Soil depth and grassland origin cooperatively shape microbial community co-occurrence and function

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Abstract
Many soils are deep, yet soil below 20 cm remains largely unexplored. Exotic plants can have shallower roots than native species, so their impact on microorganisms is anticipated to change with depth. Using environmental DNA and extracellular enzymatic activities, we studied fungal and bacterial community composition, diversity, function, and co-occurrence networks between native and exotic grasslands at soil depths up to 1 m. We hypothesized (1) the composition and network structure of both fungal and bacterial communities will change with increasing depth, and diversity and enzymatic function will decrease; (2) microbial enzymatic function and network connectedness will be lower in exotic grasslands; and (3) irrigation will alter microbial networks, increasing the overall connectedness. Microbial diversity decreased with depth, and community composition was distinctly different between shallow and deeper soil depths with higher numbers of unknown taxa in lower soil depths. Fungal communities differed between native and exotic plant communities. Microbial community networks were strongly shaped by biotic and abiotic factors concurrently and were the only microbial measurement affected by irrigation. In general, fungal communities were more connected in native plant communities than exotic, especially below 10 cm. Fungal networks were also more connected at lower soil depths albeit with fewer nodes. Bacterial communities demonstrated higher complexity, and greater connectedness and nodes, at lower soil depths for native plant communities. Exotic plant communities’ bacterial network connectedness altered at lower soil depths dependent on irrigation treatments. Microbial extracellular enzyme activity for carbon cycling enzymes significantly declined with soil depth, but enzymes associated with nitrogen and phosphorus cycling continued to have similar activities up to 1 m deep. Our results indicate that native and exotic grasslands have significantly different fungal communities in depths up to 1 m and that both fungal and bacterial networks are strongly shaped jointly by plant communities and abiotic factors. Soil depth is independently a major determinant of both fungal and bacterial community structures, functions, and co-occurrence networks and demonstrates further the importance of including soil itself when investigating plant–microbe interactions.

Keywords
bacteria, exotic plant species, fungi, invasive species, irrigation, native grasslands, network analysis, soil depth

Disciplines
Biodiversity | Ecology and Evolutionary Biology | Plant Sciences | Soil Science

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Soil depth and grassland origin cooperatively shape microbial community co-occurrence and function

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INTRODUCTION

Exotic plant species are increasing in abundance, often forming novel ecosystems with species that lack an evolutionary history (Hobbs et al. 2006, 2013, Wilsey et al. 2009, Cook-Patton et al. 2011). Novel ecosystems are especially prevalent in grasslands, both because introduced species are abundant and grasslands have been subject to intensive disturbances, including modifications for pasture and urban development (Samson et al. 2004, Hoekstra et al. 2005, Stephens et al. 2008).

Native and exotic plant species can differ in tissue nutrient quality, net primary productivity, carbon (C)-to-nitrogen (N) ratios, and rooting depths, all of which can impact soil microbial communities. Native plant species can have greater root biomass below the soil surface (20–45 cm) compared with exotic plant species (Wilsey and Polley 2006). Thus, a shift from native to exotic plant species could have large and long-lasting impacts on soil microorganisms and elemental cycling by altering substrate inputs (Seastedt et al. 2008, Pries et al. 2018). These differences could be exacerbated if native and exotic plant species respond differently to climatic events, including extreme rainfall or drought events, due to their different rooting depths (Singh et al. 1998, Heisler-White et al. 2009, Hodge 2009, Maestre et al. 2012, Hoover et al. 2015, Bernardo et al. 2016). Differences in native and exotic rhizosphere responses to changes in rainfall patterns alter plant-microbe interactions both directly and indirectly, with possible differences occurring with soil depth. Relatively few studies have sampled microbial communities beyond the top 30 cm of the soil rhizosphere (Jackson et al. 1996, Fierer et al. 2003, Eilers et al. 2012). The median depth for soil C studies was only 15 cm in 2001, and this value increased only slightly to 20 cm when updated in 2017 (Conant et al. 2001, 2017). Yet, it is known that high levels of soil organic matter (SOM) can persist up to 1 m or deeper in soil orders that commonly support grasslands (Mollisols and Vertisols). Interactions between plants and microbes deeper in the soil profile may contribute to C accumulation, resulting in over two-thirds of soil C occurring below 30 cm (Balesdent et al. 2018), as evidenced in the large pool (~2000 Pg) of organic C in the top 1 m of Earth’s soil (Janzen 2005).

The vertical distribution of microbial communities in soils is hypothesized to be strongly correlated with differences in soil pH, redox potential, water, oxygen, porosity, and nutrient distribution with depth (Wynn 2007, Manzoni and Porporato 2009, Kempen et al. 2011). Therefore, microbial communities, intercommunity interactions, and their associated biogeochemical processes could persist at lower soil depths, but this remains unresolved in grassland ecosystems.

Co-occurrence network analysis is able to bridge species abundance data in the environment to geographic distributions of biotic relationships and is particularly useful in microbial ecology (Baselga and Aratújo 2009, Barberán et al. 2012, Cazelles et al. 2016). Microbial networks measure co-occurrence among species, using correlations among OTUs (operational taxonomic units) to develop a graphical representation of potential species interactions. Microbial network analysis can be used to generate ecosystem-wide associations on a microbial scale (Faust and Raes 2012). Negative correlations represent competitive and interference interactions, and positive interactions can occur due to shared resource use or facilitation (Lidicker 1979, Faust and Raes 2012, Berry and Widder 2014). How microbial networks will be altered due to changes in species composition of plant communities and varied precipitation remains understudied. We investigated how this change from native to exotic plant species as well as novel precipitation regimes altered components of microbial co-occurrence networks, including connectedness, complexity, node distribution, and the abundance of individual microbes with significant correlative interactions across soil depths.

We compared microbial composition, diversity, enzyme activity, and network structure between native and exotic plant communities, with and without summer irrigation at four soil depths to 100 cm. We studied the long-term Maintenance of Exotic vs. Native Diversity (MEND) experimental site in central Texas (Temple, Texas, USA), which is composed of plots with mixtures of either all native or all exotic perennial plant species (Wilsey et al. 2011). Exo-
tic plant species were from multiple continents (Africa, Asia, South America, and Europe). Change in both plant species treatment and watering regime created novel conditions compared with native and nonirrigated conditions. We hypothesized that (1) with increasing depth, the composition and network structure of both fungal and bacterial communities will change, and diversity and enzymatic function will decrease; (2) microbial enzymatic function and network connectedness will be weaker in exotic plots due to legacy effects of plants and microbes historically co-occurring together in native grasslands; this effect should be most pronounced at deeper soil depths; and (3) irrigation will alter both native and exotic microbial networks, increasing the overall connectedness of the system.

