Soil microbial responses to drought and exotic plants shift carbon metabolism

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Abstract

Significant gaps in our understanding of how global change drivers interact to affect the resistance and functioning of microbial communities hinders our ability to model ecosystem responses and feedbacks to co-occurring global stressors. Here, we investigated the effects of extreme drought and exotic plants, two of the most significant threats to Mediterranean-type ecosystems, on soil microbial community composition and carbon metabolic genes within a four-year field rainfall manipulation experiment. We combined measurements of bulk microbial and soil properties with high-throughput microbial community analyses to elucidate microbial responses and microbial-mediated alterations to carbon cycling. While microbial responses to experimental droughts were weak, scant rainfall periods resulted in decreased microbial biomass and activity, and relative abundances of bacterial groups such as Proteobacteria, Verrucomicrobia, and Acidobacteria decreased concomitantly with increases in Actinobacteria, Chloroflexi, and Firmicutes abundance. Soils under exotic plants had increased temperatures, enhanced infiltration during rainfall events, and decreased water retention and labile carbon in comparison to soils under native plants. Higher peaks and more seasonally variable microbial activity were found under exotic plants and, like drought periods, the microbial community shifted towards osmotic stress life-strategies. Relationships found between microbial taxonomic groups and carbon metabolic genes support the interpretation that exotic plants change microbial carbon cycling by altering the soil microclimate and supplying easily decomposed high-quality litter. Soil microbial community responses to drought and exotic plants could potentially impact ecosystem C storage by producing a smaller, more vulnerable C pool of microbial biomass that is prone to increased pulses of heterotrophic respiration.

Introduction

Exotic plants and climate disturbances are among the most significant threats to Mediterranean-type ecosystems (MTEs) [1, 2]. This is the case for shrub-dominated MTEs distributed along coastal and southern California (CA) that have been invaded extensively by exotic forbs and

grasses [3] and have recently experienced 5 consecutive years (2012–2016) of high temperatures and scant rainfall, including three consecutive years of record warmth (2014–2016) (NOAA-NCEI). With projected future scenarios of increased drought severity [4], there is a need to improve our understanding of the interactions of these co-occurring stressors and potential consequences for ecosystem functions. Losses in biodiversity and carbon (C) storage are of particular concern as MTEs have been underscored as global reservoirs of biodiversity [5] and play an important role in the interannual variability of global C sequestration [6].

Numerous studies of the effects of exotic plants and rainfall shifts on CA shrublands have had an aboveground focus, highlighting the role of the competitive advantages of exotic plants [7–11] and soil nitrogen feedbacks in ecosystem alterations [12, 13]. However, fewer studies have considered belowground communities [13, 14] and impacts on soil C cycling [15]. While often overlooked, soil communities represent one of the largest reservoirs of

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biodiversity [16] and comprise a significant fraction of soil carbon [17–20]. Additionally, microbial heterotrophic respiration is one of the largest avenues of C loss from ecosystems [21]. Thus, assessments of the response of MTE soil communities to global stressors can enhance our understanding of community dynamics and how these responses influence ecosystem C functions [22].

A growing body of belowground-based studies have reported the resistance and resilience of semi-arid soil microbial communities to extreme weather events due to their routine exposure to seasonal drought and ability to recover upon rewetting [23–28]. While mounting evidence suggests that changes in community structure can occur as adaptive responses to drying-rewetting cycles [29, 30], responses by microbial communities to multiple global change disturbances remain poorly understood [31].

Exotic plants can interact with climatic changes to affect soil microbial communities by physically altering soil microclimates and by altering plant-microbe interactions [22, 32, 33]. Exotic species common to CA MTEs have been reported to have shallower roots [34, 35], shorter life spans, earlier seasonal activity [11], and higher litter quantity and quality [15] when compared to dominant native shrubs. The differentiation in plant structures as well as the timing, quantity, and quality of C sources between CA MTEs native and exotic plants can alter microbial communities and hence their responses to extreme droughts [36].

In MTEs, microbial responses to co-occurring global stressors must be considered in light of temporal variation, as intra-annual and inter-annual variability are key drivers of ecosystem processes [37]. As shown in previous studies that examined the response of microbial communities over multiple temporal scales [23–25, 27], there is a need to gather experimental evidence that incorporates temporally explicit assessments. Furthermore, recent high-throughput DNA sequencing technology presents an opportunity to characterize and quantify soil microbial functional attributes in greater depth, providing insight into microbial resistance [38] and ecosystem functions [39].

