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Highlights

- We measured drought and N impact on litter enzyme activities and microbial biomass.
- Despite semi-arid conditions, the litter was bacterially dominated.
- Enzyme efficiencies declined with drought.
- Enzyme efficiencies in the N treatment suggest microbes adapt to local environment.
- Measuring enzyme efficiencies could help predict *in-situ* enzyme activity.

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Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland

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ABSTRACT

Microbial enzymes play a fundamental role in ecosystem processes and nutrient mineralization. Therefore understanding enzyme responses to anthropogenic environmental change is important for predicting ecosystem function in the future. In a previous study, we used a reciprocal transplant design to examine the direct and indirect effects of drought and nitrogen (N) fertilization on litter decomposition in a southern California grassland. This work showed direct and indirect negative effects of drought on decomposition, and faster decomposition by N-adapted microbial communities in N-fertilized plots than in non-fertilized plots. Here we measured microbial biomass and the activities of nine extracellular enzymes to examine the microbial and enzymatic mechanisms underlying litter decomposition responses to drought and N. We hypothesized that changes in fungal biomass and potential extracellular enzyme activity (EEA) would relate directly to litter decomposition responses. We also predicted that fungal biomass would dominate the microbial community in our semi-arid study site. However, we found that the microbial community was dominated by bacterial biomass, and that bacteria responded negatively to drought treatment. In contrast to patterns in decomposition, fungal biomass and most potential EEA increased in direct response to drought treatment. Potential EEA was also decoupled from the decomposition response to N treatment. These results suggest that drought and N alter the efficiencies of EEA, defined as the mass of target substrate lost per unit potential EEA. Enzyme efficiencies declined with drought treatment, possibly because reduced water availability increased enzyme immobilization and reduced diffusion rates. In the N experiment, the efficiencies of β-glucosidase, β-xylosidase, and polyphenol oxidase were greater when microbes were transplanted into environments from which they originated. This increase in enzymatic efficiency suggests that microbial enzymes may adapt to their local environment. Overall, our results indicate that drought and N addition may have predictable impacts on the efficiencies of extracellular enzymes, providing a means of linking enzyme potentials with *in-situ* activities.

1. Introduction

Microbes play an important role in ecosystem function because they contribute to the cycling of key nutrients such as carbon and nitrogen (N). This cycling is largely dependent on extracellular enzymes that microbes produce to breakdown complex organic matter. The breakdown products become available for microbial metabolism and growth (German et al., 2011). Because extracellular enzyme activity (EEA) represents a direct expression of microbial function, it can indicate how microbial communities and ecosystems respond to environmental changes (Sinsabaugh et al., 1993).

Understanding microbial enzymatic responses to global change is critical for predicting rates of decomposition and nutrient cycling. Changes in precipitation and N deposition are particularly relevant for the southwestern United States (Fenn et al., 1998; Seager et al., 2007; Solomon et al., 2007). Here, multiyear droughts are expected to occur more frequently in the future (Seager et al., 2007), and ecological impacts of pollution-related N deposition are among the most severe in the United States (Fenn et al., 2003, 2005).

Previous studies have shown that environmental changes can alter EEA in soil and plant litter, particularly with N amendment.

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Glycosidase activities often increase with N fertilization (Bandick and Dick, 1999; Saiya-Cork et al., 2002; Waldrop et al., 2004; Grandy et al., 2008). However, the N response of C-acquiring enzymes may depend on the chemical composition of plant litter (Fog, 1988; Carreiro et al., 2000). Nitrogen-acquiring enzyme activities have shown mixed responses to N amendment. For instance, Saiya-Cork et al. (2002) found that in forest soil, leucine aminopeptidase (LAP) activity decreased by 47%, while N-acetyl-β-D-glucosaminidase (NAG) activity increased. Conversely, Waldrop et al. (2004) found that NAG activity declined with N addition in forest soil. In these studies, oxidative EEA was found to decrease slightly with N amendment (Saiya-Cork et al., 2002).

Fewer studies have examined the responses of EEA to reduced precipitation. Soil moisture is generally thought to be positively correlated with EEA, at least until soil becomes anaerobic (Baldrian et al., 2010; Henry, 2012). In empirical studies, drought generally decreases or does not change enzymatic activities. A decrease in soil enzyme activity with drought was found in both desert and forest ecosystems (Li and Sarah, 2003; Sardans and Peñuelas, 2005; Sardans and Penuelas, 2010; Steinweg et al., 2012), while no significant response was found at a Chihuahuan desert site despite changes in bacterial and fungal carbon utilization (Bell et al., 2009). This decrease in activity could perhaps be due to lower microbial biomass (Baldrian et al., 2010) or adsorption of enzymes to soil particles in drier conditions that limit catalytic rates while reducing enzyme turnover (Steinweg et al., 2012).

Changes in EEA can result from shifts in microbial communities (Ramirez et al., 2012). Such shifts can occur due to both direct and indirect processes in response to climate change (Allison et al., 2013). Direct responses may include changes in microbial physiology in response to abiotic drivers. On the other hand, indirect responses to change, such as shifts in the composition of microbial and plant communities, could also lead to altered ecosystem function (Manning et al., 2006). For example, microbial communities may shift due to changes in the biochemical composition of litter in which they reside or shift because certain microorganisms are better adapted to the new environmental regime (e.g., lower water potential due to drought) (Fierer et al., 2003; Schimel et al., 2007). However, specialization on chemical resources could constrain the function of microbial communities in new environments, a form of local adaptation known as home field advantage. In support of this idea, microbial communities sharing a common history with a litter type or environmental treatment often carry out decomposition more rapidly than microbial communities transplanted into new conditions (Gholz et al., 2000; Strickland et al., 2009).

In a previous study in a southern California grassland, we used a reciprocal transplant design to separate out direct versus indirect effects of drought and N addition on litter decomposition (Allison et al., 2013). We found that drought reduced litter decomposition directly, through reductions in water availability, and indirectly through changes in the abundance and/or composition of the litter microbial community. In contrast, N addition had minimal effects on litter decomposition through direct or indirect mechanisms. We also tested for home field advantage in decomposer communities. Consistent with this idea, we found that litter mass loss was significantly lower when microbes previously exposed to N fertilization were transplanted into unfertilized plots.

The goal of our current study was to examine the microbial and enzymatic mechanisms underlying the changes in decomposition that we previously observed (Fig. 1). We measured the potential activities of nine extracellular enzymes involved in litter decomposition to determine if changes in litter mass loss were proportional to changes in potential EEA. Our initial hypothesis was that treatment effects on enzyme potentials would relate directly to changes in mass loss. We expected drought to have a direct negative effect on potential EEA, whereas we expected N-adapted microbes to show higher potential EEA when transplanted into N-fertilized plots.

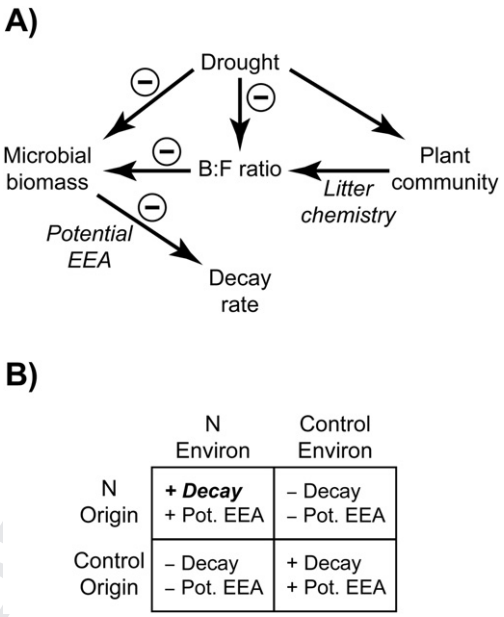

Fig. 1. Conceptual model for responses of microbial biomass and extracellular enzyme activities (EEA) to drought (A) and nitrogen treatment (B). In (A), negative signs represent hypothesized responses based on Allison et al. (2013). Italicized text indicates mechanisms of response. B:F = Bacterial:fungal. (B) Represents the home field advantage hypothesis whereby litter decay rates and potential EEA should be relatively higher when litter and microbes are transplanted into their home environments. The bold italic decay response was observed in Allison et al. (2013). N = nitrogen.

