

The US National Mall Microbiome: A Census of Rhizosphere Bacteria Inhabiting Landscape Turf

Jo Anne Crouch,[★] Zakiya Carter, Adnan Ismaiel, and Joseph A. Roberts

ABSTRACT

Turf contains living assemblages of not only grass plants, but also a plethora of microorganisms that may play critical roles in plant health through interactions such as predation, mutualism, and resource competition. Little is known about the turf microbiome and how it might change in response to renovations such as new sod installation or overseeding. In this work, we survey bacteria inhabiting the rhizosphere of mature and recently renovated turf stands at the United States National Mall in Washington, DC. We contrasted these data with the microbiomes of sod soon to be planted at the National Mall, and from the lawn of a nearby government facility. The soil-inhabiting microbiome was quantified through high-throughput next generation sequencing of the 16S ribosomal DNA. Over 1600 operational taxonomic units were identified from 9.2 million sequence reads, of which 751 could be diagnosed at the genus level. Over 50% of the 751 identifiable bacterial taxa were members of the phylum Proteobacteria, predominately in the genus *Rhodoplanes*. Phenotypic mapping of predicted microbial function on the basis of taxonomy showed all four locations were diverse and, in general, contained an abundance of Gram-negative, aerobic, free-living thermophilic organisms. Ordination plots showed clustering of samples according to location. However, this pattern of differentiation between locations was not recapitulated using hierarchical clustering analysis, α diversity measurements, or analysis of similarities (ANOSIM), with no significant differences between locations identified. Given the commonality of bacterial communities between locations, these data suggest that the resident National Mall microbiome may not experience appreciable change due to the renovation process.

J.A. Crouch, Z. Carter, and A. Ismaiel, USDA-ARS, Systematic Mycology and Microbiology Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705; Z. Carter, Eleanor Roosevelt High School, 7601 Hanover Parkway, Greenbelt, MD 20770; Z. Carter, current address, Johns Hopkins Univ., Baltimore, MD 21218; J.A. Roberts, Dep. of Plant Science and Landscape Architecture, Univ. of Maryland, 2110 Plant Sciences Building, College Park, MD 20742. Received 1 Oct. 2016. Accepted 31 Mar. 2017. [★]Corresponding author (joanne.crouch@ars.usda.gov). Assigned to Associate Editor Karen Harris-Shultz.

Abbreviations: ANOSIM, analysis of similarities; OTU, operational taxonomic unit; PCA, principal component analysis; PCR, polymerase chain reaction; rDNA, ribosomal DNA; SSU, small subunit.

AGROECOSYSTEMS are increasingly at risk due to the movement of nonindigenous microorganisms into new environments (Waage and Mumford, 2008). More than half of all documented plant pathogen invasions are directly attributable to human-mediated transfer of nonindigenous microorganisms (Anderson et al., 2004). It is generally accepted that heightened levels of microbial invasions in recent years are correlated with intensified globalization, specifically due to growing levels of world trade in agricultural commodities, tourism, transportation, and travel (Waage and Mumford, 2008). In turfgrass ecosystems, the introduction of nonindigenous microorganisms occurs through numerous human-mediated processes, including foot traffic and recreational use, machinery and irrigation systems, and the use of commercially sourced materials such as sand, organic matter, sod, etc. (Fleming et al., 2008; Murakami and Ray, 2008; Entwistle et al., 2014). Sand is a common source of nonindigenous nematodes and plant pathogenic microorganisms during golf course green construction (Entwistle et al., 2014). Reclaimed water use in turf irrigation systems may lead to the influx of human pathogenic

Published in Crop Sci. 57:S-341–S-348 (2017).
doi: 10.2135/cropsci2016.10.0849

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA
This is an open access article distributed under the CC BY-NC-ND license
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

microorganisms, such as the presence of fecal bacteria documented from golf courses in Hawaii (Murakami and Ray, 2008). The rapid emergence of the novel phytopathogen *Labyrinthula terrestris*, the causal agent of turf rapid blight disease, is thought to have occurred due to increased use of reclaimed water in golf course irrigation systems (Olsen, 2007). Living plant material used for turf renovation and establishment, including commercial sources of grass seed and sod, provides an abundant source of latent microbial pathogens, nematodes, endophytes, and other microscopic organisms (Schardl et al., 2004; Crouch et al., 2009; Entwistle et al., 2014; Rioux et al., 2014).

In this study, we set out to understand how bacteria within the rhizosphere microbiome—the ecosystem of microorganisms inhabiting a given environment—changes when turfgrass renovation practices such as seeding or sodding occur. To address this question, we made use of the lawn renovation project recently undertaken at the National Mall in the US capitol city of Washington, DC. Starting in 2010, renovation was undertaken to systematically remove and replace the existing turfgrass at the National Mall and install an underground irrigation and filtration system. Prior to the turfgrass renovation, the National Mall lawn was made of a heterogeneous mixture of numerous grass species, dicots, and in some places, bare ground, rather than a true grass-based turf. The turfgrass used to renovate the National Mall was custom-grown in the form of sod (50/50 by weight *Festuca arundinaceae* L. [cv. ‘Turbo’, ‘Falcon V’, and ‘third Millennium’] and *Poa pratensis* L. [‘Barvette HGT’, ‘Bararri’, ‘Barimpala’ and ‘Barrister’]) by a farm in the southern part of New Jersey, ~277 km northeast of the National Mall location. The new turfgrass installation is intended to restore beauty and utility to the National Mall, but at the same time, this largescale planting of new sod holds the potential to unintentionally introduce a plethora of nonindigenous microbes.

