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Microbial abundance and composition influence litter decomposition response to environmental change

Steven D. Allison,[1,2,3] Ying Lu,[1] Claudia Weihe,[1] Michael L. Goulden,[2] Adam C. Martiny,[1,2] Kathleen K. Treseder,[1] and Jennifer B. H. Martiny[1]

[1]*Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA*
[2]*Department of Earth System Science, University of California, Irvine, California 92697 USA*

Abstract. Rates of ecosystem processes such as decomposition are likely to change as a result of human impacts on the environment. In southern California, climate change and nitrogen (N) deposition in particular may alter biological communities and ecosystem processes. These drivers may affect decomposition directly, through changes in abiotic conditions, and indirectly through changes in plant and decomposer communities. To assess indirect effects on litter decomposition, we reciprocally transplanted microbial communities and plant litter among control and treatment plots (either drought or N addition) in a grassland ecosystem. We hypothesized that drought would reduce decomposition rates through moisture limitation of decomposers and reductions in plant litter quality before and during decomposition. In contrast, we predicted that N deposition would stimulate decomposition by relieving N limitation of decomposers and improving plant litter quality. We also hypothesized that adaptive mechanisms would allow microbes to decompose litter more effectively in their native plot and litter environments. Consistent with our first hypothesis, we found that drought treatment reduced litter mass loss from 20.9% to 15.3% after six months. There was a similar decline in mass loss of litter inoculated with microbes transplanted from the drought treatment, suggesting a legacy effect of drought driven by declines in microbial abundance and possible changes in microbial community composition. Bacterial cell densities were up to 86% lower in drought plots and at least 50% lower on litter derived from the drought treatment, whereas fungal hyphal lengths increased by 13–14% in the drought treatment. Nitrogen effects on decomposition rates and microbial abundances were weaker than drought effects, although N addition significantly altered initial plant litter chemistry and litter chemistry during decomposition. However, we did find support for microbial adaptation to N addition with N-derived microbes facilitating greater mass loss in N plots than in control plots. Our results show that environmental changes can affect rates of ecosystem processes directly through abiotic changes and indirectly through microbial abundances and communities. Therefore models of ecosystem response to global change may need to represent microbial biomass and community composition to make accurate predictions.

Key words: bacteria; community composition; drought; fungi; global change; grassland; home field advantage; litter decomposition; microbes; nitrogen fertilization; precipitation; reciprocal transplant.

Introduction

Human activities are causing environmental changes that may influence ecosystem processes. For example, changes in climate and nutrient inputs can alter plant productivity, decomposition rates, and ecosystem C storage (Mack et al. 2004, Dukes et al. 2005). Therefore, a major goal in ecology is to predict ecosystem responses to human-induced environmental change. However, making these predictions is challenging because ecosystems respond to environmental change through multiple mechanisms at a range of timescales (Luo 2007). Often these mechanisms occur simultaneously, making it difficult to identify the most important drivers of ecosystem response.

Most environmental changes affect both abiotic and biological parameters that ultimately determine rates of ecosystem processes. For instance, global climate change is expected to alter abiotic conditions such as temperature and soil moisture that have direct and immediate effects on the organisms that control ecosystem processes (IPCC 2007). Organisms often respond to abiotic drivers through physiological mechanisms, such as the closure of plant stomata during hot, dry conditions. These physiological responses can have immediate consequences for process rates, such as photosynthesis, which declines when plants close their stomata. Thus one important component of ecosystem response to environmental change involves the direct, physiological response of functionally relevant organisms.

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[3] E-mail: allisons@uci.edu

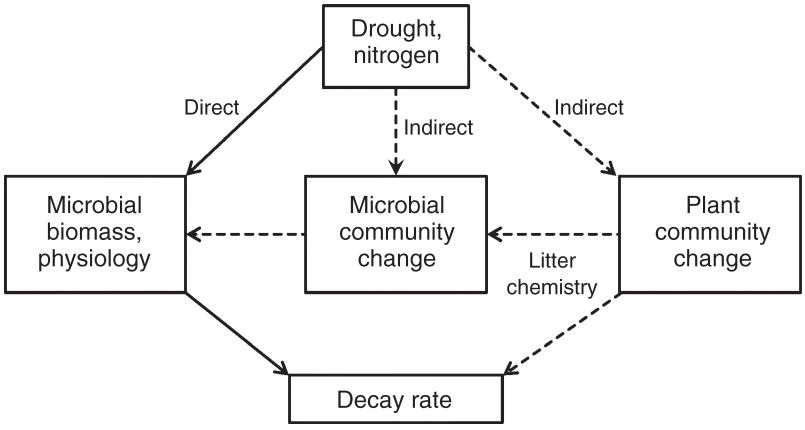

FIG. 1. Conceptual model of drought and nitrogen effects on litter decomposition. These environmental drivers alter decay rates through direct effects on microbial biomass and physiology (solid arrows), as well as indirect effects mediated through changes in plant communities, litter chemistry, and microbial communities (dashed arrows).

Shifts in the abundance and composition of biological communities contributing to a process represent another route by which an ecosystem can respond to environmental change (Manning et al. 2006, Sheik et al. 2011). Community composition may shift as organisms that are physiologically better adapted to the new conditions become more abundant and vice versa. Even if relative abundances of the taxa within a community do not change, ecosystem processes could be affected by changes in absolute abundance of the community, such as a change in total plant or fungal biomass. These indirect mechanisms, whereby process rates respond to environmental changes through an altered community, could therefore influence ecosystem responses to environmental change on generational timescales (Kardol et al. 2010).

Because physiological responses and indirect processes can occur simultaneously, teasing apart these mechanisms requires independent manipulation of abiotic conditions and biological communities. Although such manipulations have been done with plants (Reich et al. 2001), fewer studies have examined indirect effects on ecosystem processes driven by microorganisms. For some processes such as litter decomposition, changes in both plant and microbial communities may contribute to the environmental response. Thus there is a need for studies that explicitly examine the importance of the microbial community in mediating ecosystem responses to environmental change (Reed and Martiny 2007, Strickland et al. 2009).