A likely alternative hypothesis is that drought and N treatments alter the efficiency of enzymatic decomposition. Changes in decomposition may not relate to changes in potential EEA if treatments alter the physical and chemical environment for enzyme activity. For example, drought may limit rates of diffusion, which could limit the efficiency of enzymatic catalysis (Wallenstein et al., 2011). In addition, environmental treatments could affect substrate concentrations, further decoupling enzyme potentials from actual decomposition rates (Wallenstein et al., 2012).

In our previous study, we observed that bacterial but not fungal abundances declined in response to drought, and that bacterial abundance increased in litter from N-fertilized plots (Allison et al., 2013). For our current study, we converted abundances into biomass to determine if changes in microbial biomass were related to changes in EEA. Given that our study system is semi-arid, and fungi may be more drought-tolerant than bacteria, we expected litter microbial biomass and EEA responses to be dominated by fungi.

2. Materials and methods

2.1. Study site

The study site is a grassland located in Loma Ridge National Landmark of the Santa Ana foothills in Southern California (33° 44′ N, 117° 42′ W, 365 m elevation). Analyses of long-term records, including historical aerial photographs and transect surveys, revealed a relatively stable vegetation distribution since at least the 1930s. The soil is of the Myford Series and is a deep, moderately well-drained sandy loam with a pH of 6.8 (German et al., 2012). Surrounding series include clay loams. The pH of the litter layer was determined to be 6.0. The plant community is dominated by exotic annual grasses and forbs (De Vries et al., 2006).

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2.2. Field manipulation

Treatments were established in February 2007 as part of a broader experiment to examine the combined effects of N addition and precipitation variability on ecosystem processes (Potts et al., 2012; Allison et al., 2013). For the current study, we used a subset of the plots established in 2007. "Control" plots received ambient levels of precipitation and N deposition; "drought" plots received reduced precipitation and ambient N deposition; and "N" plots received ambient precipitation and added N. Each plot was 3.3 × 9.3 m, and there were a total of 24 plots arranged in 8 experimental blocks. The drought treatment was imposed by covering the drought plots with clear polyethylene during a subset of winter rainstorms. Rainfall was reduced from 369 to 194 mm during winter 2009–2010 and from 540 to 213 mm during winter 2010–2011. N plots received 20 kg N ha^{-1} as soluble $CaNO_3$ before the growing season and 40 kg N ha^{-1} as 100-day release $CaNO_3$ during the growing season.

2.3. Reciprocal transplant

In late fall 2010, we established a reciprocal transplant experiment within the field manipulation to isolate the effects of plot treatment, microbial origin, and litter origin on decomposition processes and EEA (Allison et al., 2013). Plot treatment represents the direct manipulation of abiotic inputs of precipitation or inorganic N. Microbe origin captures indirect changes in microbial abundance and composition, and litter origin represents indirect changes in plant community composition and litter chemistry. These main effects were crossed in a fully factorial design using either control and drought plots (the "drought experiment") or control and N plots (the "N experiment"). Thus we did not examine any drought × N interactions, and we treat the drought and N experiments as statistically independent. Both experiments were replicated in each block of the field manipulation (i.e. $n = 8$).

Litter origin and microbial origin were manipulated as described in Allison et al. (2013). Briefly, we manipulated litter origin by collecting senesced plant material from control and treatment plots after the 2009–2010 growing season. Plant litter was collected from each plot, combined within treatments (control, drought, or N), and homogenized by hand. Thus litter may originate from the control or drought treatment in the drought experiment and from the control or N treatment in the N experiment. Plant litter (2 g air dry weight) was placed in litter bags and sterilized with >22 kGy gamma irradiation. The bags were made of nylon membrane material with 0.45 μm pores such that water, solutes, and small bacteria (but not fungi) could pass through.

We manipulated microbial origin by re-inoculating sterile litter bags with microbes collected from control, drought, or N treatments. In the drought experiment, microbes originate from either the control or drought treatment, and in the N experiment microbes originate from either the control or N treatment. Microbes were collected by taking litter samples from each plot on November 30, 2010, and combining within treatments to make 3 batches (control, drought, or N). Each batch of inoculum was ground in a Wiley mill to 1 mm and added in 50 mg aliquots to the sterilized litter bags. Although it is likely that some bacteria moved in and out of our litter bags, potentially affecting our results, we observed microbial origin effects for up to 11 months. Therefore, bacterial exchange was probably restricted throughout the experiment.

Litter bags were deployed on December 15, 2010, and collected in batches of 120 on March 3, 2011, June 14, 2011, and November 14, 2011. Each bag was analyzed for percent mass loss and concentrations of lignin, starch, protein, cellulose, hemicellulose, sugars, and phosphorus by near infrared spectroscopy as described in Allison et al. (2013). Litter subsamples were also analyzed for bacterial cell counts by flow cytometry and fungal hyphal lengths by staining and microscopy (Allison et al., 2013). We converted bacterial cell counts to biomass (μg C g^{-1} dry litter) assuming spherical cells with radius 0.6 μm and C density of 2.2 × 10^{-13} g $μm^{-3}$ (Bratbak, 1985). Hyphal lengths were converted to biomass (μg C g^{-1} dry litter) assuming a fresh density of 1.1 g cm^{-3}, 33% dry mass, 40% C in dry mass, and hyphal diameter of 5.2 μm (Killham, 1998). Hyphal diameter was measured using the ruler in Adobe Photoshop 12.1 with images of stained hyphae on microscope slides. Diameters were measured on a subset of 9 samples, each represented by 2 images. The 9 samples included 3 samples from each collection date where one of the samples received the control level of all factors, the second received the drought level of all factors, and the third received the N level of all factors. Diameters were measured at locations where hyphae intersected gridlines spaced at 40 μm intervals over a total area of ~0.63 mm^2 per image. Each sample was represented by at least 34 measurements that were averaged, and these averages were used to calculate an overall mean for the 9 samples since there were no significant differences in hyphal diameter across treatments or dates. Total microbial biomass was computed as the sum of bacterial and fungal biomass.

2.4. Extracellular enzyme activity assays

Litter samples collected in March, June, and November 2011 were kept in a −80 °C freezer for up to 8 weeks before being processed (Wallenius et al., 2010). Litter homogenates were assayed for the activity of nine enzymes involved in decomposition or cycling of organic N, carbon, or phosphorus (Table 1). Sample homogenates were prepared by adding 0.1 g of litter to 60 mL of 25 mM maleate buffer (pH 6.0) and homogenizing with a Polytron automated homogenizer (12 mm generator) or a Biospec Tissue Tearor (14 mm

Table 1
Extracellular enzymes assayed in litter decaying in a southern California grassland, and their abbreviations, functions, corresponding substrates, and final substrate concentrations.

Enzyme	Abbreviation	Function	Substrate	Substrate concentration
α-glucosidase	AG	Starch degradation	4-MUB-α-D-glucopyranoside	200 μM
Acid phosphatase	AP	Mineralizes organic P into phosphate	4-MUB Phosphate	800 μM
β-glucosidase	BG	Cellulose degradation	4-MUB-β-D-glucopyranoside	400 μM
β-xylosidase	BX	Hemicellulose degradation	4-MUB-β-D-xylopyranoside	400 μM
Cellobiohydrolase	CBH	Cellulose degradation	4-MUB-β-D-cellobioside	200 μM
Leucine aminopeptidase	LAP	Peptide breakdown	L-leucine-7-amido-4-methylcoumarin hydrochloride	200 μM
N-acetyl-β-D-glucosaminidase	NAG	Chitin degradation	4-MUB-*N*-acetyl-β-D-glucosaminide	400 μM
Polyphenol oxidase	PPO	Degrades lignin and other aromatic polymers	Pyrogallol	1000 μM
Peroxidase	PER	Catalyzes oxidation reactions	Pyrogallol	1000 μM

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generator) for four 30 s pulses, with 30 s between pulses. The resulting homogenates were continuously stirred using a magnetic stir plate while 200 μl per well was dispensed into 96-well microplates with eight replicate wells per sample per assay.

