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Interactive effects of precipitation manipulation and nitrogen addition on soil properties in California grassland and shrubland

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ABSTRACT

Soil microbial communities and pools of carbon (C) and nitrogen (N) play an important role in ecosystem responses to precipitation variability and N deposition. In southern California, ecosystem vulnerability to these environmental change drivers may differ for grassland versus shrubland vegetation types. We hypothesized that (1) these vegetation types would differ in their responses to precipitation and N manipulation; (2) reduced precipitation ("drought treatment") would have a negative effect on soil microbial abundance and alter microbial community composition, (3) these changes would be associated with reductions in soil C and N pools, (4) N addition would increase microbial abundance as well as soil C and N pools, and (5) combined drought and N deposition would have offsetting effects on soil properties. We tested these hypotheses at the Loma Ridge Global Change Experiment in southern California. Across vegetation types, we found that microbial biomass based on phospholipid fatty acids declined with drought and N addition. Microbial composition differed more strongly by vegetation type than with environmental change treatments. Added precipitation had little effect on microbial biomass but reduced labile C and N pools; these reductions were mitigated by N addition. Drought reduced labile forms of soil C and N, whereas N addition increased labile soil C pools and all soil N pools. Negative effects of drought and N addition were additive for microbial biomass, which could inhibit soil C cycling if both of these environmental changes occur together. Drought interacted with N addition to significantly increase the most labile N pool under the drought + N treatment, which suggests a build-up of available N under these conditions. These results imply that multiple environmental changes may combine non-additively to affect below-ground microorganisms and soil C and N pools, which may have important consequences for ecosystem services such as productivity, biodiversity, and soil quality in Mediterranean climate regimes of North America.

1. Introduction

Belowground communities in soils are expected to respond to human-induced environmental changes with consequences for soil carbon (C) and nitrogen (N) pools (Bardgett et al., 2008; Ogunseitan, 2005). Large-scale environmental change can produce local effects on soil moisture patterns, N availability, and soil temperature. In addition, changes in plant community composition, C allocation patterns, or the quantity and quality of plant-derived organic matter can alter the supply of C and N to soil as well as the structure and activity of microbial communities involved in biogeochemical processes (Allison et al., 2013; Balser et al., 2010). Shifts in microbial community composition and functioning can also feedback to affect other soil biogeochemical processes (Carreiro et al., 2000; Treseder et al., 2012; Todd-Brown et al., 2012; Henry et al., 2005).

In grasslands and shrublands of southern California, climate change may lead to reductions in winter season precipitation and increases in the duration and severity of drought (Seager and Vecchi, 2010; Cayan et al., 2010). However, precipitation projections are uncertain, and rainfall events may become more extreme with climate change (IPCC, 2013). These changes may have implications for microbial communities and nutrient cycling (Cregger et al., 2012; Castro et al., 2010). For example, Fierer et al. (2013) found a strong correlation between taxonomic diversity of soil microbial communities and precipitation amount. Precipitation changes can also influence soil microbial

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communities and biogeochemical cycles indirectly, via vegetation change (Bragazza et al., 2015; Tiemann and Billings, 2011; Harper et al., 2005). Allison et al. (2013) found that drought reduced litter decomposition directly, through water limitation, and indirectly through changes in microbial population size, community composition, and litter chemistry. Previous studies have shown that increased precipitation can increase soil light fraction C and N, whereas total soil C and heavy fraction C is relatively stable under climate change (Song et al., 2012; Rosenstein et al., 2011).

Nitrogen deposition in some areas of California is among the highest in the United States, with 20 kg ha^{-1} y^{-1} or greater in southern California (Fenn et al., 2010). Nitrogen limits primary production in many terrestrial ecosystems (LeBauer and Treseder, 2008; Niboyet et al., 2011). The effect of chronic N enrichment on soil microbial community structure and activity varies with ecosystem type, duration of N addition, and rate of N addition. Often, N enrichment alters osmotic potentials in soil solution, reduces soil pH and soil magnesium/calcium availability, and alters the availability of soil C and N through aboveground litter production, which could alleviate microbial C limitation (Treseder, 2008; Carreiro et al., 2000; Gutknecht et al., 2012).

The impacts of global change stressors affecting southern California may also depend on vegetation type. Grasslands and shrublands differ substantially in species composition as well as ecosystem C and N dynamics (Wolkovich et al., 2010). Coastal sage shrub species show higher vulnerability to xylem cavitation than chaparral or desert shrubs (Kimball et al., 2014), implying that shrubland species may be particularly sensitive to drought. Such differences in drought or N vulnerability could cause vegetation-specific changes in plant input chemistry or quantity with consequences for soil C and N pools (Harpole et al., 2007; Follett et al., 2012).

In southern California, both precipitation change and N deposition may occur simultaneously. Therefore, we investigated the responses of microbial communities and soil C and N pools to altered precipitation and N deposition with a factorial design in grassland and shrubland vegetation types at the Loma Ridge Global Change Experiment, CA, USA. We hypothesized that (1) The grassland and the shrubland should differ in their soil responses to precipitation and N manipulations; (2) Declining precipitation levels should have a negative effect on soil microbial abundance and soil C and N pools owing to declines in net primary production (Parolari et al., 2012); (3) Changes in microbial community composition are associated with changes in plant community composition under precipitation manipulation (Allison et al., 2013; Parolari et al., 2012); (4) N addition should increase soil microbial abundance, C pools, and N pools based on previous results at Loma Ridge showing that N availability increases above-ground net primary production (Parolari et al., 2012); and (5) Assuming the responses to manipulation are additive, the positive effects of N addition should offset the negative effects of drought in the combined manipulation.

2. Materials and methods

2.1. Field site and experimental design

The Loma Ridge Global Change Experiment is located in the Santa Ana foothills within the Irvine Ranch National Landmark in Orange County, California (33.742 N, 117.704 W, elev. 365 m) on a northeast-facing slope (<10%). Soils are loamy, mixed, thermic *Typic Palexeralfs* sandy loams (California Soil Resource Lab, http://casoilresource.lawr.ucdavis.edu) formed on a several-meter-deep colluvial deposit eroded from sedimentary rock of the Vaqueros formation (Potts et al., 2012). The vegetation at the site is a mosaic of non-native annual grassland (e.g. *Bromus diandrus*, *Avena fatua* and *Lolium multiflorium*) and perennial, drought deciduous shrubland (e.g. *Artemisia californica*, *Salvia melifera*) (Potts et al., 2012). The grass to shrub boundary is not obviously related to differences in soil texture, depth or pH. The grassland and shrubland soils have a consistent texture of 85.3% sand and fine gravel, 7.6% silt, and 7.1% clay, with reduced clay content near the surface presumably due to leaching (Goulden, unpublished data).

