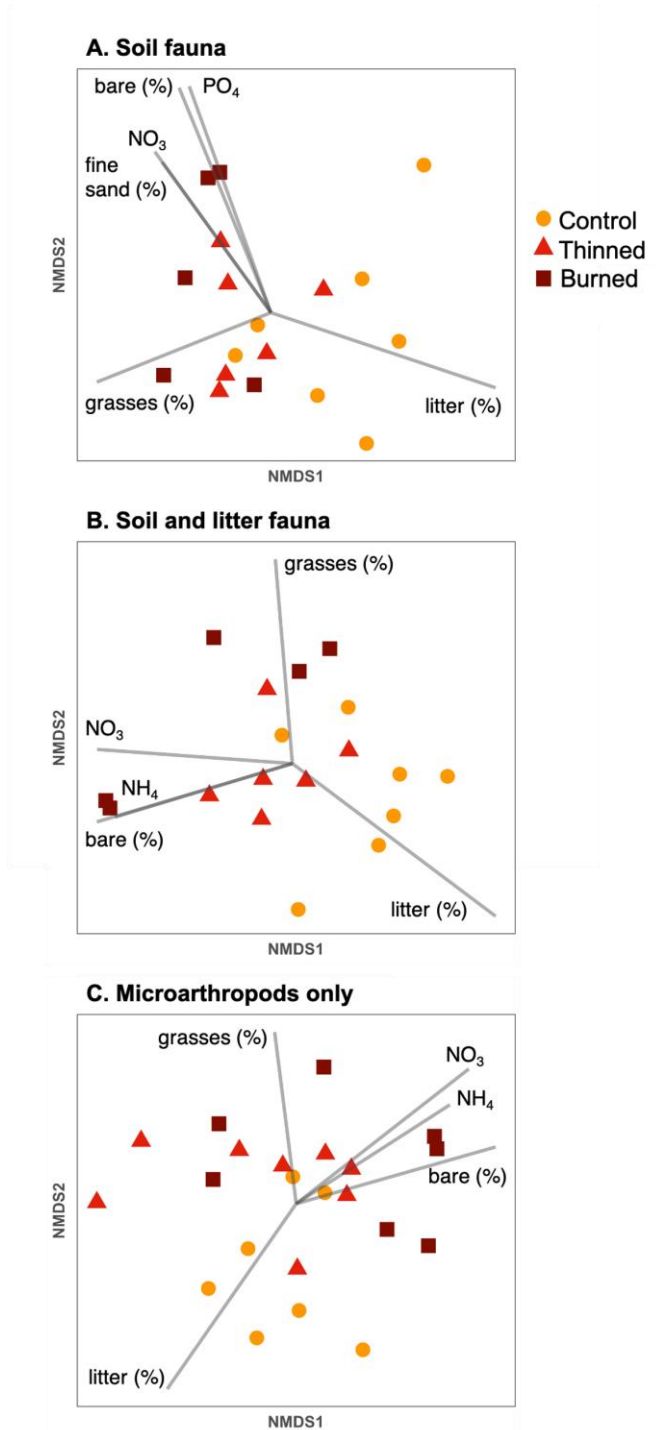


Fig. 4. Non-metric multidimensional scaling ordinations of A) all soil fauna (including mites, collembolans, nematodes, and other taxa) (final stress= 0.089); B) all litter and soil fauna (final stress=0.134); and C) soil and litter mites and collembolans only (final stress= 0.133). Displayed resource and habitat vectors have a correlation with community dissimilarity of $R^2 \geq 0.2$.

Table 3. Summaries of models predicting abundances of faunal groups. Optimal models were selected using an automated stepwise procedure based on Akaike information criterion (AIC) values.

	Parameter Estimate	Std. Error	<i>t</i> value	Parameter <i>P</i> -value	Adjusted <i>R</i> ²	Overall model <i>P</i> -value
ln (Soil mites / m²)						
Intercept	8.52	0.91	9.404	<0.001		
% Litter cover	0.01	0.01	1.823	0.085		
Fine AM hyphae (m/g)	0.15	0.07	2.222	0.039		
ln (% SOM)	0.72	0.39	1.854	0.080	0.32	0.017
ln (Litter mites / m²)						
Intercept	8.89	2.81	3.164	<0.01		
% Litter cover	0.02	0.01	1.804	0.092		
Bulk density	-5.96	1.29	-4.618	<0.001		
% Clay	-0.23	0.09	-2.695	0.017		
ln (% Bare ground)	-0.68	0.21	-3.275	<0.001		
ln (NO ₃ µg/g)	-0.94	0.31	-3.000	<0.001		
% Wood cover	-0.03	0.01	-1.739	0.104		
ln (% Forb cover)	0.46	0.31	1.510	0.153	0.82	<0.001
ln (Total mites / m²)						
Intercept	13.64	0.89	15.364	<0.001		
% Litter cover	0.02	0.01	4.012	<0.001		
Bulk density (g/cm ³)	-2.42	0.91	-2.645	0.016		
% Clay	-0.12	0.06	-1.891	0.074	0.50	<0.01
ln (Soil collembolans / m²)						
Intercept	12.38	1.67	7.472	<0.001		
% Clay	-0.21	0.05	-3.936	<0.01		
ln (% Grass cover)	0.43	0.12	3.684	<0.01		
% Wood cover	0.03	0.01	2.997	<0.01		
ln (NO ₃ µg/g)	0.27	0.19	1.449	0.165	0.67	<0.001
ln (Litter collembolans / m²)						
Intercept	14.90	5.71	2.611	0.019		
Bulk density (g/cm ³)	-8.81	2.58	-3.419	<0.01		
ln (% Forb cover)	-1.97	0.61	-3.227	<0.01		
% Clay	-0.45	0.17	-2.694	0.016		
Fine AM hyphae (m/g)	-0.43	0.16	-2.672	0.017		
ln (% Bare ground)	-0.73	0.29	-2.527	0.023		
ln (NO ₃ µg/g)	-0.87	0.60	-1.452	0.167	0.62	<0.01
ln (Total collembolans/ m²)						
Intercept	11.62	0.88	13.236	<0.001		
% Litter cover	-0.02	0.01	-2.295	0.034		
% Clay	-0.23	0.06	-3.674	<0.01		
ln (% Bare ground)	-0.39	0.14	-2.820	0.011		

ln (% SOM)	0.80	0.32	2.518	0.022	0.62	<0.001
Nematodes / g dry soil						
Intercept	-1.31	1.82	-0.716	0.482		
ln (% SOM)	2.78	1.00	2.783	0.012	0.26	0.012

Discussion

Overall patterns

Soil faunal groups exhibited different associations with forest restoration treatments. Our hypotheses that resource and habitat availability would be enhanced by thinning and reduced by burning were partly supported. We observed that nematodes and collembolans were more abundant in the thinned unit (nematodes and soil collembolans strongly, litter collembolans weakly); however, mite abundances were similar in the thinned unit and in the control unit. As expected, nematode and mite densities were lower, and collembolans tended also to be reduced, in the burned unit relative to the thinned unit. Although the burned unit hosted the fewest mites, densities of nematodes and collembolans in this unit were similar to those in the untreated control, suggesting that, as broad groups, these animals are resilient to restoration treatment disturbances. Also, as predicted, faunal community composition and abundances of individual faunal groups were correlated with physicochemical and biological indicators of faunal habitat and resource availability that were likely impacted by the restoration treatments.

Physicochemical predictors of soil fauna responses

Each successive restoration treatment in this study tended to be associated with higher nutrient availability; thinning and mastication promote mineralization of organic nutrients previously bound up in living and dead plant material, and low intensity burning may increase mineral N and P through several mechanisms, including direct conversion of organic to mineral N and P during combustion and changes to microbial communities (Certini, 2005; Covington and Sackett, 1986). Mineral N and P were consistently robust correlates of faunal community variation. In the untreated control and the thinned unit, higher nutrient availability likely corresponded with greater abundance of microbial prey. Nematodes were strongly negatively correlated with pH, although collinearity of pH and SOM makes it difficult to disentangle the effects of these two habitat variables on nematode abundance. Modeling by van den Hoogen et al. (2019), however, suggests that pH is an important driver of nematode abundance globally. As pH strongly influences bacterial diversity and richness (Fierer and Jackson, 2006; Rousk et al., 2010), changes in nematode abundance with pH could be related to changes in basal food resources, or to direct physiological effects, or both.

Because accessible pore space and water availability are two of the most important parameters governing soil faunal activity and trophic interactions (Erktan et al., 2020), modification of soil physical structure and water holding capacity are potentially the most consequential impacts of forest restoration treatments for these animals. Thinning and mastication were associated with reduced bulk density and increased SOM relative to the untreated control unit, while soils in the burned unit had less SOM and higher bulk density. Despite minor soil texture differences among treatment units, bulk density was not correlated with soil texture, but it was related to SOM, indicating restoration treatments were primarily responsible for changes in pore space. Logging machinery simultaneously compacts soil and churns fragmented organic material deeper into soil layers, so it is not possible to distinguish the effects of compaction versus bulk density changes due to organic matter addition (in the thinned unit) and combustion (in the burned unit); shallow surface soils are likely more subject to churning by equipment wheels, thereby reducing bulk density and increasing SOM, whereas deep soils would be more susceptible to compaction without increases in SOM.

Bulk density was selected as an important covariate in both mite and collembolan abundance models. On average, collembolans and mites have much wider bodies than nematodes, and thus require larger pore sizes for movement. Pore size and connectivity changes following restoration treatments may be particularly important for large-bodied taxa “commuting” from the litter layer to lower soil horizons. Selected models also indicated sensitivity of mites and especially collembolans to soil texture: coarse, sandy soils hosted larger microarthropod populations than fine, clay-rich soils. Because slightly coarser soils were present in the thinned unit and the correlation with collembolans was particularly strong ($r^2=0.44$, $P<0.001$ for the bivariate correlation), the patterns we observed in collembolan treatment responses may also be partly explainable by soil texture. Soil texture was also correlated with soil faunal communities, but not with litter and total faunal communities.

Although not correlated with community composition, SOM was the only habitat variable determined to be an important predictor of nematode abundance by the model selection procedure. Organic carbon has been demonstrated to track strongly with nematode densities on a global scale (van den Hoogen et al., 2019). In xeric forest types especially, the higher water holding capacity of more organic soils should dramatically extend periods of activity for aquatic organisms like nematodes.

Biotic drivers: plant inputs are key

Soil fauna responded to changes in ground cover associated with restoration treatments. Litter cover, grass cover, and bare ground were orthogonal correlates of all three faunal community sets, indicating that resources and habitat associated with these ground cover classes drove distinct changes in faunal communities. More litter was associated with more mites in the litter and below it, and with greater total abundance of collembolans, while absent or patchy ground cover reduced densities of microarthropods active above the soil surface. Grass cover was positively associated with soil collembolan abundance. Previous studies suggest that collembolans may prefer plant roots over fungal hyphae when given a choice (Endlweber et al., 2009). Grass roots, which tend to be poorly defended in comparison to conifer roots, may be especially palatable, and fine roots are probably more abundant beneath grasses than beneath other types of ground cover in this system. A meta-analysis of studies examining nematode trophic groups indicated that energy flow through the herbivory channel is greater in grassland than in forest soil food webs (Zhao and Neher, 2014).

Although declines of fungal biomass after fire have been widely reported (reviewed in Pressler et al., 2019), we did not observe a decrease in septate hyphae or fine AM hyphae with burning. Fine AM hyphae were positively correlated with soil mites, however, and the broad morphological categories we employed in quantification of hyphae do not allow us to discriminate taxa that may vary in their appeal to soil fauna, so it is possible that burning may have reduced fungi representing important food sources for mites. Alternatively, a decline in mite abundance in the burned unit and their failure to increase in the thinning and mastication unit may stem from predominant life history characteristics in this group rather than changes in resource availability. Mite assemblages in temperate forests often contain a high proportion of taxa which move and reproduce slowly, and these may be slower than most nematodes or collembolans to recolonize more severely burned patches from unimpacted refugia in a burn-severity mosaic. More taxonomically detailed investigations are needed to uncover species-specific and mechanistic responses to restoration of ponderosa pine forests.

Conclusions and next steps

Our findings indicate variable responses of soil fauna to restoration treatments. We observed nematodes and collembolans to be relatively resilient to restoration treatments, while mite populations did not appear to have recovered one year after burning. Investigations in other systems have variably found rapid recovery of mite abundances (Hutchins et al., 2011; Jacobs et al., 2015) after low-intensity fire, or persistent depression of populations for at least several years (Malmström et al., 2008); further surveys are needed to assess the longevity of changes to mite communities after prescribed burning in ponderosa pine forests. Despite high heterogeneity in communities of soil and litter fauna, the treatment-related habitat variables we measured

explained a relatively large proportion of the variation in nematode, collembolan, and mite abundances. These patterns suggest some potential indicators of restoration-related changes to soil meso- and microfauna communities: bulk density, SOM, pH, grass cover, and litter cover and depth. These variables are all easy and inexpensive for non-specialists to measure, offering potential utility to land managers who may not be equipped to directly quantify micro- and mesofauna responses to treatment decisions. Future studies are needed to test the generality of these indicators in other forest restorations.

It is important to note that both the untreated control unit and the thinned and burned unit in our study represent departures from historic conditions for ponderosa pine forests in the region (Touchan and Swetnam, 1995). Critically, prescribed fires that follow more than a century of fire suppression probably burn much more intensely than did historic fires in this area (Roos et al., 2020). The absence of a true reference system complicates judgement of the “desirability” of observed changes in micro- and mesofauna communities, while the functional significance of these changes is also currently unknown. However, we submit that if the goal is to restore forest communities (including soil faunal assemblages) and their ecological functions to putative pre-Euro-American settlement conditions, managers should consider the ways in which restoration activities mirror or depart from historic disturbances to soil and litter habitats, and strive to minimize non-historic habitat modifications when crafting treatment plans.

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Study 2: Soil mechanical disturbance study

In preparation for *Forest Ecology and Management*

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Introduction

Logging machinery can cause considerable soil disturbance, but the potential consequences for forest soil fauna of these habitat alterations remain largely unexplored, especially for nematodes. Because soil micro- and mesofauna are unable to physically shift soil particles, soil structure is among the most important parameters governing resource access and trophic interactions (Erktan et al., 2020). Two main types of soil disturbance can result from use of tracked logging equipment: compaction and churning. We hypothesized that compaction would reduce pore space accessible to nematodes, negatively affecting nematode abundance, and that larger-bodied taxa would be disproportionately impacted. We anticipated that the effects of soil churning on nematode communities would be more complex. Although soil churning by tracked equipment increases total pore space (at least temporarily, until soil particles settle), this might or might not translate to additional habitat availability, as churning also destroys aggregates and may not create pores of optimal size for soil microfauna. We also expected that churning (and, to a lesser degree, compaction) would liberate resources for primary decomposers, with potentially positive effects on disturbance-tolerant R-selected nematodes. We presumed that populations of K-strategist nematode species, however, were more likely to respond negatively to mechanical disturbance. Alteration of ground cover by logging machinery was also predicted to affect soil nematodes, as living plants and litter are the primary sources of matter and energy flowing through soil food webs, and ground cover also modulates microclimate.

We examined the effects of the number of feller buncher passes on soil bulk density, penetration resistance, shear strength, ground cover, and nematode communities, with the aim of determining thresholds for impacts. In addition, we quantified responses of the aforementioned soil abiotic characteristics to disturbance from a single pass by a wheeled skidder, a single pass by a wheeled skidder plus a single pass by a tracked feller buncher, and heavy traffic from multiple equipment types on a logging road.

Methods

Study site, experimental design, field measurements, and sample collection

This study was located at management unit Seco 5. Soils at this site are volcanic loams to silt loams classified primarily as Vitrandic Hapludalfs, Vitrandic Hapludolls, and Vitrandic Argiudolls (Hibner et al., 2010). Overstory vegetation consists of mixed conifer forest including *Picea engelmannii* (Engelmann spruce), *Picea pungens* (blue spruce), *Abies concolor* (white fir), *Pseudotsuga menziesii* (Douglas-fir), and *Pinus ponderosa* (ponderosa pine), with occasional *Populus tremuloides* (quaking aspen). Mean temperatures range from ~15 °C in July to ~ -5 °C in December and January, and the area receives an average of ~690 mm in precipitation annually (30 year normals from 1981-2010; PRISM Climate Group, 2021).

In November 2017, we established five experimental transects along natural corridors between trees to assess soil compaction and disturbance by logging machinery and impacts to soil nematode communities. Sections of each of the first three transects received treatments of one, three, and nine passes by a feller-buncher. The fourth

transect received only one pass by a skidder, and the fifth was treated with one skidder pass and one feller-buncher pass. Trees surrounds the transects were left intact to minimize impacts of changing light and temperature on the soil surface. Soil properties within these transects were also compared to those of an old logging road which received extensive traffic during thinning of the unit. Three replicate control and treated sample pairs were collected at evenly spaced points along the one, three, and nine pass sections of the three feller-buncher transects, respectively, and from the skidder, skidder and feller-buncher, and road transects. Treated samples were collected from the center of the machine track, and control samples were collected from between the tracks. Sampling and surface measurements occurred at three evenly spaced points per transect section. Prior to sampling, we characterized ground cover for 0.25 m² areas centered on each sample collection point, quantifying percent cover by grasses, shrubs, forbs, mosses, lichens, pine litter, spruce litter, forb litter, moss litter, thatch, woody debris, sticks, scat, and bare ground. All sampling and *in situ* soil measurements were performed at consistent points marked on our 0.25 m² cover frame. We measured soil surface resistance to penetration (three pocket penetrometer readings), litter depth, shear strength using a TORVANE (Durham Geo Slope Indicator, Tucker, GA), and trench depth (the depth of the nearest indentation formed by feller buncher tracks). We collected soil cores for determination of bulk density at depth increments of approximately 2-6 cm, 9-13 cm, 16-20 cm, and 23-27 cm (for a total of 292 samples). Cores were retrieved using a bulk density sampling cup with liner ring (AMS, Inc., American Falls, ID) designed to minimize compaction during sampling. Because most soil fauna reside in the uppermost 0-10 cm, we sampled this interval for nematodes. Overlying litter, if present, was cleared away, and soil cores for nematode extraction were removed using pipe segments with an internal diameter of 5.08 cm.

Sample processing

Bulk density soil samples were dried at 65 °C and weighed. Bulk density was calculated as soil sample dry weight/90.59 cm³, the internal volume of the sampling cup liner ring. To ensure that no confounding correlation with soil organic matter (SOM) and bulk density existed, and to examine relationships between SOM and nematode communities, we determined soil organic matter (SOM) content by loss on ignition for the 2-6 cm and 9-13 cm depth increments overlapping or partially overlapping the nematode sampling depth interval. We analyzed soil organic matter content only for feller buncher transect samples where nematode communities were also characterized. Samples were sieved to 2 mm and homogenized, then 5 g subsamples were dried at 105 °C, weighed, heated at 450 °C for 24 hours, and reweighed (Bisutti et al., 2004).

Nematode soil samples were kept on ice for transportation to Northern Arizona University and were stored at 4 °C until processing. Nematodes were extracted by centrifugal flotation with Ludox colloidal silica solution using a method modified from Griffiths et al. (1990) as described here. Soil samples were sieved gently to 6.3 mm and thoroughly homogenized, and a 5 g subsample was dried for 48 hours at 105 °C to determine gravimetric water content. An 80 cc soil subsample for nematode extraction was weighed and transferred to a 500 mL centrifuge tube, which was filled with tap water and shaken. Subsamples were then centrifuged at 2110 rpm (~700 g) for 12 minutes. Floating organic matter was removed from the centrifuge tubes with a spoon and the supernatant was decanted and discarded. Nematodes and soil particles in the remaining pellet were resuspended in 300 mL of Ludox (diluted to a specific gravity of 1.17 g/cm³ with DI water) and centrifuged again at the same speed for 7 minutes. Nematodes were retrieved from the Ludox supernatant by pouring it over a 20 µm sieve, then were backwashed into a beaker with tap water. The original Ludox solution which passed through the sieve was then returned to the centrifuge tube and the Ludox centrifugation and sieving steps were repeated two times. Collected animals were preserved in DESS solution (Yoder et al., 2006) and refrigerated at 4 °C until examination. Eight randomly selected control/treated sample pairs per disturbance level were included in nematode analyses (48 total samples).

As nematode yields were very high, we estimated total sample abundance based on ~10% subsamples. We used a Hensen-Stempel pipette, developed to avoid sampling fractionation of plankton suspensions, to obtain representative 4 mL subsamples of 40 mL nematode suspensions. Samples were mixed gently by repeated

inversion prior to subsampling, and subsampled nematodes were examined using an inverted compound microscope at 100X-400X magnification. We validated the accuracy of this abundance estimation method for 11 samples by comparing abundances calculated by subsampling to those obtained by direct examination of all nematodes present. Enumeration of entire nematode samples was performed using a stereomicroscope at a magnification of 40X-78.8X. Correlation between these abundance estimation methods was high ($R^2=0.932$).

The first 200 nematodes encountered in each subsample were identified to the taxonomic level necessary for classification to feeding group and position on the colonizer-persister scale (cp class (Bongers, 1990)). This scale represents the continuum from r-strategists (cp1) to K-strategists (cp5): Nematodes in colonizer-persister classes cp1 and cp2 are considered indicators of organic enrichment and/or basal fauna, while nematodes in the maturity indicator classes cp3, cp4, and cp5 are associated with increasing food web stability, complexity, and connectance (Ferris et al., 2001). Assignments were made according to the NEMAPLEX Nematode Ecophysiological Parameter database (Ferris, n.d.). Because specimen condition sometimes made determination of family impossible, and this information is necessary for assignment of cp4 and cp5 Dorylaimida to both cp class and feeding group, all Dorylaimida were grouped together as cp4/cp5 omnivores, predators, and fungivores. We distinguished the groups cp1 bacterivores, cp2, cp3 bacterivores, cp4 bacterivores, cp2 fungivores, cp4/cp5 Dorylaimida, cp4 predators, cp2 plant associates (the ubiquitous and enigmatic Tylenchidae), and strict herbivores (which were not assigned to cp class, as plant parasitic taxa are not commonly used as indicators of disturbance). Group assignments for taxa are listed in **Table 2.1**. We hereafter refer to these groups as functional guilds (*sensu* Ferris et al., 2001), but it should be noted that Dorylaimida actually encompasses multiple functional guilds.

Table 2.1. Feeding group, cp class, and functional guild assignments for nematode taxa at Seco 5.

Taxon	Feeding group(s)	Colonizer-persister class(es)	Functional guild assigned
Panagrolaimidae	Bacterivore	cp1	cp1 bacterivore
Rhabditidae	Bacterivore	cp1	cp1 bacterivore
<i>Wilsonema</i> sp.	Bacterivore	cp2	cp2 bacterivore
<i>Acrobeles</i> sp.	Bacterivore	cp2	cp2 bacterivore
<i>Cervidellus</i> sp.	Bacterivore	cp2	cp2 bacterivore
Other Cephalobidae	Bacterivore	cp2	cp2 bacterivore
Other Plectidae	Bacterivore	cp2	cp2 bacterivore
Tylenchidae	Plant associate	cp2	cp2 plant associate
Criconematidae	Herbivore	NA	Herbivore
<i>Helicotylenchus</i> sp.	Herbivore	NA	Herbivore
Hemicyclophoridae	Herbivore	NA	Herbivore
Other Tylenchoidea (apart from Tylenchidae)	Herbivore	NA	Herbivore
<i>Trichodorus</i> sp.	Herbivore	NA	Herbivore
Aphelenchidae	Fungivore	cp2	cp2 fungivore
Aphelenchoididae	Fungivore	cp2	cp2 fungivore
Prismatolaimidae	Bacterivore	cp3	cp3 bacterivore
Teratocephalobidae	Bacterivore	cp3	cp3 bacterivore
Rhabdolaimidae	Bacterivore	cp3	cp3 bacterivore
cf. Chronogasteridae	Bacterivore	cp3	cp3 bacterivore

Alaimidae	Bacterivore	cp4	cp4 bacterivores
Mononchidae	Predators	cp4	cp4 predators
Dorylaimida	Omnivores, predators, and fungivores	cp4/cp5	cp4/cp5 omnivores, predators, and fungivores

Statistical analyses

Treatment differences in soil physical properties and abundances of nematode groups were assessed by Student's *t*-tests where parametric model assumptions could be met, or by Wilcoxon rank sum tests where they could not. Relationships between nematode groups and soil habitat characteristics were examined with bivariate regressions. One 9 pass treated sample with anomalously high bulk density (presumably due to the presence of rocks) was excluded from these regressions. Predictor and response variables were log transformed where necessary to achieve normality and constant variance. Wilcoxon rank sum tests, Student's *t*-tests, and linear regressions were executed in R (R Core Team, 2020) and visualized with package *ggplot2* (Wickham, 2011). We tested multivariate treatment group differences using multi-response permutation procedure (MRPP) and visualized community distances with non-metric multidimensional scaling ordination (NMDS) in PC-ORD (McCune and Mefford., 2006). All NMDS and MRPP analyses were performed using Bray-Curtis distance.

Results and Discussion

Soil physical properties

Surface resistance to penetration decreased markedly relative to controls for all treatments except one feller-buncher pass and along the old logging road (**Fig. 2.1 A**). This accords with our observations of extensive churning and disruption of surface soil structure, especially at treatment levels above one feller-buncher pass. Shear stress tended to be increased by treatment also, although control vs treatment differences were only significant for 1 feller bunch pass (**Fig. 2.1 B**). Effects of logging machinery on bulk density appeared negligible in the uppermost interval (likely also due to churning disturbance) but were discernible at lower depth intervals (**Fig. 2.2** and **Fig. 2.3**). The magnitude of compaction differences did not appear to attenuate with depth. This is contrary to most previous studies, which have generally found that compaction impacts are greatest near the surface (Cambi et al., 2015). However, exceptions to this pattern have been reported, including for loam to silt loam forest soils (Jourgholami et al., 2014) such as occur at Seco 5. Volcanic parent material may also render these soils more vulnerable to compaction (reviewed in Cambi et al., 2015). Consistent with this, the majority of compaction occurred with 1 pass, and additional passes produced only negligible additional compaction (although variability in bulk density was higher, likely because additional passes subjected more fine-scale locations to stress). While considerably lower replication limits inference regarding the skidder, skidder/feller-buncher, and logging road transects, our data suggest that bulk density impacts from these treatments did not exceed those from the feller-buncher alone. Treated and control samples from the two uppermost sampling depths in the feller buncher transects did not differ in soil organic matter content (2-6 cm: $t = -0.39312$, $df = 42.946$, $p\text{-value} = 0.696$; 9-13 cm: $t = -0.96179$, $df = 40.472$, $p\text{-value} = 0.341$), indicating that treatments likely were not confounded with pre-existing bulk density differences. Mean SOM in control samples collected from the 2-6 cm depth interval was 8.05% (SE= 0.418), vs. 8.27% in the treated samples (SE= 0.394); in the 9-13 cm depth interval, mean SOM in controls was 5.87% (SE= 0.166), vs. 6.13% for treated samples (SE= 0.202).

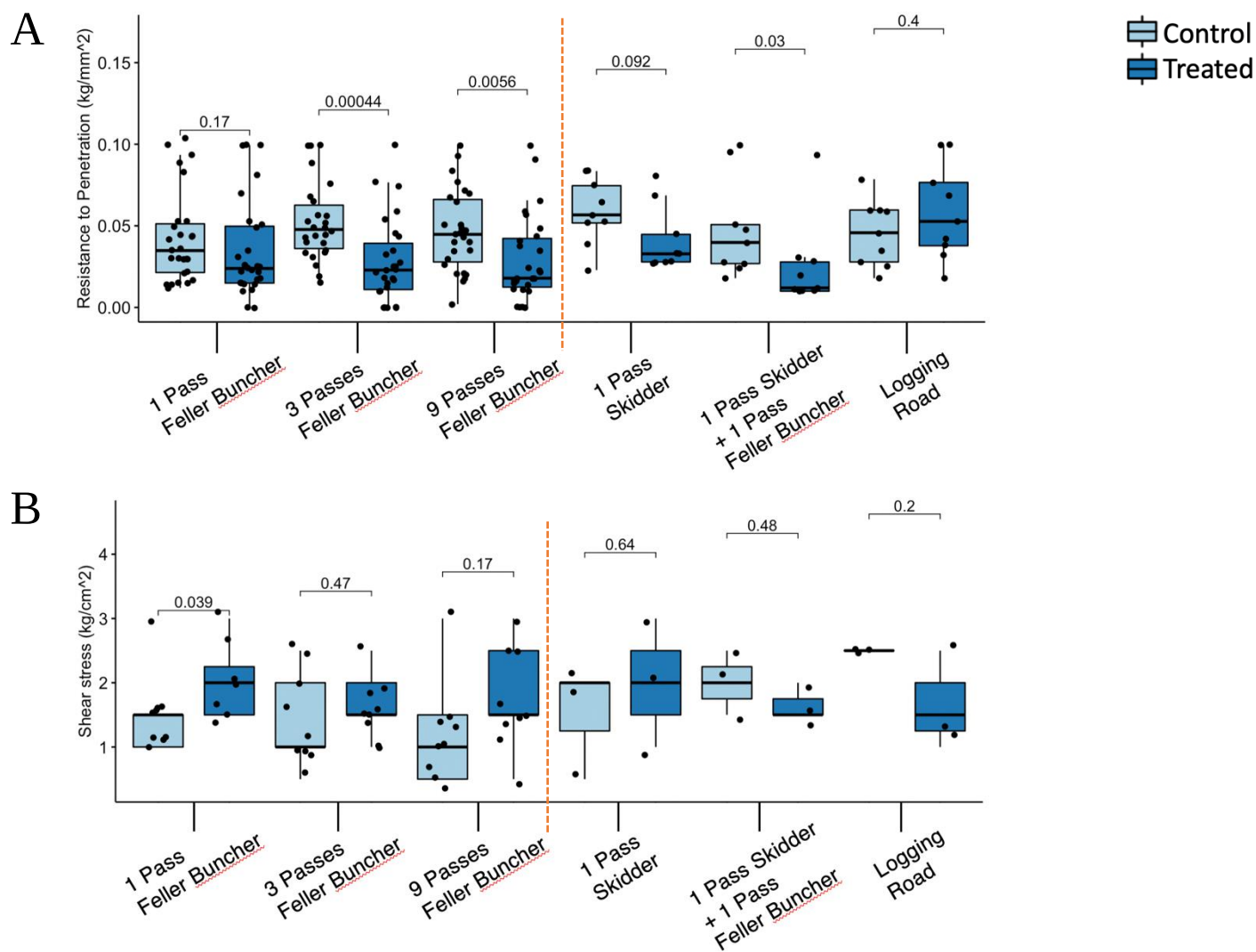


Fig. 2.1. A) Soil surface resistance to penetration. **B)** Shear stress as measured by TORVANE. P-values above brackets are calculated from Wilcoxon rank sum tests and have not been corrected for multiple comparisons. Treatments to the left of the orange dashed line were replicated at three transects ($n=3$ per transect); treatments to the right were replicated at only one transect ($n=3$ total).

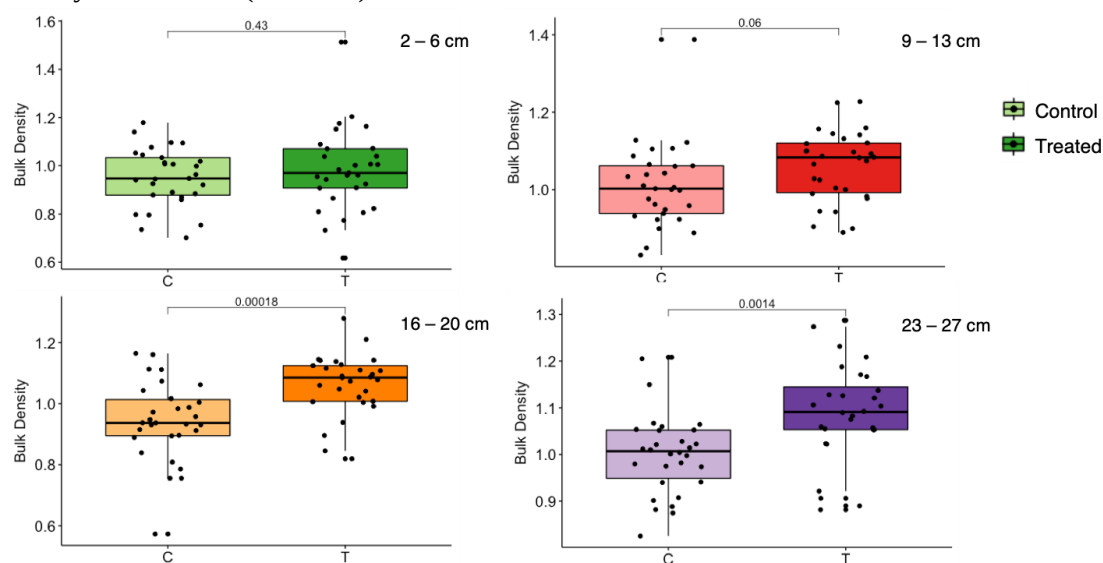


Fig. 2.2. Soil bulk density (g/cm^3) comparisons across all experimental treatments and adjacent untreated transects (excluding the old logging road, which had been heavily disturbed prior to sampling). P-values above brackets are calculated from unpaired Student's t -tests and have not been corrected for multiple comparisons.

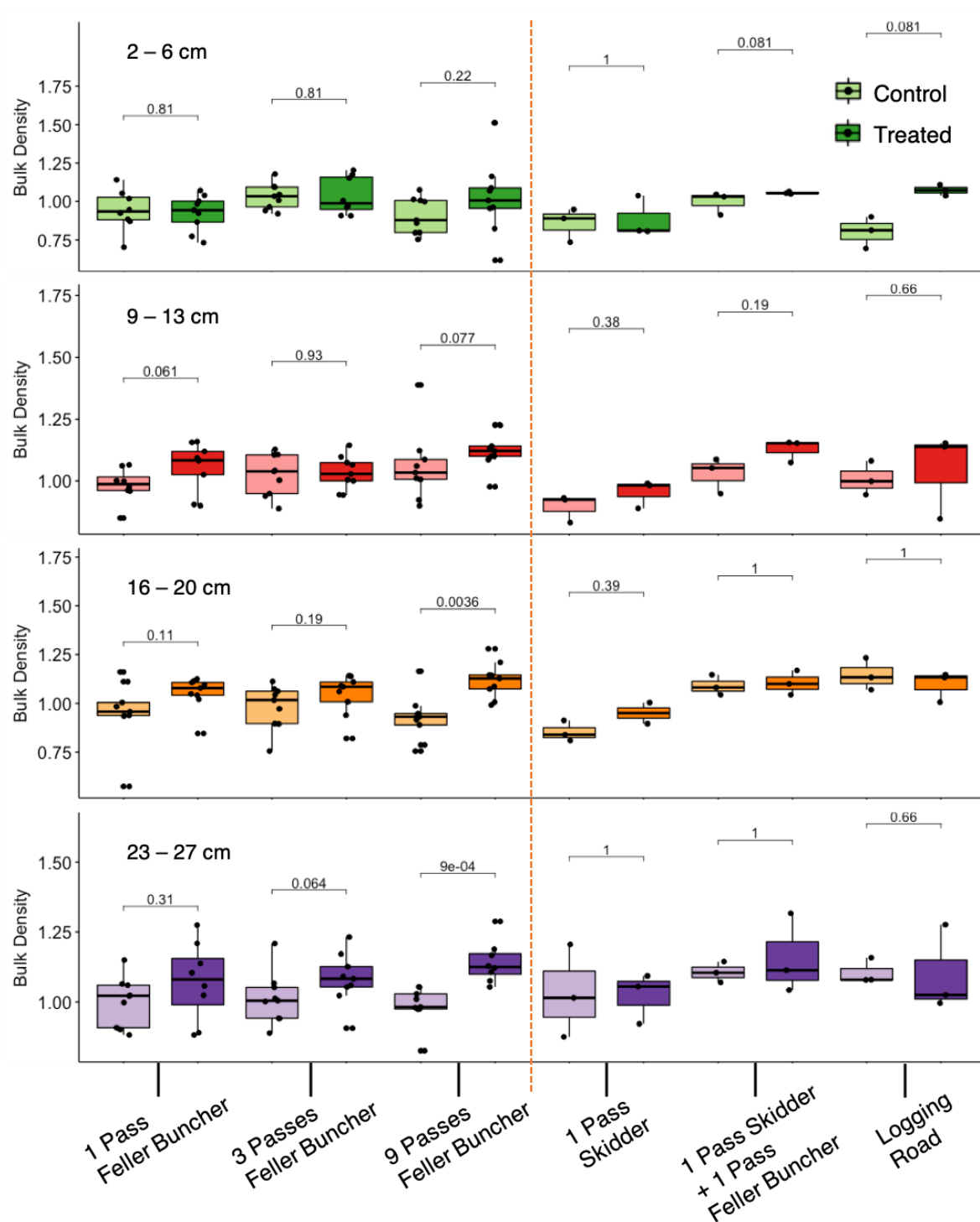


Fig. 2.3. Soil bulk densities (g/cm³) from treated and adjacent untreated experimental transects. *P*-values above brackets are calculated from Wilcoxon rank sum tests and have not been corrected for multiple comparisons. Treatments to the left of the orange dashed line were replicated at three transects (*n*=3 per transect) and were also used for nematode community analysis; treatments to the right were replicated at only one transect (*n*=3 total).

Total nematode abundance was unaffected by any level of feller buncher disturbance we investigated (**Fig. 2.4**), and total nematodes were only weakly correlated with bulk density (averaged over the two depths overlapping or partially overlapping with the nematode sampling interval). However, enrichment and basal indicator nematodes, herbivore and root associate nematodes, and maturity indicator nematodes responded differently to disturbance treatments.

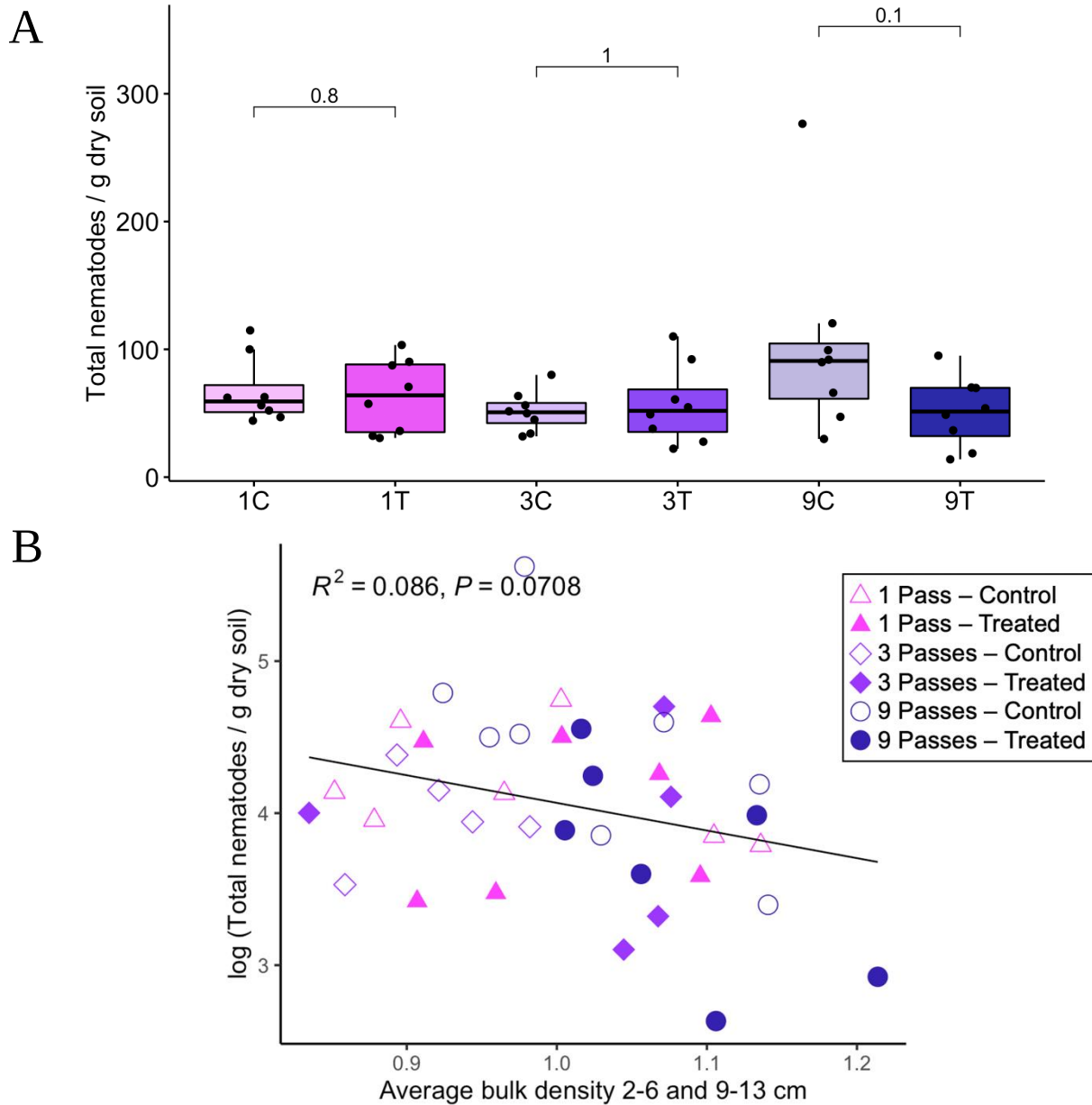


Fig. 2.4 A) Total nematode abundances in treated tracks (T) and control center (C) transect areas which received 1, 3, or 9 passes. P-values above brackets are calculated from Wilcoxon rank sum tests and have not been corrected for multiple comparisons. **B)** Bivariate correlation between total nematode abundance and average bulk density for depths 2-6 cm and 9-13 cm.

Neither herbivore nematodes (**Fig. 2.5 A**) nor plant associate nematodes in the family Tylenchidae (**Fig. 2.5 B**) were sensitive to measured levels of feller buncher traffic. Herbivore nematodes were, however, correlated with ground cover plot dissimilarity (**Fig. 2.6, Fig. 2.5 C**), and effects of feller buncher disturbance on ground cover were detectable with 9 passes (**Table 2.2**). Herbivore nematodes were more abundant in plots with higher grass and thatch cover, and less numerous in plots with more bare ground (**Fig. 2.5 C**). When examined as bivariate relationships, only the correlation between herbivore nematodes and grass cover was significant ($R^2=0.2$, $p=0.0016$). Tylenchidae comprised a median ~27% of nematode individuals across samples, in line with proportions frequently reported from natural systems (Ferris and Bongers, 2006), and were not correlated with grass or any other cover class ($R^2<0.1$, $p>0.1$ for all relationships). Feeding habits in this likely polyphyletic family remain poorly resolved; members have been variously reported to feed on plant roots, fungi, mosses, or algae (Okada et al., 2005; Qing and Bert, 2019). Variability in food preferences within the Tylenchidae may explain their lack of correlation with ground cover.