MATERIALS AND METHODS

Research site

The Maintenance of Exotic vs. Native Diversity (MEND) experiment was established in 2008 at the Grassland, Soil, and Water Research Laboratory (Wilsey et al. 2011, 2014, Polley et al. 2016) in Temple, Texas, USA. A detailed description of the experiment can be found in Wilsey et al. (2011, 2014). Briefly, the design had a factorial treatment of all native or all exotic plant species (plant community treatment) crossed with a summer irrigation treatment (128 mm per year added from 15 July to 15 August or no additions as a control) with 16 plots per treatment, 191 m in size. The 64 experimental mixtures were planted to identical plant densities, functional group proportions, and species diversity to determine whether differences develop over time between native (U.S. origin species) and exotic (Africa, Asia, South America, and Europe) plant communities with and without altered water input. Either all native species or all exotic species were assigned to 9 species mixtures from a species pool of 18 native and 18 exotic species. Each exotic was paired with a phylogenetically related native species. We executed eight random species draws per treatment, with two true replicates per random draw and treatment (n = 16 mixtures in each of the four treatments). Plots of plant mixtures were separated by 2-m alleyways seeded with Bouteloua curtipendula (Daneshgar et al. 2013).

Soil sampling and DNA extraction

One soil core (4.2 cm diameter) to 100 cm depth was collected from each mixture plot (n = 64) at peak biomass in October 2015. The soil samples were stored on dry ice and immediately shipped to Iowa State University (Ames, Iowa, USA), and then separated into four layers: 0–10, 10–30, 30–60, and 60–100 cm. The separated soil depths were subsampled for soil pH analysis (air-dried), DNA extraction (stored at −80°C), and enzymatic assays (stored at −20°C). Microbial DNA was isolated from 0.25 g of soil using the PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, USA) following the manufacturer’s instructions. DNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). The DNA samples were stored at −80°C prior to further processing.

Library preparation and amplicon sequencing

Ampliﬁcations were prepared and paired-end Illumina MiSeq sequencing was performed at Argonne National Laboratory (Lemont, Illinois, USA) using the Earth Microbiome Project methodologies. The amplification of the V4 region of 16S rRNA gene and internal transcribed spacer (ITS) region for construction of amplicon libraries was performed using separate primers 515F/806R (bacterial, EMP, http://www.earthmicrobiome.org/protocols-and-standards/16s/) and ITS1F/ITS2 (fungal, Smith and Peay 2014, EMP, http://www.earthmicrobiome.org/protocols-and-standards/its/), respectively, following the sequencing protocol by Caporaso et al. (2010). Following the multiplexing protocol, agarose gel electrophoresis was used to determine the presence of PCR products, and DNA concentration was measured with Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, California, USA).

Raw sequence processing

Fungal and bacterial sequences were processed through the open-access “hundo” v1.1.6 pipeline (Brown et al. 2018). Fungal and bacterial sequences were demultiplexed with EA-Utils (Aronesty 2013) with zero mismatches allowed in
the barcode sequence followed by quality filtering with BBDDuk2 (Bushnell 2014) for removal of adapter sequences and PhiX with matching kmer length of 31 bp at a hamming distance of 1. Any reads shorter than 51 and 175 bp were removed for bacterial and fungal samples, respectively. USEARCH was used to merge reads (Edgar 2013) with a minimum length threshold of 175 bp and maximum error rate of 1%. After sequences were dereplicated (minimum sequence abundance of 2), they were clustered using USEARCH (Edgar 2013) at 97% pairwise sequence identity among operational taxonomic unit (OTU) member sequences. Concurrently, de novo prediction of chimeric sequences was performed during clustering. BLAST was used to assign taxonomy to OTU sequence (Camacho et al. 2009) alignments followed by least common ancestor assignments across UNITE v.7 database for assigning the taxonomy clustered at 97% (Quast et al. 2012). OTU seed sequences were filtered against UNITE v.7 clustered at 97% to identify chimeric OTUs using USEARCH. Sequences were aligned using Clustal Omega (Sievers et al. 2011), and FastTree2.0 (Price et al. 2010) was used to generate the phylogenetic tree in a Newick format.

**Extracellular enzyme activity assays**

Microbial decomposition was measured by analysis of extracellular enzyme activities (EEA). The studied EEA were involved in C, N, and phosphorus (P) cycling. We measured ß-1,4-glucosidase (EC 3.2.1.21, BG, which hydrolyzes cellulose into glucose), ß-1,4-xylosidase (EC 3.2.1.37, BX, which degrades hemicellulose), cellobiohydrolase (EC 3.2.1.91, CB, an exocellulase), 1,4-ß-N-acetyl-glucosaminidase (EC 3.2.1.52, NAG, which breaks down chitin and peptidoglycan, and hydrolyzes glucosamine from chito-bose), and acid phosphatase (EC 3.1.3.2, AP, which liberates phosphate). Extracellular enzyme activities were measured using the modified protocol from German et al. (2011) and Hargreaves and Hofmockel (2015). Briefly, 1 g of soil was homogenized with 125 mL of 100 mmol/L Tris buffer titrated to pH 8.0. Enzyme activity was induced with the addition of methyumbelliferone (MUB)-linked substrates, and all plates were incubated for 3 h at 23°C. At the end of the incubation, 10 μL of 1M NaOH was added and plates were read using a fluorometer after a 2-min development period (360-nm excitation and 460-nm emission; BioTek Instruments, Winooski, Vermont, USA). Eight analytical replicates per sample and substrate combination were run, and each plate included replicate MUB standard curves, substrate controls, and homogenate controls. Enzyme activity potential was calculated as nmol/g of dry soil/h based on MUB standard curves and accounting for the quench of each sample (German et al. 2011). Linearity of the reaction was confirmed for the 3-h incubation, and all reactions were run at saturating substrate concentrations (400 μmol/L for BG, BX, CB, and NAG; 800 μmol/L for AP).

**Soil pH**

Soil pH was determined by using an air-dried soil sample from each plot. Ten grams of soil was combined with 20 mL of deionized water. The suspension was stirred intermittently for 30 min and then let to stand for 1 h at room temperature. Soil pH was then measured using a pH meter electrode, and the reading was recorded once constant (Carter 1993).

**Statistical analyses**

The effects of plant community treatment (native vs. exotic), irrigation (0 or 128 mm), and soil depth (0–10, 10–30, 30–60, 60–100 cm) on soil pH and EEA were determined using a split-plot ANOVA in R version 3.3 (R Core Team 2018) where depth was considered a subplot term and draw and rep were random terms. Plant community treatment (native vs. exotic) and irrigation (0 or 128 mm) were considered fixed effects main plot terms with draw and rep as random terms. Extracellular enzyme measurements were log-transformed as necessary to meet normality requirements, and samples with activity measurements below detection were removed prior to statistical analysis.