The grassland manipulation site is immediately northeast of the coastal sage shrubland manipulation site (Kimball et al., 2014). The sites experience a Mediterranean-type climate, with cool, wet winters and dry, hot summers and annual mean precipitation of 281 mm that falls mostly from November through April (Tustin Irvine Ranch weather 1902–2003 from http://www.wrcc.dri.edu) (Parolari et al., 2012). The timing and size of precipitation events vary markedly both within and between years. Ambient precipitation ranged from 72 mm to 540 mm over the 7 years of the experiment.

Factorial manipulations at Loma Ridge were initiated in 2007 and include altered precipitation and N input. We used a randomized split-plot design with eight replicate blocks in each vegetation type. Three levels of precipitation input were applied within each block: ambient (control), ambient minus 40% (drought), and ambient plus 40% (added) (Fig. 1). Rainfall was excluded from the drought plots with retractable polyethylene roofs that were closed during approximately half of the rain events (closed <5% of the days during a year); this approach reduced potential climate artifacts (measurements show no effect on air temperature or humidity when open) (Parolari et al., 2012; Kimball et al., 2014). Water draining from the roofs was collected in polyethylene tanks for subsequent application to the added precipitation plots using pressure compensated drip tubing. All plots were burned in 2007, shortly after the start of the experiment. Each plot was split lengthwise and half of each plot was fertilized while the other half remained unfertilized. Plots were fertilized with 2 g N m^{-2} immediate-release calcium nitrate (15.5−0−0 + 19% Ca) prior to the growing season and 4 g N m^{-2} 100-day release calcium nitrate during the growing season (Parolari et al., 2012).

2.2. Soil sampling and soil moisture

Soil samples were collected from all of the grassland and shrubland plots on August 20th, 2012. Three replicate cores (2.5 cm diameter and 15 cm deep) were taken from each plot, combined, homogenized, and used for soil analysis. A sub-sample of the homogenized core was frozen and freeze dried for lipid analysis (Microbial ID, Inc., Newark, DE).

The effect of precipitation treatment on volumetric soil water content (q) was measured using Frequency Domain Reflectometry

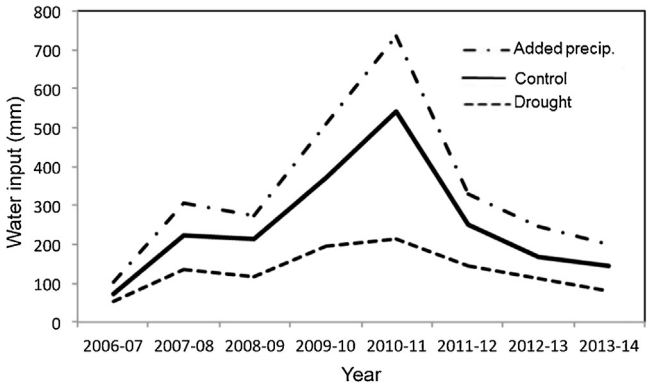

Fig. 1. Annual water input in the control, reduced and added precipitation treatments.

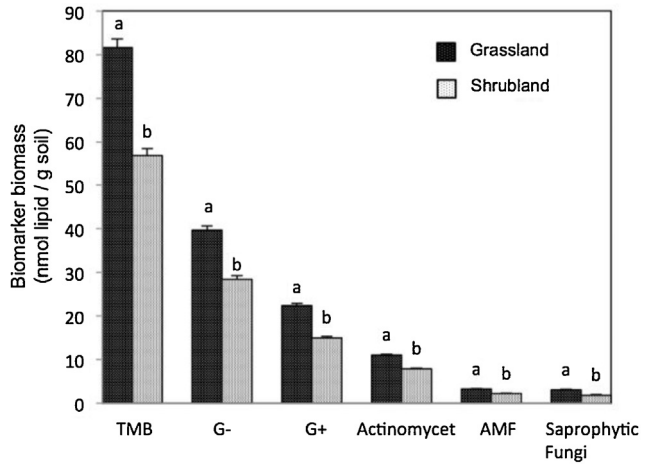

Fig. 2. Effect of vegetation types (grassland and shrubland) on biomarker classes. Error bars indicate standard errors (n = 6). Means within a group sharing the same letter are not significantly different. TMB = total microbial biomass; G− = gram negative bacteria; G+ = gram positive bacteria; AMF = arbuscular mycorrhizal fungi.

(FDR) or time-domain reflectometry (TDR; Mini-Trase, Santa Barbara, CA USA). Probes were installed vertically at the soil surface, providing a measure of water content throughout the upper 15 cm of soil (θ, $m^3\,m^{-3}$) (Parolari et al., 2012).

2.3. Labile and recalcitrant C and N pools

We used a two-step acid hydrolysis with H_2SO_4 as the extractant to determine the labile and recalcitrant C and N pools (Oades et al., 1970; Rovira and Vallejo, 2007). 500 mg soil was hydrolyzed with 20 mL of 2.5 M H_2SO_4 in sealed Pyrex tubes at 105 °C for 30 min; the hydrolysate was then recovered by centrifugation. This hydrolysate was analyzed for labile C and N pool I (LCPI, LNPI) with a Shimadzu TOC/TN analyzer (Shimadzu Corporation, Kyoto, Japan). The residue was washed with water and dried. The remaining residue was hydrolyzed with 2 mL of 13 M H_2SO_4 overnight at room temperature under continuous shaking. After diluting the acid with de-ionized water to 1 M, the residue was hydrolyzed for 3 h at 105 °C and the hydrolysate was recovered by centrifugation. This second hydrolysate was taken as labile pool II and analyzed for C (LCPII) and N (LNPII) with a Shimadzu TC analyzer. The labile pool I is composed mainly of polysaccharides of plant (hemi-cellulose and starch) and microbial origin (mostly microbial cell walls) while the labile pool II is composed primarily of cellulose (Oades et al., 1970; Rovira and Vallejo, 2007). We also measured total C and total N with a Carlo Erba elemental analyzer (Carlo-Erba, Milan, Italy). Recalcitrant C (RC) and N (RN) pools were calculated as the difference between the total concentration of the elements and the labile pools (LPI and LPII summed together).

