UNIVERSITY OF CALIFORNIA,
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Climate drivers of microbial decomposition in southern California

DISSERTATION

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for the degree of

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in Ecology and Evolutionary Biology

by

Nameer R. Baker

Dissertation Committee:
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DEDICATION

To

my loving mother and father

the best parents and friends a child could ask for

And to

Sarah

without a doubt

the best thing to happen to me

in my time here

Without education, you are not going anywhere in this world.

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Microbial responses to climate change

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ABSTRACT OF THE DISSERTATION

Climate drivers of microbial decomposition in southern California

By

Nameer R. Baker

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2016

Associate Professor Steven D. Allison, Chair

The overall aim of my dissertation was to determine how microbial decomposers may respond to future climate change in the American Southwest. In my first chapter, I investigated how attenuation of ambient ultraviolet radiation (UV) affected microbial litter decomposition during a one-year field study. Using flow cytometry to quantify bacteria, microscopy to quantify fungi, and assays to quantify potential extracellular enzyme activity, I determined that attenuation of ambient UV reduced decomposition rates and the effectiveness of extracellular enzymes produced by microbial decomposers in plant litter.

In my second chapter, I observed how microbial decomposer communities varied with climate and litter chemistry across a regional climate gradient that extends from cold, wet conditions to hot, dry conditions. Changes along this gradient emulate the potential impacts of future climate change in the American Southwest. I determined that differences in microbial activity were not driven directly by precipitation, but rather by differences in protein degradation rates, enzyme turnover, and the availability of carbon substrates.

In my third chapter, I transplanted grassland microbial communities into five sites along the same regional climate gradient to simulate climate change effects on microbial properties and decomposition rates in the field. The transplant design allowed me to determine if grassland microbial communities were constrained in their ability to respond to climate forcing. I found that temperature and precipitation interact to limit decomposition rates in the coldest and driest sites. I also found that grassland microbial communities were not constrained in their ability to degrade litter in any site along the gradient.

Together, the results of my dissertation research indicate that transitions from forests to both grasslands and scrublands in southern California marks a significant shift in litter chemistry and enzyme dynamics, while the transitions from grasslands to drier scrublands and deserts and from cold subalpine sites to milder montane forests may be the most significant for rates of C-cycling. In a more arid future, shifts in climate and resulting shifts in plant communities in these transition zones are therefore likely have the most significant effects on future decomposer activity and C-cycling rates.

INTRODUCTION

Human impacts on the biosphere have become a significant driver of the interacting processes that make up the Earth System (Crutzen, 2002). As a result of these rapid changes across the globe, communities of organisms are likely to experience novel climates and environments in the future (IPCC, 2014). Improving our ability to accurately predict future climates and environments has therefore become an overarching goal of much ecological research. Microbial communities mediate crucial pathways in the biogeochemical cycles that drive the Earth System, making their response to climate change a vital question in need of answering if we are to accurately forecast future climate scenarios (Singh et al., 2010). Resolving the consequences of interactions between climate change and microbe-mediated process rates will be especially important in the American Southwest, where future climate is consistently predicted become more arid (Garfin et al., 2014; Seager et al., 2007). This is of note because decomposition dynamics in drier ecosystems are difficult to predict using established decomposition models that have primarily been developed through the study of more mesic ecosystems (Meentemeyer, 1978; Throop and Archer, 2009; Whitford et al., 1981).

Soil and litter microbial communities mediate the flux of carbon dioxide (CO_2) from terrestrial ecosystems back into the atmosphere. Microbial decomposers break down dead plant tissues (litter) and soil organic matter into more labile carbon (C) compounds that are eventually respired as CO_2 (Anderson, 2011). During litter decomposition, microbes secrete extracellular enzymes that attack specific substrates, breaking them down into accessible forms of carbon, nitrogen, and phosphorus that can then be absorbed and assimilated into microbial biomass (Allison et al., 2007). The kinetics of these enzymatic

reactions should lead to increased reaction rates as temperature increases, causing a positive feedback to the global C cycle (Davidson and Janssens, 2006). However, the exact response of different enzyme classes to temperature change is not well understood. This is in part because the two parameters that determine the rate of an enzymatic reaction, V_{max} and K_m, can have opposing effects on the temperature response of a given enzyme-catalyzed reaction. V_{max} describes the maximum rate of enzyme activity at a given temperature, and usually increases with increasing temperature. K_m is inversely proportional to the enzyme's affinity for its substrate at a given temperature, and may increase as temperature increases (Davidson et al., 2006).