The goal of our study was to assess the importance of short-term physiological responses vs. indirect microbial and plant community changes in determining the rate of litter decomposition under environmental change (Fig. 1). Rates of litter decomposition depend on many biotic and abiotic factors such as microbial activity, processing by invertebrates, chemical composition of the decaying material, and climate (Swift et al. 1979). Although microbes, especially fungi and bacteria, play a major role in the decomposition of plant litter (Waksman 1927), it is not clear how the environmental responses of microbial communities will ultimately affect decomposition in most ecosystems (Hättenschwiler et al. 2005, Gessner et al. 2010).

The environmental changes we studied were drought and nitrogen (N) deposition in southern California. Climate models predict substantially drier conditions for this region during the 21st century (Seager and Vecchi 2010). Drought could affect decomposition by limiting decomposer activity through physiological stress or constraints on enzyme and substrate diffusion (Manzoni et al. 2012). Indirect effects on decomposition might operate through the microbial community if drought adaptation leads to changes in the abundance and composition of decomposer microbes (Schimel et al. 2007). In addition, drought-induced changes in plant community composition and leaf chemistry could affect the chemical quality and decomposition rate of litter inputs (Morecroft et al. 2004).

Emissions from automobiles, industry, and agriculture have substantially increased reactive N deposition in southern California (Fenn et al. 2010). This deposition could directly stimulate decomposition by allowing microbial decomposers to produce more extracellular enzymes or shift allocation toward carbon-acquiring enzymes (Carreiro et al. 2000, Sinsabaugh et al. 2002, Allison et al. 2009). As with drought, N addition may indirectly alter decomposition through effects on plants and microbes. Additional N could shift plant communities and increase leaf and litter N concentrations, thereby enhancing litter quality and decomposition rates (Vitousek 2004, Suding et al. 2005). In the microbial community, N addition could select for nitrophilic microbes that require more N but decompose recalcitrant carbon compounds more efficiently (Treseder et al. 2011). Changes in microbial communities resulting from

these adaptive mechanisms could allow decomposition rates to increase, decrease, or remain constant with environmental change.

To examine the mechanisms underlying decomposition responses to environmental change, we used a reciprocal transplant design to manipulate abiotic conditions (precipitation and N), the microbial community, and plant litter in a California grassland ecosystem. We hypothesized that drought would have a negative effect on litter decomposition rates through moisture limitation of decomposer physiological processes (Fig. 1). Over time, we predicted that the altered abiotic environment would also select for microbial communities that were more effective at decomposition under drought conditions. Furthermore, we expected that drought-induced changes in plant community composition and leaf physiology would alter litter chemistry and have negative effects on decomposition. In contrast to the drought response, we predicted that decomposition rates would increase with N addition because litter decomposers are often N-limited (Allison et al. 2009). We also expected that N addition would influence decomposition through the plant community by increasing leaf and litter N concentrations. Finally, we hypothesized that microbial communities would adapt to drought and N addition through changes in composition. If adaptation occurs, then microbial communities derived from a given treatment should drive faster decomposition in litter and plots receiving that treatment, a mechanism known as "home field advantage" (Gholz et al. 2000).

Methods

Site description and field manipulation

Our experiment took place in a California grassland ecosystem 5 km north of Irvine, California, USA (33°44′ N, 117°42′ W, 365 m elevation), dominated by exotic annual grasses and forbs (Potts et al. 2012; M. L. Goulden, G. C. Winston, S. Parker, K. Suding, and D. Potts, *unpublished manuscript*). In spring 2010, the site was dominated by the annual grass genera *Avena*, *Bromus*, and *Lolium*; the annual forb genera *Erodium* and *Lupinus*; and the native perennial grass *Nassella pulchra*. The climate is mediterranean with a mean annual temperature of 17°C, mean annual precipitation of 325 mm, and little rainfall between April and October. Estimates of total annual N deposition in the region are $\sim$15 kg·ha^{-1}·yr^{-1} (Fenn et al. 2010).

For our experiment, we used a subset of plots from an existing field manipulation of precipitation and N inputs that began in February 2007 (see Plate 1). The field manipulation involves three levels of precipitation (ambient, reduced, or added) applied at the plot scale and two levels of N (ambient or added) applied to subplots within precipitation treatments. This design is replicated in eight experimental blocks. Within each block, we used only subplots with (1) ambient precipitation and N ("control" plots), (2) reduced precipitation + ambient N ("drought" plots), and (3) ambient precipitation + added N ("N" plots). Thus we did not study any effects of added precipitation or interactions between precipitation and N. Rainfall was reduced by covering the 6.7 × 9.3 m whole plots with clear polyethylene during a subset of the rainstorms each winter to achieve an $\sim$50% reduction in annual precipitation. This treatment reduced rainfall from 369 to 194 mm during the 2009–2010 winter and from 540 to 213 mm during the 2010–2011 winter. Every year, added N subplots received 20 kg N/ha as soluble $CaNO_3$ prior to the growing season and 40 kg N/ha as 100-day release $CaNO_3$ during the growing season.

Reciprocal transplant

After the treatments were in place for 3.5 years, we set up a reciprocal transplant within the field manipulation to isolate the effects of plot environment, microbe origin, and litter origin on decomposition rates (Reed and Martiny 2007). Plot environment represents the direct manipulation of abiotic conditions (precipitation or inorganic N inputs). Microbe origin represents indirect changes in microbial abundance and composition, and litter origin represents indirect changes in plant community composition and litter chemistry (Fig. 1). These main effects were crossed in a fully factorial design within either the drought or N experiment (Fig. 2). Thus the drought and N experiments are statistically independent, and we did not examine any drought × N interactions. There were two levels of each factor (control and drought or N), and the design was replicated within each block of the field experiment.