2.4. Microbial community analysis

Phospholipid fatty acids from microbial cell membranes were extracted, purified, and characterized following Buyer and Sasser (2012). Soil samples were dried overnight and then a Bligh-Dyer lipid extraction was performed. The extract was dried, dissolved in chloroform and loaded onto a solid phase extraction plate. Phospholipids were eluted into glass vials, dried and trans-esterified. The resulting fatty acid methyl esters were analyzed by GC and quantified relative to an internal standard. The total nmol lipid g^{-1} dry soil (sum of all lipids present, 20 or less C atoms in length) was used as an index of microbial biomass (Zelles and Bai, 1993; Frostegård and Bååth, 1996). Individual lipids were used as biomarkers of broad microbial taxa: 16:1 ω5c for arbuscular mycorrhizal fungi (Balser et al., 2005), 18:2 ω6,9c for saprophytic fungi (Balser et al., 2005), 10-methyl fatty acids for actinobacteria (Zelles, 1999), monounsaturated fatty acids and cyclopropyl such as 18:1 ω9c for Gram-negative bacteria (Zelles, 1999), and iso and anteiso saturated branched fatty acids such as 15:0 iso for Gram-positive bacteria (Zelles, 1999). The fungal to bacterial ratio was calculated as the average biomass of fungal lipid biomarkers (16:1 ω5c and 18:2 ω6,9c) divided by the average biomass of bacterial lipid biomarkers (15:0 iso, 15:0 anteiso, 16:0 iso, 16:1 ω7c; 16:0 10 methyl; 17:0 iso, 17:0 anteiso, 17:0 cyclo, 18:1 ω7c, 18:1 ω9c, 18:0 10 methyl, and 19:0 cyclo ω9c).

2.5. Statistics

Multivariate and univariate statistical analyses were performed on the microbial community profiles for the factorial treatment with a randomized split plot design for N and precipitation treatments (N factor levels assigned randomly as the subplot, nested within precipitation treatments assigned as the main plots). Data were analyzed by analysis of variance (three-way ANOVA) using PROC MIXED, SAS Institute Inc., Cary, NC, USA, to examine the effects of vegetation type (grassland vs. shrubland), precipitation regime (reduced, ambient and added) and N (ambient vs. addition), and their interactions, on the observed soil properties. The dependent variables were total microbial biomass, fungal to bacterial ratio, Gram positive bacteria, Gram negative bacteria, actinomycetes, AM fungi, and saprophytic fungi phospholipid fatty acids, as well as soil total C and N and their labile and recalcitrant pools. Mean comparisons using Least Significant Difference (LSD) values were conducted if significant treatment effects were found.

Fatty acid profiles were classified and discriminated by principal components analysis (PCA) using XLSTAT Software (XLSTAT, 2012, Addinsoft, New York, NY). The factor loading scores for the individual fatty acid biomarkers were used to assess the relative importance of each individual biomarker in the calculation of the principal component axes. PCA plots mapped locations and treatment combinations through loadings and scores in

Table 1
Analysis of variance P-values for ecosystem, precipitation manipulation, and nitrogen addition effects on soil microbial communities.

Factor	Multivariate	Total microbial biomass	Fungi:bacteria	G^+ bacteria	G^- bacteria	Actinomycetes	AM[a] Fungi	Saprophytic Fungi
Ecosystem (E)	**<0.0001***	**<0.0001**	**0.0500**	**<0.0001**	**0.0002**	**<0.0001**	**0.0007**	**0.0007**
Precipitation (P)	**<0.0001**	**0.0003**	**<0.0001**	**0.0003**	**0.0007**	**0.0003**	**<0.0001**	**0.0133**
Nitrogen (N)	**0.0024**	**0.0385**	**0.0130**	**0.0137**	0.0972	**0.0426**	**0.0029**	0.1450
E × P	**0.0062**	0.5939	0.3222	0.7918	0.4501	0.7690	0.5475	0.5940
E × N	0.6657	0.1605	0.5703	0.0931	0.1767	0.1784	0.2838	0.4725
P × N	0.3295	0.1163	0.7801	0.0657	0.1308	0.0841	0.3270	0.3874
E × P × N	0.8543	0.7664	0.7801	0.8983	0.6876	0.6563	0.9714	0.8171

* Bold numbers are significant at $P < 0.05$.
[a] AM = arbuscular mycorrhizal.

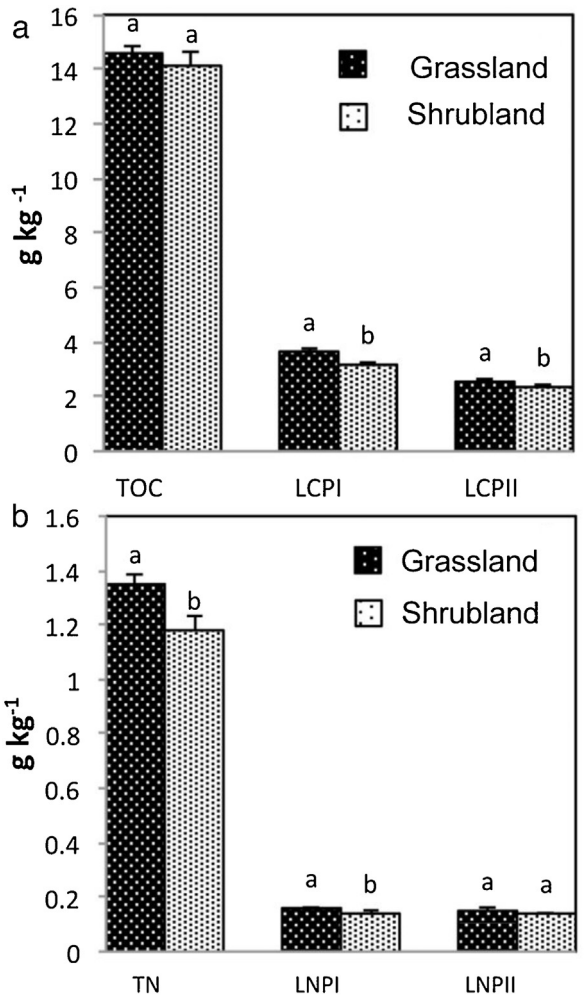

Fig. 3. (a) Effect of vegetation types (grassland and shrubland) on soil carbon pools, (b) Effect of vegetation types (grassland and shrubland) on soil nitrogen pools. Error bars indicate standard errors (n = 3). Means within a group sharing the same letter are not significantly different. TOC = total organic carbon; LCPI = labile carbon pool I; LCPII = labile carbon pool II; TN = total N; LNPI = labile nitrogen pool I; LNPII = labile nitrogen pool II.

