



Plant communities mediate the interactive effects of invasion and drought on soil microbial communities

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Abstract

Soil microbiomes could play a major role in ecosystem responses to escalating anthropogenic global change. However, we currently have a poor understanding of how soil microbes will respond to interacting global change factors and if responses will be mediated by changes in plant community structure. We used a field experiment to assess changes in soil fungal and bacterial communities in response to plant invasion, experimental drought, and their combination. In addition, we evaluated the relative importance of direct versus indirect pathways of invasion and drought through changes in associated plant communities with structural equation models. We found that fungal communities were interactively structured by invasion and drought, where fungal richness was lowest with invasion under ambient conditions but highest with invasion under drought conditions. Bacterial richness was lower under drought but unaffected by invasion. Changes in the plant community, including lower plant richness and higher root biomass, moderated the direct effects of invasion on microbial richness. Fungal and bacterial functional groups, including pathogens, mutualists, and nitrogen metabolizers, were also influenced by plant community changes. In sum, plant communities mediated the effects of interacting global change drivers on soil microbial community structure, with significant potential consequences for community dynamics and ecosystem functions.

Introduction

Soil microbiomes provide essential ecosystem services that may be altered by interacting global change factors [1]. Changes in microbial community composition in response to multiple global change factors, such as elevated CO₂

concentration, temperature, and precipitation may be non-additive [2, 3], there may be no change [4], or fungi and bacteria may respond differently [5]. These responses are even more uncertain when considering interactions between biotic (e.g., plant invasion) and abiotic (e.g., precipitation) global change factors [6]. Furthermore, microbial communities may be changed directly by global change factors, such as invasion or drought [7], or indirectly through shifts in plant community structure or function in response to altered conditions [8–11]. Alteration of plant communities by multiple global change factors may have indirect consequences for soil microbial communities that are difficult to predict.

Plant communities can play a major role in shaping soil microbial communities via root structure and exudation as well as litter inputs and microclimate effects [9, 12–16]. Non-native invasive plants may change microbial communities directly (e.g., through production of allelochemicals in the rhizosphere that inhibit mutualists) [17]. However, invasive plants also can have impacts on plant communities (e.g., reduced plant diversity) that could indirectly alter microbial communities through, for example, changes in root exudate profiles or availability of host plants [12, 16, 18]. The indirect effects of invasion on microbial

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communities could act in the same direction as the direct effects of the invader or they could counteract the direct effects of the invader.

Greater frequency and severity of regional droughts is likely to produce major changes in soil microbial communities, ecosystem functions, and plant–microbe interactions [19, 20]. Drought can cause rapid declines in microbial activity as well as shifts in the abundance of different microbial taxa, which may influence the rates of decomposition and nutrient cycling [2, 21–23]. Invasive plants could mediate drought effects either through physiological (e.g., water-use) or biophysical (e.g., shading, infiltration) effects [6, 24, 25]. Conversely, drought could modify the effects of invasive plants on soil microbes via changes in invader abundance or altered invader physiology (e.g., root exudates, litter inputs) [26]. Furthermore, changes in abundance of certain fungal and bacterial groups can mediate plant responses to drought [27, 28]. Altogether, plant–microbe interactions are likely to be modified by drought and interactions with plant invaders; however, our ability to predict these changes is limited.

Longleaf pine (*Pinus palustris*) forests with highly diverse understory plant communities historically covered ~30 million hectares of the southeastern US, but only 3% of their original extent remains [29]. Invasive species such as *Imperata cylindrica* (cogongrass), a C₄ grass from Asia [30], can greatly reduce plant diversity of longleaf pine forests and threaten rare native plant species [31, 32]. Climate models suggest that *I. cylindrica* invasion will accelerate as the southeastern US experiences greater frequency and duration of droughts over the next several decades [20, 33]. Therefore, to improve ecosystem management under global change, it is critical to understand the individual and combined impacts of invasion and drought on soil microbial communities.

Modern sequencing technologies allow for assessments of microbial community changes at greater taxonomic resolution and organization of microbes into functional groups, which can improve our understanding of the ecological interactions occurring belowground [34–36]. Consequently, it is possible to evaluate microbial responses to global change, and potential effects on ecosystem functions [37]. Microbes with different functional roles are likely to show divergent responses to interacting global change factors. For example, the invader *I. cylindrica* is thought to produce allelopathic chemicals that reduce ectomycorrhizal colonization of pines [38], but *I. cylindrica* associates with arbuscular mycorrhizal (AM) fungal mutualists, suggesting that AM fungal abundance may increase with invasion [39]. Thus, an improved understanding of the responses of microbial taxonomic and functional groups to invasion and drought is essential for moving towards a mechanistic understanding of plant–microbe–ecosystem interactions.

To evaluate the potential interactive effects of plant invasion and drought on soil microbial communities, we sampled soil from a long-term field experiment to investigate the soil microbiome. Our aims were: (1) to determine how plant invasion and drought independently and interactively affect fungal and bacterial community structure and the relative abundance of taxonomic and functional groups, and (2) to parse out the importance of direct effects of invasion and drought versus the indirect effects through changes in plant community structure. We hypothesized that there would be direct negative effects of both invasion and drought on microbial richness, as well as indirect effects of the global change drivers through changes in the associated plant communities. Specifically, we expected that reductions in plant diversity, pine abundance, and root biomass would reduce microbial richness. Altogether, we aimed to provide an improved mechanistic understanding of soil microbial responses to multiple interacting global change drivers.

Methods

Experimental design

The Bivens Arm Research Site (BARS) was established at the University of Florida in Gainesville, Florida, USA (29°37'42" N, 82°21'14.4" W). Mean annual temperature and precipitation are 20.5 °C and 1300 mm, respectively. Soils are primarily Portsmouth sandy loam composed of Blichton sand (25%; 2–5% slope) and Bivans sand (75%; 5–8% slope; Natural Resources Conservation Service, Web Soil Survey) with soil textures ranging from loamy sand to sandy loam. The study area of ~30 m × 60 m was mowed and tilled to prepare the site. Soils across the plots were similar in particle size distribution and bulk density (Tables S1, S2). In May 2012, plant communities were established in forty 4 m × 4 m plots with 36 native perennial grass and forb seedlings (12 spp. × 3 individuals; The Natives Inc., Davenport, FL) and 20 longleaf pine seedlings per plot (Florida Forest Service, Chiefland, FL). Many additional species colonized the plots from the soil seedbank and the surrounding area [32].

In spring 2013, a blocked factorial combination of *I. cylindrica* “invasion” and precipitation reduction (hereafter referred to as “drought”) was applied (ten replicates per treatment combination). Nine *I. cylindrica* ramets were added into each invaded plot. The drought treatment consisted of rainout shelters with 89% cover of polycarbonate roofing (TUFTEX PolyCarb, Fredericksburg, VA) and gutters connected to pipes to move the water off site. Around each drought plot, belowground plastic barriers were inserted to 1 m depth to inhibit subsurface flow of water, and aluminum flashing extended 10 cm above the

soil surface to reduce overland flow. Structures were constructed over no-drought control plots (hereafter “ambient”) with 22% white shade cloth to mimic shading by the rainout shelters [24]. As extreme climate events are expected to increase in the Southeast US, we aimed to create a severe drought scenario [20, 40]. Based on local historical data, a 100-year drought in the region would be a 66% reduction in precipitation compared with the long-term average. It has been shown that for high levels of rain exclusion, the precipitation removed with rainout shelters tends to be less than the percent cover of roofing [41]. Furthermore, heavy precipitation events and strong winds are common in Florida, so we expected the precipitation reduction to be considerably less than the roofing cover. Volumetric soil moisture was on average 30–50% lower in the drought plots compared with the ambient precipitation plots over the first 3 years of the experiment [24]. The drought treatment remained in place from March 2013 through the time of soil sampling in June 2016.