Here, we monitored soil microbial biomass, activity, community composition, and C functional genes under native-dominated and exotic-dominated, rainfall manipulated plots in a CA MTE community experiencing a multi-year drought to assess the impacts of extreme drought, exotic plants and temporal variability on soil microbial communities and carbon cycling. We hypothesized that physiological stress caused by extreme drought conditions could impact microbial population size, shift microbial community structure, and alter microbial functions. Alternatively, while microbial communities are generally constrained by droughts, the adaptive capabilities and phenotypic plasticity of soil microbes may yield

mechanisms to tolerate and resist periods of droughts. We expected complex interactions between temporal variability, plant community types, and microbial communities. For example, the lack of aboveground structures in exotic annuals can exacerbate the impacts of a drying climate to promote a more stressful environment that is detrimental for soil microbes [40]. On the other hand, higher quality exotic litter can promote microbial abundance and metabolism, altering microbial C processes [41].

Materials and methods

Site description

This study was conducted at the Santa Margarita Ecological Reserve (SMER), located in the upper part of the Santa Margarita River watershed (33°26′28.6″N, 117°09′51.89″W), in Riverside County, CA, USA. The site is dominated by native mature shrubs, such as *Artemisia californica*, *Salvia mellifera*, and *Salvia apiana*, interspersed with native perennial herbs including *Calochortus splendens* and *Dichelostemma capitatum* and exotic annual species, such as *Centaurea melitensis*, *Hirschfeldia incana*, and *Bromus madritensis*. These exotic species likely became established after the site was disturbed in the 1970s during the construction of a water pipeline. The region has a 30-year (1981–2010) rainfall average of 358 mm year⁻¹.

Thirty 3 m × 3 m experimental plots with a dominant plant community composition of either native shrub ($n = 15$) or exotic annuals ($n = 15$) were established prior to the 2013 rainy season. *Salvia mellifera* and *Artemisia californica* dominated the native plots and exotic annuals, such as *Centaurea melitensis*, *Hirschfeldia incana* and *Bromus madritensis*, dominated the exotic plots. All plots were located on similar soil, classified as Las Posas loam, *Fine, smectitic, thermic Typic Rhodoxeralfs*, which are well drained with an argilic horizon under topsoil that ranges from 15 to 46 cm (<http://websoilsurvey.sc.egov.usda.gov>). Soil analyses at the beginning of the experiment showed no differences in pH, soil organic matter, and texture between plots (Supplementary Fig. 1), indicating that these parameters, the major variables responsible for shaping soil microbial communities [42–44], were consistent among treatments. We are therefore reasonably confident that the effects observed in this experiment did not arise from unintentional historical artifacts. Using rainfall shelters, three rainfall treatments (50, 100, and 150% of ambient rainfall) were imposed on experimental plots of native vs. exotic plant communities. For each combination of rainfall and plant community type, five replicates were allocated in a randomized block design. The rainfall exclusion shelters were constructed with clear corrugated acrylic roofing and

were removed from May to September each year (dry season). Rainfall was collected from the roofs by gutters and stored in on-site tanks for redistribution to plots to achieve each respective rainfall treatment level using an overhead irrigation system. The irrigation system was controlled by volume flow meters. Daily rainfall was monitored from the NOAA Fallbrook 5 NE weather station (<http://www.ncdc.noaa.gov/qcld/QCLCD?prior=N>) located 2.4 km east of the study site. After each rain event, the appropriate amount of watering was applied to plots to achieve the rainfall treatments.

Soil sampling and analyses

To evaluate how intra-annual and inter-annual variations influence belowground microbial dynamics, soils were sampled during the winter (rainy season), spring (peak plant biomass), and summer (dry season) over a 4-year period from 2013 to 2016, resulting in 12 sampling events (Supplementary Table 1). Soil was sampled to a depth of approximately 15 cm using a 5 × 15 cm slide hammer soil core sampler (ASM Inc.). Samples were stored in a cooler for transportation and processed within 24 h of collection. All visible stones, roots and litter were removed with forceps. To assess whether rainfall treatments and plant community type influenced the soil microclimate, we measured the gravimetric water content (GWC) of soil samples by drying 5-g of soil at 65 °C for 24 h and measured soil temperature in the field during 4 of the 12 sampling times (Summer 2013 and 2015 and Winter and Spring of 2014) using the temperature sensor of an EGM-4 Environmental Gas Monitor (PP Systems). To evaluate alterations in soil C, dissolved organic carbon (DOC) was measured in 0.5 M potassium sulfate extracts, determined by oxidation with Mn^{3+} , and estimated on a spectrophotometer [45].