Microbial communities in turfgrass and other grassland ecosystems can differ significantly on the basis of site conditions such as climate, plant species composition, soil chemistry, and management inputs (Elliot and Des Jardin, 1999; Elliott et al., 2004, 2008; Bartlett et al., 2008; Yao et al., 2011; Jenkins et al., 2009; Beirn et al., 2017). For example, Beirn et al. (2017) recently observed significant differences in the complete archeal and bacterial microbiome inhabiting a single golf course green across a growing season. Given the geographical distance and differences in plant species composition in our study system, we predicted that vast differences in the microbiome would exist between the US National Mall lawn in Washington, DC, and the New Jersey farm where replacement sod was custom grown. To test our hypothesis, in this study, we conducted a census of the bacterial communities inhabiting the rhizosphere of turf ecosystems from the pre- and post-renovation lawn at the National Mall site, in addition

to the southern New Jersey sod farm and the front lawn of a government research facility. We used next-generation sequencing tools to simultaneously barcode the diagnostic bacterial 16S ribosomal DNA (rDNA) small subunit (SSU) to provide an environmental snapshot of bacterial community composition inhabiting these turf ecosystems.

MATERIALS AND METHODS

Sample Sites and Soil Sampling

Soil samples were taken from turfgrass grown at three different locations in the eastern mid-Atlantic region of the United States, as summarized in Table 1. The two locations—the US National Mall in Washington, DC (38.9233497° N, 76.9961253° W), and a commercial sod producer located in southern New Jersey (39.6803276° N, 74.7846638° W)—are separated by ~277 km and were selected to address our main question of whether these two sites were inhabited by similar bacterial communities. The third location at the USDA-ARS facility in Beltsville, MD (39.0262543° N, 76.9255522° W), located ~27 km northeast of the National Mall, was sampled to provide a third community comparison within the same geographic and climactic zone. Renovation of the turfgrass site at the National Mall commenced during 2015, and two sample sets were included to address our question of how bacterial communities might change as new turfgrass became established at this location. The first sample taken from the National Mall was collected prior to renovation during June 2015 (with this location abbreviated hereafter as “DC”), and a second sample was collected post renovation in October 2015 (with this location abbreviated hereafter as “ODC”). The pre-renovation turf collected from the DC site was predominately inhabited by annual bluegrass (*Poa annua* L.). The post-renovation ODC turf included a mixture of *F. arundinaceae* and *P. pratensis*. An additional set of turf samples was collected from the other two locations during October 2015: the New Jersey sod farm (with this location abbreviated hereafter as “NJ”) and the USDA location in Maryland (with this location abbreviated hereafter as “UA”). The soil collected from the NJ site was also planted with a mixture of *F. arundinaceae* and *P. pratensis*. The UA turf was inhabited by a diverse community of grass and dicot plants maintained as an unirrigated lawn (Supplemental Fig. 1). Soil cores were collected using a 15.9-mm-diameter handheld auger to a depth of ~4 to 10 cm; deeper samples were taken at the NJ and ODC site due to more permeable rootzones. The auger was brushed clear of soil using a fresh paper towel and then surface sterilized by wiping with a 70% ethanol solution between samples. After extraction, samples were immediately stored in sterile 50-mL Falcon tubes (BD-Biosciences) on ice and were then stored at –20°C until processing.

Each soil sample was processed individually in the laboratory in fresh disposable sterile Petri dishes placed on top of fresh Thermo Scientific Nalgene Versi-Dry Surface Liners. Gloves were changed between samples, and all tools and surfaces were cleaned with 70% ethanol prior to and between sample processing. Visual examination of soil cores showed that the majority of root material extended to ~2.5 cm from the surface, so this section was excised from the core using a sterile razor blade and was used for all subsequent processing. The entire core sample

Table 1. Summary of sample locations.

Site†	Total samples	Total sequences	Avg. sequences per sample	Biological replicate IDs	No. technical replicates	Sampling date
DC	12	2,496,530	208,044	DC1	4	June 2015
				DC2	4	
				DC3	4	
ODC	16	2,814,415	175,901	ODC1	2	Oct. 2015
				ODC2	2	
				ODC3	2	
				ODC4	2	
NJ	8	1,108,752	138,594	NJ1	8	Oct. 2015
				NJ2	8	
UA	12	2,787,456	232,288	UA1	4	Oct. 2015
				UA2	4	
				UA3	4	

† DC and ODC = US National Mall lawn, Washington, DC; NJ = New Jersey sod farm, Hammonton, NJ; UA = USDA lawn, Beltsville, MD.

analyzed was therefore at the interface between soil and roots, within the sphere of influence from the roots (Hartmann et al., 2008), and will be referred to hereafter as the rhizosphere. Soil was first separated from plant material, insects and rocks, and the remaining soil by hand using tweezers. The resultant soil sample was passed through a #20 sieve (Newark Wire Cloth Company) to remove any residual large particulate matter. Sieved soil was homogenized using a sterile mortar and pestle and then stored in autoclaved, sealed paper coin envelopes at -20°C until use for DNA extractions.