Rising CO_2 concentrations in the atmosphere will contribute to future warming and altered precipitation patterns in regions around the globe, though the exact magnitudes and locations of changes in climate are not certain (IPCC, 2014). These climate changes could have significant effects on such microbial community functions as litter decomposition (Anderson, 2011; Bissett et al., 2013; Raich and Schlesinger, 2002). Microbial decomposer communities are known to be sensitive to changes in temperature and moisture (Allison and Treseder, 2008; Frey et al., 2013; Schimel and Schaeffer, 2012), but are also expected to be exposed to novel litter chemistries in the future as plant communities shift with changing climate (Keiser et al., 2013). Changes in litter inputs could potentially alter the structure or functional capabilities of microbial decomposer communities (Saleska et al. 2002), especially since microbial communities appear adapted to degrade specific litter chemistries (Keiser et al., 2011; Strickland et al., 2009).

For my dissertation, I investigated effect of climate on microbial decomposer communities and decomposition rates in southern California. In my first chapter, I

manipulated the amount of ultraviolet radiation that decomposing litter samples experienced over the course of one year to determine how photodegradation affects decomposition as well as decomposer communities. In my second chapter, I observed how microbial communities varied with climate along a regional elevation gradient that extends from cool, wet sites to hot, dry sites in a manner that realistically emulates the predicted future shift to a more arid climate in the American Southwest. In my third chapter, I transplanted microbial communities from the intermediate grassland site to all of the sites along the gradient to compare how climate and microbial community composition drive the response of microbial decomposers to simulated climate change.

CHAPTER 1

Ultraviolet photodegradation facilitates microbial litter decomposition in a Mediterranean climate

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Introduction

Litter decomposition is a key contributor to the global annual flux of ~68 Pg carbon (C) that enters the atmosphere from heterotrophic respiration (Raich and Schlesinger, 2002). Much early work on litter decomposition was performed in mesic ecosystems, where temperature, moisture, and litter chemistry are primary drivers of decomposition rates (Meentemeyer, 1978; Parton et al., 1987). However, models built around these three drivers consistently underestimate rates of decomposition in more xeric "dryland" ecosystems, such as semi-arid Mediterranean grasslands and arid deserts (Whitford et al., 1981). Multiple hypotheses have been proposed for the unexplained mechanisms contributing to this discrepancy: foraging by subterranean micro-arthropods (Johnson and Whitford, 1975), persistence of microbe-sustaining microclimates as a result of high overnight humidity (Dirks et al., 2010; Nagy and Macauley, 1982; Whitford et al., 1981), and photodegradation by solar radiation (Moorhead and Reynolds, 1989; Pauli, 1964). Photodegradation in terrestrial ecosystems as a result of ultraviolet radiation (UV), in particular, has become the focus of a growing body of literature in the last decade (reviewed in King et al. 2012 and Song et al. 2013).

Photodegradation is thought to take on added importance in dryland ecosystems through a variety of mechanisms. First, litter in dryland ecosystems is subject to a greater intensity of solar radiation because there are fewer days of cloud cover and lower levels of shade than in more productive ecosystems (Pauli, 1964). Second, in grassland ecosystems, litter is formed through the senescence of standing grass. This standing litter may be subject to photodegradation before it comes in contact with the soil microbial community (Austin and Vivanco, 2006). Third, the presumed inhibition of microbial activity by dry climates should reduce the importance of microbial decomposition and increase the importance of abiotic drivers such as photodegradation (Gallo et al., 2009). On the other hand, the elevated intensity of photodegradation in more xeric ecosystems may lead to microbial communities that are adapted to the effects of UV (Caldwell et al., 2007). If photodegradation can facilitate microbial decomposition through its effects on litter chemistry, it could enhance decomposition of litter by microbial communities that are adapted to dryland climates (Gallo et al. 2006, Henry et al. 2008, Foereid et al. 2010).

Though solar radiation in general (Gallo et al., 2009; Henry et al., 2008) and UV in particular have been found to increase rates of litter mass loss in previous studies (Austin and Vivanco, 2006; Brandt et al., 2010; Day et al., 2007; Lin and King, 2014), the exact mechanism has yet to be established. It is thought that lignin-like compounds in litter should be the most susceptible to photodegradation, due to the presence of aromatic rings that can absorb UV wavelengths. There has been some evidence for this mechanism in the lab (Austin and Ballaré, 2010; Brandt et al., 2009; Lee et al., 2012) and in the field (Day et al., 2007; Gallo et al., 2009; Gehrke et al., 1995; Rozema et al., 1997), but recent field studies

have shown mixed (Brandt et al., 2010, 2007) or nonexistent (Lin and King, 2014) effects of UV on the lignin fraction in litter.