Total enrichment and basal indicator nematodes (a group comprising cp1 bacterivores, cp2 bacterivores, and cp2 fungivores) did not differ between treated and control samples at any treatment level (**Fig. 2.7 A**). Only ~20% of samples yielded any cp1 bacterivores, and where they occurred, these nematodes usually were present in low numbers (< 1 nematode / g dry soil). The two exceptions with appreciable cp1 bacterivore populations were collected from treated 3 pass and 9 pass transect sections, respectively, but treated and control populations of this group did not differ for any number of feller buncher passes. Cp2 bacterivores were elevated in several control samples from the 9 pass section (**Fig. 2.7 B**), while cp2 fungivores tended to be more numerous in treated 3 and 9 pass transect sections relative to their paired controls (**Fig. 2.7 C**). Cp2 fungivores were weakly correlated with plot ground cover dissimilarity in an NMDS (**Fig. 2.6**).

Negative impacts of mechanical disturbance on maturity indicator nematodes were detectable only with 9 feller buncher passes (**Fig. 2.8 A**). The predominant members of this group, cp3 and cp4 bacterivores (**Fig. 2.8 B**) and cp4 and cp5 omnivore, predator, and fungivore Dorylaimida (**Fig. 2.8 C**), showed similar patterns, but the negative response of bacterivores to 9 passes appeared stronger than that of Dorylaimida. However, Dorylaimida were more strongly correlated with bulk density (averaged for the depths included or partially included in the nematode sampling depth interval) than maturity indicator bacterivores (**Fig. 2.9 A and B**). This finding supports our hypothesis that larger-diameter nematodes would be more negatively impacted by compaction, as maturity indicator bacterivores in this study were far more slender than Dorylaimida on average.

Nematode community differences between matched treated and control sampling locations were detectable only with 9 passes (**Table 2.3**), and these differences were modest (MRPP $A < 0.08$). Ordination of nematode communities based on functional guilds revealed correlations with ground cover, litter, depth, and trench depth (**Fig. 2.10**). Communities in 9 pass treated and 9 pass control samples were more variable than communities in 1 pass and 3 pass treated and control samples. The strongest difference in nematode communities was between the 1 pass control samples and the 9 pass treated samples (**Table 2.3**), which may indicate effects of soil disturbance on adjacent “undisturbed” areas—for example, due to horizontal stress or severed roots and/or hyphae—or may be attributable to spatial autocorrelation. This pattern was also evident in comparisons of ground cover (**Table 2.2**).

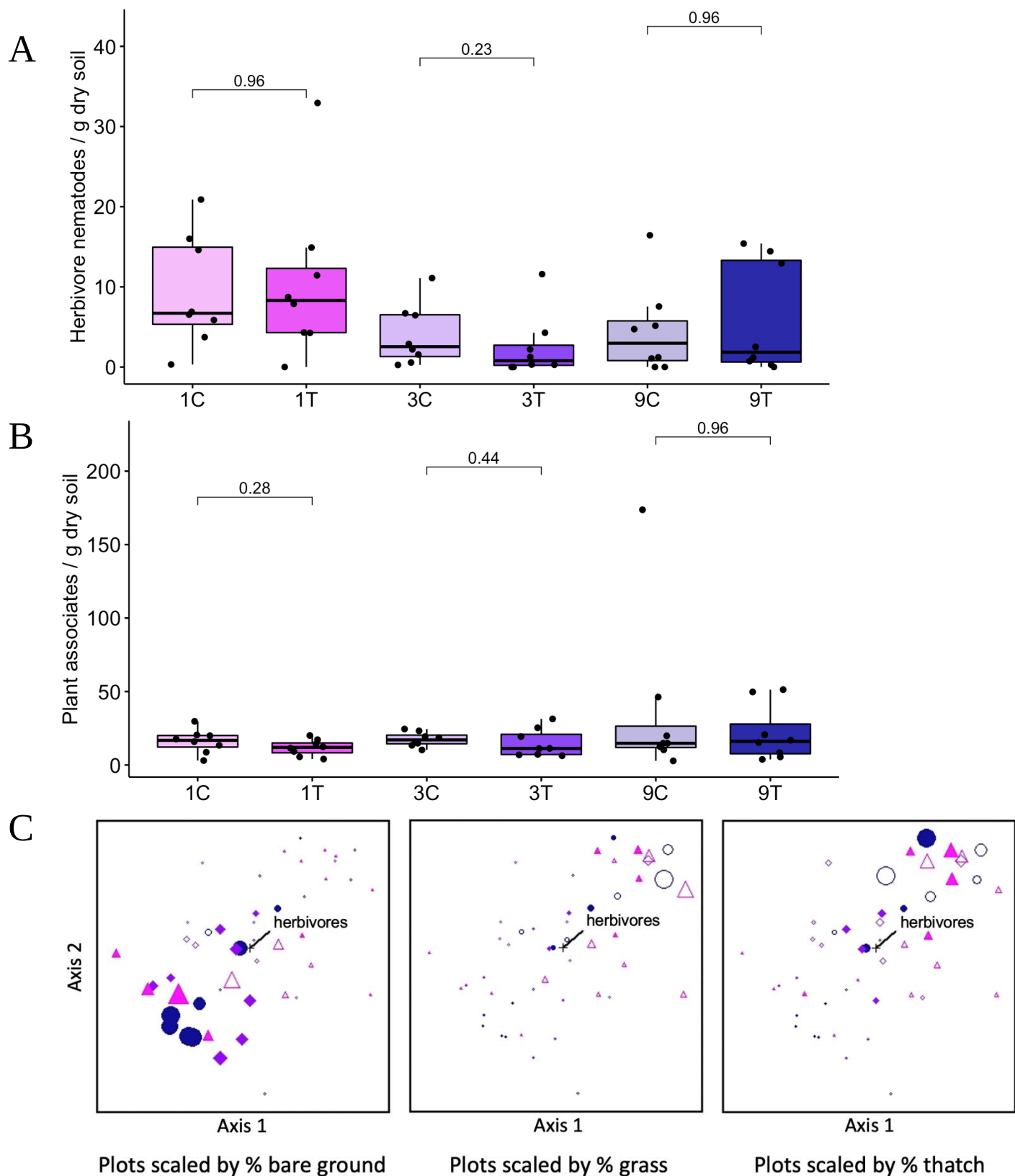


Fig. 2.5 Densities of **A**) herbivore nematodes and **B**) plant associates (Tylenchidae). **C**) Correlation of herbivore abundance with plot ground cover dissimilarity along axes 1 and 2 of a three-dimensional non-metric multidimensional scaling ordination. Points are scaled according to percent bare ground, grass cover, and thatch cover, respectively.

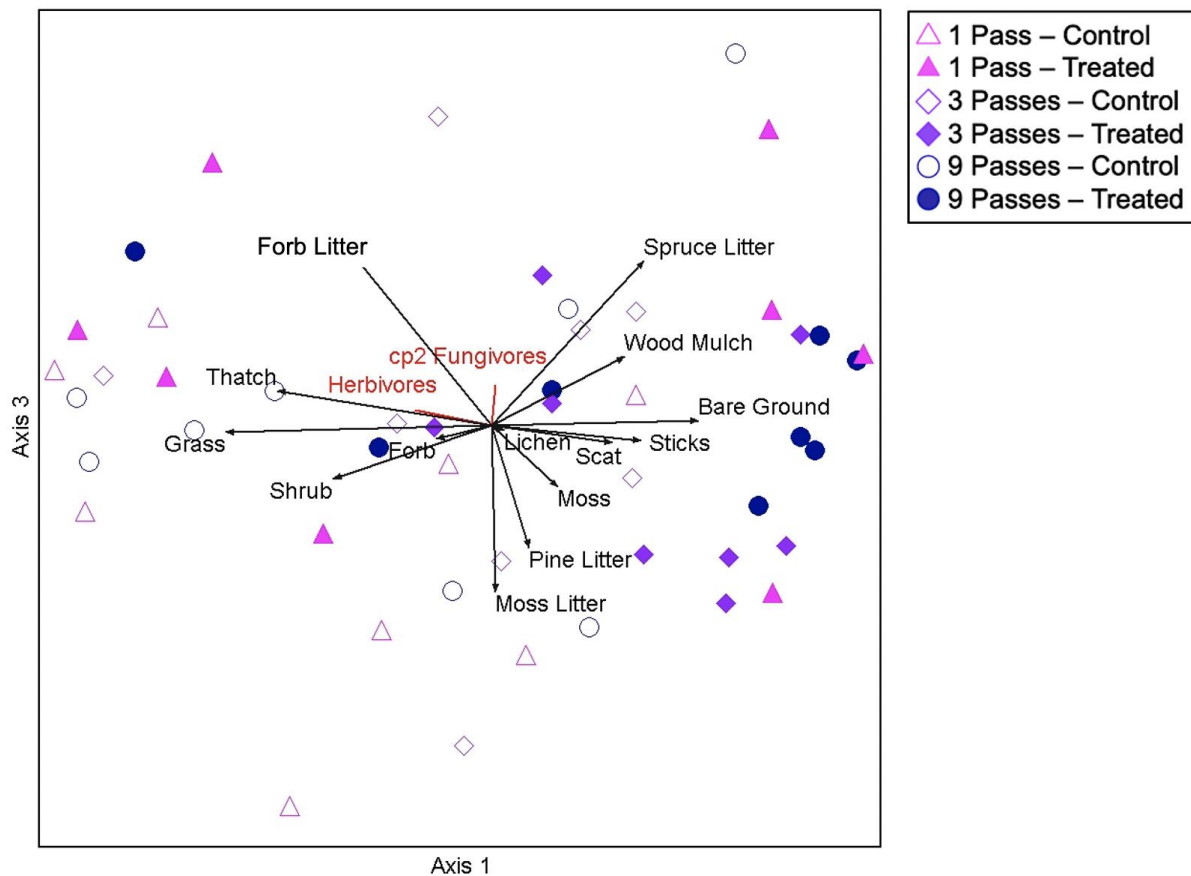


Fig. 2.6. Axes 1 and 3 of a three-dimensional non-metric multidimensional scaling ordination (stress=11.428) of plot ground cover. Cover classes are shown as black vectors, and nematode groups correlated with ground cover dissimilarity ($r^2 > 0.1$) are shown as red vectors.

sdf

Table 2.2. Multi-response permutation procedure (MRPP) results for ground cover.

Ground cover	A	p
Overall group differences	0.0292	0.0521
1 pass (control vs. treated)	-0.0306	0.9603
3 passes: control vs. treated	0.0038	0.3708
9 passes: control vs. treated	0.0524	0.0272*
1 pass control vs. 9 pass treated	0.0629	0.0126*

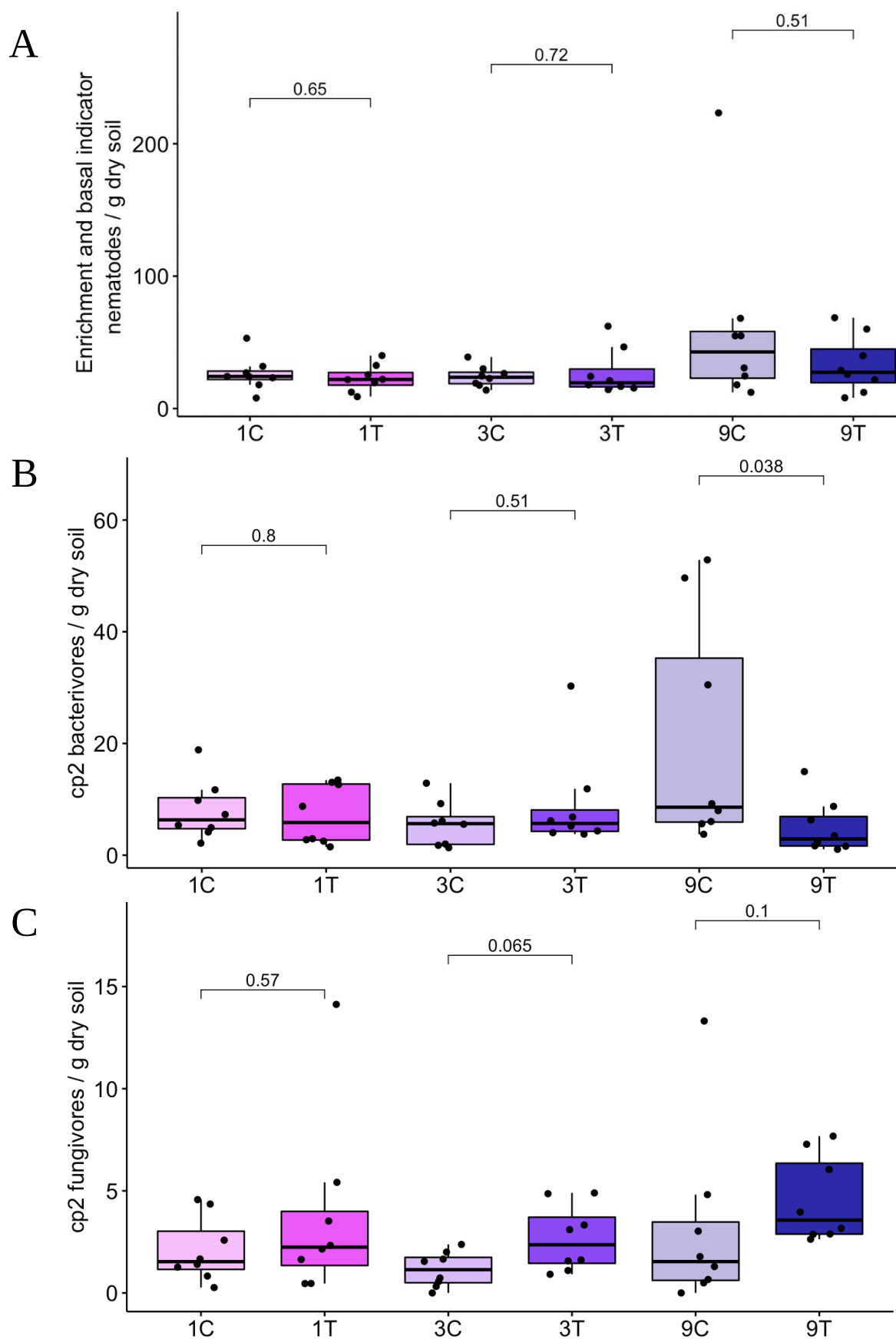


Fig. 2.7 Densities of **A)** total enrichment and basal indicator nematodes (cp1 and cp2); **B)** cp2 bacterivore nematodes; and **C)** cp2 fungivore nematodes.

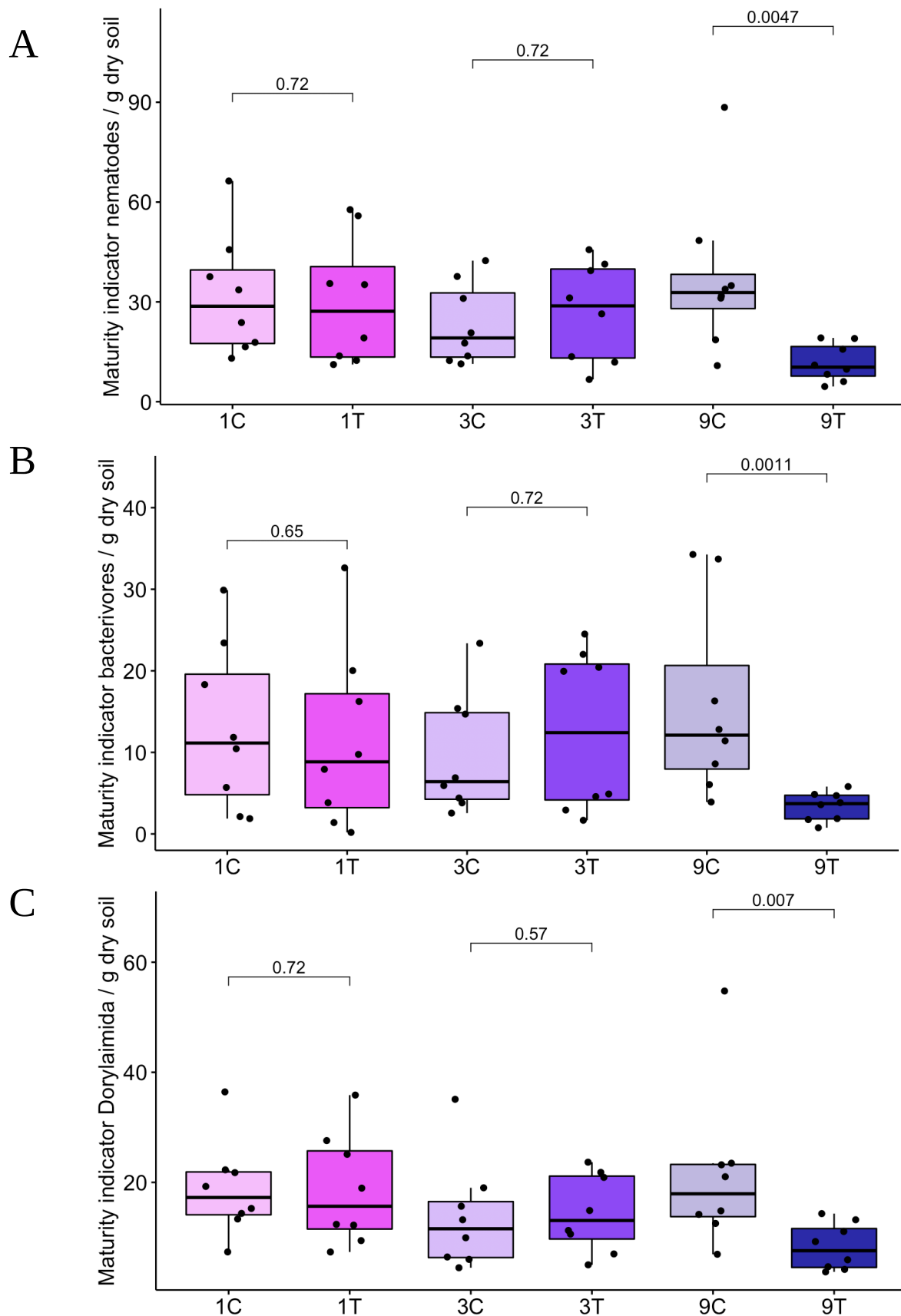


Fig. 2.8 Densities of **A)** total maturity indicator nematodes; **B)** maturity indicator bacterivores; and **C)** maturity indicator Dorylaimida (including predators, omnivores, and fungivores) in soil samples collected from treated (T) and adjacent control (C) areas of transect segments which received either 1, 3, or 9 passes from a feller buncher. P-values are from pairwise Wilcoxon rank-sum tests and have not been corrected for multiple comparisons.

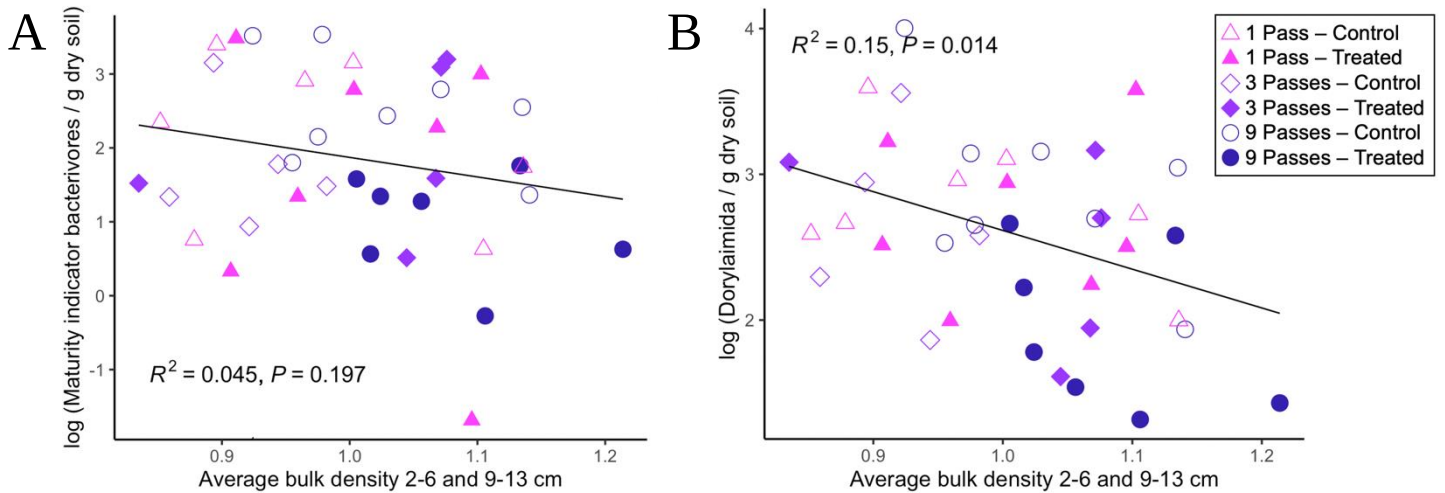


Fig. 2.9 Bivariate correlations between bulk density (averaged for sampling depths 2-6 cm and 9-13 cm) and **A)** maturity indicator bacterivore nematode densities; **B)** Dorylaimida densities.

Table 2.3. Multi-response permutation procedure (MRPP) results for nematode communities from the compaction experiment. Overall group differences are for all treatment level comparisons (15 in total).

Functional guilds	A	p
Overall group differences	0.0292	0.0521
1 pass (control vs. treated)	-0.0306	0.9603
3 passes: control vs. treated	0.0038	0.3708
9 passes: control vs. treated	0.0524	0.0272*
1 pass control vs. 9 pass treated	0.0629	0.0126*

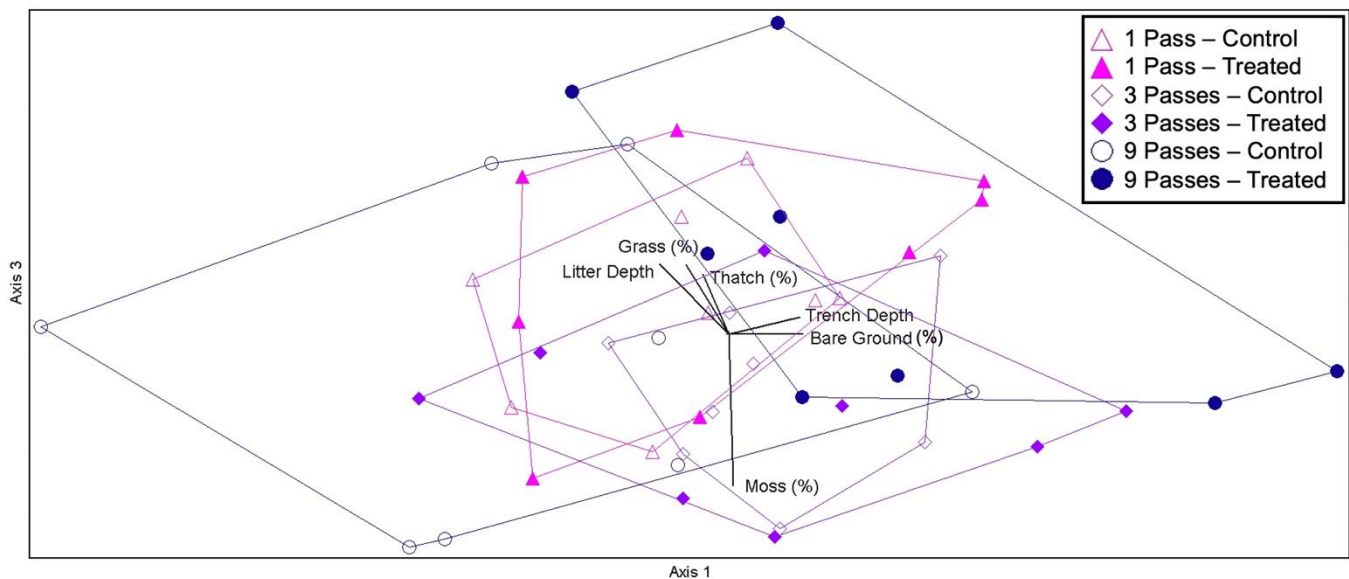


Fig. 2.10 Non-metric multidimensional scaling ordination based on nematode functional groups discriminated in this study. Axes 1 and 3 of a 3-dimensional solution (stress=10.870) are shown. R^2 cutoff for vectors is 0.1.

Conclusions

Our results highlight the vulnerability of some soil types to deep compaction with even one pass of logging machinery. Although we measured bulk density to a depth of 27 cm, it is probable that compaction extends deeper into the soil profile. This has potential implications for preservation of the endangered endemic Jemez Mountains salamander (*Plethodon neomexicanus*) which spends the majority of the year belowground. Care should be taken to avoid compaction by using the lightest adequate machinery, minimizing areas subject to logging machinery traffic, driving over brush mats, and thinning only when soils are dry or frozen (Cambi et al., 2015). Ideally, the efficacy of these techniques for avoiding deep compaction of sensitive soils should be verified prior to treatment of large areas in *P. neomexicanus* habitat.

Although significant compaction occurred with one pass from a feller buncher, nematode community changes were not detectable until 9 passes. Only maturity indicator nematodes (colonizer-persister classes cp3, cp4, and cp5) appeared adversely affected. While other types of disturbance (e.g. churning) are likely responsible for the majority of impacts to maturity indicator nematodes, modest correlation between bulk density and large-bodied, K-selected nematodes (Dorylaimida) supports our hypothesis that compaction most negatively impacts nematodes of larger diameter. However, other types of soil disturbance from logging machinery, including churning, may be more disruptive than compaction to soil food webs, as evidenced by the similarly negative response of slender maturity indicator bacterivores to 9 passes. Churning tended to stimulate *r*-selected cp2 fungivores, however, as we hypothesized (although *r*-selected cp1 and cp2 bacterivores were not affected). Overall, our data suggest that logging machinery passes should be limited to three or fewer to minimize impacts to nematode communities, which perform important ecological functions (Neher et al., 2012; Trap et al., 2016) and support higher trophic levels in soil food webs. Further work is needed, however, to elucidate how these patterns vary with operator, logging machinery type, and soil type.

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Study 3: Fuel loading experiment

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This study is ongoing under our new cooperative agreement, which includes two additional years of post-fire monitoring and continued processing of previously collected soil animal samples. Findings presented here are thus preliminary.

Few data are available linking ground fuels characteristics with post-fire changes to soil biotic communities. In collaboration with Rachel Loehmann (USGS), we modified our initial monitoring plans for Cerro Seco in order to examine the effects of ground fuels type and load on soil heat intensity, depth of heat penetration, and residence time during prescribed fire. We will couple these measurements with pre- and post-fire observations of soil micro- and mesofauna communities, soil fungal communities, and soil chemical properties. We hypothesized that both thinning and burning would alter fungal and faunal communities, and that responses of fungi would partially mediate impacts of management treatments on fauna. We further anticipated that post-burn changes to faunal and fungal communities would scale with fuel loading and heat dose during fire. Because soil temperatures during fire rapidly attenuate with depth, we predicted that assemblages of deep-dwelling soil fauna would be minimally altered by burning, while those at the surface would be most strongly affected. Finally, our new agreement will add in unburned meadow comparisons to determine if the more open structure of the treated unit leads to community shift more reflecting that of a meadow community.

Methods

Study site and experimental design

We have installed 1 m² experimental plots representing five ground fuels types within treatment unit Seco 5 (see site description in Study 2) that was mechanically thinned in November 2017. Four replicate plots with either high woody fuel loading (4-7 kg/m²); moderate woody fuel loading (0.37-0.77 kg/m²); heavy grass cover and no woody fuels (herbaceous fuels loads between 0.1-0.15 kg/m²); or with decayed *Pseudotsuga menziesii* logs (important cover objects for the federally endangered Jemez Mountains salamander found in the area) were instrumented with thermocouples and XR5-SE data loggers (Pace Scientific, North Carolina) prior to prescribed burning in October of 2019. At each plot, two replicate thermocouples were placed at the litter/soil O horizon interface and at depths of 2.5 cm, 7.5 cm, and 15 cm, corresponding to the midpoints of our sampling intervals for soil biota. Four replicate uninstrumented plots were also established at the driplines of *Pinus ponderosa* trees to establish relationships between fuel loading and post-fire soil biotic characteristics within this important zone for tree performance. Soil biotic and abiotic characteristics within these 20 total plots will be compared to 12 additional plots (four replicates of heavy grass, *P. ponderosa* dripline, and log plots) within an adjacent unthinned and unburned control unit (the same control unit sampled in 2016, shown in Appendix A.D.) across four sampling timepoints. For monitoring years 2021 and 2022, we will sample an additional 12 plots established in a nearby pre-existing meadow to assess whether post-treatment soil faunal communities converge with meadow communities after restoration of an open stand structure.

Post-thinning, pre-burn soil and litter samples and ground cover data were collected in September 2018, and initial post-burn monitoring and sampling for soil biota was conducted in May of 2020. At each timepoint,

sampling was performed within an undisturbed 0.25 m² quadrant of each plot. Three plots were excluded from re-sampling in 2020 due to disturbance from fire line construction (one treated moderate fuels plot) and windthrow (one control dripline plot and one treated dripline plot). Cores for extraction of microarthropods and nematodes, respectively, were retrieved from 0-5 cm, 5-10 cm, and 10-20 cm below the soil surface. For one microarthropod core replicate of each fuel type, we sampled 10-15 cm and 15-20 cm increments separately to enhance our understanding of the vertical distribution of microarthropods. Litter overlaying the microarthropod coring location was collected for microarthropod extraction. Two additional cores were taken from the 0-10 cm interval for fungal and bacterial community sequencing and for determination of available nitrogen and total organic carbon, respectively. Because implementation of the prescribed burn originally scheduled for autumn of 2018 was delayed, and because burning ultimately occurred too late in 2019 to collect soil fauna before cold temperatures reduced their activity, we added two limited sampling efforts in September (pre-burn) and October (post-burn) 2019 for analysis of available nitrogen in instrumented plots only. Soil cores for these additional quantifications of nitrogen availability were collected from 0-10 cm using a 1.9 cm diameter sampling probe to minimize disturbance within the plots.

Samples for determination of soil abiotic properties were dried at 38 °C. Soil ammonium and nitrate were extracted according to Keeney and Nelson (1982) and quantified with colorimetry (Wendt, 1999) on a QuikChem 8500 Series 2 (Lachat Instruments, Milwaukee, WI). Microarthropods were extracted over 7 days using a modified Tullgren funnel apparatus and stored in 70% ethanol, and nematodes were extracted by colloidal silica centrifugation as described above. Cores for fungal and bacterial community sequencing were stored at -80 °C until processing. We extracted DNA from soil samples using Qiagen DNeasy Powersoil kits. Next-generation fungal community sequencing was completed at Arizona State University using the MiSeq Illumina platform. Amplicon sequencing of the ITS region was performed with the barcoded primer set ITS1f-ITS2 designed by Smith and Peay (2014) and following the protocol by the Earth Microbiome Project (<http://www.earthmicrobiome.org/emp-standard-protocols/>) for the library preparation. PCR amplifications for each sample were done in duplicate, then pooled and quantified using the Accublock High Sensitivity dsDNA Quantitation Kit (Biotium). A no template control sample was included during the library preparation as a control for extraneous nucleic acid contamination. 200 ng of DNA per sample was pooled and then cleaned using QIA quick PCR purification kit (QIAGEN). The pool was quantified by Illumina library Quantification Kit ABI Prism (Kapa Biosystems). The DNA pool was diluted to a final concentration of 4 nM, then denatured and diluted to a final concentration of 4 pM with a 25% of PhiX. Finally, the DNA library was loaded in the MiSeq Illumina sequencer and run using the version 2 module, 2x250 paired-end, following the directions of the manufacturer.

Statistical analyses

Nitrogen availability and soil organic matter content for fuel loading levels were compared between timepoints by Wilcoxon rank sum tests. *P*-values calculated with Wilcoxon rank sum tests are approximate where ties occurred. Microbiome bioinformatics were performed with QIIME 2 2020.8 (Bolyen et al., 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al., 2016) (via q2-dada2). All amplicon sequence variants (ASVs) were aligned with mafft (Katoh et al., 2002) (via q2-alignment) and used to construct a phylogeny with fasttree2 (Price et al., 2010) (via q2-phylogeny). Alpha-diversity metrics (observed features and Faith's phylogenetic diversity (Faith, 1992)) and Bray-Curtis dissimilarity for PERMANOVA, indicator species analysis, and principle coordinate analysis (PCoA) were estimated using q2-diversity after samples were rarefied (subsampling without replacement) to an average of 15,000 sequences/sample. Taxonomy was assigned to ASVs using the q2-feature-classifier (Bokulich et al., 2018) classify-sklearn naïve Bayes taxonomy classifier against the UNITE database (Nilsson et al., 2019). Six separate indicator species analyses were performed in PC-ORD (McCune and Mefford., 2006) based on sequence assignment to class (27 taxa) or to lowest level possible (112 taxa) for management units, management units x timepoint, and management units x timepoint x fuel load level. One pre-burn grass plot from the treated unit was excluded from post-sequencing statistical analyses due to inferred DNA contamination

with a post-burn high fuel load plot in an adjacent sample well. Including this contaminated sample does not alter our main findings regarding treatment group differences, but would bias the indicator species analyses. Bioinformatic analyses are preliminary and will be further refined under our new agreement.

Results to date

Nitrogen availability and soil organic matter

Ammonium increased immediately after burning (although this change was not always significant) and scaled with fuel loading in instrumented plots, while nitrate was reduced (or tended to be reduced) relative to pre-burn levels (**Fig. 3.1**). These changes were expected, because a portion of the N volatilized from organic compounds condenses as ammonium in soil (Busse et al. 2014), while existent nitrate is volatilized at temperatures as low as 80 °C. Fire effects on nitrogen did not, however, exceed variability observed between the September 2018

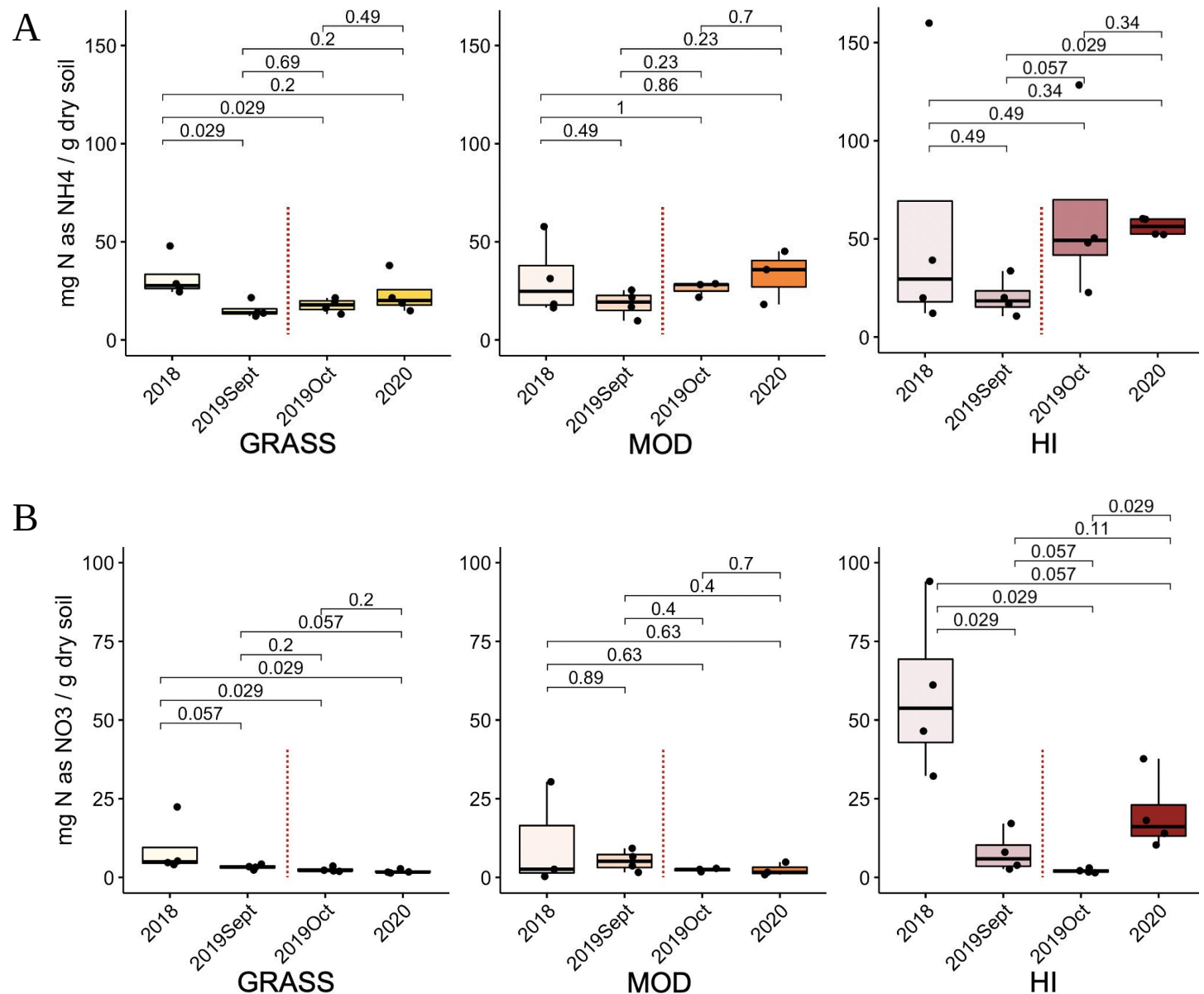


Fig. 3.1. Ammonium and nitrate concentrations in soil at two pre-burn and post-burn sampling timepoints in plots with grass/no woody fuels (GRASS), moderate woody fuels (MOD), or high woody fuels (HI) at

Seco 5. Timepoints to the left of the dashed red line are pre-burn, timepoints to the right are post-burn. P-values are from pairwise Wilcoxon rank-sum tests and have not been corrected for multiple comparisons.

and September 2019 pre-burn sampling timepoints. Some of this variability in available nitrogen between pre-burn sampling years may be due to interannual climate variation, as control dripline plots also varied between 2018 and 2020 (**Fig. 3.2**), but decomposition of slash and soil disturbance would also be expected to elevate mineral nitrogen shortly after thinning. Release of ammonium and nitrate from unburned logging residues should correlate with abundance of recently deposited organic material, in line with our observations of increased pre-fire nitrogen availability in plots with higher fuel loading levels. In 2020, one year after burning, ammonium did not differ from either 2018 pre-burn or 2019 post-burn levels in any of the instrumented plot treatments, while nitrate remained reduced for grass and high fuel load plots (nitrate levels were also nearly undetectable in the moderate fuel loading plots in 2018). High fuel load plots had increased nitrate relative to the 2019 sampling points, however. Activities of nitrifying bacteria—which convert ammonium to nitrate—might be expected to produce a delayed pulse of nitrate after burning in proportion to the initial pulse of ammonium, but different sampling seasons (fall in 2019, vs. spring in 2020) and higher organic debris loads make it difficult to pinpoint mechanisms for increased nitrate in burned plots in 2020. We detected no significant effects of fire on SOM, although burning tended to reduce SOM in grass and dripline plots (**Fig. 3.3**).

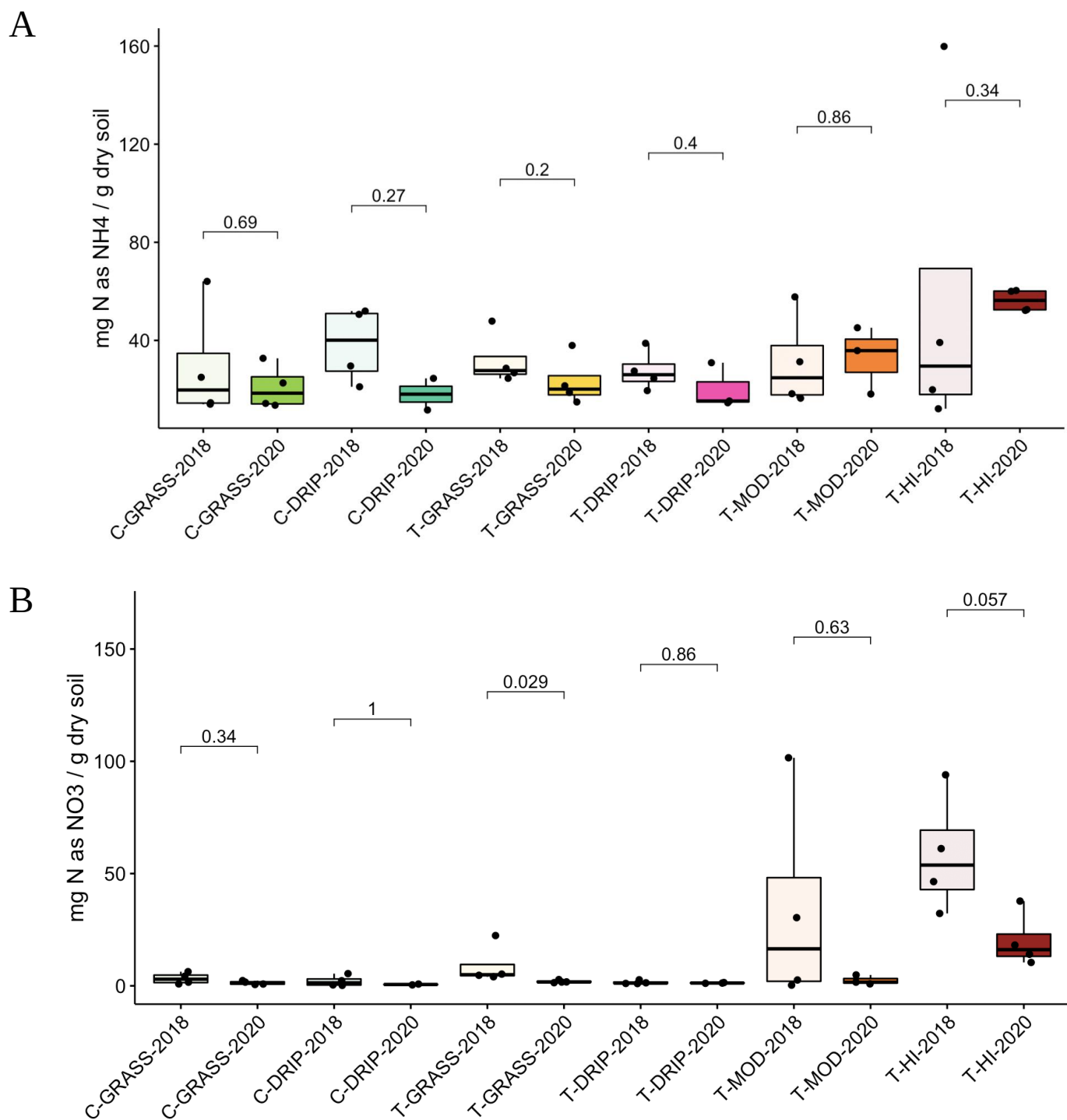


Fig. 3.2. Soil ammonium and nitrate concentrations one year pre-burn (2018) and one year post-burn (2020) in plots with grass/no woody fuels (GRASS), at *P. ponderosa* driplines (DRIP), with moderate woody fuels (MOD), and with high woody fuels (HI) at Seco 5. P-values are from pairwise Wilcoxon rank-sum tests and have not been corrected for multiple comparisons.