DNA Extraction and Library Construction

Genomic DNA was extracted from 40 g of prepared soil per sample using the PowerSoil DNA Isolation Kit (Mo-Bio) according to the manufacturer's protocol. Two DNA extractions were performed per soil sample. Quality and quantity of DNA was assessed through visualization of 1% agarose gels and a Nano Drop spectrophotometer (Nano Drop Technologies), respectively. DNA libraries for sequencing were prepared by generating polymerase chain reaction (PCR) amplicons from the 16S rDNA using the Ba9F/Ba515Rmod1 primers designed for bacteria (~500 bp; Weisburg et al., 1991; Kittelmann et al., 2013) with 5' modification with Illumina adaptor sequences, as described (Beirn et al., 2017). Polymerase chain reactions were performed using 10 nM of each primer in 25- μL volumes using GoTaq Flexi DNA polymerase (Promega) with reactions and cycling conditions as described by Beirn et al. (2017). After PCR, amplicons were visualized and quantified in a high-resolution gel cartridge using the QIAxcel Advanced capillary gel electrophoresis instrument (QIAGEN). Amplicons were purified on a 96-well MIDI plate/Invitrogen Ambion Magnetic Stand 96 (Thermo Fisher Scientific) using Agencourt AMPure XP beads (Beckman Coulter) to remove salts, unincorporated primers, and nucleotides. Illumina Nextera v2 XT Indices (Set A) were added to the purified amplicons via PCR to facilitate pooling and multiplexing. Indexed libraries were purified using AMPure XP beads, normalized to 4 nM, denatured, diluted to 10 pM, and sequenced as paired end reads on an Illumina MiSeq using a 600-cycle v3 cartridge.

Data Analyses

Binning and barcode removal from fastq reads generated from the MiSeq run was performed using the MiSeq Reporter (Illumina).

Fastq reads were imported into CLC Genomics Workbench (QIAGEN), where adapters, low-quality reads ($<Q20$), and runs of ambiguous bases >2 were removed. Trimmed reads were stitched together as paired end sequences using CLC Genomics Workbench and subsequently exported as fasta files. The two sets of individual reads generated from DNA extractions from each soil sample were concatenated into a single fasta file. Fasta sequences for each soil sample were given unique identifiers within the header line using an awk script in Unix. Chimeric sequences were identified and removed from the datasets using USEARCH (Edgar et al., 2011) launched within the QIIME bioinformatics pipeline (Caporaso et al., 2010). After concatenation of the 48 individual soil sample datasets into a single fasta file, sequences were clustered into operational taxonomic units (OTUs) using a de novo picking strategy at a 97% similarity threshold using QIIME. The OTUs were identified at the lowest taxonomic rank possible in QIIME (kingdom to species) with the RDP Classifier 2.2 (Wang et al., 2007) and the GreenGenes database version 13_8 (McDonald et al., 2012; Werner et al., 2012). A census of predicted bacterial phenotypes for oxygen requirement, temperature range, biotic relationship, Gram stain, and cell shape were mapped onto OTUs identified to the genus level using METAGENassist, which makes use of a database containing phenotypic information for $>11,000$ microbial species based around BacMap (Stothard et al., 2005). Data analyses were performed in the R statistical computing environment (R Core Team, 2015). Where calculated, a p -value ≤ 0.05 was considered significant for all analyses. The Shannon index was used to assess rarified datasets to calculate community diversity (α diversity). Rank abundance curves were plotted to visualize difference in OTU abundance profiles between samples (Hughes et al., 2001). Nonparametric Kruskal Wallis test was used to test for differential abundance between locations, with false discovery rate correction applied to account for multiple comparisons. Principal component analysis (PCA) was conducted from the genus-level OTU dataset using the vegan package (Oksanen et al., 2013) to visualize clustering among samples. Hierarchical clustering was performed and heat maps were generated using a Spearman's rank correlation coefficient as a distance measure. Analysis of similarities (ANOSIM) calculated from a Bray-Curtis dissimilarity matrix was used to test if samples within categories were more similar to one another than samples in different categories using 999 permutations.

RESULTS AND DISCUSSION

Sequencing Summary

A total of 48 samples of DNA were prepared from rhizosphere soil extracted from turf at four locations in the mid-Atlantic region of the eastern United States (Table 1). Illumina sequencing of bacterial 16S SSU PCR amplicons from the DNA yielded 10.6 million reads after adaptor and quality-control trimming, of which 13% were predicted to be chimeric and were discarded. From the final dataset of 9.2 million reads, the average read length was 272 bp. The average number of sequencing reads per sample ranged from 138,594 to 232,288, with an overall average read count of 170,833 per sample (Table 1). Rarefaction curves plotted using the Shannon index were similar for all 48 samples (data not shown), with curves approaching but not fully reaching a plateau, reflecting a partial sampling of the predicted resident microbial communities.

Rank abundance curves showed that, depending on the sample site, overall species richness (number of taxa) fell between 10^4 and 10^5 . Community evenness (relative abundance of taxa) exhibited the same overall rank abundance curve for all 48 samples (Supplemental Fig. 2). Statistically significant differences were not observed between α diversity indices ($p = 1$).

Qualitative Summary of Bacterial OTUs

At the genus level of 97% sequence similarity, 1656 OTUs were identified from the sequence reads. These data are summarized in Supplemental Table 1, where OTUs are quantified in terms of their relative abundance in a given sample. Twenty-six percent of the OTUs were present in at least 90% of the 48 samples. On the opposite end of the spectrum, 27.4% of OTUs were identified from <10% of the 48 samples. On average, each OTU was identified from 47% of all samples.