To manipulate litter origin, we collected senesced plant material from treatment and control plots. During the 2009–2010 growing season, drought increased relative abundances of *Erodium*, *Lolium*, and the native forb *Phacelia distans*, while reducing relative abundances of *Bromus*, *Lupinus*, and *Nassella*. N addition increased relative abundances of *Bromus*, *Lolium*, and *Phacelia*, but reduced *Avena*, *Lupinus*, and *Nassella* relative abundances (M. Goulden, *unpublished data*). We collected litter from haphazardly located 0.07-m^2 quadrats in each plot (drought, N, or control) on 29 June and 2 July 2010, and returned to the same quadrats on 14 September 2010, to sample plant material that had not yet senesced in June (mainly deep-rooted annual forbs). In each block, we sampled two, two, or four quadrats in the drought, N, or control plots, respectively (we needed twice as much control litter to transplant into both drought and N plots). Litter from all plots within a treatment was pooled and homogenized by hand.

We made litterbags containing litter from the drought, N, or control plots by placing 2 g (air dry mass) litter into nylon membrane bags with 0.45-μm pores. We sterilized all bags and their contents with at least 22 kGy gamma irradiation. The bags allow water, nutrients, and possibly small bacteria to pass through.

Preliminary work showed that environmental conditions (particularly moisture levels) were similar inside and outside the bags, and that fungi could not move through the nylon material. Sterility was verified by plating out litter extracts on LB (lysogeny broth) and fungal media.

We manipulated microbe origin by reinoculating the sterile litterbags with non-sterile litter collected from either drought, N addition, or control plots. Three haphazardly located litter samples (~5 g each) from each of the eight drought, N, or control plots were collected by hand on 30 November 2010, and combined within each treatment to generate three batches of litter inoculum. The inoculum litter was air dried, ground (Wiley mill, 1-mm mesh), and added in 50-mg aliquots to bags containing sterilized litter.

Mass loss and litter chemistry

A total of 360 litterbags were placed in the field on 15 December 2010 and retrieved in batches of 120 (15 treatment combinations × 8 blocks; Fig. 2) on 3 March 2011, 14 June 2011, and 14 November 2011, for analysis of percentage mass loss and litter chemistry. We weighed the fresh litter in each bag and dried a subsample to constant mass at 65°C to obtain dry mass; all mass losses are reported as percentage initial dry mass. This subsample was sent to Cumberland Valley Analytical Services (Maugansville, Maryland, USA) for near infrared (nIR) spectroscopy analysis (Shepherd et al. 2005). The following chemical fractions are reported here as a percentage of dry mass: lignin, starch, protein equals crude protein, cellulose equals acid detergent fiber minus lignin, hemicellulose equals neutral detergent fiber minus acid detergent fiber, sugars equal ethanol soluble sugars, and fat equals crude fat. Percentages of each chemical fraction were obtained by matching the nIR spectrum for each sample to a database of spectra from plant materials with known chemical composition based on wet chemistry assays. Another subsample was ground in a ball mill and analyzed for percentage C and percentage N by combustion on an elemental analyzer. Chemical parameters were also analyzed in 8–10 subsamples of each initial litter treatment.

Fungal hyphal length and bacterial cell density

Fungal hyphal lengths were determined using a modified procedure of Sylvia (1992). Litter subsamples (0.1 g) were ground to 1–2 mm, dried at 60°C, and dispersed in 10-mL sodium hexametaphosphate solution (0.395% mass/volume) with vigorous stirring. A 1.5-mL subsample of this solution was vacuum-filtered through a 0.2-μm nylon filter (Millipore, Billerica, Massachusetts, USA) and stained with acid fuchsin. This process was repeated with a second 1.5-mL subsample, and both filters were dried and mounted on a microscope slide with Permount (Fisher Scientific, Pittsburgh, Pennsylvania, USA). After drying at 20°C overnight, hyphal lengths (m/g dry litter) were determined with a Nikon Eclipse E400 microscope (Nikon Instruments, Melville, New York, USA) in phase contrast mode under 100× magnification using the grid-intercept method (Newman 1966, Giovannetti and Mosse 1980) and 50 grids per filter.

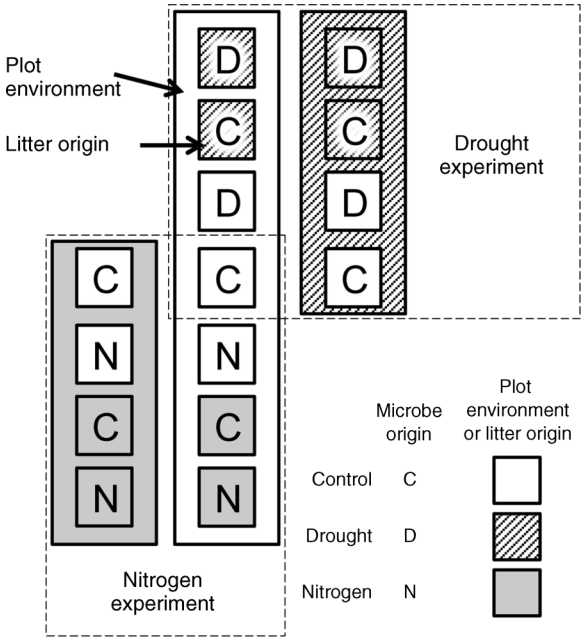

FIG. 2. Reciprocal transplant design for one block ($n = 8$ blocks) in the plant litter manipulation. For each experiment (drought or nitrogen), the three main effects of plot environment, litter origin, and microbe origin were crossed in a fully factorial design with two levels of each factor (control and treatment). The control plots (3.3 × 9.3 m) were common to both drought and nitrogen experiments, but each experiment was treated separately (i.e., no drought × nitrogen interactions were examined). The design was sampled once on each of three dates in 2011 (3 March, 14 March, and 14 November) for a total of 360 litterbags. Control plots were unmanipulated, drought plots received an ~50% reduction in rainfall, and nitrogen plots were fertilized with 60 kg $N \cdot ha^{-1} \cdot yr^{-1}$.