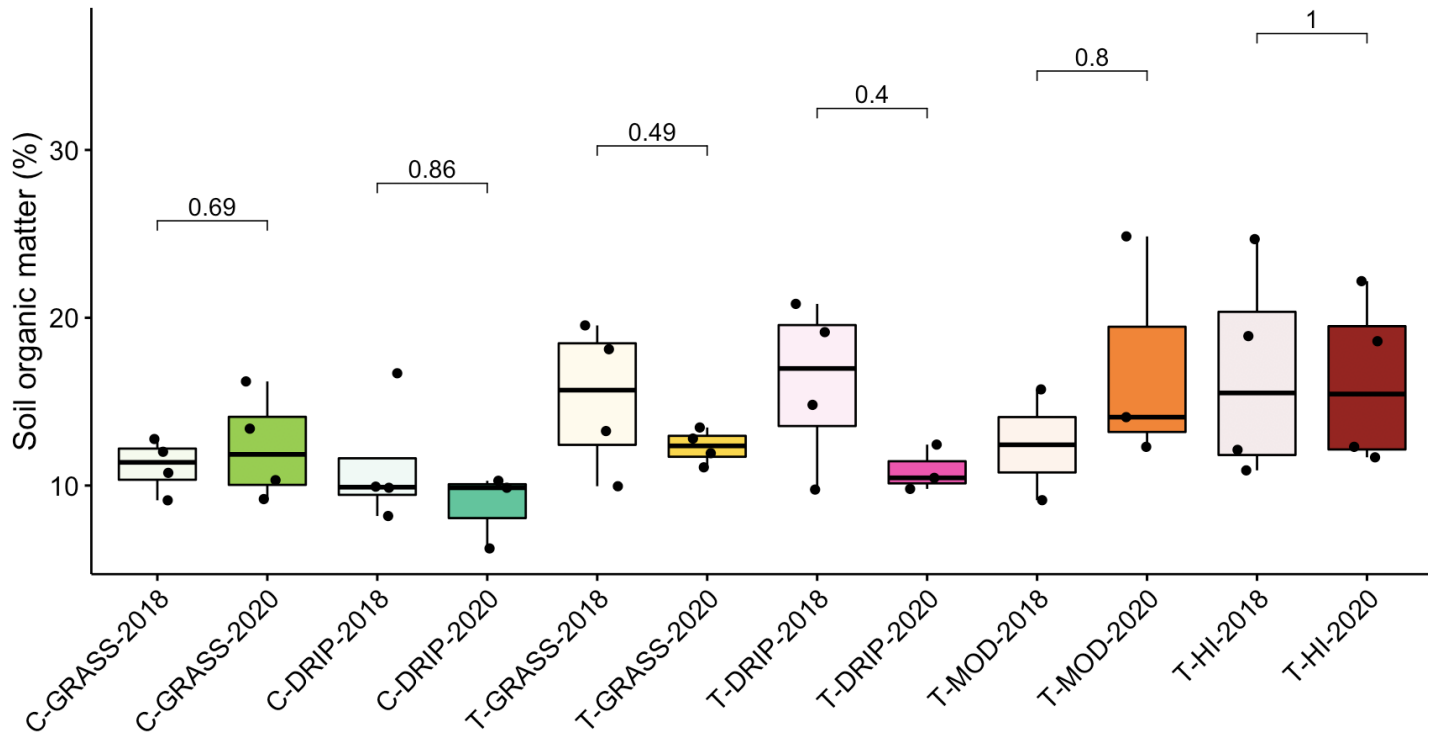


Fig. 3.3. Soil organic matter content one year pre-fire (2018) and one-year post-fire (2020) in plots with grass/no woody fuels (GRASS), at *P. ponderosa* driplines (DRIP), with moderate woody fuels (MOD), and with high woody fuels (HI). P-values are from pairwise Wilcoxon rank-sum tests and have not been corrected for multiple comparisons.

Fungal communities

Fungal communities differed in control and treated management units pre-fire in 2018 (**Table 3.1, Fig. 3.4**), supporting our hypothesis that fungal communities respond to thinning. Fungal community composition within the control plots appeared stable between the autumn 2018 pre-burn sampling and the spring 2020 post-burn sampling date, but contrary to our expectations, fungal communities in the treated management unit were also similar pre- and post-burn (**Table 3.2**). Burning significantly altered fungal communities only in plots where fuel loads were high, in partial support of our hypothesis that fire effects on fungal communities would scale with fuel loading. Post-fire plots with high fuel loads diverged from other plots along a similar trajectory in a principal coordinates analysis (**Fig. 3.4**). Overall, phylogenetic diversity was higher for the treated management unit (median Faith's phylogenetic diversity=78.08) than the control unit (median Faith's phylogenetic diversity=78.08; Kruskal-Wallis: $H=7.533$, $p=0.006$), as was evenness according to Pielou's metric (median Pielou's evenness=0.453 for the control unit vs. 0.599 for the treated unit; $H=5.342$, $p=0.020$). Post-burn fungal phylogenetic diversity and evenness did not differ from pre-burn within the treated unit ($H=0.043$, $p=0.835$). Indicator species analysis by fungal class yielded 4 indicator classes for the thinned management unit, and 2 for the control unit (including one class that was an indicator for a single timepoint) (**Table 3.3**). At the lowest level of taxonomic classification, more than twice as many indicator fungi were detected for the treated unit or for treated timepoint x fuel loading sample subsets (20 total indicators) than for the control unit (8 total indicators) (**Table 3.4**). Of the indicator fungi classified to a taxonomic level permitting assignment to functional group, 9/11 indicators for the treated unit (entire or any plot/timepoint subset) were obligate or facultative saprotrophs (the remaining two indicators were an ectomycorrhizal (EcM) fungus and a plant pathogen). Ectomycorrhizal fungi and saprotrophs respectively comprised 2/5 indicator taxa of known function for plots in the control unit, with the remaining indicator a lichenized fungus. Dominant ectomycorrhizal (EcM) fungi may suffer from

thinning as host trees are removed, while many saprotrophic taxa should benefit from the addition of root necromass and slash. No strong relationships were apparent between overall fungal community composition and soil organic matter, however (**Fig. 3.5 A**), within either the control or treated plots. Overall fungal community composition also did not vary predictably with soil ammonium or nitrate content (**Fig. 3.5 B and C**).

Conclusions to date

Effects of fire on nitrogen availability were modest and did not exceed mineral nitrogen released by decomposition of thinning residues, even at high fuel loads. Fungal communities also were not significantly affected by fire, except when fuel loads were high. This partially supports our hypothesis that fire effects on fungi would scale with fuel loads. Overall, however, thinning appeared to have a stronger effect on fungal communities than burning when fuel loading levels were low to moderate. Saprotrophic fungi appeared to drive community changes with thinning, but ectomycorrhizal taxa may also be adversely affected by thinning treatments.

Table 3.1 PERMANOVA pairwise comparisons of pre-burn (2018) and post-burn (2020) fungal communities in treated (thinned) and control management units, based on Bray-Curtis distance. Total sample size includes samples in both compared groups.

	<i>Control post-burn</i>			<i>Treated pre-burn</i>			<i>Treated post-burn</i>		
	Total n	pseudo- F	p	Total n	pseudo- F	p	Total n	pseudo- F	p
<i>Control pre-burn</i>	15	0.956	0.539	23	1.391	0.002*	22	1.281	0.006*
<i>Control post-burn</i>				22	1.286	0.01*	21	1.236	0.012*
<i>Treated pre-burn</i>							29	1.063	0.258*

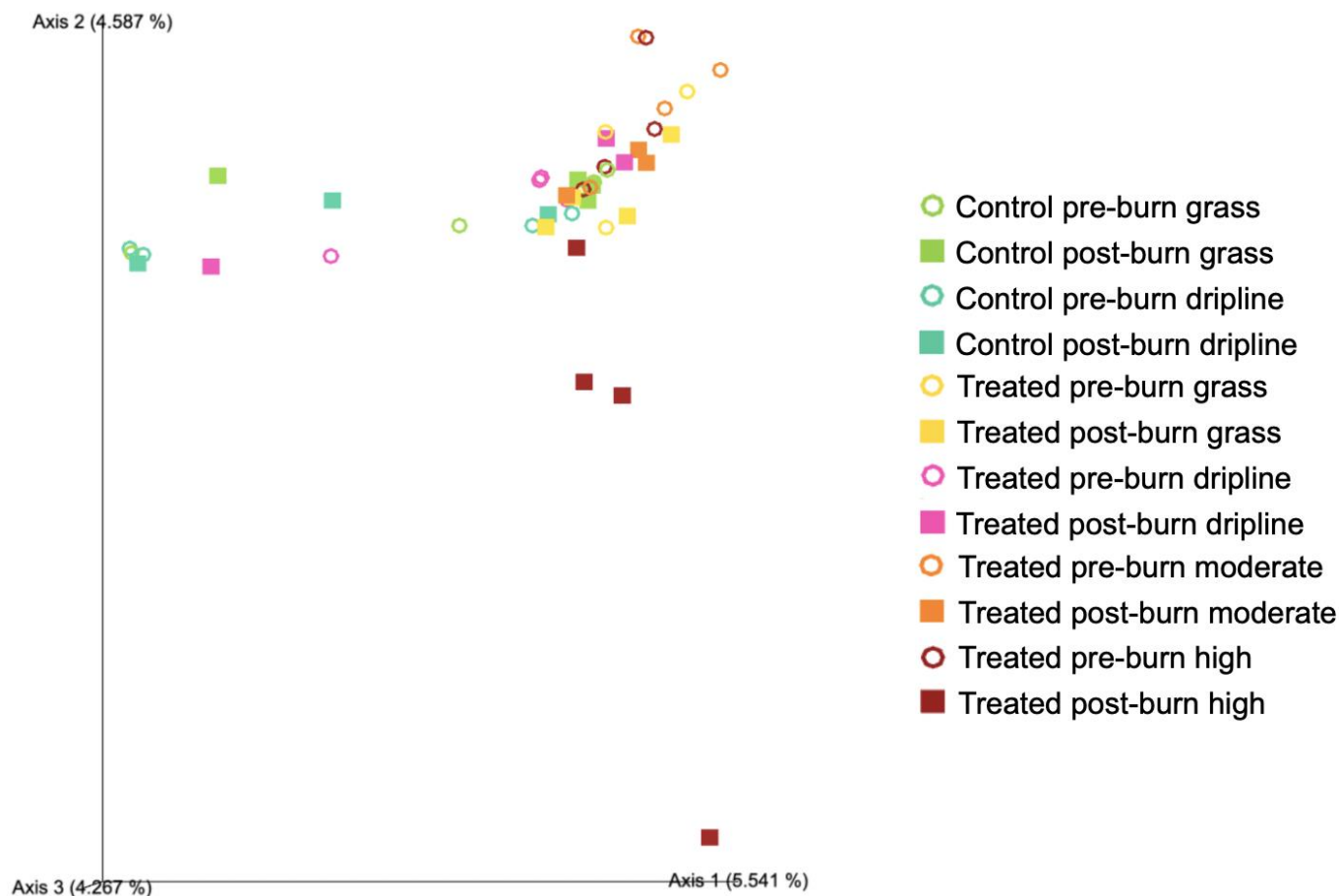


Fig. 3.4 Axes 1 and 2 of a three-dimensional principal coordinates analysis of fungal communities in pre- and post-burn grass (no woody fuels), dripline, moderate woody fuels, and high woody fuels plots. Ordination was performed using Bray-Curtis distance.

Table 3.2. PERMANOVA pairwise comparisons of pre-burn (2018) and post-burn (2020) fungal communities in each fuel loading/management unit combination, based on Bray-Curtis distance. Total sample size includes both pre- and post-burn samples.

Management unit/fuel load	Total <i>n</i>	pseudo- <i>F</i>	<i>p</i>
<i>Control grass/no woody fuels</i>	8	0.792	0.916
<i>Control dripline</i>	7	0.892	0.911
<i>Treated grass/no woody fuels</i>	7	0.930	0.975
<i>Treated dripline</i>	7	0.678	0.846
<i>Treated moderate woody fuels</i>	7	1.037	0.401
<i>Treated high woody fuels</i>	8	1.254	0.026*

Table 3.3. Results of indicator taxa analysis for fungal communities at the class level for management units and management units x timepoint. No significant indicators were detected at this taxonomic level for management unit x timepoint x fuel loading.

Indicator taxon	Management unit			Management unit x timepoint		
	Max group	IV	p	Max group	IV	p
Ascomycota: Lecanoromycetes				Control pre-burn	30.8	0.0482
Ascomycota: Eurotiomycetes	Treated	94.5	0.0002			
Ascomycota: Sordariomycetes	Treated	80.4	0.0002			
Basidiomycota: Agaricomycetes	Control	73.3	0.0178			
Basidiomycota: Microbotryomycetes	Treated	74.8	0.008			
Basidiomycota: Tremellomycetes	Treated	67.9	0.0436	Treated post-burn	76.6	0.0022

Table 3. Indicator fungal taxa with IV > 25 and p<0.05 for management units, management units x timepoint, and management units x fuel load x timepoint. For assignments of OTUs to genus or species level, confidence ranges for assignment of operational taxonomic units are given in parentheses. Functional groups (Funct.) are based on genus data in the FungalTraits database (Pölme et al., 2020). Sapro=saprotrophic, EcM=ectomycorrhizal, DSE=dark septate root endophyte, Lichen=lichenized fungi, Path=plant pathogen. Foliar endophytes and epiphytes with a secondary lifestyle as saprotrophs are listed as the latter.

Indicator taxon	Funct.	Management unit			Management unit x timepoint			Management unit x fuel load x timepoint		
		Max group	IV	p	Max group	IV	p	Max group	IV	p
Ascomycota: Dothideomycetes: Dothideales: Aureobasidiaceae: <i>Aureobasidium</i> indet. (0.80-0.98)	Sapro	Treated	64.1	0.004	Treated post-burn	72.2	0.0004	Treated moderate post-burn	53.7	0.0088
Ascomycota: Dothideomycetes: Mytilinidales: Gloniaceae: <i>Cenococcum</i> indet. (0.77-0.99)	EcM				Treated post-burn	48.3	0.0104			
Ascomycota: Dothideomycetes: Pleosporales indet.		Treated	31	0.0458						
Ascomycota: Eurotiomycetes indet.		Treated	73.8	0.0162						
Ascomycota: Eurotiomycetes: Chaetothyriales: Chaetothyriales inc. sed.: <i>Sarcinomyces</i> indet. (0.72-0.98)	Sapro	Treated	75	0.0012						
Ascomycota: Eurotiomycetes: Chaetothyriales: Herpotrichiellaceae: <i>Cladophialophora</i> indet. (0.75-0.99)	Sapro/ DSE	Treated	57.8	0.0096				Treated moderate post-burn	62.6	0.0058
Ascomycota: Eurotiomycetes: Chaetothyriales indet.		Treated	89	0.0054						
Ascomycota: Eurotiomycetes: Eurotiales indet.		Treated	92.1	0.01						
Ascomycota: Lecanoromycetes: Pertusariales: Ochrolechiaceae: <i>Ochrolechia androgyna</i> (0.73-0.83)	Lichen				Control pre-burn	30.8	0.049			
Ascomycota: Leotiomyces indet.		Treated	86.9	0.0018				Treated moderate post-burn	45.2	0.012
Ascomycota: Leotiomyces: Helotiales indet. 1		Treated	79	0.012						

Ascomycota: Leotiomyces: Helotiales: Dermateaceae indet.					Control post-burn	82.2	0.0082	Control dripline post-burn	92.3	0.0134
Ascomycota: Leotiomyces: Helotiales indet. 2		Treated	92.9	0.0002	Treated post-burn	62	0.0254			
Ascomycota: Leotiomyces: Thelebolales: Pseudeurotiaceae: <i>Gymnostellatospora japonica</i> (0.72- 0.99)	Sapro	Treated	86.7	0.0038	Treated post-burn	62.8	0.0184	Treated high post- burn	40.7	0.0324
Ascomycota: Pezizomycetes: Pezizales: Pyronemataceae: <i>Pseudaleuria</i> indet. (0.90-0.90)	Sapro	Treated	43	0.024						
Ascomycota: Pezizomycetes: Pezizales: Pyronemataceae: <i>Trichophaea</i> indet. (0.99-0.99)	Sapro	Control	67	0.0002	Control post-burn	54.5	0.0108			
Ascomycota: Sordariomycetes: Hypocreales: Hypocreaceae indet.		Control	69.4	0.0002	Control pre-burn	70.1	0.002			
Ascomycota: Sordariomycetes: Sordariales: Lasiosphaeriaceae indet.		Treated	82.7	0.0008						
Ascomycota: Sordariomycetes: Xylariales: Amphisphaeriaceae: <i>Lepteutypa</i> indet. (0.87-0.99)	Path				Treated pre-burn	60.6	0.0168			
Basidiomycota: Agaricomycetes: Agaricales: Inocybaceae: <i>Inocybe</i> indet. (0.72-0.99)	EcM	Control	71.3	0.0058	Control post-burn	63.6	0.0148			
Basidiomycota: Agaricomycetes: Agaricales: Strophariaceae; <i>Hypholoma fasciculare</i> (0.70-0.92)	Sapro	Control	46.6	0.0054						
Basidiomycota: Agaricomycetes: Agaricales: Strophariaceae: <i>Psilocybe</i> <i>yungensis</i> (0.82-0.87)	Sapro							Treated high pre- burn	72.1	0.0096
Basidiomycota: Agaricomycetes: Cantharellales: Ceratobasidiaceae: Thanatephorus: <i>Thanatephorus</i> <i>cucumeris</i> (0.72-0.99)	Path/ Sapro				Treated pre-burn	37.8	0.0356			
Basidiomycota: Agaricomycetes: Thelephorales: Thelephoraceae:	EcM	Control	97.7	0.003	Control post-burn	74.3	0.0414			

Tomentella: *Tomentella stuposa* (0.70-0.99)

Basidiomycota: Agaricomycetes:		Control	33.3	0.0034						
Thelephorales: Thelephoraceae indet.										
Basidiomycota: Microbotryomycetes:		Treated	72.6	0.0488						
Kriegeriales: Camptobasidiaceae indet.										
Basidiomycota: Microbotryomycetes:	Sapro	Treated	63.1	0.0054	Treated pre-burn	60.1	0.0054	Treated moderate pre-burn	45.7	0.0192
Sporidiobolales: Sporidiobolaceae:										
<i>Rhodosporeidiobolus</i> indet. (0.70-0.86)										
Basidiomycota: Tremellomycetes:	Sapro	Treated	68.2	0.0436	Treated post-burn	77.3	0.0042			
Tremellales: Bulleribasidiaceae;										
<i>Vishniacozyma</i> (0.76-0.86)										

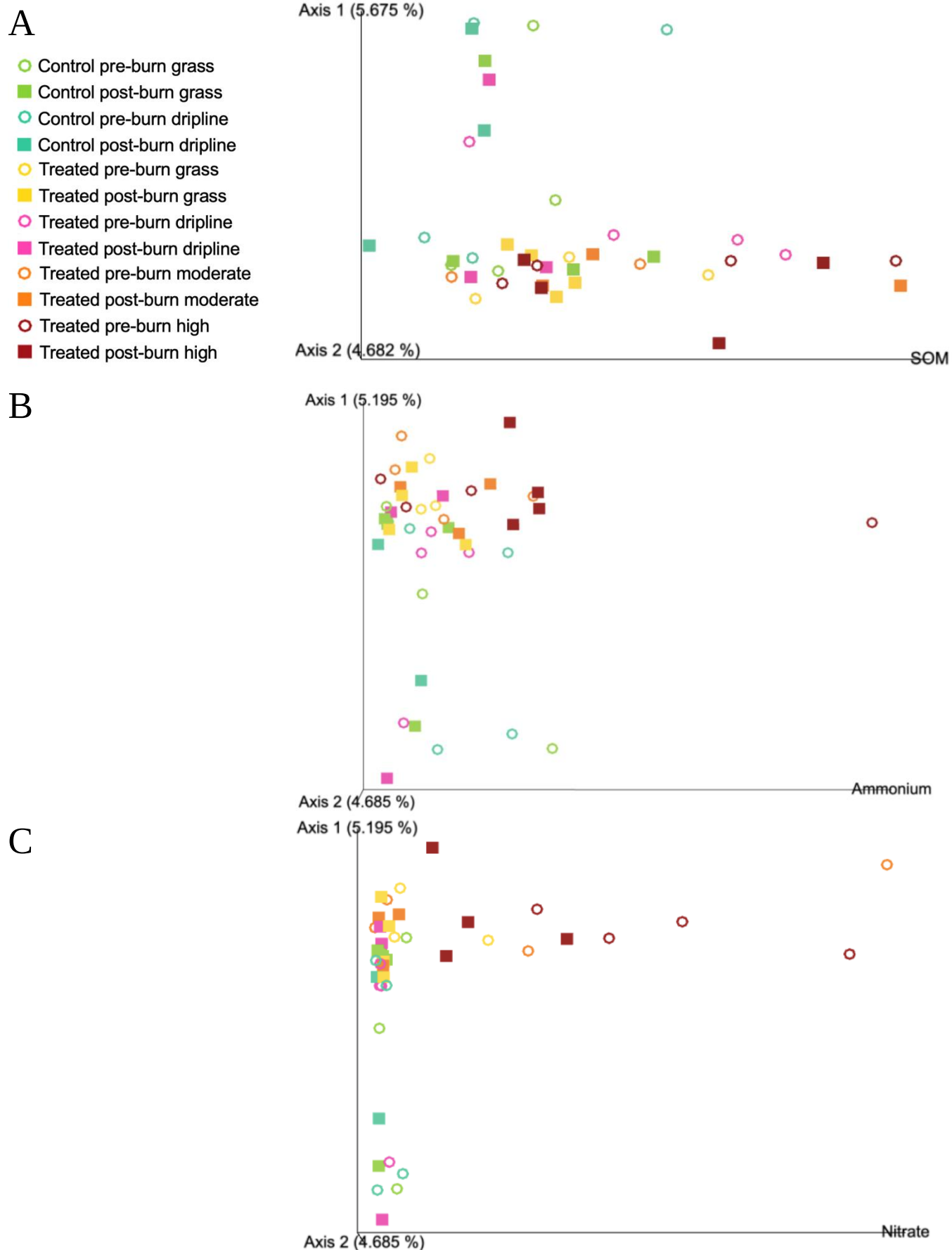


Fig. 3. Three dimensional principal coordinates analysis ordination showing Axis 1 of fungal community dissimilarity and (A) soil organic matter (SOM), (B) ammonium, and (C) nitrate, respectively, as a third axis.

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Objective 2: Contributions to the All Taxa Biodiversity Inventory

Our contributions to the All Taxa Biodiversity Inventory are ongoing under our new cooperative agreement and our lists of taxa are thus preliminary. Our efforts to date have focused mainly on Acari (especially Oribatida) and Nematoda. In addition to the sample collections completed during the studies described above, we have obtained nematode and microarthropod samples from various microhabitats within two vegetation types on Redondo Peak: a *Festuca thurberi*-dominated subalpine meadow, and the Engelmann spruce-dominated subalpine forest at the summit. We also received samples collected by Valles Caldera National Preserve staff from Redondo Peak in 2014. Redondo Peak's summit hosts vegetation types which are especially vulnerable to climatic change, and its soil biota likely will be altered in the near future by warming and precipitation shifts. Finally, we collected samples from History Grove and South Mountain as part of the cross-ecosystem comparison study (described in the next section).

Methods

In addition to the above sample collections, we have collected soil and litter samples from 11 microhabitats in the subalpine forest at Redondo Peak, and 5 in the subalpine meadow. At each sampling point, we recorded litter depth and photographed the ground cover. Microarthropod soil and litter samples from the subalpine forest were obtained in the same manner as at the Banco North and Cerro Seco sites. In microhabitats where the ground cover was lichen, moss, or decomposing wood, we collected these with the large corer in the same manner as litter. Microarthropod litter samples were combined with soil samples for the subalpine meadow. We modified our nematode sampling technique slightly for the forested site, including the O_e layer and mosses in our cores, when present, since these substrates likely support nematode assemblages.

Voucher specimens will be archived with Valles Caldera National Preserve upon completion of our new cooperative agreement. With the assistance of Dylan Fedor, an undergraduate student, we have also imaged select oribatid specimens with scanning electron microscopy. Photographs and scanning electron micrographs of selected nematode and oribatid mite taxa encountered thus far are shown in Appendix B.

Findings to date

Taxa from Redondo Peak, Banco North, Seco 5, and South Mountain are listed in **Table 4.1**. As some taxa have not been sorted below the family level (or sometimes above), a “sp.” may actually comprise multiple species in some cases. We used “spp.” only where we were confident that multiple species were present within a group. Note that taxa marked as not present at a site are not necessarily absent, as we have attempted to identify more specimens from Redondo Peak, Seco 5, and Banco North than from South Mountain. We have not yet commenced identification of specimens from History Grove. This list likely contains novel species, especially in groups with high endemism that have not been reported from New Mexico. However, as mite taxonomist Beaulieu et al. (2019) have commented:

“Reliable identification of any given mite to species is difficult because of the general lack of identification tools, including species-level keys, adequate species descriptions and illustrations, as well as published checklists. This is in stark contrast with major groups of insects and spiders for which there are usually checklists available at the national or provincial levels for the entire order as well as many species-level identification keys. The slow progress in mite systematics reflects the dearth of acarological taxonomists in Canada and North America, the relatively rare inclusion of mites in biological surveys and student projects, and the small number of amateur collectors (Lumley et al. 2013).”

Table 4.1. Preliminary list of microarthropod and nematode taxa encountered to date.

	Redondo Peak	Seco 5	South Mountain	Banco North
Oribatida				
<i>Camisia</i> sp.	X			
<i>Ceratoppia</i> sp.	X			
Damaeidae (>3 spp.)	X			X
<i>Dentizetes rudentiger</i>	X			
<i>Eremaeus</i> cf. <i>boreomontanus</i>		X		X
<i>Eremaeus oresbios</i>		X		
<i>Eremaeus plumosus</i>	X			
Euphthiracaroida sp.				X
<i>Eupterotegaeus</i> sp.	X			X
<i>Gustavia</i> sp.			X	
Gymnodamaeidae sp. 1				X
Liacaridae sp. 1	X			
Liacaridae sp. 2	X			
<i>Nothrus</i> sp.				X
<i>Odontodamaeus</i> sp.				X
<i>Opiella nova</i>				X
<i>Oribatella</i> sp. (nov.?)	X			
cf. Oribatulidae sp.				X
Peloppiidae sp.	X			
Phenopelopoidea sp.	X			
Phthiracaridae sp.	X			
<i>Propelops</i> cf. <i>canadensis</i>				X
<i>Protoribates</i> sp.				X
<i>Scapheremaeus</i> sp.	X			
<i>Scheloribates</i> sp. (or spp.)	X			X
<i>Tectocepheus velatus</i>	X	X		
Trhypochthoniidae sp.				X
<i>Trichoribates</i> sp.				X
Brachypilina sp. 1	X			
Brachypilina sp. 2	X			
Brachypilina sp. 3	X			
Unidentified Oribatida spp.	X	X	X	X

Table 4.1 cont.

	Redondo Peak	Seco 5	South Mountain	Banco North
Prostigmata				
<i>Bdella</i> cf. <i>muscorum</i>				X
Bdellidae sp.	X			
Bdelloidea sp. 1				X
cf. <i>Biscirus</i> sp.				X
Cunaxidae sp. 1				X
Cunaxidae sp. 2				X
Cunaxidae sp. 3				X
Cunaxidae sp. 4				X
Eupodidae sp.				X
<i>Linopodes</i> sp.	X			
Prostigmata sp. 1				X
Prostigmata sp. 2				X
Prostigmata sp. 3				X
Prostigmata sp. 4				X
Prostigmata sp. 5				X
Prostigmata sp. 6				X
Prostigmata sp. 7				X
Prostigmata sp. 8				X
Prostigmata sp. 9				X
<i>Spinibdella</i> sp.				X
Unidentified Prostigmata spp.	X	X	X	X
Mesostigmata				
Unidentified Mesostigmata spp.	X	X	X	X
Uropodidae sp.				X
Zerconidae sp.				X
Nematoda				
<i>Acrobeles</i> sp.	X	X		X
Actinolaiminae sp.				X
Alaimidae sp.		X		
Aphelenchidae spp.		X		X
Aphelenchoididae spp.	X	X		X
Bunonematidae sp.	X			
Cephalobidae spp.	X	X		X
<i>Cervidellus</i> sp.	X	X		X
cf. Chronogasteridae		X		X
Criconematidae		X		

cf. *Dorylaimellus* sp.

X

Table 4.1 cont.

	Redondo Peak	Seco 5	South Mountain	Banco North
Dorylaimida spp.	X	X		X
Dorylaiminae sp. 1	X			
Dorylaiminae sp. 2	X			
Dorylaiminae sp. 3	X			
Dorylaiminae sp. 4	X			
Dorylaiminae sp. 5	X			
Dorylaiminae sp. 6	X			
<i>Helicotylenchus</i> sp.	X	X		
Hemicyclophoridae				X
Leptonchidae sp.	X	X		X
Mononchidae sp.	X	X		X
cf. Nordiidae sp.		X		
Panagrolaimidae sp.		X		
Plectidae (>3 spp.)	X	X		X
Plectidae spp.		X		
<i>Pratylenchus</i> sp.		X		
Prismatolaimidae sp.	X	X		X
Qudsianematidae spp.	X	X		X
Rhabditidae sp.		X		X
Rhabdolaimidae sp.		X		
cf. <i>Teratocephalus</i> sp.	X	X		
<i>Trichodorus</i> sp.		X		
Tylenchidae (>3 spp.)	X	X		X
Tylenchoidea spp.		X		
cf. <i>Tylencholaimus</i> sp.				X
<i>Wilsonema</i> sp.		X		X
cf. <i>Zeldia</i> sp.		X		
Collembola				
Entomobryidae spp.	X	X		X
Entomobryomorpha spp.	X	X		X
Hypogastruridae spp.	X	X		X
Isotomidae spp.	X	X		X
Onychiuridae sp.	X	X		X
Sminthuridae spp.	X	X		X
Tullbergiidae sp.	X	X		X

Cross-site comparison

We originally intended to compare restoration treatment responses of soil biota across forest types within VALL. To this end, in addition to our sampling of untreated, thinned, and thinned/burned treatment units at Banco North, we conducted pre-treatment sampling and established control and treated plot pairs in 2016 within a xeric mixed conifer stand at Seco 5 and a mesic mixed conifer fringe forest on the north side of South Mountain. Both these mixed conifer forest units were slated for thinning with mastication (as at Banco North) followed by prescribed fire. Unfortunately, the Hidden Valley wildfire, a windthrow event, and altered management decisions disrupted our plans to complete these pre- and post-treatment comparisons. Our pre-treatment data from these mixed conifer sites are still useful, however, in understanding the range of variation present across untreated forests. We combine it here with data from a limited sampling of History Grove sites that were affected or unaffected by the 2013 Thompson Ridge fire. We expected forest type to play a major role in determining soil fertility, carbon, and hydrology, and thus in structuring assemblages of soil biota. We hypothesized that soil organic matter (SOM) would play an important role in driving soil nematode abundance, as SOM is strongly linked to water holding capacity, and nematodes are aquatic organisms active only in water films. We will continue to identify soil fauna from these sites for the All Taxa Biodiversity Inventory under our new agreement.

Between late August and early September of 2016, at each sampling site, we selected areas with approximately matching slope and aspect, then randomly selected origin points for arrays of quadrats as described in **Fig. 5.1**. In order to assess plot-scale variability, and to determine how soil ecological functioning and biodiversity might change across the landscape with restoration-related changes in correlated aboveground variables, we measured ground cover, canopy cover, and litter depth at each quadrat. A 0.5 m x 0.5 m gridded frame was used to estimate percentage cover by grasses, forbs, litter, bare ground, and other less frequent features. Litter depth was measured as near as possible to the center of the quadrat, and canopy cover was measured using a spherical densiometer at ground height to measure the cover experienced by soil biota. Overall slope and aspect were also recorded for each quadrat. A subset of quadrats from these arrays were randomly selected for collection of soil cores (**Fig. 5.1**). Four 5.08 cm diameter soil cores were collected from the uppermost 10 cm of mineral soil and the O_a layer, if present. One core each was reserved for extraction of nematodes, microarthropods, and for analyses requiring dried and frozen soil, respectively.

At the Cerro Seco sites, as at Banco North, we used a 10.16 cm diameter circular corer to collect a litter sample (if litter cover was present) for extraction of microarthropods before sampling the underlying soil for these organisms. At the South Mountain and History Grove sites, heavy grass cover in many quadrats made this sampling approach impractical, so a combined soil and litter (or root mat) microarthropod sample was collected using the 5.08 cm diameter core. Microarthropod soil samples were transported intact in the PVC cores to reduce animal mortality. We also collected a smaller soil core (2.5 cm diameter, to a depth of 9.5 cm) from each randomly selected quadrat for quantification of hyphal lengths. Between uses, these smaller corers were rinsed with water and then sanitized with 90% isopropyl alcohol. Cores intended to be dried for abiotic analyses were stored in open air while in the field, then were dried at 47 °C for at least one week upon return to Northern Arizona University. Nematode and microarthropod cores were stored in coolers on ice, and cores for hyphae extraction were frozen on dry ice, until arrival at NAU. Nematode and microarthropod cores were then refrigerated at 4 °C and processed within ten days of collection. Samples for hyphae extraction were stored at -20 °C.

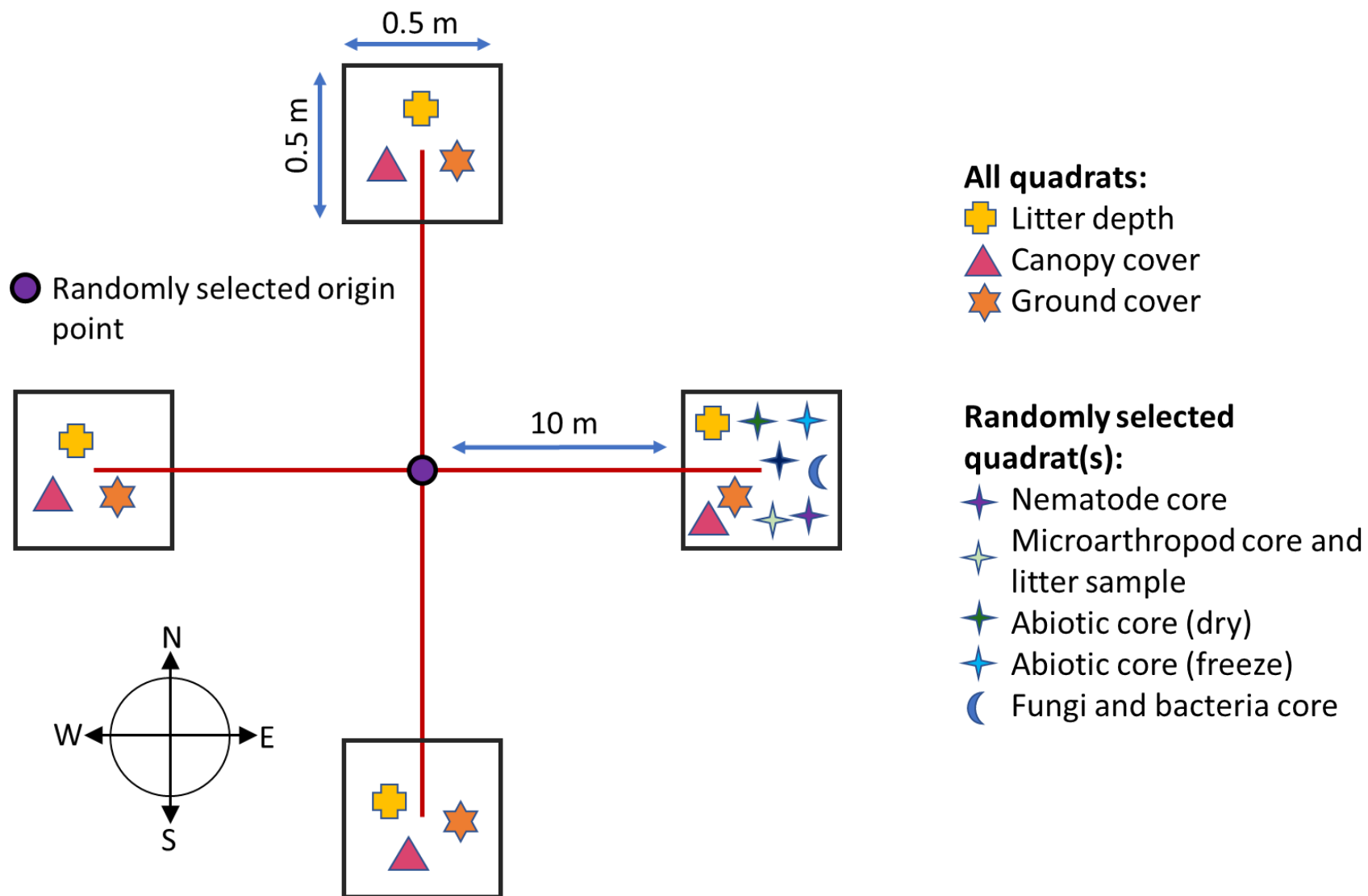


Fig. 5.1. A. Quadrat array sampling design used at the Cerro Seco xeric mixed conifer, Banco North ponderosa pine, and History Grove ponderosa pine-dominated sites. At Banco North, sampling points were stratified by slope and aspect (flat or gentle south-facing (the predominant aspect) slopes). We acknowledge that this stratification choice may have introduced uncontrolled variation in received runoff, as these units featured rolling hills; however, steeper slopes in the thinned, and thinned and burned, areas often had been left untreated due to difficult access or archeological sites. The random origin points were unstratified at the other sites, where slope and aspect were relatively consistent across the study area.

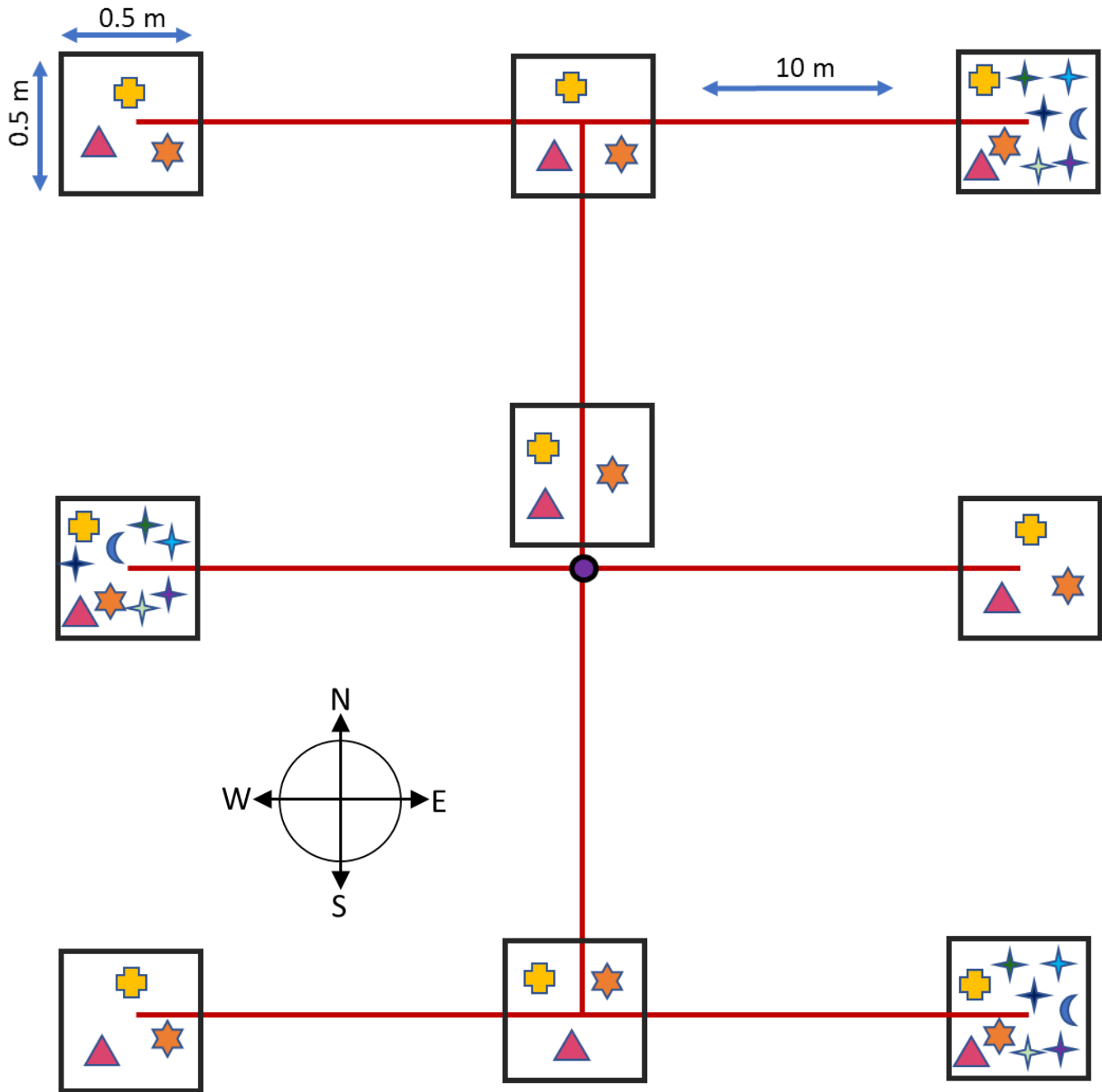


Fig. 5.1. B. Quadrat array sampling design used at the South Mountain fringe forest site. One array was laid in each of the six subdivisions of the site, which were too small to accommodate replication of our other quadrat array layout. To avoid trampling the center quadrat, we located it 2 m north of the center point.

Microarthropods

Microarthropods were extracted from soil and litter samples over six days using modified Tullgren funnels. Soil or litter samples were spread on cheesecloth within funnels, which emptied into scintillation vials holding tap water. Light was gradually intensified over 4 days, followed by a 2-day period of full light intensity. To produce a steeper temperature gradient, chambers housing collection vials beneath the funnels were insulated and cooled with ice. Following extraction, microarthropods were preserved in approximately 70% ethanol and refrigerated,

and soil and litter samples were dried at 60 °C for at least one week to determine animal densities on a per weight basis. Mites, collembolans, and other microarthropods in these samples have been enumerated using a dissecting microscope at 20-63x magnification.

Nematodes

Nematodes sampled in 2016 were extracted using the “raft method” (Gibson 2016), an adaptation of Baermann trays. Approximately thirty grams of well-homogenized soil were weighed and spread in a floating frame on a single layer of Kimwipe tissue, which was partially submerged in tap water. Active nematodes swim through the layer of tissue and settle at the bottom of the basins. After 48 hours, nematodes were collected from the basins using a 25 µm sieve, preserved with DESS solution (Yoder et al. 2006), and stored at 4 °C. A 5 g subsample of soil from each original nematode core was also dried for 3 days at 105 °C to enable determination of nematode densities by dry soil weight.

Modification: Prior to our first field season, we performed limited preliminary extractions to determine whether nematode abundances were sufficient to permit placing a small soil subsample directly on the rafts, or if a larger subsample (requiring pre-extraction by decanting and sieving) would be needed. Our results suggested that nematode abundances were high enough to yield the requisite 200 individuals from a small 30 g subsample, so we elected to omit pre-extraction with decanting and sieving. However, nematode abundances from the 2016 sampling campaign were ultimately lower than our preliminary estimates indicated, so we repeated extractions on remaining soil from the original nematode soil samples using decanting, sieving, and sucrose centrifugation. This method extracts live, inactive, and dead nematodes. Approximately 100 cc of soil (unless less than this amount remained from the previous extraction) were used for each sucrose centrifugation extraction. Nematodes were counted using a dissecting microscope.