There were 19,044 unique bacterial OTUs and 4993 fungal OTUs. The number of sequences per sample was normalized by rarefaction to 2200 sequence reads (bacteria) and 1047 sequence reads (fungi). The normalized data were used in all statistical tests. Diversity was estimated by calculating Simpson’s diversity (1/∑p2), Chao1 richness estimator, Shannon’s diversity index (Σplnp), and phylogenetic diversity (PD whole tree). The results were used for a split-plot
analysis of variances (ANOVA) where depth was considered a subplot term in the split plot as explained above. Fungal Simpson’s reciprocal measures were log_{10}-transformed prior to ANOVA analysis to meet normality requirements. The effects of plant community treatment (native vs. exotic), irrigation (0 or 128 mm), and soil depth (0–10, 10–30, 30–60, 60–100 cm) on the relative abundance of bacterial and fungal phyla were determined using the same split-plot ANOVA as described for soil pH.

Permutational multivariate analysis of variance (PERMANOVA) analysis was performed on Bray-Curtis dissimilarity measures to determine the main effects on the normalized OTU abundances for fungal and bacterial communities separately using the vegan package (Oksanen et al. 2007, 2013) and Adonis function in R Studio version 1.0.136 (R Core Team 2018). In order to be consistent with a split-plot design, the analysis was performed with two different models. The first model was performed with a two-way factorial design on the main plot treatments (native vs. exotic × 2 levels of summer irrigation, 0 or 128 mm, and their interaction) with 999 permutations with OTU abundance averaged across depth treatments. A second model was then run on the subplot treatments to determine how depth (0–10, 10–30, 30–60, 60–100 cm) and interactions between depth and main plot treatments impacted OTU abundance. Beta diversity, indicative of community composition turnover between plots, was tested across soil depths with a permutation test of homogeneity of multivariate dispersion using the betadisper function and average distances to the group centroids (Oksanen et al. 2013). Beta diversity was then analyzed with the mixed model ANOVA to determine whether depth or depth’s interactions with main plot treatments (plant community treatment and irrigation) had a significant effect on average centroid distance. Tukey’s HSD (honestly significant difference) post hoc test was used to determine significant differences in beta diversity measurements in different soil depths. Soil pH was used to determine whether there were significant correlations to fungal and bacterial community composition along the soil vertical gradient. Pearson’s correlation was performed with the eigenvalues generated from OTU abundance variability of the fungal and bacterial communities separately with soil pH using the envfit function.

The rarefied fungal data were used to determine fungal ecological guilds with FUNGuild (https://github.com/UMNFuN/FUNGuild) for native and exotic fungal communities and in individual soil depths. The same split-plot ANOVA procedure as described for soil pH was performed on the distribution of fungal guilds.

**Network analyses**

All network analyses were performed on fungal and bacterial sequence abundance separately. Two taxonomic levels were explored, individual OTUs and families. Prior to network analysis, microbial sequence abundance data were limited to include only OTUs or families (dependent on taxon level analyzed) with at least 2 reads in 10% of samples for each treatment, and this removed <1% of OTUs found in each sample. Unassigned OTUs, at either individual OTU level or family level, were pooled into one group. Co-occurrence relationships were determined by performing a pairwise Spearman’s ranked correlation on microbial abundance following normalization using the rcorr function in Hmisc package. Following, the fdrtool function was used to detect false discovery relationships, q-values. Only strong microbial co-occurrence relationships with a q-value of <0.05 and a rho (r-value ranked) of >0.7 or <-0.7 for OTUs and >0.5 or <-0.5 for families were included in network analyses. For each network, a random network generator was used to create random networks (1000 permutations) of the same size, same edge (the number of unique relationships in a network), and node count (the number of unique individuals, dependent on taxonomic level, that have a significant relationship included in the network), to assure the observed networks are nonrandom (Ju et al. 2014). Using igraph function graph.edgelist, each treatments’ network was generated and the betweenness (number of geodesics going through a node or edge), transitivity (probability measurement that the adjacent node or nodes are connected, clustering coefficient), mean distance (mean of the shortest paths to and from one node to another in a network, weighted on correlation strength), all distances (shortest path based on weight, correlation strength to one node from another), and closeness (distance or how many
steps from each individual node within the network to every other node within the network in all directions) were determined. Modularity (the number, strength, and size of subnetworks within each treatment) was calculated using the Louvain function in iGraph and resulted in modules and modularity measures for each treatment. We utilized a bootstrapping function using 1000 permutations to determine the 95% CI on network distance and closeness measurements. Following that, a split-plot ANOVA, as described for soil pH, was performed to determine whether plant community treatment (native vs. exotic), irrigation (0 or 128 mm), and soil depth (0–10, 10–30, 30–60, 60–100 cm) had a significant effect on network distances and closeness. Prior to the split-plot ANOVA, network measurements were log_{10}-transformed as needed to meet normality requirements.

**RESULTS**

**Fungal diversity and composition**

Shannon’s fungal diversity was significantly greater in exotic than native plant communities ($F_{1,60} = 7.22, P = 0.008$). However, no other fungal diversity measure was significantly impacted by plant community treatment (Fig. 1a, b) or

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![Fig. 1](https://www.esajournals.org/)

Fig. 1. Fungal and bacterial diversity measurements (a) fungal Chao1 richness, (b) fungal Simpson's 1/D, (c) bacterial Chao1 richness, and (d) bacterial Simpson's 1/D (error bars ± 1 SE) by plant community and soil depth treatments.
irrigation. Fungal OTU relative abundance was significantly affected by plant community treatment and accounted for approximately 5.8% of variance (Bray-Curtis, $F_{1,60} = 7.22$, $P = 0.015$), but neither irrigation nor the irrigation–plant community interaction was significant ($P > 0.05$; Table 1).

All diversity measures of fungal taxa declined with soil depth, with an approximate 50% decrease in richness between 0–10 and 60–100 cm (Chao1; $F_{3,220} = 46.58$, $P < 0.001$, Fig. 1a, Simpson’s; $F_{3,220} = 13.495$, $P < 0.001$, Fig. 1b, Shannon’s; $F_{3,220} = 13.495$, $P = 0.008$, PD; $F_{3,220} = 39.7$, $P < 0.001$). Fungal beta diversity (average distance from group centroids) was higher in surface layers and declined with depth ($P < 0.001$, Fig. 2a). The taxonomic composition of the fungal community was also significantly affected by soil depth (Bray-Curtis, $F_{3,203} = 36.51$, $P < 0.001$). The dominant fungal phyla at all four depths were as follows: Ascomycota, Basidiomycota, Zygomycota, and taxonomically unclassified OTUs (Table 2).