Microbial community analyses

To test for alterations in microbial population size, microbial biomass carbon (MBC) was evaluated by the fumigation-extraction (FE) method [46]. After extractions (in 0.5 M potassium sulfate), the C concentrations in the extracts were determined [45]. The MBC contents were calculated from the difference between fumigated and non-fumigated samples. Substrate-induced respiration (SIR) (Anderson and Domsch 1978) was used to assess microbial activity as SIR is a simple and rapid method that provides a suitable index of potentially active biomass [47]. Bacterial community structure and C functional genes analyses were performed on a subsample of soils ($n = 37$) collected in the spring months of 2013 (4 native and 4 exotic), 2015 (6 native and 5 exotic) and 2016 (9 native and 9 exotic) (Supplementary Table 2). Soil DNA was extracted using

Power Soil DNA isolation kit (MoBio, Carlsbad, CA USA) according to the manufacturer's instructions. Extracted DNA was quantified by PicoGreen fluorometry and diluted with sterile water to $30 \text{ ng } \mu\text{L}^{-1}$ DNA based on the lowest sample concentration. The diluted DNA was sheared to 350 base pairs (bp) using a Covaris ultrasonicator (Covaris Inc., Woburn, MA, USA) according to manufacturer's recommendations. The sheared DNA was repaired, ligated, and indexed using the Accel-NGS 2S Plus DNA Library Kit (Swift Biosciences, Inc. Ann Arbor, MI, USA). The prepared library was quantified using Agilent 2100 Bioanalyzer System and sequenced using an Illumina MiSeq (300 bp). Quality control and preprocessing of metagenomic data was performed using PRINSEQ to eliminate sequences with less than 75 bps, less than 25 mean quality scores, and replicated reads [48]. The sequences in each taxon or function were calculated and normalized by using Metagenomic rapid annotations (MG-RAST Version 4) against RefSeq Subsystems databases with an e-value cutoff of 10^{-5} , a minimum identity cutoff of 60%, and a minimum alignment length cutoff of 15 aa [49].

Statistical analyses

Data analysis was performed in R version 3.3.3 [50]. To determine the effects of rainfall treatment, plant community type, season, and year and their interactions on MBC, SIR, GWC, and DOC, mixed-effects repeated measures analysis of variance (ANOVA) was employed using the lme function within the nlme package. Linear model assumptions of normality and homoscedasticity were tested using Shapiro-Wilk and Levene's test and observed visually using Q-Q plots and plotting residuals against fitted values, respectively. When significant effects were found, multiple comparisons between treatments, seasons, and years were performed using the glht function within the multcomp package. To test significant correlations, Pearson correlation tests were performed using cor.test function. *P*-values less than 0.05 were considered significant.

To determine the effects of plant community type, rainfall treatment, and interannual variability on bacterial community structure and carbon cycling genes, taxonomic profiles were compared at the phylum, class and genus levels, and functional profiles were compared at the level 2 subsystems that belonged to carbohydrate metabolism [51]. Bray-Curtis dissimilarities were compared by permutational multivariate analysis of variance (PerMANOVA) using the adonis function of the vegan package [52]. When significant effects were found, multiple comparisons between treatments and years were performed using the pairwise.perm.manova function within the RVAideMemoire package. Non-metric multidimensional scaling (NMDS) analysis was also used to visualize

community similarity between metagenomes [53] using the function ‘metaMDS’ in the vegan package. Component scores were used to generate 95% confidence ellipses around metagenomes using the ordiellipse() function within vegan. Also within vegan, a Pearson’s correlation matrix was constructed for percent abundances of gene hits corresponding to most abundant phyla and abundances of level 2 carbohydrate metabolism using as .matrix() and cor() functions [54]. Statistical analysis of the Metagenomic Profiles (STAMP) (version v2.0.3) was also used to identify statistically significant taxonomic and functional groups among year, rainfall treatments, and plant community type [55]

Results

Effects of droughts and exotic plants on soil microclimate

During the 42-month sampling period (December 2012–June 2016), CA was under natural drought conditions. Our field site received 209, 218, 285, and 207 mm of rainfall during the water years of 2013–2016, respectively, compared to a long-term average of 358 mm year⁻¹ (Table 1). Additionally, a dry year also occurred the year before the experiment (2012), when the site received 285 mm of rainfall. Due to the lack of rainfall, our 50 and 100% rainfall treatments represented two levels of drought (mean = 32 and 64% of normal rainfall, respectively) and the 150% treatment corresponded to a normal rainfall year. Water year 2014 had the driest winter, receiving only 122 mm from October to February (Table 1). Water year 2015 was the wettest year during the study, receiving major Pacific storms and summer rains associated with the 2015–2016 El Niño event.