Bacterial cell densities were measured by extraction and flow cytometry. A 0.1-g subsample of fresh litter was ground to 1–2 mm and fixed with 5-mL phosphate-buffered glutaraldehyde solution within 8 h of collection. The solution contained 0.9% NaCl, 1% glutaraldehyde, and 0.12 mol/L phosphate. Fixed samples were stored at 4°C up to four weeks. Samples were extracted by adding 0.55 mL of 0.1 mol/L tetrasodium pyrophosphate solution and gently sonicating for 30 min at 4°C. The extract was pushed through a 2.7-μm GF/D syringe filter to remove large particles, stained with SYBR Green (1×), and incubated for 15 min at 20°C in the dark. Particles in stained extracts and unstained controls were counted by flow cytometry (BD Accuri C6; BD Biosciences, San Jose, California, USA), and cell densities are reported as the number of stained counts minus unstained counts per gram dry litter. Unstained counts correspond to particles that auto-fluoresce at the same wavelength as SYBR Green but may not contain

PLATE 1. Rainfall exclosures (in the open position) at the field site in Irvine, California, USA. Most vegetation in this annual grassland ecosystem was senescent when this image was taken in June 2010. Photo credit: Y. Lu.

DNA; they were usually orders of magnitude lower than stained counts. Flow cytometer gating parameters were optimized to count fluorescent particles in the size range typical for bacterial cells. We used a 2.7-μm filter to avoid counting nonbacterial cells; however, total counts increased by a factor of 2 if we used a larger (5-μm) filter pore size. Therefore our method may not detect some particle-attached bacteria.

Statistical analyses

We analyzed percentage mass loss, litter chemistry data, and microbial abundances using a factorial mixed-model ANOVA with repeated measures (hereafter "overall ANOVA"). The model included plot environment, litter origin, microbe origin, and date as fixed effects (along with their interactions) and two random effects: block and subject nested within block. We define subject as each batch of three litterbags with the same block, plot, litter origin, and microbe origin. Each subject was sampled once on each date (the repeated measurement). This design accounts for nonindependence within blocks and across repeated measurements (within subjects). If the fixed effects or their interactions were significant, we used ANOVAs on each date with block as a random effect to test for significant treatment effects within dates (hereafter "post hoc ANOVA"). Finally, post hoc contrasts were used to test for differences among levels of the fixed effects if the ANOVAs were significant. We used Welch's t tests to identify significant differences in initial litter chemistry. Data were square root- or log-transformed where necessary to improve normality and reduce heteroscedasticity. All analyses were conducted in the R software environment (R Development Core Team 2011).

RESULTS

Temporal patterns

In both drought and N experiments, litter mass loss was ~1–5% in March, and ~15–22% in June (Fig. 3; Appendix: Tables A1 and A2). Very little additional mass was lost between June and November, a time period corresponding to the dry season at our site (Fig. 3C). The dominant chemical fractions in the litter were cellulose and hemicellulose with ~11% lignin content (Appendix: Tables A1 and A2). Although there were statistically significant changes in most of the chemical fractions over time, the overall chemical composition of litter was generally consistent throughout the study period. However, there was a pronounced increase in litter percentage N from March to November ($P < 0.001$ for post hoc contrast in both drought and N experiments). Litter protein concentrations showed a similar