Soil samples were collected in mid-June 2016 from the experimental plots using a 5 cm diameter hammer corer (AMS Inc., American Falls, ID). Surface plant litter was removed, and three cores per plot were extracted to a depth of 15 cm. The 5–15 cm fraction of the three cores per plot was composited and homogenized through a 2 mm sieve. Separate sieves were used for each treatment and sieves were washed in between samples to reduce the chance of cross-contamination. A 5 g subsample of fresh soil was dried at 105 °C for 72 h and weighed to calculate gravimetric soil moisture. All roots were hand sorted from the soil, washed, dried at 60 °C for 48 h, and weighed. Soils were then frozen at –10 °C for transport and then at –80 °C until DNA extraction.

DNA extraction, PCR, and Illumina sequencing

Genomic DNA was extracted from each soil sample (0.25 g) using MoBio PowerSoil DNA extraction kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA). The fungal ITS2 and bacterial 16S rRNA genes were amplified for each sample using primer sets of 5.8S-FUN/ITS4-FUN [42] and F515/R806 [43], respectively. We used the 5.8S-FUN/ITS4-FUN primers because they were designed to capture the broadest array of fungi and have been shown to work well for specific groups of fungi such as the Glomeromycota [44]. The primers were modified for the Illumina platform by fusing CS1 and CS2 linker primers for forward and reverse primers, respectively.

Polymerase chain reactions were conducted with 50 µL assays. For the fungal ITS2 amplification, 25 µL of GoTaq® Colorless Master Mix, 5 µL of BSA (5 ng µL⁻¹), 0.8 µL of each primer (10 µM), 8 µL of PCR-grade water, and 10.4 µL of a genomic DNA template (5 ng µL⁻¹) were mixed in a

200-µL PCR tube for each sample. The following thermal profile was used for the fungal PCR: an initial denaturation and enzyme activation step of 96 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 40 s, and 72 °C for 120 s, with a final extension of 72 °C for 10 min. For the 16S amplification, 25 µL of GoTaq® Colorless Master Mix (Promega, Madison, Wisconsin, USA), 5 µL of BSA (5 ng µL⁻¹), 1 µL of each primer (10 µM), 15 µL of PCR-grade water, and 3 µL of a genomic DNA template (5 ng µL⁻¹) were mixed in a 200-µL PCR tube for each sample. The following thermal profile was used for bacterial PCR: an initial denaturation and enzyme activation step of 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, with a final extension of 72 °C for 10 min. Qualities of PCR products were evaluated by agarose gel electrophoresis. Additional rounds of PCR were performed to fuse CS1/CS2 linker primers to the indices and adapters (an initial denaturation and enzyme activation at 95 °C for 10 min, followed by 15 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 60 s with a final extension at 72 °C for 10 min) before Illumina MiSeq PE250 sequencing at Génome Québec (Montréal, Québec, Canada). The Illumina sequences were deposited at NCBI (accession number SAMN09623609 to SAMN09623688).

Sequence data processing

QIIME 1.9.0 toolkit [45] was used to process the Illumina sequences using the standard protocol for bacteria, and a protocol developed for fungal sequences amplified by the 5.8-FUN/ITS4-FUN primers [42]. Chimeric 16S and ITS2 sequences were identified using reference-based (May 2013 version of Greengenes database, [46]) and abundance-based methods, respectively, via USEARCH [47], and removed for downstream analyses. Operational taxonomic units (OTUs) were determined at the ≥97% similarity level of the nucleotide sequences [48] using the open-reference OTU picking option. ITS2 sequences were first extracted from representative OTU sequences via ITSx [49]. Taxonomy was assigned to each OTU via BLAST [50] against the UNITE [51] and Greengenes [46] databases for ITS2 and 16S sequences, respectively.

For the fungal ITS2 sequences, non-fungal sequences, which accounted for 38.6% of OTUs and 34.6% of the total sequences, were removed for downstream analyses. We defined “non-fungal sequences” as those that could not be categorized as fungi at the kingdom level against the UNITE database. Because 5.8S-FUN/ITS4-FUN can amplify non-fungal sequences, we used only sequences that we could confidently identify as fungi [52]. The fungal data were rarefied at 13,300 sequences per sample, resulting in a data set with a total of 3562 OTUs. Numbers of identified fungal phyla, classes, orders, families, and genera in the

rarefied fungal data set were 10, 32, 91, 229, and 458, respectively. FUNGuild was employed to categorize the OTUs into trophic modes and guilds, including plant pathogens, mutualists, and saprotrophs [35]. We used only taxa where the confidence ranking for the guild assignment was rated either “probable” or “highly probable”.

For the 16S sequences, *de novo* sequences, which accounted for 90.1% of OTUs but only 18.6% of the total sequences, were removed for downstream analyses. After non-bacterial sequences and singletons were removed, remaining 16S sequences were aligned using PyNAST [45] to build a phylogenetic tree using FastTree [53]. The bacterial sequences were rarefied at 71,166 sequences per sample, resulting in a data set with a total of 8534 OTUs. Numbers of identified bacterial phyla, classes, orders, families, and genera in the rarefied fungal data set were 36, 111, 173, 209, and 284, respectively. The FAPROTAX database was used to extrapolate bacterial taxa into putative functional roles [54]. Further studies would be required to assess gene expression for these putative functions.

Statistical analyses

Statistical analyses were conducted in R 3.4.1 [55]. Mixed-effect models using the nlme package were conducted with the invasion and drought treatments and their interaction as fixed effects and block as a random effect [56]. OTU richness, Shannon diversity index, Pielou’s evenness, and Chao1 index of fungi and bacteria were used as dependent

variables [57]. Plant, fungal, and bacterial community composition were assessed using ordination by nonmetric multidimensional scaling (NMDS) calculated with the “metaMDS” function of the vegan package [58]. Plant community data were taken from an associated publication [32]. The bacterial community was assessed with the weighted and unweighted UniFrac distance matrix, and the fungal community with the Bray–Curtis dissimilarity matrix. We tested for differences in community composition with invasion, drought, and their interactions with permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and permutations constrained within each block using “adonis” from the vegan package [58]. We also tested for homogeneity of group dispersions with the multivariate analog of Levene’s test using the “betadisper” function in the vegan package [59].

The effect of invasion and drought on relative abundance of fungal and bacterial taxa and functional groups that occurred in at least 25% of the sample units was tested using the analysis of composition of microbiomes (ANCOM) with a cut off of $W > 0.75$ [60].