Table 1 Ambient seasonal rainfall (100% treatment) in mm for water years 2012–2016 and percent normal calculated with the normal average of 358 mm year⁻¹ during the fall/winter (October to February), spring (March to April) and summer (May to September) seasons

Water Year	Fall/Winter	Spring	Summer	Total	Normal (%)
2012 ^a	178	102	5	285	80
2013	161	36	12	209	58
2014	122	47	49	218	61
2015	182	21	82	285	80
2016	159	41	7	207	58

Water year starts on October 1 and ends on September 30. The experimental rainfall treatment was established prior to the 2013 winter season

^a2012 presents the rainfall statistics for the year prior to sampling

Rainfall treatment effects on surface (0–10 cm) gravimetric soil moisture were observed more strongly during the winter and spring seasons than during summer dry seasons, when GWC values fell to similar levels for all treatments (rainfall treatment × season interaction $P = 0.002$, Table 2). For example, during the winters GWC values in native-dominated plots averaged 9, 11, and 12% (based on average values across all winters) in the 50, 100, and 150% rainfall treatments and 8, 12, and 14% in the exotic-dominated plots. During the summers GWC dropped to approximately 3% for all rainfall treatments in both plant community types (Fig. 1a). Post-hoc comparisons detected differences between the 50 and 150% treatments especially during the winter seasons of 2015 and during the spring seasons of 2013 and 2014 (rainfall treatment × season × year interaction, $P = 0.04$), potentially associated with high rainfall events (Table 1).

Plant community type influenced soil microclimate through direct and interactive effects. The effects of plant community on soil moisture varied by season and year (plant × season × year interaction $P = 0.002$). During the first year (2013) higher levels of GWC were found during the winter and summer in native-dominated plots when seasons were evaluated separately (Fig. 1a). As drought duration increased, higher levels of GWC were observed in exotic-dominated plots throughout the winter seasons (although only significant in 2015). During the springs, significantly higher GWC values were found in native-dominated plots in 2014 and in exotic dominated plots in 2016. Plant community composition also affected soil temperature significantly. On average, a 3-degree difference was detected between plant community types (estimated mean ± SD, native-dominated: 20.3 ± 6.5 vs. exotic-dominated: 23.3 ± 7.6 °C, based on average values across all observations).

Microbial population size and activity responses

Microbial bulk properties (MBC, SIR) responded more strongly to temporal variability than to rainfall treatments or plant community type (Table 2). Given the complex interactions among plant type, season and year, direct treatment effects were weak, but were seen more clearly when years were evaluated separately. For example, the overall effect of plant type on MBC was marginal ($P = 0.066$), but during the first two years of the experiment (2013–2014), MBC was significantly higher in native-dominated plots ($P = 0.03$ when 2013 and 2014 were evaluated separately) (Fig. 1b). During 2015 and 2016 no significant differences were found between plant community types ($P = 0.77$ when 2015 and 2016 were evaluated separately). Similarly, the overall rainfall treatment effect on MBC was weak ($P = 0.049$), but a highly significant increase in MBC in the 150% treatment

Table 2 Summary of *P*-values for the linear mixed model for effects of rainfall treatments, plant community type, season, year and the interaction on soil and microbial characteristics (there were no significant 4-way interaction)