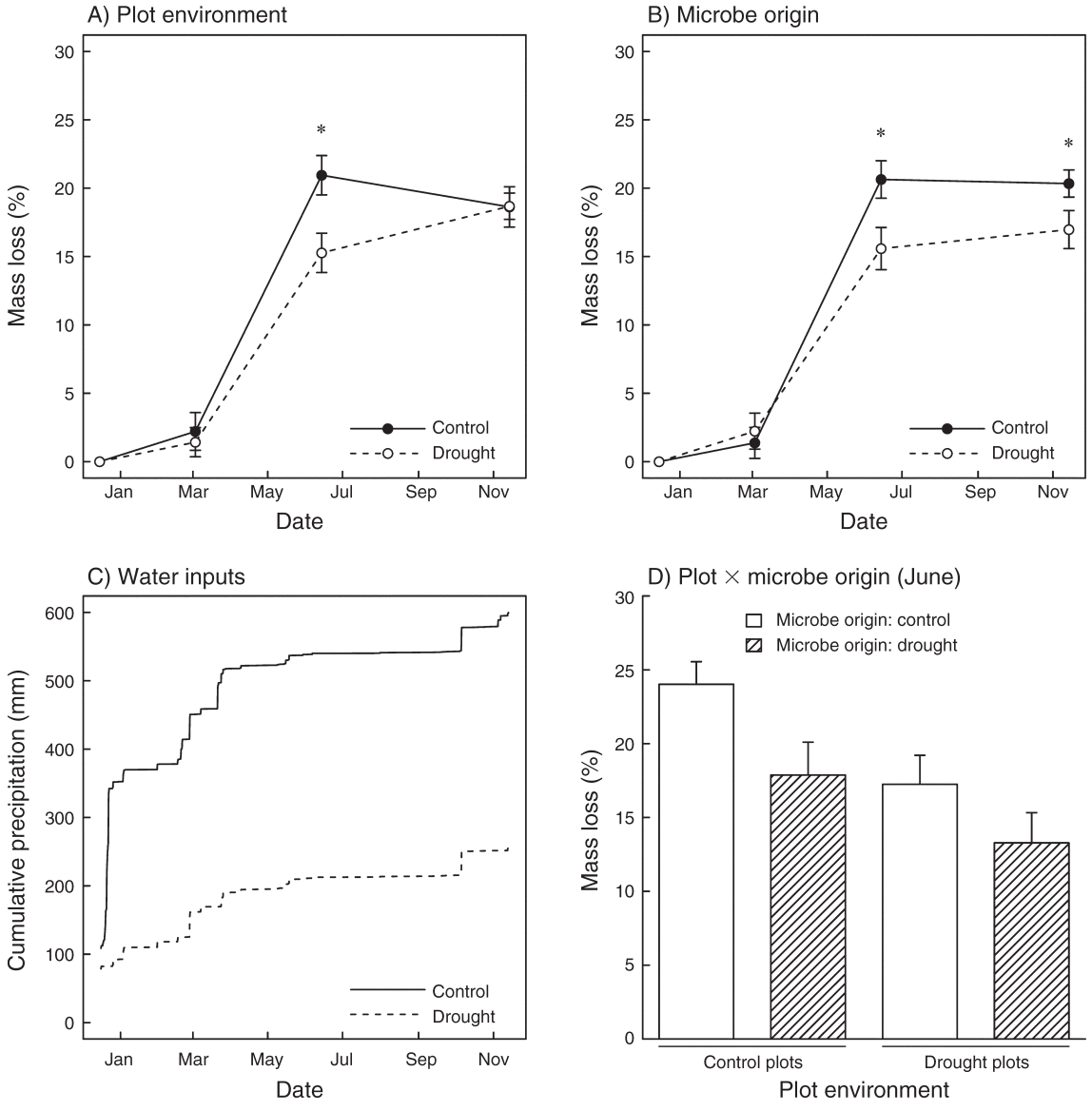

FIG. 3. Percentage mass loss over time (mean ± SE) in response to (A) drought manipulation for the plot environment or (B) microbe origin. An asterisk denotes a significant difference on a given date ($P < 0.05$, post hoc ANOVA). (C) History of water inputs over the same time period. (D) Combined effect of plot environment and microbe origin on mass loss (mean ± SE) on 14 June 2011 (statistics in Table 1).

pattern of increase. Fungal hyphal lengths increased steadily from ~10 to ~20 m/g between March and November in the drought experiment (Fig. 4A–C; $P < 0.001$ for post hoc contrast), with a similar pattern in the N experiment (Appendix: Fig. A1). Bacterial densities in the drought experiment were highest in November, nearly an order of magnitude lower in June ($P < 0.001$ for post hoc contrast to November), and intermediate in March (Fig. 4D–F). In the N experiment, bacterial densities were high in both March and November, but significantly lower in June ($P < 0.001$ for both post hoc contrasts; Appendix: Fig. A1).

Drought response: decomposition

Drought treatment in the plot environment had a negative effect on decomposition, and we also observed a negative effect of drought mediated through the microbial community. In June, mass loss was 20.9% ± 1.4% in control plots but only 15.3% ± 1.4% in drought plots ($P < 0.01$, post hoc ANOVA); however, mass loss was similar again by November (Table 1, Fig. 3A). Mass loss was also lower in litter inoculated with microbes from the drought treatment, with the strongest effect in June and November (Table 1, Fig. 3B). This microbial origin effect was similar in magnitude to the effect of

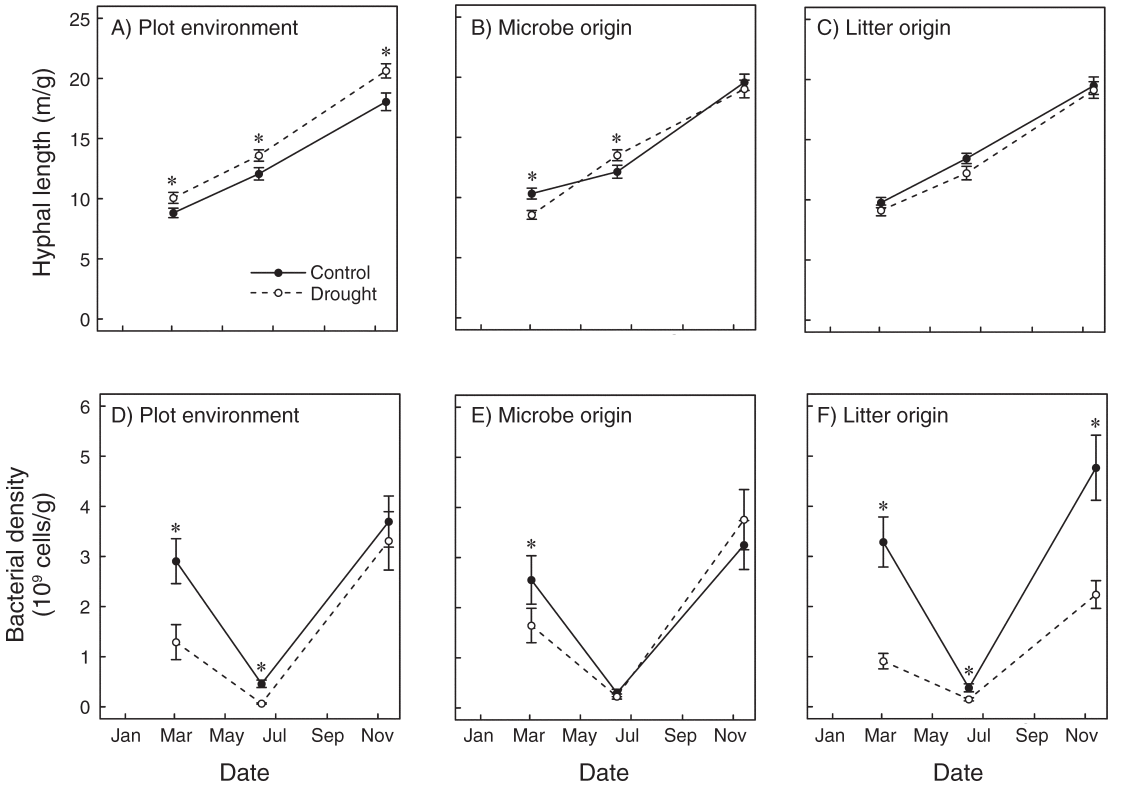

FIG. 4. Plot environment, microbe origin, and litter origin effects on (A–C) fungal hyphal lengths and (D–F) bacterial densities (in billions of cells) over time in the drought experiment. The asterisk denotes a significant difference on a given date ($P < 0.05$, post hoc ANOVA). Symbols represent means (± SE) pooled across other factors.

drought on the plot environment. Because there were no interactions, plot environment and microbial origin effects were essentially additive in June, meaning that litter in the drought plots with drought-derived microbes lost only 13% of its mass compared to 24% mass loss in the control plots with control-derived microbes (Fig. 3D). There were no significant effects of litter origin on decomposition in the drought experiment.