Soil abiotic properties

Dried soil from the abiotic core was sieved to 2 mm and the fine fractions were weighed to provide an estimate of bulk density. Most samples contained few particles > 2 mm. We have measured total organic carbon (TOC) and total inorganic carbon (TIC) by loss on ignition and analyzed particle size distribution, pH, nitrate, ammonium, and phosphate content. We also have determined total C, N, δC^{13} , and δN^{15} . Dried soil was subsampled using a microsplitter to ensure that the minute quantities required for analysis of TOC, TIC, particle size distribution, and C and N isotopes were representative fractions. Particle size analysis by laser diffraction was completed using a Beckman-Coulter LS-230 Particle Size Analyzer.

Extramatrixal hyphae

We quantified extramatrical hyphal lengths in samples obtained during our first field campaign with the help of Andrea Raya (National Autonomous University of Mexico (UNAM)), a visiting graduate student who wished to learn mycorrhizal techniques in our lab. Extramatrical hyphae were classified as fine and coarse arbuscular mycorrhizal (AM) fungi, heavily melanized “dark” septate fungi, and other septate fungi. The septate fungi (primarily Basidiomycota and Ascomycota) cannot be separated to functional groups (i.e., saprotrophic or mycorrhizal); however, this analysis has provided an estimate of the fungal mycelium density in the soil. We use the extraction method of Abbott et al. 1984.

Data analysis

Univariate analyses of variance (ANOVA) and Tukey’s tests of honestly significant differences were conducted in R (v.3.3.2; R Core Team 2016) to compare soil abiotic properties, litter depth, and canopy cover among forest types and treatments. We used non-metric multidimensional scaling (NMS) ordination and multi-response permutation procedures (MRPP), both performed using PC-ORD 6.0 (McCune and Mefford 2011), to

look for community differences between forest types and among treatments and examine how community dissimilarity correlates with soil abiotic properties and microhabitat characteristics. Molecular analyses (community composition, alpha and beta diversity) will be performed in QIIME and R.

Results

Summary statistics and ANOVA results for soil abiotic characteristics, litter depth, and canopy cover are shown in **Table 5.1**. Lengths of fine and coarse AM hyphae, dark septate hyphae, and other septate hyphae were highly variable within treatments at Banco North, and when all six hyphal categories (including stained and unstained AM hyphae) were ordinated (**Fig. 5.2**), the treatments did not differ. Forest types tended to have marginally different hyphal compositions (with statistically significant *p*-values, but *A* values < 0.1, i.e., below the widely used threshold for ecological significance), with the exception of the mesic mixed conifer site and History Grove, which had similar hyphal communities. In an NMS, community dissimilarities were correlated with soil moisture, soil texture, and canopy cover (**Fig. 5.2**). Interestingly, dissimilarities in hyphal composition among treatment units at Banco North were comparable to differences among forest types (**Fig. 5.2**). Comparisons of hyphal lengths for each forest type are shown in **Table 5.2**. We anticipated that soils with higher organic carbon would support the highest abundance and diversity of soil nematodes, and our data indicate that nematode abundance is indeed positively correlated with soil organic carbon, both within Banco North and across the preserve (**Fig. 5.4**). In general, more organic soils have higher water holding capacity, which should facilitate nematode movement for longer periods between precipitation events (nematodes are aquatic organisms and rely on water films for movement). Abundance of prey items (e.g., bacteria, fungi, and protozoans) for non-parasitic nematodes most likely also increases with total organic carbon in this ecosystem. Finally, soils with higher organic carbon content had lower bulk density ($R^2=0.72$, $p<0.001$ across our preserve study sites), and should provide nematodes with more utilizable pore space.

Table 5.1. Field observations from surveyed quadrats and soil properties from analyses completed thus far. Groups with different letters are significantly different according to univariate ANOVA ($p < 0.05$ after Tukey's HSD adjustment).

	South Mountain			Cerro Seco			History Grove						Banco North								
	Mesic mixed conifer			Xeric mixed conifer			Ponderosa pine-dominated						Ponderosa pine								
	<i>Pre-Thin/Control</i>			<i>Pre-Thin/Control</i>			<i>Unburned</i>		<i>Burned</i>		<i>Untreated</i>		<i>Thinned</i>		<i>Thinned/Burned</i>						
n surveyed	54			80			20		20		32		32		32						
n cored	18			20			5		5		8		8		8						
	Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD	
Litter depth (cm)	2.33	1.97	ab	3.26	3.08	ab	1.83	0.99	ab	1.84	1.12	ab	3.92	3.32	a	3.03	2.85	ab	1.44	1.57	b
% Canopy cover	83.07	11.23	a	81.35	16.29	a	49.26	17.34	bc	37.8	20.19	c	55.89	27.94	b	39.3	23.01	c	31.85	20.28	c
Bulk density (g/cm ³)	0.45	0.12	d	0.69	0.14	c	0.74	0.08	bc	0.62	0.10	cd	0.92	0.14	ab	0.77	0.11	bc	1.02	0.19	a
pH	5.63	0.44	a	5.11	0.79	abc	5.15	0.16	abc	5.73	0.52	ab	4.85	0.46	bc	4.56	0.62	c	5.25	0.66	abc
% TOC	19.95	7.66	a	12.32	5.69	b	12.76	4.25	ab	9.56	2.77	b	6.00	2.19	b	8.66	4.11	b	5.46	3.57	b
% TIC	0.88	0.18	a	0.65	0.13	b	0.58	0.12	ab	0.57	0.11	ab	0.45	0.08	c	0.44	0.12	c	0.35	0.11	c
% C	NA			NA			NA		NA		NA		2.82	1.54	a	3.34	2.29	a	2.34	2.02	a
δC ¹³	NA			NA			NA		NA		NA		-	0.40	a	-	0.60	a	-	0.66	a
													24.24			24.48			24.25		
PO ₄ µg/g	12.42	5.78	a	3.23	1.90	b	5.98	2.14	b	7.02	5.97	ab	3.44	1.82	b	3.73	2.13	b	6.43	4.43	b
NH ₄ µg/g	22.65	7.74	a	12.41	3.61	b	23.66	7.08	ab	16.56	7.38	ab	5.90	1.02	b	7.92	3.22	b	18.48	26.82	ab
NO ₃ µg/g	4.16	3.22	a	0.68	0.61	b	4.26	2.47	a	2.51	1.37	ab	0.21	0.06	b	0.33	0.27	b	0.59	0.59	b
% N	NA			NA			NA		NA		NA		0.12	0.06	a	0.13	0.07	a	0.11	0.10	a
δN ¹⁵	NA			NA			NA		NA		NA		2.79	0.51	a	2.25	1.06	a	2.72	1.81	a
% Clay	12.83	1.51	a	12.90	1.10	a	15.13	0.66	a	14.21	0.34	a	9.28	2.66	b	5.10	1.13	c	6.43	2.55	c
% Silt	58.15	5.75	a	62.08	5.47	a	65.19	1.23	a	60.68	1.58	a	43.71	11.82	b	25.78	6.33	c	32.06	12.19	c
% Very fine sand	17.30	1.91	a	18.71	1.22	a	18.60	0.86	a	18.55	1.36	a	19.60	3.94	a	12.50	2.51	b	16.08	4.84	ab
% Fine sand	7.58	3.89	b	4.46	3.13	b	0.92	1.21	b	3.69	0.34	b	14.32	5.71	a	20.17	4.40	a	19.33	5.25	a

% Medium sand	2.44	2.54	bc	0.84	1.91	c	0.06	0.13	c	1.18	0.92	bc	7.67	6.46	b	18.91	4.54	a	13.92	7.87	a
% Coarse sand	1.70	2.17	b	0.98	2.45	b	0.09	0.20	b	1.69	1.33	b	5.00	5.65	b	16.41	5.31	a	11.29	6.88	a

Table 5.2. Hyphal lengths (m/g) of arbuscular mycorrhizal (AM) and septate fungi. Groups with different letters are significantly different according to univariate ANOVA ($p < 0.05$ after Tukey's HSD adjustment).

	South Mountain			Cerro Seco			History Grove						Banco North								
	Mesic mixed conifer			Xeric mixed conifer			Ponderosa pine-dominated						Ponderosa pine								
	<i>Pre-Thin/Control</i>			<i>Pre-Thin/Control</i>			<i>Unburned</i>		<i>Burned</i>		<i>Untreated</i>		<i>Thinned</i>		<i>Thinned/Burned</i>						
n surveyed	54			80			20		20			32		32		32					
n quantified	18			20			5		5			8		8		8					
	Mean	SD		Mean	SD		Mean	SD	Mean	SD		Mean	SD		Mean	SD	Mean	SD			
Fine AM	8.67	2.57	ab	13.60	6.38	a	6.23	1.65	b	8.10	2.76	ab	7.49	2.69	b	8.01	3.39	ab	7.22	4.28	b
Coarse AM	2.87	1.12	a	2.64	0.77	a	2.32	0.69	a	3.11	0.97	a	1.81	0.94	a	3.19	1.14	a	2.31	0.97	a
Dark septate	0.83	0.65	c	1.23	0.76	bc	0.40	0.27	c	0.21	0.06	c	2.00	1.65	abc	2.45	1.21	ab	2.79	1.73	a
Other septate	0.65	0.32	a	1.42	0.70	a	1.02	1.20	a	0.60	0.25	a	1.41	0.87	a	1.66	1.03	a	1.80	1.31	a

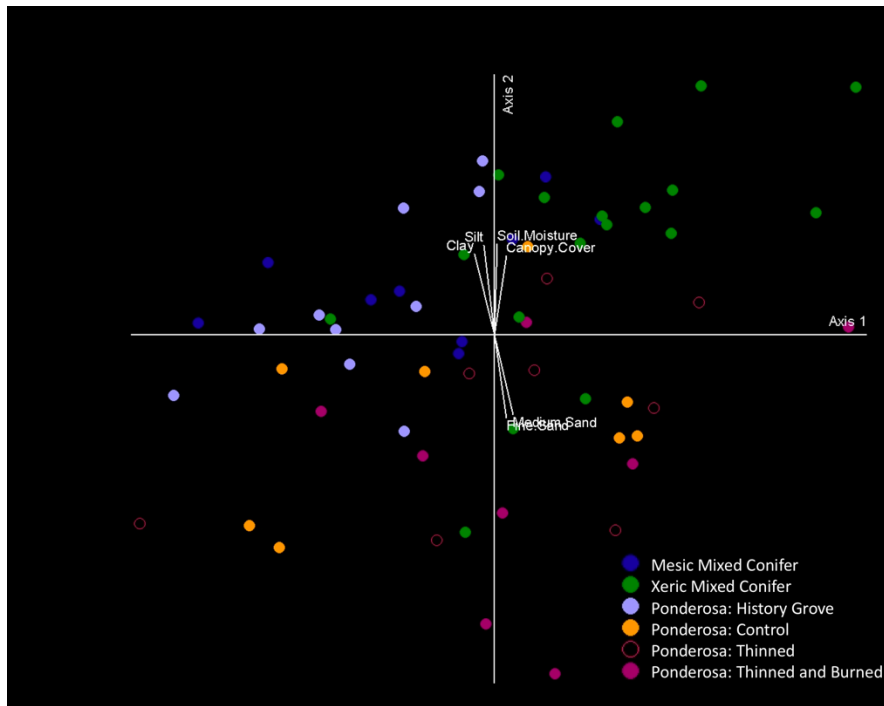


Fig. 5.2. Non-metric multidimensional scaling ordination based on Bray-Curtis distance showing differences in abundance of six hyphal types (stained and unstained fine and coarse arbuscular mycorrhizal hyphae and “dark septate” and other septate hyphae) among forest types and treatments at the ponderosa site (Banco North).

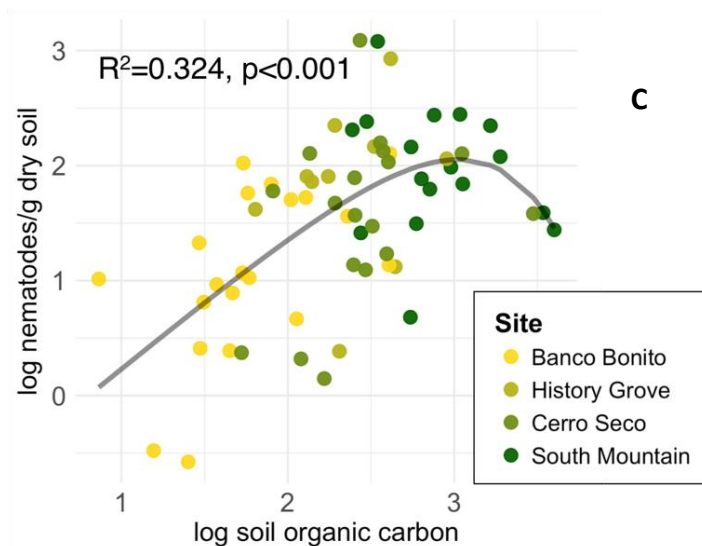


Fig. 5.4. Nematode abundance in control, thinned, and thinned/burned units at Banco North (**A**), and its correlation with total soil organic matter (**B**) across four sites at the preserve ranging in mesicness from (Banco North (yellow, driest) to South Mountain (darkest green, wettest).

Value added work:

II. Methods Development: how long can one store nematode samples before extraction?

Storage experiment addition: Publications are scarce addressing the effect of storage duration on recovery of free-living soil nematodes, so we have performed an experiment to determine how nematode abundance changes over time in samples stored at 4 °C. In May of 2017, we collected 7 soil cores each from four locations within the control and thinned / burned treatment units at Banco North, sieved them to 4 mm, and homogenized them to produce control and thinned / burned soil mixtures. Soil from each mixture sufficient for two extractions was portioned out into the Ziploc bags used for nematode soil sample storage and refrigerated at 4 °C, with the bags left partially unzipped to facilitate gas exchange. One control mixture bag and one thinned / burned mixture bag were randomly selected for extractions every week for the first eight time points, then every two weeks for the next six time points, then every three weeks for the final four time points, for a total of 32 weeks. Extractions were performed on two 100 cc subsamples from each bag using sucrose centrifugation, and nematodes from each subsample were enumerated. These data indicate that nematode densities and variance decrease slowly with increasing storage time (**Fig. 6.1**). While there tended to be more nematodes in samples from the control mixture than from the thinned and burned mixture, the trend for the two units was the same. Based on these results, we feel comfortable extracting stored samples up to four weeks after collection.

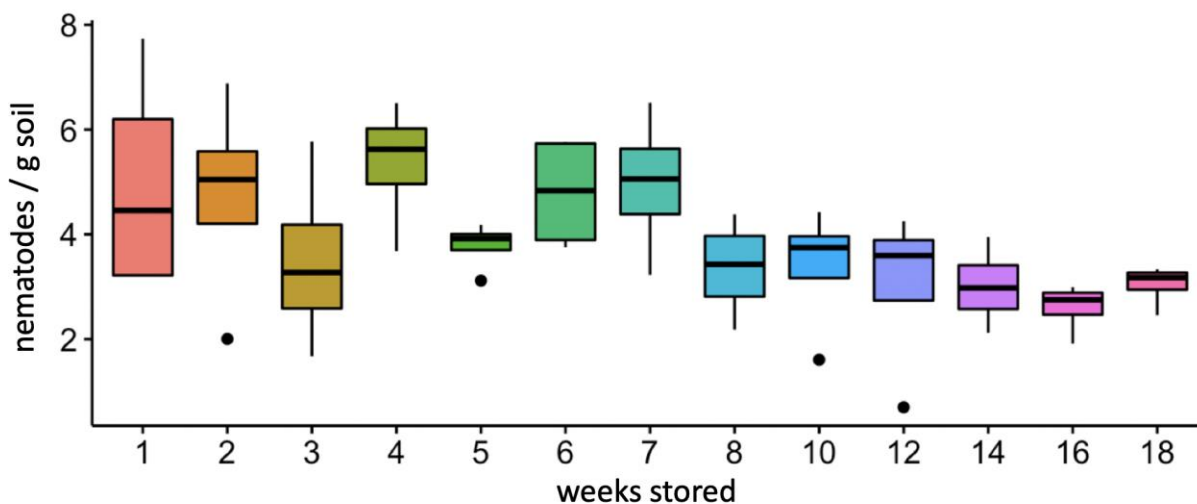


Fig. 6.1. Nematodes extracted from samples stored between one and eighteen weeks at 4 °C.

III. Methods Development: *Development of a colloidal silica centrifugation nematode extraction protocol suitable for large soil samples*

Extraction efficiencies for the most widely used techniques—sucrose centrifugation and Baermann funnels/Baermann trays, with or without pre-extraction by decanting and sieving—likely often fall below 50% (Viglierchio and Schmitt, 1983). Further, methods requiring pre-extraction are labor intensive and provide many opportunities for inadvertent variation across operators. These limitations spurred us to develop a new protocol for high-efficiency extraction of nematodes without pre-extraction using colloidal silica, which unlike sucrose solution does not subject nematodes to high osmotic stress. We adapted Griffiths et al.’s (1990) colloidal silica centrifugation method, developed for extraction from very small subsamples, for use on subsamples as large as 100 cc. Extra-large 500 mL centrifuge tubes accommodate soil subsamples without pre-extraction, and industrial-grade Ludox HSA (available through Remet Corp. from W.R. Grace and Co.) is used instead of laboratory-grade Ludox, which would be prohibitively expensive at the volumes required for this protocol. Our comparisons of this novel method with the standard decanting, sieving, and sucrose centrifugation protocol, and with an identical protocol substituting MgSO₄ solution (which has an osmotic effect greater than that of colloidal silica but lower than sucrose solution) for Ludox HSA, indicate that it recovers considerably more nematodes (**Table 7.1**).

Table 7.1. Nematodes extracted by three methods from 100 cc subsamples of soil collected at two locations near Flagstaff, AZ in the summer of 2018.

	Ludox	Sucrose	MgSO ₄	Percent increase with Ludox
Humane Society 1	2320	671		246%
Aspen Corner 1	785	202		289%
Humane Society 2	1522		1016	50%
Aspen Corner 2	611		383	60%

Objective 3. In an effort to determine the potential of fire mosses as a BAER restoration treatment, a field experiment on the Cajete Fire was set up in November of 2017 by Henry Grover.

Paper in review at Ecological Engineering: H.S. Grover, M.A. Bowker, P.Z. Fule, C. Seig, A. Antoninka. 2021. Pelletized inoculation overcomes ant predation and enhances fire moss establishment in severely burned conifer forests. Ecological Engineering

Land managers use Burned Area Emergency Response (BAER) hillslope treatments to mitigate devastating post-fire impacts and protect values at risk. However, the effectiveness of BAER seeding hillslope treatments has been debated in the literature and by managers (Peppin et al. 2010; 2014, Robichaud et al. 2000). Perhaps a focus on early successional, functionally different species could help increase seeding success. With wildfires increasing in extent and severity through the Western US (Westerling et al. 2006), land managers need new tools to facilitate post wildfire soil stabilization as well as ecosystem recovery (Robichaud et al. 2013).

One unexplored BAER seeding approach is using the fire increasing, early successional mosses *Ceratodon purpureus*, *Funaria hygrometrica*, and *Bryum argenteum* as hillslope treatments. These so-called Fire Mosses have functional differences which could complement existing seed mixes and mulching efforts (Peppin et al. 2010): (1) They are desiccation tolerant (Stark et al. 2010), (2) they can be propagated and dispersed vegetatively (During 1979), (3) they can aggregate disturbed soils, reducing erosion and increasing infiltration (Bowker et al. 2010), (4) and Fire Mosses have global distributions and are native plants virtually everywhere. Thus, they may be a valuable restoration material throughout the world (Longton 1981).

To date, we have rapidly cultivated fire mosses in a greenhouse setting, explored the drivers of fire moss colonization on recent wildfires in the Southwest US, and inoculated Fire Mosses on high severity fire surrogates. These successes combined with input from local BAER practitioners and leading BAER researchers give us confidence that testing the effectiveness of fire moss inoculation on a severely burned landscape is a valuable next step in our research.

With this study we will explore 3 major research questions:

1. What is Fire Mosses' potential to stabilize and restore severely burned soils in Valles Caldera National Preserve (VC)? We hypothesize that inoculation of fire moss gametophytes will increase colonization of moss, vascular plant, and soil microbial colonization of severely burned soils and could rapidly improve post-fire ecosystem stability and recovery when compared with uninoculated controls.
2. Can we improve fire moss colonization by applying it in a pelletized form? This will be achieved by mixing moss with diatomaceous earth and organic matter and rolling it into pellets before applying it to the soil. We hypothesize that that mixing moss into pellets will improve colonization by increasing the connection between the soil and moss and reducing the sensitivity of moss to climatic fluctuations.
3. How will ex-situ growth affect fire moss function in the field? To understand this impact, we will inoculate plots with either field collected moss or greenhouse grown moss in every block. We hypothesize that ex-situ growth will not have a large impact on fire moss function however it could affect soil microbial communities dramatically.

Methods

Study Area and Plot Selection: The study has been setup on the northern edge of the Cajete Fire on the Santa Fe National Forest just north of Highway 4 and south of Valles Caldera National Preserve. We selected a roughly 200 m x 200 m patch of high burn severity where 100% overstory tree mortality had occurred (**Fig. 17**, Lat 35.818208 Lon -106.554691). This wildfire allows us to test the effectiveness of inoculating moss in a post-fire setting similar to a real BAER (Burned Area Emergency Response) treatment. Sixty 1 m x 1 m plots were

marked with rebar in two opposing corners (**Fig. 18**). Inoculation occurred on 11/4/2017 roughly 4 months after the wildfire (**Fig. 17**). There was some initial colonization of moss prior to inoculation during the monsoon season, and these patches, and locations downslope of them were avoided during plot setup.

Treatments

Using a completely randomized design we inoculated levels of: greenhouse grown moss inoculation, field collected moss inoculation, two concentrations of greenhouse grown moss rolled into pellets, and no inoculation in November 2017. Variability of fire moss colonization from a previous study was used to conduct a power analysis, allowing us to determine adequate replication for this experiment. Treatments were replicated 12 times for a total of 60 experimental units, and a study wide ground impact of 60 m². Greenhouse grown moss and field collected moss were dried, ground through a sieve, and inoculated at an initial cover of 10%. The greenhouse grown pellet treatments were also inoculated at a cover of 10% as well as a cover of 50%.

Measurements

Sampling of plots occurred every Spring and Fall for two years post-inoculation (**Fig. 18**). Sampling consisted of cover estimates of: plant functional type, moss to species, bare ground, rock, and litter taken using standardized ocular estimates. Moss colonies to species were counted in every plot, this allowing us to measure inoculation survivorship at a higher resolution. Infiltration (3 per plot) and soil aggregate stability (4 per plot) measurements were taken at randomly generated positions on every plot in fall 2019.

Results

Pelletization at 50% cover was the most successful treatment with *B. argenteum* reaching a maximum mean cover of 10.5% in June 2019 ($F = 2.73$, $p = .046$, **Fig. 19 A**). The sieve and pellet 10% treatments had similar cover values throughout the experiment and performed only slightly better than the controls (**Fig. 19 A**). Natural recruitment on control plots of *B. argenteum* was high, reaching a maximum cover of 6.8% in June 2019. Moss cover at the first monitoring visit, six months after inoculation, was undetectable on any plot; however, pellets were still visible. This is most likely due to the exceptional drought that occurred over the winter of 2017/2018 (**Fig. 19 C**). As treatment cover did not strongly diverge from controls in this experiment, we did not see effects of moss cover on infiltration ($F = .18$, $p = .67$) or soil aggregate stability ($F = .46$, $p = .50$). *C. purpureus* was detected on one pelletized and one sieve plot at the final monitoring visit. No *F. hygrometrica* was found at any time.

The number of colonies for all treatments increased through time (**Fig. 19 B**, $F = 3.96$, $p = .01$). In spring 2018 there had been so little precipitation that pellets were intact, and diatomaceous earth was still visible making it difficult to assess colony counts. Strong monsoon precipitation in 2018 lead to a large increase in colony counts by the fall 2018 monitoring (**Fig. 19 B-C**). The colony count number plateaued from fall 2018 to spring 2019; however, there was a relatively large increase in cover during that time period (**Fig. 19 A**).

Study Conclusion: At the final monitoring timepoint (Fall 2019) plots were left intact to allow for the possibility of monitoring at longer timescales. All soil samples taken from the site will be consumed during analysis.



Fig. 17. Location of inoculation plots just north of NM highway 4 in the Cajete Fire. (White polygon.)



Fig. 18. Fire moss plots post inoculation. White squares are plots inoculated with pellets.

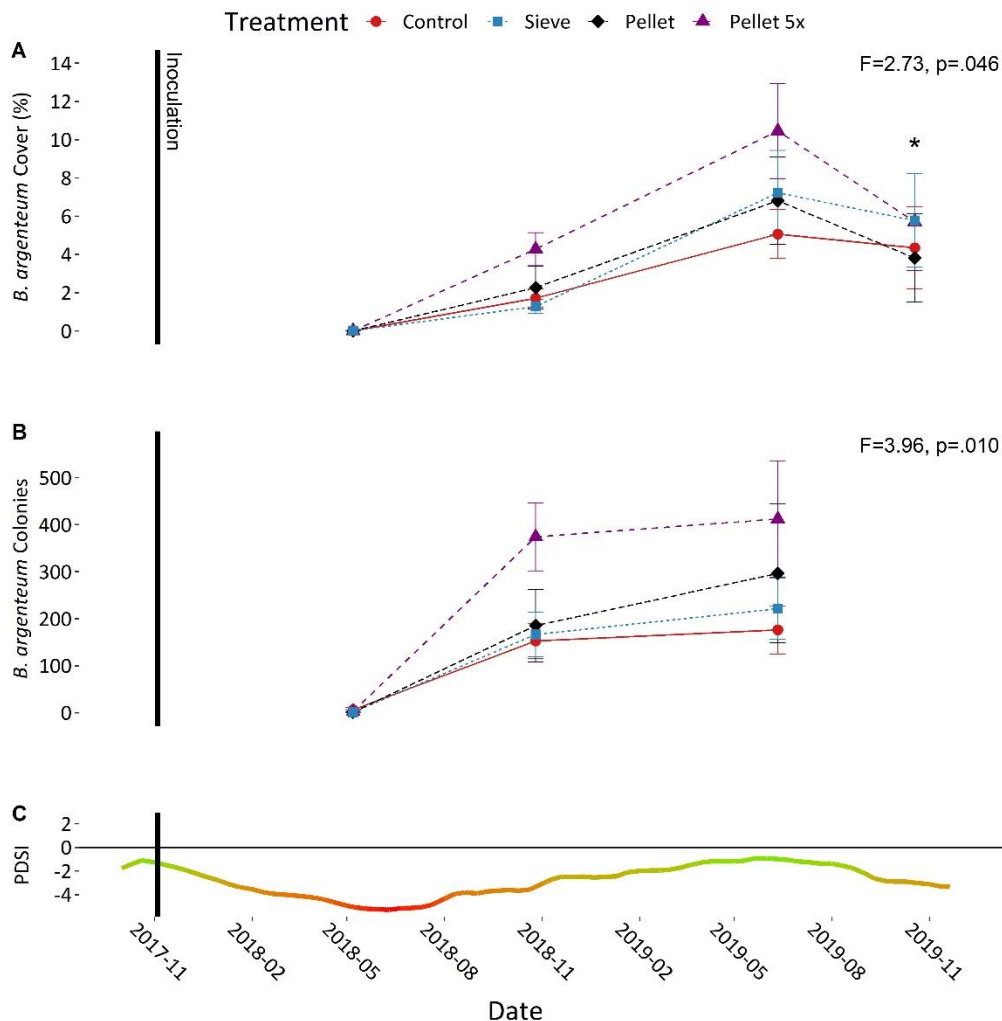


Fig. 19. *B. argenteum* (A) cover and (B) colony count at monitoring visits. Vertical line indicates date of inoculation. Error bars represent $\pm$ SE. *Cover methodology changed, and no colony count data was taken in final monitoring visit due to extensive vascular plant colonization. (C) Palmer drought severity index (PDSI) throughout the experiment, negative values indicate drought while positive indicate moisture.

5. A survey of fire moss distribution and associated function in the southwestern US was published in the International Journal of Wildland Fire.

A portion of this study was conducted within Valles Caldera National Preserve on the Thompson Ridge Fire. Funding from this grant was used for travel to fires within the Jemez Mountains and technician wages for both field and lab work.

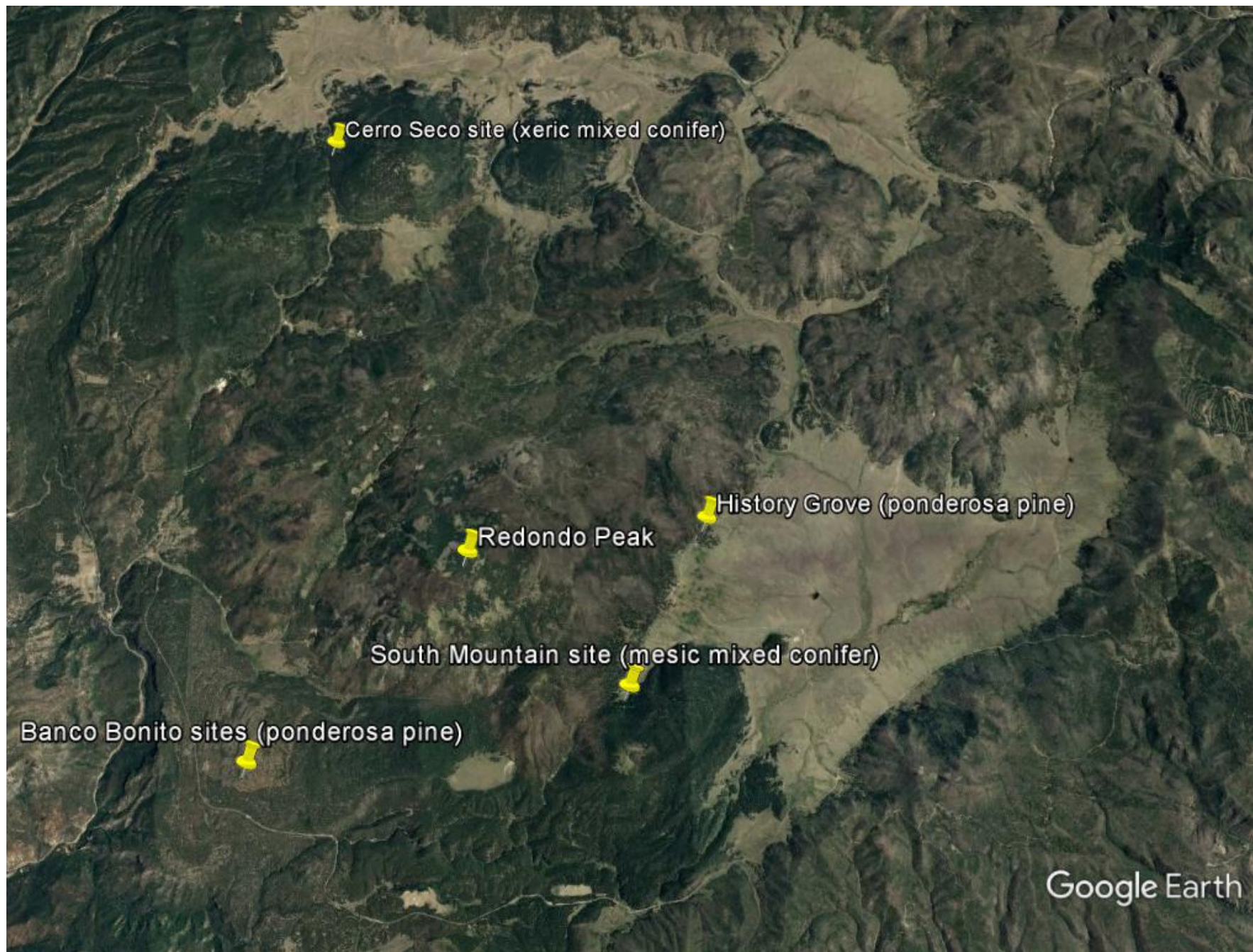
Reference: Grover HS, Doherty KD, Bowker MA, Fulé PZ, Sieg CH, Antoninka AJ (2020) Post wildfire moss colonisation and soil functional enhancement in forests of the southwestern USA. International Journal of Wildland Fire <https://doi.org/10.1071/WF19106>.

Abstract: Fire mosses, including *Ceratodon purpureus*, *Funaria hygrometrica* and *Bryum argenteum*, can achieve high cover within months to years after high-severity fire, but do so heterogeneously across space and time. We conducted a survey of moss cover and erosion-related functions after 10 wildfires in *Pinus ponderosa* and mixed-conifer forests of the southwestern USA. We sampled 65 plots in high-severity patches, stratifying by elevation and insolation over each fire. Using three landscape-scale predictor variables and one temporal predictor, we explained 37% of the variance in fire moss cover using a random forest model. The predictors in order of importance were: equinox insolation (sunlight/day), pre-fire vegetation type, pre-fire soil organic

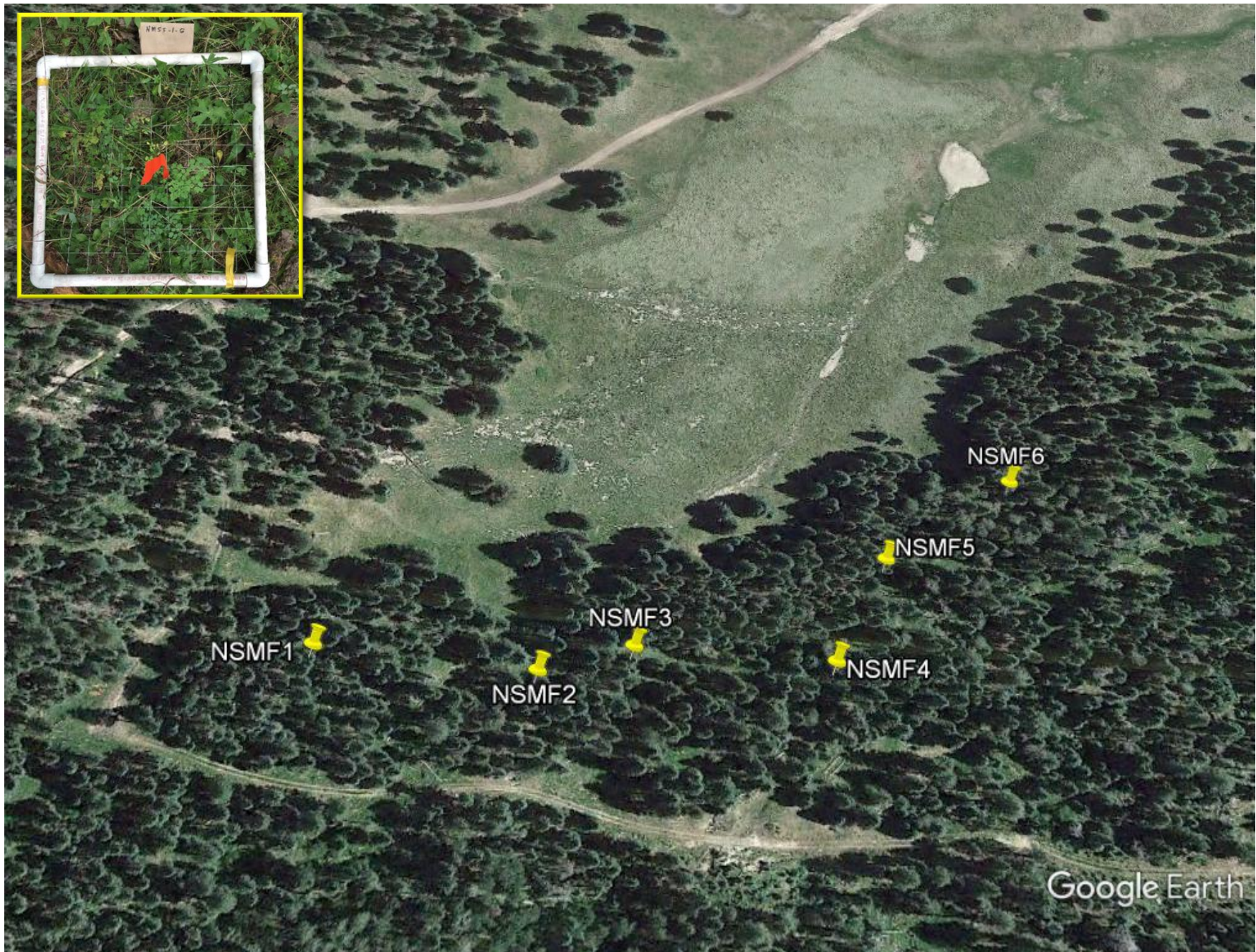
carbon and time since fire. Within each plot we examined differences between bare and moss-covered soil surface microsites and found moss-covered microsites had a mean increase of 55% water infiltration, 106% shear strength, 162% compressive strength and 195% aggregate stability. We tested a suite of nutrients, finding 35% less manganese in the moss-covered soil. This research demonstrated that post-fire colonisation by moss is predictable and that colonisation improves soil surface erosion resistance and hydrological function, with implications for managing severely burned landscapes.

Research Mentoring and Dissemination:

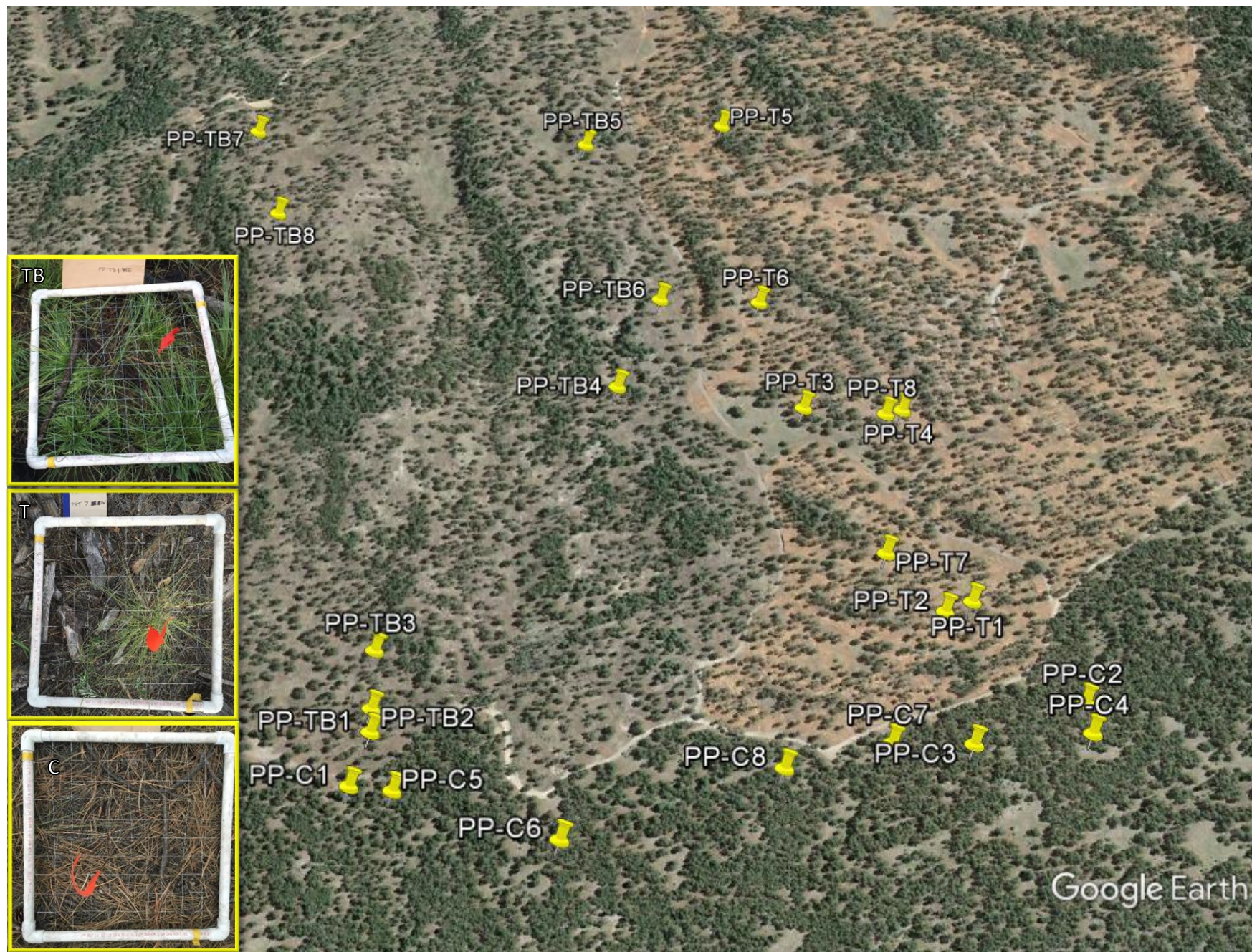
1. Thirteen undergraduate students and recent graduates have been mentored on this project.
2. Data from this project were presented by Kara Gibson and Channing Laturno at the Soil Ecology Society meeting in Fort Collins, Colorado (2017); by Channing at the NASA Research Symposium in Phoenix, Arizona (2017); by Kara at the Biennial Conference on the Colorado Plateau in Flagstaff, Arizona (2017 and 2019); in a poster presentation by Channing at the Undergraduate Research Symposium in Flagstaff (2017); and at the Jemez Collaborative Forest Landscape Restoration Program (CFLRP) All Hands Meeting by Kara Gibson in 2017 and 2019 (she also presented a poster at the CFLRP in 2018).
3. Henry Grover presented fire moss BAER tool data at Biennial 2019 (invited talk); Society for Ecological Restoration Southwest, Tucson AZ, Nov 2019 (invited talk); Association for Fire Ecology, Tucson AZ, Nov 2019 (Contributed talk).
4. Currently we have one published paper and two under review from this work. We expect a minimum of three more peer review papers.



