RESEARCH ARTICLE

Microbial community response to a decade of simulated global changes depends on the plant community

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Global changes such as increased drought and atmospheric nitrogen deposition perturb both the microbial and plant communities that mediate terrestrial ecosystem functioning. However, few studies consider how microbial responses to global changes may be influenced by interactions with plant communities. To begin to address the role of microbial–plant interactions, we tested the hypothesis that the response of microbial communities to global change depends on the plant community. We characterized bacterial and fungal communities from 395 plant litter samples taken from the Loma Ridge Global Change Experiment, a decade-long global change experiment in Southern California that manipulates rainfall and nitrogen levels across two adjacent ecosystems, a grassland and a coastal sage scrubland. The differences in bacterial and fungal composition between ecosystems paralleled distinctions in plant community composition. In addition to the direct main effects, the global change treatments altered microbial composition in an ecosystem-dependent manner, in support of our hypothesis. The interaction between the drought treatment and ecosystem explained nearly 5% of the variation in bacterial community composition, similar to the variation explained by the ecosystem-independent effects of drought. Unexpectedly, we found that the main effect of drought was approximately four times as strong on bacterial composition as that of nitrogen addition, which did not alter fungal or plant composition. Overall, the findings underscore the importance of considering plant–microbe interactions when considering the transferability of the results of global change experiments across ecosystems.

Introduction

Ongoing human-driven global changes are altering species distributions and interspecific interactions (Parmesan, 2006; Rosenzweig et al., 2008). Many field experiments have considered the responses of plant and/or microbial communities to simulated global changes (Stylinski and Allen, 1999; Vilà et al., 2003; Allison et al., 2013; Martiny et al., 2017). These studies aim to predict how global changes such as altered precipitation, nitrogen availability, CO_2 concentration, and temperature affect community composition and ecosystem processes (Stylinski and Allen, 1999; Cione et al., 2002; Vilà et al., 2003; Allison and

Martiny, 2008; Cruz-Martinez et al., 2009; Gaertner et al., 2009; Castro et al., 2010; Gutknecht et al., 2012). However, fewer studies consider how the impact of a global change treatment on microbial composition is influenced by interactions with the plant community (Classen et al., 2015; Sayer et al., 2017). Such interactions will influence the transferability of the results of global change experiments as plant community composition, among other factors, varies across ecosystems.

Here, we focus on one half of plant–microbe interactions—specifically, the ways in which plants may influence microorganisms. One way in which plants influence microorganisms is through decomposition. Bacteria and fungi are the primary decomposers of dead plant biomass, and this process regulates the amount of soil carbon exchanged with the atmosphere (Swift et al., 1979; Adair et al., 2008; Schimel and Schaeffer, 2012). More broadly, plant communities can influence microorganisms through plant species and tissue composition (influencing nutrients and secondary compounds), changes in the abiotic environment (plant architecture influencing canopy and

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moisture of the soil), and relationships with other organisms such as endophytes and herbivores (Wardle et al., 2006; Tintjer and Rudgers, 2006; Kara et al., 2008; Kominoski et al., 2009; Rodriguez et al., 2009; Chapman and Newman, 2010; Santonja et al., 2017; Graff et al., 2020). Through such associations, the impact of a global change treatment on plant-litter microbial communities may depend on the plant community in at least three ways. First, initial differences in plant communities across ecosystems will select for different microbial communities. Thus, the microbial response to global change may be driven by taxa that are uniquely prominent in an ecosystem. Second, some microbial taxa may be more sensitive to global change depending on the plant resources available to them in an ecosystem (Wood et al., 2018; Malik et al., 2020a). Specifically, litter substrate quality may impact the ability of litter microbes to respond to stressful conditions (Malik et al., 2020b). Finally, the extent to which the plant litter substrates and resources are altered by global change, and thereby indirectly influence litter microbial communities, will depend on the plant community (Aerts, 1997; Rouifed et al., 2010; Fernández-Alonso et al., 2018). For instance, drought typically reduces the germination of annual plants, ground cover, and primary productivity of arid grasslands, whereas plants with deeper root systems are less impacted (Le Houérou, 1996; Shinoda et al., 2010; Kinugasa et al., 2012).

The Loma Ridge Global Change Experiment (LRGCE) simulates the increased frequency of drought and the increased availability of nitrogen. Drought is an extreme climatic event that occurs in most climatic zones, and its frequency and severity are projected to increase, along with atmospheric nitrogen deposition (Mishra and Singh, 2010; Dai, 2011; Intergovernmental Panel on Climate Change, 2014). A unique feature of the LRGCE is that treatments are applied to two adjacent ecosystems (Figure S1A), a grassland and a coastal sage scrubland (CSS). Much is known about the plant and litter microbial communities at the LRGCE. Within the first 5 years of the experiment, both the grassland and CSS plant communities responded to drought and nitrogen addition (Potts et al., 2012; Kimball et al., 2014; Kimball et al., 2016). In the grassland, drought reduced non-native annual grass cover, while nitrogen addition reduced native grasses and increased non-native annual grasses (Kimball et al., 2014; Kimball et al., 2016). In the CSS, drought reduced shrub cover and increased grass cover, and added nitrogen further reduced shrub cover and native grasses (Kimball et al., 2014). Bacterial and fungal community composition on surface plant litter also responded to these treatments in the grassland (Allison et al., 2013; Berlemont et al., 2014). Moreover, reciprocal transplant experiments within LRGCE revealed shifts in microbial community composition due to direct, abiotic effects of the global change treatments, and as indirect effects of drought on the grassland plant litter (Martiny et al., 2017). However, the response of the microbial communities within the CSS treatment plots at the Loma Ridge research site have not yet been investigated. Additionally, drought and added nitrogen treatments have been ongoing for more than

a decade; thus, it is important to assess the long-term effects of simulated drought and added nitrogen.