Structural equation models

Piecewise structural equation models (SEMs) were used to compare the direct and indirect effects of the treatments using the piecewiseSEM package [61]. The specific hypothesized interactions included in the models are outlined in Fig. 1. For the indirect effects via changes in the plant

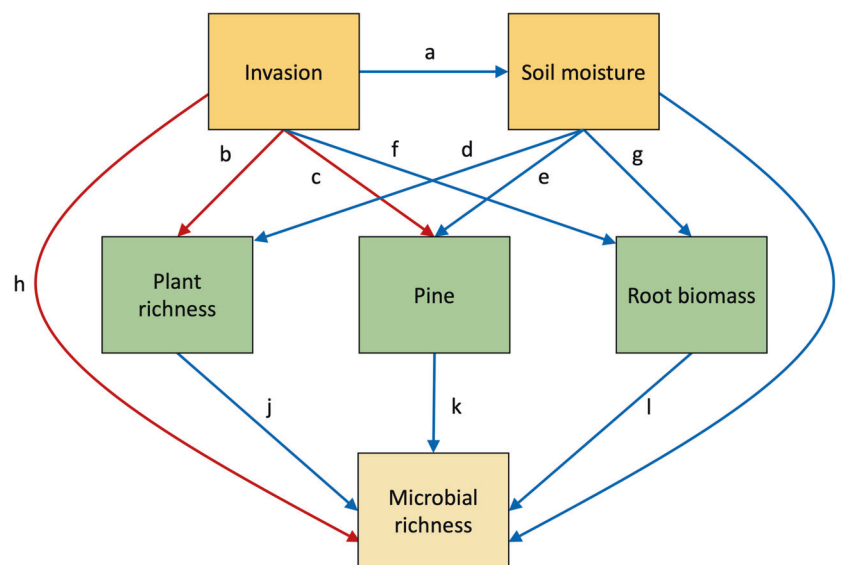
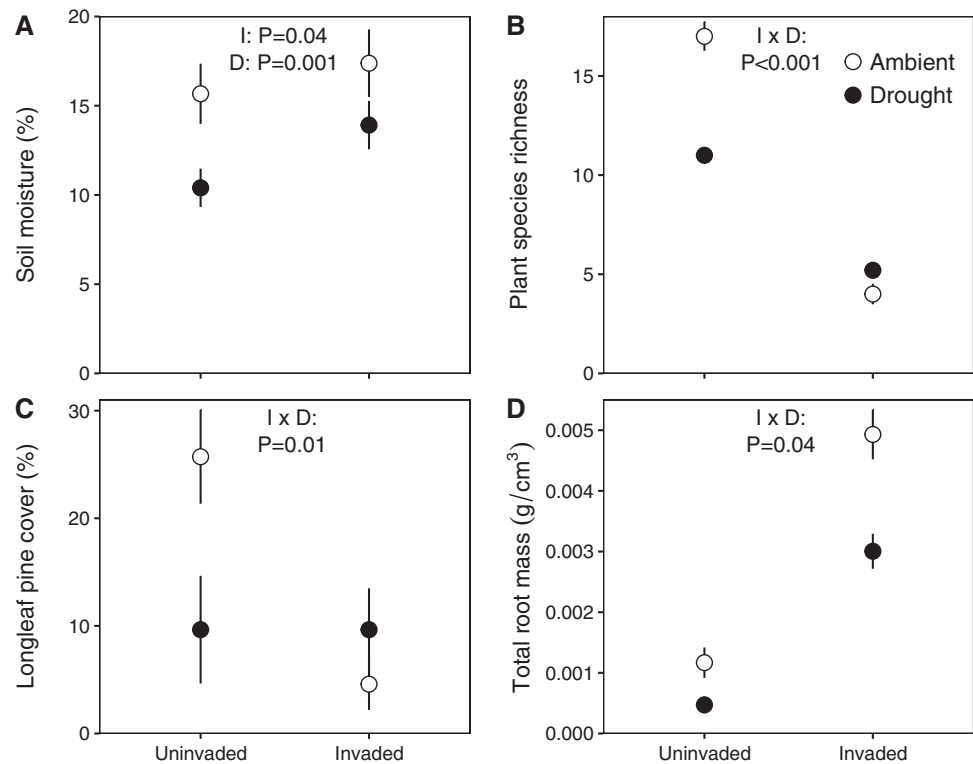


Fig. 1 Hypothesized direct and indirect effects of invasion and soil moisture on soil microbial richness, with indirect effects through changes in the plant community (green boxes). Blue arrows indicate hypothesized positive effects and red arrows indicate negative effects. In previous studies from this experiment, we showed that invasion resulted in higher soil moisture, especially under drought conditions

(a), and that invasion and drought both reduced plant species richness and longleaf pine abundance (b–e). We predicted that invasion would promote but drought would reduce root biomass (f, g); invasion and drought would directly reduce microbial diversity (h, i); and greater plant diversity, pine abundance, and root biomass would increase microbial diversity (j–l).

Fig. 2 Environmental and plant community variables used in the structural equation models. (a) Percent soil moisture, **(b)** plant species richness, **(c)** percent cover of longleaf pine, **(d)** total root biomass in the 5–15 cm depth soil fraction, as affected by *I. cylindrica* invasion and drought treatment with ambient precipitation shown in white and drought treatment in black (mean \pm SE, $N = 10$).



community, plant richness provided an estimate of the complexity of the plant community; the cover of longleaf pine represented a foundation species and a functionally distinct species in the community; and root biomass estimated the belowground productivity of the system. Invasion was specified as a categorical exogenous variable, and gravimetric soil moisture, plant species richness, cover of longleaf pine, and root biomass as endogenous variables. Paths were included from invasion and soil moisture to all other endogenous variables. A path from invasion to soil moisture was included because previous research showed that *I. cylindrica* increased soil moisture, particularly under drought conditions [24]. Paths were included from plant species richness, root biomass, and pine abundance to the microbial community variables. Microbial community variables were fungal and bacterial richness and functional groups. These variables were used in linear mixed effects models with block as a random effect in the piecewise SEM. The initial test of *d*-separation (Fisher's $C = 11.6$, $P = 0.07$) suggested that longleaf pine cover and plant species richness were not independent ($P = 0.005$). This correlation was likely due to similar responses of longleaf pine and herbaceous plants to the treatments [32, 62], thus, covariance between these variables was included in the final model. The final test of *d*-separation (Fisher's $C = 0.91$, $P = 0.92$) showed a good model fit. A stepwise removal of the least significant paths from the models was conducted until either the AIC did not decrease with removal of the next parameter or there were no remaining parameters with $P > 0.1$.

Results

At the time of sampling, soil moisture was 26% lower in drought plots than ambient plots, and 20% higher in invaded plots than uninvaded plots (D: $F_{1,27} = 12.6$, $P = 0.001$; I: $F_{1,27} = 4.5$, $P = 0.04$; Fig. 2a). Plant species richness was 76% lower under invasion alone, 35% lower under drought alone, and 69% lower in invaded drought plots than uninvaded ambient plots (I \times D: $F_{1,27} = 79.2$, $P < 0.001$; Fig. 2b). Plant community composition was strongly affected by invasion (I: Pseudo- $F_{1,39} = 12.3$, $P < 0.01$) and marginally affected by the interaction of invasion and drought (I \times D: Pseudo- $F_{1,39} = 2.1$, $P = 0.06$; Fig. 4a). Longleaf pine cover was 82% lower under invasion alone and 62% lower under drought both with and without the invader compared with the uninvaded ambient plots (I \times D: $F_{1,27} = 7.6$, $P = 0.01$; Fig. 2c). Total root biomass was over four times higher with invasion alone, 40% lower with drought alone, and 2.6 times higher in invaded drought plots than in uninvaded ambient plots (I \times D: $F_{1,27} = 4.8$, $P = 0.04$; Fig. 2d; Table S3).