dimensional spaces determined by PCs with eigenvalues >1.0 based on Kaiser's rule.

3. Results

3.1. Effects of vegetation type on microbial communities and C and N pools

Total microbial biomass, G^+ bacteria, G^- bacteria, AM fungi, saprophytic fungi and actinomycete lipid biomarkers and fungal to bacterial ratio were significantly greater in the grassland than the shrubland (Fig. 2, Table 1). Except for TOC and LNPII, all C and N pool sizes were significantly lower in shrubland than in grassland soils (Fig. 3a and b, Table 2).

3.2. Effects of precipitation manipulation and N addition on microbial communities

In general, microbial abundances in both ecosystems displayed qualitatively similar responses to precipitation and N manipulations (Fig. 4). Precipitation treatment significantly affected the fungal:bacterial ratio and all metrics of microbial abundance (Table 1) with lower values in the drought treatment than in the ambient and addition treatments (Fig. 4). Particularly in shrubland, drought had a pronounced negative effect on total microbial biomass, AM fungi, and G- bacteria (Fig. 4). Nitrogen addition had a significant negative effect on fungal:bacterial ratios and all microbial abundances except G- bacteria and saprotrophic fungi (Table 1, Fig. 4).

The additive effects of drought and N tended to reduce microbial abundance the most in the drought + N treatment. Compared to controls in the grassland and shrubland, the drought + N treatment had a significant ($P < 0.05$) negative effect on total microbial biomass, G+ bacteria, G^- bacteria, AM fungi, and actinomycetes compared to the control treatment in both the grassland and shrubland, with the greatest decline in AM fungi abundance. Total microbial biomass decreased from 86.9 nmol lipid g^{-1} dry soil in the control to 68.7 nmol lipid g^{-1} dry soil in the drought + N treatment in the grassland, and from 63.4 nmol lipid g^{-1} dry soil to 47.0 nmol lipid g^{-1} dry soil in the shrubland (Fig. 4). There was also a significant negative effect of drought + N treatment on the fungal to bacterial ratio ($P < 0.05$) in both vegetation types (Fig. 4). Saprotrophic fungi abundance in the drought + N treatment was lower than in the control, although the decline was not statistically significant (Fig. 4).

Principal components analysis reduced the PLFA biomarkers variables to two factors (Fig. 5). PC 1 explained 75.04% of the variance and was closely related to vegetation type. The second PC, which accounted for 7.1% of the variance, separated the drought treatment from the ambient and added precipitation treatment.

3.3. Treatment effects on C pools

Total organic C pools were similar in the grassland and shrubland, although LCPI values were significantly lower in the shrubland (Table 2, Fig. 6). Nitrogen addition significantly increased LCPI, and both drought and added water treatments reduced LCPI, although there was an interaction between precipitation and ecosystem type (Table 2, Fig. 6). Treatment effects on LCPII were minor, although there was a precipitation x N interaction whereby N had opposing effects in ambient and added precipitation treatments. For recalcitrant C, there was a three-way interaction (Table 2) whereby N addition increased values in the shrubland under ambient precipitation (Fig. 6). There were also

Table 2
Analysis of variance *P*-values for ecosystem, precipitation manipulation, and nitrogen addition effects on carbon and nitrogen pools. LCPI = labile carbon pool I; LCPII = labile carbon pool II; TN = total N; LNPI = labile nitrogen pool I; LNPII = labile nitrogen pool II.

Factor	LCPI	LCPII	Recalcitrant C	Total C	LNPI	LNPII	Recalcitrant N	Total N
Ecosystem (E)	**0.0175**	**0.0364**	0.1074	0.3757	**0.0467**	0.1180	**0.0130**	**0.0031**
Precipitation (P)	**0.0016**	0.1592	0.0766	<**0.0001**	**0.0003**	**0.0009**	0. 0515	**0.0002**
Nitrogen (N)	<**0.0001**	0.2490	**0.0274**	<**0.0001**	**0.0039**	**0.0034**	**0.0024**	<**0.0001**
E × P	**0.0129**	0.2258	0.1475	**0.0008**	0.9505	**0.0386**	0.1119	**0.0068**
E × N	0.0909	0.8696	0.5510	**0.0012**	**0.0017**	0.1973	0.0692	**0.0207**
P × N	0.1192	**0.0035**	0.2251	**0.0390**	<**0.0001**	<**0.0001**	0.4966	0.0701
E × P × N	0.0767	0.1522	**0.0187**	**0.0442**	**0.0010**	**0.0006**	0.2372	0.2407

Bold numbers are significant at $P < 0.05$.