Here, we tested the hypothesis that the response of microbial communities to global change depends on the plant community using a decade-long global change experiment in Southern California. To address our hypothesis, we ask: Does the response of microbial communities to global change depend on the ecosystem (grassland vs. CSS)? The two ecosystems are immediately adjacent to each other at our study site, without major differences in slope, aspect, soil type, or climate, and subtle bulk soil differences seem unlikely to affect the plant litter microorganisms on the soil surface. Thus, we presume that any differences in the treatment responses across ecosystems are likely due to differences in the plant communities, rather than other abiotic factors. As observed in the grassland within the first 5 years of treatments, we expect a treatment response would occur in both ecosystems, resulting in a significant main effect of the treatments. However, we also predict that the microbial community response will result in ecosystem-specific compositional shifts, as reflected in significant treatment-by-ecosystem effects. Given that the surrounding abiotic conditions are similar in these adjacent ecosystems, such interactive effects provide evidence that microbial responses are dependent on the plant community.

Materials and methods
Field site, sample collection, and experimental design
The LRGCE was established in February 2007 and is located 5 km north of Irvine, CA, USA (117.704°W, 33.742°N; 365 m elevation), on a sloping (<10%) deep colluvial deposit from layers of sedimentary rock and soil mapped as Myford Sandy Loam (Potts et al., 2012; Kimball et al., 2014). The grassland plots are dominated by the native perennial grass *Stipa pulchra*; the annual grass genera *Avena*, *Bromus*, and *Festuca*; and the annual forb genera *Erodium* and *Lupinus*. The CSS plots are dominated by *Artemisia californica*, *Salvia mellifera*, *Eriogonum fasciculatum*, and *Acmispon glaber* (Kimball et al., 2014). The climate is Mediterranean with an annual precipitation of 30 cm. The "wet" season is typically from November to April, while the "dry" season is from May to October (Figure S2). Air temperatures are moderate in the wet season with an average high and low of 21.1°C and 7.1°C, respectively, and increase in the dry season with an average high and low of 27.6°C and 14.4°C, respectively (Tustin Irvine Ranch weather 1981–2010; Western Regional Climate Center, https://wrcc.dri.edu/).

Surface litter samples were collected at seven time points (approximately every 3 months) from August 2016 to March 2018, and from four replicate plots receiving four different treatments: control, drought, added nitrogen, and drought plus added nitrogen. Thus, up to 16 samples in each ecosystem were collected at each time point in the lower half of the LRGCE plots; however, after quality checks, some samples were excluded for a total of 108 grassland and 111 CSS samples. The LRGCE implements a randomized split-plot design in both grassland

(12.2 x 6.1 m) and CSS (18.3 x 12.2 m) sites, where the nitrogen treatment is nested within drought treatment plots. Drought control plots received ambient rainfall, while drought plots were exposed to approximately a 50% reduction in rainfall, and either ambient or added nitrogen. Drought was simulated by covering rain shelters with manually retractable, clear, 6-mil polyethylene roofs before predicted large rain events and removed promptly afterwards (Figure S1B and C). Added nitrogen was applied at 60 kg N ha^{-1} year^{-1} as fast release calcium nitrate, CaNO$_3$, and in two amounts per year, 20 kg prior to the first rains of the wet season and 40 kg in December coinciding with the start of the plant growing season (Potts et al., 2012; Kimball et al., 2014).

DNA isolation, PCR, and microbial community sequencing

DNA was extracted from approximately 0.05 g of senescent leaf material from treatment plots in grassland and CSS sites using ZymoBiomics DNA isolations kits (Zymo Research, Irvine, CA, USA) and processed for 5 min of bead beating at maximum speed (6.0 m/s, FastPrep-24 High Speed Homogenizer, MP Biomedicals, Irvine, CA, USA). To avoid batch effects, the plant litter samples were randomized prior to DNA extraction.

To characterize bacterial composition of the leaf litter communities, PCR amplification of the V4 and partial V5 region of the bacterial 16S rDNA (approximately 411 bp) was carried out following the Earth Microbiome protocol (Lane et al., 1985; Caporaso et al., 2012; Parada et al., 2016). The barcoded forward primers contain the 5′ Illumina adapter (AATGATACGGCGACCACCGAGATCTA-CACGCT), a unique 12-base error correcting Golay barcode, a pad (TATGGTAATT), a linker sequence (GT), and the 515fb primer (GTGYCAGCMGCCGCGGTAA), and reverse primers that contain the reverse complement of the 3′ Illumina adapter (CAAGCAGAAGACGGCATACGA-GAT), a pad (AGTCAGCCAG), a linker sequence (GG), and the 926r primer (CCGTCAATTCCTTTRAGTTT).