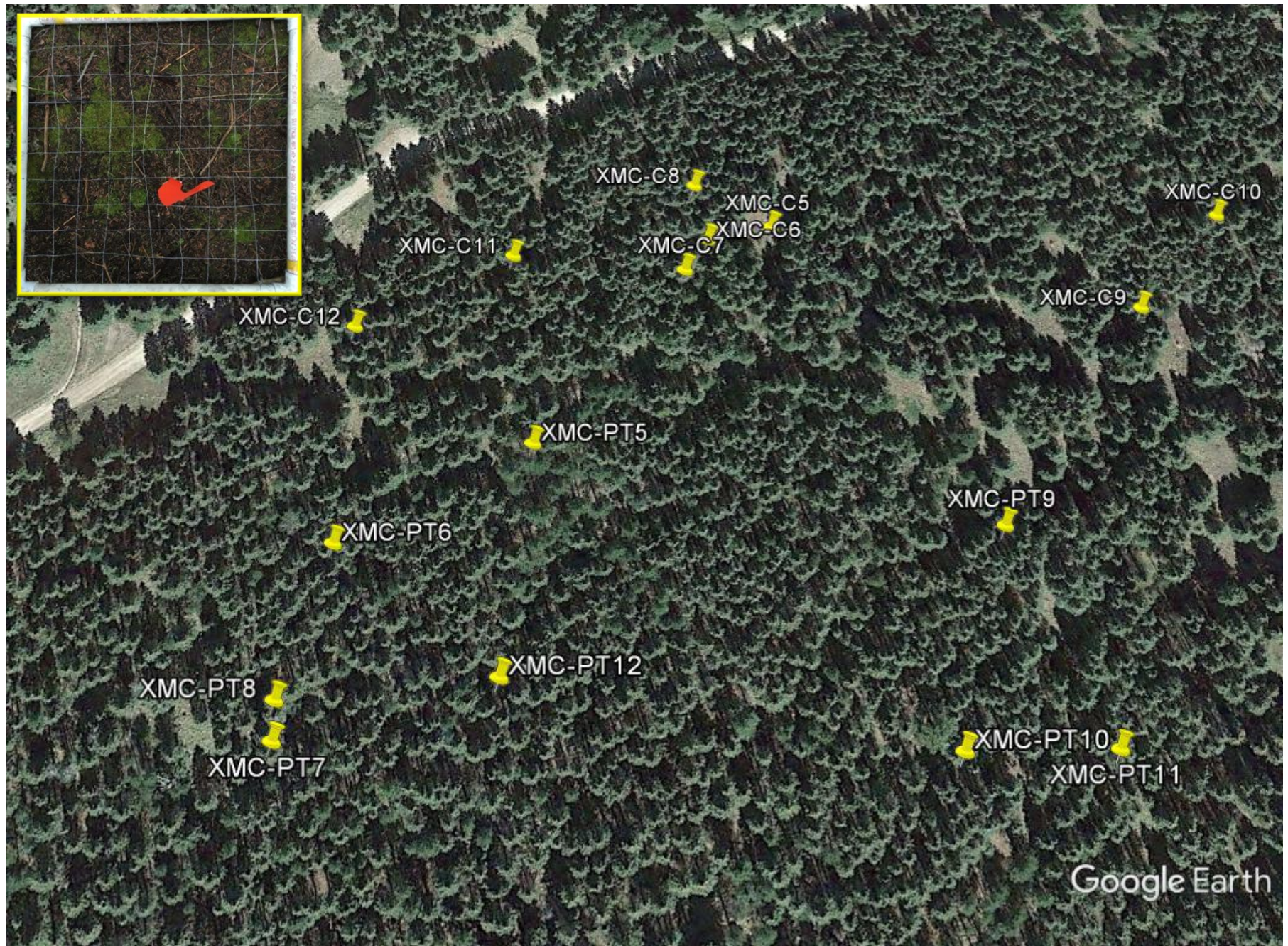
Appendix A. A. Valles Caldera locations sampled in 2016.



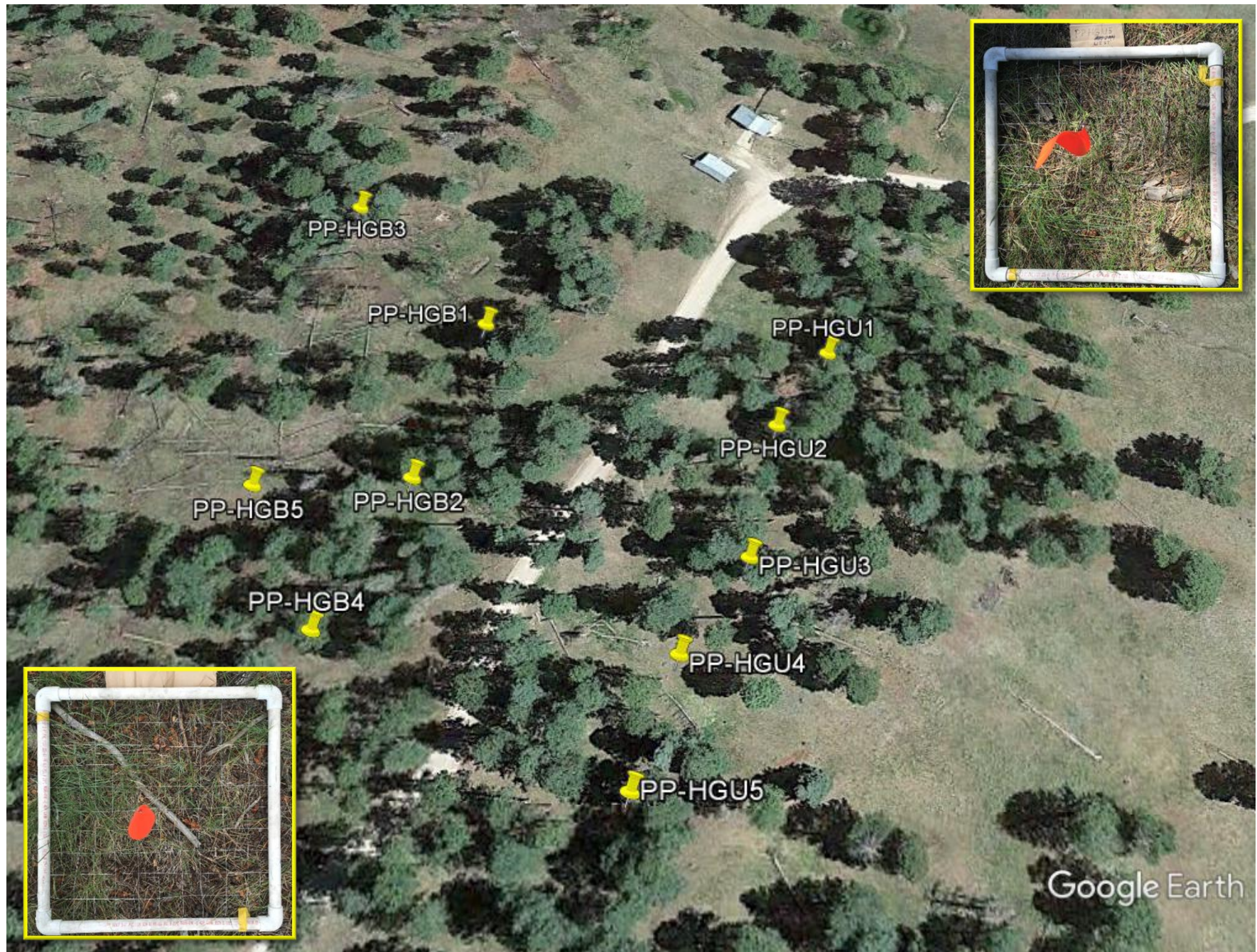
Appendix A. B. Quadrat array centerpoints at the South Mountain mesic mixed conifer fringe forest site. Each array was comprised of 9 quadrats, of which 3 were sampled for soil and litter.



Appendix A. C. Quadrat array locations in Banco North's thinned (PP-T), thinned and burned (PP-TB), and untreated control (PP-C) units. Each array included 4 quadrats, of which one was sampled.



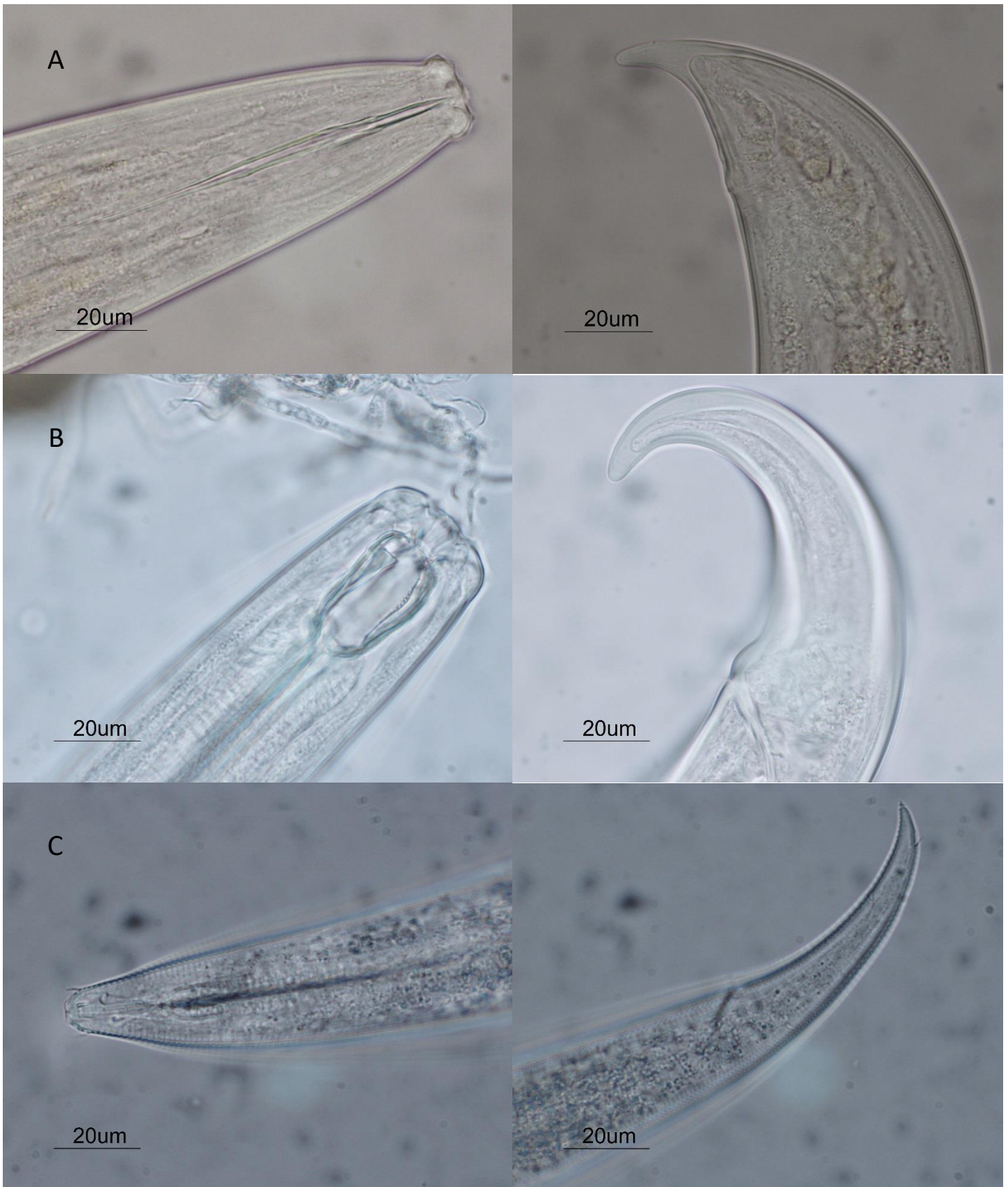
Appendix A. D. Quadrat array locations in the pre-thinning (XMC-PT) and control (XMC-C) areas at the Cerro Seco site. Each array included 4 quadrats, of which one was sampled.



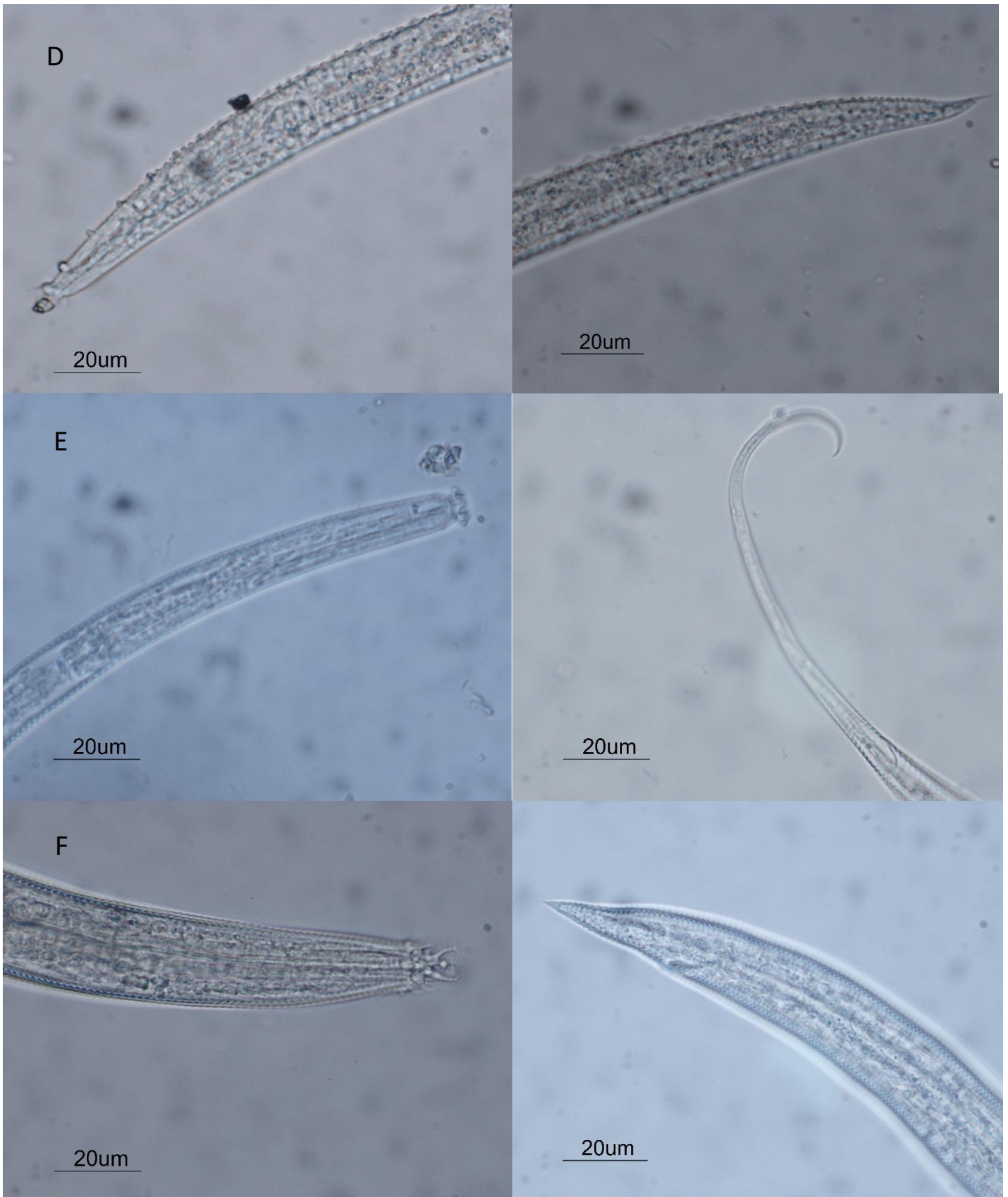
Appendix A. E. Quadrat array locations at the History Grove site. The left side of the road (PP-HGB) burned during the Thompson Ridge Fire in 2013; the right side (PP-HGU) did not.



Appendix A. F. Redondo Peak nematode and microarthropod sampling locations.



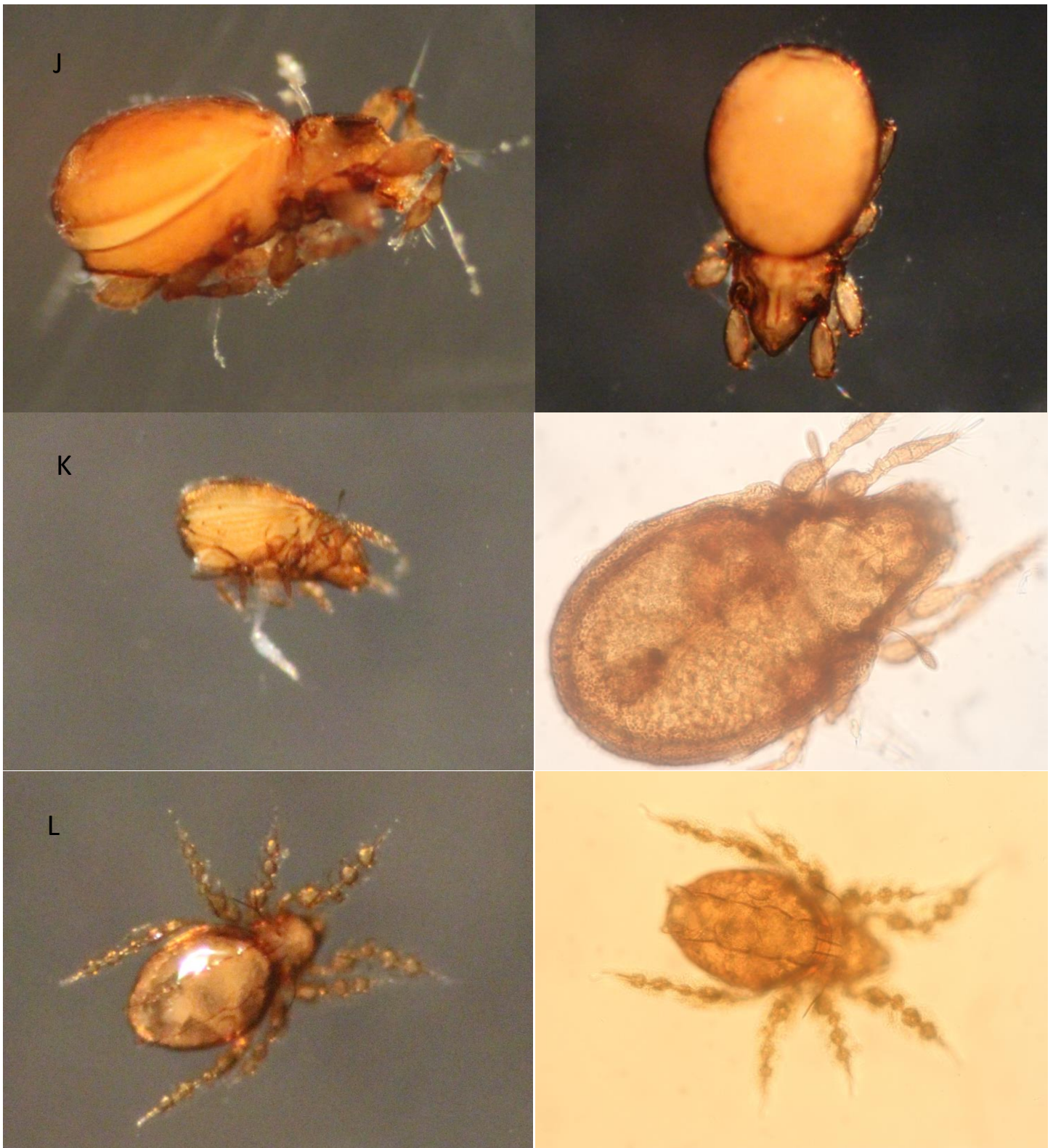
Appendix B. Heads and tails of nematode morphotypes from the sub-order Dorylaimina (**A**); family Mononchidae (**B**), and genus *Plectus* (Plectidae) (**C**).



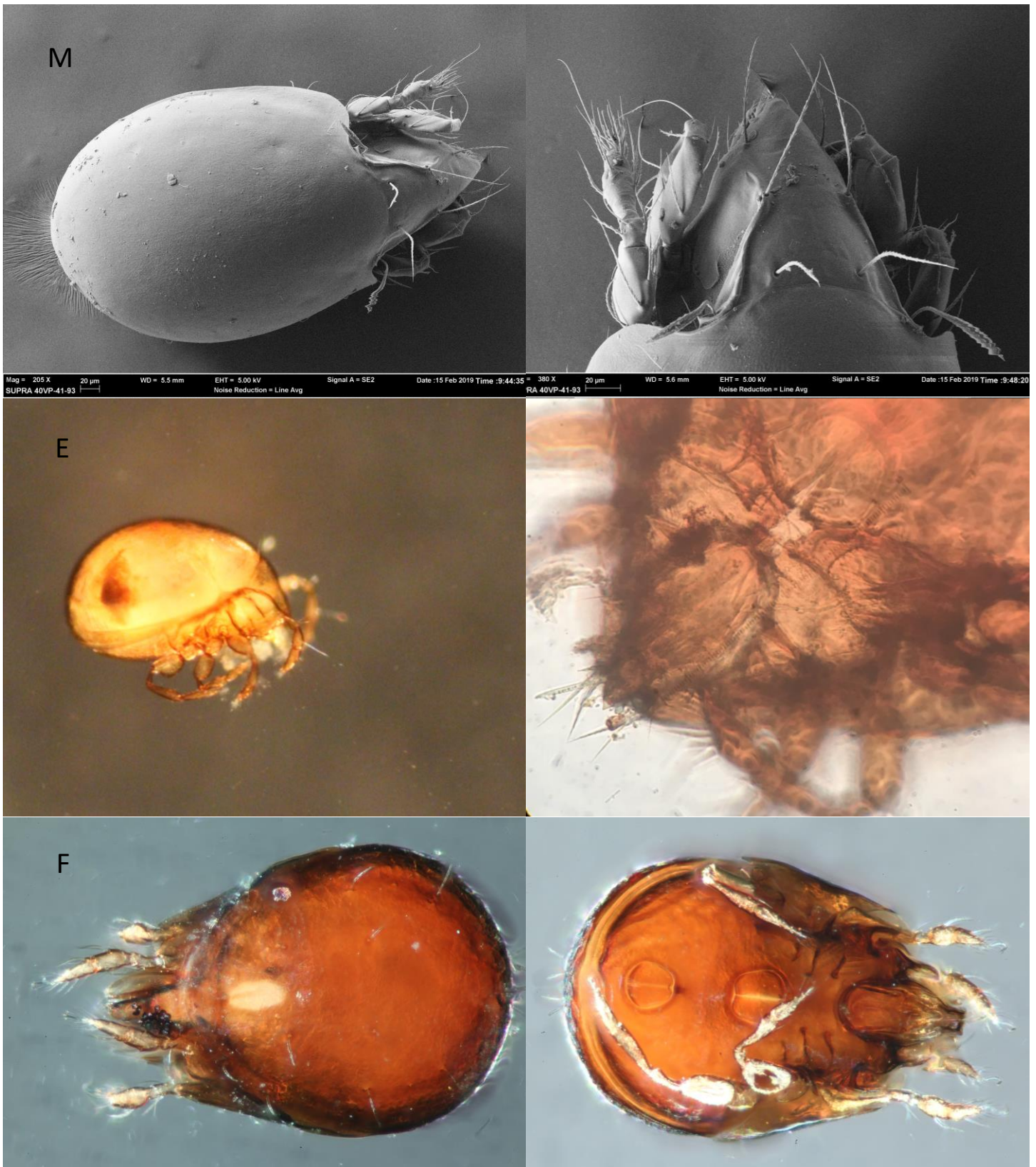
Appendix B. Heads and tails of nematode morphotypes from the family Bunonematidae (**D**); family Teratocephalidae (**E**), and genus *Acrobeles* (Cephalobidae) (**F**).



Appendix B. Heads and tails of nematode morphotypes from the family Aphelenchoididae (**G**), sub-order Dorylaimina (**H**), and family Cephalobidae (**I**).



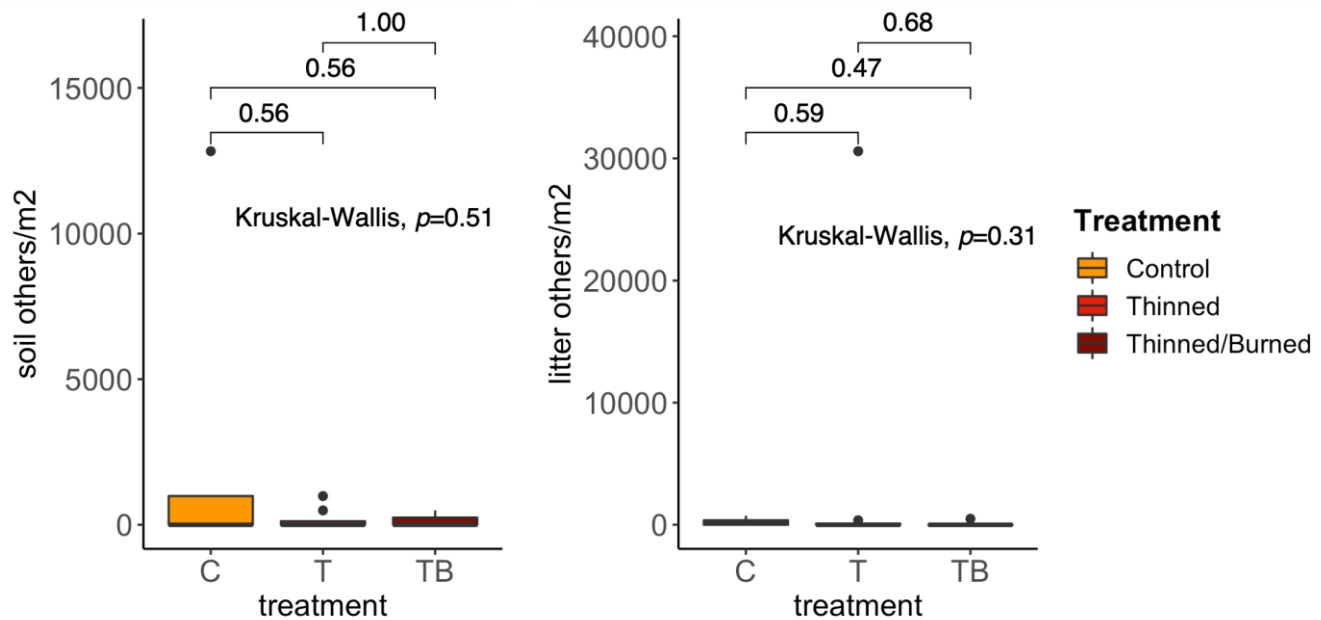
Appendix B. Microarthropod morphotypes from Cerro Seco. *Eremaeus oresbios* (J), *Tectocephus velatus* (K), and a *Damaeidae* sp. (L).



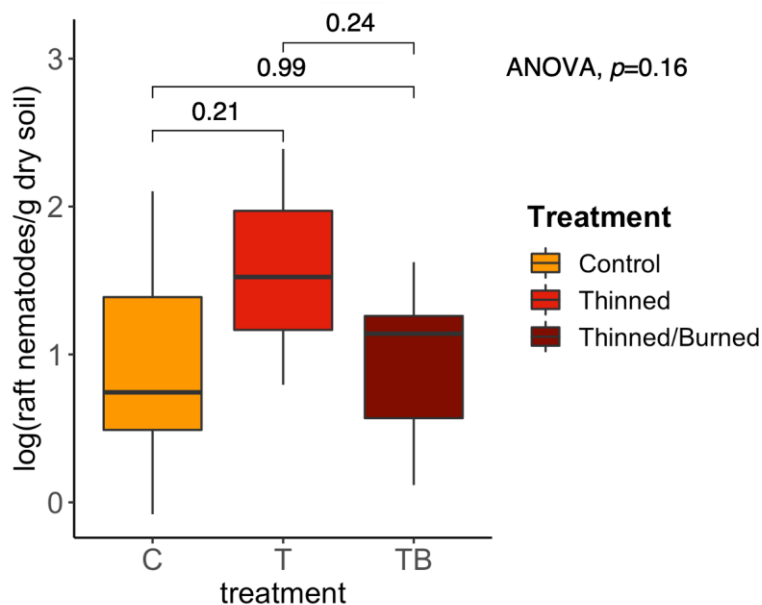
Appendix B. Mites from Redondo Peak. *Scheloribates* sp. (collected in Redondo Peak subalpine forest) (M); *Gustavia* sp. (collected in South Mountain mesic mixed conifer fringe forest) (N); *Phenopelopoidea* sp. (collected in Redondo Peak subalpine meadow) (O).

Supplementary Information for:

Collembolans and nematodes, but not mites, are resilient to restoration of a *Pinus ponderosa* forest with thinning, mastication, and prescribed fire

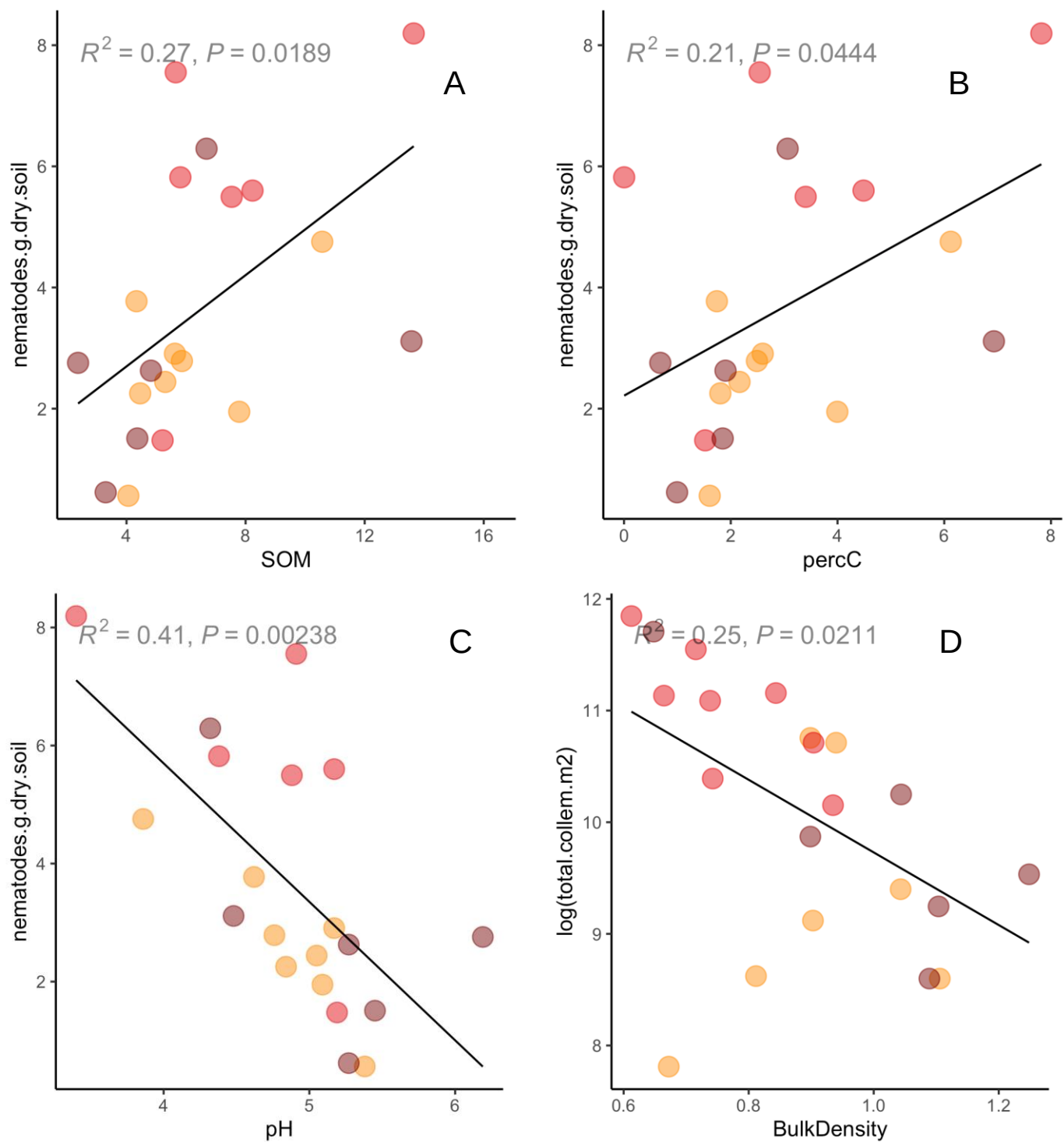


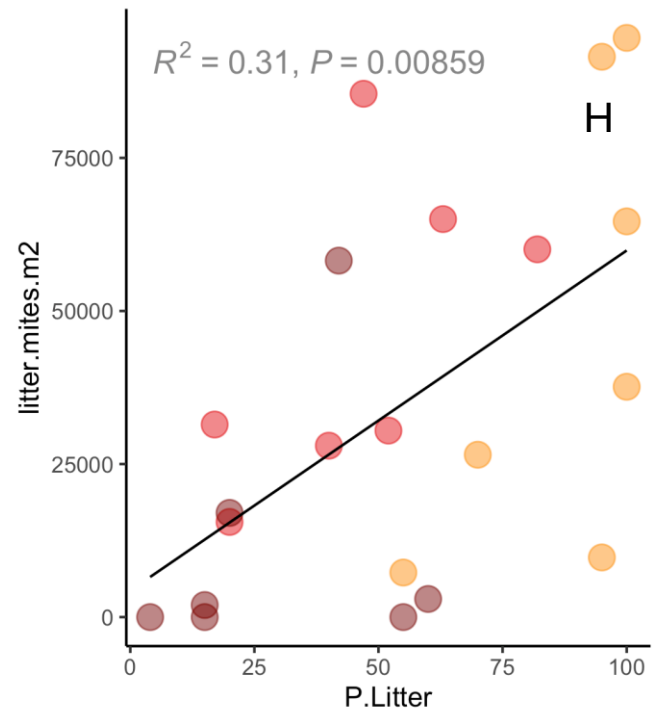
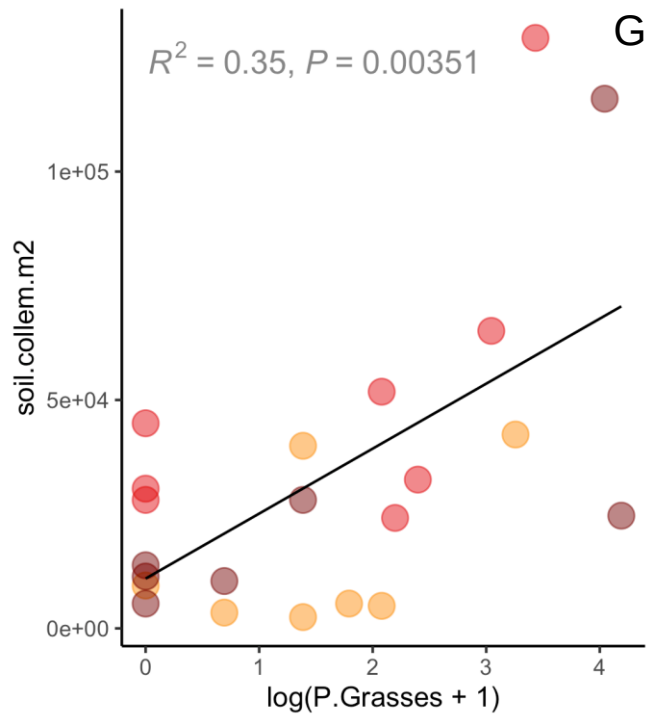
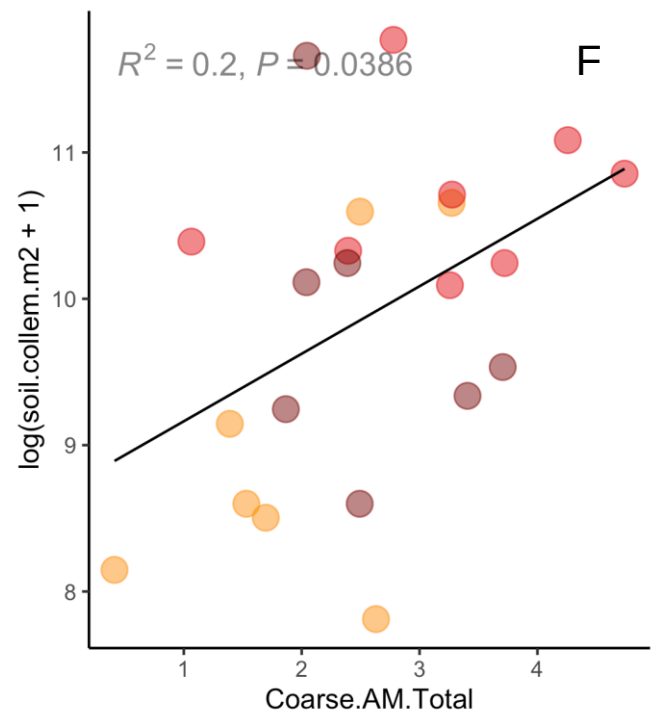
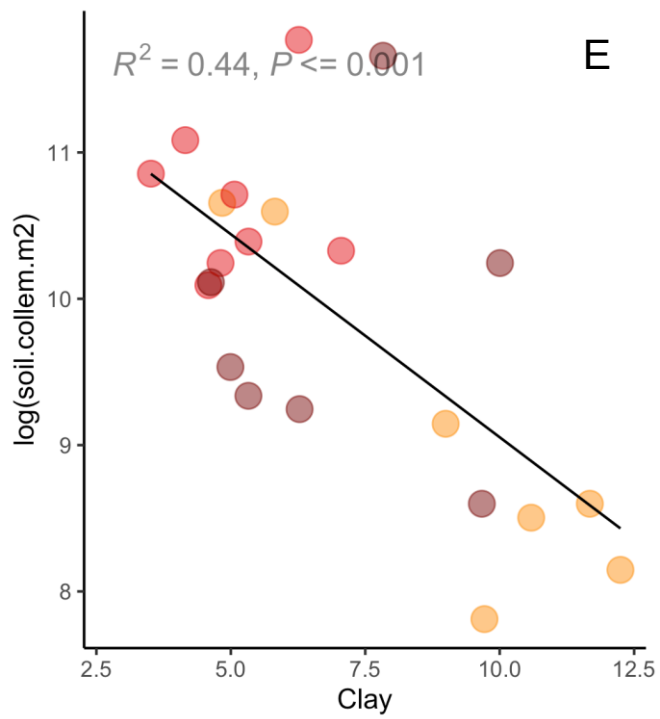
Supplemental Fig. 1. Abundance of arthropods other than mites and collembolans (chiefly ants, macroarthropod larvae, and enchytraeids) extracted from soil and litter samples with Tullgren funnels.

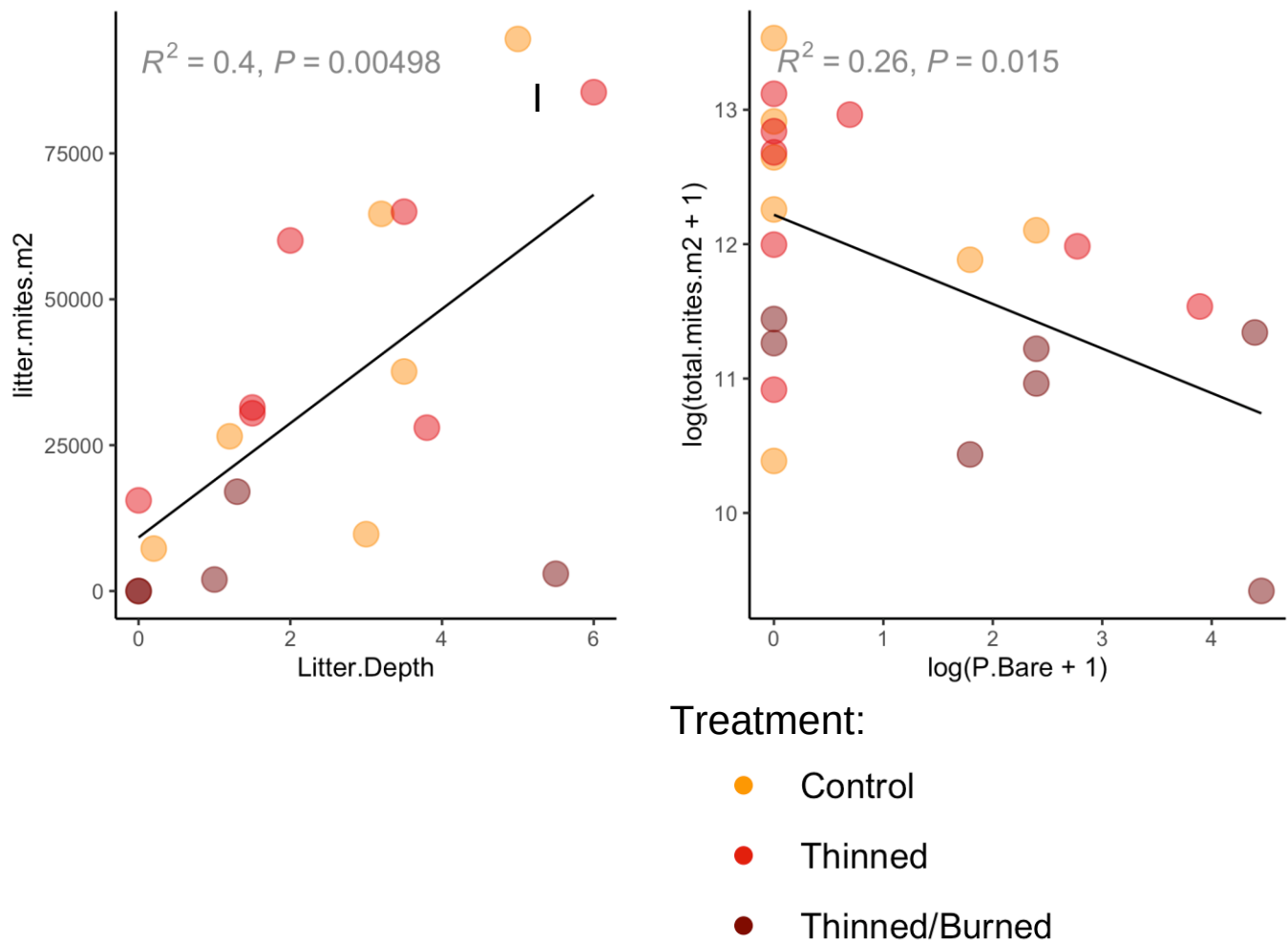


Supplemental Fig. 2. Densities of nematodes extracted with modified floating Baermann trays ("nematode rafts"), without preliminary decanting and sieving. Nematodes extracted with this less-efficient method showed the same overall pattern in restoration treatment responses as did those re-extracted later with the more efficient sucrose centrifugation method, although the variance was higher and treatment effects were not significant. Although nematode densities were likely reduced by extended storage, we feel that the nematode abundance data

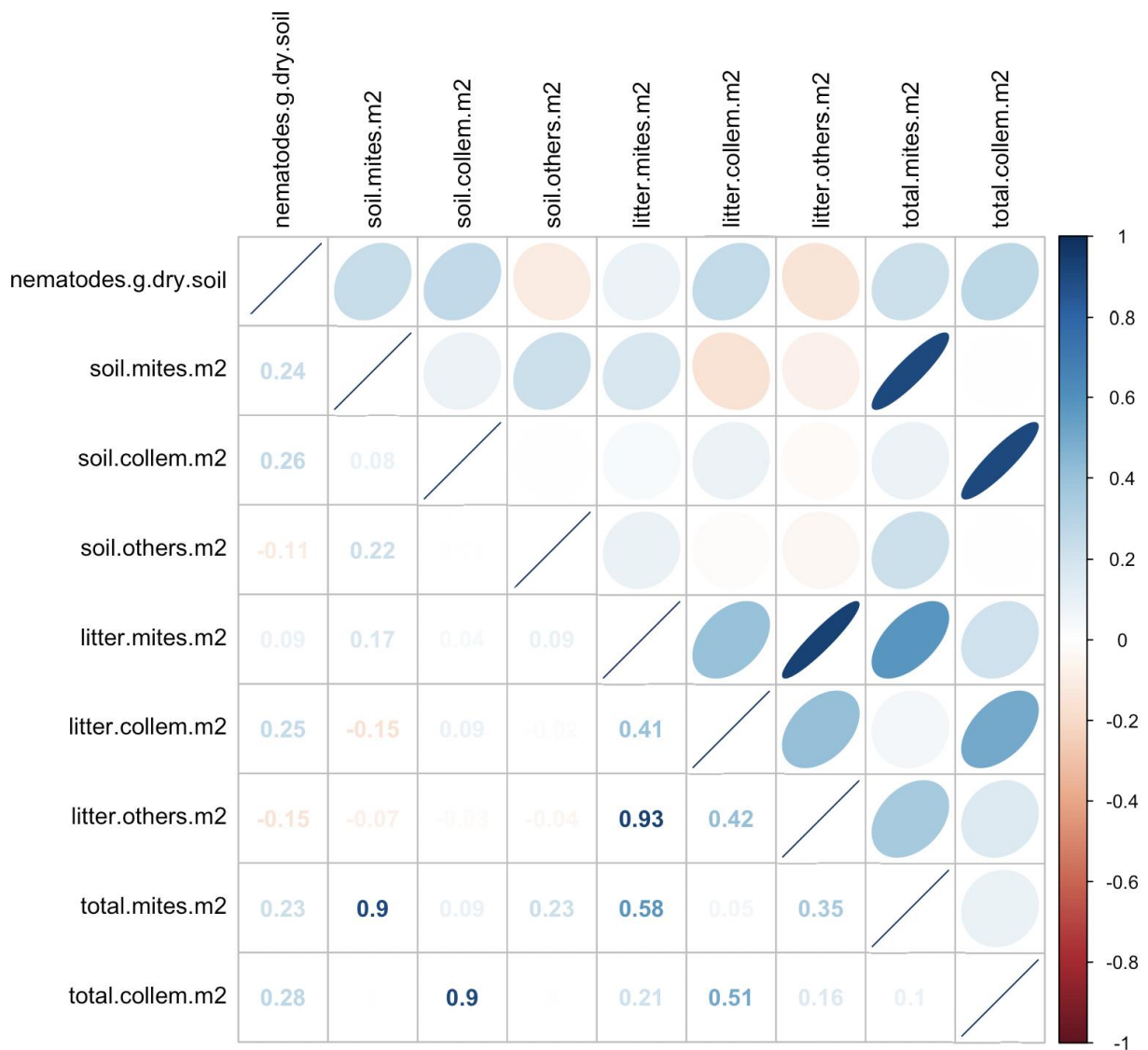
obtained using the more efficient method are better suited to assessing differences across management treatments, so these data are presented in the main text.