The average relative abundance of unclassified OTUs increased with depth from 21.84 ± 1.81% in 0–10 cm to 48.67 ± 2.2% in 60–100 cm (Table 2). Soil depth accounted for 15.4% of variance in fungal community composition with no interaction with plant community or irrigation (PERMANOVA, $P < 0.001$).

**Fungal guilds**

FUNguild was used to categorize the fungal OTUs into ecological guilds. Unfortunately, the majority of the fungal OTUs found in this study were not assigned to ecological guilds, which is very common in grassland studies due to smaller, less complete fungal databases than in other ecosystems. Out of 2076 fungal OTUs detected, 523 OTUs were assigned to various ecological guilds. Unassigned fungal OTUs were not included in further statistical analyses. Based on the number of reads, the majority of the guilds were classified as saprotrophs (unidentified, wood) and plant pathogens (Table 3), followed by multifunctional groups. Wood saprotrophs were significantly higher in exotic plant communities ($F_{1,27} = 4.16$, $P = 0.05$) and varied with depth ($F_{3,171} = 4.77$, $P = 0.003$) with peak abundance occurring in 30–60 cm. A subset of animal and plant pathogens (animal pathogen–endophyte–plant pathogen–wood saprotroph) was significantly higher in native plots than exotic plant communities ($F_{1,27} = 5.79$, $P = 0.017$). The most prevalent group, undefined saprotrophs, was significantly affected by soil depth with an increase in relative abundance at greater depth ($F_{3,127} = 8.51$, $P < 0.0001$).

**Bacterial diversity and composition**

Bacterial diversity was similar between plant community and irrigation treatments ($P > 0.05$). The average relative abundance of bacterial phyla did not differ between native and exotic plots or with irrigation. Bacterial OTU abundance was not significantly impacted by plant community treatment, irrigation, or their interaction, when averaging over 1 m of soil (PERMANOVA, $P > 0.05$; Table 1).

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**Table 1. The results of PERMANOVA analyses for fungal and bacterial community OTU abundance.**

| Source                        | df | F   | P    |
|-------------------------------|----|-----|------|
| **Fungi**                     |    |     |      |
| Plant community               | 1  | 1.49| 0.02 |
| Irrigation                    | 1  | 0.86| 0.8  |
| Plant community × irrigation  | 1  | 0.80| 0.89 |
| Main plot residual            | 59 |     |      |
| Depth                         | 3  | 10.2| 0.001|
| Depth × plant community       | 3  | 0.93| 0.67 |
| Depth × irrigation             | 3  | 0.81| 0.92 |
| Depth × plant community × irrigation | 3 | 0.99 | 0.44 |
| Subplot residual              | 230|     |      |
| Total                         | 245|     |      |
| Total                         | 62 |     |      |
| **Bacteria**                  |    |     |      |
| Plant community               | 1  | 1.0 | 0.39 |
| Irrigation                    | 1  | 0.55| 0.86 |
| Plant community × irrigation  | 1  | 1.62| 0.12 |
| Main plot residual            | 60 |     |      |
| Total                         | 65 |     |      |
| Depth                         | 3  | 14.9| 0.001|
| Depth × plant community       | 3  | 0.83| 0.82 |
| Depth × irrigation             | 3  | 0.79| 0.9  |
| Depth × plant community × irrigation | 3 | 0.99 | 0.42 |
| Subplot residual              | 208|     |      |
| Total                         | 223|     |      |

*Notes:* Plant community treatment (native vs. exotic), irrigation (0 or 128 mm), and their interaction were all main effects in the PERMANOVA, and OTU abundance was averaged across depth for main plot tests. Subplot effects of soil depth and its interaction with main plots were also tested.
Bacterial diversity and composition differed among soil depths but not between plant communities or irrigation treatments as shown by Bray-Curtis dissimilarity measurement and subsequent PERMANOVA. Diversity declined significantly with soil depth for all measures, up to 20% decrease at 60–100 cm comparatively to shallow soil depths (Chao1; $F_{3,218} = 26.08$, Fig 1c, Simpson’s; $F_{3,218} = 35.02$, Fig 1d, Shannon’s; $F_{3,218} = 31.7$, PD; $F_{3,218} = 20.25$, all, $P < 0.001$). Soil depth had a significant impact on bacterial phyla abundance for all phyla except Cyanobacteria, Fusobacteria, Gracillibacteria, and low-abundance phyla group together as other (Table 2). Depth significantly impacted bacterial OTU abundance (PERMANOVA, $P < 0.0001$; Table 1, Fig. 2b), but unlike fungi there was no significant difference in average centroid distance ($P > 0.05$, Fig. 2b). Distinct communities were found at each depth, with little overlap (Bray-Curtis, $P < 0.0001$).

**Enzyme activities**

Of the five enzymes tested, only C-cycling enzymes, β-1,4-glucosidase (BG) and β-1,4-xylosidase (BX), showed significantly higher activities in shallow soil layers compared to deeper soil (BG, $F_{3,122} = 21.49$, $P < 0.001$; BX, $F_{3,29} = 13.58$, $P = 0.06$; Appendix S1: Table S1). β-1, 4-glucosidase and β-1,4-xylosidase activities were highest in the top layer of the soil and dropped significantly when measured at 30 cm and below, remaining at a similar level between 30 and 100 cm (Fig. 3); neither BG nor BX activity was significantly affected by plant community treatment, irrigation, or their interaction. Plant community treatment, irrigation, depth, or their interactions did not have a significant effect on CB, NAG, or AP, all of which exhibited relatively low activity (Appendix S1: Table S1).

**Soil pH**

Soil pH values were slightly basic and increased significantly with depth ($P < 0.0001$; Fig. 4). There was no interactive effect of depth, plant community, or irrigation on pH. In addition, pH significantly correlated with OTU abundance, and community composition, for both fungal and bacterial communities ($P < 0.001$; Fig. 2).

**Network analyses**

**Fungi.—** All networks were found to be nonrandom. Fungal networks were more highly connected (increased linkages, lower distance) in
native plots than exotic plots (Appendix S1: Table S2). Distance and closeness were all significantly impacted by plant treatment and plant treatment by irrigation interaction, but not irrigation (Table 4). Irrigation had opposing effects dependent on plant community; in native communities, irrigation increased connectedness, whereas in exotic communities, there was a decrease in linkages (Fig. 5). Soil depth had large effects on co-occurrence networks, distance \( (F_{3,47} = 3.34; \ P = 0.03) \) and closeness \( (F_{3,47} = 9.009; \ P < 0.0001; \ Table 4) \). Across all main plot treatments, plant community and irrigation, there was increased closeness and complexity, in lower soil depths, reflected in the significant interactions between depth and the main plot effects \( (F_{3,47} = 1,441; \ P < 0.0001) \). In all treatments, node count decreased with depth, while edge count increased, creating simpler but more connected networks (Fig. 6; Appendix S1: Table S3). Family-level analyses revealed greater connectedness in deeper soil depths under native plots, but lower connectedness with exotic plots, reflecting the same patterns as seen at the OTU level.