No single OTU dominated the overall dataset. The most abundant OTU—making up 7.5% of the complete dataset and an average of 0.15% of each individual sample community—was an assemblage of sequences that could not be identified below the level of the kingdom Bacteria. Only four other OTUs made up >1% of the overall community across all samples. Three of these OTUs were also assemblages of sequences that could not be fully resolved to the genus level: a group OTU from the Gaiellaceae family, and two group OTUs from the order Solirubrobacterales and phyla Acidobacteria. Only one genus-level OTU, the *Rhodoplanes*, comprised >1% of the total microbial community. Overall, each of these four OTUs made up just 0.03 to 0.04% of each individual sample's bacterial community.

Of the 1656 OTUs, only 45% could be named to the genus level (Supplemental Table 2). The OTUs for which a genus-level identification was not possible were assigned a name at higher taxonomic levels (family through kingdom). This is generally consistent with known difficulties

associated with applying names to bacteria genera from soil using rDNA sequences. For some genera, 16S data has low discriminatory power (Bosshard et al., 2006; Mignard and Flandrois, 2006). More importantly, genus- and species-level identification is reliant on the quality and completeness of reference. However, the cultured part of soil bacterial diversity—from which genera are named and described—are shown to only make up 2.7 to 3.7% of total soil bacteria known from molecular data (Floyd et al., 2005).

In contrast with the complete OTU dataset, the set of 751 named OTUs were far more conserved across the 48 sample sites, in that these were present in 82% of samples on average (range = 71 to 100%), versus just 47% from the full OTU assemblage.

Microbial Community Structure and Phenotype of Named OTU Genera

To evaluate the bacterial community composition and phenotype at the four locations, a reduced dataset was constructed from the 751 OTUs for which a genus-level identification could be made (Supplemental Table 2). Across all four locations, members of the Gram-negative Proteobacteria phylum dominated, making up 52.2 to 58.6% of the identifiable bacterial community (Fig. 1a–1d). The large cohort of Proteobacteria was largely attributable to the relatively high abundance of organisms in the phototrophic genus *Rhodoplanes*, comprising 26.4 to 37.3% of the identifiable community inhabiting the four locations (Fig. 1e–1h). Members of the genus *Rhodoplanes* are widespread and abundant in several rhizosphere ecosystem types, including turfgrass lawns and golf course greens (Jesus et al., 2009; Uroz et al., 2009; Rosenzweig et al., 2013; Zhalnina et al., 2015; Beirn et al., 2017), including *P. annua* golf course turf. In general, the ecological role of *Rhodoplanes* is poorly understood (Hiraishi and Imhoff, 2005), and the significance of their abundance at the four locations surveyed in this study is unknown. Dinitrification is a defining feature for all known *Rhodoplanes*, but the importance of these organisms for nitrogen breakdown in natural systems is not well studied (Hiraishi and Imhoff, 2005). Other Proteobacteria present at appreciable levels included members of the genus *Bradyrhizobium* (8.0–8.9% abundance), which are best known for their role in nitrogen fixation. The Proteobacteria genus *Pedomicrobium*—a group of ubiquitous organisms found as biofilms within manmade water environments such as water distribution systems (Cox and Sly, 1997)—also made up an abundant component of the identified bacterial community. The abundance of *Pedomicrobium* was variable between the four sites, making up 5.5% of the identified community at the DC location, but only 1.4% at ODC; however, the difference was not significant.

In addition to the Proteobacteria, members of the Gram-positive phylum Actinobacteria were also found at