Fluorimetric enzyme assays were performed according to the methods described in German et al. (2011) for AG, AP, BG, BX, CBH, LAP, and NAG. Fifty microliters of substrate solution were added to each sample well (final concentrations shown in Table 1). Homogenate control wells received 50 μl of maleate buffer and 200 μl of sample suspension. Substrate control wells received 50 μl substrate solution and 200 μl of maleate buffer. Quench wells received 50 μl of standard (25 μM 4-Methylumbelliferone or 25 μM 7-Amino-4-methylcoumarin) and 200 μl of sample suspension. Reference standard wells received 50 μl of standard and 200 μl maleate buffer. There were 8 replicate wells for each homogenate control, substrate control, reference standard, and quench. Samples were incubated in covered black microplates for one hour. Preliminary assays confirmed the increase in fluorescence was linear for 60 min for all enzymes. After incubation, 10 μl of 1.0 M NaOH was added to each well to stop the reaction, and fluorescence was measured immediately at 365 nm excitation and 450 nm emission. The enzymatic activity was then calculated using the following equation:

$$\text{Activity}\left(\mu\text{mol}\, g^{-1}\, h^{-1}\right) = \frac{\text{Net fluorescence} \times \text{Buffer volume(mL)}}{\text{Emission coefficient} \times \text{Homogenate volume(mL)} \times \text{Time(h)} \times \text{Litter mass(g)}} \quad (1)$$

where net fluorescence is ~~sample fluorescence − (substrate control − homogenate control fluorescence)~~ and the emission coefficient is fluorescence μmol^{-1} standard in the reference well.

The oxidative enzymes, PPO and PER, were only measured in November using a colorimetric assay described in Allison and Jastrow (2006). ~~Fifty microliters of pyrogallol was~~ added to each sample well with 200 μl of sample suspension. Blank wells received 50 μl of water and 200 μl of sample suspension. Negative control wells received 50 μl pyrogallol and 200 μl of maleate buffer. For the PER assay, sample and control wells also received 10 μl of 0.3% hydrogen peroxide. There were eight replicate wells for each type of sample and control. Samples were incubated in covered clear microplates for 24 h. Absorbance was measured at 410 nm. Activity was calculated using Equation (1), but substituting net absorbance for net fluorescence and extinction coefficient for emission coefficient.

2.5. Statistical analysis

To examine the link between enzyme activity and degradation of litter chemical constituents, we calculated enzyme efficiencies. Enzyme efficiency is defined as the mass loss of a chemical compound per unit enzyme activity. Since most litter mass loss occurred between March and June, we calculated the mass loss of each litter constituent during this time period and divided by the mean enzyme activity for the March and June time points. However, we used the November time point for PPO and PER because these enzymes were not measured on the earlier dates. For some replicates, mass increased from March to June, which resulted in negative enzyme efficiency values; these values were set to zero. The efficiencies of BG, CBH, and NAG were calculated relative to cellulose loss; BX relative to hemicellulose loss; AG relative to starch loss; LAP relative to protein loss; PPO and PER relative to lignin loss; and AP relative to phosphorus loss. Using the same time points, we also calculated microbial efficiency as overall litter mass loss mg^{-1} microbial biomass. Likewise, we calculated protein efficiency as overall mass loss mg^{-1} litter protein. Protein efficiency measures the effectiveness of the total protein pool (including all enzymes) in catalyzing litter decomposition.

We first analyzed mass loss, microbial biomass, and enzyme potentials using a factorial mixed-model ANOVA with repeated measures ("overall ANOVA", (Allison et al., 2013)). The model included 4 fixed effects (plot treatment, litter origin, microbe origin, and date), interactions among fixed effects, and 2 random effects: block and subject nested within block. Subject is defined as each unique combination of block, plot, litter origin, and microbe origin. Each subject was sampled once on each of the 3 dates (the repeated measurement). If the fixed effects or their interactions were significant, we ran post-hoc ANOVAs on each date with block as a random effect to test for significant treatment effects within dates ("single-date ANOVA"). We also used single-date ANOVAs to test for treatment effects on enzyme efficiencies and PPO and PER activities that were determined on only one time point. Tukey post-hoc contrasts were used to test for significant differences among treatment means from the single-date ANOVAs. If there were significant date effects in either drought or N experiments, we used Tukey post-hoc contrasts to test for significant differences in means across dates. These contrasts were run on a subset of the litter bags receiving control levels of all factors to avoid pooling date effects across other treatments. The ANOVAs preceding these contrasts included date as a fixed effect and block as a random effect. Data were square root- or log-transformed where necessary to improve normality and reduce heteroscedasticity. All analyses were run in the R software environment.

3. Results

3.1. Decomposition and microbial biomass

Compared to controls, mass loss was 5 percentage points lower in the drought plots ($P = 0.035$, overall ANOVA) and 6 percentage points lower when litter was inoculated with microbes from the drought treatment ($P = 0.009$, overall ANOVA, Table 3, Fig. 2). Nitrogen had no direct effect on mass loss (Table 4), although microbes from the N treatment generated 6 percentage points more mass loss in the N plots relative to control plots in June (Fig. 3A).

Drought had a negative effect on microbial biomass (Fig. 4) that was driven by changes in bacterial abundance (Fig. 2). Although fungal abundance increased by 13% in drought plots (Fig. 2), the community was dominated by bacterial biomass as indicated by bacterial:fungal ratios approaching 30:1 (Fig. 4, Table 2). Plot treatment with drought reduced microbial biomass by up to 50%, and drought-derived litter also showed steep declines in microbial biomass (Fig. 4). Microbes derived from the drought treatment showed lower biomass, but only in March (Fig. 4B). Most of these microbial biomass responses were also reflected in bacterial:fungal ratios (Fig. 4). Nitrogen treatment had few significant effects on microbial biomass; however, bacterial abundances increased by 6% overall in N-derived litter (Fig. 5C). This pattern was mainly driven by the June time point when microbial biomass and bacterial:fungal

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Table 2
Mean ± SEM microbial biomass (mg C g^{-1} dry litter), bacterial:fungal ratios, protein concentrations (mg g^{-1} dry litter), and enzyme activities (μmol h^{-1} g^{-1} dry litter) on 3 sampling dates in 2011. Means were calculated for litter bags receiving control levels of plot treatment, microbe origin, and litter origin ($n = 8$) and compared using Tukey post-hoc contrasts. Values sharing the same letter within a row are not significantly different ($P > 0.05$). Abbreviations in Table 1.

	March 3	June 14	November 14
Microbial biomass	1.03 ± 0.21^{a}	0.19 ± 0.04^{b}	0.89 ± 0.22^{a}
Bacterial:fungal ratio	31.8 ± 6.7^{a}	4.1 ± 1.1^{b}	16.3 ± 4.8^{a}
Protein concentration	61.1 ± 1.3^{a}	66.0 ± 1.5^{ab}	75.4 ± 3.0^{c}
AG	0.89 ± 0.11^{a}	0.96 ± 0.12^{a}	2.71 ± 0.33^{b}
BX	4.78 ± 0.76^{a}	4.45 ± 0.39^{a}	9.62 ± 1.15^{b}
BG	32.0 ± 3.2^{a}	23.3 ± 2.3^{a}	48.0 ± 5.4^{b}
CBH	12.4 ± 1.7^{a}	9.3 ± 0.8^{a}	19.6 ± 2.7^{b}
NAG	12.2 ± 1.3^{ab}	9.9 ± 1.0^{a}	14.9 ± 1.4^{b}
LAP	3.5 ± 0.6^{a}	5.2 ± 0.7^{a}	3.7 ± 0.6^{a}
PPO			0.20 ± 0.02
PER			2.6 ± 0.7
AP	12.2 ± 2.3^{ab}	10.5 ± 1.9^{a}	19.5 ± 3.3^{b}

ratios were ~50% greater in N-derived litter relative to control litter (Fig. S1).

3.2. Temporal patterns

We observed seasonal effects on most microbial and enzyme variables. Microbial biomass and bacterial:fungal ratios dropped by at least a factor of 4 in June relative to the other 2 dates (Fig. 4, Table 2). Litter protein concentrations increased steadily from 61 to 75 mg g^{-1} from March to November (Table 2). Most potential enzyme activities were slightly but not significantly higher in March than in June, but then increased by at least 50% in November (Table 2). However, LAP did not show a significant temporal trend, and oxidases were only measured in November. Most litter mass loss occurred between March and June, with little additional mass loss by November (Allison et al., 2013).