Drought response: litter chemistry

Litter derived from the drought plots was chemically distinct from control-derived litter, although these initial differences did not affect mass loss. Drought-derived litter had a significantly higher C:N ratio but significantly less cellulose and hemicellulose (Table 2; Appendix: Table A1). It also contained significantly more lignin, sugar, starch, and fat. Most of these initial

TABLE 1. Overall ANOVA statistics for factors affecting mass loss in the drought and nitrogen manipulations.

Factor	Drought			Nitrogen		
	F	*P*	df	*F*	*P*	df
Plot environment (P)	4.70	**0.035**	1, 49	1.78	0.188	1, 49
Microbe origin (M)	7.50	**0.009**	1, 49	1.09	0.302	1, 49
Litter origin (L)	0.07	0.787	1, 49	0.41	0.525	1, 49
Date (D)	109.39	**<0.001**	2, 110	134.34	**<0.001**	2, 112
P × M	0.50	0.484	1, 49	0.65	0.424	1, 49
P × L	3.67	0.061	1, 49	2.61	0.112	1, 49
P × D	3.05	0.051	2, 110	1.45	0.239	2, 112
M × L	1.30	0.260	1, 49	0.06	0.801	1, 49
M × D	2.93	0.058	2, 110	0.94	0.395	2, 112
L × D	0.84	0.434	2, 110	1.01	0.368	2, 112
P × M × L	2.04	0.159	1, 49	0.05	0.828	1, 49
P × M × D	0.42	0.660	2, 110	4.25	**0.017**	2, 112
P × L × D	0.46	0.633	2, 110	7.30	**0.001**	2, 112
M × L × D	0.06	0.941	2, 110	0.20	0.820	2, 112
P × M × L × D	0.44	0.648	2, 110	0.29	0.752	2, 112

Note: Significant *P* values (<0.05) are shown in bold.

TABLE 2. Litter chemistry changes in response to the main effects of plot environment (env.), microbe origin, and litter origin; "+" indicates a significant ($P < 0.05$, overall ANOVA) increase and "−" indicates a significant decrease.

Litter chemistry, by treatment	Plot env.	Microbe origin	Litter origin	Initial litter
Drought treatment				
C:N	−			+
%C				+
%N	+			
Protein	+			+
Cellulose	−		−	−
Hemicellulose	−		−	−
Lignin	−		+	+
Sugars			+	+
Starch	+		+	+
Fat				+
Nitrogen treatment				
C:N	−		−	−
%C				
%N	+		+	+
Protein	+			
Cellulose	−		+	+
Hemicellulose	−	+	+	+
Lignin	+		−	−
Sugars				−
Starch			−	
Fat		+†	−†	

Note: The effect of the treatments (increase or decrease) on each constituent of the initial litter is shown in the last column (Welch's t test).

† Significant ($P < 0.05$) interaction with date observed.

differences persisted throughout the experiment, such that there were significant litter origin effects on cellulose, hemicellulose, lignin, sugars, and starch (Table 2).

The effect of drought in the plot environment altered litter chemistry during decomposition. Litter decaying in the drought plots contained significantly more N and protein, which reduced the litter C:N ratio by up to 10% (Table 2; Appendix: Table A1). Concentrations of cellulose, hemicellulose, and lignin declined significantly in response to drought. In contrast, starch concentrations increased significantly in response to drought. There were no significant effects of microbial origin on litter chemistry in the drought experiment.

Nitrogen response: decomposition

Overall, there were fewer significant responses of decomposition to N addition compared to drought. None of the main effects (plot environment, microbe origin, litter origin) were significant for mass loss in the N experiment. However, we found some evidence for home field advantage. Specifically, there was a significant plot environment × microbe origin interaction in June ($P < 0.01$, post hoc ANOVA), whereby microbes derived from N plots were associated with higher mass loss in N plots (the home environment) than in control plots (Fig. 5). However, control-derived microbes decomposed litter equally in N and control plots, in contrast to the prediction of home-field advantage.

Nitrogen response: litter chemistry

As with drought, N addition caused significant changes in the initial chemistry of plant litter (Table 2; Appendix: Table A2). Litter derived from the N treatment initially contained significantly more N, cellulose, and hemicellulose, but significantly lower concentrations of lignin and sugars. The initial differences in sugar concentrations diminished with time, whereas the other chemical differences generally persisted throughout the experiment, leading to significant litter origin effects for percentage N, cellulose, hemicellulose, and lignin (Table 2; Appendix: Table A2). Litter derived from the N plots retained significantly less starch during decomposition than litter from the control plots. Fat concentrations were also lower in litter from N plots, but only in March ($P < 0.001$, post hoc ANOVA). However, these litter origin effects on litter chemistry were not associated with changes in decomposition, similar to the drought experiment.

There were modest effects of plot environment and microbial origin on litter chemistry during the N experiment (Table 2). Protein and N concentrations were higher in litter decaying in the N plots, resulting in a significantly lower C:N ratio. Cellulose and hemicellulose concentrations were significantly lower in litter decaying in the N plots, whereas lignin concentrations were significantly greater. Litter inoculated with N-derived microbes had significantly greater hemicellulose concentrations (Table 2). Fat concentrations were also higher in litter with N-derived microbes, but only in November ($P < 0.05$, post hoc ANOVA).