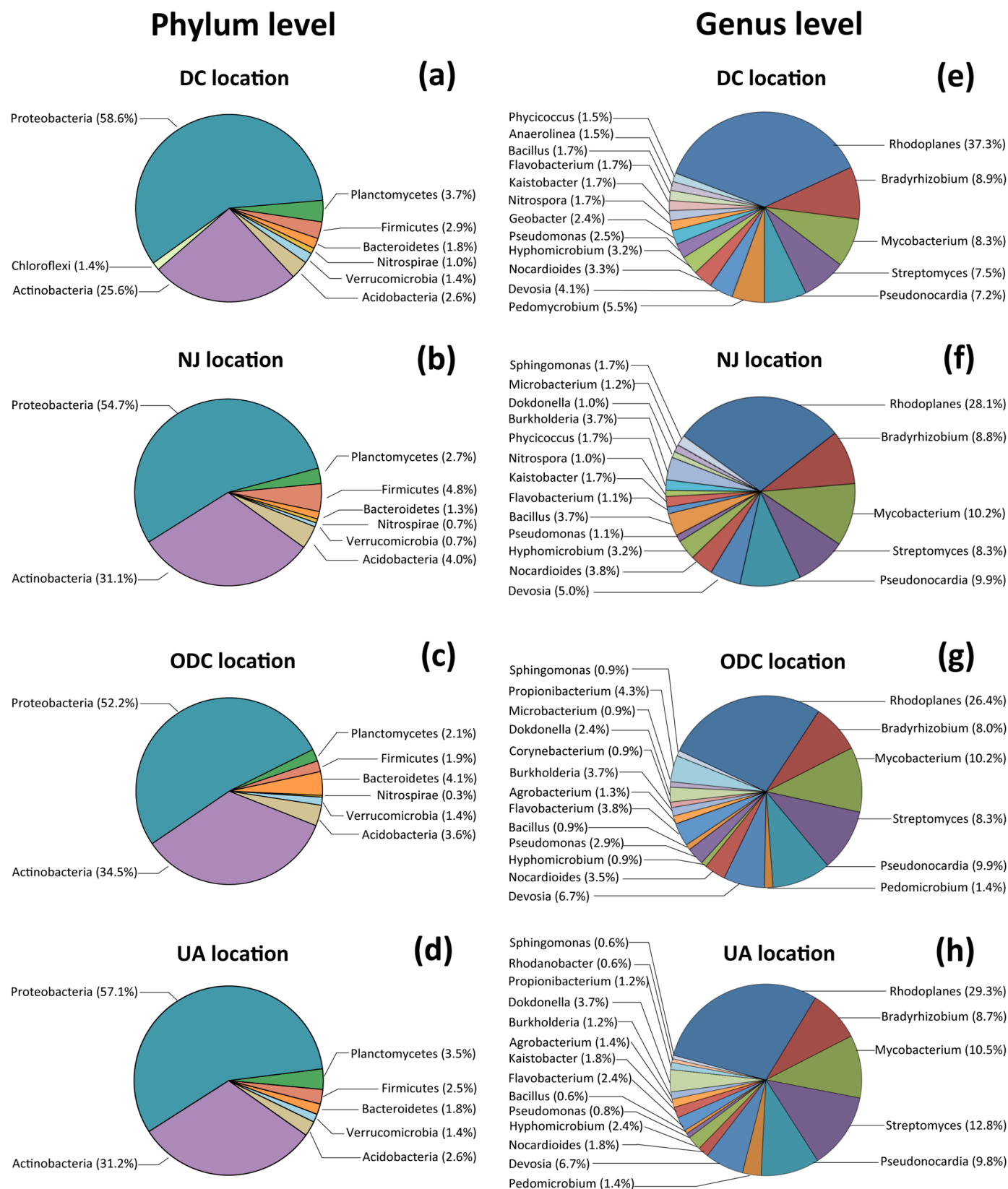


Fig. 1. Pie charts illustrating the abundance of identifiable bacteria observed from the four sampling locations. Figures 1a–1d show the abundance of 751 operational taxonomic units (OTUs) identified at the phylum level; Fig. 1e–1h show the abundance of bacterial genera. Only taxa present at 1% or above are shown. Location abbreviations: DC and ODC = US National Mall lawn, Washington, DC, sampled June 2015 and October 2015, respectively; NJ = New Jersey sod farm, Hammonton, NJ, sampled October 2015; and UA = USDA lawn, Beltsville, MD, sampled October 2015.

relatively high abundance from the overall microbiome, making up 25.6 to 34.5% of the total sample (Fig. 1a–1d). Actinobacteria is a functionally and metabolically diverse group of microorganisms well known for their involvement in carbon cycling and nutrient processing in soils (Holmalahti et al., 1994). This abundance of Actinobacteria was due to the presence of a few genera. Members of the species-rich genus *Streptomyces*, which are well known for their production of secondary metabolites (Busti et al., 2006), made up 7.5 to 10.8% of the Actinobacteria community (Fig. 1e–1h). *Mycobacterium*, a genus of diverse and widely distributed microorganisms (Parish and Brown, 2009), comprised 8.3 to 10.6% of the Actinobacteria community. Interestingly, the genus *Pseudonocardia*, a group of Actinobacteria known for their mutualistic association with leaf cutter ants (genera *Atta* and *Acromyrmex*; Poulsen et al., 2005), was identified from the soil communities at relatively high abundance (7.2–9.9% of the identifiable bacterial community).

We mapped the 751 identifiable OTUs to predicted phenotypes for five main categories: oxygen requirement, temperature range, biotic relationships, Gram stain, and cell shape (Supplemental Table 3). According to PCA, no association was observed between four of the five phenotypic characteristics and the four sample locations. Only one category, temperature range, showed appreciable differences in the abundance of sequence reads among the four locations, allowing for some discrimination of communities in the PCA based on location (Supplemental Fig. 3), but without statistical support. Despite the observed clustering differences in this category, of the phenotypically characterized OTUs, the majority of OTUs were

identified as temperate-adapted mesophilic genera, consistent with the climatic profile of the sample sites. In general, approximately half of all OTUs were members of aerobic genera, and about a quarter of the OTUs were assigned anaerobic phenotypes (Supplemental Table 3). The majority of OTUs were from free-living genera (average = 61.5%), whereas 33.1% were from symbiotic organisms, and the community was primarily made up of Gram-negative bacilli (Supplemental Table 3).

Microbiome Cluster Analysis

To investigate whether there was an association between location and bacterial community structure, we performed hierarchical sample clustering and a PCA (Fig. 2, Supplemental Fig. 4). The hierarchical analysis, visualized as a heat map (Supplemental Fig. 4), showed partial clustering of samples on the basis of location but overall demonstrated no clear separation of the communities from the four locations. Principal component analysis plots (Fig. 2a and 2b) illustrated the degree of separation and relationships between samples made from the four locations. In the two-dimensional PCA diagram, the first two ordination axes only explained 41.4% of the variation, with samples from all locations except DC tightly clustered. The three-dimensional PCA accounted for 61.0% of the observed variation and allowed for visualization of distinct location microbiomes to a large extent. However, ANOSIM and Kruskal–Wallis testing showed that the distinction between the four locations was not significant (test statistic = -0.0147331 , $p = 0.601$; Supplemental Table 4).