Each 16S PCR reaction contained: 9.5 µL PCR grade water (Fisher Scientific, Hampton, NH, USA), 12.5 µL of 2x concentrated AccuStart II PCR ToughMix (Quanta bio, Beverly, MA, USA) for a final 1x concentration, 0.5 µL of 10 µM 926r primer (final concentration of 0.2 µM), 1 µL of 10 mg/mL bovine serum albumin (final concentration of 1 µg/mL; New England BioLabs, Ipswich, MA, USA), 0.5 µL of 10 µM barcoded 515f primers (final concentration of 0.2 µM), and 1 µL of genomic DNA. Reactions were held at 94 °C for 3 min to denature DNA, with amplification proceeding for 30 cycles at 94 °C for 45 s, 55 °C for 30 s, and 72 °C for 1 min, followed by a final extension for 10 min at 72 °C to ensure complete amplification.

To determine fungal community composition, and improved accuracy of amplicon cluster detection and resolution during sequencing, a staggered primer design was used to amplify the internal transcribed spacer (ITS) region (approximately 340 bp) of the 5.8S rRNA gene. The ITS primers used are as follows: ITS9f primer (AATGATACG GCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT CCGATCTNNNNNGAACGCAGCRAAIIGYG), along with

a barcoded reverse ITS4 primer (CAAGCAGAAGACGGCA-TACGAGATAGTCAGTCAGCCTCCTCCGCTTATTGATATGC), which contained the reverse complement of the 3′ Illumina adapter, a unique 12-base barcode, and a pad with a linker sequence (Tremblay et al., 2015; Looby et al., 2016).

Each ITS PCR reaction contained: 9 µL PCR grade water (Fisher Scientific, Hampton, N, USA), 12.5 µL of 2x concentrated AccuStart II PCR ToughMix (Quanta bio, Beverly, MA, USA) for a final 1x concentration, 0.75 µL of 10 µM ITS9f primer (final concentration of 0.3 µM), 1 µL of 10 mg/mL bovine serum albumin (final concentration of 1 µg/mL; New England BioLabs, Ipswich, MA, USA), 0.75 µL of 10 µM barcoded ITS4 primers (final concentration of 0.3 µM), and 1 µL of genomic DNA. Reactions were held at 94 °C for 5 min to denature DNA, with amplification proceeding for 34 cycles at 95 °C for 45 s, 50 °C for 1 min, and 72 °C for 1 min 30 s, followed by a final extension for 10 min at 72 °C.

Sequencing libraries were prepared with pooled 16S or ITS amplicons from each sample after purification using Speed Bead Magnetic Carboxylate (GE Healthcare UK Limited, Buckinghamshire, UK) to remove primers. A composite library with equimolar ratios of the purified pooled 16S and ITS amplicons was prepared, and DNA size and quality for sequencing was determined by Qubit and Bioanalyzer (450 ng/ml and average amplicon size of 532 bp, respectively). Custom sequencing primers for 16S and ITS libraries were used as described in Caporaso et al., 2012 and Looby et al., 2016. The libraries were sequenced by the UCI Genomics High Throughput Sequencing Facility using an Illumina MiSeq platform with paired end reads at 300 bp.

Analysis of 16S and ITS sequencing

The forward reads of amplicon sequences were demultiplexed using QIIME2 version 2018.11 toolkit (Caporaso et al., 2010; Bolyen et al., 2018). Five samples were excluded from the bacterial analysis because of poor sequencing quality, and five samples were removed from the fungal analysis because of duplicated barcodes (Table S1 and S2). Demultiplexed sequences were denoized using DADA2, with operational taxonomic units, OTUs, picked at 100% identity level (amplicon sequence variants) using UCLUST within the QIIME2 pipeline (Caporaso et al., 2010; Edgar, 2010; Callahan et al., 2016; Bolyen et al., 2018). Resulting OTU tables were rarefied via randomized sampling of sequences without replacement over 300 iterations at a depth of 1,090 and 1,064 sequences per sample for bacteria and fungi, respectively, and using the "EcolUtils" package in R version 3.6.3 (R Core Team, 2018; Salazar, 2020). Taxonomy was assigned to OTUs using bacterial representative sequences and the q2-feature-classifier, classify-sklearn naïve Bayes taxonomy classifier against the Greengenes 13_8 99% OTUs reference sequences (McDonald et al., 2012; Bokulich et al., 2018). Taxonomy for fungal representative sequences were assigned using a dynamic threshold (97%–99% identity to reference), which is based on the most accurate assignment for a given lineage, and determined manually by experts in the field from the UNITE v7.2 database, release date December 1, 2017 (Nilsson et al., 2018; UNITE

Community, 2019). Unassigned OTUs at the Kingdom level, or assigned as chloroplasts, mitochondria, and Archaea were excluded from analysis.