3.3. Drought response: potential enzyme activity

Plot treatment with drought had a positive effect on potential activity for the carbohydrate-degrading enzymes BG, BX, and CBH with increases of at least 50% (Fig. 2A). NAG activity also increased by 54%, but LAP activity decreased by 26% in response to plot treatment with drought. These enzyme changes were accompanied by a 13% increase in litter protein concentration (Fig. 2A). In litter inoculated with drought-derived microbes, PPO declined by 36% and NAG increased by 9%, but none of the other enzymes varied with microbe origin (Fig. 2B). In litter derived from the drought treatment, all enzyme activities decreased by 12–38% except AP, which increased by 31%, and BG and the oxidases, which did not respond (Fig. 2C). Aside from a weak plot × date interaction for CBH ($P = 0.049$, overall ANOVA), there were no interactions among the main effects or with date observed for EEA in the drought experiment.

3.4. Drought response: enzyme efficiency

Since the loss of litter chemical components generally declined in the drought treatment, but most enzyme activities increased, there were declines of at least 63% in the efficiencies of carbohydrate-degrading enzymes and NAG (Table 3). NAG efficiency also declined by one-third with drought-derived microbes. These declines in enzyme efficiency were paralleled by ~30% declines in protein efficiency with drought in the plot treatment and microbe origin (Table 3). There were no significant interactions among the main effects of our experimental design that influenced enzyme efficiency.

3.5. Nitrogen response: potential enzyme activity

In response to plot treatment with N, the only significant enzyme responses were increases of 11% for CBH, 9% for NAG, and 52% for PPO (Fig. 5A). This treatment also caused a small but significant 4% increase in litter protein concentration (Fig. 5A). In litter inoculated with microbes from the N treatment, 5 of the 9 enzyme activities were lower by 11–27% (Fig. 5B). In litter derived from the N treatment, all enzymes except the oxidases were higher by 12–24% (Fig. 5C). Aside from a weak plot treatment × litter origin × date interaction for BX ($P = 0.046$, overall ANOVA), there were no significant interactions with date for enzymes in the N experiment. There were significant ($P < 0.05$, overall ANOVA) microbe origin × litter origin effects for BG, BX, and LAP, but only LAP showed a pattern consistent with home field advantage. However, LAP also showed a plot treatment × microbe origin interaction that was inconsistent with home field advantage ($P = 0.036$, overall ANOVA). Likewise, NAG showed a weak plot treatment × litter origin interaction ($P = 0.044$, overall ANOVA) that was inconsistent with home field advantage.

3.6. Nitrogen response: enzyme efficiency

Enzyme efficiency responses to the main effects in our N experiment were generally not significant (Table 4). Although

Table 3
Mean ± SEM percent mass loss at 6 months and decomposition efficiencies for microbes, protein, and enzymes in the drought experiment. Each mean was pooled across other factors ($n = 32$). Efficiencies are expressed as g mass loss mg^{-1} microbial biomass C (eMicrobe), mg mass loss mg^{-1} protein (eProtein), or mg mass loss × h μmol^{-1} (eAG–eAP).

	Plot treatment		Litter origin		Microbe origin	
	Control	Drought	Control	Drought	Control	Drought
Mass loss (%)	20.9 ± 1.4	15.3 ± 1.4*	19 ± 1.6	17.2 ± 1.5	20.6 ± 1.4	15.6 ± 1.5*
eMicrobe	0.91 ± 0.17	1.5 ± 0.3	0.87 ± 0.17	1.6 ± 0.30*	1.2 ± 0.19	1.3 ± 0.3
eProtein	3.1 ± 0.3	2 ± 0.3*	2.7 ± 0.3	2.3 ± 0.3	3 ± 0.3	2.1 ± 0.3*
eAG	6.3 ± 1.4	7.1 ± 1.7	6.4 ± 1.3	7.1 ± 1.8	7.8 ± 1.3	5.7 ± 1.7
eBX	9 ± 1.1	3.1 ± 0.7*	6 ± 0.9	5.9 ± 1.3	6.9 ± 1.1	5 ± 1.1
eBG	3.2 ± 0.4	1.3 ± 0.2*	2.4 ± 0.3	2.1 ± 0.4	2.6 ± 0.4	2 ± 0.4
eCBH	9.5 ± 1.7	2.4 ± 0.4*	5.5 ± 1	6.3 ± 1.7	6.4 ± 1.1	5.5 ± 1.6
eNAG	8.1 ± 1.2	3 ± 0.5*	5.8 ± 0.9	5.3 ± 1	6.7 ± 1.1	4.5 ± 0.9*
eLAP	4.1 ± 0.9	51.2 ± 41.3	5.5 ± 2.5	53 ± 44.1	6.5 ± 1.6	49 ± 41.4
ePPO	2300 ± 1700	1400 ± 880	520 ± 360	3100 ± 1700	3400 ± 1800	350 ± 140
ePER	17.1 ± 5.5	20.4 ± 8.7	14.2 ± 4.6	22.9 ± 8.8	19 ± 6.1	18.5 ± 8.3
eAP	0.038 ± 0.009	0.032 ± 0.006	0.037 ± 0.006	0.033 ± 0.009	0.039 ± 0.006	0.03 ± 0.009

*$P < 0.05$ for difference from control (single-date ANOVA).

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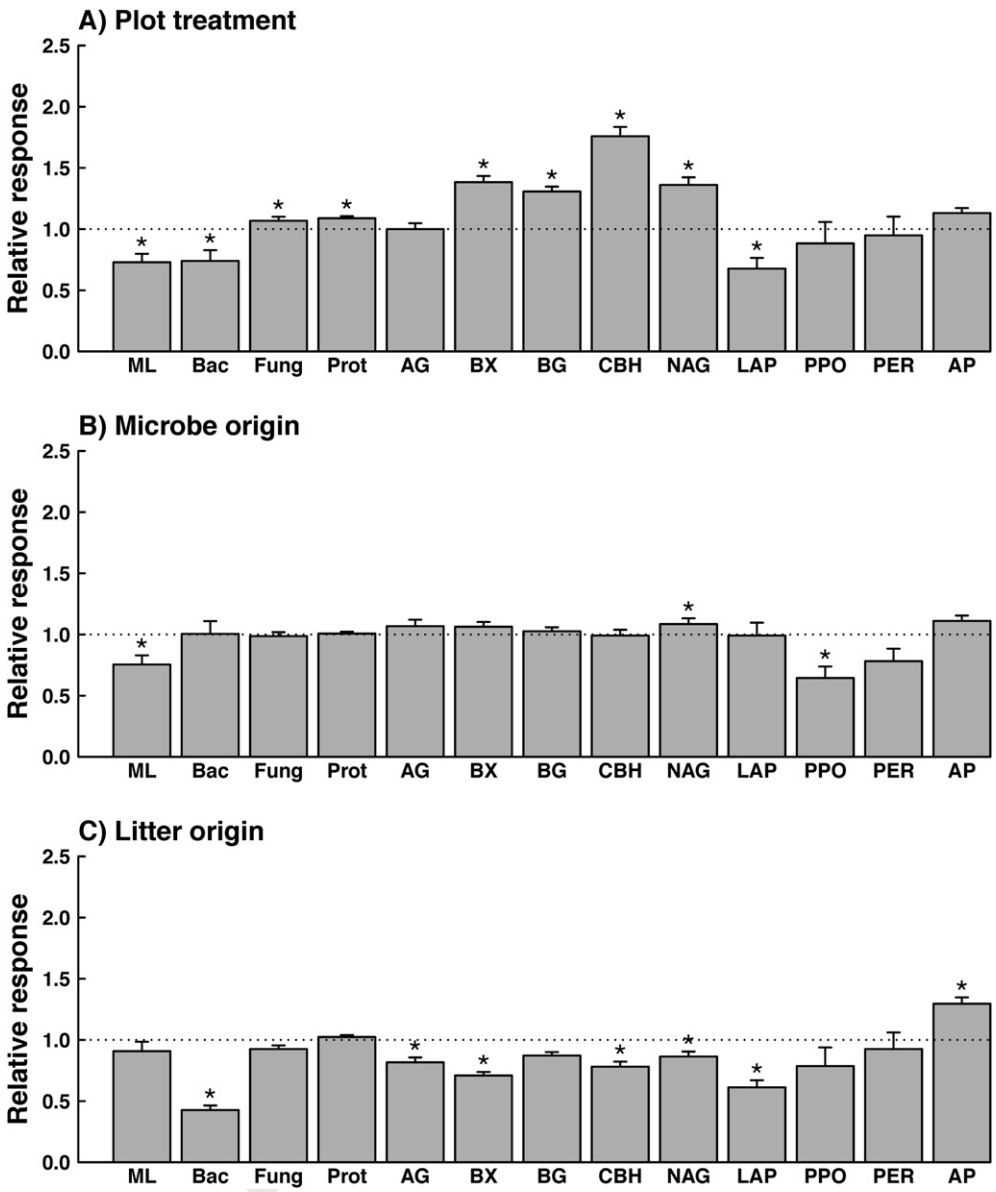

