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RESEARCH ARTICLE

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

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Global changes suchas increased drought andatmospheric nitrogen depositionperturbboththe microbial and plant communities that mediate terrestrial ecosystem f1.11ctioning. 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 395plant 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 comm1.11ity 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 importarce of considering plant-microbe interactions when considering the transferability of the results of global changeexperiments across ecosystems.

Keywords: Drought, Nitrogen addition, Soil microbiome, Global change, Mediterranean ecosystem, Decomposition

Introduction

Ongoing human-driven global changesareal ring species distributions and int.erspecific interactions (Parmesan, 2006: Rosenzweig et al., 2008). Many field e,q>erimems have considtred the responses of plant and/or microbial communities LO simulated global changes (Stylinslci and Allen, 1999; Vila et al., 2003: Allison et al., 2013; Martiny et al, 2017). These studies aim to predict how global changessuch asaltered precipi1alion, nitrogen availability, CO_2 concentration, and temperature affect community composition and ecosystem processes (Stylinski and Allro, 1999; Cione et al., 2002; Vila 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 impan of a global change treatment on microbial oomposition is innuenced 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 fO(lJSon one half of plant-microbe interactions-specifically, the ways in which plants mayinfluence miaoorganisms. One way in which plants influenr.e microorganisms is through decomposition. Bacteria and fungiare the primary decomposers of dead plant biomass, and this process regulates the amount of soil carbon exchanged with the atmosphere (Swift ct al, 1979; Adair et al., 2008; Schimel and Schaeffer, 2012). More broadly, plantcon1munitiescan intlucnce microorganisms I,hrough plant species and lissue composition (influencing nutrients and secondary compounds), changes in the abiotic en..;ronment (plant architecture influencing canopy and

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moisture of the soil), and relationships with other organisms such as endophyies and herbivores (Wardle el al., 2006; Tintjer and Rudgers, 2006: Kara et al., 2008: Kominoski et al. 2009; Rodriguez et al. 2009; Chapman and Newman, 2010; Santonja el al., 2017; Graff et al. 2020). Through such associations, lhe impae1or a global change treatment on plant-litter microbial communities may depend on the plant community in at least three ways. Firs initial differences in plant communities across ecosystems will select for diffenmt microl:ial communities. Thus, the microbial response to global change may be driven by r.axa that are uniquely prominent in an ecosystern. 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, liuersubstrate quality may impaa the al:ility of liner microbes to respond to stressful conditions (Malik et al, 2020b). Finally, the extent to which the plant liuer substrates and resources are altered by global change. and thereby indirectly influence liner microbial communities, will depend on the plant community(Aerts,1997; Rouifed etal. 2010; Fernandez-Alonso et al, 2018). For instance. drought typicallyreduces the germination of aMual plants, ground cover, and primary productivity ofaridgrasslands,whereas plants with deeper root systems are less impaaed (Le Houerou, 1996; Shinoda et al, 2010; Kinugasaet al., 2012).

The Loma Ridge Global Change E.xperiment (LRGCE) simulates the increased frequency or 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; Intergovemm,ntal Panel on Climat, Change, 2014). A uniqu, feature of the LRCCE is that treatments areapplied to two adjacent ecosystems (Figure S1A), a grassland and a coastalsagescrubland (CSS Much is known about the plant and litttr microl:ial communities at the LRGCE. Within the first 5 years or the experiment, both the grassland and CSS plant communities respond,d to drought and nitrogen addition (Potts et al., 2012; Kimball et al. 2014; Kimball et al., 2016). In the grassland, drought reduced non-nativeannual grass CCNer, while nitrogen addition reduced native grass,s and increased non-native annual grasses (Kimball et al., 2014; Kimball et al. 2016). In the CSS, drought reduced shrub coverand increased grass cover, and added nitrogen further reduced shrub cover**and** native grasses(Kimball et al, 2014 Baaerial and fungal community composition on surface plant litter also responded IO these 1rea1menIS in th, grassland (Allison ,1 al., 2013; 8'rlemont **et** al., 2014i Moreover, reciprocal transplant experimen1Swithin LRCCE revealed shif1S in microbial community composition due to dirtct, al:iotic effects of the global chang, treatmen1S,and asindirecteffeClS or droughton the grassland plant liuer (Martiny CL al., 2017). However, the response or 1he microbial communi1ies within the CSS ueatm,nt plOtsat the Loma Ridge research site have not yet been investigated. Additionally, drought and added ni11ogen treatments have been ongoing for more than a decade; thus, it is important to assess the long-term effeclS 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.Toaddressour hypothesis, we aslc Does the response of microbial communities to global change depend on the e,:osystem (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 dimate, and subtle bulk soil differencesseem unlikely to affect the plant litter microorganisms on the soil surface.Thus, **we** presume !hat any differences in the treatment responses across ecosystems are likely due LO differences in the pl.lnt communities, rather than other abiotic faaors. 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 effeCl or the treatments. However, *we* also predict that the microbial community response will result in ecosystem-specific compositional shifts, as reflee1ed in significant ireatment•by-ecosystem effects. Given that the surrounding abiotic conditions are similar in these adjacent ecosystems, such interactive efferu prll'lide evidence that microbial responsesaredepen•dent on the plant communiiy.