Microbial diversity and community composition

Fungal OTU richness and Chao 1 index were interactively affected by invasion and drought. Under ambient conditions invasion reduced richness by 56 OTUs but under drought conditions invasion promoted richness by 48 OTUs (I \times D: $P = 0.02$; Fig. 3a). Fungal diversity and evenness showed

Fig. 3 Effects of invasion and drought treatments on microbial richness. Richness of (a) fungal and (b) bacterial OTUs with ambient precipitation shown in white and drought treatment in black (mean \pm SE; $N = 10$).

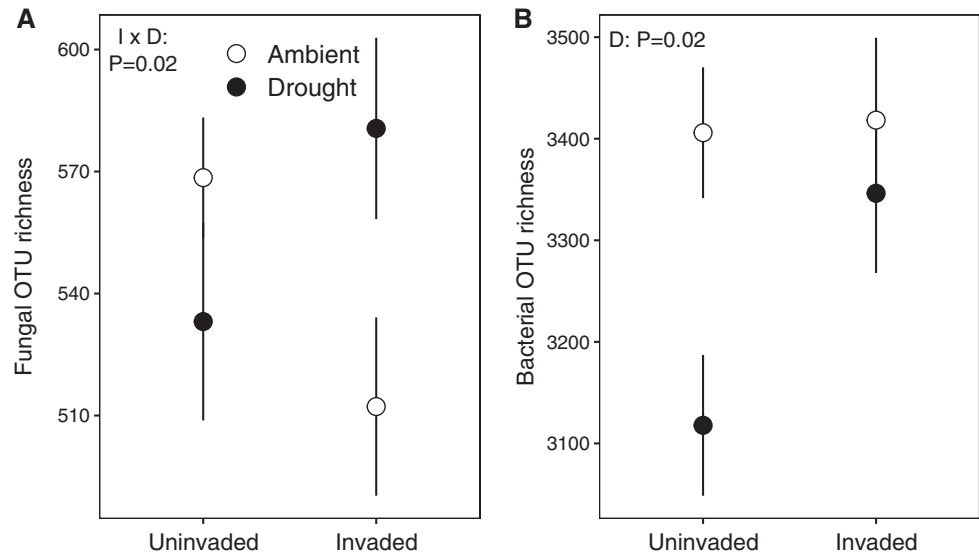


Table 1 Results of mixed effects models of invasion and drought treatment effects on fungal and bacterial richness, Shannon diversity index, Pielou's evenness, and Chao 1 index.

Treatment	numDF	denDF	Richness		Diversity		Evenness		Chao1	
			<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>
Fungi Invasion	1	27	0.04	0.84	0.5	0.49	0.77	0.39	0.14	0.71
Fungi Drought	1	27	0.61	0.44	0.66	0.42	0.52	0.48	1.1	0.3
Fungi Invasion: drought	1	27	6.03	0.02	2.72	0.11	1.89	0.18	6.45	0.02
Bacteria Invasion	1	27	2.68	0.11	8.91	0.01	11.07	0.003	2.59	0.12
Bacteria Drought	1	27	5.98	0.02	16.3	<0.001	19.37	<0.001	4.29	0.05
Bacteria Invasion: drought	1	27	2.15	0.15	0.86	0.36	0.37	0.55	1.28	0.27

the same trend as richness ($I \times D$: $P = 0.11$, $P = 0.18$, respectively; Fig. S1, Table 1). Invasion resulted in greater Shannon diversity index and evenness of soil bacteria with a similar trend for richness and Chao 1 index (I : $P = 0.01$, 0.003, 0.11, 0.12, respectively; Table 1; Fig. 3b). The effects of the drought treatment were evident in lower bacterial Shannon diversity index, evenness, OTU richness, and Chao 1 index (D : $P = 0.001$, 0.001, 0.02, 0.048, respectively, Fig. S2). On average, drought plots had 180 fewer bacterial OTUs than ambient precipitation plots.

Invasion and drought interactively affected fungal community composition where uninvaded ambient plots and invaded drought plots had similar composition, while invaded ambient plots and uninvaded drought plots were similar in composition ($I \times D$: $P = 0.001$; Fig. 4b; Table S4). For bacteria, drought was the strongest driver of community composition with a weak interaction between invasion and drought (weighted UniFrac: D : $P = 0.002$, $I \times D$: $P = 0.26$; unweighted UniFrac: D : $P = 0.001$, $I \times D$: $P = 0.06$; Fig. 4c, d; Table S4). There were significant differences in dispersion between treatments for the plant community ($P = 0.003$) [32], but neither fungal nor

bacterial communities showed differences in treatment group dispersion (Fungi: $P = 0.96$; Bacteria unweighted UniFrac: $P = 0.37$; Bacteria weighted UniFrac: $P = 0.59$).