Fig. 2. Relative responses of mass loss (ML), bacterial cell density (Bac), fungal hyphal length (Fung), protein concentration (Prot), and enzyme activities (abbreviations in Table 1) to drought treatment in the plot (A), microbe origin (B), and litter origin (C). Bars represent means with SEM pooled across dates ($n = 96$) except for mass loss which is shown for June 24, 2011, and PPO and PER which were only measured on November 14, 2011 ($n = 32$). (*) $P < 0.05$ for comparison to control (ANOVA).

Table 4
Mean ± SEM percent mass loss at 6 months and decomposition efficiencies for microbes, protein, and enzymes in the nitrogen experiment. Each mean was pooled across other factors ($n = 32$). Efficiencies are expressed as g mass loss mg^{-1} microbial biomass C (eMicrobe), mg mass loss mg^{-1} protein (eProtein), or mg mass loss × h μmol^{-1} (eAG-eAP).

	Plot treatment		Litter origin		Microbe origin	
	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen
Mass loss (%)	19.2 ± 1	22.3 ± 1.1	20 ± 0.8	21.5 ± 1.3	21.2 ± 1.1	20.3 ± 1.1
eMicrobe	0.29 ± 0.05	0.39 ± 0.06	0.36 ± 0.07	0.31 ± 0.04	0.34 ± 0.04	0.33 ± 0.06
eProtein	2.6 ± 0.3	3 ± 0.3	2.5 ± 0.2	3.1 ± 0.3	2.9 ± 0.3	2.6 ± 0.3
eAG	5.2 ± 1.2	4.9 ± 1.2	6.8 ± 1.3	3.2 ± 1.0*	6.2 ± 1.2	3.9 ± 1.2
eBX	6.8 ± 0.9	8 ± 1.1	6.6 ± 0.8	8.2 ± 1.2	6.5 ± 0.7	8.3 ± 1.2
eBG	2.5 ± 0.4	2.9 ± 0.3	2.4 ± 0.3	3 ± 0.4	2.6 ± 0.3	2.8 ± 0.4
eCBH	6.5 ± 1.1	6.8 ± 0.8	6.2 ± 0.9	7.1 ± 1.1	6.2 ± 0.9	7.1 ± 1.1
eNAG	6.6 ± 1	7.1 ± 0.8	6.5 ± 1	7.2 ± 0.9	6.6 ± 0.9	7.1 ± 1
eLAP	1.6 ± 0.3	2 ± 0.4	2 ± 0.4	1.7 ± 0.3	2.2 ± 0.4	1.4 ± 0.3
ePPO	750 ± 620	920 ± 720	210 ± 98	1300 ± 790	760 ± 640	900 ± 690
ePER	13.4 ± 4.2	7 ± 1	10.8 ± 3.4	10 ± 3.1	9.9 ± 2.9	10.8 ± 3.6
eAP	0.039 ± 0.005	0.047 ± 0.007	0.049 ± 0.006	0.036 ± 0.005	0.036 ± 0.005	0.049 ± 0.006

*$P < 0.05$ for difference from control (single-date ANOVA).

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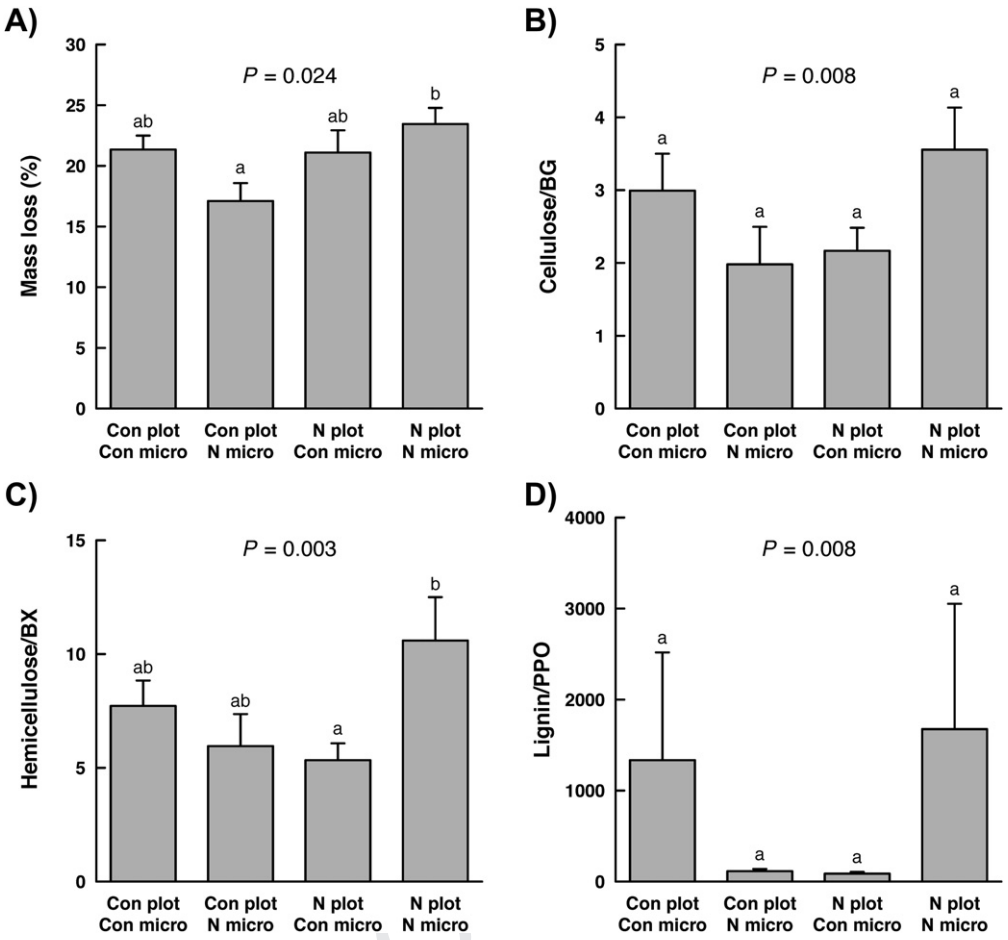

Fig. 3. Barplots of the interaction between plot treatment and microbe origin for the nitrogen experiment. (A) Mass loss at 6 months; (B) efficiency of cellulose degradation by β-glucosidase; (C) efficiency of hemicellulose degradation by β-xylosidase; (D) efficiency of lignin degradation by polyphenol oxidase. Efficiency units are mg mass loss × h μmol^{-1}. Bars represent means with SEM ($n = 16$). *P*-values are for the single-date ANOVA interaction, and means sharing the same letter are not significantly different (Tukey post-hoc contrasts).

most enzyme activities were higher in litter derived from the N treatment, mass loss of their chemical substrates also increased, leading to little change in enzyme efficiency. Only AG efficiency declined significantly by 53% in litter derived from the N treatment.