Materials and methods

Field site, sample collection, and experimental design

The LRCCE 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 coUuvial deposit from layers of sedimentary rock and soil mapped asMyford Sandy Loam (Potts et al, 2012; Kimball et al. 2014 The grassland plots are dominated by the native perennial grass*Stipa puldua;* the annualgrass genera *A\t'na, Bromus,* and *Festuca;* and the aMual forb genera *Erodium* and *Lupinus.* The CSS plotsare dominated b'f *Artemisia coliforniai, Salvia mellifera, Eriogonum fasciculatum,* and *Aanispon g/aber* (Kimball et al., 2014). The climate is Mediterranean with anannual precipitation of 30 cm.The "wet"season is typically from November to April, while the "dry"season is from May to October (Figure $2). Air ttmperatures are moderate in th, *w,t* season with an average high and low of 21.l°C and 7.1°C. respectively, and increase in the dry season wilh an average high and lowof27.6°Cand 14.4°C, respectively (Tustin Irvine Ranch weather 1981-2010; Western Regional Climate Center, https://wrcc.dri.edu/).

Surface litter samples were colleaed at stven time points (approximately every 3 months) from August 2016 to March 2018, and from four replicate plots rer.civing four different treatmentS: control, droughl, added nitrogen, and drought plus addtd nitrogen. Thus. up to 16 samplesin each ecosysrem were collected at each time point in the lower half of the LRGCE plots:however, a er quality checks, som, samples were excluded for a total of 108 grassland and 111 CSS samples. The LRGCF.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 bycovering rain shelters with manually retractable. dear, 6-mil polyethylene roofs before predicted rain events and removed promptly afterwards (Figure S1B and C). Added nitrogen was applied at 60 kg N ha-• yea,-•as last release calcium nitrate. CaN01, and in two amounts per year, 20 kg prior to thefirst rains of thewet 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 plolS in grassland and CSS sites using Zymolliomics DNA isolations kits (Zymo Research, Irvine, CA, USA)and processed for5 minof 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 characterire bacterial composition of the leaf litter communities, PCR amplifiration of the V4 and partial VS region of the bacte.rial 16S rONA(appro.ximately 411 bp) wa.s rarried out following the Earth Mia-obiome proLOCOI (Lane et al., 1985: Capo,aso et al., 2012; Parada et al., 2016 The barcoded forward primers contain the 5' lllumina adapter (MTGATACGGCGACCACCGAGATCTA· CACGCT), a unique 12-base error correcting Golay barcode, a pad (TATC.CTAAJT), a linker sequence (G11. and the 51Sfb primtr (GTGYCAGCMGCCGCGGTM), and reverse primers that contain the re rse complement of the 3' lllumina adapter {CAAC.CAGAAGACGC.CATACGA· GAT), a pad (AGTCAGCCAq a linker sequence (GG), and the 926r primer (CCGTCMTTCCTTTRAGTTT).

Each 16S PCR reaction contained: 9.5 µL PCR grade water (Fisher Scientific, Hampton, NH, USA), 12.S µLof 2x concentrated Aa:uSlart II PCR ToughMix (Quanta bio, Beverly, MA, USA) fora final lx concentration,0.5 µLof10 µM 926r primer (final concentration of*02* µM). 1 µLof 10 mg/ml bovine serum albumin (final concentration of l µg/ml; New England Biol.ab.s, Ipswich, MA. USA 0.5 µl. of 10 µM barcoded 51Sf primers (final concentra1fon of *02* µM), and l µL of genomic DNA. Reactions were held at 94 •C for 3 min to denature DNA, with amplification proceeding for 30 cydes at 94 •C for 45 *s,* 55 •C for 30 s,and 72 •C for l min,followed by a final extension for 10 min at 72 •C to ensure complcte amplifiration.