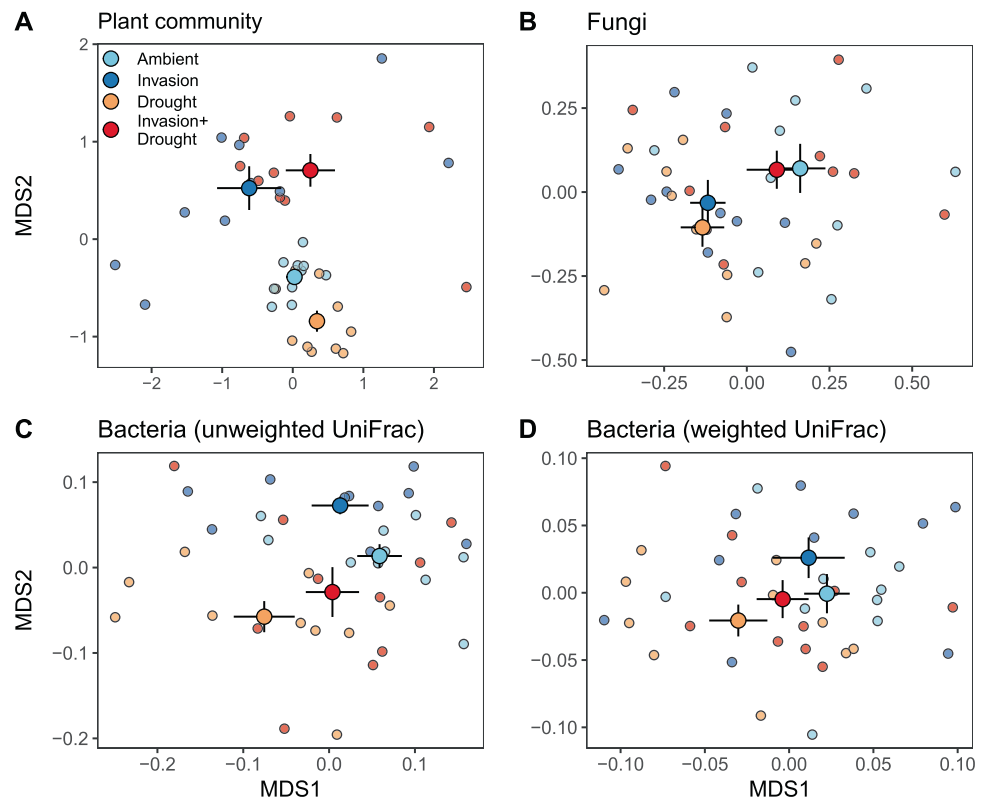
Fungal groups

No fungal phyla or classes were differentially abundant under the treatments (Figs. S3 and S4). All of the fungal orders and families that responded to the treatments were rare (i.e., <1% relative abundance), except for Atheliales/Atheliaceae. Six of 62 fungal orders that occurred in at least 25% of the samples were affected by the treatments (Fig. S5). Sebaciniales was more abundant with invasion (I : $W = 0.89$). Glomerales and Umbelopsidales were less abundant under drought (D : $W = 0.79$, 0.77, respectively). Phacidiales, Atheliales, and Venturiales were all less abundant in invaded ambient plots and uninvaded drought plots than the other treatments ($I \times D$: $W = 0.95$, 0.94, 0.79, respectively). Five fungal families out of 157 were affected by the treatments (Fig. S6). Serendipitaceae was more abundant in invaded plots (I : $W = 0.94$) and Umbelopsidaceae and Glomeraceae were less abundant under drought

Fig. 4 Nonmetric multidimensional scaling ordinations of community dissimilarities among invasion and drought treatments. (a)

Bray–Curtis dissimilarity of plant community composition (stress = 0.164), (b) Bray–Curtis dissimilarity of the fungal community (stress = 0.199), (c) unweighted UniFrac distance of the bacterial community (considers presence–absence; stress = 0.152), (d) weighted UniFrac distance of the bacterial community (considers relative abundance; stress = 0.169).

Larger points with error bars indicate the mean \pm SE of the 10 plots per treatment and smaller background points show NMDS values for each plot. Light blue points indicate uninvaded plots with ambient precipitation, dark blue points indicate invaded plots with ambient precipitation, orange points indicate uninvaded drought plots, and red points indicate invaded drought plots.



(D: $W = 0.87$). Phaciaceae and Atheliaceae were less abundant with invasion and drought individually compared with uninvaded ambient plots and invaded drought plots ($I \times D$: $W = 0.97, 0.96$, respectively).

In the fungal dataset, 69% of OTUs were identified as having known guilds based on the FUNGuild database and 81% of those OTUs had a confidence ranking of probable or highly probably. There were no significant effects of the treatments on fungal guilds; however, there was a marginal effect of drought on the guild “Endophyte, Undefined Saprotroph, Wood Saprotroph” and a marginal interaction between invasion and drought on the guild “Animal Pathogen, Fungal Parasite, Undefined Saprotroph” ($W = 0.74$; Fig. S7).

Bacterial groups

Only relatively rare bacterial taxa (i.e., <1% relative abundance) were affected by the treatments, including 4/32 phyla (Fig. S8). Fibrobacteres were over seven times and Spirochaetes over three times more abundant in invaded plots (I: $W = 0.88, 0.81$, respectively). Bacteria in candidate division TM7 were 2.4 times more abundant under drought ($W = 0.90$), while FCPU426 were 73% less abundant under drought than ambient conditions (D: $W = 0.77$). Ten out of 91 bacterial classes were affected by the treatments (Fig. S9). Fibrobacteria were 7.5 and Spirochaetia were 4.3

times more abundant with invasion (I: $W = 0.92, 0.85$). Holophagae, Pla4, OM190, Chlamydia, Cytophagia, and PRR-12 were less abundant under drought (D: $W = 0.96, 0.91, 0.89, 0.80, 0.79, 0.79$, respectively), while TM7-1 and TM7-3 were more abundant under drought (D: $W = 0.96, 0.89$, respectively). Four of 136 bacterial orders and seven of 152 families were affected by drought, two orders and one family by invasion, and one order and one family by the interaction of invasion and drought (Figs. S10 and S11, and Table S5).

In the bacterial dataset, 20% of OTUs were identified as having at least one known putative function in the FAPROTAX database. Most bacterial groups had several assigned functions, so we refer to the functional groups by number (based on rank of relative abundance) and a single key function. A complete list of bacterial functional groups is given in Table S6. Five out of 48 bacterial functional groups were affected by drought, where FG2-denitrifiers and FG13-human pathogens were more abundant under drought while, FG6-intracellular parasites, FG10-cyanobacteria, and FG16-fermenters were less abundant with drought ($W = 0.96, 0.81, 0.77$, respectively). FG8-nitrate reduction was marginally less abundant under drought ($W = 0.72$). No functional groups responded to the invasion treatment. FG9-ureolysis responded interactively to invasion and drought where abundance was lower with invasion and drought independently but higher with invasion and drought together

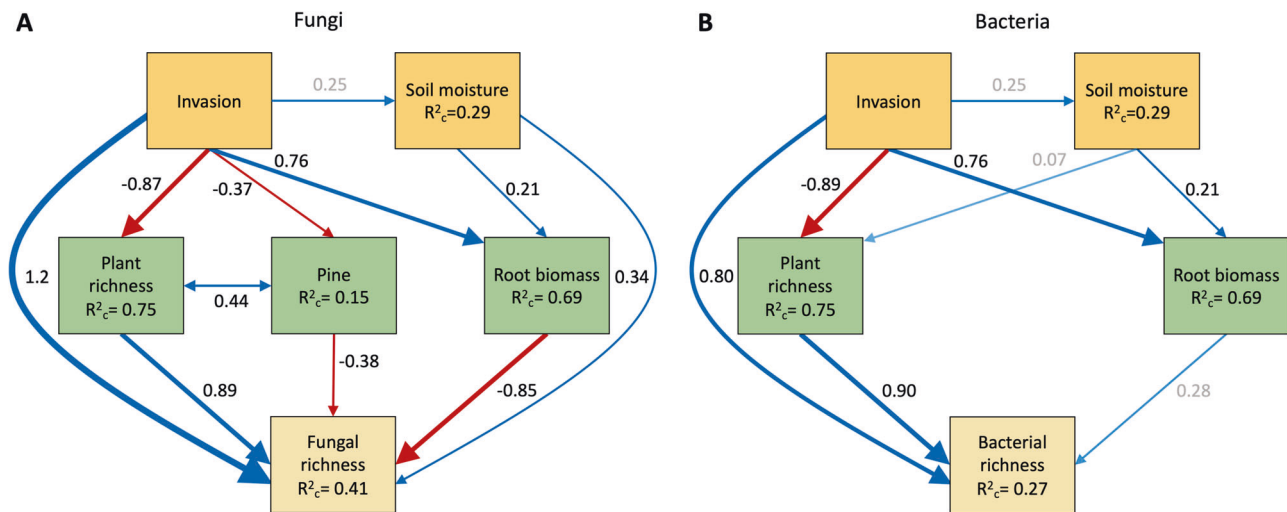


Fig. 5 Path diagrams showing the relationships between invasion and drought treatments, plant communities, and microbial richness.