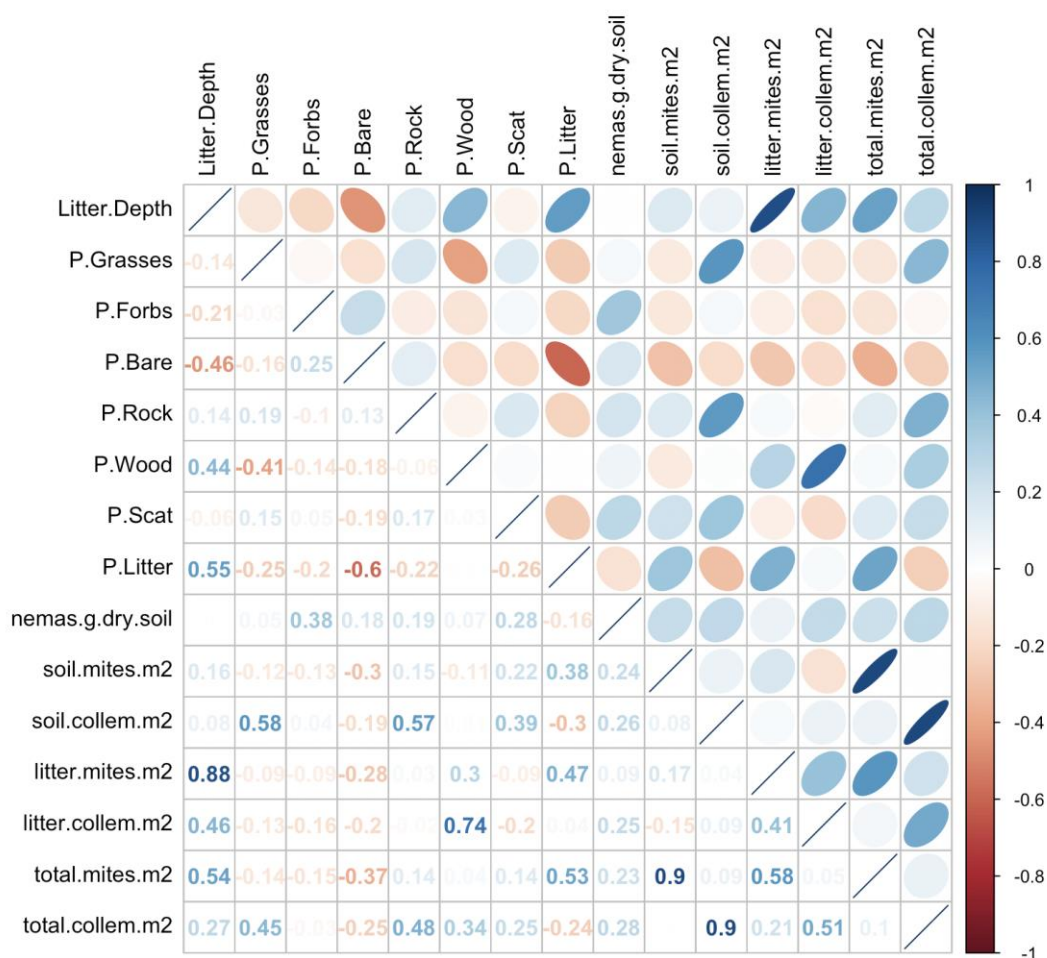




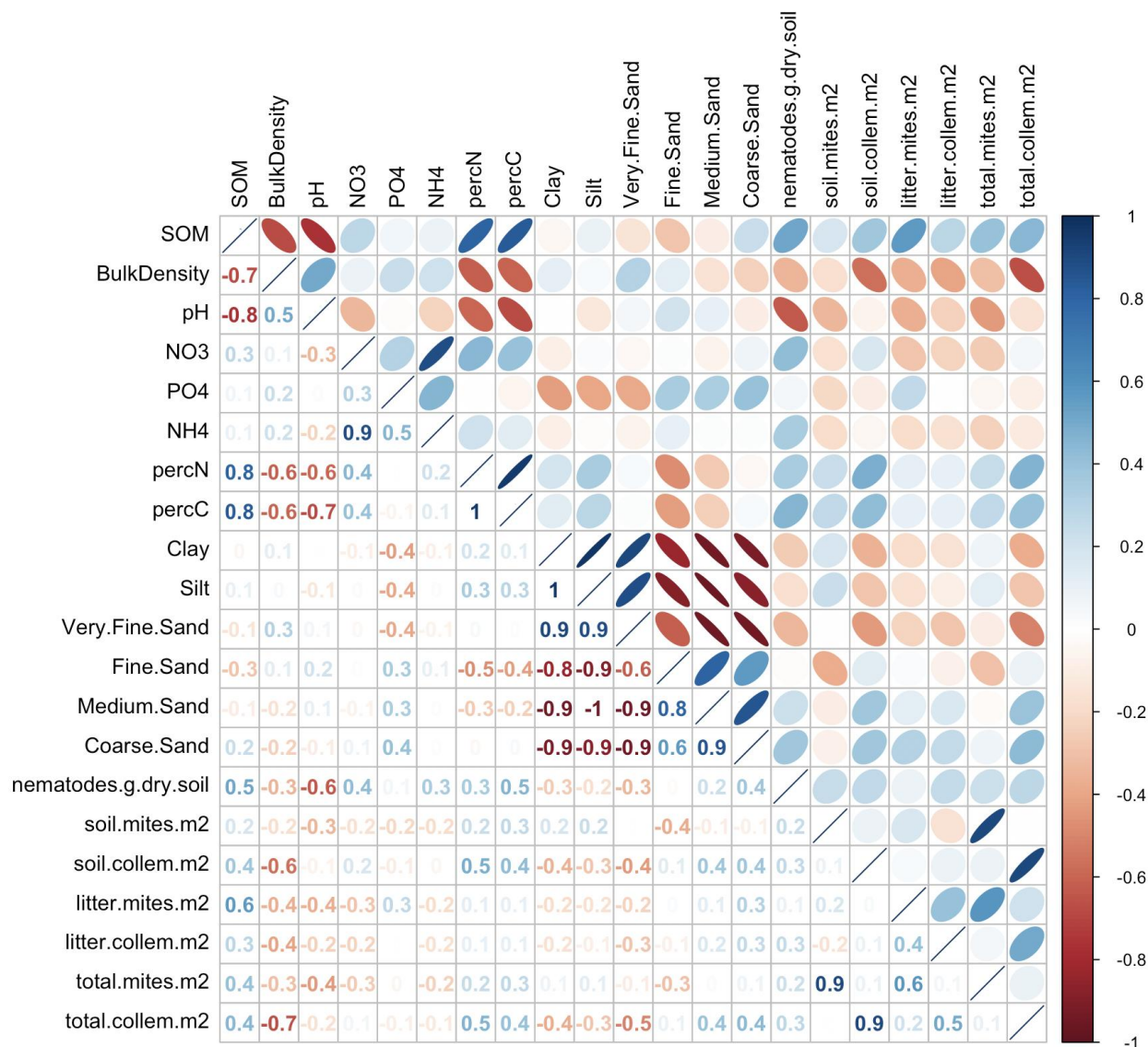
Supplemental Fig. 3. Correlations between micro- and mesofauna groups and the habitat characteristics which best predicted their abundance. A. Nematodes per g dry soil and percent soil organic matter. B. Nematodes per g dry soil and percent soil carbon. C. Nematodes per g dry soil and pH. D. Total collembolans per m² and soil bulk density. E. Soil collembolans per m² and percent clay. F. Soil collembolans per m² and lengths of coarse arbuscular mycorrhizal hyphae (m per g dry soil). G. Soil collembolans per m² and percent grass cover. H. Litter mites per m² and percent litter cover, after removing an outlier sample with both extremely high litter depth and extremely high mite abundances. I. Litter mites per m² and litter depth (cm), after removal of the same outlier.



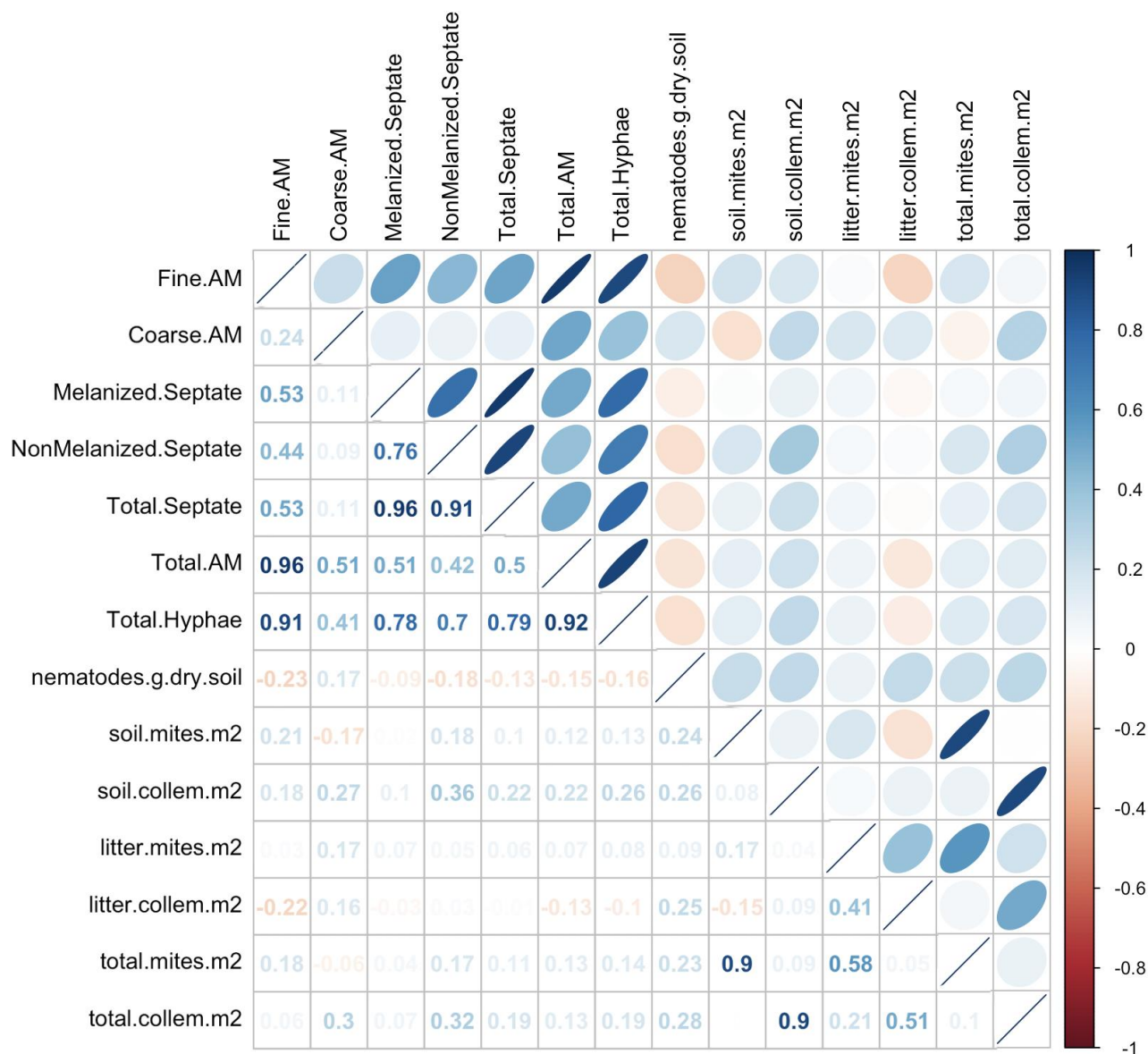
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Supplemental Fig. 5. Matrix of Pearson correlations between micro- and mesofauna groups and ground cover, color coded by strength and direction.



Supplemental Fig. 6. Matrix of Pearson correlations between micro- and mesofauna groups and soil abiotic properties, color coded by strength and direction



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Appendix D. Faunal complexity mesocosm experiment

We leveraged funding from a Joint Fire Science Program Graduate Research Innovation Award to conduct a field mesocosm experiment examining the contribution of soil faunal complexity to ecosystem functions in thinned/burned vs. untreated control ponderosa pine forest units at Banco North. Our report on this study is included below.

FINAL REPORT

Title: Assessing the contribution of soil
faunal complexity to ecosystem
services after restoration with thinning
and fire

JFSP PROJECT ID: 18-1-01-49

July 2021

Anita Antoninka
Northern Arizona University

Kara Gibson
Northern Arizona University



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Research Supporting Sound Decisions



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List of Abbreviations/Acronyms

MRPP: Multi-response permutation procedure

PCA: Principal components analysis

VALL: Valles Caldera National Preserve

Keywords

Soil, Mites, Collembolans, Ponderosa Pine, Thinning, Prescribed Fire, Faunal Complexity, Decomposition, Soil Organic Matter, Nitrogen, Fungal Communities, Field Mesocosms

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Abstract

While managed fire often produces clear changes in aboveground functional diversity, we know little about how fire affects belowground fauna and their mediation of biogeochemical processes. Because soil micro- and mesofauna, particularly nematodes, collembolans and mites, are significant contributors to nutrient and carbon cycling in forest systems, understanding how managed fire changes soil faunal assemblages and their contributions to ecosystem function is important. We successfully manipulated soil faunal complexity over two growing seasons in field mesocosms installed in thinned/burned and untreated control management units within a second-growth ponderosa pine forest at Valles Caldera National Preserve, New Mexico. The method we developed was designed to minimize treatment side effects while allowing repeated internal measurements of mesocosms, and it should aid future field investigations of faunal communities. These studies are sorely needed, both to illuminate the roles of soil fauna in forests and to adequately assess forest management impacts on soil ecological functions. Five years post-fire, densities and species richness of microarthropods $>300\ \mu\text{m}$ were markedly reduced in mesocosms within the thinned/burned unit relative to the untreated control unit. However, we did not find evidence that faunal complexity influenced fungal community composition, nitrogen mineralization, or soil organic matter formation in either forest management unit. Decomposition appeared to be affected by faunal complexity only within the thinned/burned unit, despite reduced faunal complexity in that unit. We speculate that the biotic and abiotic context of faunal complexity may be more important for modulating decomposition than faunal complexity per se, or that abundance of fauna must be severely reduced before decomposition effects are evident in this system.

1. Objectives

While managed fire often produces clear changes in aboveground functional diversity, we know little about how fire affects belowground fauna and their mediation of biogeochemical processes. Because soil micro- and mesofauna, particularly nematodes, collembolans and mites, are significant contributors to nutrient and carbon cycling in forest systems, understanding how managed fire changes soil faunal communities and their contributions to ecosystem function is important. **Our objective was to assess what concomitant changes in ecological function may occur with shifts in soil food web structure after restoration of a ponderosa pine forest.** This objective was met: **we successfully manipulated soil faunal complexity** in field mesocosms installed in adjacent thinned/burned and untreated control ponderosa pine management units at Valles Caldera National Preserve (VALL), New Mexico, **and we elucidated how faunal complexity modulates fungal community structure, decomposition, soil organic matter, and nitrogen availability** in the treated and untreated units. Since thinning and burning alter both the abiotic context of faunal interactions with decomposer organisms and the composition of faunal assemblages, **we hypothesized that the functional importance of faunal complexity would differ between untreated and thinned/burned units.** We further predicted that lignin decomposition would be primarily affected by modulation of competitive interactions between saprotrophic and ectomycorrhizal fungi (the Gadgil effect (Fernandez and Kennedy, 2016)), while cellulose decomposition would be primarily affected by comminution (Neher and Barbercheck, 2019). Our predictions regarding the direction and relative strength of faunal complexity effects on focal ecosystem functions are summarized in **Table 1**.

This project was undertaken to address the JFSP topic area of *managed fire effects and post-fire recovery*. Factors governing decomposition rates, nitrogen cycling, and fungal community dynamics are of interest to land managers, as these processes help determine tree growth rates, forage nutritional quality, ground fuel accumulation, water holding capacity, and carbon storage. Our study aids in evaluating restoration of soil ecological function with thinning and managed fire, focusing on key players in soil food webs that are often neglected in forest restoration research. In addition, comparing ecosystem services provided by simple soil faunal communities to those provisioned by more complex communities can allow us to make inferences about impacts to ecosystem services when soil fauna are decimated by high-severity wildfire.

Table 1. Predictions regarding the effects of increasing soil faunal complexity (more microarthropod species and individuals) on ecological functions in untreated and thinned/burned ponderosa pine management units.

More complex faunal assemblages will:

Untreated Control	Thinned/Burned
<u>Inhibit decomposition of recalcitrant substrate (lignin)</u> if microarthropods preferentially graze on saprotrophic over ectomycorrhizal hyphae, influencing competitive interactions among fungi (the Gadgil effect). Effect expected to be stronger because more microarthropods are likely present in this unit, and because competition between ectomycorrhizal and saprotrophic fungi is predicted to be greater.	<u>Inhibit decomposition of recalcitrant substrate (lignin)</u> if microarthropods preferentially graze on saprotrophic over ectomycorrhizal hyphae. Effect expected to be weaker because there are likely fewer microarthropods and less competition between ectomycorrhizal and saprotrophic fungi. Alternatively, if low fungal abundance is limiting decomposition and fauna are dispersing fungi to the substrate, increased faunal complexity could promote decomposition.
<u>Increase decomposition of labile substrate (cellulose)</u> because comminution (fragmentation) increases substrate surface area, and because a wide range of saprotrophic microorganisms (including many bacteria) can break down cellulose. This effect is expected to be stronger because there are more microarthropods.	<u>Increase decomposition of labile substrate (cellulose)</u>. Effect expected to be weaker because there are likely fewer microarthropods.
<u>Decrease nitrogen availability</u>, if there are more bacterivore nematodes in mesocosms with less faunal complexity (in other words, if there are top-down effects of faunal complexity on nematode abundance). Nematodes are the most important soil fauna in mineralizing nitrogen, and more nitrogen is immobilized in microbial biomass in the control unit.	<u>Not affect nitrogen availability</u>, because top-down effects on nematodes should not be strong (few predators, few nematodes) and because mineral nitrogen is abundant, with relatively little nitrogen immobilized in microbial biomass.
<u>Affect fungal communities</u>, mainly because of grazing impacts on competitive outcomes (due to preferential grazing) and on hyphal morphology (stimulation of hyphal growth/branching).	<u>Affect fungal communities</u>, mainly because of dispersal.

2. Background

The potentially millions of nematodes (Yeates, 2007) and tens to hundreds of thousands of collembolans and mites (e.g., Marra and Edmonds, 2005) which can, along with other soil fauna, inhabit a square meter of forest soil contribute both directly and indirectly to the fates of carbon and nutrient inputs to the pedosphere. Directly, comminution of litter by mesofauna increases the surface area available to microbial decomposers and enhances leaching of low-molecular weight compounds into the soil, which may increase formation of stable SOM fractions (Soong, 2014). Bacterivores (especially nematodes) and fungivorous micro- and mesofauna liberate nutrients from immobilization in bacterial and fungal biomass (Chen and Ferris, 1999; Freckman, 1988). Indirectly, soil fauna can regulate carbon and nutrient cycling by altering microbial abundance, community composition, and function (e.g., Kaneda and Kaneko, 2008; Trap et al., 2016). Their feeding may favor unpalatable, poisonous, or structurally-protected microbial taxa, altering the enzymatic capabilities of soil communities. For instance, many fungivores appear to prefer saprotrophic to mycorrhizal hyphae, and melanized over hyaline hyphae (Klironomos and Kendrick, 1996). Fungivore preferences thus have the potential to intensify the Gadgil effect - the oft-cited but highly variable depression of decomposition rates by ectomycorrhizal fungi (reviewed in Fernandez and Kennedy, 2016). Grazing at low intensities, however, may stimulate growth of fungal taxa (Janoušková et al., 2018) and/or modify mycelial morphology and resource allocation (Ngosong et al., 2014). Bacterivores, while usually observed to decrease bacterial biomass, may also increase it by liberating resources bound in senescent cells (Trap et al., 2016). Finally, the feces, exuviae, eggs, and corpses of mesofauna can serve as nuclei for aggregate formation, sequestering occluded particulate organic matter and increasing soil carbon storage (Maaß et al., 2015).

The magnitude and even the direction (positive or negative) of net faunal effects on soil processes is dependent upon biotic and abiotic context, but environmental drivers of functional outcomes still remain poorly understood (Briones, 2014). In fire-adapted dry forests of the Southwest, restoration with thinning and low-severity burning is likely to alter many biotic and abiotic characteristics relevant to determination of food web function. Soil nutrient availability and other physicochemical characteristics changed by fire may govern the magnitude and functional consequences of top-down controls (Lenoir et al., 2007). As faunal size classes can have opposing effects on fungal community composition and decomposition (Crowther et al., 2011), net outcomes may also depend on the relative resilience of broad faunal groups. Soil fauna could perhaps play an oversized role in structuring microbial communities in recently burned soils by facilitating propagule dispersal as they return to scorched soil patches from unburned or little-affected patches in the typical post-fire habitat mosaic, providing a recolonization advantage to favored taxa or specialized hitchhikers.

3. Materials and Methods

3.1 Study sites

The study was conducted at VALL in the Banco Bonito area (**Fig. 1**). Soils in this area are sandy loams to loamy sands formed by a rhyolite lava flow ~68,000 years ago (Goff, 2009) and belonging to the Totavi-Jemez-Rock Outcrop association (Hacker and Banet, 1987). Annual

precipitation averaged 590 mm from 1981–2010, and the mean annual temperature for this period was 6.6 °C, with monthly mean temperatures ranging from -2.3 °C in January to 17 °C in July (PRISM, 2021). Mesocosms were installed within two ponderosa pine forest management units, hereafter referred to as the thinned/burned unit and the untreated control unit. In 2012, the thinned/burned unit was thinned to an average tree density of ~60 trees ha⁻¹. Marketable timber was removed, and remaining woody residues were masticated. A low-intensity broadcast burn occurred in October of 2015. The untreated control unit was located across an old logging road and retained ~300 trees ha⁻¹. Adjacent study areas with similar topography and soil texture, measuring approximately 0.75 ha and 1.25 ha, respectively, were established within these units.

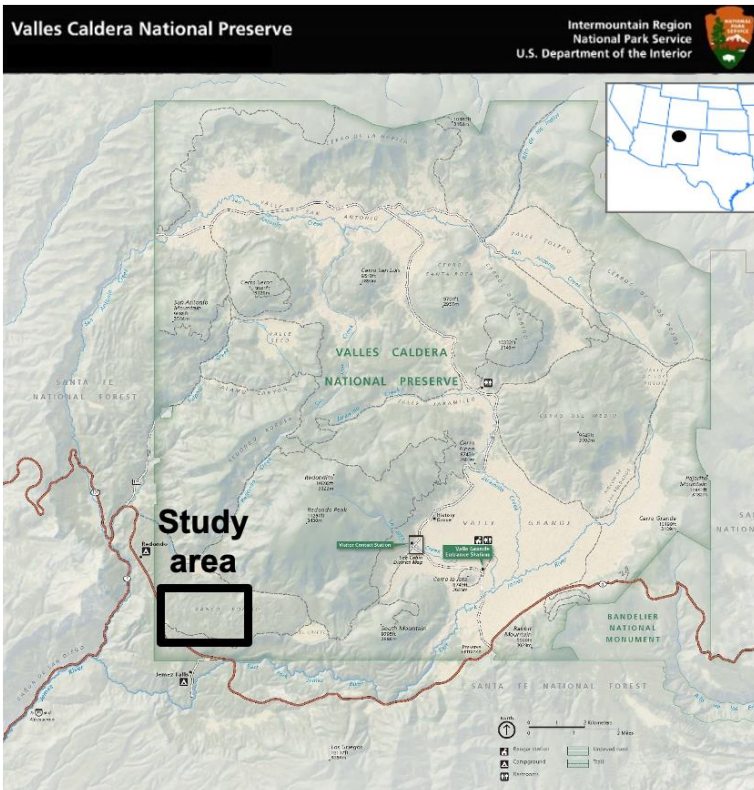


Fig. 1. Location of study area within Valles Caldera National Preserve and New Mexico.

3.2 Experimental design

Twelve trees per study area with diameters at breast height between 34.4–55.4 cm were selected to serve as blocks. At each of these trees, we installed defaunated mesocosms designed to permit recolonization by either small microfauna only, small and medium sized-microfauna (including most nematodes, but not mites), all micro- and mesofauna but not roots, or all micro- and mesofauna and roots. One replicate of each mesocosm type was installed at each tree for a total of 96 experimental mesocosms. Six of these trees per study area were randomly selected to receive one additional replicate of each mesocosm type for periodic destructive monitoring of recolonization by soil biota (hereafter “sacrificial mesocosms”, 48 in total). Four of the remaining trees in each study area were selected for installation of three types of controls (24 control units in total) to quantify any side effects of mesocosm physical structure and root

severing on soil properties and processes. Severed disturbed controls and unsevered disturbed controls consisted of mesocosm-sized holes refilled with defaunated mineral and organic horizons and covered with defaunated litter, with or without root severing, and undisturbed controls consisted of intact forest floor equivalent in area to a mesocosm.

3.3 Mesocosm construction and installation

Five large windows were cut in the sides of 25 cm long sections of PVC sewer pipe with an internal diameter of 20.32 cm, then polyester mesh with hole sizes of 21 μm , 41 μm , or 1 mm was wrapped around the exterior of the mesocosms (**Fig. 2 A**). These mesh sizes were selected because diameters of common small nematode taxa at this site range from 12.5-15 μm and most nematodes are at least 25 μm in diameter, while the smallest mites are approximately 46 μm in diameter, and no mite or collembolan taxa measuring > 1 mm in diameter were encountered in the course of our earlier work at this site. The bottoms and caps of all mesocosms were constructed identically to equalize drainage, infiltration, and albedo. Mesocosm bottoms were fitted with 21 μm mesh and covered with PVC-coated fiberglass window screen to provide additional structural support (**Fig. 2 B**). Tight-fitting removable caps (**Fig. 2 C** and **Fig. 2 D**) were assembled from vinyl flashing and 41 μm mesh, chosen for its superior permeability to precipitation versus 21 μm mesh and because few soil microfauna were expected to colonize mesocosms from aboveground. Mesh was glued to surfaces with all-weather 100% silicone caulk. A ring of Fluon PTFE insect barrier (byFormica, Warner Robins, Georgia, USA) was applied above the windows to reduce the probability of arthropods entering the tops of the mesocosms.

Mesocosms were installed in June 2019 at tree driplines in semi-circular arrays centered on their southern aspects, at intervals of 50 cm, or greater if necessary to avoid understory vegetation (**Fig. 3**). If woody debris longer than mesocosm width but not covering more than half of the mesocosm footprint was present, the debris was moved. At each mesocosm location, litter and soil O horizon depths were measured, then these layers were collected separately using a segment of the sewer pipe with a sharpened end as a coring guide. Holes for the mesocosms were dug to 15 cm below the mineral soil surface with a 20.32 cm soil auger, and excavated soil was sieved to 5.6 mm and homogenized. Mineral and O horizon soil and litter were defaunated following a modified version of Franco et al.'s (2017) method for nematode exclusion. Soil and litter were placed in aluminum steam table pans, were pre-wetted and allowed to incubate for 24 hours (12 hours if already well wetted by rain) at ambient temperature, then were heated at 65 $^{\circ}\text{C}$ for 3 days. Following this treatment, litter and O horizon soil were additionally frozen to -20 $^{\circ}\text{C}$ for at least 48 hours, since we expected fauna in these layers would be more resistant to heating than fauna in mineral soil. Mineral soil was sieved once more to 1.25 cm to break up hardened blocks that resulted from heating.

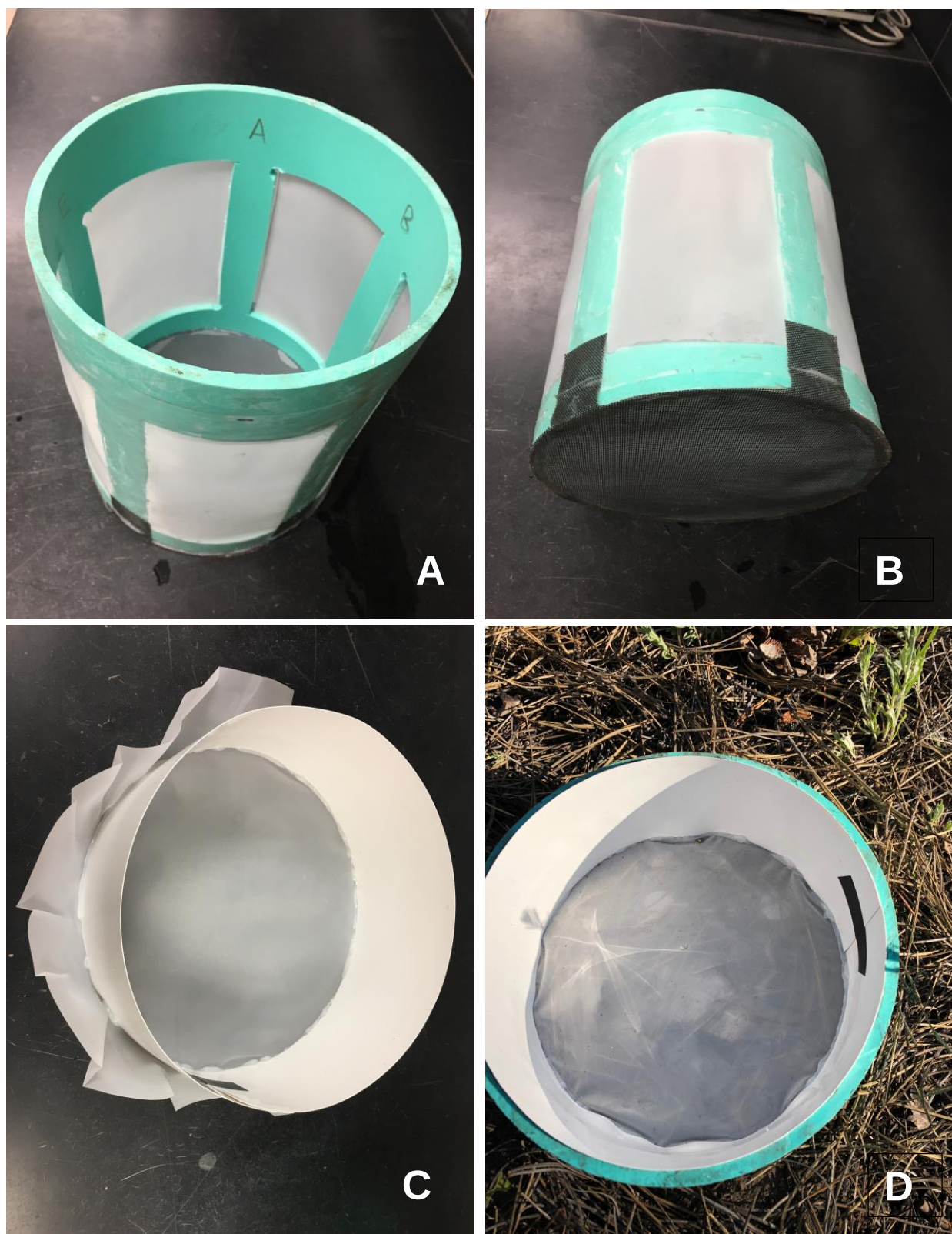


Fig. 2. Top (A) and bottom (B) views of mesocosms assembled from PVC sewer pipe, polyester mesh, and PVC-coated fiberglass window screen. Caps constructed from 40 μm mesh and vinyl flashing before (C) and after (D) installation of mesocosms.



Fig. 3. Sacrificial and experimental mesocosms at the dripline of a tree in the thinned/burned study area. Other trees in the study, flagged with orange tape and marked with stars, are visible in the background.

Mesocosms were buried to a depth of 15 cm below the mineral soil surface and capped until defaunation of soil and litter was completed. Immediately prior to refilling, the empty interiors of the mesocosms were sprayed with 70% ethanol to ensure no live fauna were present inside. Defaunated mineral soil, O horizon soil, and litter layers were returned to their trees of origin and used to fill mesocosms and holes designated for disturbed controls. Soil was leveled and litter was distributed so as to maximize contact between interior and exterior soil and litter layers. Decomposition bags containing standard substrates composed predominantly of lignin or cellulose (described below) were placed between the litter and O horizon layers. Finally, the mesocosm caps were replaced, and petroleum jelly was applied between the flashing and the interior walls of each mesocosm to prevent fauna from entering through this crevice. During the growing season, we severed roots monthly to a depth of 20 cm around the root-exclusion treatment 1 mm mesh mesocosms (smaller mesh sizes did not allow root entry) and around disturbed severed controls.

We verified defaunation efficacy by extracting nematodes (which were presumed would be more resistant to the defaunation procedure than microarthropods) from subsamples of mineral soil. Subsamples from each tree were retained during mesocosm installation, transported on ice to Flagstaff, Arizona, and stored at 4 °C until extraction 10-19 days after mesocosms were filled. Nematodes were extracted from 100 cc soil using a combination of decanting and sieving and modified Baermann trays (“nematode rafts”, see Gibson et al. (2019)) and were collected after 48 and 72 hours. Resulting samples were refrigerated unpreserved and examined for nematodes within one week of extraction. No nematodes were detected in 22 of the 24 samples, but the two remaining samples each contained one nematode. The effects of sieving and defaunation on nitrogen availability were determined from analysis of three replicate samples of unsieved, sieved, and defaunated soil collected from each study site. Ammonium and nitrate were extracted according to Keeney and Nelson (1982) and analyzed via colorimetry (Wendt, 1999).

We used balsa wood (predominantly lignin) and museum board (predominantly cellulose) as standard labile and recalcitrant substrates (Neher et al., 2003) to illuminate the influence of faunal complexity on decomposition processes. Balsa wood (~1 mm thick) and museum board (~1.75 mm thick) disks with a diameter of 2.22 cm were dried at 60 °C, weighed, and sealed in polyester mesh bags with openings of 1 mm. Each bag contained one disk of each type. We buried five decomposition bags in each mesocosm (except sacrificial units) beneath the litter layer. Decomposition bags were also placed at control locations, over which we secured chicken wire to reduce disturbance of the bags.

3.4 Mesocosm monitoring and measurement of response variables

One replicate decomposition bag was collected from within each mesocosm and control in September 2019 (T1; the end of the monsoon season), April 2020 (T2; the end of winter), July 2020 (T3; the end of the dry season), August 2020 (T4; the height of the monsoon season), and September 2020 (T5; one year after the first sampling). All measurements and sampling activities were performed with tools and gloved hands that were sanitized with 70% ethanol between mesocosms. To quantify potential microclimate differences across mesocosm treatments, we measured soil moisture at each of these timepoints using a portable probe (ML3 ThetaProbe with HH2 soil moisture meter; Delta-T Devices, Cambridge, UK), and soil

temperature at the first timepoint. (No temperature differences were detected, so we did not measure temperature at subsequent timepoints.) We also destructively sampled sacrificial mesocosms for nematodes and microarthropods at T1 and T3. Samples for nematode extraction were collected from the uppermost 10 cm of soil (including mineral and O horizons, but not litter) using a 2.54 cm diameter probe, and samples for microarthropod extraction including the top 10 cm of soil and the overlying litter layer were obtained using a 5.08 cm diameter corer. Microarthropod and nematode soil samples were transported on ice to Northern Arizona University and stored at 4°C until processing. Nematodes were extracted as described above and preserved in DESS solution (Yoder et al., 2006), then enumerated under a dissecting microscope at 40X magnification. Microarthropods were extracted using Tullgren funnels with 15 watt bulbs over 7 days. Light intensity was gradually increased over 5 days, then held at maximum brightness for two days. Extracted animals were preserved in 70% ethanol. Microarthropods were counted at 40-50X and categorized as mites, collembolans, or others (all other arthropods); additionally, we noted whether mites in the suborder Brachypylina were present (this is a diverse and often numerically dominant group of oribatids with adult body sizes >200 µm).

We destructively sampled all mesocosms and controls (with the exception of two mesocosms in the thinned/burned management unit which were compromised by mammal activity) at T5 to determine final faunal complexity, fungal community composition, and ammonium, nitrate, and soil organic matter (SOM) content. We used diversity of microarthropods >300 µm as a proxy for soil faunal complexity. This size cutoff is used by the Alberta Biodiversity Monitoring Institute (2009) in their microarthropod inventories, and separates juveniles from identifiable adults for most taxa in the most speciose order of mites, the Oribatida. Microarthropods were extracted and preserved as detailed previously, then were filtered using a 300 µm sieve. Microarthropods captured on this sieve were counted, and mites and collembolans were sorted to morphospecies. Soil samples for SOM, ammonium, and nitrate content determination were collected in envelopes and stored in closed containers with silica beads to air dry in the field, then were sieved to 2 mm prior to analysis. Soil organic matter was measured by loss on ignition of 5 g soil at 450 °C for 24 hours (Bisutti et al., 2004). Soil for fungal community analysis was transported from the field on dry ice and stored at -20 °C upon return to Northern Arizona University. We extracted DNA from these samples using Qiagen DNeasy PowerSoil kits.

Fungal community analysis was performed at the Arizona State University Genomics Core via next generation sequencing of the ITS region using the MiSeq Illumina platform. We used the barcoded primer set ITS1f-ITS2, designed by Smith and Peay (2014), and followed the Earth Microbiome Project protocol (<http://www.earthmicrobiome.org/emp-standard-protocols/>) for library preparation. PCR amplifications for each sample were done in duplicate, then pooled and quantified using Accublock® High sensitivity dsDNA Quantitation Kit (Biotium). A no-template sample was included during the library preparation as a control for extraneous nucleic acid contamination. 200 ng of DNA per sample was pooled and then cleaned using QIA quick PCR purification kit (QIAGEN). The pool was quantified by Illumina library Quantification Kit ABI Prism® (Kapa Biosystems); diluted to a final concentration of 4 nM; then denatured and diluted to a final concentration of 4 pM with a 25% of PhiX. Finally, the DNA library was loaded in the MiSeq Illumina and run using the version 2 module, 2x250 paired-end, following the directions of the manufacturer.

3.4 Statistical analyses

All analyses were performed in R (R Core Team, 2020) unless otherwise noted. Effects of mesh treatment, ponderosa restoration treatment, and mesh x ponderosa restoration treatment combinations were assessed with ANOVA, if assumptions could be met, or Kruskal-Wallis H tests, if they could not be met. Where necessary, response variables were log transformed (after adding 1 to all observations if there were 0 values) to meet normality requirements for parametric analysis. P-values for multiple pairwise comparisons were adjusted using Tukey's HSD for ANOVA or with the Benjamini-Hochberg method for Wilcoxon rank sum tests. Data were visualized with ggplot2 (Wickham, 2011).

Multivariate analyses of faunal communities, and of interactions between fauna and fungi, were executed in PC-ORD version 5.1 (McCune and Mefford., 2006). We used multi-response permutation procedure (MRPP) to test whether mesocosm mesh treatments had produced different faunal communities. This non-parametric test for differences among *a priori* groups yields the statistic *A*, the chance-corrected proportion of between-sample distances explained by group identity. *A* will equal 1 when all samples within groups are internally identical, but groups are distinct, and 0 if heterogeneity within groups is equal to the chance expectation. $A < 0$ indicates more within-group heterogeneity than should be expected to occur by chance, while $A > 0$ if there is more agreement within groups than should occur by chance (i.e., if differences between groups are likely to exist). Mantel tests of correlation between fungal and faunal communities were conducted using the Monte Carlo method with 999 randomized runs. For microarthropod communities, oth MRPP and Mantel tests were performed using Bray-Curtis distance after adding a dummy variable column containing 1 for all rows, since some samples contained no microarthropods > 300 μm . Weighted UniFrac was used as the distance measure for fungal communities.

Analysis of ITS sequence data was accomplished using Qiime2 at the Arizona State University Biodesign Institute's Bioinformatics Core. Briefly, the DADA2 tool was used to merge paired-end fastq files, denoise them, purge chimeras, and infer sample sequences. Group differences were visualized with principal components analysis and tested with PERMANOVA using weighted UniFrac distance, and Faith phylogenetic diversity values were calculated.

We assessed the effects of forest restoration treatment, mesocosm treatment, and soil characteristics (moisture, ammonium, and organic matter) on decomposition using analysis of covariance models. To address non-normality, we applied the logit transformation to the proportion of original mass remaining for both substrates (Warton and Hui, 2011). This transformation requires positive values between 1 and 0 noninclusive. For balsa disks, approximately 14.4% of which gained an average of 2.42% of their original mass despite our cleaning procedure, we removed observations with mass gain >6.7% (outliers which were at least 1.5 times the interquartile range below the 25th percentile of the distribution). These observations represented 3.3% of the 590 balsa disks collected. We then subtracted the next highest value of mass gain (6.5%) from the remaining balsa mass loss observations prior to transformation to constrain proportions between 0 and 1. One outlying museum board disk observation which had gained a significant amount of weight was also discarded, and values for 5 museum board disks which had gained a small amount of mass were changed to 0% mass loss

(these constituted <1% of the data set). Finally, we subtracted 0.025 from each final mass proportion so that all values were below 1.