**Bacteria.** In general, there were higher numbers of bacterial edges and nodes, as well as a greater number of negative interactions than

### Table 2. The mean relative abundance of bacterial and fungal phyla by soil depth (phyla with less than a mean 0.1% abundance in any soil depth were not included).

| Phylum                | Soil depth          | Mean | SE   | Mean | SE   | Mean | SE   | Mean | SE   |
|-----------------------|---------------------|------|------|------|------|------|------|------|------|
|                       | 0–10 cm             |      |      |      |      |      |      |      |      |
| Bacteria              |                     |      |      |      |      |      |      |      |      |
| Proteobacteria        | 26.95               | 0.45 | 24.21| 0.38 | 23.5 | 0.36 | 23.69| 0.46 |      |
| Actinobacteria        | 20.9                | 0.62 | 25.33| 0.58 | 27.01| 0.51 | 29.83| 0.69 |      |
| Acidobacteria         | 17.61               | 0.4   | 16.62| 0.4  | 14.52| 0.36 | 12.75| 0.26 |      |
| Verrucomicrobia       | 9.81                | 0.25 | 9.51 | 0.25 | 8.55 | 0.22 | 6.56 | 0.2  |      |
| Bacteroidetes         | 6.49                | 0.44 | 3.68 | 0.22 | 2.74 | 0.22 | 1.64 | 0.19 |      |
| Chloroflexi           | 5.77                | 0.25 | 7.03 | 0.27 | 8.78 | 0.32 | 10.82| 0.27 |      |
| Planctomycetes        | 4.9                 | 0.14 | 4.36 | 0.09 | 3.65 | 0.12 | 2.77 | 0.09 |      |
| Firmicutes            | 1.84                | 0.08 | 1.63 | 0.12 | 1.17 | 0.07 | 1.09 | 0.13 |      |
| Gemmatimonadetes      | 1.33                | 0.12 | 2.52 | 0.17 | 3.87 | 0.25 | 3.54 | 0.22 |      |
| Nitrospirae           | 1                   | 0.09 | 1.41 | 0.1  | 2.51 | 0.16 | 4.04 | 0.18 |      |
| Unknown bacteria      | 0.97                | 0.05 | 1.3  | 0.04 | 1.37 | 0.04 | 1.2  | 0.03 |      |
| Latiescibacteria      | 0.47                | 0.03 | 0.54 | 0.03 | 0.38 | 0.03 | 0.22 | 0.01 |      |
| Elusimicrobia         | 0.32                | 0.02 | 0.23 | 0.01 | 0.2  | 0.02 | 0.14 | 0.01 |      |
| Armatimonadetes       | 0.31                | 0.01 | 0.32 | 0.01 | 0.32 | 0.01 | 0.28 | 0.01 |      |
| Cyanobacteria         | 0.22                | 0.05 | 0.18 | 0.02 | 0.22 | 0.05 | 0.16 | 0.04 |      |
| TA06                  | 0.16                | 0.01 | 0.22 | 0.01 | 0.25 | 0.01 | 0.26 | 0.01 |      |
| TM6                   | 0.14                | 0.02 | 0.1  | 0.01 | 0.07 | 0.01 | 0.04 | 0.01 |      |
| Chlamydiae            | 0.13                | 0.01 | 0.1  | 0.01 | 0.07 | 0.01 | 0.06 | 0.01 |      |
| Chlorobi              | 0.12                | 0.02 | 0.09 | 0.01 | 0.1  | 0.01 | 0.15 | 0.02 |      |
| Paracutes              | 0.11                | 0.01 | 0.11 | 0.01 | 0.14 | 0.03 | 0.1  | 0.01 |      |
| Saccharibacteria      | 0.1                 | 0.02 | 0.09 | 0.01 | 0.06 | 0.01 | 0.06 | 0.01 |      |
| Microgenomates        | 0.07                | 0.01 | 0.09 | 0.01 | 0.09 | 0.01 | 0.11 | 0.01 |      |
| OP3                   | 0.05                | 0.0  | 0.07 | 0.01 | 0.1  | 0.01 | 0.07 | 0.01 |      |
| Fungi                 |                     |      |      |      |      |      |      |      |      |
| Ascomycota            | 47.41               | 1.66 | 50.09| 1.87 | 41.78| 1.86 | 34.01| 1.75 |      |
| Unknown Fungi         | 21.84               | 1.81 | 27.15| 1.78 | 38.45| 2.19 | 48.67| 2.20 |      |
| Basidiomycota         | 16.04               | 1.56 | 16.54| 1.39 | 15.70| 1.14 | 15.57| 1.38 |      |
| Zygomycota            | 12.77               | 1.55 | 7.33 | 0.68 | 3.57 | 0.71 | 1.25 | 0.35 |      |
| Blastocladimycota     | 0.91                | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |      |
| Glomeromycota         | 0.56                | 0.08 | 0.39 | 0.04 | 0.38 | 0.05 | 0.35 | 0.05 |      |
| Rozellomyces          | 0.37                | 0.10 | 0.05 | 0.02 | 0.04 | 0.01 | 0.12 | 0.11 |      |
| Chytridiomycota       | 0.10                | 0.03 | 0.05 | 0.01 | 0.08 | 0.06 | 0.03 | 0.01 |      |
Table 3. The mean relative abundance (%) for each FUNguild in individual soil depths and plant community treatments, native vs. exotic (rare guilds, <0.10% relative abundance, were combined into one category, other, rare).