UV is thought to affect litter mass loss through two primary pathways that lead to depolymerization – direct photolysis and indirect photolysis. During direct photolysis, a photosensitive organic molecule such as lignin absorbs photons and is fragmented or rearranged by the infusion of energy, potentially resulting in a less chemically complex compound that is easier to degrade or leach out of the system (King et al., 2012). Indirect photolysis is similar, except that after absorbance of photons by photosensitive compounds, the resulting energy is transferred to "reactive intermediates" such as O, OH-, H_2O_2 or reduced metals, which can then alter organic compounds such as cellulose (reviewed in Lanzalunga and Bietti 2000). Both direct and indirect photolysis could affect litter mass loss by making organic compounds in litter more bio-available for microbial decomposers (King et al 2012).

In dryland ecosystems in particular, extended dry periods should result in the build-up of microbially-available substrates in litter (Hon and Feist, 1981), potentially facilitating wet season decomposition (Henry et al. 2008). Foereid et al. (2010) found evidence for facilitation in a lab study, but field studies have yet to determine how UV affects microbial properties in litter. UV could have detrimental effects on microbial communities, as it is known to damage microbial DNA (Rohwer and Azam, 2000) and suppress growth of terrestrial microbes (Hughes et al., 2003). On the other hand, UV facilitation of microbial communities could be especially important in semi-arid Mediterranean ecosystems with marked seasonality. In the dry summer months, UV might alter litter chemistry and stimulate mass loss while inhibiting microbial activity. These changes in litter chemistry

could then facilitate microbial decomposition during the wet winter months with lower UV radiation. Microbial communities in dryland ecosystems might also be adapted to UV radiation, and there is some evidence that UV exposure alters microbial community composition (Caldwell et al., 2007). Long-term exposure could select for microbes that are more capable of withstanding UV radiation or better able to use photodegraded litter compounds.

We tested three hypotheses in a litterbag experiment whereby UV exposure and litter chemistry were both manipulated at two levels. First, we hypothesized that UV photodegradation would enhance litter mass loss in a Mediterranean ecosystem, potentially as a result of direct or indirect photolysis of organic compounds in litter (King et al., 2012). Second, we hypothesized that UV would preferentially degrade the lignin fraction in litter, as its aromatic structure is known to absorb UV wavelengths (Austin and Ballaré, 2010) and is thought to undergo chemical changes when exposed to solar radiation (Lanzalunga and Bietti, 2000). Finally, we hypothesized that the net result of UV is inhibition of microbial activity, given previous observations that UV can damage microbial DNA (Rohwer and Azam, 2000), slow the growth of microbial communities (Hughes et al., 2003), and result in altered microbial community composition (Caldwell et al., 2007).

Materials and Methods

Site description and field manipulation

To test our hypotheses, we used a litterbag study with a split-plot design. Twelve 1 m^2 plots were paired into six split-plots at the UCI Arboretum in Irvine, CA, USA (33°39′ N, 117°51′ W). The Arboretum is situated 30 m above sea level and has a mean annual

temperature of 17°C and mean annual precipitation of 30 cm. Local vegetation consists of coastal sage scrub.

Each set of paired plots consisted of one ambient plot (hereafter referred to as the "UV-pass" treatment) and one plot covered with polyester UV-blocking film supported by a PVC frame (hereafter referred to as the "UV-block" treatment). This film blocked 68% of all UV while allowing 90% transmittance of visible light, as measured by a UV photometer on-site. PVC frames were 1 m on each side and set up 40 cm above the soil surface, with strips of UV-blocking film 20 cm wide used to cover the plot area under the frame. Gaps 1 cm wide between strips of film allowed precipitation to infiltrate to the plot area, and the distance between the frame and soil surface was chosen to limit the potentially strong greenhouse effects of film coverage found by Uselman et al. (2011). Ambient plots had no PVC frame or film covering.

Within each paired plot, two types of litterbags were deployed – four containing litter of *Avena* species (*A. barbata* and *A. fatua)* with 7.38% (± 0.05%) lignin by mass, and four containing litter of *Elymus condensatus* (Giant wild rye), a grass species with 13.05% (± 0.08%) lignin by mass. *Avena* litter contained 4.79% (± 0.10%) crude protein and 4.08% (± 0.03%) ethanol soluble carbohydrates, while *Elymus* litter contained 3.81% (± 0.09) and 2.44 % (± 0.19), respectively. Both litter types were more similar in cellulose and hemicellulose content than they were in lignin content (**Table 1.2**). Hereafter, *Avena* litter is referred to as "low lignin" and *Elymus* litter is referred to as "high lignin." Both litter types were collected in late June of 2012 as standing, senesced litter from Loma Ridge (33°44′ N, 117°42′ W, 365 m elevation), a Mediterranean grassland managed by the Irvine Ranch Conservancy 16 km northeast of the field site in Irvine. Litter of each type was

collected by clipping standing litter at least 20 cm above the soil surface to minimize prior soil contact, then homogenized by clipping to <5 cm lengths and mixing. A sub-sample was weighed and oven-dried to determine moisture content. The equivalent of 1.9 g dry weight of litter (including ash content) was then added to litterbags for each litter type and deployed in the field on July 18, 2012. Each litterbag was made of two types of mesh: a 1.5 mm aluminum mesh used for the side exposed to the sun, and a 0.5 mm nylon bridal mesh used for the side exposed to the soil surface.