Factor(s)	GWC	Soil T	DOC	MBC	SIR	Phylum	Genus	Level 2 carbohydrates
Rainfall treatment	0.0003	0.2076	0.2121	0.0487	0.4643	0.076	0.02	0.128
Plant community type	0.6482	<.0001	0.0002	0.0661	0.7022	0.013	0.01	0.012
Season	<.0001	<.0001	0.0046	0.0002	0.0084	N/A	N/A	N/A
Year	<.0001	N/A	0.0156	<.0001	<.0001	0.001	0.01	0.001
Rain:plant	0.8478	N/A	0.2381	0.9234	0.9769	0.437	0.71	0.162
Rain:season	0.002	N/A	0.8841	0.3024	0.4744	N/A	N/A	N/A
Plant:season	0.0725	N/A	0.2302	0.042	0.0435	N/A	N/A	N/A
Rain:year	0.0504	N/A	0.875	0.2624	0.394	0.144	0.32	0.817
Plant:year	0.0019	N/A	0.8232	0.2311	0.0117	0.37	0.3	0.887
Season:year	<.0001	N/A	<.0001	<.0001	<.0001	N/A	N/A	N/A
Rain:plant:season	0.4392	N/A	0.1798	0.5809	0.777	N/A	N/A	N/A
Rain:plant:year	0.5517	N/A	0.5549	0.8238	0.2946	0.69	0.84	0.383
Rain:season:year	0.0428	N/A	0.8232	0.1842	0.8321	N/A	N/A	N/A
Plant:season:year	0.0024	N/A	0.6068	0.0444	0.1207	N/A	N/A	N/A

Statistically significant values are in bold

was found during 2015 (the wettest year) when compared to the other rainfall treatments (Supplementary Fig. 2).

The highly significant seasonal effect on MBC ($P = 0.0002$) reflects a trend towards highest biomass in winter and lowest in summer, though these patterns varied by year (Fig. 1b). During 2016, low MBC values were observed during the winter and summer seasons (Fig. 1b). The lack of the 2016 winter pulse could have been associated with the scant rainfall during that winter (Table 1).

The highly significant effect of year on MBC ($P < 0.0001$) reflects a trend of decreasing biomass over the course of the experiment. This is seen most clearly during the winter seasons when average values of MBC significantly decreased from 2013 to 2014 (in native-dominated plots) and from 2015 to 2016 (in both plant communities) (Supplementary Fig. 3). This pattern correlates with annual rainfall decreasing in consecutive years (Table 1). For both plant communities, a weak but significant correlation was found between MBC and GWC (native-dominated: $P = 0.0002$ and exotic-dominated: $P = 0.004$, Fig. 2a).

SIR, a measure of potentially active microbial biomass, was not significantly affected by rainfall treatment or plant type, but showed a similar long-term decline to that seen for MBC. Mean annual SIR decreased over time (2013 > 2014 = 2016 > 2015 in native-dominated plots and 2013 > 2014 = 2015 > 2016 in exotic-dominated plots, Supplementary Fig. 4). Soils in exotic-dominated plots had more pronounced SIR seasonal variation, with higher spikes during spring (2013 and 2014) or summer (2015) (plant $\times$ season interaction $P = 0.044$, Fig. 1c). In native plots, SIR was positively

correlated with MBC (native-dominated: $P = 0.002$ and exotic-dominated: $P = 0.2$, Fig. 2b) and DOC (native-dominated: $P = 0.01$ and exotic-dominated: $P = 0.3$, Fig. 2c).

Bacterial community composition responses

The soil bacterial community, as revealed by metagenomic sequencing, was most strongly influenced by annual variability, but effects of plant community and experimental rainfall treatment were also observed (Table 2). A total of 27 bacterial phyla were detected, with Proteobacteria (36%), Actinobacteria (31%), Bacteroidetes (6%), Firmicutes (6%), Acidobacteria (4%), Chloroflexi (4%), and Verrucomicrobia (3%) accounting for 90% of the sequences. The PerMANOVA results from phylum relative abundances detected significant differences between years and plant community types but not between rainfall treatments (Table 2). Consistent with the PerMANOVA post-hoc comparisons, NMDS analysis showed differences between 2015 communities when compared to 2013 and 2016 (Fig. 3). STAMP showed that 14 bacterial phyla differed significantly between years. Among the most abundant, Actinobacteria and Deinococcus-Thermus were more abundant in 2013, Acidobacteria, Bacteroidetes, Planctomycetes, Proteobacteria, and Verrucomicrobia in 2015 (wettest year) and Actinobacteria, Chloroflexi and Firmicutes in 2016 (Supplementary Fig. 5). At the class level, alpha-Proteobacteria, beta-Proteobacteria, delta-Proteobacteria, and gamma-Proteobacteria, Acidobacteria, Solibacteres, Spartobacteria, and Opitutae were overrepresented in

Fig. 1 Intra-annual and inter-annual variations of **a** soil moisture, **b** MBC, **c** SIR, and **d** DOC for each plant community type from winter (Wi) 2013 to summer (Su) 2016 across rainfall treatments (no plant

community type $\times$ rainfall treatment interaction). Error bars represent the standard error of the mean

2015, while Bacilli (Firmicutes) and Dehalococcoidetes (Chloroflexi) were overrepresented in 2016.