(a) Fungal and (b) bacterial richness where blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardized path coefficients are shown next to the arrows and arrow size is scaled to the strength of the coefficients. Solid arrows and black coefficients

indicate a significant relationship ($P < 0.05$) while faded arrows and gray coefficients indicate a non-significant relationship ($P > 0.05$) and the level of transparency of the arrows corresponds to the P value. The conditional R^2 (based on the variance of both the fixed and random effects) for the component mixed effects models are shown in the boxes for the response variables.

($W = 0.79$; Fig. S12). Table S5 provides the complete ANCOM results.

Drivers of microbial community structure

The structural equation models explained 41% and 27% of the variability in fungal and bacterial richness, respectively. Invasion had a negative effect on plant richness (standardized coefficient = -0.87 , $P < 0.001$) and pine abundance (std. coefficient = -0.37 , $P = 0.02$) but a positive effect on root biomass (std. coefficient = 0.76 , $P < 0.001$; Fig. 5a). Soil moisture had a positive effect on root biomass (std. coefficient = 0.21 , $P = 0.03$).

Invasion had a direct positive effect on fungal richness (std. coefficient = 1.2 , $P < 0.001$) and a positive indirect effect through pine cover (std. coefficient = 0.14). However, invasion also had negative indirect effects through plant richness (std. coefficient = -0.77) and root biomass (std. coefficient = -0.65 ; Fig. 5a), resulting in a small negative overall effect of invasion (std. coefficient = -0.07). Soil moisture had a direct positive effect on fungal richness (std. coefficient = 0.34 , $P < 0.001$) but an indirect negative effect through root biomass (std. coefficient = -0.18), resulting in a positive overall effect of soil moisture (std. coefficient = 0.17).

Invasion had a direct positive effect on bacterial richness (std. coefficient = 0.80 , $P = 0.04$), but there was also an indirect negative effect of invasion on bacterial richness through plant species richness (std. coefficient = -0.80), resulting in a more moderate overall positive effect of invasion on bacterial richness (std. coefficient = 0.24).

There was no direct effect of soil moisture on bacterial richness, and the indirect effects of soil moisture on bacterial richness were not significant (std. coefficient = 0.06 ; Fig. 5b).

The structural equation model for fungal guilds explained 48% of the variability in plant pathogen relative abundance, 30% of AM fungal relative abundance, 63% of ectomycorrhizal fungal relative abundance, and 58% of saprotroph relative abundance. Plant pathogen relative abundance was directly positively affected by higher soil moisture (std. coefficient = 0.38 , $P < 0.01$) but negatively affected by root biomass (std. coefficient = -0.59), resulting in indirect negative effect of soil moisture (std. coefficient = -0.12). Invasion had opposing indirect effects on the abundance of plant pathogens through abundance of pines and root biomass (std. coefficients = 0.22 , -0.45), creating an overall negative effect of invasion on plant pathogens (std. coefficient = -0.23 ; Fig. 6a). There was no direct effect of invasion or soil moisture on AM fungi (Fig. 6b). Their abundance was greater with higher root biomass, which was promoted by invasion and soil moisture, creating positive indirect paths from invasion and soil moisture to AM fungal abundance (std. coefficients = 0.47 , 0.13). Invasion also had an indirect negative effect on AM fungi through plant richness (std. coefficient = 0.44). Ectomycorrhizal fungal abundance was positively correlated with pine abundance (std. coefficient = 0.80 , $P < 0.001$), resulting in a negative indirect effect of invasion on ectomycorrhizae (std. coefficient = -0.30 ; Fig. 6c). Saprotrophs were less abundant with greater pine abundance (std. coefficient = -0.69 , $P < 0.001$), creating a

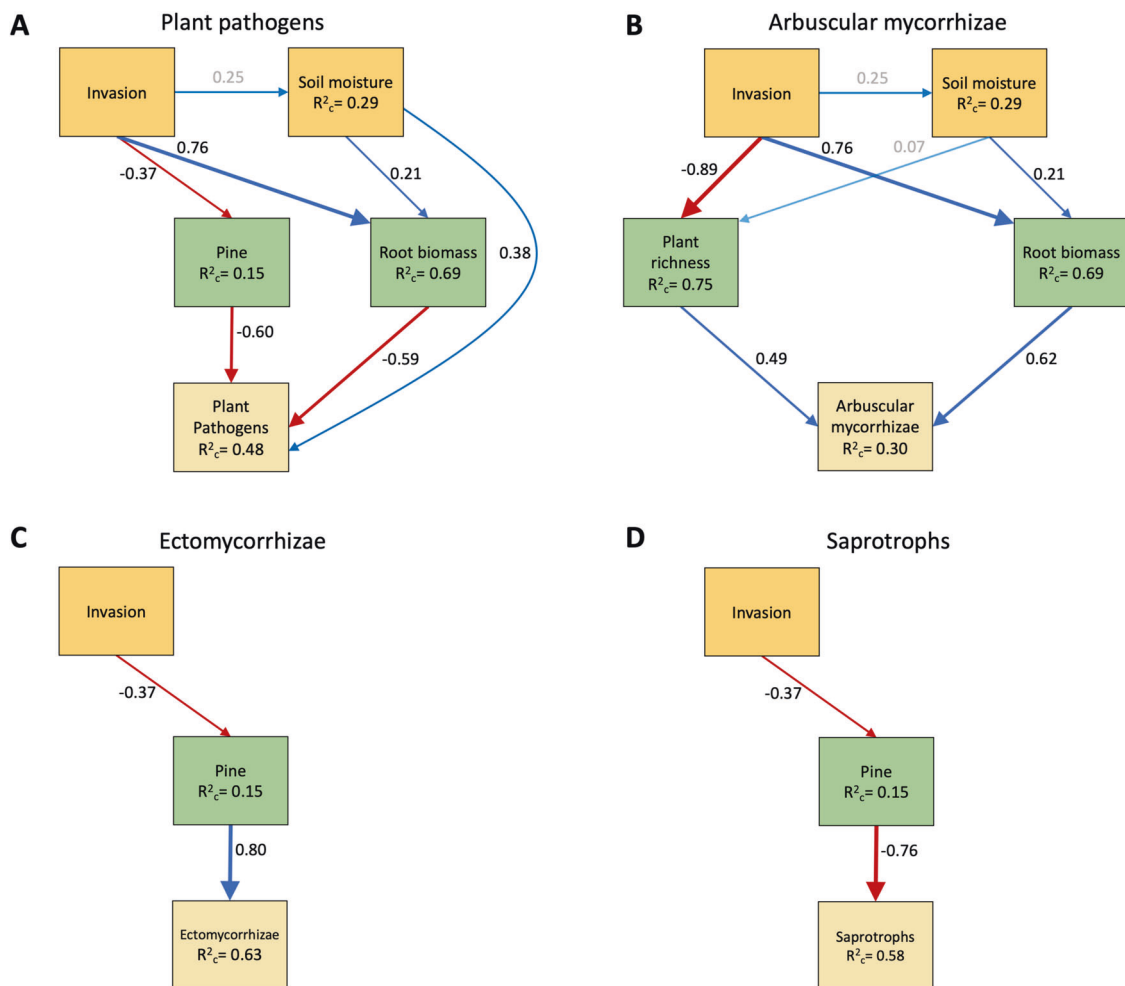


Fig. 6 Path diagrams showing the relationships between invasion and drought treatments, plant communities, and fungal guilds. Relative abundance of (a) plant pathogens, (b) arbuscular mycorrhizae, (c) ectomycorrhizae, (d) saprotrophs where blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardized path coefficients are shown next to the arrows and arrow size is scaled to the strength of the coefficients. Solid arrows and black

coefficients indicate a significant relationship ($P < 0.05$) while faded arrows and gray coefficients indicate a non-significant relationship ($P > 0.05$) and the level of transparency of the arrows corresponds to the P value. The conditional R^2 (based on the variance of both the fixed and random effects) for the component mixed effects models are shown in the boxes for the response variables.

positive indirect effect of invasion on saprotroph abundance (std. coefficient = 0.28; Fig. 6d).