Four litterbags of both litter types were deployed into each of the six paired plots, resulting in 4 x 2 x 6 x 2 = 96 total litterbags. One litterbag of each litter type was then collected randomly from each of the paired plots at the end of the first dry season (October 2, 2012), the middle of the wet season (January 18, 2013), the end of the wet season (June 4, 2013), and the end of the second dry season (September 17, 2013), for a total of five time points (including the initial deployment) over a period of 15 months.

Collected litter was weighed to determine mass loss before being ground into fragments <0.5 cm in length and sub-sampled for extracellular enzyme assays, a bacterial cell count assay, and a fungal hyphae staining assay. The remainder of the litter was weighed and oven-dried to determine moisture content.

Extracellular enzyme assays

Litter was assayed for potential activity of eight enzyme classes using fluorescently labeled substrates (for hydrolytic enzymes) or colorimetric assays (for oxidative enzymes) according to methods detailed in German et al. (2012). The enzyme classes assayed consisted of hydrolytic cellulose and starch degradation (β-glucosidase, cellobiohydrolase,

and α-glucosidase; or BG, CBH, and AG), hydrolytic hemicellulose degradation (β-xylosidase; or BX), hydrolytic chitin-degradation (N-acetylglucosaminidase; or NAG), peptide degradation (leucine-aminopeptidase; or LAP), and oxidative degradation (peroxidase and phenol oxidase; or PER and PPO). Negative potential activities were converted to zero values for statistical analyses.

Bacterial cell density

Methods for estimating bacterial cell density were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in a phosphate-buffered, 1% glutaraldehyde solution on the day of sample collection to "fix" bacterial cells for storage. Within two weeks, 0.1 M tetrasodium pyrophosphate was added to each sample, and samples were sonicated to dislodge bacterial cells. Filtered extracts of sonicated litter were stained with 1x SYBR-Green and then analyzed with an Accuri flow cytometer to determine cell counts from fluorescing bacterial cells.

Fungal hyphal length

Methods for measuring fungal hyphal length were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in 0.395% (w/V) sodium hexametaphosphate and vigorously stirred before being vacuum-filtered and stained with acid fuchsin. Two filters were made for each litter sample and affixed to a glass slide. Hyphae were counted with a Nikon Eclipse E400 microscope at 100X magnification using the grid-intercept method (Giovanetti and Mosse, 1980; Newman, 1966) and 50 grids per

filter. Hyphal counts were converted to estimates of hyphal length in meters per gram of dry litter using a modified procedure of Sylvia (1992).

Litter chemistry

Oven-dried litter was sent to Cumberland Valley Analytical Services for near-IR spectroscopy, whereby reflectance of near-infrared wavelengths of light from each sample are matched to a verified database of spectra for plant materials with known chemical composition as determined by wet chemistry (Shepherd et al., 2005). Relative amounts of the following organic compounds were determined as proportions of total dried litter mass: lignin, cellulose (acid detergent fiber – lignin), hemicellulose (neutral detergent fiber – acid detergent fiber), ash, and non-ash dry mass (1 – ash fraction). The proportion of total litter mass attributable to different C compounds will be referred to as *concentration* in the text. The concentration of non-ash dry mass was multiplied by the recovered dry mass at each time point to determine the mass loss from the organic portion of litter. The same calculation was used to determine mass loss for each carbon compound (lignin, cellulose, and hemicellulose). The total mass or mass lost from each carbon compound will be referred to as *content* in the text.

Statistical methods

Effects of UV treatment, litter type, and sampling date on non-ash dry mass, litter chemistry, litter moisture, and bacterial cell counts were analyzed using mixed-model ANOVA with the identity of each pair of split-plots as a random factor. We originally compared this simple model with a more complex model whereby plot identity was a

random factor within which UV, litter type, and time were nested, but AIC comparison showed no significant differences between the two models. The simpler model had a lower AICc (51.7 vs. 60.3), AIC (42.6 vs. 47.5), BIC (85.0 vs. 97.0), and log-likelihood (-3.3 vs. -2.7), allowing us to employ it with a high degree of confidence. Tukey contrasts were used to determine the effect of UV within litter types at each time point.