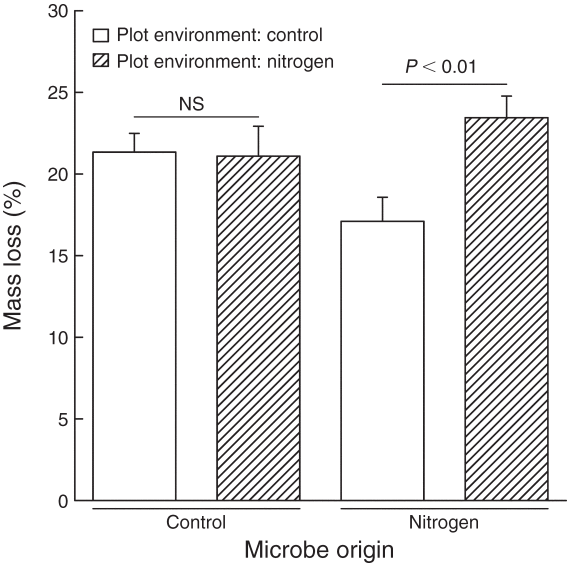

FIG. 5. Percentage mass loss for the plot environment × microbe origin interaction (mean + SE) in the nitrogen experiment on 14 June 2011. P values are from post hoc contrasts; NS stands for not significant.

Microbial abundance responses

Statistically significant responses of microbial abundance were more common for drought than for N addition, with fungi responding positively and bacteria responding negatively to drought effects. Hyphal length increased by 13–14% in response to drought in the plot environment ($P < 0.001$, overall ANOVA; Fig. 4A). In addition, there was a significant microbe origin × date interaction ($P < 0.05$, overall ANOVA), whereby hyphal lengths were 17% lower in March but 11% higher in June in litterbags inoculated with microbial communities from the drought plots (Fig. 4B). In contrast, litter origin had no significant effect on fungal abundance.

Bacterial cell densities were 56% and 86% lower in drought vs. control plots in March and June, respectively, but there was no drought effect in November ($P < 0.05$ for plot environment × date interaction, overall ANOVA; Fig. 4D). In March, bacterial densities were 36% lower in litter inoculated with drought-derived microbial communities, but this effect disappeared on the later dates ($P < 0.05$ for microbe origin × date interaction, overall ANOVA; Fig. 4E). Drought-derived litter had significant negative effects on bacterial densities, with declines >50% on all dates ($P < 0.001$ for litter origin effect, overall ANOVA; Fig. 4F). The only significant effect of N on microbial abundances was that bacterial densities were twofold higher on N-derived litter in June, although there was no date interaction ($P < 0.05$ for litter origin effect, overall ANOVA; Appendix: Fig. A1).

Discussion

Changes in precipitation and N availability are expected to occur as a result of human activities over the next century. Our study is among the first to test whether these environmental changes have indirect effects on plant and microbial communities that influence litter decay and therefore, C cycling rates. By independently manipulating the plot environment, plant litter origin, and microbial community origin, we were able to tease apart the mechanisms that contribute to changes in litter decomposition rates under drought and N deposition. Furthermore, we measured these effects and their interactions under field conditions, meaning that our results can inform future predictions of grassland responses to environmental change. Consistent with prior work in Mediterranean grasslands (Sanaullah et al. 2011), our results indicate that litter decomposition is particularly sensitive to precipitation inputs, with weaker responses to N, although both environmental drivers affected microbial communities and litter chemistry.

Drought response

We did not find support for our hypothesis that microbial communities would quickly adapt to drought conditions. The microbial origin effect observed in June and November suggests that microbial communities from the drought treatment have lower decomposition potential even under ambient conditions. Although there were probably changes in microbial community composition, changes in microbial biomass can explain most or all of this effect. Fungal abundance was 17% lower and bacterial abundance was 36% lower in March litter inoculated with drought-derived microbes (Fig. 4B, E). Assuming that the microbes present in March caused the mass loss observed in June–November, these reductions in abundance may account for the ~25% reduction in mass loss associated with drought-derived microbes.

In June, the plot environment effect of drought on decomposition coincided with large declines in bacterial (though not fungal) abundance (Fig. 4A, D). Thus drought in the plot environment did not solely manipulate abiotic conditions, but also triggered changes in microbial abundance and composition (i.e., the ratio of bacteria to fungi). This result suggests that in our system, environmental change responses of the microbial community can operate on monthly timescales. The prominent changes in microbial abundance associated with microbe origin and plot environment together suggest that microbial biomass is closely linked to decomposition rate. Such a link would simplify efforts to model decomposition responses to environmental change (Todd-Brown et al. 2012), although we cannot rule out the possibility that undetected shifts within bacterial or fungal communities contributed to differences in decomposition rates.

Lasting effects of differences in microbe origin imply that historical legacies impact ecosystem processes. A historical legacy of precipitation manipulation was observed with fungal : bacterial ratios and soil respiration in a Kansas, USA, grassland study (Evans and Wallenstein 2012). Our results imply that drought conditions can reduce bacterial abundances and may also alter microbial communities such that decomposition rates remain low even when water availability increases. The microbe origin effect shown in Fig. 3B represents the legacy of a microbial community exposed to drought prior to our litter manipulation. Regardless of where the drought-derived microbes were transplanted (control or drought treatments), they continued to show reduced rates of decomposition (no significant interactions in Table 1). The duration of this legacy effect implies that reduced rainfall in any given winter is likely to constrain litter decomposition for the next 11 months. This effect would persist until microbes with higher decomposition potentials are able to grow again or disperse from unaffected ecosystems and colonize the litter layer.