The plant community significantly influenced microbial community structure; a significant reduction of Proteobacteria and Verrucomicrobia and an increase in the relative abundance of Chloroflexi, Cyanobacteria and Deinococcus-

Thermus were found in exotic-dominated plots (Supplementary Fig. 6). Among these phyla, 84 genera were significantly over- or under-represented in native-dominated and exotic-dominated plots, with 58 belonging to the phylum Proteobacteria, 14 to Cyanobacteria, 5 to Chloroflexi, 4 to Verrucomicrobia, and 3 to Deinococcus-Thermus

Fig. 2 Relationships between **a** MBC and GWC, **b** SIR and MBC, and **c** SIR and DOC for each plant community type across rainfall treatments (no plant community type $\times$ rainfall treatment interaction)

Fig. 3 Non-metric multidimensional scaling (NMDS) analyses of taxonomic (left) and functional (right) composition with 95% confidence ellipses by plant community type and year. Taxonomic profiles represent the phylum level and gene functions are level 2 carbohydrate subsystems

(Supplementary Fig. 7). The PerMANOVA results at the genus level detected significant differences between rainfall treatments (Table 2). The NMDS analyses shows this effect is driven by the 150% treatment clustering apart from the 50 and 100% treatments (Supplementary Fig. 8).

C cycling

Soil DOC was mostly influenced by plant community type ($P = 0.0002$) and season and year interactions ($P < 0.0001$) (Table 2). DOC was reduced in exotic plots by approximately 25% based on average values of all plots across the entire time of observations (estimated mean $\pm$ SD, native-dominated: 61.3 ± 35.6 vs. exotic-dominated: 45.8 ± 23.8 $\mu\text{g C g}^{-1}$ soil). Seasonal effects were detected after post-hoc comparisons during 2013 and 2014, when DOC peaked during the winter season, and in 2015 during the summer season for both plant community types (Fig. 1d).

Carbon metabolism represented 15% of the broad scale (level 1) bacterial functional genes with a total of 11 functional attributes at the level 2 (Fig. 4). PerMANOVA results detected significant differences between years and plant community types but not between rainfall treatments (Table 2). Contrary to the annual trends for taxonomy, in which 2015 was distinct from the other years, post-hoc comparisons and NMDS analysis of functional gene abundance showed differences between 2013 vs. 2015 and 2016 (Fig. 3).

There was a striking commonality between the impacts of wet vs. dry years and the influence of native vs. exotic plant communities on the microbial community structure. Hence, the annual and vegetation-driven variations in microbial community were generalized into a single axis with two groups, with group 1 representing exotic plots and the driest years (2013 and 2016) and group 2 representing native plots and the most mesic year (2015) (Fig. 4). Correlation analyses of these groups and C cycling genes showed two distinct taxonomic/functional clusters (Fig. 4). Group 1, composed of bacterial groups Actinobacteria, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, and Firmicutes, were positively correlated with aminosugars and sugar alcohols, and Group 2, composed of Acidobacteria, Bacteroidetes, Planctomycetes, Proteobacteria, and Verrucomicrobia, were mostly positive correlated with polysaccharides and glycoside hydrolases (Fig. 4).

Discussion

The results of this experimental study showed that: (1) microbial population size, structure, and functioning responded more to temporal rainfall variability than experimental droughts, (2) in comparison to soils under native plants, soils under exotic plants differed in microbial activity, structure, and functioning through complex interactions, and (3) microbial responses to climate variability and exotic plants can influence soil carbon cycling processes.

Fig. 4 Pearson's correlation matrix between carbon cycling functional gene abundances (x-axis) and microbial phyla abundances (y-axis). Functional genes were clustered by Bray–Curtis dissimilarity and Ward's distance, and groupings informally annotated. Pearson's correlation is color coded with blue being positive and red being negatively correlated. Summary statistics (boxplot) for each row are provided