Because litter moisture content was found to be significantly affected by UV treatment (**Figure A1**, **Table A1**) and is known to be a strong control on decomposition processes in Mediterranean ecosystems, the model was run for all variables as an ANCOVA with litter moisture content as the covariate. Data were checked for normality using the Shapiro-Wilk test, and non-normal data were log-transformed or square root-transformed to improve normality when possible. Litter non-ash dry mass, lignin content, bacterial cell abundance, and observed potential β-glucosidase activity met assumptions of normality after being log transformed. Potential activities for β-xylosidase, phenol oxidase and N-acetylglucosaminidase met assumptions of normality after being square root transformed. Potential peroxidase activity exhibited improved normality after square-root transformation, although a significant but tolerable deviation from normality was still evident. Litter hemicellulose content and potential activities of cellobiohydrolase and leucine-aminopeptidase were still not normally distributed after transformation, but visual inspection of residuals suggested that deviations from normality were tolerable for untransformed data. Litter cellulose content, hyphal lengths, and potential α-glucosidase activity met assumptions of normality without data transformation.

To analyze enzymatic controls over decomposition, we tested for correlations between potential activities of different enzyme classes and the concentrations of their

target carbon compounds at each time point. We also tested for correlations between potential enzyme activities at one time point and the rate of change in the content attributable to their target carbon compounds by the next time point. If there was a significant correlation, then we used linear regressions to determine how much variation in the rate of change in the content of each carbon compound could be attributed to potential extracellular enzyme activity. These regressions assume that enzyme activity causes the change in mass of target substrate. All statistical analyses were conducted in the R software environment version 3.0.2 (R Development Core Team, 2016).

Results

Litter mass loss and moisture content

Litter mass loss was affected by both litter type ($p<0.001$, $F_{1,72}=112.1$) and by UV treatment ($p<0.001$, $F_{1,72}=60.9$) (**Table 1.1**, **Figure 1.1**). Low lignin litter lost the most mass across both UV treatments, with an average of 23.2% mass loss by June 2013. High lignin litter lost an average of 11.0% of original mass across both UV treatments over the same time period. There was no significant interaction between litter type and UV treatment. High lignin litter lost 16.2% of original mass by June 2013 under UV-pass, but exhibited negligible (<1%) mass loss under UV-block. Low lignin samples showed a similar pattern (29.0% mass loss in UV-pass samples vs. 17.4% mass loss in UV-block samples), but post-hoc tests within dates were only marginally significant (**Figure 1.1**). Mass loss was not significantly affected by UV treatment after the first dry season (July 2012 to September 2012), for either high lignin or low lignin samples, with differences in litter mass only appearing during or after the wet season (January 2013 and later time points).

With the exception of the high lignin, UV-block treatment, all litter samples exhibited mass loss at each time point over the course of the experiment until the final September 2013 time point. September 2013 samples had higher concentration of ash, indicating that soil deposition over the course of the second dry season obscured mass loss from litter and likely introduced organic compounds into litterbags, with the net result being increased similarity between all treatments at the final time point.

Litter moisture content ranged from 7 to 14% of litter mass (**Figure A1**). UV-block significantly reduced litter moisture content by 0.47 percentage points ($p=0.04$, $F_{1,73}=4.4$), and moisture content was significantly lower by 0.67 percentage points in low lignin litter compared to high lignin litter ($p<0.001$, $F_{1,73}=13.1$; **Table A1**, **Figure A1**). Litter moisture also varied significantly over time with the lowest values in January 2013 ($p<0.001$, $F_{3,73}=94.3$).

Carbon fractions

High lignin litter began the study with 13.05 ± 0.08% lignin by mass and low lignin litter began the study with 7.38 ± 0.06%. Lignin content was significantly affected by litter type ($p<0.001$, $F_{1,72}=371.9$), but UV treatment had only a marginally significant effect ($p=0.090$, $F_{1,72}=2.96$). The significant increase in lignin content over time (**Tables 1.1**, **1.2**) is likely due to the deposition over time of particulate matter containing organic compounds, either microbial by-products or plant detritus, that have a lignin-like near-IR signal. Litter cellulose content was significantly affected by litter type ($p<0.001$, $F_{1,72}=137.9$) and UV treatment ($p<0.001$, $F_{1,72}=74.5$). There was a significant interaction between UV treatment and litter type on cellulose content because UV had a stronger effect on cellulose content in

high lignin litter than in low lignin litter (Tukey p=0.017 for UV effect in low lignin litter, p<0.001 in high lignin litter, **Figure A2**). Litter hemicellulose content was not significantly affected by litter type, but was significantly affected by UV treatment (p<0.001, $F_{1,72}$=25.5) (**Table 1.1**). Both litter types had reduced cellulose and hemicellulose content under UV-pass compared to UV-block (Table 1.2).