Plant community composition and tissue chemistry depend on precipitation (Henry et al. 2005), so it was somewhat surprising that we did not observe an effect of litter origin on decomposition rates. Drought altered plant community composition, with increased relative abundances of some forbs and *Lolium*, but lower relative

abundances of *Bromus*, *Lupinus*, and *Nassella pulchra*. Although these plant shifts were associated with increased litter lignin concentrations, drought litter also contained higher concentrations of decomposable compounds such as sugars, starches, and fats (Table 2). Thus changes in plant litter chemistry may have offset one another during decomposition. Alternatively, the chemical differences, although statistically significant, may have been too small to significantly affect decomposition rates. For example, lignin is known to have substantial impacts on litter decay rates (Melillo et al. 1982), but initial lignin concentrations differed by less than one percentage point in drought vs. control litter (Appendix: Table A1). Although litter chemistry differences did not affect decomposition in the drought experiment, it is possible that the microbial communities used in our inocula were affected by litter chemical changes caused by prior drought treatment (Fig. 1), which may have contributed to the microbial origin effect we observed.

The observed response of microbial abundance to drought in our study is consistent with the idea that fungi are more drought tolerant than bacteria (Lennon et al. 2012). Interestingly, fungi were able to maintain elevated hyphal lengths under drought despite being unable to translocate water from outside the litterbags (e.g., from soil). Increased hyphal lengths may have resulted from increased growth under dry conditions, or reduced turnover, perhaps due to reduced activity of fungal grazers (Treseder et al. 2010, Blankinship et al. 2011). In contrast, bacteria responded negatively to drought in the plot environment in March and June. They also declined sharply across all treatments at the onset of the dry season in June before recovering again in November after the first winter rains (Fig. 4). These results are consistent with a study that showed a decline in bacterial abundance following the onset of drought conditions in tallgrass prairie soils (Sheik et al. 2011). Lower bacterial abundances on drought-derived litter suggest that drought also changes plant chemistry in ways that negatively impact bacteria.

Nitrogen response

Contrary to our initial hypothesis, there were no significant main effects of N on litter decomposition, despite differences in litter chemistry. A lack of response to N in the plot environment is not surprising because fungi could not translocate nutrients into the litterbags. Also, fertilizer was added to the soil surface, and most litterbags did not contact the soil until later in decomposition. As with drought, plant community composition shifted with N addition, but the associated litter chemical changes did not clearly improve initial litter quality. Lignin concentrations were slightly lower, but so were starch and sugar concentrations, and initially elevated litter N disappeared rapidly during decomposition. Our results are in line with previous studies showing that differences in internal and external N availability have inconsistent effects on litter decomposition rates (Carreiro et al. 2000, Hobbie 2005, Knorr et al. 2005).

We found limited evidence for home field advantage in the N experiment, with N-derived microbial communities decomposing litter more rapidly in their home plots in June (Fig. 5). Home field advantage was only observed on one date and did not occur with control-derived microbes. This result implies that most microbial communities may rapidly acclimate, adapt, or shift under new conditions, resulting in similar decomposition rates in different nutrient environments. Rapid decomposer responses were proposed as a mechanism to explain a lack of home field advantage in a recent litter transplant experiment at the global scale (Makkonen et al. 2012). However, the home field advantage that we observed in June is consistent with another reciprocal transplant experiment in which microbial communities decomposed litter from their home environment more rapidly (Strickland et al. 2009). In our study, N fertilizer addition may have selected for decomposer taxa with high mineral N requirements that were unable to decompose litter as rapidly in the control plots. Shifts in microbial composition probably played a role in this response because abundances did not vary in control vs. N plots (Appendix: Fig. A1). In contrast, microbe origin effects in the drought experiment were driven by changes in microbial abundance.

Although we found evidence for drought and N effects on litter decomposition that are mediated by microbes, our results should be interpreted with caution for several reasons. We focused on the initial stage of litter decomposition (<25% mass loss), and different patterns might be observed at later stages. Furthermore, our litterbag design probably slowed decomposition compared to unmanipulated litter in the plots. Litterbags were sterilized and inoculated with a small amount of potentially disturbed microbial biomass, so there was probably a lag in microbial growth following bag deployment. Fungal hyphae in particular were likely disrupted by the grinding of the microbial inoculum, possibly resulting in altered fungal biomass and community composition. The bags also block UV radiation, which may contribute to litter decay at our site, especially during the dry season (Austin and Vivanco 2006). Thus our manipulation of microbe origin necessarily results in a somewhat artificial environment in the litterbags. Finally, our study took place during a single year, and interannual variability in climatic variables at our site could lead to different patterns in different years. For example, decomposition may have been less sensitive to rainfall reduction in a wetter year.

Conclusion

Our study shows that differences in microbial abundances and potentially community composition influence rates of litter decomposition independent of plant chemistry changes and the physiological response of

decomposers to drought in the plot environment. These differences are ecologically meaningful, as the reduction in decomposition rate resulting from the historical legacy of microbial abundance response was similar in magnitude to the reduction associated with drought in the plot environment. Although responses to N addition were not as strong, we found evidence for adaptation to differing levels of mineral N availability within the microbial community, lending support for the hypothesis of home field advantage. Taken together, these findings imply that differences in microbial abundances and community composition induced by environmental change can alter ecosystem process rates governed by broad groups of microbial taxa (Schimel 1995). Our study supports the emerging viewpoint that predictions from ecosystem models could be improved by explicitly representing microbial biomass and communities (Singh et al. 2010, Todd-Brown et al. 2012). Furthermore, understanding the trade-offs between microbial traits such as drought tolerance, N requirement, and decomposition potential could facilitate these modeling efforts (Treseder et al. 2011, Lennon et al. 2012).

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Supplemental Material

Appendix

Supplementary results: microbial abundance responses to nitrogen and litter chemistry responses to drought and nitrogen (*Ecological Archives* E094-062-A1).