The structural equation models of bacterial functional groups explained 51% of the variability in relative abundance of FG2-denitrifiers, 20% of FG3-aerobic chemoheterotrophs, 66% of FG7-hydrocarbon degraders, and 16% of FG8-nitrate reducers. We also tested FG4-aerobic nitrite oxidizers but there were no significant paths in the final reduced model. There was a direct negative effect of invasion on FG2 (std. coefficient = -0.95 , $P < 0.01$) and a negative effect of plant richness (std. coefficient = -0.85 , $P < 0.01$) creating a positive indirect effect of invasion (std. coefficient = 0.74; Fig. 7a). There was also a direct negative effect of invasion on FG3 (std. coefficient = -0.53 , $P = 0.04$) and a positive effect of root biomass (std. coefficient = 0.69, $P = 0.01$), creating indirect positive

effects of invasion and soil moisture (std. coefficient = 0.52, 0.15; Fig. 7b). There was also a direct negative effect of invasion on FG7 (std. coefficient = -0.27 , $P = 0.03$) and a negative effect of pine (std. coefficient = -0.49 , $P < 0.01$) creating a positive indirect effect of invasion (std. coefficient = 0.18; Fig. 7c). There was a positive effect of root biomass on FG8 (std. coefficient = 0.40, $P = 0.01$) creating positive indirect effects for both invasion and soil moisture (std. coefficient = 0.30, 0.08; Fig. 7d).

Discussion

We quantified the individual and interactive effects of plant invasion and drought on the soil microbiome and evaluated the direct versus indirect pathways of these effects through

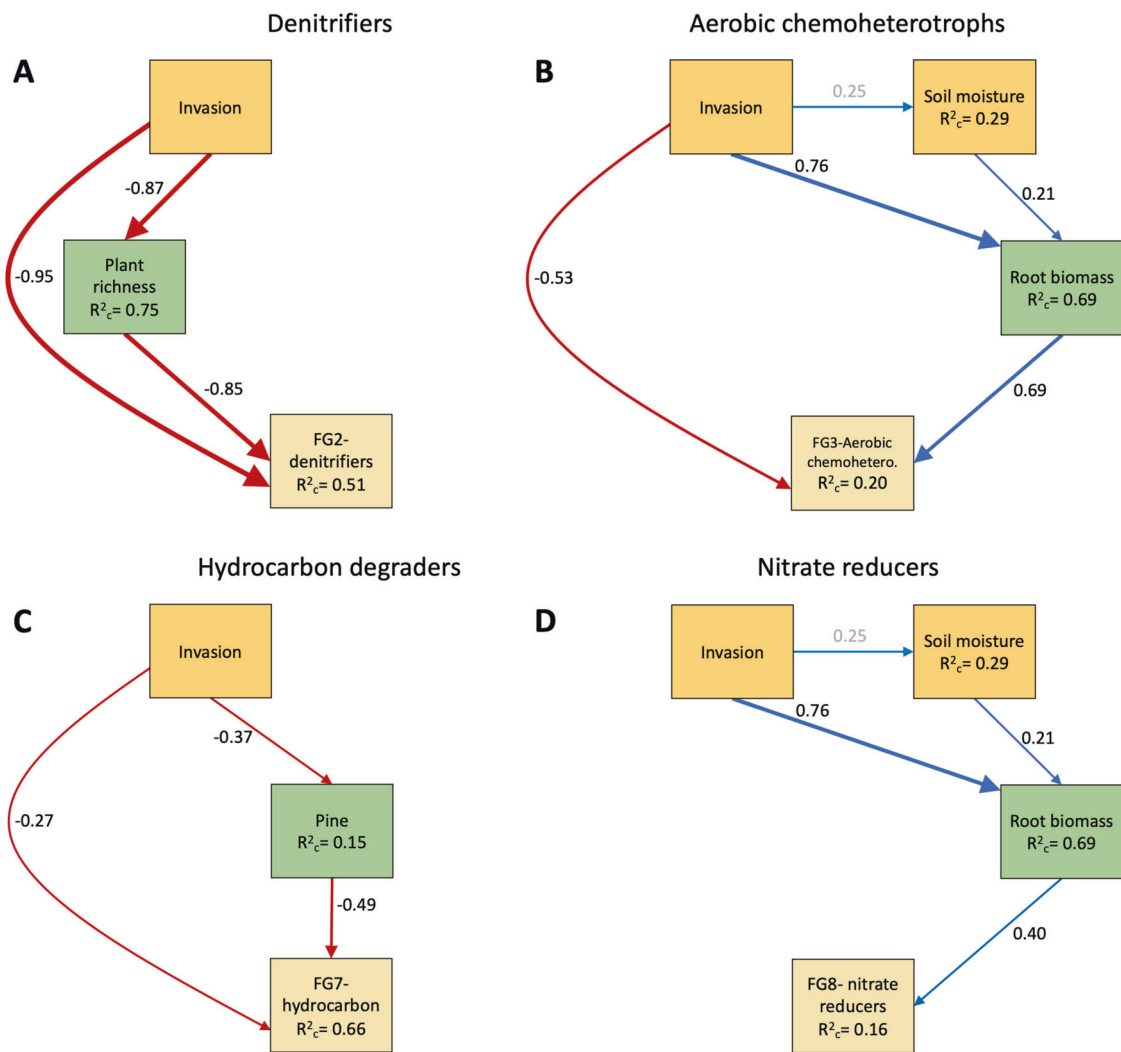


Fig. 7 Path diagrams showing the relationships between invasion and drought treatments, plant communities, and bacterial functional groups. Relative abundance of (a) functional group 2 (includes functions: aerobic chemoheterotrophy, anoxygenic photoautotrophy, chemoheterotrophy, denitrification, nitrate reduction, nitrate respiration, nitrite respiration, photoautotrophy, photoheterotrophy, and phototrophy), (b) functional group 3 (includes functions: aerobic chemo-heterotrophy, and chemoheterotrophy), (c) functional group 7 (includes functions: chemoheterotrophy, hydrocarbon degradation, methanotrophy, and methylotrophy), (d) functional group 8 (includes functions:

chemoheterotrophy, fermentation, and nitrate reduction). Blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardized path coefficients are shown next to the arrows and arrow size is scaled to the strength of the coefficients. Solid arrows and black coefficients indicate a significant relationship ($P < 0.05$) while faded arrows and gray coefficients indicate a non-significant relationship ($P > 0.05$) and the level of transparency of the arrows corresponds to the P value. The conditional R^2 (based on the variance of both the fixed and random effects) for the component mixed effects models are shown in the boxes for the response variables.

changes in plant community structure. Our results showed that microbial communities were interactively affected by invasion and drought. Specifically, invasion and drought together had less of an effect on microbial communities than either treatment alone, suggesting alleviation of one treatment by the other. In addition, invasion had strong positive direct effects on both fungal and bacterial richness but also opposing indirect effects through the plant community that moderated overall invasion effects. We demonstrated that the interactive effects of invasion and drought on microbial communities can be nonadditive and that plant communities

play a major role in mediating the effects of global change factors on microbial communities.