Bacterial cell counts

Neither litter type nor UV treatment had a significant effect on bacterial cell counts (**Table 1.1**, **Figure 1.2A**). Bacterial abundance across and within all treatments was significantly higher on June 4, 2013 when compared to all other time points (Tukey p<0.001).

Fungal hyphal length

In contrast to bacteria, fungal hyphal length was significantly affected by both UV treatment (p=0.024, $F_{1,72}$=5.3) and litter type (p=0.004, $F_{1,72}$=8.7) (**Table 1.1**, **Figure 1.2B**). The UV-pass treatment did not, in general, have a negative effect on fungal hyphal length across litter types when compared to UV-block, and UV treatment had no discernible effect on fungal hyphal length in high lignin litter. We did find a significant three-way interaction between UV treatment, litter type, and sampling date (p=0.017, $F_{3,72}$=3.6), likely because fungal hyphal length in UV-pass samples was greater in our low lignin samples during the wet season compared to UV-block samples (25.0 ±1.7 m/g hyphae in low lignin litter under UV-pass vs. 15.4 ± 1.8 m/g under UV-block in January 2013; Tukey p<0.001). By contrast, UV-block samples did not attain peak fungal abundance until the end of the wet season.

Potential extracellular enzyme activities

Potential extracellular enzyme activities varied with time, litter type, and occasionally by UV treatment (**Table 1.1**). In general, potential enzyme activities were lower in high lignin litter compared to low lignin litter, and lower during the dry season compared to the wet season (**Figure 1.3**). The main effect of UV treatment was only significant for potential leucine-aminopeptidase activity ($p=0.017$, $F_{1,72}=6.0$). There were significant interactions with UV for the four enzymes depicted in **Figure 1.3**, in addition to peroxidase (trends similar to phenol oxidase) and N-acetylglucosaminidase (trends similar to leucine-aminopeptidase). Leucine-aminopeptidase exhibited significantly higher (Tukey $p<0.001$) potential activity under UV-pass compared to UV-block across all time points in high lignin litter only (**Figure 1.3D**). UV-block had marginally significant negative effects on potential activity of α-glucosidase ($p=0.070$, $F_{1,72}=3.4$) and N-acetylglucosaminidase ($p=0.085$, $F_{1,72}=3.0$).

Potential activities of β-glucosidase and cellobiohydrolase were significantly positively correlated with percent mass of cellulose in low lignin litter, but only under UV-pass. Potential activities of peroxidase and phenol oxidase were significantly positively correlated with percent mass of lignin in both litter types under UV-pass, but, with the exception of phenol oxidase in low lignin litter, not under UV-block (**Table 1.3**).

Potential activities of three enzymes were significantly positively correlated with the rate of change in the content of their target carbon compounds, but only in litter under

UV-pass. β-xylosidase was positively correlated with the rate of change in hemicellulose content in low lignin litter, and phenol oxidase was positively correlated with the rate of change in lignin content in high lignin litter. Peroxidase activity was positively correlated with the change in lignin content in both litter types (**Table 1.4**).

Discussion

Our first hypothesis was that UV-block would reduce mass loss in both high and low lignin litter. Our results supported this hypothesis: reducing UV transmittance by 68% in the UV-block treatment significantly reduced mass loss in high lignin litter, and reduced mass loss to a marginally significant extent in low lignin litter (Fig. 1). This effect was significant even after accounting for a slight but significant negative effect of UV-block on litter moisture. Several previous studies have shown that attenuating solar radiation through shading can reduce litter mass loss in arid (Gallo et al., 2009) and semiarid (Henry et al., 2008) ecosystems. A number of studies have also found, as we did, that reducing UV can reduce litter mass loss rates in semiarid ecosystems. Austin and Vivanco (2006), Day et al. (2007), Brandt et al. (2007, 2010), and Lin and King (2014) all found that blocking UV reduced litter mass loss in the field anywhere from 3% over 5 months (Day et al., 2007) to 33% over 18 months (Austin and Vivanco, 2006). Taken together, these results confirm that UV can increase litter mass loss in dryland ecosystems.