We fit the linear models *balsa wood mass remaining* ~ *timepoint* + *restoration treatment* and *museum board mass remaining* ~ *timepoint* + *restoration treatment*. We used the timepoint number (1, 2, 3, 4, 5) instead of days in the field for these analyses, as we did not expect daily decomposition rates to be consistent across seasons. If restoration treatment was a significant predictor of mass loss for a substrate, we assessed the advisability of including an interaction between *restoration treatment* and *timepoint* using an F-test. We then compared the preferred model to models additionally including all possible combinations of *average soil moisture Z score* (the average Z score of soil moisture for each mesocosm across five measurement timepoints), *soil organic matter*, *ammonium concentration*, and/or *mesh treatment* using the Akaike information criterion (AIC). Mesocosm controls were excluded because they were not replicated at all trees and were subject to the influence of UV and additional disturbance, which appear to have speeded mass loss; however, selected models run with and without controls yielded broadly similar parameter estimates (**Table S3**). Prior to beginning the selection procedure for each model, we also tested for an interaction between mesh size and timepoint, but the additional explanatory power did not justify inclusion in the model. Predictor variables were checked for collinearity prior to inclusion in candidate models, and soil organic matter and ammonium concentration were log transformed to address non-normality. In the event that models tied for minimum AIC (i.e., AIC values differed by <2), we chose the most parsimonious model that also included soil moisture (which we knew to be confounded with mesh size).

We investigated faunal complexity effects on soil organic matter and ammonium content with linear mixed effects modeling using the packages “lme4” (Bates et al., 2015) and “lmerTest” (Christensen et al., 2017). We did not model nitrate content, because this nutrient was very low in most mesocosms and could not be transformed to meet model assumptions. In addition, we expected ammonium to better reflect mineralization by soil fauna (Chen and Ferris, 1999). We modeled *mesh treatment* (excluding mesocosm controls) and *average soil moisture Z score* as fixed effects, and *tree* as a random effect (since mesocosms in each tree block had identical soil characteristics at the outset of the study). We fit a version of this model for all trees, untreated control trees only, and thinned/burned trees only. We then tested each model for significance against a simpler model omitting mesh treatment with the “anova()” function.

4. Results and Discussion

4.1 Manipulation of soil faunal complexity

4.1.1 Monitoring of sacrificial mesocosms

Monitoring of sacrificial mesocosms at T1 and T3 showed some colonization of fine mesh mesocosms by very small microarthropods but indicated continued efficacy of mesh treatments at maintaining microarthropod community complexity differences (**Table 2**). Importantly, mites in the speciose and abundant oribatid suborder Brachypylina were absent from all but one of the fine mesh mesocosms. Examination of nematodes collected at T1 revealed that 21 µm mesh mesocosms were already contaminated by large nematode taxa, and nematode community

composition did not differ among mesocosm mesh treatments (MRPP based on morphospecies: $A < 0.01$, $P > 0.3$ for pairwise comparisons of 21 μm vs. larger mesh sacrificial mesocosms in untreated control and thinned/burned units). Nematode communities were therefore not compared at subsequent sampling timepoints, and subsequently we focused on microarthropod community differences.

Table 2. Abundances of microarthropods in all size classes and presence of adult higher oribatids (number of units in which Brachypylina occurred/total units sampled) in sacrificial mesocosms sampled in September 2019 (T1) and July 2020 (T3). $N=3$ per restoration treatment unit.

	<i>T1 Microarthropods</i>			<i>T3 Microarthropods</i>		
	Mean mites	Mean collembolans	Presence of Brachypylina	Mean mites	Mean collembolans	Presence of Brachypylina
21 μm	84.3	11.2	0/6	51.2	6.8	0/6
41 μm	225.8	0.5	0/6	220.3	16	1/6
1 mm Sev	239.2	56.2	6/6	139.8	9.2	6/6
1 mm	310.8	79.2	6/6	208.3	8.8	5/6

4.1.2 Final community complexity

Examination of microarthropods $> 300 \mu\text{m}$ at the conclusion of our study confirmed that the mesocosm treatments had successfully manipulated soil faunal complexity, producing more complex microarthropod communities in 1 mm mesh mesocosms and in mesocosm controls than in mesocosms with fine mesh sizes (21 μm and 41 μm). Abundance and species richness of mites $> 300 \mu\text{m}$ was higher in 1 mm mesh mesocosms and controls than in fine mesh mesocosms (**Fig. 4 A and B**), and fine meshes were especially successful at excluding mites in the order Oribatida (**Fig. 4 C**). Densities of other microarthropods (including Protura, minute spiders, termites, and macroarthropod larvae) also tended to be lower in fine mesh mesocosms, although these animals were encountered infrequently in general (**Fig. 4 D**). Mesh treatments were less effective at excluding collembolans, however, and species richness and abundance of these animals did not differ across mesocosm treatments (**Fig. 4 E and F**).

Multi-response permutation procedure (MRPP) analysis of microarthropod species abundances revealed that communities in fine mesh mesocosms were distinct from those of coarse mesh mesocosms and controls (**Table 3**). Of the 43 mite and collembolan taxa observed in the $>300 \mu\text{m}$ fraction (**Table S1**), only one was a strong indicator ($IV > 25$) for any of the mesh treatments: a collembolan in the family Entomobryidae, which had maximum IV values in the 21 μm mesh treatment. We suspect that this species, which has a very large furcula (spring), was especially well equipped to colonize mesocosms through small tears in lids that appeared between maintenance trips, or while lids were temporarily removed for soil moisture monitoring and collection of decomposition disks.

Table 3. Multiresponse permutation procedure (MRPP) results for soil fauna species $>300 \mu\text{m}$, grouped by mesocosm mesh treatment and based on Bray-Curtis distance. Note that mesocosm controls were not replicated at all trees, and p -values are not corrected for multiple comparisons.

All trees

	41 μ m		1 mm Sev		1 mm		Control	
	A	p	A	p	A	p	A	p
21 μ m	0.015	0.098	0.052	0<.01*	0.063	<0.001*	0.064	<0.001*
41 μ m			0.059	<0.001*	0.077	<0.001*	0.061	<0.001*
1 mm Sev					-0.006	0.903	-0.001	0.449
1 mm							0.002	0.273

4.1.3 Non-target treatment effects

Defaunation, but not sieving, increased soil ammonium concentrations immediately after treatment, especially in soil from the untreated control units (**Fig. S1 A**). Nitrate was below detectable levels in all but two defaunated samples from the thinned/burned unit. This pulse in available nitrogen had dissipated by the end of the study, at which time ammonium concentrations were very similar in mesocosm controls with and without defaunated soil (**Fig. S1 B**). No differences in soil temperature between mesocosm mesh treatments were detected at T1, and temperature was not monitored at later sampling points. Mesocosm mesh size and root severing did influence moisture retention, however, with 21 μ m mesh mesocosms retaining the most, and 1 mm unsevered mesocosms the least, soil moisture on average (**Fig. S2**). Median soil moisture differences between 21 μ m and 1 mm unsevered mesocosms ranged from 1.2% at T3 to 6.2% at T5. We accounted for these moisture differences to the best of our ability in our models of decomposition as described below.

4.2 Forest management treatment effects

4.2.1 Effects of thinning/burning on soil fauna

Five years after burning, microarthropod communities differed in the two management units (MRPP: $A=0.081$, $p<0.001$). Both abundance and species richness of mites and collembolans $>300 \mu$ m, as well as abundance of other microarthropods in this size class, were reduced in thinned/burned mesocosms and controls relative to those in the untreated control management unit (**Fig. 5**). Oribatids, which comprised approximately two thirds of examined microarthropods in 1 mm mesocosms and in mesocosm controls, appeared more sensitive to thinning and burning than mites as a whole (**Fig. 5 C**). This finding supports those of Camann et al. (2012), who found evidence of continuing oribatid decline two years after prescribed fire in ponderosa pine-dominated stands in the California Cascade Range. Four microarthropod species were strong indicators for the untreated management unit ($IV>25$), but none appeared to prefer mesocosms in the thinned/burned unit (**Table S1**).

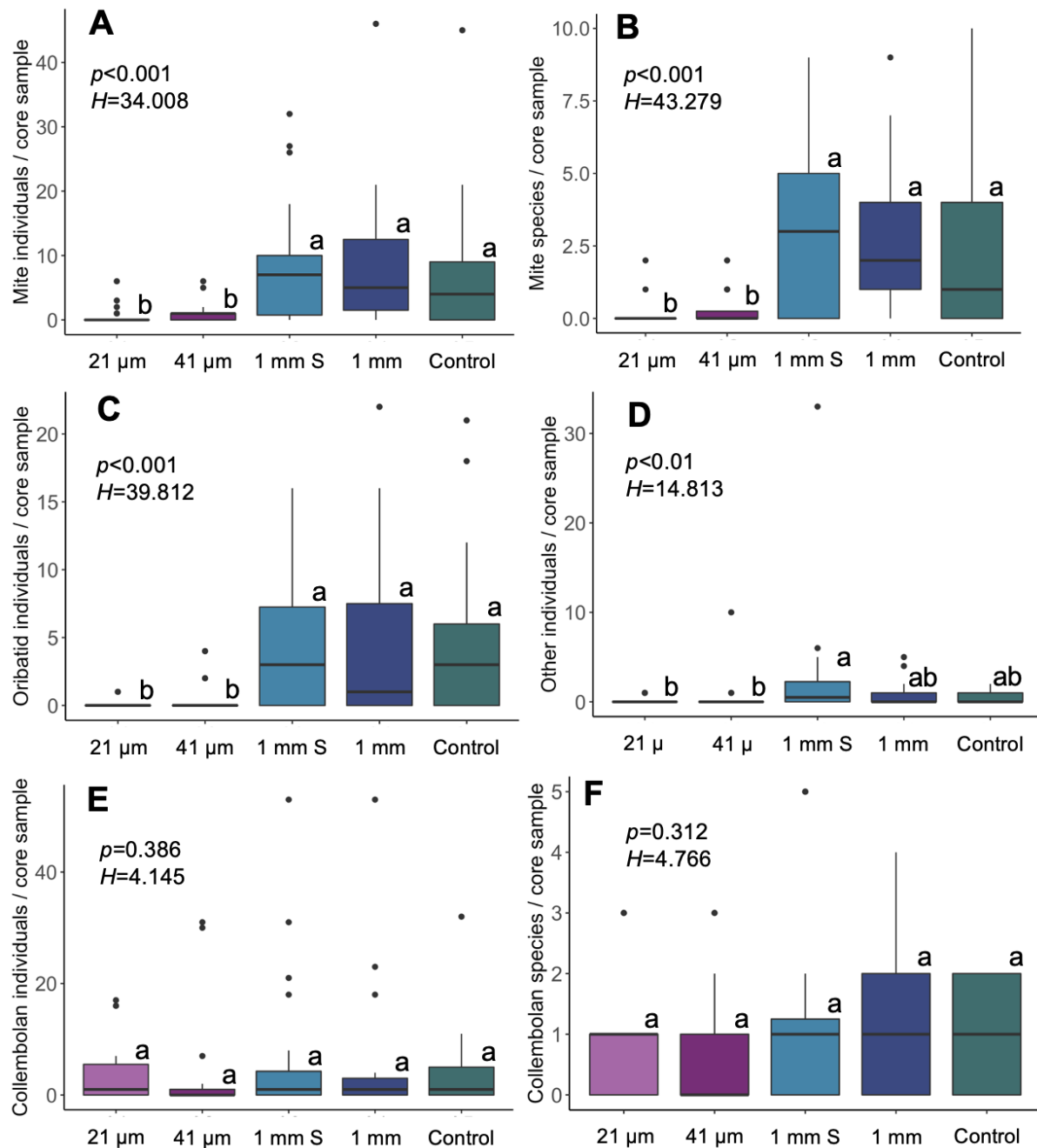


Fig. 4. Abundance and species richness of soil fauna >300 µm extracted from combined soil/litter core samples, grouped by mesocosm mesh treatment: 21 µm, 41 µm, 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe. **(A)** Abundance and **(B)** species richness of mites; **(C)** abundance of oribatids; **(D)** abundance of other microarthropods not belonging to Acari or Collembola; **(E)** abundance and **(F)** species richness of collembolans. Boxes with different letters are significantly different at $\alpha < 0.05$ according to Wilcoxon rank sum tests, after application of the Benjamini-Hochberg correction for multiple comparisons. Model P -values were calculated from Kruskal-Wallis H -tests.

4.2.2 Effects of thinning/burning on soil fungi

Fungal communities diverged in mesocosms in the thinned/burned and untreated control management units (**Fig. 6**; PERMANOVA based on weighted UniFrac distance: pseudo- F : 7.579, $p=0.001$). Sequences belonging to the fungi in the phylum Basidiomycota were observed more frequently in mesocosms within the untreated control unit, while subphylum Glomeromycotina sequences were more abundant in the thinned/burned unit and phylum Ascomycota sequences were similarly common in both management units (**Fig. S3**).

Glomeromycotina comprises arbuscular mycorrhizal fungi (AMF) which are obligate symbionts of graminoids and many forbs. Host species for AMF typically increase following restoration treatments in ponderosa pine forests (indeed, promoting herbaceous understory vegetation is often an explicit goal of these restoration treatments), and positive responses of AMF to thinning and prescribed burning of ponderosa forests have been documented by others (Korb et al., 2003). The Basidiomycota and Ascomycota include both saprotrophic and ectomycorrhizal taxa. Ascomycota in ponderosa pine forests may be more resilient to fire effects than Basidiomycota, as Reazin et al. (2016) also reported rapid replacement of dominant Basidiomycota by Ascomycota after burning small-scale experimental plots in this forest type.

In contrast to our findings regarding restoration treatment effects on diversity of microarthropod communities, the thinned/burned management unit hosted more diverse fungal communities as quantified by Faith's phylogenetic diversity index (**Fig. S4**), a measure of the total branch length of the phylogenetic tree formed by operational taxonomic units in a sample (Faith, 1992). (Note, however, that this is a fundamentally different measure of diversity than that used to compare microarthropod communities.) Increased phylogenetic diversity in the thinned/burned mesocosms may be the product of an interaction between mesocosm artifacts and restoration treatments, because mesocosm controls in the thinned/burned and untreated units did not differ. On the other hand, mesocosm controls were replicated at only three trees per management unit, so our power to detect differences in diversity between the management treatments was reduced for these experimental units.

4.2.3 Effects of thinning/burning on soil properties and ecological functions

Soil organic matter was reduced (**Fig. S5 A**), and soil ammonium tended to be lower (**Fig. S6 A**), in mesocosms within the thinned/burned unit than in those within the untreated control unit. Median nitrate concentration was an order of magnitude lower than ammonium concentration and did not differ between restoration treatment units ($W=1970$, $p=0.156$). Soil moisture was reduced by thinning and burning ($F=4.678$, $p=0.032$). Parameter estimates for selected models explaining decomposition of balsa wood and museum board substrates are shown in **Table 4** and **Table 5**. Decomposition of both balsa wood and museum board was lower on average in the thinned/burned management unit than in the untreated control management unit (significant intercept offset), but interactions between restoration treatment and timepoint were not significant and were not included in the models.

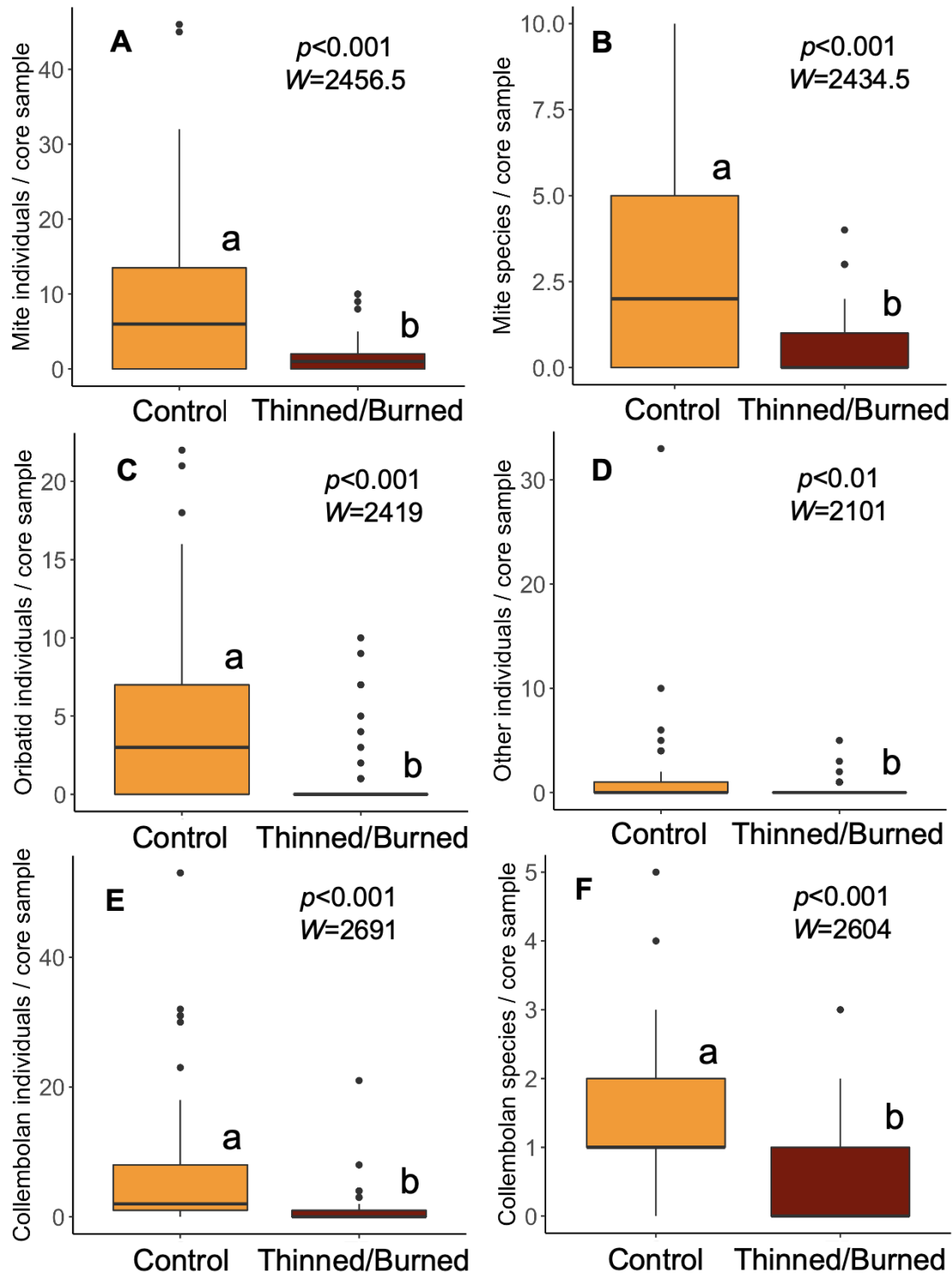


Fig. 5. Abundance and species richness of microarthropods in combined soil and litter core samples from mesocosms and mesocosm controls in the thinned/burned management unit and the untreated control management unit. (A) Species richness and (B) abundance of collembolans. (C) Species richness and (D) abundance of mites. (E) Abundance of mites in the order Oribatida. (F) Abundance of other microarthropods apart from mites and collembolans. P -values and test statistics are from Wilcoxon rank-sum tests.

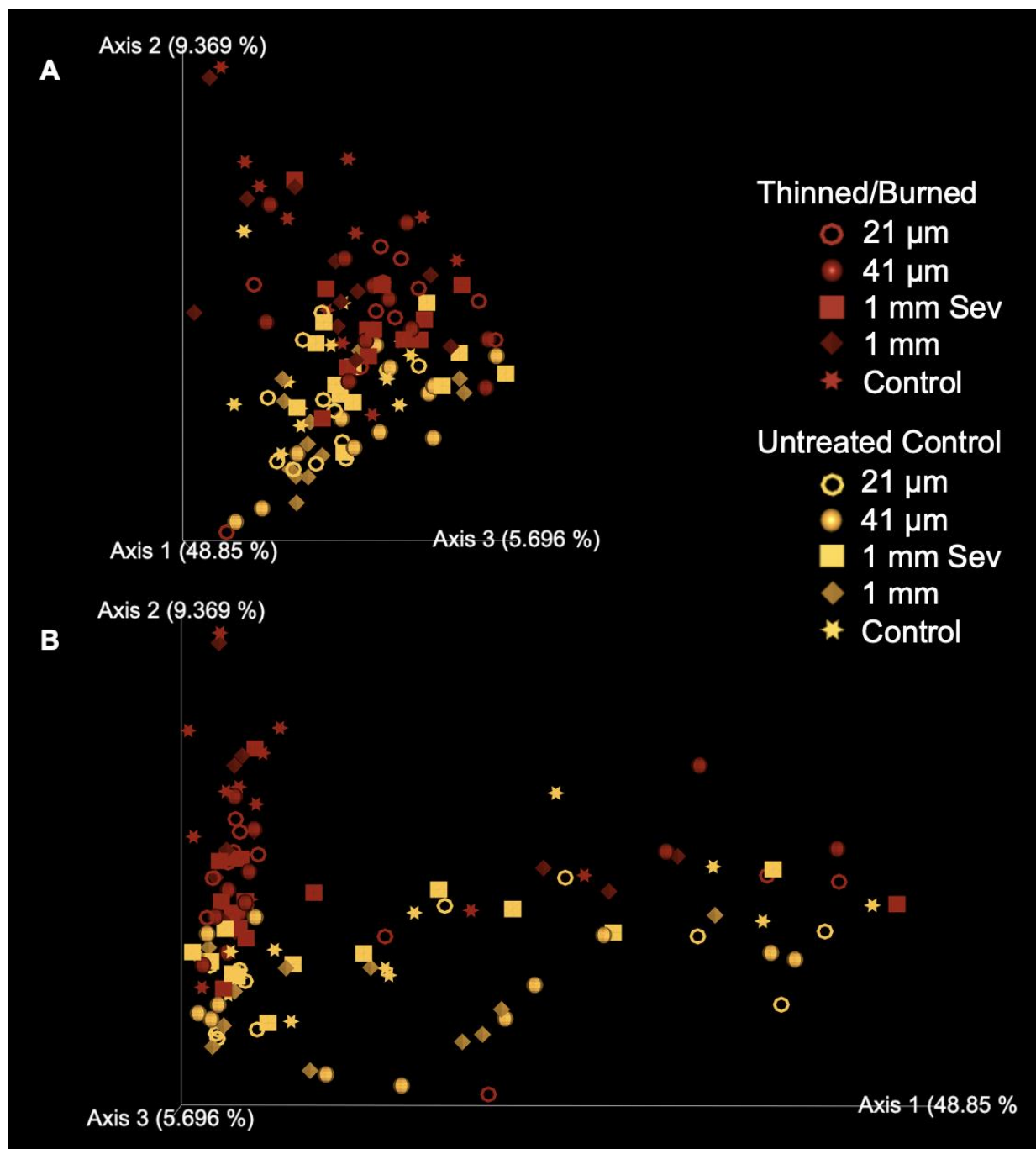


Fig. 6. Three dimensional principal component analysis (PCA) ordination of fungal communities based on weighted UniFrac distance, visualized with Emperor Qiime2View. Colors correspond to ponderosa restoration treatment, and shapes designate mesh treatment: 21 µm, 41 µm, 1 mm with root severing (1mm Sev), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe. **(A)** Variation along PCA axes 2 and 3. **(B)** Variation along PCA axes 1 and 2.

In addition to warmer and drier conditions, altered microbial community composition and decreased fungal biomass (we observed more hyphae within the untreated control unit when harvesting balsa and museum board disks in the field) may have contributed to slower mass loss

in the thinned/burned unit. Others comparing decomposition in thinned and/or burned and untreated second growth ponderosa pine forests have observed conflicting patterns, from increased mass loss of a lignin-rich standard substrate in burned and thinned/burned plots (Gundale et al., 2005; up to two years post-fire) to slightly faster decomposition of ponderosa pine needles in untreated units (Monleon and Cromack Jnr, 1996; along a chronosequence ranging from months to 12 years post-fire). Higher N availability immediately after fire may ephemerally enhance decomposition (Gundale et al., 2005), especially of more recalcitrant substrates, but mineral nitrogen was no longer elevated in the soils of our thinned/burned mesocosms 5 years after fire. The artificiality of our mesocosms does limit our inferences regarding effects of thinning and burning on decomposition at the stand level, however.

Restoration treatment unit was the strongest predictor of decomposition across all trees for balsa wood, but for museum board, the model indicated a strong positive effect of soil moisture and a strong negative effect of soil organic matter in addition to the effect of restoration treatment. Increased soil moisture enhances activity of saprotrophs where moisture is limiting, as it is in xeric forest types. We are uncertain what mechanism may underlie the apparent retardation of cellulose decomposition with increasing soil organic matter. This pattern seemed to be driven by trees in the thinned/burned management unit, and soil organic matter was not selected as an important covariate in the cellulose decomposition model for the untreated control unit alone. One possibility is that locations with more soil organic matter were less impacted by fire and hosted more ectomycorrhizal fungi relative to strictly saprotrophic fungi. If so, the Gadgil effect could be at play, where decomposition rates are suppressed by competition of ectomycorrhizal with saprotrophic fungi.

4.3 Faunal complexity effects on fungi and ecosystem functions

Mantel tests based on weighted UniFrac distance revealed that fungal and faunal communities were correlated across all trees ($r=-0.087$, $p=0.019$) and within the thinned/burn unit alone ($r=-0.188$, $p=0.009$) but not within the untreated control management unit alone ($r=-0.033$, $p=0.208$). However, fungal community composition did not differ according to mesh treatments at either the stand level (PERMANOVA based on weighted UniFrac distance: pseudo- F : 0.9578, $p=0.448$) or management treatment level ($p>0.10$ for all pairwise comparisons among mesh treatments within thinned/burned and control stands, respectively) (**Fig. 6**). Although phylogenetic diversity did vary by mesh treatment (**Fig. S4 B** and **Fig. S4 C**), these differences did not accord with measured variation in the complexity of faunal communities among the mesh treatments. Together, these findings suggest that faunal community complexity is not a strong force regulating fungal communities in this system, and that instead fauna simply respond to fungal community characteristics, or that common factors drive fungal and faunal community differences, or both.

Table 4. Results from selected analysis of covariance models for decomposition of balsa wood disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only. Note that unsevered 1 mm mesh mesocosms are the reference group for models including mesh size.

Balsa wood (predominantly lignin)
logit (proportion mass remaining)

<i>All trees</i>				
	Estimate	Std. error	t value	Probability < t
Intercept	2.09179	0.06050	34.575	< 2e-16***
Timepoint	-0.08587	0.01357	-6.330	5.97e-10***
Restoration treatment: TB	0.14734	0.03978	3.704	0.000239***
1 mm mesh severed	0.08061	0.05679	1.419	0.156514
41 µm mesh	-0.02786	0.06234	-0.447	0.125520
21 µm mesh	-0.10027	0.06533	-1.535	0.655147
Soil moisture (average Z score)	-0.02518	0.03148	-0.800	0.424267
	Residual standard error			0.4105
	Adjusted R²			0.1254
	F (d.f.=)			11.83 (6, 447)
	Model P			2.519e-12***
<i>Untreated control trees only</i>				
	Estimate	Std. error	t value	Probability < t
Intercept	2.09912	0.06791	30.908	< 2e-16 ***
Timepoint	-0.08853	0.02050	-4.319	2.34e-05 ***
Soil moisture (average Z score)	-0.07205	0.03230	-2.231	0.0266 *
	Residual standard error			0.442
	Adjusted R²			0.08487
	F (d.f.=)			11.76 (2, 230)
	Model P			1.375e-05***
<i>Thinned/burned trees only</i>				
	Estimate	Std. error	t value	Probability < t
Intercept	2.31056	0.07785	29.680	< 2e-16***
Timepoint	-0.08329	0.01717	-4.850	2.36e-06***
1 mm mesh severed	0.08373	0.07212	1.161	0.24693
41 µm mesh	-0.15799	0.08413	-1.878	0.06175 .
21 µm mesh	-0.25929	0.07911	-3.278	0.00122**
Soil moisture (average Z score)	0.08791	0.05441	1.616	0.10761
	Residual standard error			0.3646
	Adjusted R²			0.1648
	F (d.f.=)			9.681 (5, 215)
	Model P			2.367e-08***

Table 5. Results from selected analysis of covariance models for decomposition of museum board disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only.

Museum board (predominantly cellulose)
logit (proportion mass remaining)

<i>All trees</i>				
	Estimate	Std. error	t value	Probability < t
Intercept	1.14731	0.29954	3.830	0.000146 ***
Timepoint	-0.44262	0.03482	-12.712	< 2e-16 ***
Restoration treatment: TB	0.22057	0.10416	2.118	0.034747 *

Soil moisture (<i>average Z score</i>)	-0.37166	0.06604	-5.628	3.17e-08 ***
log % Soil organic matter	0.49691	0.15530	3.200	0.001470 **
Residual standard error				1.062
Adjusted R²				0.3148
F (d.f.=)				54.53 (4, 462)
Model P				< 2.2e-16

Untreated control trees only

	Estimate	Std. error	t value	Probability < t
Intercept	2.10192	0.16810	12.504	< 2e-16 ***
Timepoint	-0.46827	0.05048	9.276	< 2e-16 ***
Soil moisture (<i>average Z score</i>)	-0.43692	0.07841	-5.572	6.84e-08 ***
Residual standard error				1.101
Adjusted R²				0.3246
F (d.f.=)				58.2 (2, 236)
Model P				< 2.2e-16***

Thinned/burned trees only

	Estimate	Std. error	t value	Probability < t
Intercept	0.21269	0.42094	0.505	0.613866
Timepoint	-0.41536	0.04656	-8.920	< 2e-16 ***
1mm mesh severed	0.21460	0.19390	1.107	0.269621
41 µm mesh	0.34738	0.22667	1.533	0.126820
21 µm mesh	0.68006	0.21434	3.173	0.001725 **
Soil moisture (<i>average Z score</i>)	-0.49196	0.14702	-3.346	0.000963 ***
log % Soil organic matter	0.97593	0.23223	4.202	3.83e-05 ***
Residual standard error				0.9931
Adjusted R²				0.3266
F (d.f.=)				19.35 (6, 221)
Model P				< 2.2e-16***

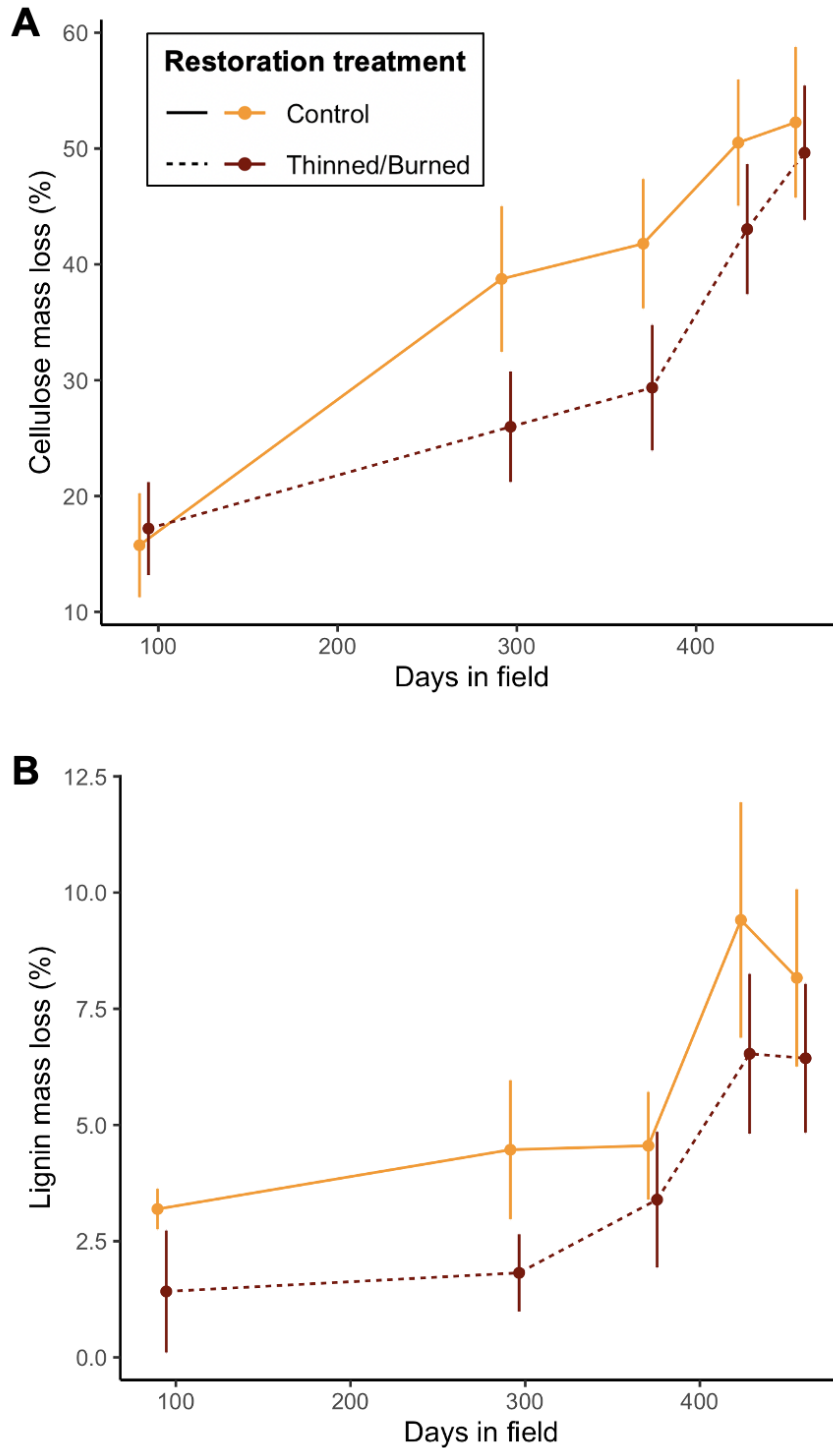


Fig. 7. Decomposition of recalcitrant and labile standard substrates in thinned/burned and untreated control ponderosa pine forest management units. **(A)** Mass loss of museum board (predominantly cellulose) over time. **(B)** Mass loss of balsa wood (predominantly lignin) over time. Error bars represent 95% confidence intervals.

The importance of faunal complexity for decomposition varied between untreated control and thinned/burned forest management units (**Table 4**, **Table 5**, **Fig. 8**, **Fig. 9**). Faunal complexity

does not appear to have affected decomposition of either the lignin-rich or the cellulose-rich substrates in the untreated control unit: our model selection procedure indicated that mesh treatment differences in decomposition were better explained by soil moisture within this forest management unit. Mesh size did emerge as an important predictor of decomposition of both substrates in the thinned/burned unit, however. For both balsa wood and museum board decomposition in this forest management unit, only the 21 μm mesh treatment had a significant effect on mass loss. Compared to 1 mm mesh mesocosms, the 21 μm mesh treatments increased remaining museum board by an estimated 15.6% of the initial mass, and decreased remaining balsa wood by an estimated 2.36% of the initial mass. The 41 μm mesh treatment produced weaker effects of the same directionality as the 21 μm mesh. Faunal complexity in the 21 μm and 41 μm mesh treatments did not differ according to our assessment of microarthropods $>300\ \mu\text{m}$, but data from the sacrificial mesocosms suggests that the 41 μm mesh mesocosms may have hosted considerably more small microarthropods than did the 21 μm mesh mesocosms.

We also saw no evidence that faunal complexity influenced mineral ammonium content (**Fig. S6 C**) or soil organic matter (**Fig. S5 C**): mesh treatment was not a significant predictor of either of these soil characteristics in mixed models accounting for between-tree variation ($p>0.3$ for all null model comparisons). We expected that nematodes (and especially bacterivorous nematodes) would influence nitrogen mineralization more strongly than microarthropods (Neher et al., 2012), and our mesocosms do not appear to have directly manipulated nematode community complexity. We would not expect to see an influence of faunal complexity on soil organic matter in the absence of effects on fungal communities, nitrogen mineralization, or decomposition—at least not over the timespan of this study. Given that we observed faunal complexity effects on decomposition only in the thinned/burned unit, where relatively little organic material was available for saprotrophs to break down, more sensitive analyses may have been required to detect changes in soil organic matter there. On the other hand, Grandy et al. (2016) argue that faunal effects on decomposition may not necessarily translate to effects on soil organic matter dynamics.

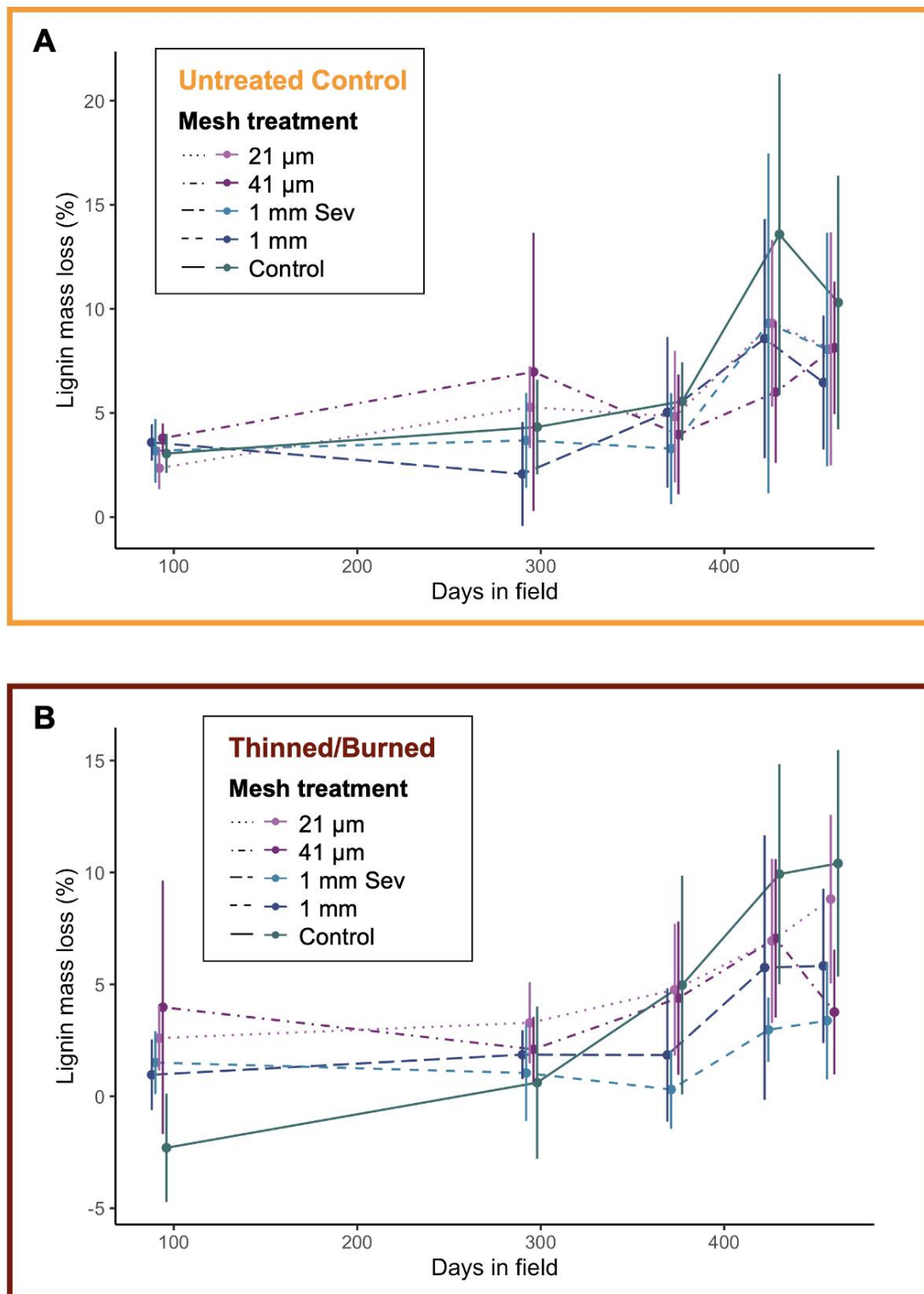


Fig. 8. Decomposition of balsa wood (predominantly lignin) in (A) untreated control and (B) thinned/burned ponderosa pine forest management units. Error bars represent 95% confidence intervals.

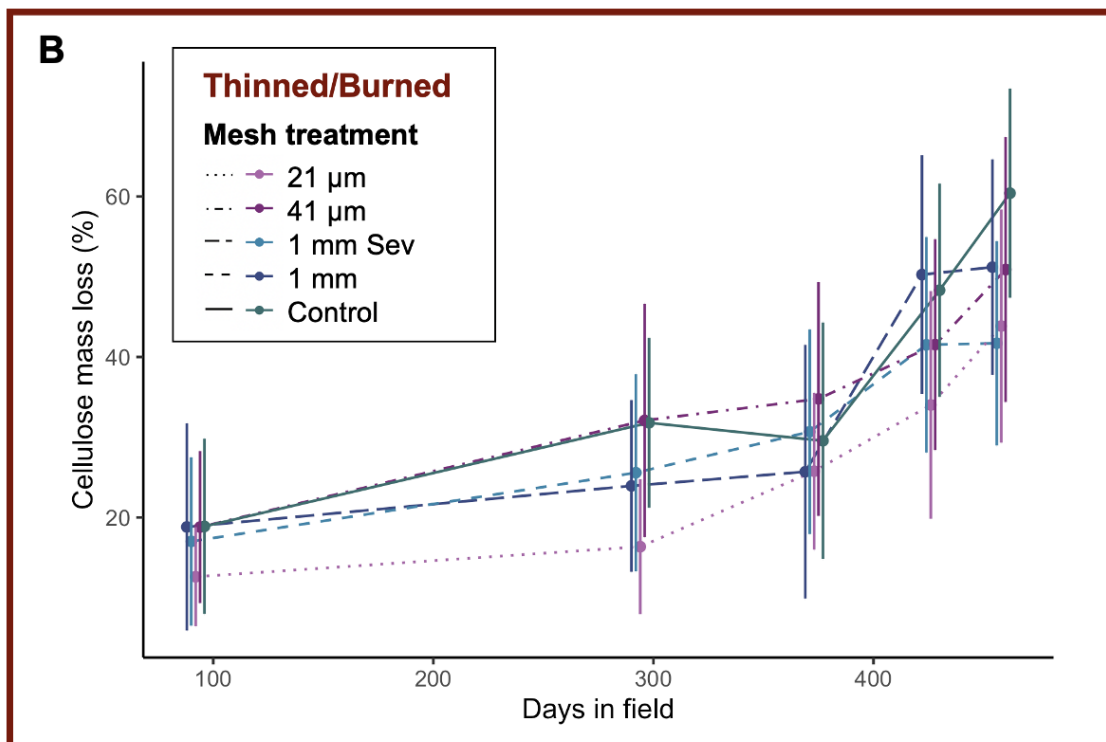
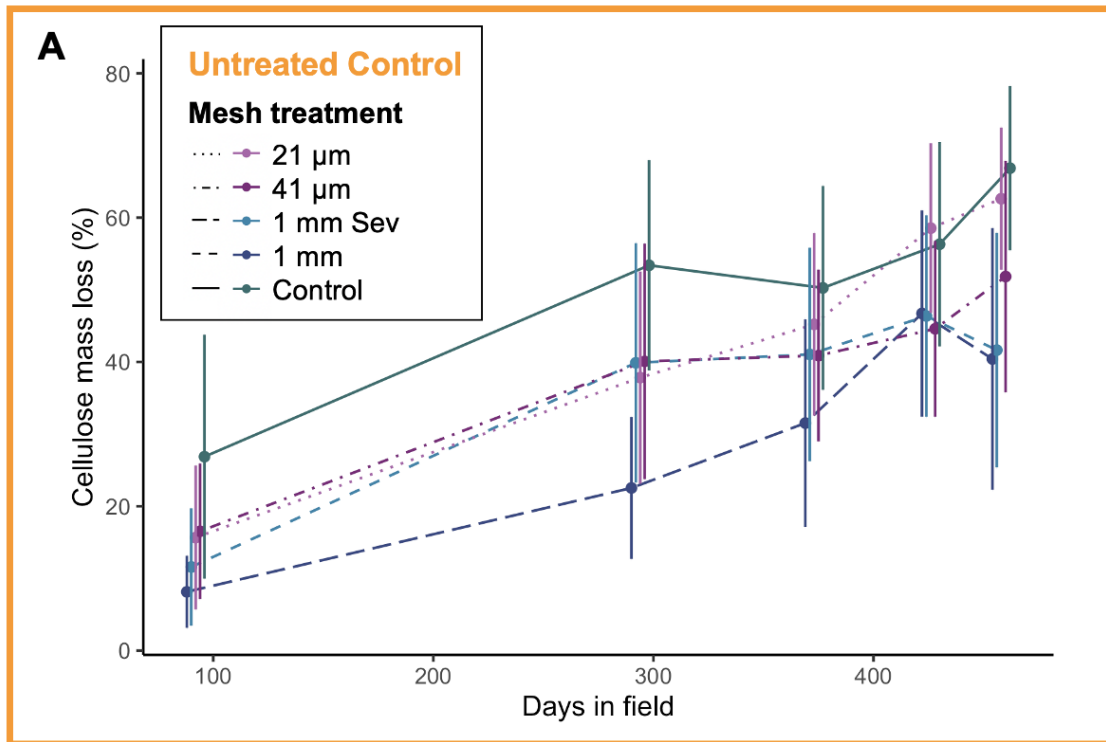


Fig. 9. Decomposition of balsa wood (predominantly lignin) in **(A)** untreated control and **(B)** thinned/burned ponderosa pine forest management units. Error bars represent 95% confidence intervals.