To determine fungal community composition, and improved accuracy of amplicon clus1er de1ec1ion and res• elution during sequencing, a staggered primerdesign was used toamplify theinternal transcribed spacer(ITS) region (approximately 340 bp) of the 5.8S rRNA gene. The ITS primers used are as follows: ITS9f primer (AATCATACC GCGACCACCGAGAICTACAOC111CCCTACACGACGCTCTT CCGATCTNNNNNGAACGCAGCRAAIIGYG), along with a barcoded reveise ITS4 primer (CMGCAGAAGACGGCA-TACGAGATAGTCAGTCAGCCTCCTCCGffiATTGATATGC), whichcontained the reverse complement of the3' illumina adapter,**a** unique 12-basebarcode,and a **pad** with a linker sequence (Tremblayetal., 2015:Loobyet al., 2016).

Each ITS PCR reaction contained: 9 µLPCR grade water (Fisher Scientific, Hampton, N, USA), 12.5 µLof 2x concentrated AccuStart II PCR ToughMix(Quanta bio, Beverly, MA, USA) for a final lx concentration, 0.75 µLof 10 µM rJS9f primer (final concentration of 0.3 µM), 1 µL of 10 mg/ml bo\line serum albumin (final concentration of l µg/mL;New England Biol.abs, Ipswich, MA, USA), 0.75 µL of 10 µM barcoded llS4 primers (final concentration of 0.3 µM1and 1 µL of genomicDNA. Reactions were heldat 94 •C for 5 min to denature DNA, with amplification proceeding for 34 cydes at 95 •C for 45 s, SO•C for 1 min, and 72 •C for l min 30 s, followed by a final extension for 10 min at 72•C.

Sequencing libraries were prepared with pooled 16Sor ns amplicons from each sample after purification using Speed Bead Magnetic Carboxylate (GE Healthcare UK Limited, Buckinghamshire, UK) to remo primers.A compos• itelibrarywith equimolar ratios of the purified pooled 16S and nsampliconswas prepared.and DNAsizeand qualiiy for sequencing was determined by Qubit and Bioanalyzer (450 ng/ml and average amplicon size of 532 bp, respec• *Lively* Cus10msequencing primersfor16Sand ITS libraries were used asdescribed in Caporaso et al, 2012 and Looby etal., 2016.The libraries were sequenced by the UO Geno• mies HighThroughputSequencing Facility usingan illumina MiSeq platform with paired end readsat 300 bp.

Analysis of 16S and ITS sequencing

The forward reads of amplioon sequences were demultiplexed using QIIM£2 version 2018.11 toolkit (Caporaso et al. 2010; Bolyen eta I, 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 lhe 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 ror bacteria and fungi, respectively, and using the "Eco!Utils" package in R version 3.6.3 (R Core Team, 2018; So1la1.ar, 2020). Taxonomy was assigned to 011.Js using bacterial representative sequences and the q2-feature-classifier. dassify-skleam naive Bayes taxonomy dassifier against the Greengenes 13_8 99% OTUs rererence sequences (McDo• nald et al., 2012; Bokulich et al., 2018). Taxonomy ror fungal representative sequences were assigned using a dynamic threshold {97o/o,-99% idcntily 10 reference), which is based on 1he most accura1e assignment for a given lineage, and determined manually by expcrlS in the field from the UNITE v7.2 database, release date December 1, 2017 (Nilsson et al.. 2018: UNITE

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

Plant community

Species composition and fractional cover was det.ennined in all plots by point intercept during mid-April of 2015, coinciding with late flowering and maximum seed set. Briefly, two 160 x 60 cm^2 polyvinyl chloride frames with lOon intervalgrids 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 CCNer. Fractional cover data of all species observed (32 in tOl:31) were used to generate a Bray-Curtis dissimilarity matrix. Fractional cCNer data for all species were further tal.elll)rized into five functional groups induding nal.ive grasses. non-nal.ive grasses, native faro. non-native forb, and natr.<eshrub(KimbaUet al, 2014;Matulichet al. 2015).

