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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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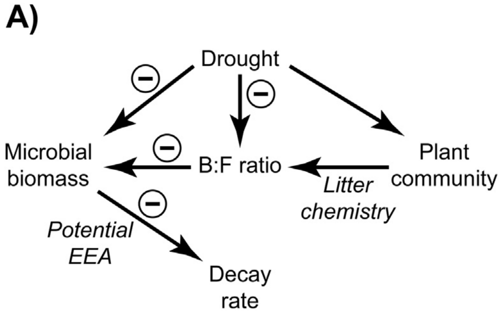
Glycosidase activities often increase with N fertilization (Bandick and Dick, 1999; Saiya-Cork et al., 2002; Waldrop et al., 2004; Grandy et al., 2008). However, the N response of C-acquiring enzymes may depend on the chemical composition of plant litter (Fog, 1988; Carreiro et al., 2000). Nitrogen-acquiring 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.



B)

	N Environ	Control Environ
N Origin	***+ Decay*** + Pot. EEA	– Decay – Pot. EEA
Control Origin	– Decay – Pot. EEA	+ Decay + Pot. EEA

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).