Plant community

Species composition and fractional cover was determined in all plots by point intercept during mid-April of 2015, coinciding with late flowering and maximum seed set. Briefly, two 160×60 cm^2 polyvinyl chloride frames with 10-cm interval grids were positioned within each plot. A wire was dropped from each grid point, and the first-intercepted species was recorded. The point was recorded as plant litter or bare soil if live plant material was not encountered. The number of interceptions for each plant species was summed within a plot to calculate fractional cover. Fractional cover data of all species observed (32 in total) were used to generate a Bray-Curtis dissimilarity matrix. Fractional cover data for all species were further categorized into five functional groups including native grasses, non-native grasses, native forb, non-native forb, and native shrub (Kimball et al., 2014; Matulich et al., 2015).

Statistical analysis

To determine the effects of ecosystem, drought, added nitrogen, sample collection date, and all interactions on microbial composition, PERMANOVAs using Bray-Curtis dissimilarity matrices generated with rarefied OTU tables were performed using PRIMER-e version 6 (Clarke and Gorley, 2006; Anderson et al., 2008). Microbial and plant mixed models included plot treatment (ambient rainfall or nitrogen, drought, added nitrogen, and drought with added nitrogen), ecosystem, and sample collection date as fixed factors. The block factor was nested within ecosystem as a random effect to account for the split-plot design of the experiment. The estimated percentage of variance explained was determined by dividing terms with significant P-values by the sum of the estimates of components of variation given as output from PRIMER-e. Post-hoc comparisons of PERMANOVAs for drought and collection dates given community dissimilarities were performed using PRIMER-e. Multivariate homogeneity of variances for drought and nitrogen treatments by ecosystem were tested in R using the "betadisper" function of the "vegan" package, calculating distance to group centroid and accounting for sampling bias (Anderson, 2006; Anderson et al., 2006; R Core Team, 2018; Oksanen et al., 2019). To determine which taxa associated with OTU identifiers were key contributors to compositional differences in bacterial and fungal communities, SIMPER tests were conducted in PRIMER-e. To visualize factors influencing bacterial, fungal, and plant communities, ordinations of rarefied Bray-Curtis matrices were performed using non-metric multidimensional scaling using the "vegan" and "ggplot2" packages in R (Wickham, 2009).

Results

We investigated the response of microbial communities on plant litter to drought and nitrogen addition in a decade-long global change experiment carried out in adjacent grassland and CSS ecosystems. From samples taken over 3 years (beginning near the end of year nine of the LRGCE) and across 32 treatment plots, 2.48 million bacterial sequences were clustered into 1,197 OTUs (defined at 100% sequence similarity; Table S1). The majority of bacterial OTUs were associated with four phyla: Proteobacteria (40.2%), Bacteroidetes (29.6%), Actinobacteria (17.9%), and Firmicutes (2.9%). Correspondingly, 5.69 million fungal sequences clustered into 4,190 OTUs from two main phyla: Ascomycetes (71.4%) and Basidiomycetes (28.2%).

Main effects of ecosystems and time

The composition of both bacterial and fungal communities varied significantly between the grassland and CSS. Ecosystem, including its interactive effects with collection date (encompassing annual and seasonal variation), explained the largest amount of compositional variation, approximately 15% and 10% of the variation for bacterial and fungal communities, respectively (**Figure 1A** and **B**; Table 1; PERMANOVA: $P \leq 0.001$). These compositional differences were apparent at the genus level. Within bacteria, *Sphingomonas*, *Hymenobacter*, and *Curtobacterium* tended to be relatively more abundant in the grassland, whereas *Janthinobacterium*, *Methylobacterium*, and *Agrobacterium* were relatively more abundant in CSS (**Figure 2A**). Within the fungi, *Alternaria*, and *Vishniacozyma* tended to be relatively more abundant in the grassland, whereas *Cylindroseptoria* and *Coleophoma* were more abundant in CSS (**Figure 2B**). The differences in microbial composition between ecosystems paralleled distinctions in plant community composition, with ecosystem accounting for 38% of the variation in plant composition (Figure 1C and D; **Table 1**; $P \leq 0.01$). The grassland was dominated by non-native grasses, whereas CSS was predominantly comprised of native grasses, shrubs, and forbs (Figure 2C).

Microbial composition also varied temporally over the three sampling years, as expected from previous studies in the grassland at LRGCE (Matulich and Martiny, 2015). After ecosystem, collection date explained the most variation in both bacterial and fungal community composition, approximately 8% and 2%, respectively ($P \leq 0.001$; Table 1; Figure 1D). Indeed, microbial communities from sample collection dates coinciding with the peak wet season (e.g., January and March typically have the highest amounts of rainfall) differed in composition from that of collection dates in June and September months coinciding with the peak of the dry season (post-hoc pairwise comparisons: $P < 0.01$; Figure S2 and S3).