While instantaneous gravimetric soil moisture was independently affected by invasion and drought, long-term measurements of volumetric soil moisture demonstrated that soil moisture was elevated under invasion in the drought treatment but less so with ambient precipitation [32]. Bacterial richness closely mirrored differences in instantaneous soil moisture across the treatments with lower richness under drought and community composition most altered by drought [23]. Conversely, fungal richness was

lower under invasion and drought individually but showed complete offsetting of the effect with invasion and drought together, indicating that the interactive effects of invasion and drought on fungal richness were antagonistic [63] (also referred to as mitigating synergism [64]). Similar patterns were observed for fungal community composition, where communities with invasion and drought together were more similar to the uninvaded ambient plots than plots with either treatment alone. These results may indicate that bacteria are more strongly driven by instantaneous soil moisture, while fungi are more sensitive to long-term patterns in soil moisture [65].

Both direct and indirect effects of the treatments on the plant community had major effects on microbial richness. Invasion had a strong positive direct effect but negative indirect effects on both fungal and bacterial richness. Effects of soil moisture followed the same pattern for fungal richness with a positive direct effect and negative indirect effect. As expected, plant richness was positively correlated with both fungal and bacterial richness, suggesting that the complexity of the plant community is a primary driver of microbial richness [9]. Contrary to our hypothesis, pine abundance was negatively correlated with fungal richness suggesting that a dominant native species can homogenize the fungal community [12]. Root biomass was also negatively correlated with fungal richness, perhaps because much of the variation in root biomass was associated with invader roots. This relationship suggests that higher belowground productivity associated with the invader can reduce fungal richness, while the reduction in root biomass with drought can increase fungal richness [12, 15, 66]. Overall, the direct effects of the invasion and drought treatments on microbial richness were balanced by the indirect effects through alterations to the plant community.

Despite the rise in studies assessing microbial responses to environmental conditions, there is still considerable debate about which microbial taxa respond to drought stress [21, 67–69]. In this study, restructuring of fungal communities occurred mainly at lower taxonomic levels. Relative abundance of the order Sebaciales and specifically the family Serendipitaceae was greater in invaded plots, suggesting that they may associate with *I. cylindrica*. These taxa can have complex interactions with plants, as saprotrophs or as plant endophytes and mycorrhizal fungi [70, 71]. Relative abundance of Glomeraceae, which is considered to be the most ruderal AM fungal family, was unaffected by invasion but was lower under drought [5, 21, 72]. AM fungi as a whole were negatively affected by drought through lower root biomass [73]. They were also negatively affected by invasion through plant richness but positively through root biomass, and these indirect effects counteracted each other. Results from previous studies on AM fungal response to invasion have been mixed, with

some studies showing either higher or lower AM fungal abundance and diversity, and others showing no response to invasion [17, 39, 74–77]. Some of this variability across studies could be due to the mycorrhizal status of the invader. This hypothesis is supported by the positive effect of *I. cylindrica* invasion through greater root biomass on AM fungi. However, the negative effect of invasion through lower plant richness indicates that plant interactions can mediate invader impacts on AM fungi.

Ectomycorrhizal abundance was driven by the abundance of pine, which was negatively affected by invasion. Contrary to previous studies suggesting a direct allelopathic effect of *I. cylindrica* on ectomycorrhizae [38], our results indicated an indirect negative pathway through the abundance and possibly vigor of pine trees. Saprotrophs were also affected by invasion through pine abundance but with a positive indirect effect. This trade-off in relative abundance of saprotrophs and ectomycorrhizae could indicate a switch from saprotroph to ectomycorrhizal dominance in the role as decomposers with pine abundance [78, 79].

Some invasive plants modify the soil microbiome in ways that have negative impacts on native plants, including reductions in beneficial mutualists and accumulation of harmful pathogens [80–83]. *Imperata cylindrica* invasion had indirect negative effects on fungal plant pathogens through reduction of root biomass and pine abundance. The negative association of plant pathogens with root biomass and pine abundance suggests that *I. cylindrica* and pine may inhibit pathogens, perhaps because of associations with mycorrhizal fungi [84]. Soil moisture had a positive direct effect on plant pathogens, confirming previous studies showing pathogen susceptibility to desiccation [85, 86]. Differences in the responses of pathogens, mutualists, and saprotrophs to invasion and drought could play a role in plant community dynamics. Future work should test whether changes in abundance of these fungal groups have differential effects on the invader versus native species [87].

In regards to bacterial taxa, some studies suggest that Bacteroidetes and Planctomycetes are susceptible to drought, while Firmicutes, Chloroflexi, and Actinobacteria are more abundant under drought conditions [23]. However, we found that only relatively rare bacterial taxa responded to drought. Most previous studies on the impacts of drought on microbial communities have been conducted in dry systems (e.g., [6, 88–90]). More studies in mesic ecosystems are needed because microbes could show considerably different responses to drought based on acclimation to particular abiotic conditions [91, 92].

We found that bacteria associated with denitrification and other functions (FG2) were more abundant under drought particularly in uninvaded plots. While denitrification is a primarily anaerobic process, denitrifying bacteria are facultative anaerobes and denitrification can occur in

anaerobic microsites such as within soil aggregates [93]. Bacteria associated with nitrate reduction (FG8) were more abundant under drought and those associated with ureolysis (FG9) were interactively affected by invasion and drought [23, 94]. While additional work is needed to determine if the genes for these processes are active, previous studies suggest that changes in nitrogen cycling have the potential to create positive feedbacks with invader growth [95]. Furthermore, we found that all of the most abundant bacterial functional groups were influenced by some aspect of the plant community (i.e., plant richness, pine abundance, or root biomass). These results suggest the potential for changes in nitrogen cycling under invasion and drought driven by changes in the soil bacterial communities and mediated by plant communities.

We showed that both abiotic and biotic factors influenced soil microbial community structure. We expected that both invasion and drought would have negative direct effects on the microbial community, but instead we found that invasion had direct positive effects on microbial richness. However, reductions in plant richness associated with invasion created a strong negative indirect effect of invasion on microbial richness. These results indicate that loss of plant diversity with invasion could drive loss of microbial diversity. Contrary to our hypothesis, pine abundance and root biomass were negatively associated with fungal richness, while for bacteria neither were significantly related to richness. Soil moisture was positively associated with fungal but not bacterial richness, and the negative indirect effect of soil moisture through belowground biomass counteracted the positive direct effect. Overall, plant communities proved to be essential mediators of both biotic and abiotic global change factors on the soil microbiome, and by assessing these indirect effects we can begin to tease apart the complexity of the interactive effects of anthropogenic environmental change.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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