Several enzyme efficiencies showed significant interactions consistent with home-field advantage, mainly for the carbohydrate-degrading enzymes BG, BX, and CBH, but also for NAG and PPO. All five of these enzyme efficiencies showed significant plot treatment × microbe origin interactions ($P < 0.05$, single-date ANOVAs) whereby enzymes were more efficient at degrading their substrates when microbes were transplanted into their home treatment plots. However, post-hoc contrasts among means were not always significant; patterns for BG, BX, and PPO efficiencies are shown in Fig. 3. Similarly, efficiencies of BG and BX were higher when microbes were transplanted onto litter from their home treatment ($P < 0.05$, single-date ANOVAs), although differences among individual means were not significant (Fig. 6). Regardless of microbe origin, efficiencies of BG, BX, CBH, and NAG were higher when litter was transplanted into its home treatment plot ($P < 0.01$, single-date ANOVAs). This effect was most evident with N-derived litter transplanted into N plots (i.e. doubling of BG and BX efficiencies, Fig. 7). Consistent with the enzyme efficiencies, protein efficiency also showed a pattern consistent with home-field advantage for microbes and litter transplanted into home plots ($P = 0.005$ for plot treatment × microbe origin and $P < 0.001$ for plot treatment × litter origin, single date ANOVA).

4. Discussion

4.1. Summary of mechanisms

In contrast to our initial hypothesis, we found little correspondence between litter mass loss and potential enzyme responses to drought and N addition. Whereas litter mass loss declined in drought plots, most potential enzyme activities increased (Fig. 2A). Only PPO showed a response to microbial origin in the drought experiment that coincided with a change in mass loss (Fig. 2B). In the N experiment, significant changes in potential enzyme activities were not accompanied by changes in litter mass loss (Fig. 5). Microbial biomass was dominated by bacteria and did not consistently respond to drought and N addition in parallel with potential enzyme activity. Together these results suggest that responses of potential enzyme activities are unreliable predictors of decomposition responses to drought and N addition. Rather, our direct measurements of litter substrate decay (e.g. cellulose, lignin, protein) show that drought and N addition clearly alter the efficiencies of enzymes degrading specific litter compounds. In the N experiment, there was often evidence for home-field advantage with enzyme efficiency but not with potential enzyme activity.

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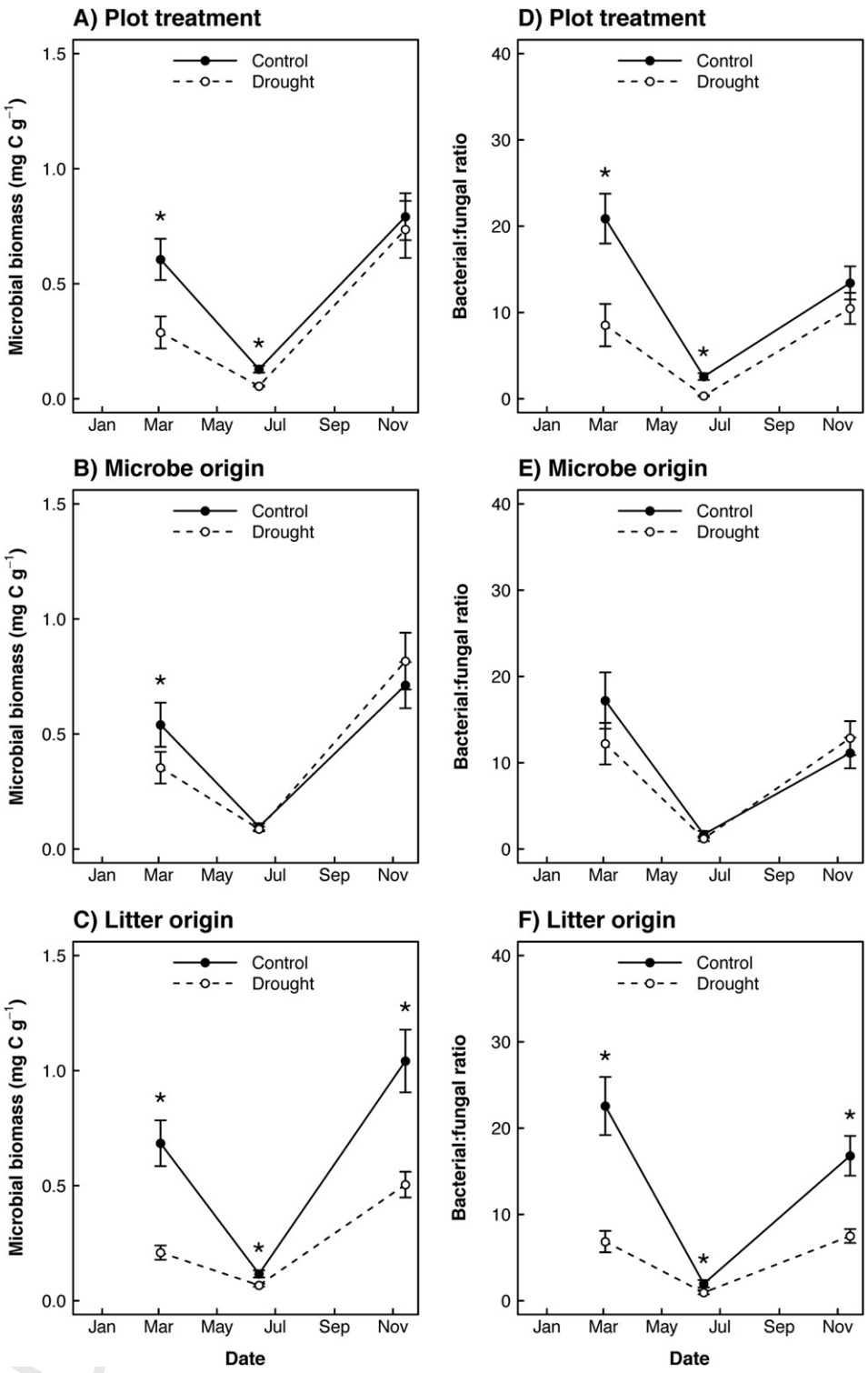

Fig. 4. Plot treatment, microbe origin, and litter origin effects on microbial biomass (A–C) and bacterial:fungal ratios (D–F) over time in the drought experiment. (*) denotes a significant difference on a given date ($P < 0.05$, single-date ANOVA). Symbols represent means (±SEM) pooled across other factors ($n = 32$).

4.2. Microbial biomass and composition

In contrast to our initial expectation, bacteria dominated the microbial communities in this experiment. Bacteria often dominate systems with high nutrient availability and low soil organic matter concentration, such as our study site (Van Der Heijden et al., 2008). These factors may have been more important than annual precipitation in determining bacterial versus fungal abundance in our system. Fungi were drought tolerant, as expected, but fungal responses to drought and N treatments were overwhelmed by bacterial responses. Nonetheless, bacterial:fungal ratios declined with drought, as indicated by lower bacterial biomass in the drought plots and in litter from the drought treatments. This pattern is consistent with the prediction that fungi are resistant to