Recent evidence suggests that extreme seasonal changes to Mediterranean-type ecosystems may have a larger impact on soil communities than observed in experimental droughts [24, 39]. In this study, we found that microbial communities were more responsive to temporal variability than to experimental droughts. However, we observed responses of microbial communities to multi-year droughts and rainfall shifts. For example, we observed microbial biomass and activity declining over the extended (4-year) period of drought, with significant declines during summer seasons, with the exception of 2015, which experienced high summer rainfall. During 2015 (the wettest year), microbial biomass significantly increased in the 150% treatment when compared to the other rainfall treatments. Other rainfall manipulation studies have also shown a positive response in microbial characteristics with high rainfall [25, 56]. Zhao et al. [56] detected an increment in microbial biomass with increased rainfall up to 40%+ of ambient rainfall in a semi-arid steppe, and Cruz-Martínez et al. [25] detected microbial community differences between rainfall treatments when the watering event exceeded normal conditions. Water is clearly a strong factor controlling microbial activity and diversity, but its effect is

strongly dependent on timing. While MBC was correlated with GWC, this relationship explained only a small amount of variation (Fig. 2a). The relatively large influence of seasonal and annual variation in rainfall compared to the effects of individual rainfall event size and soil water content suggest that the timing of rainfall events can be more important than rain intensity [57]. The annual effects could also arise from variations in temperature, humidity and their resulting impacts on the plant community. The exotic plant community in this ecosystem is particularly dynamic, varying annually in relative dominance of grasses and forbs (E.E.C., unpublished data).

Due to sampling frequency and the methods employed, our experimental design did not address immediate, short-term responses of microbes to soil drying and rewetting. While FE methods can include inactive microbes, the detection of seasonal and annual changes in MBC (Fig. 1 and Supplementary Fig. 3) suggests that FE measurements capture microbial turnover at seasonal and annual time scales. Correlations between MBC and the physiologically active biomass (SIR) also support this conclusion.

Bacterial community composition behaved similarly to microbial biomass, responding more strongly to year than to

rainfall treatments, despite the possibility that persistence of relic DNA may have limited our ability to detect changes over time. Higher abundance of gram-positive bacteria, such as Actinobacteria, Chloroflexi and Firmicutes, found during 2013 and 2016 (drier years) vs. gram-negative groups, such as Proteobacteria, Verrucomicrobia, and Acidobacteria, that were found in higher abundance in 2015 (wetter year) may indicate that the composition shifted due to osmotic stress. Gram-positive bacteria are known to be more drought tolerant (stronger cell wall and drought avoidance strategies such as spore formation) than gram-negative bacteria [58]; thus we speculate that these microbial taxonomic shifts were likely due to osmotic stress caused by low rainfall during 2013 and 2016. This is consistent with Castro et al. [59], Cruz-Martínez et al. [60], and Neilson et al. [61] which found similar relationships between moisture and bacterial communities. However, Castro et al. [59] found Acidobacteria were more abundant under dry conditions which has been also found in a long-term drought treatment [39]. Variance in the responses of Acidobacterial groups has been explained in terms of distinct classes within this phyla [62], and consistent with our finding, the group Solibacteres has been found to decrease with drying in a summer desiccation experiment [63]. Additionally, while this study observed compositional changes among years, we observed several phyla-level responses to annual rainfall variability that are also consistent with drying and rewetting studies [26, 30]. For example, Barnard et al. [30] found Actinobacteria and Firmicutes to decrease while Acidobacteria and Verrucomicrobia increased during soil rewetting, a pattern that we found during our wettest year. Similar to our findings, Drigo et al. [26] found *Deinococcus* taxa resistant to drought conditions.

Our results showed that soils under exotic plants had increased temperatures, enhanced infiltration during rainfall events, decreased water retention during spring and summer, and decreased labile carbon in comparison to soils under native plants. Similar impacts have been reported in shrubs vs. grasses studies, where increases in runoff and evaporation due to the lack of soil retention capacity and shading conditions were found in grass-dominated systems [64–66]. We propose that these soil hydraulic alterations under exotic plants represent an amplification of the rewetting-drying cycles of MTEs and result in a more heat and osmotically stressed environment. Reductions in microbial biomass, enhanced seasonal fluctuations in MBC and SIR, and reductions in less tolerant bacterial groups such as Proteobacteria and Verrucomicrobia in exotic dominated plots support this theory. Overlaps in taxonomic and functional composition between exotic plots and 2016 (hotter and 5th consecutive drought year) and between native plots and 2015 (wetter year) (Fig. 3) further

support our interpretation that soils under exotic plants stimulate osmotic and heat stresses. Additionally, increases of di- and oligosaccharide metabolic genes during 2016 and in exotic plots can be attributed to osmotic stress adaptations since disaccharides, such as trehalose, have been previously reported as compatible solutes in osmoregulation [67, 68].