Table 6. Evidence for hypotheses regarding the effects of increasing soil faunal complexity (more microarthropod species and individuals) on ecological functions in untreated and thinned/burned ponderosa pine management units.

<i>More complex faunal assemblages will:</i>	
Untreated Control	Thinned/Burned
<u>Inhibit decomposition of recalcitrant substrate (lignin)</u> Effect expected to be stronger <i>Not supported:</i> no effect of mesh treatment on mass loss of balsa wood in untreated control unit after accounting for variation in soil moisture	<u>Inhibit decomposition of recalcitrant substrate (lignin)</u> Effect expected to be weaker <i>Partially supported:</i> mass loss was slower in coarse than in fine mesh mesocosms in thinned/burned unit
<u>Increase decomposition of labile substrate (cellulose)</u> Effect expected to be stronger <i>Not supported:</i> no effect of mesh treatment on mass loss of museum board after accounting for variation in soil moisture	<u>Increase decomposition of labile substrate (cellulose)</u> Effect expected to be weaker <i>Partially supported:</i> mass loss was faster in mesocosms with more faunal complexity
<u>Decrease nitrogen availability</u> <i>Not supported:</i> no effect of mesh size on soil ammonium content	<u>Not affect nitrogen availability</u> <i>Supported:</i> no effect of mesh size on soil ammonium content
<u>Affect fungal communities</u> <i>Not supported:</i> no effect of mesh size on fungal communities, and faunal and fungal communities were not correlated	<u>Affect fungal communities</u> <i>Not supported:</i> although fungal and faunal communities were correlated, mesh size was not a significant predictor of fungal communities

4.4 Methods development

In a meta-analysis of 40 years of litterbag experiments testing microarthropod effects on decomposition, Kampichler and Bruckner (2009) made the disconcerting observation that none of the 101 experiments surveyed had taken potential side effects of faunal exclusion treatments into account. Noting that the mean effect size estimate from studies employing insecticide treatments was approximately twice that of those using mesh size differences, and that applying a correction factor for mesh size side effects could change the sign of microarthropod effects, they concluded that the real contribution of microarthropods to decomposition could not be assessed. A later meta-analysis (García-Palacios et al., 2013) of faunal exclusion litterbag studies downplayed the importance of potential treatment side effects (which researchers had evidently continued to ignore), noting that exclusion method (mesh or insecticide) and, for mesh size-based exclusion experiments, mesh size choices did not alter the finding that soil fauna increased

decomposition. However, this meta-analysis did not distinguish between studies testing the effects of mesofauna from those examining macrofauna. (In fact, many of the faunal exclusion treatments included in this broader meta-analysis used fine mesh sizes that would have included all microarthropods.) This discrimination is important, because these faunal size classes differ in their predominant functional roles. To our knowledge, no relevant meta-analyses have been published since, but it appears that these issues remain largely unaccounted for in studies concerning mesofauna effects on decomposition. Minimizing and controlling for side effects in manipulative studies of faunal functions is especially important when working in high elevation xeric forests, where water is a limiting resource and UV radiation is strong.

Our study endeavored to address the most likely side effects resulting from manipulation of faunal complexity by mesh size: 1) altered infiltration, leaching, and UV penetration (by using the same mesh size for lids and bottoms); 2) relatedly, differences in microclimate between substrates with and without focal fauna (by repeatedly measuring soil moisture); and 3) the increased loss of litter fragments through coarse mesh (by controlling the presence or absence of fauna in an entire soil/litter system, not just on the substrate). Our novel mesocosm design enabled us to perform repeated measurements within mesocosms while maintaining differences in faunal complexity over two growing seasons. Although side effects were not eliminated, they were quantified, and we are confident that they were lower than would have occurred in this system with use of other faunal exclusion mesocosm designs (e.g. Vedder et al., 1996). Side-effects could be further reduced if these mesocosms were installed in locations that allowed frequent servicing, in which case any measured soil moisture differences could be equalized through water addition or rain-out shelters. Moisture differences may be negligible, however, if these mesocosms are used in a more mesic forest type. We also note that our mesocosms are compatible with LI-COR chambers, facilitating measurement of gas fluxes. We hope that development of this mesocosm method will invite future field manipulations of soil faunal complexity.

4.4 Planned and completed science delivery activities

Some of our science delivery activities were delayed due to the COVID-19 pandemic.

1. Presentation at CFLRP All Hands Meeting (cancelled in 2020 and 2021, will present at next meeting in 2022)
2. Presentation at relevant forest management conference (delayed)
3. JFSP fact sheet
4. Dissertation chapter (forthcoming)
5. Peer-reviewed publication (forthcoming)

5. Conclusions, Management Implications, and Future Research

We successfully manipulated faunal complexity within thinned/burned and untreated control ponderosa pine management units over two growing seasons using mesocosms of novel design. These mesocosms were constructed to minimize side effects of manipulation while permitting repeated sampling and measurement of internal conditions. We are hopeful that the method we developed can aid in resolving relationships between soil faunal communities and ecological functions across forest types and management treatments. Field data on these relationships that

can be causally dissected are scarce, but critical for informing soil biogeochemical models (Grandy et al., 2016).

Our study revealed persistent effects of thinning and burning on microarthropod assemblages five years after treatment, but did not indicate significant ramifications for several soil ecological functions known to be influenced by faunal communities: regulation of fungal communities, nitrogen mineralization, soil organic matter formation, and decomposition (Kanters et al., 2015; Soong and Nielsen, 2016; Verhoef and Brussaard, 1990). The sole exception was decomposition, which appears to have been increased by faunal complexity for a labile substrate (cellulose) and decreased by faunal complexity for a recalcitrant substrate (lignin)—but only in the thinned/burned unit. These differences in decomposition were only marked when comparing the simplest communities (21 μm mesh mesocosms) to the most complex communities (1 mm mesh mesocosms). We can think of two possible explanations for this:

1. Paradoxically, faunal complexity may be more important for decomposition after restoration treatments (despite simpler microarthropod communities) than in untreated stands (where microarthropod diversity and abundance are higher) because the biotic and abiotic context of faunal activities matters, and that context changes with restoration treatments. For example, in thinned/burned forests the ratio of faunal to microbial biomass could be higher, strengthening grazing effects; the ratio of saprotrophic to ectomycorrhizal fungi could be higher, enabling fauna to intensify the Gadgil effect (Fernandez and Kennedy, 2016); and spatial and temporal connectivity of resources could be lower, increasing the importance of micro-scale propagule dispersal and nutrient translocation by microarthropods which “wake up” soil microbes (Briones, 2018).
2. An extreme reduction in microarthropod densities, not merely a simplification of faunal communities, must occur in this system before decomposition is significantly impacted. If this is the case, the effects of thinning and burning on faunal mediation of decomposition may be negligible. Although we did not enumerate microarthropods < 300 μm in all mesocosms at the end of the study, data collected earlier from a subset of sacrificial mesocosms indicate that 21 μm mesh mesocosms in the untreated control unit had total mite densities similar to those of the 1 mm mesh mesocosms in the thinned/burned unit (**Fig. S8 A**). In other words, the most effective “microarthropod exclusion” mesh treatment in the untreated control unit achieved total microarthropod densities similar to the “microarthropod inclusion” mesh treatment in the thinned/burned unit.

Our results must be interpreted in the context of historically anomalous climate behavior in the region. While our mesocosms were deployed, the area received only an estimated ~60% of its average precipitation over that timespan, and only ~50% of its average precipitation during the growing season (PRISM Climate Group, 2021). In an already xeric ecosystem type, these precipitation deficits likely had profound implications for microarthropod communities and their performance of ecological functions. However, increasing aridity is expected in the region with climate change, so our findings may be more reflective of future than of past conditions. We also note that our fungal community data represent a single snapshot in time, and are derived from minute subsamples of soil, while our mineral nitrogen and soil organic matter analyses were probably only capable of detecting relatively large effects of faunal complexity. It is also critical to remember that the reduced complexity of faunal communities in the thinned/burned unit may or may not be representative of pre-fire-exclusion conditions, because impacts of thinning and

prescribed fire are likely more extreme than those of historic low-intensity burns (e.g. due to disturbance from logging machinery and increased heat transfer to soil from higher fuel loads). On the other hand, the untreated control unit in our study was undeniably a very unnatural ecological stage for microarthropod communities. It is thus difficult to gauge the “desirability” of these changes from a management perspective.

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GRIN Faunal Complexity Experiment Supplemental Tables and Fig.s

Table S1. Mite and collembolan species encountered in the > 300 µm size fraction and results of indicator species analyses for management treatment groups (untreated control (UC) vs. thinned/burned (TB)) and mesh treatment groups. Statistics are listed only for strong indicators for the specified group with IV>25 (Dufrene and Legendre, 1997).

	Management Treatment			Mesh Treatment		
	IV	<i>p</i>	Max Group	IV	<i>p</i>	Max Group
Collembola						
Hypogastruridae sp. 1						
Hypogastruridae sp. 2						
Onychiuridae sp.						
Tullbergiidae sp.						
Isotomidae sp.	33.6	<0.001	UC			
Entomobryidae sp.	43.8	<0.001	UC	25.6	0.0134	21 µm
Entomobryomorpha sp. 1						
Entomobryomorpha sp. 2						
Entomobryomorpha sp. 3						
Entomobryomorpha sp. 4						
Acari						
Euphthiracaroida sp.						
Trhypochthoniidae sp.						
<i>Nothrus</i> sp.						
<i>Eremaeus</i> cf. <i>boreomontanus</i>	38.4	<0.001	UC			
<i>Odontodamaeus</i> sp.	28.8	<0.001	UC			
<i>Propelops</i> cf. <i>canadensis</i>						
Damaeidae sp.						
Oribatida sp. 1						
Oribatida sp. 2						
Oribatida sp. 3						
Oribatida sp. 4						
Oribatida sp. 5						
Gymnodamaeidae sp. 1						
Eupodidae sp.						
Cunaxidae sp. 1						
Cunaxidae sp. 2						
Cunaxidae sp. 3						

Cunaxidae sp. 4
Spinibdella sp.
Biscirus sp. (?)
Bdella cf. *muscorum*
Bdelloidea sp. 1
Prostigmata sp. 1
Prostigmata sp. 2
Prostigmata sp. 3
Prostigmata sp. 4
Prostigmata sp. 5
Prostigmata sp. 6
Prostigmata sp. 7
Prostigmata sp. 8
Prostigmata sp. 9
Mesostigmata sp. 1
Mesostigmata sp. 2

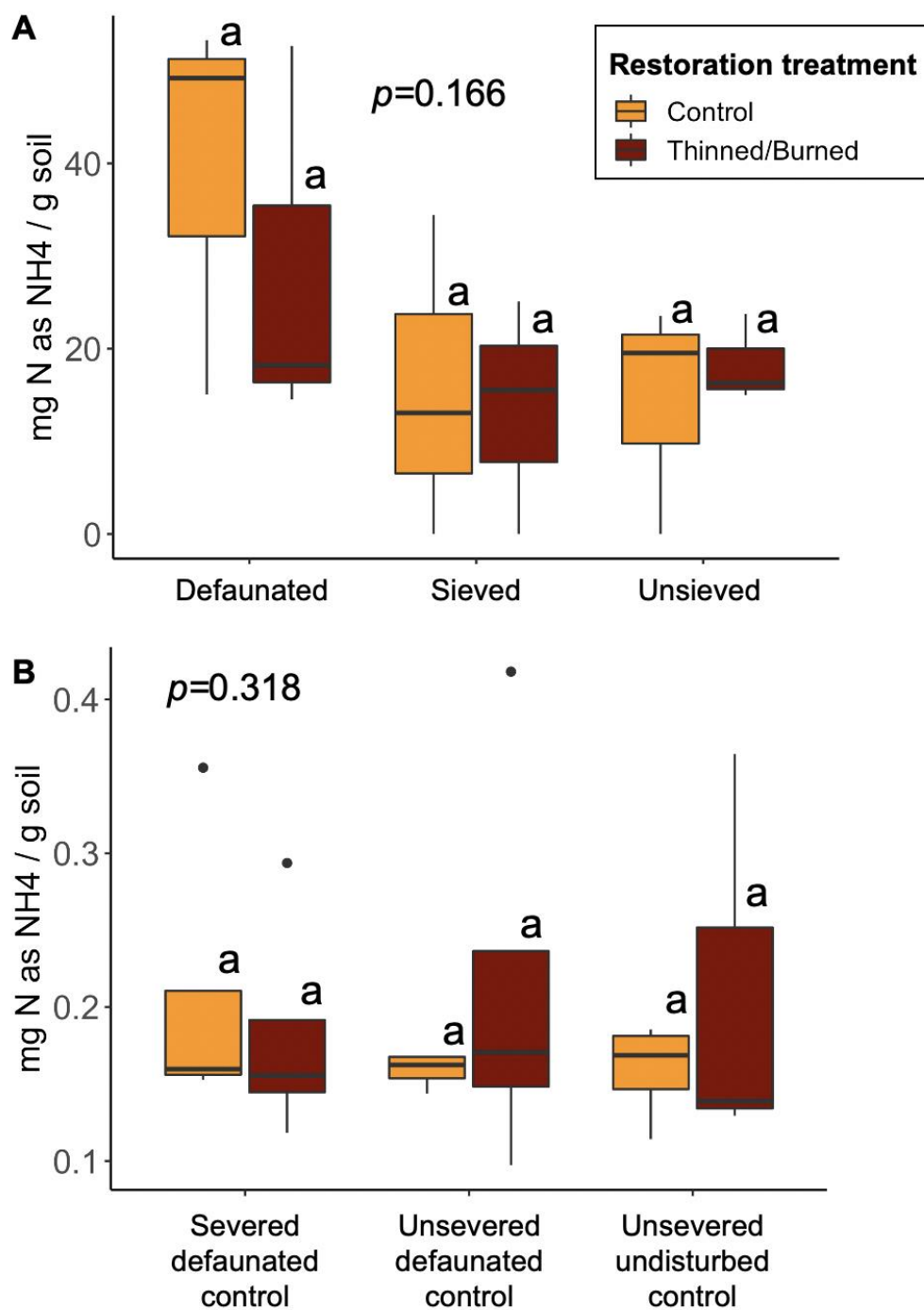


Fig. S1. Effects of soil defaunation and sieving treatments on ammonium content. **A.** Ammonium content of defaunated (sieved and heated wet), sieved only, and unsieved (and unheated) soil collected from three trees per forest management unit prior to installation of mesocosms. Model p -value was calculated by Kruskal-Wallis H test. **B.** Ammonium content of soil in the three types of mesocosm controls at the end of the study. Model P -value was calculated by ANOVA on log-transformed values.

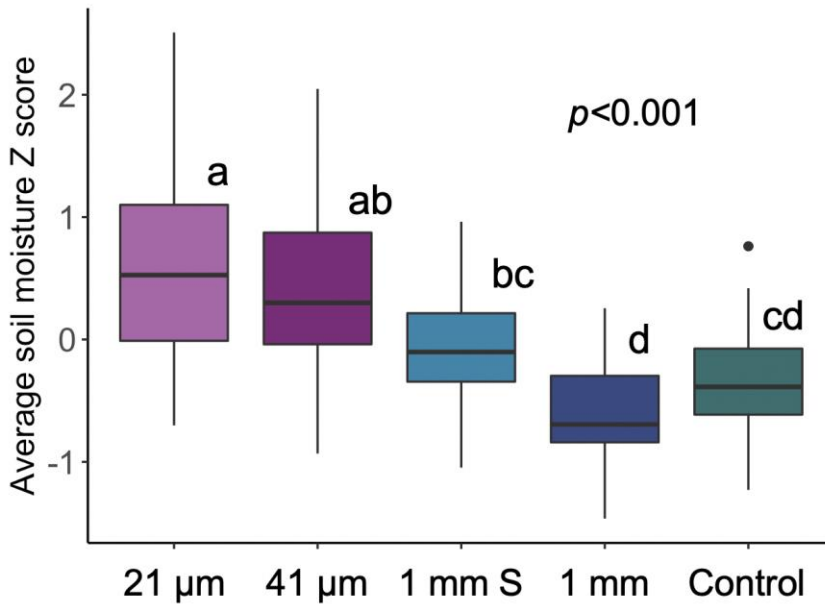


Fig. S2. Average soil moisture Z-scores for mesocosm mesh treatments (21 µm, 41 µm, 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe) across five monitoring timepoints. ANOVA: $F=14.52$.

Table S2. Multi-response permutation procedure (MRPP) results for soil fauna species >300 µm within untreated control and thinned/burned management units, grouped by mesocosm mesh treatment and based on Bray-Curtis distance. Note that mesocosm controls were not replicated at all trees, and p -values are not corrected for multiple comparisons.

Untreated control only								
	41 µm		1 mm Sev		1 mm		Control	
	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>
21 µm	0.034	0.065	0.117	$<0.001^*$	0.085	$<0.001^*$	0.165	$<0.001^*$
41 µm			0.086	$<0.01^*$	0.070	$<0.01^*$	0.155	$<0.001^*$
1 mm Sev					-	0.566	0.030	0.011*
					0.003			
1 mm							0.015	0.091
Thinned/burned only								
	41 µm		1 mm Sev		1 mm		Control	
	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>	<i>A</i>	<i>p</i>
21 µm	-	0.535	0.052	0.048*	0.101	$<0.01^*$	0.032	0.071
	0.009							
41 µm			0.054	0.034*	0.104	$<0.01^*$	0.016	0.147
1 mm Sev					-	0.787	0.010	0.222
					0.014			
1 mm							0.037	0.036*

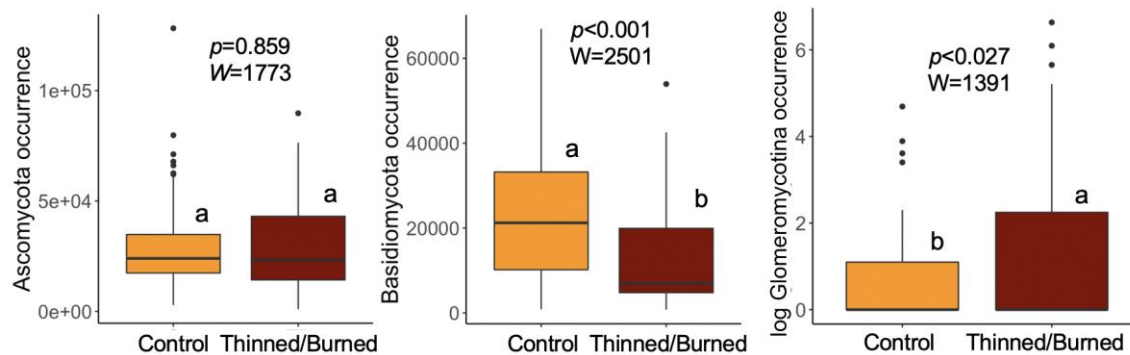


Fig. S3. Observations (per sample) of sequences belonging to the fungal phyla Ascomycota and Basidiomycota and the subphylum Glomeromycotina in mesocosm soil core samples from the untreated control management unit and the thinned/burned management unit. *P*-values and test statistics are from Wilcoxon rank-sum tests.

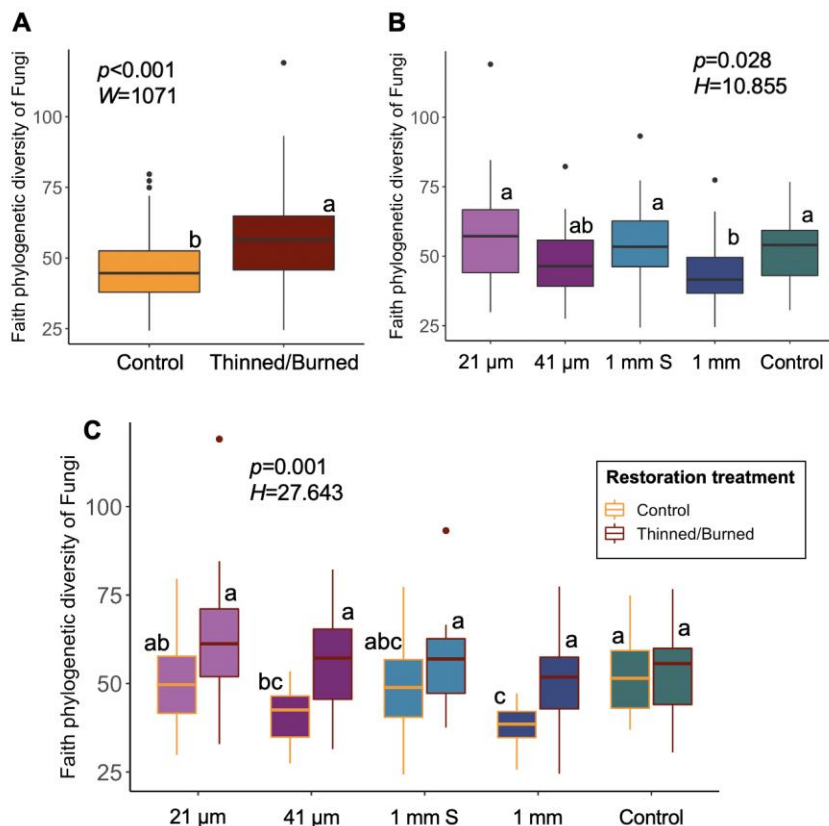


Fig. S4. Faith phylogenetic diversity of fungal communities by **A.** forest management treatment; **B.** mesocosm mesh treatment (21 μm , 41 μm , 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe); and **C.** mesh treatment by forest management treatment. Boxes with different letters are significantly different at $\alpha < 0.05$ according to Wilcoxon rank sum tests, after application of the Benjamini-Hochberg correction for multiple comparisons. Model *P*-values were calculated by Kruskal-Wallis H-test for mesh and mesh by forest management unit comparisons, and by Wilcoxon rank sum tests for forest management comparisons alone.

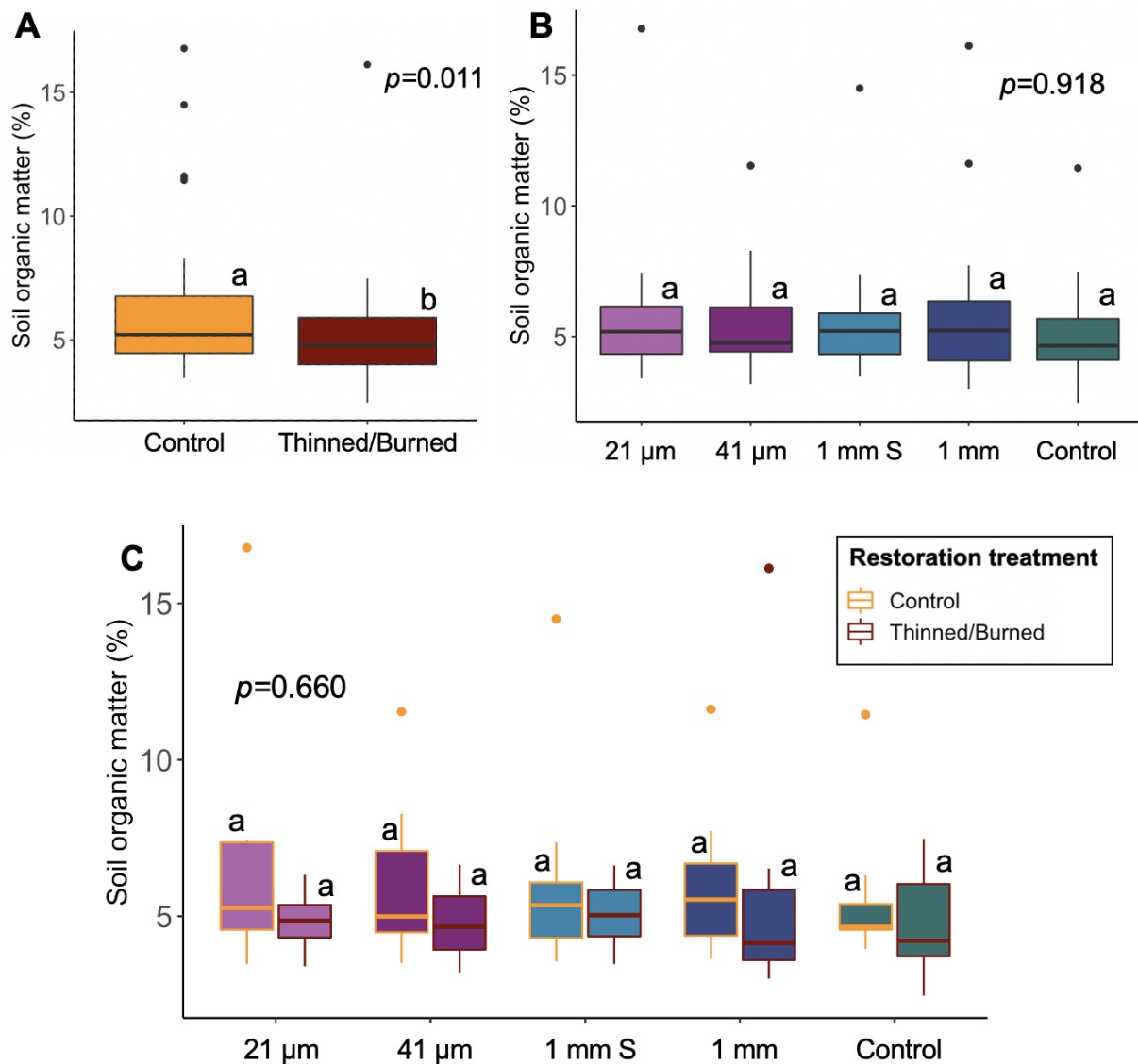


Fig. S5. Soil organic matter by (A) forest restoration treatment unit ($F=6.575$); (B) mesocosm mesh treatment ($H=0.940$); (C) forest restoration treatment x mesocosm mesh treatment ($H=6.780$). P-values are from ANOVA on log-transformed data for ponderosa restoration treatment, and Kruskal-Wallis H tests for mesh and mesh by restoration treatment comparisons.

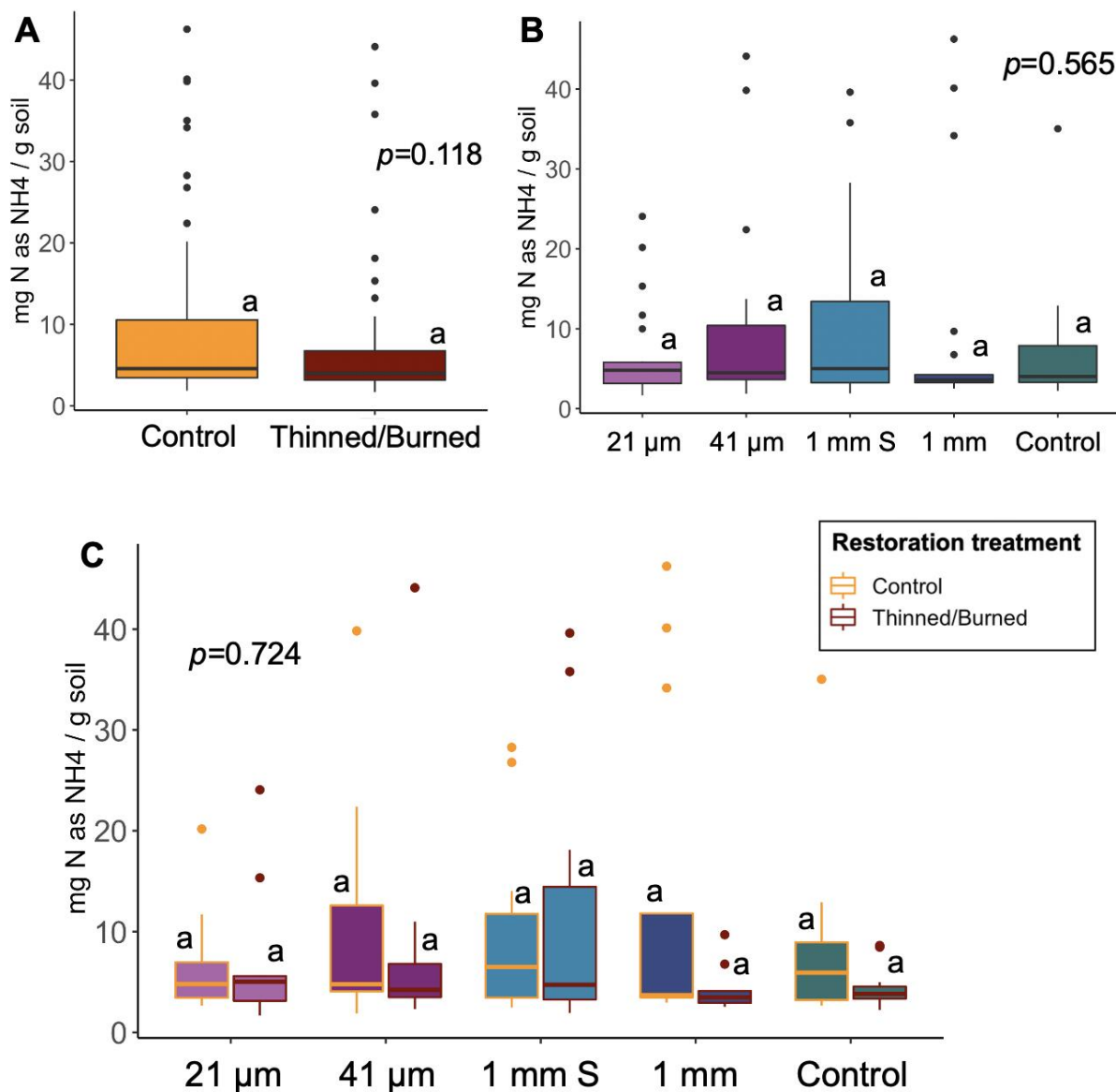


Fig. S6. Soil ammonium content by (A) forest restoration treatment unit ($H=2.432$); (B) mesocosm mesh treatment ($H=2.954$); (C) forest restoration treatment x mesocosm mesh treatment ($H=6.153$). Model p -values are from Kruskal-Wallis H tests.

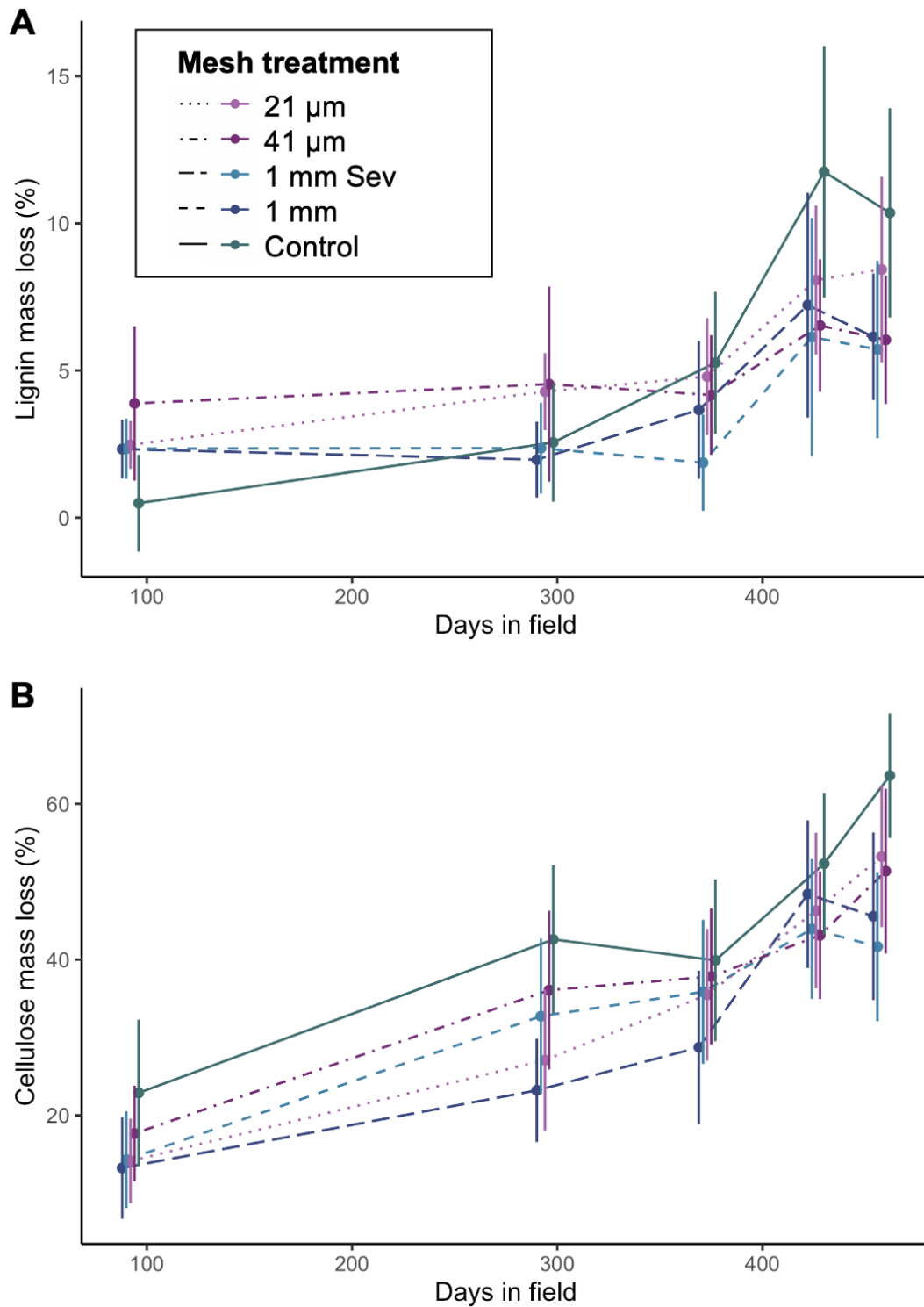


Fig. S7. Decomposition of (A) balsa wood (predominantly lignin) and (B) museum board (predominantly cellulose) in untreated control by mesocosm mesh treatment. Error bars represent 95% confidence intervals.

Table S3. Results from selected analysis of covariance models for decomposition of balsa wood and museum board disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only, run with mesocosm controls included. Note that unsevered 1 mm mesh mesocosms are the reference group for models including mesh size.

Balsa wood (predominantly lignin)				
logit (proportion remaining)				
<i>All trees</i>				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.1548	0.06076	35.463	< 2e-16 ***
Timepoint	-0.11373	0.01299	-8.752	< 2e-16 ***
Restoration treatment: TB	0.16719	0.03793	4.408	1.25e-05 ***
1 mm mesh severed	0.0891	0.0602	1.48	0.139
21 µm mesh	-0.07891	0.06827	-1.156	0.248
41 µm mesh	-0.01009	0.06539	-0.154	0.877
Control	-0.08631	0.05889	-1.466	0.143
Soil moisture (average Z score)	-0.04479	0.0313	-1.431	0.153
			Residual standard error	0.4373
			Adjusted R²	0.1625
			F (d.f.=)	16.66 (7, 558)
			Model P	< 2.2e-16***
<i>Untreated control trees only</i>				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.1073	0.05999	35.129	< 2e-16 ***
Timepoint	-0.10202	0.01822	-5.6	5.01e-08 ***
Soil moisture (average Z score)	-0.07343	0.0296	-2.481	0.0137 *
			Residual standard error	0.4371
			Adjusted R²	0.109
			F (d.f.=)	18.69 (2, 287)
			Model P	2.35E-08***
<i>Thinned/burned trees only</i>				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.44304	0.08767	27.866	< 2e-16 ***
Timepoint	-0.12647	0.01833	-6.9	3.72e-11 ***
1 mm mesh severed	0.08031	0.08522	0.942	0.34682

21 µm mesh	-0.26289	0.0928	-2.833	0.00496 **
41 µm mesh	-0.16501	0.09812	-1.682	0.09379 .
Control	-0.04931	0.08333	-0.592	0.55448
Soil moisture (<i>average Z score</i>)	0.09337	0.06108	1.529	0.12754
Residual standard error				0.4324
Adjusted R²				0.1784
F (d.f.=)				10.95 (6, 269)
Model P				6.52E-11***

Museum board (predominantly lignin)

logit (proportion remaining)

All trees

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.03566	0.27481	3.769	0.000181 ***
Timepoint	-0.44846	0.03224	-13.909	< 2e-16 ***
Restoration treatment: TB	0.32894	0.09595	3.428	0.000651 ***
Soil moisture (<i>average Z score</i>)	-0.30319	0.06289	-4.821	1.82e-06 ***
log % Soil organic matter	0.46488	0.14448	3.218	0.001365 **
Residual standard error				
Adjusted R²				0.2929
F (d.f.=)				61.46 (4 and 580)
Model P				< 2.2e-16***

Untreated control trees only

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.87849	0.15694	11.969	< 2e-16 ***
Timepoint	-0.46013	0.04739	-9.709	< 2e-16 ***
Soil moisture (<i>average Z score</i>)	-0.36824	0.07632	-4.825	2.25e-06 ***
Residual standard error				
Adjusted R²				0.2788
F (d.f.=)				58.42 (2, 295)
Model P				< 2.2e-16***

Thinned/burned trees only

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.13764	0.39395	2.888	0.00418 **
Timepoint	-0.43646	0.04331	-10.079	< 2e-16 ***

1 mm mesh severed	0.2242	0.20104	1.115	0.26571
21 µm mesh	0.65247	0.22076	2.956	0.00339 **
41 µm mesh	0.30865	0.23216	1.329	0.18478
Control	-0.14396	0.19522	-0.737	0.46147
Soil moisture (average Z score)	-0.47777	0.14449	-3.307	0.00107 **
log % Soil organic matter	0.43954	0.21052	2.088	0.03772 *
Residual standard error				
Adjusted R^2				0.2934
F (d.f.=)				17.96 (7, 279)
Model P				< 2.2e-16***

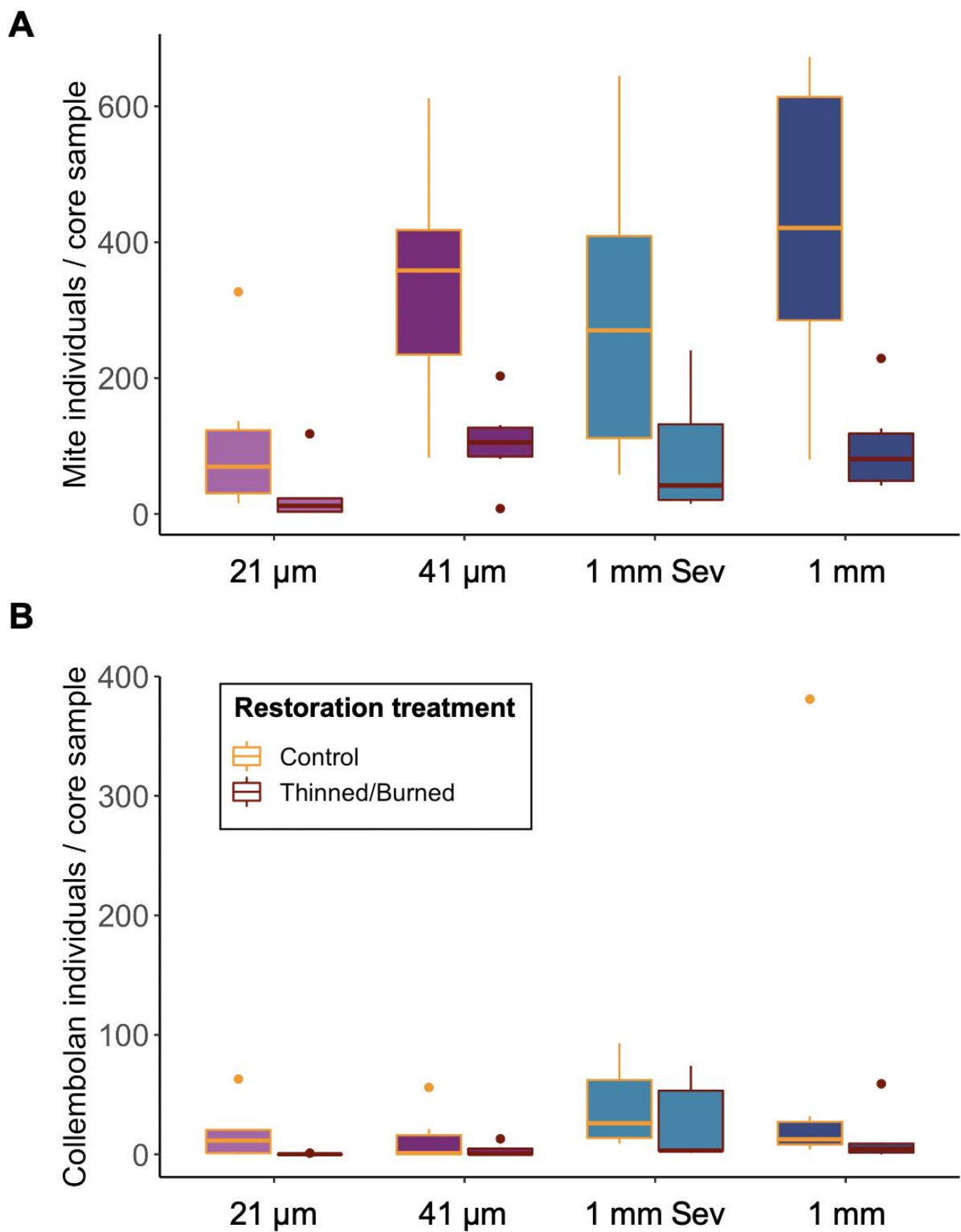


Fig. S8. Abundances of mites (**A**) and collembolans (**B**) in sacrificial mesocosms sampled at T1 and T3. Data from both timepoints are pooled in these Fig.s. N=3 mesocosms per restoration treatment per timepoint.