Main effects of drought and added nitrogen

Drought significantly altered both bacterial and fungal communities (Figure 1A and B; Table 1; main effects: $P \leq 0.01$), whereas nitrogen only altered the bacterial community. The main effect of drought, including its interactive effects with collection date, explained 5.6% and 3.6% of total variation in bacterial and fungal community composition, respectively (Figure 1D). In contrast, added nitrogen accounted for less than 1% of the variation in bacterial composition. Main effects of drought also explained 20% of variation in plant community
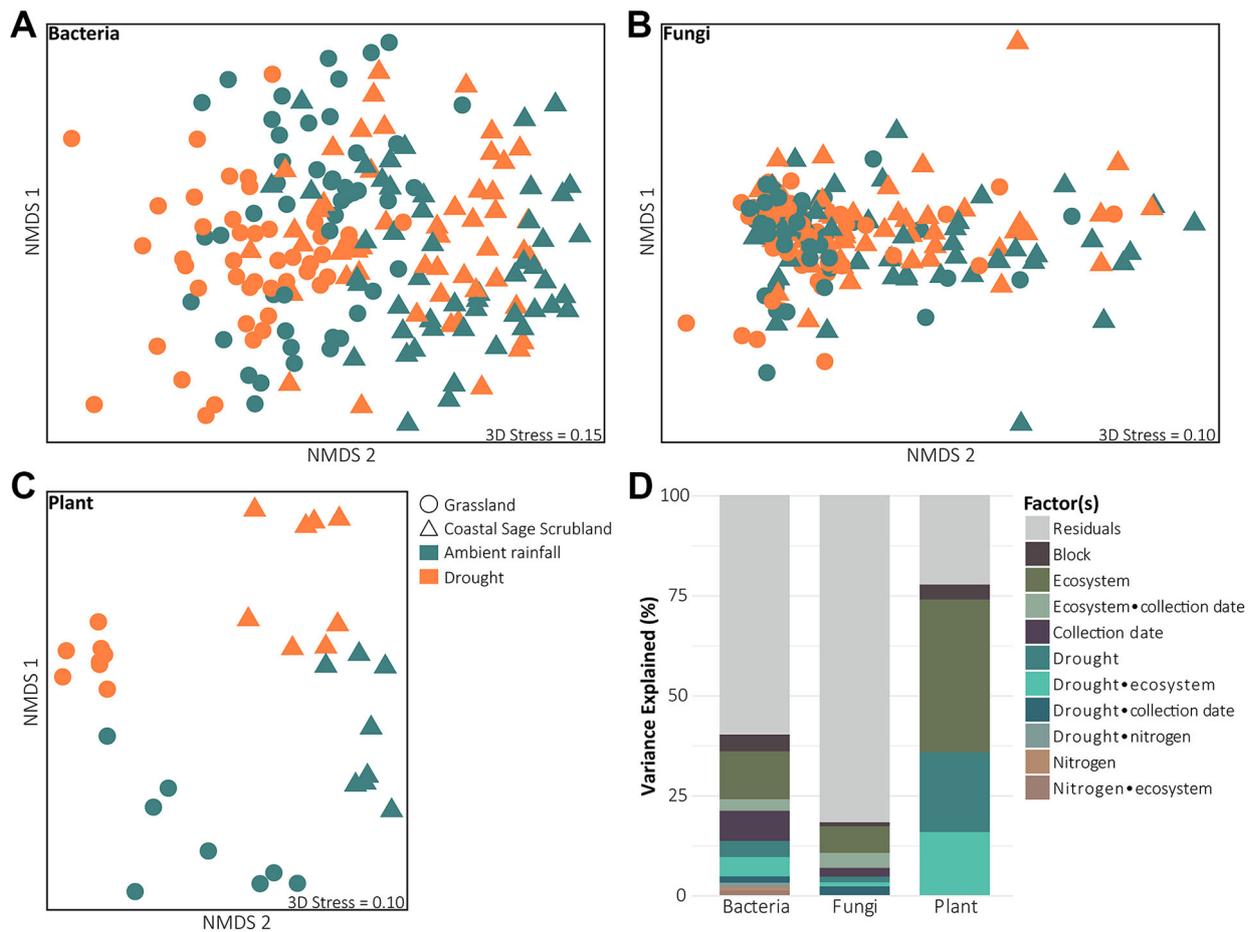
Figure 1. Microbial and plant communities vary by drought and ecosystem. Non-metric multidimensional scaling (NMDS) ordination depicting (A) bacterial (B) fungal (C) plant community composition. Symbols are defined in the legend in Panel C. Nitrogen treatment and collection date are not plotted here because their effects were not significant or applicable for all communities (see Table 1 and Figure S3). (D) The percentage of variance explained for significant ($P < 0.05$; Table 1) factors in a mixed-effects PERMANOVA for bacterial, fungal, and plant community composition. DOI: https://doi.org/10.1525/elementa.2021.00124.f1

composition across both ecosystems (Figure 1C and D). Overall, the main effects of drought were apparent across bacterial, fungal, and plant communities, while the nitrogen treatment only seemed to effect bacterial communities.

Ecosystem-dependent responses to drought and nitrogen addition

In addition to the main effects, the global change treatment altered microbial composition in an ecosystem-dependent manner, in support of our hypothesis. The interaction between the drought treatment and ecosystem explained nearly 5% of the variation in bacterial community composition, similar to the variation explained by the ecosystem-independent effects of drought (main effect plus drought-by-collection date effect = 5.54%; $P = 0.001$; Table 1). This interactive effect was apparent at the genus level; for instance, *Curtobacterium* decreased in abundance under drought conditions in the grassland, whereas it increased under drought in CSS (Figure 2A). Similarly, at the OTU level, a number of relatively abundant taxa contributed differentially to compositional

shifts under the treatments, responding in opposite directions (positively or negatively) depending on whether they were in the grassland or CSS. Further, some bacterial OTUs were observed exclusively in one ecosystem, where they contributed a large effect to the global change response. For example, OTUs belonging to *Xanthomonadaceae* and *Nesterenkonia* were only detected in grassland plots and increased in response to drought (SIMPER analysis; Table S3). These trends illustrate the ways in which bacterial responses to drought can contribute to a significant drought-by-ecosystem interaction.