Statistical analysis

To detennine the effects of ecosystem. drought, added nitrogen, sample collection date, and all interactions on microbial composition, PERMANOVAs using Bray-Cunis dissimilarity matrices generated with rarefied OT1J iables were performed using PRIMER-e version 6 (Oarlce and Gorley, 2006; Anderson et al, 2008). Microbial and plant mixed models induded plot treatment (ambient rainfall or nitrogen, drought, added nitrogen, and drought with added nitrogen1 eco.system, and sample collection date as fixed factors. The bloclcfactor was nested within ecosystemas a random effect toaccount for thesplit-plot design of the , riment The estimated percentage of variance explained was det.ennined by dividing tenns with significant P.values by the sum of the estimates of components ofvariation giv,n asoutput from PRIMER-e. Post-hoc comparisons of PERMANOVAs fordroughtand collection dates given community dissimilarities were performed using PRIMER-e. Multivariat, homogeneity of varian"s for drought and nitrogen treatments by ecosystem were tested in R using the·beiadisper" function of the •vegan· package, calculating distance 10 group centroid and accounting for sampling bias (Anderson, 2006; Anderson et al. 2006; R Core Team, 2018; Oksanen et al., 2019 To determine which Laxa associated with OT1J idenliliers were key contributors to compositional differences in **bac**terial and fungal communities, SIMPER tests were con• ducted in PRIMER-e. To visualize factors influencing bacterial, fungal, and plant communities, ordinations of rarefied Bray.Curtis matrices were perfooned using non-metric multidimensional scaling using the •vegan" and "ggploi2" packages in R (Wickham, 2009).

Results

We inves1iga1ed the response of microbial communities on plant litter to drought and nitrogen addition in a decade-long global change experiment canied out in adjacent grassland and CSS ecosystems. From samples taken over 3 years (beginning near the end of year nine of I.he 1.RGCE) and across 32 treatment plots, 2.48 million bacterial sequences were clustered into 1,197 OTUs (defined at JOO% sequence similarity; Table S1). The majority of bacterial *arus* were associated with four phyla: Proteobaaeria {40.2%). Bacteroidetes (29.6% Ac· tinobaaeria (17.9% and Firmicutes {2.9% Correspondingly, 5.69 million fungal sequences dustered into 4,190 mus from IWO main phyla: Ascomycetes (71.4%) and Ba• sidiomycetes (28.2%

Main effects of ecosystems and time

The composition of both bacterial and fungal communities varied significantly between the grassland and CSS. EcOSystem, induding its interactive effects with collection date (encompassing annual and seasonal variation), ex• plained the largest amount of compositional variation, approximately 15%and 10% of thevariation for bacterial and fungal communities, respectively (Figure 1A and **B;** Table 1; PERMANOVA: P *5* 0.001). These compositional differences were apparent at the genus level. Within bac-Leria. *Sphingomonas, Hymenobacwr,* and *Cu.rt.obacteri.um* tended to be relatively more abundant in the grassland, whereas *Janthinobactl'f'ium, 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 *C)Aindroseptoria*and*Coleoplwma*weremoreabundantin CSS (Figure 2B The differences in microbialcom• position between ecosystems paralleled distinctions in plant community composition, with ecosystem accounting for 38% of the variation in plant composition (Figure IC and D;Table 1;P50.01).The grassland wasdominated by non-native grasses, whereas CSS was predominantly comprised of native grasses, shrubs, and forbs (Figure 2Q.

Microbial compo1ition also varied temporally over the three sampling years, asexpected from previous studies in the grassland at LRGCE (Matulich and Martiny, 2015). After ecOSystem, collectjon date explained the moot vari• ation in both bacterial and fungal community composition, approximately 8% and 2%, respectively (P ::: 0.001; Table 1; Figure ID). 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 (posr-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 $ 0.01), whereas nitrogen only altered the bacterial community. The main effect of drought, including its interactive effects with collection date, explain,d 5.6% and 3.6% of 10tal variation in bacterial and fungal community composition, respectively (Figure ID). In contrast, **added nitrogen** accounted for less than 1% of the varia• lion in bacterial composition. Main effects ofdrought also explained 20% of variation in plant community