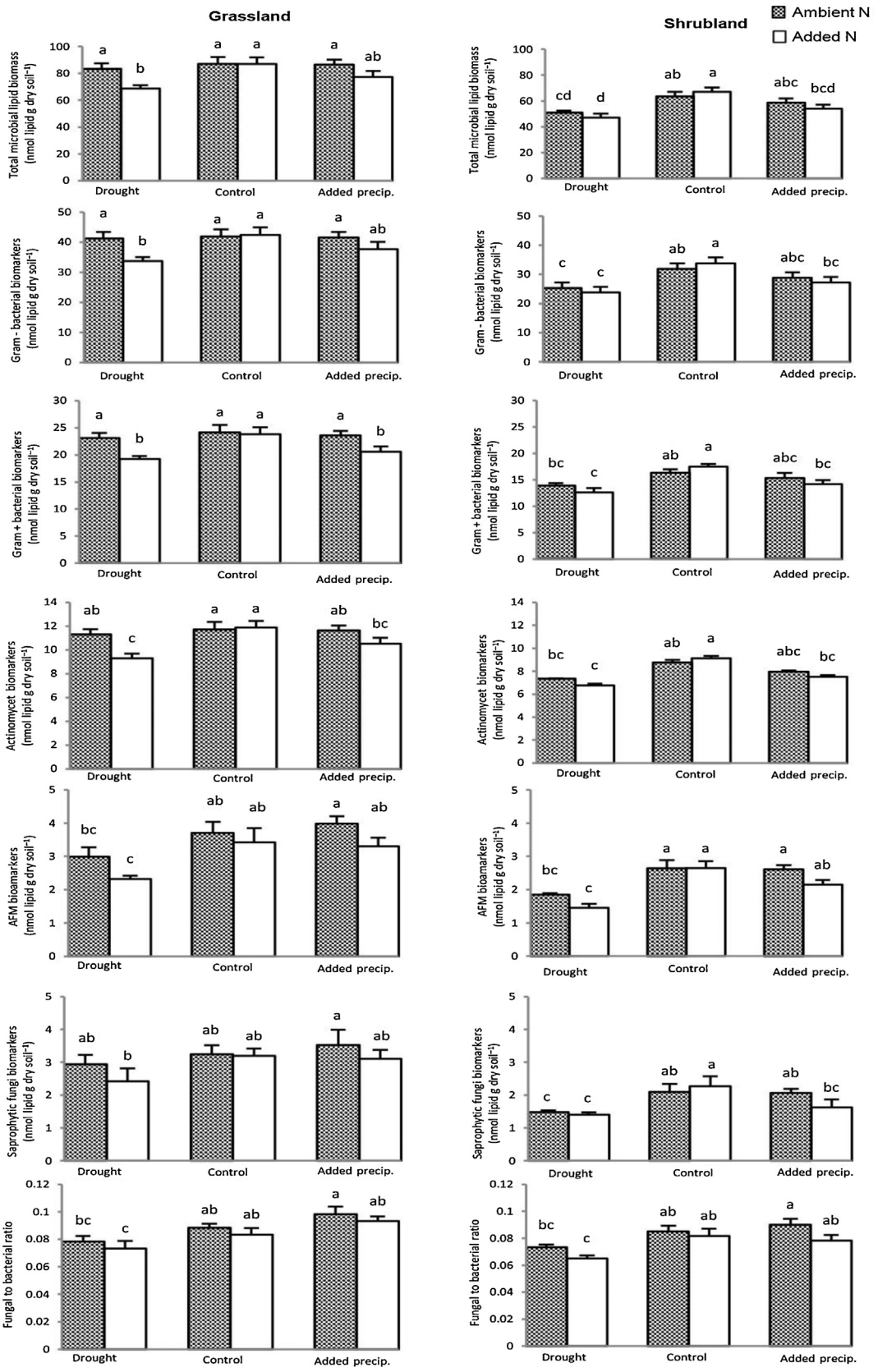

Fig. 4. Microbial lipid biomass (nmol lipid g dry soil^{-1}) in all treatments. Error bars indicate standard errors (n = 6). Filled bars are ambient nitrogen; open bars are added nitrogen. Means with the same letter are not significantly different ($P < 0.05$).

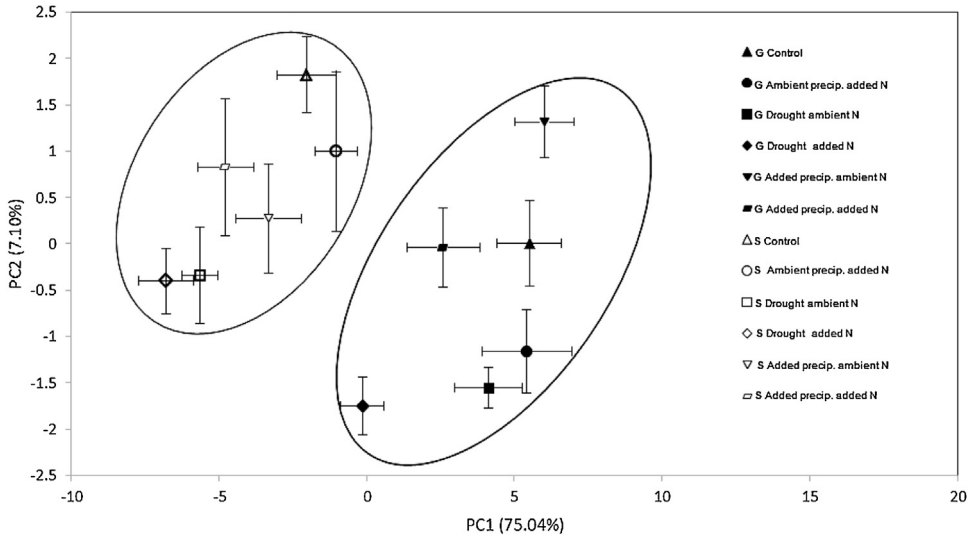

Fig. 5. Ordination of 12 treatments in 2 ecosystem types based on PLFA biomarkers using principal components analysis (varimax rotation). The variance explained by each principal component axis is shown in parentheses. Filled symbols are grassland (G), and open symbols are shrubland (S).

significant treatment interactions for total organic C (Table 2) such that N addition increased total C whereas drought and added precipitation reduced total C, particularly in the shrubland (Fig. 6).

3.4. Treatment effects on N pools

Total N and recalcitrant N were greater in the grassland than the shrubland, whereas the effect of vegetation type varied by treatment for the labile N pools (Table 2, Fig. 7). For LNPI, there were several treatment interactions (Table 2); N addition tended to increase LNPI except in the added precipitation treatment (Fig. 7). There was a negative LNPI response to drought, but only in the shrubland under ambient N. We also observed many significant treatment interactions for LNPII (Table 2), with the most notable result being that N addition reduced LNPII under ambient precipitation but increased LNPII under added precipitation (Fig. 7). Recalcitrant N increased significantly overall with N addition (Table 2, Fig. 7), as did total N, although the total N increase was greater in shrubland.