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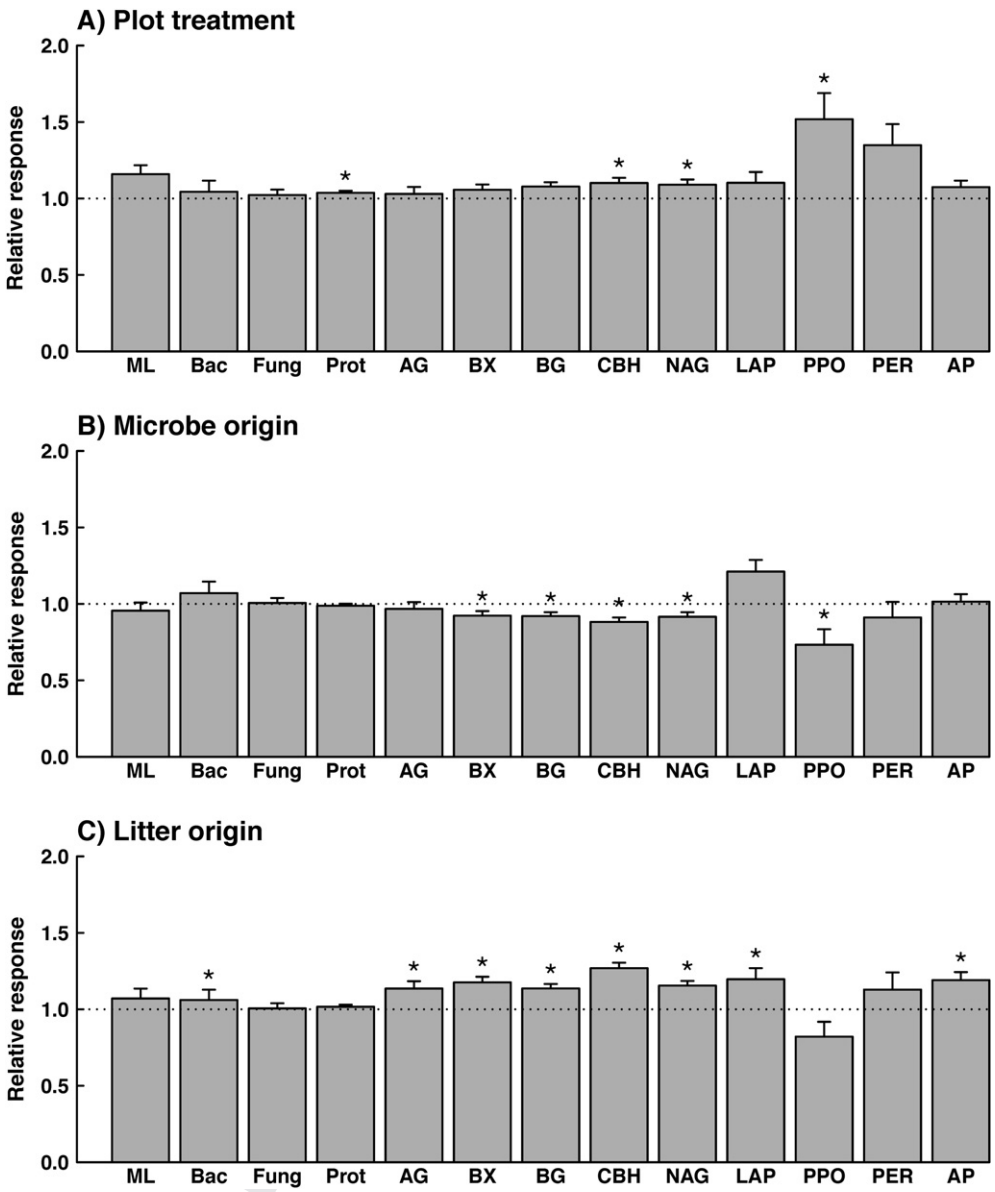

Fig. 5. Relative responses of mass loss (ML), bacterial cell density (Bac), fungal hyphal length (Fung), protein concentration (Prot), and enzyme activities (abbreviations in Table 1) to nitrogen treatment in the plot (A), microbe origin (B), and litter origin (C). Bars represent means with SEM pooled across dates ($n = 96$) except for mass loss which is shown for June 24, 2011, and PPO and PER which were only measured on November 14, 2011 ($n = 32$). (*) $P < 0.05$ for comparison to control (ANOVA).

changes in moisture availability because of their chitinous cell walls (Holland and Coleman, 1987). The temporal patterns we observed also suggest that bacteria are drought-sensitive, since bacterial:fungal ratios declined in June once precipitation inputs had ceased. Other studies have found conflicting results as to whether drought should favor fungal versus bacterial dominance (Strickland and Rousk, 2010).

The effect of N on microbial communities was generally weaker than the effect of drought. However, bacterial biomass increased in N-derived litter, yielding a greater bacterial:fungal ratio in June. This result supports the prediction that bacterial dominance increases with greater N availability since bacteria have higher nutrient requirements than fungi (Güsewell and Gessner, 2009). In contrast, other studies have found that bacterial:fungal ratios decline with N addition (De Vries et al., 2006; Rousk and Bååth, 2007).

All techniques used to measure bacterial and fungal biomass include a series of assumptions (Strickland and Rousk, 2010). We estimated biomass with direct counts because bacterial cells and fungal hyphae could be relatively easily extracted from our leaf litter and measured directly using microscopy. Even so, there are several caveats to our approach. The biomass conversions for bacteria in particular are not well constrained since we used literature estimates for C density and average cell size when converting bacterial counts into biomass. Furthermore, grinding the initial inoculum probably disproportionately affected fungal hyphae, potentially reducing fungal biomass throughout our experiment. Nonetheless, the high bacterial:fungal ratios in our data suggest an important role for bacteria in this system.

4.3. Enzyme responses: temporal patterns

Potential enzymatic activities were highest in November, followed by March and June. These results are consistent with Bell et al. (2010) who suggested that potential EEA could increase during winter months if microbes increase enzyme production to

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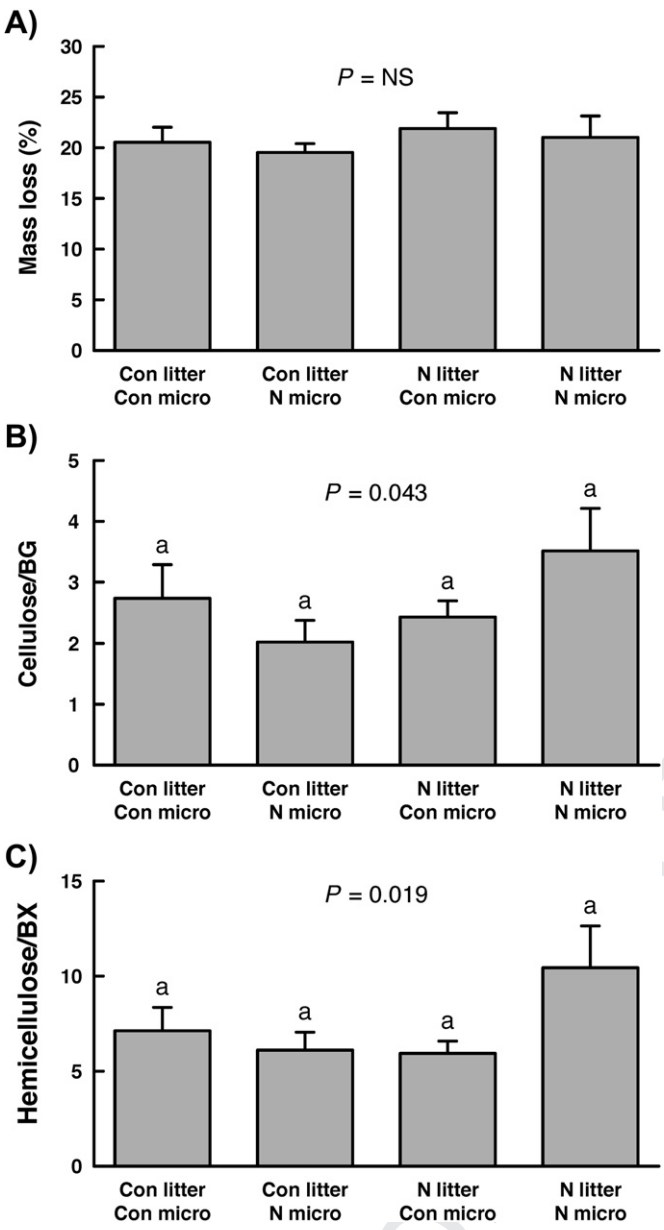

Fig. 6. Barplots of the interaction between litter origin and microbial origin for the nitrogen experiment. (A) Mass loss at 6 months; (B) efficiency of cellulose degradation by β-glucosidase; (C) efficiency of hemicellulose degradation by β-xylosidase. Efficiency units are mg mass loss × h μmol^{-1}. Bars represent means with SEM ($n = 16$). *P*-values are for the overall interaction, and means sharing the same letter are not significantly different (Tukey post-hoc contrasts).