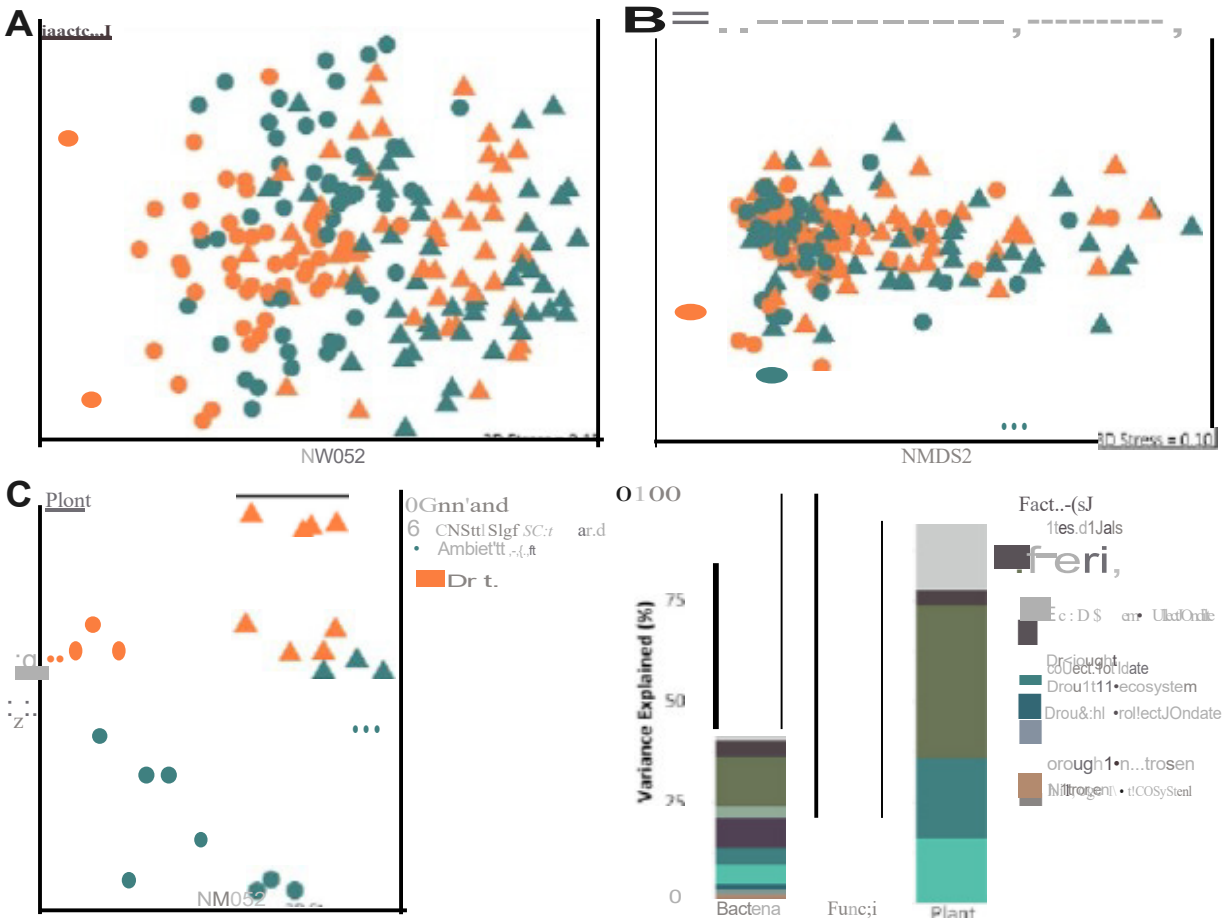

Figure 1. Microbial and plant communities vary by drought and ea:,system. Non-metric multidimensional scaling (NMDSJ ordination depicting (A) bacterial (BJ fungal (q plant community composition. Symbols are defined in the legend in Panel C. Nitrogen treatment and collection date are not plo11ed here because their effects were not significant or applicable ror all communities (see Table 1 and Figure S3). (DJ The percentage or variance explained for significant (P < 0.05; Table lJfactors in a mixed ffects P£RMANOVA for bacterial, fungal, and plant community composition. DOI: hnps://doi.org/10.1525/elementa.2021.00124.fl

composition across boc.h ecosys1ems (Figure IC and DJ. Overall, the main effects ordrought were apparent across bacterial fungal, and plant communities,while the nitrogen treatment only seemed to effect bac.terial communities.

Ecosystem-depend<>nt rl!Sponses to drought and nitrogen addition

In addi1jon 10 the main effects, 1he global change treat• ment altered microbial composition in an ecosystem-dependent manner, in suppon or our hypothesis. The interaajon beiween the drought1rea1ment andecos)'Stem explained nearly 5% of the variation in bacterial community composition, similar Lo thevariation explained by the ecosystem-independent effects or drought (main effea plus drough1-by-collee1;on date effect - 5.54%; *P* - 0.001; Table lJ.This interactive e ct was apparent at the genus level; for instance, *Curtobaacrium* 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 or relatively abun• dam taxa contributed differentially to compositional shifts under the treatments. responding in opposite directions (positivelyor negatively) depending on whetherthey were in the grassland or CSS. Funher, some bacterial OTIJs were observed exdusively in one ecosystem, where they contributed a large effect to the global change response. For example, OTIJs belonging to *Xanthomonadaceae* and *Nesterenkonia* were only detected in grassland plots and increased in response to drought (SIMPERanalysis; Tuble S3 These trends illustrate the Wil'fSin which bacterial responses to drought can contribute to a significant drought-by-ecosyslem inleraction.