4. Discussion

Across grassland and shrubland vegetation types, we quantified responses of soil microbial communities and C and N pools to two interacting global change factors: precipitation level and N addition. Our comparison of vegetation types suggests that shrublands are more sensitive to environmental change, which could possibly facilitate vegetation-type conversion of native shrubs to annual grassland (Wolkovich et al., 2010; Kimball et al., 2014). As predicted, drought reduced microbial abundance and altered microbial community composition, especially in combination with N addition. Also consistent with our initial hypotheses, C and N pools responded to precipitation manipulations and N addition. Drought and to some extent added precipitation reduced labile C and N pools, especially in the shrubland, whereas N addition and drought + N treatments increased the LNPI pool across vegetation types. N addition also increased LCPI, total C, and total N pools in the shrubland. Our hypothesis about offsetting effects drought and N addition was not supported because both drought and N addition had negative effects on microbial biomass that combined in an additive way. Also, effects on the LNPI pool were non-additive, with significantly greater pool sizes in the drought + N treatment than expected from the single factors.

4.1. Effect of vegetation type on microbial communities and C and N pools

Differences in vegetation type contributed to differences in below-ground microbial biomass and composition (Figs. 2 and 4, Table 1). This result is likely explained by plant effects on the physical and biochemical characteristics of the root zone, the quantity and quality of litter, and the availability of low-molecular-weight C and N (Wardle et al., 2004; Balser et al., 2010; Gutknecht et al., 2012; Bragazza et al., 2015). Different vegetation types may also result in differences in soil aeration, soil moisture (Fig. 8), and the quality of resources available to microbes (Allison et al., 2010; Bragazza et al., 2015). In our system, inputs from grassland vegetation to soil are likely higher in C:N ratio and carbohydrate content but lower in lignin and N concentrations compared to shrubland inputs (Allison et al., 2013; Wolkovich et al., 2010).

4.2. Effects of precipitation manipulation and N addition on microbial communities

We found that both drought and N addition reduced microbial abundances, with the two treatments combining additively to strongly reduce abundances in the drought + N treatment. Drought may negatively impact soil microbes through reduced input of plant C into the rhizosphere (Bragazza et al., 2015; Bardgett et al., 2008). Increasing the soil water osmotic pressure along with reducing plant C allocation to fine roots and mycorrhizal symbionts are mechanisms that could explain microbial biomass declines under N addition (Treseder, 2008; Carreiro et al., 2000; Gutknecht et al., 2012). Given that many California ecosystems experience high rates of N deposition (Fenn et al., 2010), and that drought may increase with climate change (Seager and Vecchi, 2010), microbial abundances could decline substantially in these systems.

In our study site, where water and N availability play a dominant role, fungal responses to drought and N treatments were much stronger than bacterial responses. Fungal:bacterial ratios declined when drought was combined with N addition, mainly due to lower fungal biomass in the drought + N treatments. The patterns we observed suggest that the sensitivity of AM fungi to N addition is important for determining fungal versus bacterial abundance in our system.

Previous work at our site has shown that drought and N addition treatments also have significant effects on litter microbial