| Guild                                                                 | 0–10 cm Native | 0–10 cm Exotic | 10–30 cm Native | 10–30 cm Exotic | 30–60 cm Native | 30–60 cm Exotic | 60–100 cm Native | 60–100 cm Exotic |
|-----------------------------------------------------------------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Undefined Saprotroph                                                 | 46.98         | 59.79         | 55.61          | 49.45          | 59.94          | 47.89          | 63.65          | 65.64          |
| Plant Pathogen                                                       | 12.95         | 7.12          | 15.99          | 9.50           | 10.11          | 9.66           | 11.52          | 10.08          |
| Plant Pathogen–Soil Saprotroph–Wood Saprotroph                      | 10.29         | 11.01         | 7.33           | 5.58           | 3.02           | 3.72           | 1.53           | 4.54           |
| Wood Saprotroph†                                                     | 7.92          | 7.59          | 5.78           | 17.42          | 12.20          | 24.04          | 5.04           | 6.06           |
| Dung Saprotroph–Soil Saprotroph–Wood Saprotroph                     | 3.90          | 2.40          | 0.69           | 0.38           | 0.11           | 0.22           | 0.00           | 2.54           |
| Animal Pathogen–Endophyte–Fungal Parasite–Plant Pathogen–Wood Saprotroph | 3.69          | 0.92          | 1.32           | 0.41           | 0.86           | 0.74           | 0.33           | 0.20           |
| Animal Pathogen–Endophyte–Plant Pathogen–Wood Saprotroph†           | 3.37          | 1.19          | 3.86           | 1.59           | 5.74           | 2.70           | 5.47           | 3.89           |
| Dung Saprotroph–Endophyte–Undefined Saprotroph                      | 3.21          | 2.21          | 2.21           | 6.13           | 2.48           | 4.39           | 0.50           | 0.86           |
| Arbuscular Mycorrhizal                                               | 2.52          | 2.14          | 2.38           | 2.26           | 2.68           | 2.16           | 2.68           | 3.06           |
| Lichenized                                                           | 1.76          | 0.59          | 0.77           | 1.34           | 0.02           | 0.38           | 0.00           | 0.02           |
| Ectomycorrhizal                                                      | 0.78          | 0.60          | 0.35           | 0.74           | 1.04           | 1.37           | 0.72           | 0.74           |
| Fungal Parasite                                                      | 0.62          | 0.80          | 1.15           | 0.90           | 0.32           | 0.34           | 0.19           | 0.05           |
| Dung Saprotroph–Undefined Saprotroph–Wood Saprotroph                | 0.56          | 0.00          | 0.02           | 0.00           | 0.00           | 0.00           | 0.02           | 0.00           |
| Ectomycorrhizal–Wood Saprotroph                                      | 0.39          | 0.00          | 0.40           | 0.20           | 0.16           | 0.14           | 0.00           | 0.20           |
| Fungal Parasite–Undefined Saprotroph                                 | 0.30          | 0.14          | 0.35           | 0.41           | 0.38           | 0.31           | 0.41           | 0.42           |
| Endophyte–Plant Pathogen                                             | 0.18          | 0.29          | 1.19           | 0.19           | 0.52           | 0.18           | 1.74           | 0.32           |
| Animal Endosymbiont                                                  | 0.16          | 0.08          | 0.02           | 0.05           | 0.00           | 0.02           | 0.00           | 0.00           |
| Soil Saprotroph                                                      | 0.12          | 0.06          | 0.04           | 0.00           | 0.00           | 0.02           | 0.02           | 0.02           |
| Plant Pathogen–Wood Saprotroph                                       | 0.11          | 0.12          | 0.23           | 0.16           | 0.20           | 0.58           | 0.00           | 0.22           |
| Other, rare†                                                         | 0.14          | 2.95          | 0.27           | 3.31           | 0.23           | 1.08           | 6.17           | 0.99           |

† Denoted guilds are significantly different by plant community treatment, and italicized guilds are significantly different by depth.

Fig. 3. Enzyme activities of β-1,4-glucosidase and β-1,4-xylosidase by soil depth and plant community treatment (error bars ± 1 SE).
found in fungal communities. Bacterial communities were also highly impacted by native vs. exotic and irrigation (Appendix S1: Table S2). Irrigation decreased connectedness in both native and exotic plots, but more dynamically in native communities (Fig. 7). Within nonirrigated treatments, native plant communities supported higher connectedness, lower modularity (higher number of OTUs per module), and lower mean distance than exotic plots (Appendix S1: Table S2; Fig. 7). The opposite was found for irrigated plots with exotic plots supporting higher overall connectedness and lower betweenness and modularity (Appendix S1: Table S2; Fig. 7). The opposite was found for irrigated plots with exotic plots supporting higher overall connectedness and lower betweenness and modularity (Appendix S1: Table S2). Bacterial closeness was significantly affected by plant community treatment ($F_{1,14} = 17.4; P = 0.003$), irrigation ($F_{1,14} = 38.1; P = 0.0003$), and their interaction ($F_{1,14} = 1213.5; P < 0.0001$); however, distance was not impacted by any main effect ($P > 0.05$; Table 4). Within individual depths, exotic plant treatments had higher connectedness at shallow soil (0–10 cm) than native plant communities (Appendix S1: Table S3). Below 10 cm, native communities increased in connectedness and node counts, indicating increasing complexity in the networks with deeper depth (Fig. 8). Exotic communities had opposing network structure dependent on irrigation, with irrigated plots becoming more simplistic and less connected at lower soil depths and nonirrigated plots having higher connectedness at our deepest sampling depth 60–100 cm (Fig. 8; Appendix S1: Table S3). Bacterial closeness was significantly impacted by

![Soil pH by soil depth and plant community treatment](image)

Fig. 4. Soil pH by soil depth and plant community treatment (error bars ± 1 SE).

Table 4. The results of split-plot ANOVA for fungal and bacterial community OTU network distance and closeness.

| Source                                | df  | F      | P       |
|---------------------------------------|-----|--------|---------|
| **Fungal closeness**                  |     |        |         |
| Plant community                       | 1   | 32.89  | 0.0004  |
| Irrigation                            | 1   | 0.02   | 0.89    |
| Plant community × irrigation           | 1   | 207.57 | <0.0001 |
| Main plot residual                    | 8   |        |         |
| Depth                                 | 3   | 9009   | <0.0001 |
| Depth × plant community                | 3   | 2810   | <0.0001 |
| Depth × irrigation                     | 3   | 5400   | <0.0001 |
| Depth × plant community × irrigation   | 3   | 1441   | <0.0001 |
| Subplot residual                      | 32  |        |         |
| **Fungal distance**                   |     |        |         |
| Plant community                       | 1   | 26.7   | 0.0008  |
| Irrigation                            | 1   | 2.4    | 0.16    |
| Plant community × irrigation           | 1   | 14.0   | 0.006   |
| Main plot residual                    | 8   |        |         |
| Depth                                 | 3   | 3.34   | 0.03    |
| Depth × plant community                | 3   | 0.91   | 0.45    |
| Depth × irrigation                     | 3   | 0.42   | 0.74    |
| Depth × plant community × irrigation   | 3   | 1.2    | 0.32    |
| Subplot residual                      | 32  |        |         |
| **Bacterial closeness**               |     |        |         |
| Plant community                       | 1   | 17.4   | 0.003   |
| Irrigation                            | 1   | 38.1   | 0.0003  |
| Plant community × irrigation           | 1   | 1213.5 | <0.0001 |
| Main plot residual                    | 8   |        |         |
| Depth                                 | 3   | 7999   | <0.0001 |
| Depth × plant community                | 3   | 27215  | <0.0001 |
| Depth × irrigation                     | 3   | 1309   | <0.0001 |
| Depth × plant community × irrigation   | 3   | 8808   | <0.0001 |
| Subplot residual                      | 32  |        |         |
| **Bacterial distance**                |     |        |         |
| Plant community                       | 1   | 1.62   | 0.24    |
| Irrigation                            | 1   | 0.89   | 0.37    |
| Plant community × irrigation           | 1   | 0.22   | 0.65    |
| Main plot residual                    | 8   |        |         |
| Depth                                 | 3   | 0.14   | 0.94    |
| Depth × plant community                | 3   | 0.73   | 0.54    |
| Depth × irrigation                     | 3   | 0.11   | 0.96    |
| Depth × plant community × irrigation   | 3   | 1.55   | 0.22    |
| Subplot residual                      | 32  |        |         |