The response of fungal communities to drought also depended on the ecosystem (drought-by-ecosyslem effect: *P* - 0.04; Table 1 Like for lhe bacteria, this ecosystem• dependent response was apparent at the OTIJ levelamong the ta,ca that most contributed to the drought response; for instance, five fungal *Alternaria* OTIJs responded to drought in opposite directions for grassland comp.ired 10 CSS (Table S3). Notably, it is unclear whether these OTIJs also varied significantly among ecosystems (Beta• dispersion anal)'Sis: *P* < 0.001; Warton et al., 2012). How• *ever,* 1he fungal response to drought appeared to be less

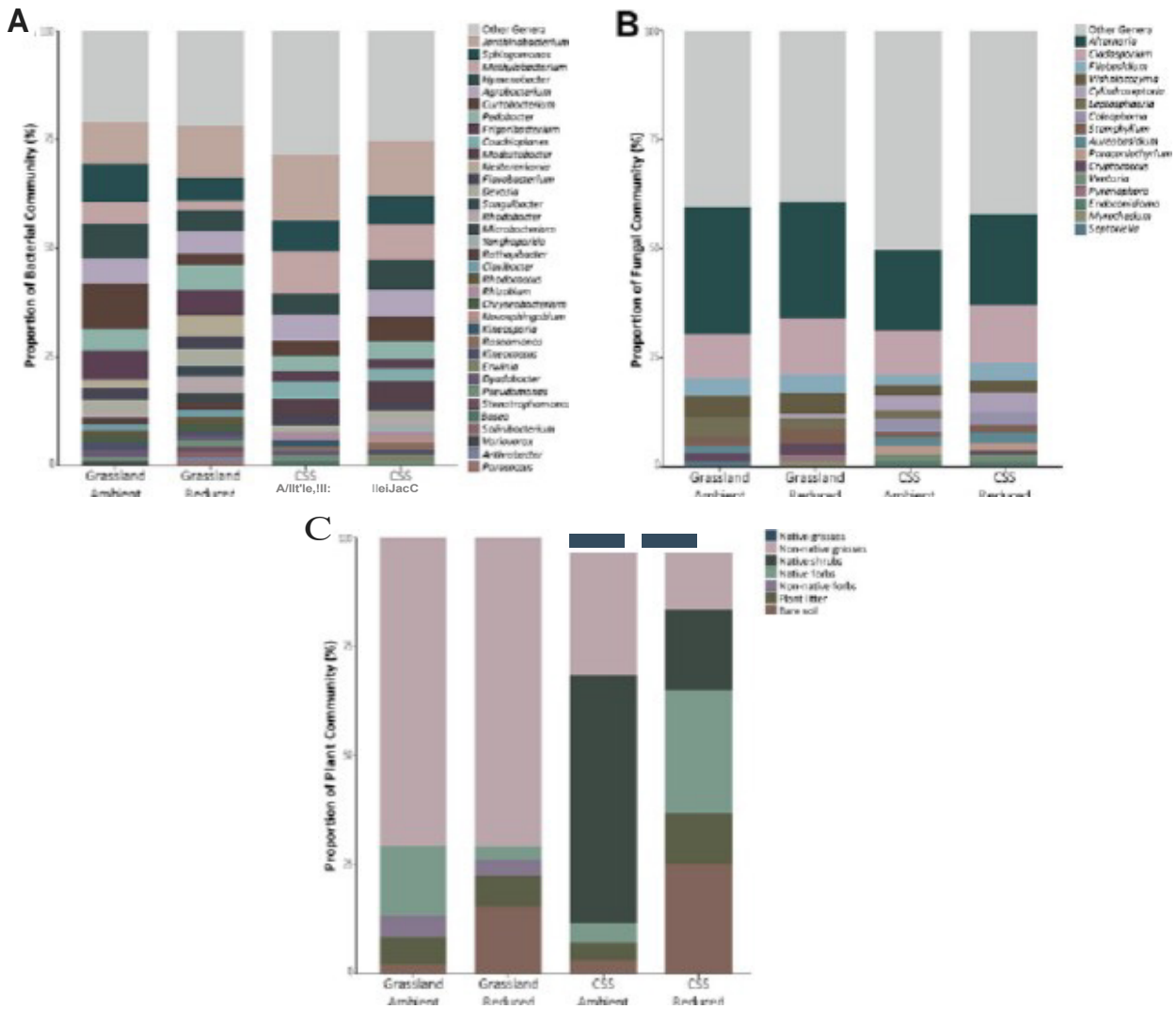

Figure 2. Microbial 1axonomy and plant function groupsdiffer across ecosy.stern and drought rre.itment. Proportional abundancesofbaaerial (Al and fungal (Bl genera and fractional ca;er of plant functional groups(Cl in grassland and CSS ambient rainfall and drought plots (nitrogtn not shown because of the minor effects; see text Microbial raxonomy assigned in QIIME2 using the Greengenes and UNITE databases for bacterial and fungal representative sequences, respectively. Allgenera under 1% relative abundanceor unidentified at the genus level were categorized as "Other Genera." DOI: htt1>5=//doi.org/10.1525/elementa.2021.00124.f2

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

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%1as the main nitrogen effect (0.9%1- In contrast, the fungal communities did notrespond to added nitrogen, either overall or in an ecosystem-dependent manner (P > 0.05; Tobie I). Finally, parallel to the bacterial and fungal communities. plant community composition shined in uniquewirys in the grassland and CSS in response to drought For example. native and non,nativeforb OM!r under drought decreased relative to ambient conditions in grassland plots approximarely 13% and lo/a. respectively (Figure 2().Whereas, native fo,b cover under drought in CSS plots increased byapproximately24% relative to ambient plots. Additionally,ground covered by plant liuer 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 noticeablyincreased bare soiloover relative to ambient plots in both ecosystems. Finally, a drought-by-ecosystem interaction explained nearly 16% of variation in plant community oomposition (P = 0.001; Table 1), while therewas nosignificant nitrogen-by-ecosys1erneffect (Table 1).