Contrary to our second and third hypotheses, our study indicates that UV photodegradation does not result in enhanced mass loss from the lignin fraction, nor does it inhibit microbial decomposition. Instead, UV photodegradation appears to facilitate microbial decomposition by increasing the efficiency of extracellular enzymes produced by

microbial communities. Lignin mass loss in our litter was not affected by UV treatment, with UV blocking instead reducing the loss of litter cellulose and hemicellulose (**Tables 1.1**, **1.2**). In addition, the net effect of UV-pass on litter microbial communities does not appear to be inhibitory - we found no effect of UV treatment on bacterial abundance (**Fig. 2A**), a potentially positive effect of UV-pass on fungal abundance (**Fig. 2B**), and no consistent effect of UV treatment on potential extracellular enzyme activity. Instead, we found that litter-degrading extracellular enzymes may be more effective under UV-pass. We found correlations between potential enzyme activity and both substrate availability and substrate degradation in litter under UV-pass, and no such correlations under UV-block. Our results indicate that the functioning of Mediterranean grassland microbial communities may be dependent on ambient UV.

Though most studies of photodegradation have hypothesized that UV acts directly upon the lignin fraction in litter, it should be noted that these studies have not established a direct link between UV exposure and lignin degradation. Although lab studies suggest that that lignin-like model compounds are photo-chemically active and absorb light in the ultraviolet range (Austin and Vivanco, 2006; Lanzalunga and Bietti, 2000), it is unclear to what extent photodegradation affects the physical and chemical structure of lignin. Kirschbaum et al. (2011) exposed grass litter and pine needles to UV equivalent to midday levels continuously for 60 days and found no direct effect of UV on either litter mass loss or concentration of lignin. Over the course of 10 weeks in the laboratory, Brandt et al. (2009) tested the effects of UV exposure on five different litter types with initial lignin concentrations varying from 6.2 to 24.6%. After standardizing for exposed surface area,

they found that lignin concentration had no effect on CO_2 efflux from litter, though mass loss was significantly greater in litter exposed to UV than in controls.

Whereas evidence for a direct effect of UV on the lignin fraction has been elusive in the lab, some field studies indicate that UV may influence the lignin content of litter. Separate studies by Gehrke et al. (1995) and Rozema et al. (1997) found that artificially enhancing levels of UVB radiation in the field caused the lignin content in litter to accumulate more slowly over time. In a California annual grassland, Henry et al. (2008) found that the rate of decrease in the concentration of lignin in grass litter over the course of a summer was roughly twice as rapid as rates of total mass loss. This indicates that mass loss during the dry season was preferentially occurring through the lignin fraction, ostensibly as a result of photodegradation. Day et al. (2007) found a similar result when exposing *Larrea tridentata* litter with very high lignin concentrations to 85% ambient and 15% ambient UVB, with greater attenuation of UVB corresponding to higher lignin content in samples at the end of the study. While it is possible that UV degraded lignin directly in these studies, it is also possible that increased microbial activity under near-ambient radiation confounded their results, given that microbial by-products can be classified as lignin-like compounds when analyzing litter chemistry (Berg and Laskowski, 2005; Berg and McClaugherty, 1987). Rather than direct photolysis, reactive intermediates resulting from indirect photolysis of litter compounds also may have contributed to lignin degradation observed in these studies (King et al. 2012).

In contrast to these field studies, we did not find that litter lignin was significantly affected by UV treatment. Instead, our results showed a significant, strong effect of UV treatment on litter cellulose and hemicellulose content. Notably, we found that this effect

was greater in high lignin litter. Cellulose could be cleaved through photo-excitation of the α-glycosidic bond linking cellulose chains to one another, producing simpler cellulose chains and releasing CO (Schade et al., 1999). Such direct photolysis of cellulose has not been extensively studied in the lab, but our results and others indicate that direct and indirect photolysis of non-lignin compounds such as cellulose and hemicellulose could be a significant mechanism through which UV affects litter decomposition. Gehrke et al. (1995), Rozema et al. (1997) and Day et al. (2007) found a significant reduction in cellulose concentration or combined cellulose and hemicellulose concentration in litter exposed to higher levels of UV. Brandt et al. (2007, 2010) also found no effect of UV treatment on the concentration of lignin in litter, but a significant, if small, negative effect of UV on the combined cellulose and hemicellulose concentration in their 2007 study, and a highly significant effect of UV on hemicellulose concentration in their 2010 study. Our results also fall in line with a study by Lin and King (2014), where attenuated UV reduced losses of hemicellulose content by 29% without having a significant effect on lignin content. Likewise, Gallo et al. (2009) found that cottonwood litter mass loss was partially driven by photo-mineralization of cellulose.