Notes: Plant community treatment (native vs. exotic), irrigation (0 or 128 mm), and their interaction were all main effects in the model, and OTU abundance was averaged across depth for main plot tests. Subplot effects of soil depth and its interaction with main plot effects were also tested.
depth and all interactions with the main plot effects (depth: $F_{3,47} = 7.999; P < 0.0001$; depth $\times$ plant: $F_{3,47} = 27.215; P < 0.0001$; depth $\times$ irrigation: $F_{3,47} = 1.309; P < 0.0001$; depth $\times$ plant $\times$ irrigation: $F_{3,47} = 8.808; P < 0.0001$; Table 4). Family-level analyses revealed the same patterns of network changes as seen at the OTU level.

**DISCUSSION**

We sought to understand how replacement of native plant communities with exotic species and altered precipitation regimes affected plant–microbe interactions in the tallgrass prairie region of Texas. Plant–microbe interactions deeper in the soil profile are often neglected in microbial ecology studies, despite the importance of root biomass below 30 cm (Jackson et al. 1996, Fargione and Tilman 2005). Our study demonstrated the strong impact soil depth has on microbial communities. Importantly, microbial communities were compositionally distinct across soil depths, with little overlap, demonstrating unique communities in deeper soils that were not merely a subset of the communities in surface layers. As we found previously (Checinska-Sielaff et al. 2018), fungal communities were more strongly impacted by the plant community treatment than bacterial communities, demonstrating that novel grassland ecosystems have altered soil fungal communities compared with the native grasslands that they replaced. Although irrigation did not alter the composition of detected microbes (active, dormant, relic), the interactions among community members responded to irrigation. Interestingly, fungi
under native and exotic plant communities responded in opposite ways to irrigation. Native plots have greater connections among community members when watered, with fewer, but more connected fungal taxa (nodes), especially below 10 cm. Exotic plots had contrasting response to watering, with fewer connections among community members, and more abundant, but less connected fungal taxa compared with native plots. Bacterial communities demonstrated higher complexity at lower soil depths for native plant communities and irrigation independently influenced bacterial networks. Lastly, microbial extracellular enzyme activity for CB, NAG, and AP did not significantly drop between soil depth layers, indicating enzymatic function continues at deeper soil depths. Soil depth is independently a major determinant of both fungal and bacterial community structures, functions, and co-occurrence networks and demonstrates further the importance of including soil itself when investigating plant–microbe interactions.

**Microbial diversity decreases with depth**

Microbial diversity and richness decreased with depth. While this diversity decrease was predicted (Jumpponen et al. 2010), we still found relatively high diversity at 60–100 cm deep, with an average of over 90 fungal and 750 bacterial
unique OTUs in both native and exotic plant communities. This is indicative of the extreme diversity and persistence of soil microorganisms deeper in the soil. Soil microbial diversity is hypothesized to be a result of many factors including substrate quality and variant C inputs, generating niche partitioning. Carbon inputs are likely greater and more variable in surface than deep soil, the result of more abundant direct litter contributions (Jobbágy and Jackson 2000, Malone et al. 2009). Deeper soils likely receive fewer C inputs, and leachate is likely partially decomposed. Differences in C input may have led to reductions in beta diversity with depth. Inactive or deceased microorganisms can generate large pools of relic DNA. Therefore, we cannot definitively conclude that this represents a pool of active microbes (Lennon et al. 2018). However, dormant microbial communities within soil have also been hypothesized to be powerful pools of potential diversity and can respond rapidly to changes in environmental inputs (Joergensen and Wichern 2018, Kearns and Shade 2018, Nelson et al. 2018). Much of the diversity at deeper soil depths consisted of unknown taxa. As climate changes continue, shifting precipitation patterns, more research is needed on microbial ecology at great soil depth, especially in grassland soil types (Mollisols, Vertisols), which are important global stocks of soil C.

Soil depth alters microbial community composition

Bacterial and fungal community composition was significantly different across soil depths. Previous studies, with targeted sequencing for protists and mycorrhizal fungi, have shown
distinct populations between topsoil and deeper soil communities (Uksa et al. 2015, Moll et al. 2016, Sosa-Hernández et al. 2018, Degrune et al. 2019). We have expanded upon these studies by examining the whole soil microbiome (fungal and bacterial) in two prairie types and have demonstrated the distinct and unique populations found in different soil depths. Deeper soil depths had an increase in Actinobacteria and Nitrospirae. Actinobacteria play important roles in humus formation (Ball et al. 1990), and their increased presence at lower depths could signify their role in the formation of long-term C storage within prairie soils and should be considered in further studies. Additionally, Actinobacteria have mycelia growth patterns in soil, which could allow for greater surface area for nutrient acquisition at lower soil depths, as well as being potential active members in soil aggregation and water and nutrient retention. Nitrospirae are an essential part of N cycle, and their increased presence at deeper depths, suggests dynamic N cycling at 30–100 cm. This is further supported by the sustained NAG enzyme activity at deeper depths, which contributes to N cycling. At lower depths, 30–100 cm, there was a decrease in abundance of several bacterial phyla including Bacteroidetes, Proteobacteria, and Verrucomicrobia. Verrucomicrobia have been hypothesized to be indicators of

Fig. 8. OTU-level bacterial co-occurrence networks by soil depth in each plant community treatment and irrigation, (a) native irrigated, (b) native not irrigated, (c) exotic irrigated, and (d) exotic not irrigated. Individual square nodes represent bacterial OTUs and lines represent significant interactions, \( q \)-value of \(<0.05 \) and rho values of \( >0.7 \) or \( <−0.7 \). Line color indicates directionality of relationship (green lines are positive relationships and red lines are negative relationships).
prairie soil health, occurring more frequently and with higher recalcitrant or more persistent C-cycling enzymatic activity in less amended soils (Ramirez et al. 2012, Fierer et al. 2013); however, given Verrucomicrobia decrease in abundance at lower depths and the unchanged CB activity in our prairie study, their effects could be limited to the surface layers of soil.