Discussion

Alier 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 or mixed-model Pf.RMANOVAs or bacterial, fungal, and plant community oomposition. DOI: httpS// doi.org/10.1525/elemenla.2021. 00124.tl

C.Ommunity a.ad Variable	*df*	SS	MS	Pseudo·F	?•	% Variance El<plained
8acleria						
Diode	6	**4.29**	0.72	2.66	0.001	4.21
Erosystem		5.53	5.53	7.98	0.001	11.97
Erosystem x Colleaion 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		1.91	1.91	7.11	0.001	3.99
Drought x Ecosystem		1.26	1.26	4.70	0.001	4.88
Drought x Collection Date	6	2.19	0.36	1.36	0.001	1.55
Drought x N n		0.52	0.52	1.94	0.001	1.12
Nitrogen		0.64	0.64	2.39	0.001	0.89
Nitrogen x Ecosystem	1	0.49	0.49	1.83	0.001	1.11
Nitrogen x Collection D.1te	6	1.67	0.28	1.04	0.346	
Fungi						
Blodc	6	1.80	0.30	1.32	0.029	1.02
Erosystem		2.12	212	7.13	0.001	6.63
Erosystem x Colleaion 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		0.62	0.62	272	0.003	1.42
Drought x Ecosystem		0.37	0.37	1.63	0.042	1.04
Drought x Collection Date	6	1.88	0.31	1.38	0.015	2.16
Drought x Ni n		0.36	0.36	1.57	0.067	
Nitrogen		0.22	0.22	0.95	0.472	
Nitrogen x Ero rn		0.22	0.22	0.97	0.4-04	
Nitrogen x Collection Date	6	1.39	0.23	1.01	0.411	
Plant						
Blodc	7	1.19	0.17	I.SO	0.046	3.74
Erosystern		2.70	2.70	15.96	0.006	38.19
Drought		1.31	1.31	11.56	0.001	19.90
Df0t'8ht x Ecosystem		0.59	0.59	5.19	0.001	15.78
Drought x Nitrogen		0.06	0.06	0.50	0.838	
Nitrogen		0.08	0.08	0.68	0.654	
Nitrogen x Erosystem		*om*	0.07	0.59	0.713	

'Significant *P.values* {**P** $ 0.0S)are in **bold.**

the microbial community responded *ID* the Jrealmcnts (except rungi to nitrogen addition), this response depended in large part on the ecosrstem (as indicated by a signilic.int treatment-by-ecosystem interaction). This result supports our hypothesis that such responses depend on the plantcommunity, assuming that*any* other differences among1he ecosys1ems do no1affec11he microbial communities in the plant liner. Although we cannot separa1e their contributions here. we suspec1 that I.his dependence is due to a oombination of the plant oommunity selecting for ini1ially divergent microbial communiLies, microbial taXa responding diffcrendy when situated in different plant communi1ies. and microbial communiLies indirectly tracking the plant community responses.