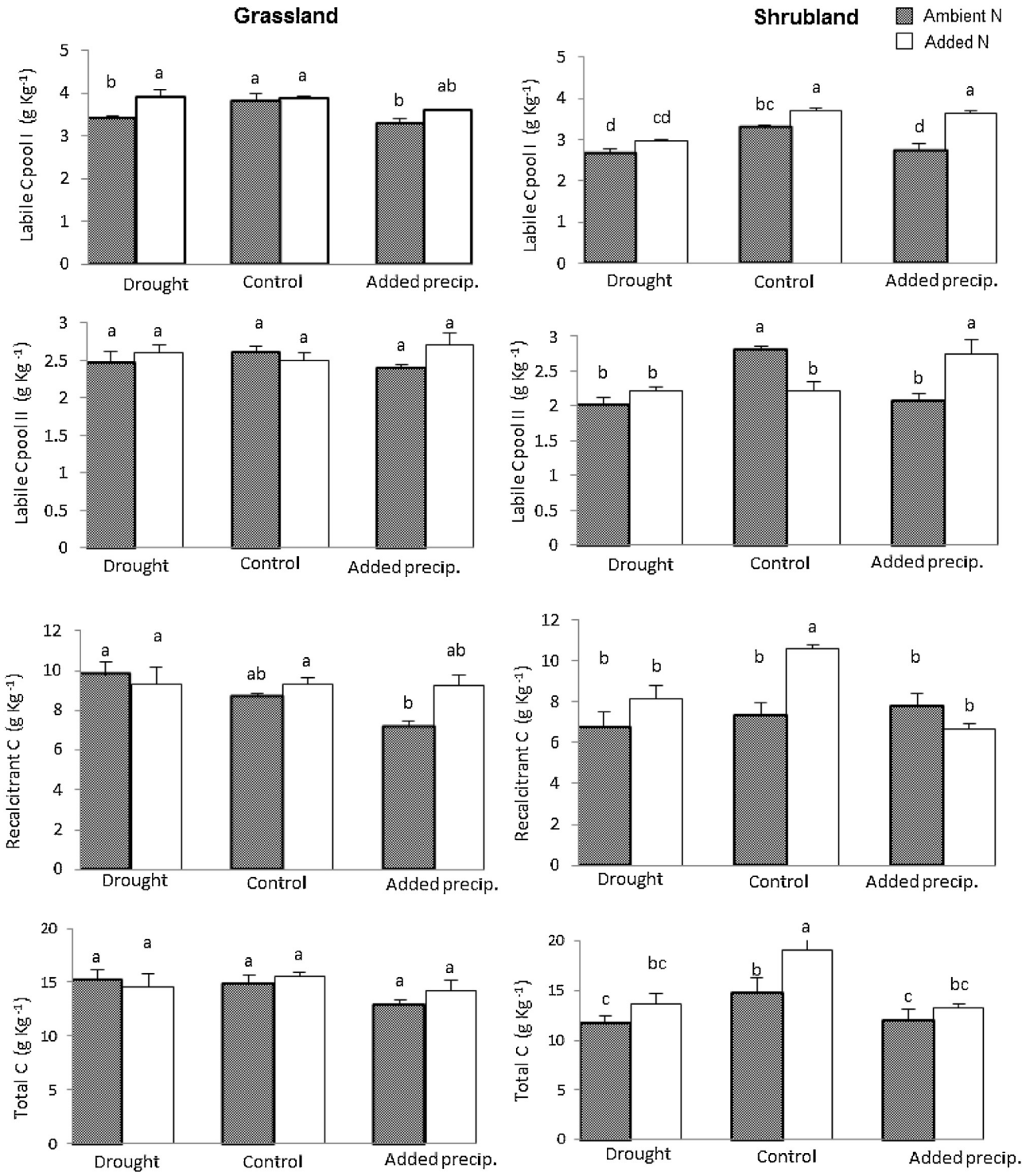

Fig. 6. Soil carbon pools (g kg^{-1}) in all treatments. Error bars indicate standard errors (n = 3). Filled bars are ambient nitrogen; open bars are added nitrogen. Means with the same letter are not significantly different ($P < 0.05$).

biomass and community composition. Based on direct cell counts, bacterial biomass declines sharply with drought in grassland litter, whereas fungal biomass increases (Alster et al., 2013). Based on taxonomic data from DNA sequencing, *Methylobacterium* was more abundant in the N addition plots but decreased in relative abundance in the drought plots (Matulich et al., 2015). Three of the most abundant fungal taxa declined significantly in relative abundance in both the N addition and drought treatments (Matulich et al., 2015).

Surprisingly, total microbial biomass and most PLFA groups did not respond positively to added precipitation (Fig. 4), even though they responded negatively to drought. It is possible that increases in soil water availability led to a temporal mismatch between resource availability and microbial demand, thereby constraining

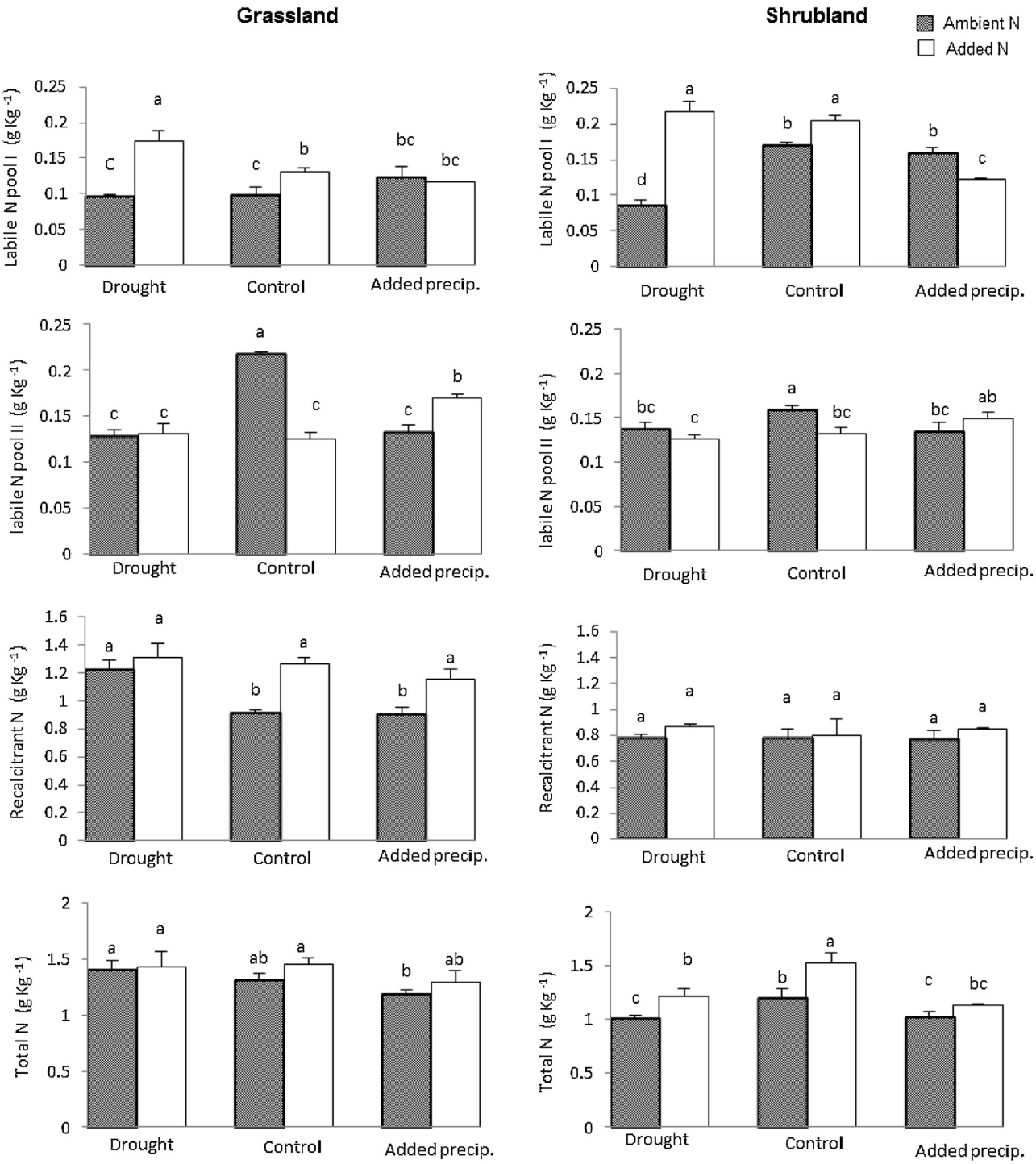

Fig. 7. Soil nitrogen pools (g kg^{-1}) at all treatments. Error bars indicate standard errors (n = 3). Filled bars are ambient nitrogen; open bars are added nitrogen. Means with the same letter are not significantly different ($P < 0.05$).

soil microbial biomass. In support of this idea, we observed reductions in total and labile C pools, particularly in the shrubland under added precipitation, consistent with reduced available C supply to microbial biomass. Labile N (pool II) was also lower with added precipitation across both vegetation types. The cause of these reductions in available C and N under added precipitation is unknown. They might be explained by declining belowground plant allocation with increasing soil moisture availability (Ye et al., 2015), but this explanation is not consistent with the observed recovery of labile C pools when N is added in combination with added precipitation (Fig. 7). Additional N is expected to reduce, not increase, belowground C allocation.