Overall, analysis of the four locations sampled showed a high degree of similarity in the microbial communities

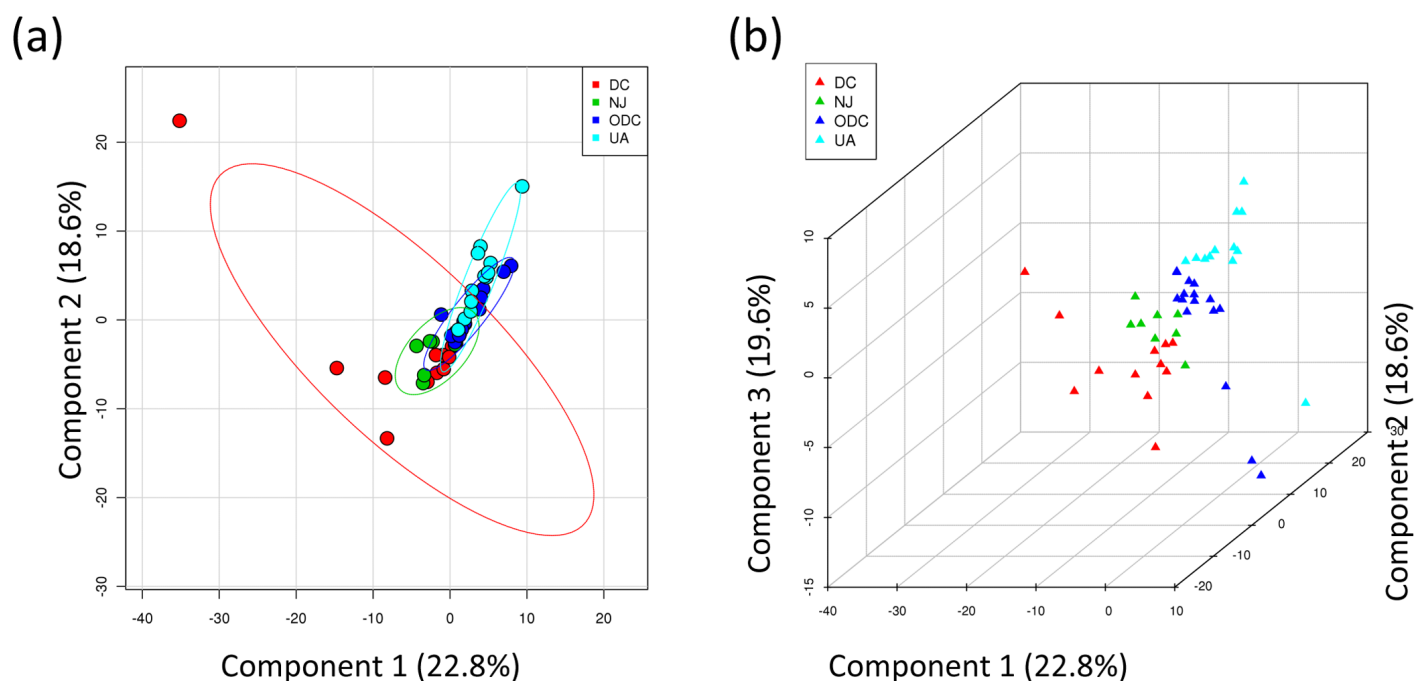


Fig. 2. Principal component analysis (PCA) plots showing the microbiome compositional relationships of genus-level taxa. (a) Two- and (b) three-dimensional visualization of PCA using the top two and three vectors, respectively.

despite geographic separation, and dissimilarity in the resident turfgrass ecosystem. The limited geographical range between sampling locations is likely a contributing factor in the similarities observed between microbial communities within our study. Previous work by Elliott et al. (2004) observed turfgrass species and location to significantly affect variation observed in culturable rhizosphere bacteria communities associated with turfgrasses sampled from golf course putting greens in the southeastern United States. In contrast with our samples, the Elliott et al. (2004) study involved golf courses separated by greater distances, and the turfgrass species used included a comparison of cool-season creeping bentgrass (*Agrostis stolonifera* L.) and warm-season hybrid bermudagrass (*Cynodon dactylon* L. × *C. transvaalensis* Burt-Davy), whereas our samples were all from cool-season turfgrass species and limited to separation by 277 km (at the furthest distance). It is believed that plant-type may affect community structure more than sampling site when comparing locations on a limited geographical scale (Grayston et al., 1998; Steer and Harris, 2000; Costa et al., 2005). Although research presented here supports this theory, future work would benefit by including additional turf species (i.e., cool- and warm-season grasses) over varying geographical distances.