After 10 years, extreme drought (imposed as an approximaiely SO% reduction of annual rainfall) continues J.o impact both microbial and plant communify composition in the LRGCE, as was observed aft.er the first 5 years of

treatment (Pol!S et at. 2012; Allison et al, 2013; Kimball et al. 2014; Matulich et al., 2015; Kimball et al. 2016; Martiny et al. 20171and is consisleflt with drought experiments from other locations (Gao et al, 2011; Sheik et al. 2011; Kinugasa et al., 2012;Schmidtet 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. 2019i

Unexpectedly, we found that the main effect of drought was approximately four times as strong on **bac**terial composition as that of niu-ogen **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 LO be narrowingsince the first 5)'!ars of the treatmenL In particular, after 5 yea15, plant communities in both the grassland and CSS responded LO nitrogen addition, albeit nocas strongly as drought; nitrogen addition reduced the cover of native grasses and shrubs and incr as d cover of non-native annual grasses (Kimball et al., 2014; Kimball et al., 2016 Nowaftera decade, only the bacterial communities **were** sensitive to nitrogen and even then, the treatment explain d <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 LO note some di rences in methodology from artier studies that predude more direct comparisons. For instance, previously **we** characterized fungal diversity using a more conserved g ne region, 28$ rONA **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; IJ!Bauer and Treseder, 2008; Kinugasa et al.,2012; Legay *et* al., 2016).We su ct that the attenuated effects of added nitrogen are due LO much larger changes in1heambient conditions at the site. Plant composition at the LRGCE is not only shifting in response to the treatments but also in the ambient plolS over time. For instance, native grasses were not de1ected in any of the grassland plots and have become rare in the CSS plots regardless of their nitrogen siatus. Indeed, Southern California has been subject to a severe long•term drought from 2012 10 2015 (Griffin and Anchukailis, 2014; Yoon et al., 2015; National Climate Prediction Center, http://www.cpc.ncep.noaa.p /produets/monitoring_and_da1a/drough1.shtml). Hence, the rela1.ively minor effects of added nitrogen may be overshadowed **by** the larger impaclSimposed **by** prolonged regional drought

Conclusion

Global changes such as drought and increased atmo•spheric nitrogen deposition are likely to alter the compo•sition 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 uncertainiy, however.is whether microbial responses influenced **by** changes in plant communities will affect plant community responses and the predictability of these re•sponses in the long term. Our results add LO growing evidence thata microbial community's response to long-term global change such as drought is dependent on biotic fac. tors such as plant communities (Sayer et al. 2017 It is important LO 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; Martinyet al., 2017;Glassman etal.,2018).Thus,ourability to predict how microbially driven terrestrial processes will change in the future will require an integrated undersianding 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 areaccessible on GitHub (001: htlps:// github.com/stevenallison). The DNA sequences are avail•able through NCBI, Sequence Read Archive accession: PRJNA615043. Weather and drought data for Southern California areavailable online at https://wrcx:.dri.edu/ and hnp://www.cpc.ncep.noaa.gov/productS/monitoring_and_data/drought.shtml, respectively.

Supplemental files

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

The SM includes Figures Sl-S3 and Tables Sl-S3, as cited in the text for additional support of the disrussion.

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Competing interests

Steve D. Allison is the EiC or the Ecology and Earth Sys• terns domain of *ELEMENTA: Science cf the Anlhropocene.* Review of this manuscript was overseen by Associate Editor Daniel Liptzin and Detlev Helmig, EiC or the Allnospheric Science domain or *ELEMEtrrA: Science of the Anlhropocene.*

Author contributions

- Contributed to conception and design: SSF,SDA. ACM, JBHM, KKT, *CN.*
- Contributed to acquisition of data: SSF,JBHM, SK, *CN.*
- Contributed to analysis and inteipretation of data: SSF, JBHM,SK,*CN.*
- Drafted and/or revised the artide: SSF, SDA, ACM, JBHM, SK,KKT, *CN.*
- Approved the submitted ver.;ion for publication: SSF, SDA.ACM, JBHM, SK, KKT, *CW.*

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