Deeper soil layers had greater relative abundance of unknown fungal taxa highlighting the vast diversity and unknownness of soil fungi in deeper soils. These unclassified OTUs might play an important role in the communities, but without a taxonomic or functional role assignment, it is not possible to determine their ecological contributions at the current time. Additionally, the relative abundance of Glomeromycota decreased with soil depth, although it should be noted that there is evidence of ITS sequencing bias against Glomeromycota, potentially explaining their detected overall low abundance (Lindahl et al. 2013). However, when the fungal community was analyzed using the FUNg guild ecological distinction, there was no change in the abundance of arbuscular mycorrhizae across soil depths, suggesting that while the phyla Glomeromycota may have been decreasing the ecological niche for mycorrhizae was being filled by other fungal organisms in deeper soils.

Plant communities alter fungal community composition

Fungal communities varied between native and exotic plant communities, but these differences were much smaller compared with the effect of soil depth. Plant communities explained <6% of variance, whereas soil depth accounted for over 15% of variance in fungal OTU composition. Native and exotic communities differed in fungal community composition in the upper soil layer (up to 10 cm) over eight years in a previous study at this site (Checinska-Sielaff et al. 2018). However, this is the first study to show that conversion from native to exotic plant species has impacts well beyond the surface soil. Soil legacy effects of exotic plant communities, even following eradication, remain unknown. However, the disruption of native soil communities to 1 m depth could indicate a larger impact on the microbiome and soil C storage than has been previously appreciated.

Depth and plant communities cooperatively shape fungal ecological guilds

The ecological guilds of fungi were dominated by unclassified saprotrophs, wood saprotrophs, and plant pathogens. Saprotrophs decompose organic matter and take part in C cycling, especially in grassland soils. The relative abundance of undefined saprotrophs increased with depth, indicating a strong fungal participation in the C cycle at deeper depths. Wood saprotrophs were found to be more abundant in exotic plots, despite the absence of woody plant species. This may reflect the lack of physiological data and the diverse lifestyle of fungal organisms even within individual fungal genera. Additionally, exotic plots had a greater contribution of C4 (warm season) grasses (Martin et al. 2014), which have higher C:N ratios in both roots and shoots, and lower nutrient contents compared with C3 species, and these differences are reflected in the differential abundance of the fungal guilds. Plant pathogen abundance was higher in the upper layers of soil and had a trend of increased abundance in native plots. Given many fungal pathogens are endophytes, their increased abundance in soil layers with greater proximity to above-ground plants is expected (Rodriguez et al. 2009). As our understanding of the function of microbial phylotypes increases, there will be more definitive answers for the ecological ramifications of exotic plant species establishment.

Plant treatment impact on microbial co-occurrence networks is dependent on depth

Bacterial co-occurrence networks had higher number of nodes and linkages than fungal networks reflecting the overall higher diversity of bacterial communities. Bacterial closeness was affected by irrigation independently of the plant community treatment. This may reflect a less complex active bacterial community when irrigated due to higher accessibility to resources, resulting in a less dependencies between individual microorganisms and consortia.

Native and exotic plant treatment effects on bacterial networks were dependent on soil depth. Exotic plant treatments had higher connectedness than native in the shallowest soil layer, 0–10 cm. In lower soil depths, native communities had higher bacterial network connectedness and node counts, demonstrating a
more complex bacterial network than in exotic plots. It is extremely important to realize that without sampling lower soil depths, we would have concluded that exotic plant communities harbored more interconnected microbial communities, which would have been incorrect. By comparing all soil depths to 1 m, we demonstrated that the differences between native and exotic plant communities continue through deeper soil depths.

In fungal communities, native plots supported higher connectedness than exotic plots. Native plant communities have deeper roots and are more diverse than exotic communities (Wilsey et al. 2011, 2014), which could increase niche space availability for soil microbial communities, altering networks (Faust et al. 2012). Fungal distance and closeness did not vary with irrigation independently of plant community treatments, indicating the fungal community responded to plant communities directly while irrigation was mediated through the plant community response.

Fungal connectedness increased with soil depth. Our findings diverge from previous studies on wheat crops, which found at lower soil depths that fungal community networks were simplistic and lacked connectedness (Schlatter et al. 2018). This may reflect the difference in rooting depth and C inputs between cropping systems and grasslands. Agricultural fields typically have lower C inputs, due to decreased root biomass and shallower rooting depth, and this may have led to less connected communities in Schlatter et al. (2018) than we discovered in deeper soils. We hypothesize that one possible mechanism behind the more connected networks in lower soil depths for microbial communities is a reduction in the number of substrates available, due to a decrease in direct root inputs and the possible metabolic by-products that remain in lower depths. This could generate more competition or co-metabolism of the less diverse substrates that are available in lower soil depths. However, co-occurrence networks are correlative and only allow us to predict ecological relationships and not activity. Extracellular enzyme measurements more directly reflect the activity at lower soil depths.

**Microbial function is altered by soil depth**

Activities of the C-cycling enzymes BG and BX were highest in the top layers of the soil where canopy input is greatest. Several factors may play important roles in the enzymatic activity of soil microorganisms across depth, including C and N inputs and soil pH. At lower soil depths, the environment becomes significantly more basic, which could inhibit microbial enzymatic activity. However, this study demonstrates that microbial activity persists at lower soil depths despite more harsh environmental conditions in some enzymes involved with N and P cycling. The continued activities of microbial extracellular enzymes at lower soil depths (up to 1 m deep) demonstrate the dynamic nature of the soil environment. Deeper soil depths should be considered when determining the ecosystem services provided by microbes within terrestrial environments. The influence of the microbial community on ecosystem functioning may continue in lower levels of soil than previously hypothesized and should be included in future biogeochemical models.

**Conclusion**

In conclusion, native and exotic plant species have differing fungal communities that were consistent across the whole 1-m soil vertical gradient. Our conclusions about microbial connectedness changed between considering the top 10 cm of soil and the whole 1-m column. Our results indicate that soil depth is independently a major determinant of both fungal and bacterial community structures, functions, and co-occurrence networks. We uniquely found more complex and highly connected networks at lower soil depths for both fungal and bacterial communities. The proportion of unknown taxa increased with depth, demonstrating that future work should be done on identifying organisms in deeper soil layers. Determining how edaphic and biotic factors impact the biology of the deeper soil horizons may be critical for maintaining terrestrial C stocks.

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DATA AVAILABILITY

The raw sequences were deposited in NCBI Sequence Read Archive (SRA) under no. SRP119532.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2973/full