CONCLUSION

The objective of this work was to conduct a census of the bacterial taxa making up the rhizosphere microbiome at the US National Mall associated with the recent turf renovation project. Our prediction—that vast differences in the microbiome would exist between the US National Mall lawn in Washington, DC, and the New Jersey farm where replacement sod was grown—was not supported by the census data. Overall, the bacterial community census at the US National Mall lawn, both pre- and post-renovation, exhibited differences in OTU frequencies relative to the sod microbiome, and from the USDA lawn microbiome. However, from a statistical standpoint, there were more commonalities than there were differences. These findings show that the census of microbes living in the National Mall lawn may not appreciably change much after establishment of the new sod during turf renovation.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

The research described in this paper was conducted as the senior year high school internship of Zakiya Carter at the USDA-ARS as part of the Maryland's Prince George's County Science and Technology Internship Program. We thank Jenny Allen and the USDA-ARS Office of Diversity and Outreach for facilitat-

ing the student internship opportunity, and Yazmín Rivera for assistance with the project. The authors would like to thank Michael Stachowicz and the National Parks Service for assistance in obtaining permits and acquiring turf samples used in this study. This work was made possible through funding provided by USDA-ARS. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

References

- Anderson, P.K., A.A. Cunningham, N.G. Patel, F.J. Morales, P.R. Epstein, and P. Daszak. 2004. Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnological drivers. *Trends Ecol. Evol.* 19:535–544. doi:10.1016/j.tree.2004.07.021
- Bartlett, M.D., I.T. James, J.A. Harris, and K. Ritz. 2008. Size and phenotypic structure of microbial communities within soil profiles in relation to different playing areas on a UK golf course. *Eur. J. Soil Sci.* 59:835–841. doi:10.1111/j.1365-2389.2008.01056.x
- Beirn, L.A., J.W. Hempfling, C.J. Schmid, J.A. Murphy, B.B. Clarke, and J.A. Crouch. 2017. Differences among soil-inhabiting microbial communities in *Poa annua* turf throughout the growing season. *Crop Sci.* doi:10.2135/cropsci2016.06.0463
- Bosshard, P.P., R. Zbinden, S. Abels, B. Böttgeringhaus, M. Altwegg, and E.C. Böttger. 2006. 16S rRNA gene sequencing versus the API 20 NE system and the Vitek 2 ID-GNB card for identification of nonfermenting gram-negative bacteria in the clinical laboratory. *J. Clin. Microbiol.* 44:1359–1366. doi:10.1128/JCM.44.4.1359-1366.2006
- Busti, E., P. Monciardini, L. Cavaletti, R. Bamonte, A. Lazzarini, M. Sosio, and S. Donadio. 2006. Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. *Microbiology* 152:675–683. doi:10.1099/mic.0.28335-0
- Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7:335–336. doi:10.1038/nmeth.f.303
- Costa, R., M.N. Gotz, J. Mroczek, G. Lottmann, G. Berg, and K. Smalla. 2005. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiol. Ecol.* 56:236–249. doi:10.1111/j.1574-6941.2005.00026.x
- Cox, T.L., and L.I. Sly. 1997. Phylogenetic relationships and uncertain taxonomy of *Pedomicrobium* species. *Int. J. Syst. Bacteriol.* 47:377–380. doi:10.1099/00207713-47-2-377
- Crouch, J.A., L.P. Tredway, B.B. Clarke, and B.I. Hillman. 2009. Phylogenetic and population genetic divergence correspond with habitat for the pathogen *Colletotrichum cereale* and allied taxa across diverse grass communities. *Mol. Ecol.* 18:123–135. doi:10.1111/j.1365-294X.2008.04008.x
- Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200. doi:10.1093/bioinformatics/btr381
- Elliott, M.L., and E.A. Des Jardin. 1999. Effect of organic nitrogen fertilizers on microbial populations associated with bermudagrass putting greens. *Biol. Fertil. Soils* 28:431–435. doi:10.1007/s003740050516
- Elliott, M.L., E.A. Guertal, and H.D. Skipper. 2004. Rhizosphere bacterial population flux in golf course putting greens in the southeastern United States. *HortScience* 39:1754–1758.