While harsher environmental conditions under exotic plants may negatively impact microbial communities, it is also possible that the higher temperatures and rainfall water pulses coupled with the more easily decomposed, high quality litter of exotics may enhance microbial respiration. The higher spikes and more pronounced microbial seasonal activity (Fig. 1c) found in this experiment can be supported by the enhancement of soil microbial respiration with increasing temperature [69] and findings of higher soil respiration with additions of exotic plant litter in CA MTEs when compared to native litter, especially under high moisture pulse scenarios [70]. These findings help explain microbial mechanisms for the reduction of soil C in invaded MTEs [71, 72]. By increasing soil temperature, water pulses, and litter quality, exotic plants can promote microbial respiration and hence, microbial-mediated C losses. Additionally, while not explicitly tested in this study, increases in soil temperature found in exotic plots also support findings of ecosystem C loss via photodegradation [73]. This effect of exotic plants was also reflected in the significant reductions of DOC in exotic dominated plots (Fig. 1d). While DOC represents only a fraction of soil C, labile C fractions have been suggested as an appropriate metric for short-term (<15 years) changes of soil C pools in semi-arid ecosystems since bulk soil C can respond relatively slowly [74].

In addition to microclimatic effects and litter quality, there are a host of other traits that differ between native shrubs and exotic herbs, such as rooting depth, C allocation [34] and plant performance under stressful conditions [75–77] that could have contributed to the observed effects of plant type. It is also possible that some residual effect of the disturbance that led to invasion of this landscape influenced our results. However, such effects are probably minimal, given the similarity of soil physical factors between the two treatments and the fact that the establishment of both native and exotic plants in this ecosystem are generally limited by dispersal of seeds to bare ground [78, 79] rather than special soil requirements.

When taken together, our results showed two distinct taxonomic/functional groups that can be associated with life-history traits, specific C sources, and microbial C uses. Group 1, composed of Actinobacteria, Chloroflexi, Cyanobacteria, Firmicutes and *Deinococcus-Thermus* (considered extremophiles), were better represented under exotic plants and were positively correlated with genes for

aminosugars, sugar alcohols and simple carbohydrate metabolic pathways, such as di- and oligosaccharides, which include many compatible solutes that confer stress tolerance. These correlations indicate that Group 1 microbes rely on recycling microbial biomass (amino sugars in cell walls) and C forms released during rainfall events (compatible solutes), especially after enhanced drought conditions [80]. Group 2, composed by bacterial groups such as Verrucomicrobia and Acidobacteria, which are generally considered K-selected [81], were better represented under native plants and correlated with complex carbohydrate metabolic genes, such as polysaccharides and glycoside hydrolases, which are included in plant structures [82–84] (Fig. 4). This indicates that Group 2 microbes rely more on plant litter and root exudates [82, 83]. This is consistent with findings of Group 1 member Verrucomicrobia correlating with polysaccharide metabolism [85].

The relationships found in this experiment between microbial community structure, functional genes and environmental factors are not strictly reflective of copiotrophic/r-selected and oligotrophic/K-selected taxa generalizations [81, 86]. A possible explanation is that more extreme pulses of rain penetration and microbial activity in exotic-dominated soils result in undetected, short-lived carbon rich conditions that are rapidly metabolized by microbes. Additionally, the more recalcitrant native litter may select for a microbial community adapted to complex carbon compounds, allowing for the accumulation of DOC, as supported by the positive relationship between Group 2 and complex carbohydrate metabolism. Other explanations for low DOC in exotic dominated plots are leaching and runoff losses promoted by the higher rainfall water pulses.

Conclusions

In this MTE, we found that temporal variability and plant traits are key factors in assessing microbial responses to extreme droughts and exotic plants disturbances. We observed extreme periods of scant rainfall to decrease microbial biomass and activity and alter microbial communities. In comparison to soils under native plants, soils under exotic plants characteristically had a microbial environment prone to increased osmotic and heat stress that enhanced microbial turnover and loss of labile soil C. Correlations of microbial taxa and C metabolic profiles in response to climate and plant disturbances suggested relationships between specific microbial traits and potential C processes. Soil microbial community responses to extreme droughts and exotic plants could potentially impact ecosystem C storage by producing a smaller, more vulnerable microbial biomass C pool prone to increased pulses of heterotrophic respiration. While this

study observed microbial responses to multi-year droughts, future studies are needed to understand the resilience of microbial communities after periods of extreme droughts and potential implications for carbon cycling [87].

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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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