The response of fungal communities to drought also depended on the ecosystem (drought-by-ecosystem effect: $P = 0.04$; Table 1). Like for the bacteria, this ecosystem-dependent response was apparent at the OTU level among the taxa that most contributed to the drought response; for instance, five fungal *Alternaria* OTUs responded to drought in opposite directions for grassland compared to CSS (Table S3). Notably, it is unclear whether these OTUs also varied significantly among ecosystems (Beta-dispersion analysis: $P < 0.001$; Warton et al., 2012). However, the fungal response to drought appeared to be less
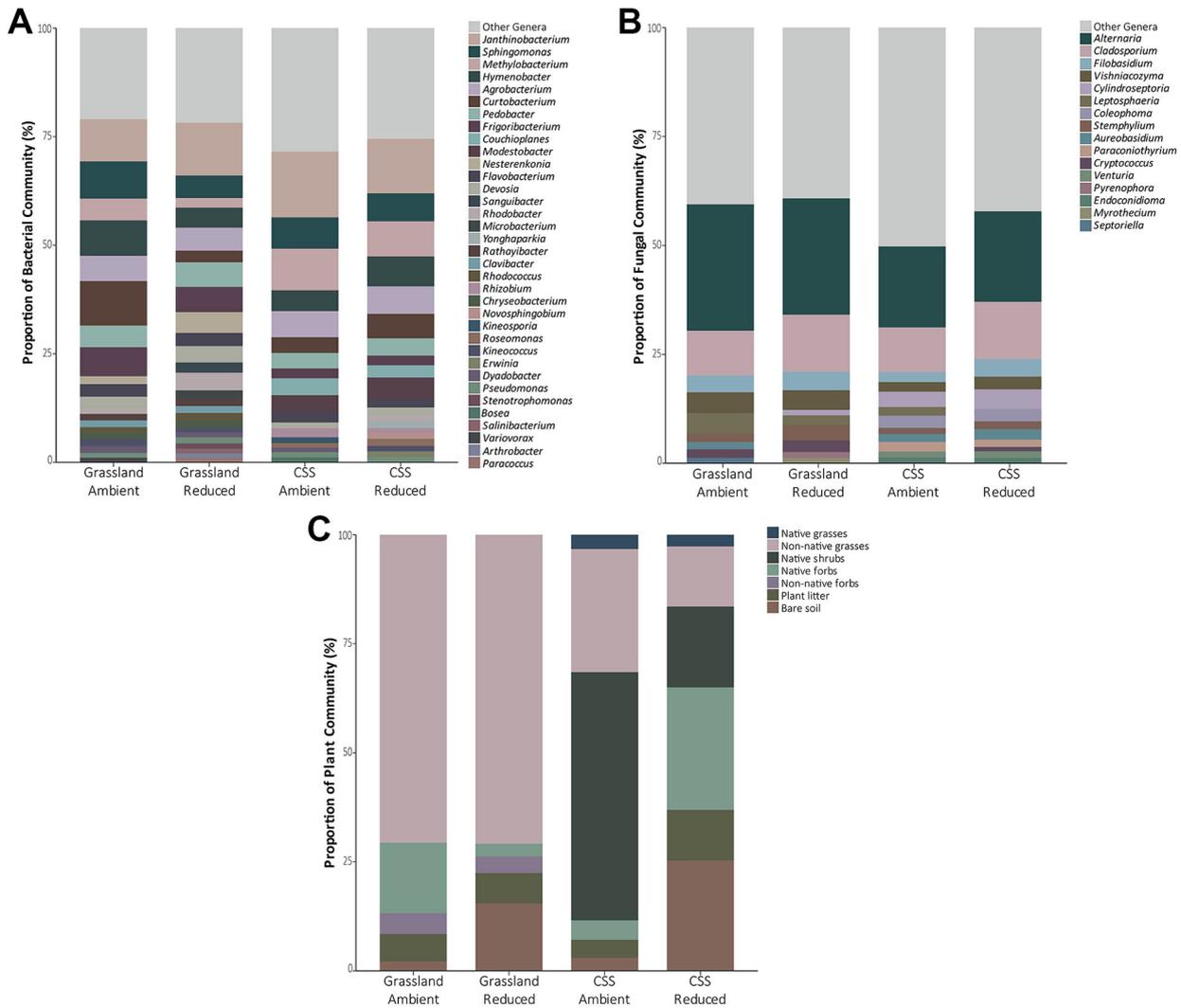
Figure 2. Microbial taxonomy and plant function groups differ across ecosystem and drought treatment. Proportional abundances of bacterial (A) and fungal (B) genera and fractional cover of plant functional groups (C) in grassland and CSS ambient rainfall and drought plots (nitrogen not shown because of the minor effects; see text). Microbial taxonomy assigned in QIIME2 using the Greengenes and UNITE databases for bacterial and fungal representative sequences, respectively. All genera under 1% relative abundance or unidentified at the genus level were categorized as "Other Genera." DOI: https://doi.org/10.1525/elementa.2021.00124.f2

dependent on the ecosystem than the bacterial response; the interactive effect only explained 1% of variation in fungal composition, lower than the variation explained by the ecosystem-independent effects of drought (main effect plus drought-by-collection date effect = 3.58%; Table 1).