- Elliott, M.L., J.A. McInroy, K. Xiong, J.H. Kim, H.D. Skipper, and E.A. Guertal. 2008. Taxonomic diversity of rhizosphere bacteria in golf course putting greens at representative sites in the south-eastern United States. *HortScience* 43:514–518.
- Entwistle, K., T. Fleming, R. Kerr, A. Maule, T. Martin, M. Hainon-McDowell, and C.C. Fleming. 2014. Biosecurity and emerging plant health problems in turf production and maintenance. *Eur. J. Hortic. Sci.* 79:S108–S115.
- Fleming, C.C., D. Craig, M. Hainon-McDowell, and K. Entwistle. 2008. Plant-parasitic nematodes: A new turf war? *Biologist* 55:76–82.
- Floyd, M.M., J. Tang, M. Kane, and D. Emerson. 2005. Captured diversity in a culture collection: Case study of the geographic and habitat distributions of environmental isolates held at the American Type Culture Collection. *Appl. Environ. Microbiol.* 71:2813–2823. doi:10.1128/AEM.71.6.2813-2823.2005
- Grayston, S.J., S.Q. Wang, C.D. Campbell, and A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* 30:369–378. doi:10.1016/S0038-0717(97)00124-7
- Hartmann, A., M. Rothballer, and M. Schmid. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil* 312:7–14. doi:10.1007/s11104-007-9514-z
- Hiraishi, A., and J. Imhoff. 2005. Genus XVII. Rhodoplanes Hiraishi and Ueda 1994b, 671 VP. In: D.J. Brenner and N.R. Krieg, editors, *Bergey's manual of systematic bacteriology*. Vol. 2: The Proteobacteria, Part 3. Springer Science and Business Media, New York. p. 545–549. doi:10.1007/0-387-29298-5_132
- Holmalahti, J.A., A. von Wright, and O. Raatikainen. 1994. Variations in the spectra of biological activities of actinomycetes isolated from different soils. *Lett. Appl. Microbiol.* 18:144–146. doi:10.1111/j.1472-765X.1994.tb00829.x
- Hughes, J.B., J.J. Hellmann, T.H. Ricketts, and B.J.M. Bonhannan. 2001. Counting the uncountable: Statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol.* 67:4399–4406. doi:10.1128/AEM.67.10.4399-4406.2001
- Jenkins, S.N., I.S. Waite, A. Blackburn, R. Husband, S.P. Rushton, D.C. Manning, and J.C. O'Donnell. 2009. Actinobacterial community dynamics in long term managed grasslands. *Antonie van Leeuwenhoek* 95:319–334. doi:10.1007/s10482-009-9317-8
- Jesus, E.d.C., T.L. Marsh, J.M. Tiedje, and F.M.d.S. Moreira. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. *ISME J.* 3:1004–1011. doi:10.1038/ismej.2009.47
- Kittelmann, S., H. Sedorf, W.A. Walters, J.C. Clemente, R. Knight, J.I. Gordon, and P.H. Janssen. 2013. Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. *PLoS One* 8:e47879. doi:10.1371/journal.pone.0047879
- McDonald, D., M.N. Price, J. Goodrich, E.P. Nawrocki, T.Z. DeSantis, A. Probst et al. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6:610–618. doi:10.1038/ismej.2011.139
- Mignard, S., and J.P. Flandrois. 2006. 16S rRNA sequencing in routine bacterial identification: A 30-month experiment. *J. Microbiol. Methods* 67:574–581. doi:10.1016/j.mimet.2006.05.009
- Murakami, G.A., and C. Ray. 2008. Turf irrigation in Hawaii using r-1 effluent: Microbial and chemical effects. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* 35:957–980.
- Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara et al. 2013. *vegan: Community ecology package*, R package version 2.0–10. R Foundation for Statistical Computing. <http://CRAN.R-project.org/package=vegan>
- Olsen, M.W. 2007. *Labyrinthula terrestris*: A new pathogen of cool-season turfgrasses. *Mol. Plant Pathol.* 8:817–820. doi:10.1111/j.1364-3703.2007.00425.x
- Parish, T., and A. Brown. 2009. *Mycobacterium: Genomics and molecular biology*. Caister Academic Press, Poole, UK.
- Poulsen, M., M. Cafaro, J.J. Boomsma, and C.R. Currie. 2005. Specificity of the mutualistic association between actinomycete bacteria and two sympatric species of *Acromyrmex* leaf-cutting ants. *Mol. Ecol.* 14:3597–3604. doi:10.1111/j.1365-294X.2005.02695.x
- R Core Team. 2015. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rioux, R.A., J. Shultz, M. Garcia, D.K. Willis, M. Casler, S. Bonos et al. 2014. *Sclerotinia homoeocarpa* overwinters in turfgrass and is present in commercial seed. *PLoS One* 9:e110897. doi:10.1371/journal.pone.0110897
- Rosenzweig, N., J.M. Bradeen, Z.J. Tu, S.J. McKay, and L.L. Kinkel. 2013. Rhizosphere bacterial communities associated with long-lived perennial prairie plants vary in diversity, composition, and structure. *Can. J. Microbiol.* 59:494–502. doi:10.1139/cjm-2012-0661
- Schardl, C.L., A.A. Leuchtmann, and M.J. Spiering. 2004. Symbioses of grasses with seedborne endophytes. *Annu. Rev. Plant Biol.* 55:315–340. doi:10.1146/annurev.arplant.55.031903.141735
- Steer, J., and J.A. Harris. 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of *Agrostis stolonifera*. *Soil Biol. Biochem.* 32:869–878. doi:10.1016/S0038-0717(99)00219-9
- Stothard, P., G. Van Domselaar, S. Shrivastava, A. Guo, B. O'Neill, J. Cruz, M. Ellison, and D.S. Wishart. 2005. BacMap: An interactive picture atlas of annotated bacterial genomes. *Nucleic Acids Res.* 33:D317–D320. doi:10.1093/nar/gki075
- Uroz, S., C. Murat, P. Frey-Klett, and F. Martin. 2009. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* 2:281–288. doi:10.1111/j.1758-2229.2009.00117.x
- Waage, J.K., and J.D. Mumford. 2008. Agricultural biosecurity. *Philos. Trans. R. Soc., B* 363:863–876. doi:10.1098/rstb.2007.2188
- Wang, Q., G.M. Garrity, J.M. Tiedje, and J.R. Cole. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73:5261–5267. doi:10.1128/AEM.00062-07
- Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173:697–703. doi:10.1128/jb.173.2.697-703.1991
- Werner, J.J., O. Koren, P. Hugenholtz, T.Z. DeSantis, W.A. Walters, J.G. Caporaso et al. 2012. Impact of training sets on classification of high-throughput bacterial 16S rRNA gene surveys. *ISME J.* 6:94–103. doi:10.1038/ismej.2011.82
- Yao, H., D. Bowman, and W. Shi. 2011. Seasonal variations of soil microbial biomass and activity in warm- and cool-season turfgrass systems. *Soil Biol. Biochem.* 43:1536–1543. doi:10.1016/j.soilbio.2011.03.031
- Zhalnina, K., R. Dias, P.D. Quadros, A. Davis-Richardson, F.A. Camargo, I.M. Clark et al. 2015. Soil pH determines microbial diversity and composition in the park grass experiment. *Microb. Ecol.* 69:395–406. doi:10.1007/s00248-014-0530-2