4.3. Effects of drought and N addition on C and N pools

The organic C and N pools were more responsive to the treatments in the shrubland than in the grassland (Figs. 6 and 7). Soil C and N pool responses may have resulted from shifts in C and N inputs and decomposer abundances and activities. Allison et al. (2013) showed that changes in vegetation cover were associated

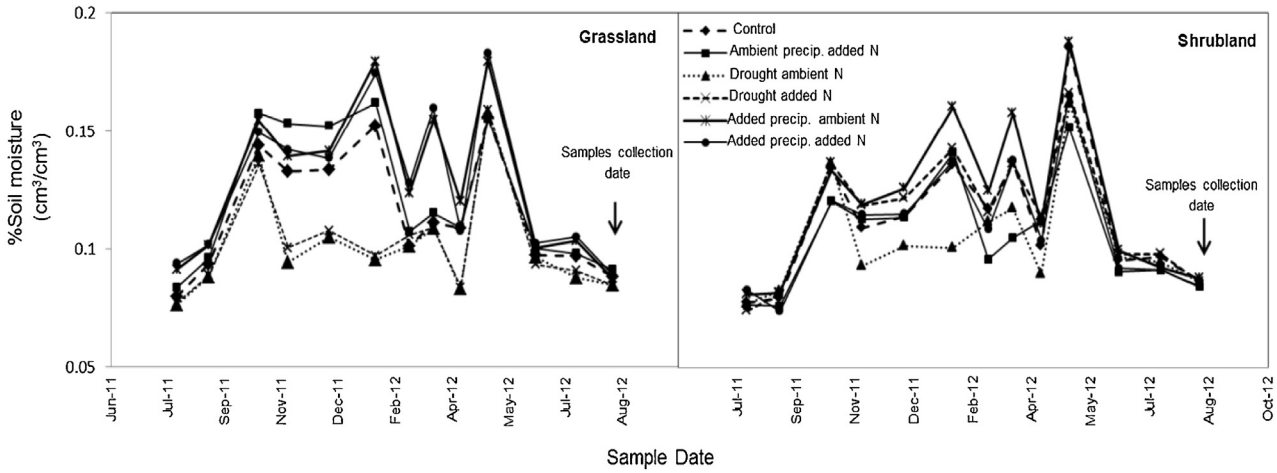

Fig. 8. Mean 15-cm integrated soil moisture in grassland and shrubland.

with shifts in litter chemistry such as lignin, cellulose, sugars and N concentrations. Size and functioning of the decomposer community may be affected by these changes in the biochemical composition of litter (Ball et al., 2014). We showed that soil microbial community abundances and composition were different in the grassland versus the shrubland. These differences could in turn affect soil C and N pools.

Although N addition tended to increase C and N pool sizes whereas drought tended to reduce them, there was a striking interaction between the factors in the most labile N pool (Table 2). Increases in labile N under drought + N may have occurred because of reduced biological demand for nutrients. In dry to average years, plant productivity responds negatively to the drought treatment, which could reduce plant N demand (Parolari et al., 2015; Kimball et al., 2014). We also observed a decline in microbial biomass under drought + N which could reduce microbial nutrient demand. In wet years, however, this accumulated N could become available to plants and microbes, thereby stimulating a pulse of productivity if sufficient moisture is available to alleviate water limitation.

The overall labile C and N pool responses to N addition may be driven by increasing aboveground litter production and higher litter quality following N fertilization (Allison et al., 2013; LeBauer and Treseder, 2008; Suding et al., 2005), which alter the supply of C and N to soils (Bardgett et al., 2008; Ogunseitan, 2005). The observed response of LCPI and LNPI pools to N addition is consistent with previous results at Loma Ridge that have reported increased vegetation production with N fertilization (Parolari et al., 2012), and altered litter chemistry, whereby litter derived from the N addition treatment had significantly higher N, cellulose, and hemicellulose, but significantly less lignin (Allison et al., 2013). Nitrogen addition could also indirectly affect soil C and N pools by shifting microbial enzyme activities toward degradation of specific litter constituents and altering microbial composition (fungal to bacterial ratios). We found a significant negative correlation between fungal to bacterial ratio and total soil N ($r = -0.88$, $p = 0.0198$). Significant increases in cellobiohydrolase, N-acetyl-glucosaminidase, polyphenol oxidase, litter decomposition rate, and cumulative respiration rate have also been reported in response to N addition in the Loma Ridge grassland (Alster et al., 2013; Amend et al., 2015). These results imply that N addition affects both the inputs and losses of soil C and N at the Loma Ridge site.

In contrast to N addition, drought generally caused a net reduction in soil C and N pools. The main mechanism by which drought might impact soil C and N pools is through reduced production of organic matter by plants. Parolari et al. (2015) showed that above-ground net primary production at Loma Ridge was strongly limited by water availability. The role of microbes in driving soil C and N losses under drought is less clear. Drought treatment has only minor effects on N cycling potential of litter microbes (Nelson et al., 2015). Reductions in soil microbial biomass under drought should lead to greater C storage, not less. In grassland litter at our site, drought reduces enzyme efficiencies and the potential for carbohydrate degradation by microbial communities (Alster et al., 2013; Berlemont et al., 2014) which could limit production of available C substrates. In addition, decomposition rates under drought may be affected by changes in the chemistry of litter inputs (van der Molen et al., 2011). Drought has altered plant community composition at Loma Ridge, leading to increased inputs of litter lignin, sugars, and starch to grassland soil pools (Allison et al., 2013).

5. Conclusions

Overall, our results suggest that the combined effect of drought and N addition, as might occur under environmental change in southern California, is to suppress microbial biomass and cause a build-up of labile N in the soil. This increase is ecologically meaningful because labile nutrients accumulated during drier years with low plant and microbial nutrient demand could stimulate productivity during wetter years. Labile C and N pools along with microbial abundance and composition showed pronounced responses to both drought and N deposition, which may contribute to the refinement of models that predict climate change vulnerability. Our findings suggest that shrublands are more sensitive to environmental change than grasslands, implying that ecosystem responses to precipitation and N deposition will depend on vegetation type.

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