Like drought, nitrogen addition altered the bacterial community in an ecosystem-dependent manner. A nitrogen-by-ecosystem interaction explained a similar amount of bacterial compositional variation (1.1%) as the main nitrogen effect (0.9%). In contrast, the fungal communities did not respond to added nitrogen, either overall or in an ecosystem-dependent manner ($P > 0.05$; Table 1).

Finally, parallel to the bacterial and fungal communities, plant community composition shifted in unique ways in the grassland and CSS in response to drought. For example, native and non-native forb cover under drought decreased relative to ambient conditions in grassland plots by approximately 13% and 1%, respectively (Figure 2C). Whereas, native forb cover under drought in CSS plots increased by approximately 24% relative to ambient plots. Additionally, ground covered by plant litter in CSS drought plots was nearly three times that of ambient CSS plots, a trend that was not observed in grassland plots (Figure 2C). However, drought noticeably increased bare soil cover relative to ambient plots in both ecosystems. Finally, a drought-by-ecosystem interaction explained nearly 16% of variation in plant community composition ($P = 0.001$; Table 1), while there was no significant nitrogen-by-ecosystem effect (Table 1).

Discussion

After a decade of global change perturbations, microbial communities on decomposing plant litter responded to both drought and nitrogen addition in adjacent ecosystems dominated by different plant communities. When

Table 1. Results of mixed-model PERMANOVAs of bacterial, fungal, and plant community composition. DOI: https://doi.org/10.1525/elementa.2021.00124.t1

Community and Variable	df	SS	MS	Pseudo-F	Pᵃ	% Variance Explained
Bacteria						
Block	6	4.29	0.72	2.66	**0.001**	4.21
Ecosystem	1	5.53	5.53	7.98	**0.001**	11.97
Ecosystem × Collection Date	6	2.60	0.43	1.61	**0.001**	2.88
Collection date	6	6.98	1.16	4.33	**0.001**	7.54
Drought	1	1.91	1.91	7.11	**0.001**	3.99
Drought × Ecosystem	1	1.26	1.26	4.70	**0.001**	4.88
Drought × Collection Date	6	2.19	0.36	1.36	**0.001**	1.55
Drought × Nitrogen	1	0.52	0.52	1.94	**0.001**	1.12
Nitrogen	1	0.64	0.64	2.39	**0.001**	0.89
Nitrogen × Ecosystem	1	0.49	0.49	1.83	**0.001**	1.11
Nitrogen × Collection Date	6	1.67	0.28	1.04	0.346	—
Fungi						
Block	6	1.80	0.30	1.32	**0.029**	1.02
Ecosystem	1	2.12	2.12	7.13	**0.001**	6.63
Ecosystem × Collection Date	6	2.26	0.38	1.65	**0.002**	3.74
Collection date	6	2.41	0.40	1.76	**0.001**	2.17
Drought	1	0.62	0.62	2.72	**0.003**	1.42
Drought × Ecosystem	1	0.37	0.37	1.63	**0.042**	1.04
Drought × Collection Date	6	1.88	0.31	1.38	**0.015**	2.16
Drought × Nitrogen	1	0.36	0.36	1.57	0.067	—
Nitrogen	1	0.22	0.22	0.95	0.472	—
Nitrogen × Ecosystem	1	0.22	0.22	0.97	0.404	—
Nitrogen × Collection Date	6	1.39	0.23	1.01	0.411	—
Plant						
Block	7	1.19	0.17	1.50	**0.046**	3.74
Ecosystem	1	2.70	2.70	15.96	**0.006**	38.19
Drought	1	1.31	1.31	11.56	**0.001**	19.90
Drought × Ecosystem	1	0.59	0.59	5.19	**0.001**	15.78
Drought × Nitrogen	1	0.06	0.06	0.50	0.838	—
Nitrogen	1	0.08	0.08	0.68	0.654	—
Nitrogen × Ecosystem	1	0.07	0.07	0.59	0.713	—

ᵃSignificant P-values ($P \leq 0.05$) are in bold.

the microbial community responded to the treatments (except fungi to nitrogen addition), this response depended in large part on the ecosystem (as indicated by a significant treatment-by-ecosystem interaction). This result supports our hypothesis that such responses depend on the plant community, assuming that any other differences among the ecosystems do not affect the microbial communities in the plant litter. Although we cannot separate their contributions here, we suspect that this dependence is due to a combination of the plant community selecting for initially divergent microbial communities, microbial taxa responding differently when situated in different plant communities, and microbial communities indirectly tracking the plant community responses.