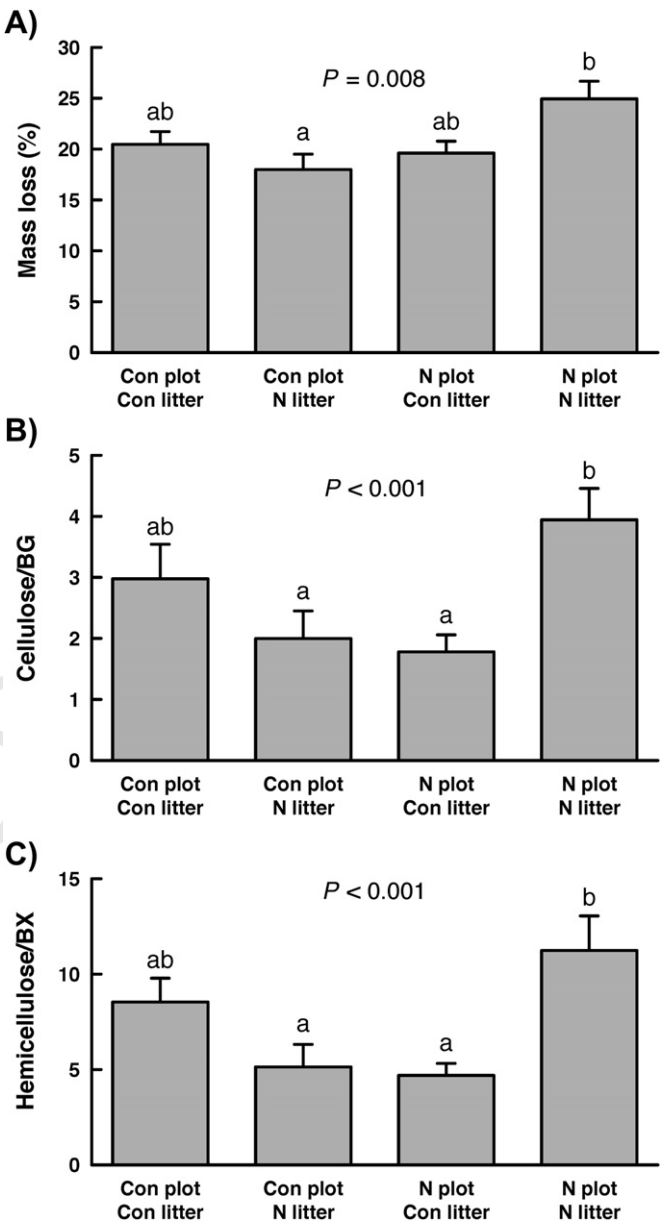

Fig. 7. Barplots of the interaction between plot treatment and litter origin for the nitrogen experiment. (A) Mass loss at 6 months; (B) efficiency of cellulose degradation by β-glucosidase; (C) efficiency of hemicellulose degradation by β-xylosidase. Efficiency units are mg mass loss × h μmol^{-1}. Bars represent means with SEM ($n = 16$). *P*-values are for the overall interaction, and means sharing the same letter are not significantly different (Tukey post-hoc contrasts).

compensate for lower temperatures that reduce enzyme efficiency. Most litter mass loss occurred between March and June, despite lower potential EEA during these months in comparison to November. However, there may not have been time for the enzymes present in November to affect mass loss, since November was preceded by the dry season during which little mass loss occurred.

4.4. Enzyme responses: drought experiment

Enzyme potentials increased in the drought plots despite a decrease in decomposition, which is inconsistent with our initial hypothesis that EEA would decline at lower litter moisture levels. Enzyme potential activity is a metric of enzyme pool size, which is positively related to enzyme production and negatively related to enzyme degradation (Geisseler et al., 2011). Higher protein concentrations in the drought plot treatment suggest that enzyme proteins were accumulating in the litter (Fig. 2A). Enzyme production might have increased if microbes living in dry litter needed to produce more enzymes to acquire sufficient resources. Alternatively, fungi may have contributed to increased enzyme production since fungal biomass increased in the drought plots.

Reduced enzyme turnover may have also increased enzyme pool sizes. In dry litter, turnover might decline if enzymes are protected through adsorption onto surfaces in the litter matrix (Burns, 1982). Furthermore, thinner water films could increase contact between enzymes and insoluble organic matter, leading to enzyme immobilization and protection from degradation (Nannipieri et al., 2002;

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Geisseler et al., 2011). Reduced proteolytic activity may have further reduced enzyme turnover. LAP catalyzes the hydrolysis of oligopeptides, and its potential activity declined in drought plots compared to control plots (Fig. 2A).

Based on measurements of potential EEA and mass loss of litter components, we calculated enzyme efficiencies that quantify the relationship between potential activity and *in-situ* decay rates. Sinsabaugh et al. (2002) quantified enzyme efficiency for several plant materials as a "turnover activity" which is essentially the inverse of our efficiency metric. Turnover activity is the amount of cumulative EEA necessary to achieve a unit of mass loss. With this approach, they established a link between potential EEA and overall mass loss for a range of different plant litter species.

In our experiment, enzyme efficiencies were lower in the drought plots because higher potential EEA did not result in greater mass loss of litter compounds. Likewise, overall mass loss per unit litter protein (protein efficiency) declined with drought (Table 3). These declines most likely reflect reduced interactions between enzyme proteins and their substrates when water is scarce. Water limitation could restrict enzyme and substrate diffusion, and enzyme immobilization may have caused lower rates of catalysis per enzyme, leading to reduced enzyme efficiency (Nannipieri et al., 2002).

Whereas potential EEA in the drought plots generally increased, EEA decreased in litter from the drought treatment. The litter origin effect most likely resulted from changes in litter chemistry in response to drought. Litter from drought plots had higher labile carbon concentrations, but also more lignin and less cellulose and hemicellulose (Allison et al., 2013). Similarly, Schimel et al. (1992) found that increased starch concentrations inhibited enzyme activities. Reductions in microbial biomass in drought-derived litter may have also resulted in lower enzyme production (Fig. 4).

PPO activity was lower in litter inoculated with drought-derived microbes, a response that supports our initial hypothesis of parallel responses for mass loss and potential enzyme activity. However, no other enzymes showed the same response to microbial origin in the drought experiment (Fig. 2B). Our results add to a body of conflicting literature on moisture effects on EEA in litter and soil (Criquet et al., 2002; Sardans and Penuelas, 2010; Bell and Henry, 2011; Geisseler et al., 2011).

4.5. Enzyme responses: nitrogen experiment

Potential enzyme activity increased in litter decaying in N fertilized plots and in litter derived from the N treatment, despite little change in mass loss. Since we saw no major shifts in enzyme efficiency (Table 3), it appears that individual litter compounds do change with the enzymes, but not enough to significantly affect overall mass loss. These results are consistent with Keeler et al. (2009) who found marginally significant decreases in decomposition rates with added N, despite increases in EEA.

In the N experiment, mass loss tended to increase in home environments whereas enzyme potentials did not. Thus enzyme efficiencies increased, such that the same amount of enzyme caused more mass loss in home environments. Protein-based efficiencies were also higher for litter and microbes transplanted into home plots. Our efficiency data support the home-field advantage hypothesis for BG and BX (Figs. 6 and 7), and also for PPO when examining the plot × microbe interaction (Fig. 3). Several other studies have reported that litter decomposes faster in its home environment (Gholz et al., 2000; Ayres et al., 2009), and our data show that changes in enzyme efficiency could drive this pattern.

Increased enzyme efficiency in home environments could be due to several mechanisms. One possibility is that the microbial community produced enzymes with enhanced substrate binding affinity (lower K_m values) through changes in the active site (Stone et al., 2012). Another possibility is that the enzymes may have been secreted closer to their substrates, therefore increasing decay efficiency. Localized changes in pH from N addition are also possible such that the pH at the enzyme active site was closer to the pH optimum for enzyme activity, thus increasing enzymatic efficiency in the home environment (Nye, 1981).

5. Conclusion

Enzymatic responses to human-induced climate change and N enrichment could influence ecosystem function and nutrient dynamics. However, we found that litter decomposition responses to environmental change were not consistently linked to changes in potential EEA. Rather, environmental factors such as moisture limitation may have obscured the relationship between potential activity and substrate degradation in the field. Processes such as enzyme immobilization and restricted diffusion probably induced greater microbial enzyme production and/or reduced enzyme turnover while simultaneously reducing litter decomposition rates. In the N fertilization experiment, we found evidence for home field advantage mainly when examining enzyme efficiencies rather than potential EEA. Enzyme efficiencies quantify the relationship between enzyme potentials and the *in-situ* decomposition of chemical substrates. Therefore, if enzyme efficiencies were measured under a range of environmental conditions, they could ultimately be used to convert enzyme potentials to *in-situ* activities.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2013.03.034.

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