After 10 years, extreme drought (imposed as an approximately 50% reduction of annual rainfall) continues to impact both microbial and plant community composition in the LRGCE, as was observed after the first 5 years of

treatment (Potts et al., 2012; Allison et al., 2013; Kimball et al., 2014; Matulich et al., 2015; Kimball et al., 2016; Martiny et al., 2017), and is consistent with drought experiments from other locations (Gao et al., 2011; Sheik et al., 2011; Kinugasa et al., 2012; Schmidt et al., 2018; Griffin-Nolan et al., 2019). Fewer studies consider how the plant communities might alter these drought impacts on microorganisms, but our study contributes to growing evidence suggesting that plant–microbe interactions might be common and play a larger role in microbial drought response than previously thought. For example, the response of bacterial and fungal communities to drought depended on plants, where plant community structure varied by allowing for the "invasion" of grasses (*Imperata cylindrica*) into a longleaf pine (*Pinus palustris*) common garden (Fahey et al., 2020). Evidence further suggests that bulk soil microorganisms influence plant growth under drought conditions. For instance, soil communities selected under drought conditions altered the growth of *Arabidopsis* (Lau and Lennon, 2012), and plant–microbe interactions prior to drought modified the stress response of the grass *Bouteloua gracilis* during drought (Ulrich et al., 2019).

Unexpectedly, we found that the main effect of drought was approximately four times as strong on bacterial composition as that of nitrogen addition, which did not alter fungal or plant composition. Indeed, the differences in plant and microbial community composition in the control and added nitrogen plots appear to be narrowing since the first 5 years of the treatment. In particular, after 5 years, plant communities in both the grassland and CSS responded to nitrogen addition, albeit not as strongly as drought; nitrogen addition reduced the cover of native grasses and shrubs and increased cover of non-native annual grasses (Kimball et al., 2014; Kimball et al., 2016). Now after a decade, only the bacterial communities were sensitive to nitrogen and even then, the treatment explained <1% of the compositional variation, as compared to 2% for both bacteria and fungi at 5 years (Matulich et al., 2015). However, it is important to note some differences in methodology from earlier studies that preclude more direct comparisons. For instance, previously we characterized fungal diversity using a more conserved gene region, 28S rDNA. The minimal effect of nitrogen fertilization is surprising as it often has large impacts on both plant and soil microbial communities (Elser et al., 2007; Allison and Martiny, 2008; LeBauer and Treseder, 2008; Kinugasa et al., 2012; Legay et al., 2016). We suspect that the attenuated effects of added nitrogen are due to much larger changes in the ambient conditions at the site. Plant composition at the LRGCE is not only shifting in response to the treatments but also in the ambient plots over time. For instance, native grasses were not detected in any of the grassland plots and have become rare in the CSS plots regardless of their nitrogen status. Indeed, Southern California has been subject to a severe long-term drought from 2012 to 2015 (Griffin and Anchukaitis, 2014; Yoon et al., 2015; National Climate Prediction Center, http://www.cpc.ncep.noaa.gov/products/monitoring_and_data/drought.shtml). Hence, the relatively minor effects of added nitrogen may be overshadowed by the larger impacts imposed by prolonged regional drought.

Conclusion

Global changes such as drought and increased atmospheric nitrogen deposition are likely to alter the composition of both plant and microbial communities (Ciais et al., 2005; Kinugasa et al., 2012; Fuchslueger et al., 2014; Preece et al., 2019; Zhao et al., 2019). A remaining uncertainty, however, is whether microbial responses influenced by changes in plant communities will affect plant community responses and the predictability of these responses in the long term. Our results add to growing evidence that a microbial community's response to long-term global change such as drought is dependent on biotic factors such as plant communities (Sayer et al., 2017). It is important to note, although we focused here on changes in microbial composition, such changes are often associated with process rates such as decomposition (Strickland et al., 2009; Allison et al., 2013; Cleveland et al., 2014; Martiny et al., 2017; Glassman et al., 2018). Thus, our ability to predict how microbially driven terrestrial processes will change in the future will require an integrated understanding of both microbial and plant communities (Ostle et al., 2009; Berg et al., 2010; Fischer et al., 2014).

Data accessibility statement

All data and scripts are accessible on GitHub (DOI: https://github.com/stevenallison). The DNA sequences are available through NCBI, Sequence Read Archive accession: PRJNA615043. Weather and drought data for Southern California are available online at https://wrcc.dri.edu/ and http://www.cpc.ncep.noaa.gov/products/monitoring_and_data/drought.shtml, respectively.

Supplemental files

The supplemental files for this article can be found as follows:

The SM includes Figures S1–S3 and Tables S1–S3, as cited in the text for additional support of the discussion.

Competing interests

Steve D. Allison is the EIC of the Ecology and Earth Systems domain of *ELEMENTA: Science of the Anthropocene.* Review of this manuscript was overseen by Associate Editor Daniel Liptzin and Detlev Helmig, EIC of the Atmospheric Science domain of *ELEMENTA: Science of the Anthropocene.*

Author contributions

- · Contributed to conception and design: SSF, SDA, ACM, JBHM, KKT, CW.
- · Contributed to acquisition of data: SSF, JBHM, SK, CW.
- · Contributed to analysis and interpretation of data: SSF, JBHM, SK, CW.
- · Drafted and/or revised the article: SSF, SDA, ACM, JBHM, SK, KKT, CW.
- · Approved the submitted version for publication: SSF, SDA, ACM, JBHM, SK, KKT, CW.

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