In addition to the aforementioned mechanisms of direct and indirect photolysis of cellulose and hemicellulose, it is likely that the effects of UV on mass loss could also result from degradation of the lignocellulose matrix without significantly affecting lignin mass loss. UV breakdown of lignin shielding other C compounds could make previously-occluded cellulose, hemicellulose, and soluble C available to microbial decomposers, facilitating enhanced microbial decomposition of litter (Gallo et al., 2006). Based on NMR analyses of litter that had been photodegraded in the field during their 2014 study, Lin et al. (*in review,*

Biogeochemistry) found that UV significantly reduced hemicellulose content, providing a mechanism for the direct photodegradation of hemicellulose by UV. Additionally, they found that inter-unit ether linkages of lignin polymers were degraded under UV *without* causing lignin mass loss, suggesting a mechanism whereby UV could weaken the lignocellulose matrix. Our results are also consistent with this potential mechanism, as cellulose mass loss was more affected by UV treatment in high lignin litter than it was in low lignin litter.

In contrast to our original third hypothesis, we found little evidence for inhibition of microbial activity by UV. Bacterial and fungal abundances did not increase in litter under UV-block. Instead, UV-block treatment negatively affected fungal abundance during the wet season in our low lignin litter. In addition, potential activities of six of eight extracellular enzymes were not affected by UV treatment, indicating that ambient UV does not generally inhibit extracellular enzyme activity (Fig. 3). These results are somewhat surprising given the known detrimental effects of UV on microbial DNA (Rohwer and Azam, 2000) and microbial community growth (Hughes et al., 2003). Rather, UV might promote microbial decomposition through biochemical interactions. We observed significant correlations between potential enzyme activities and the concentrations of their target carbon fractions, but almost exclusively under UV-pass (**Table 1.3**). We also only found significant correlations between the mass loss of a carbon fraction and its associated enzyme activity at the previous time point under UV-pass, and mainly for oxidative enzymes that target more complex organic compounds (**Table 1.4**). In other words, investment in enzymes targeting the most complex compounds in litter only had a significant effect on the mass change of those compounds when litter was exposed to ambient levels of UV. This result

falls in line with previous findings by Gallo et al. (2009) and Brandt et al. (2010) that the amount of potential enzymatic activity required to degrade a litter cohort is greater when UV is blocked.

There have been several other studies, in addition to our own, that indicate that facilitation of microbial decomposition by photodegradation may occur when microbial communities are allowed to interact with photodegraded litter. Foereid et al. (2010) found that litter exposed to light for 289 days had much higher rates of CO_2 efflux in lab incubations when compared to litter that had only been exposed to radiation treatment for 43 days. Henry et al. (2008) found that wet season decomposition was significantly greater when litter had been exposed to ambient radiation during the preceding summer dry period. Similarly, Lin and King (2014) found that shaded litter exposed to attenuated UV exhibited carbon fraction dynamics similar to shaded litter exposed to ambient UV, but with significantly slower litter mass loss rates, indicating that decomposition of shaded litter in contact with the microbial community may be facilitated by UV photodegradation and the resulting release of soluble C in the surface litter layer. The results of our study suggest a mechanism that could explain UV facilitation of litter decomposition in these studies. Photodegradation of cellulose, hemicellulose, or the lignocellulose matrix, might allow extracellular enzymes to break down their substrates more effectively.

Conclusions

Our study shows that UV photodegradation has a positive effect on both litter decomposition rates and microbial decomposer activity. UV blocking reduces litter mass loss, but does not have a significant direct effect on litter lignin content. Instead, UV

blocking significantly reduces the degradation of cellulose and hemicellulose, potentially by limiting the direct or indirect photolysis of cellulose or the lignocellulose matrix that would otherwise occur under ambient UV. UV blocking does not appear to increase bacterial or fungal abundance, and may in fact be detrimental for microbial decomposition, as extracellular enzymes produced by the microbial decomposer community were more effective at degrading their target substrates under ambient UV. These results indicate that UV photodegradation is an important driver of litter decomposition through its effects on non-lignin compounds and facilitation of microbial activity. These mechanisms of litter decomposition will likely become more important in the American Southwest if this region experiences a more arid climate in the future.

Acknowledgements

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Table 1.1 P-values from ANCOVA for each dependent variable with respect to UV treatment, litter type, time of sampling, and all possible interactions with litter moisture content as a covariate. Significant ($p<0.05$) p-values are in bold. N_{df} and D_{df} are the degrees of freedom for the numerator and denominator of the F-statistic, respectively.

Variable	H_2O	UV	Litter (L)	Time (T)	UV:L	UV:T	L:T	UV:L:T
Non-ash dry mass	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.084	0.370	0.750	0.946
Lignin (g)	**<0.001**	0.090	**<0.001**	**<0.001**	**0.016**	0.064	**<0.001**	0.346
Cellulose (g)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.025**	**0.006**	**0.023**	0.912
Hemicellulose (g)	**0.068**	**<0.001**	0.963	**<0.001**	0.300	**<0.001**	**<0.001**	**0.016**
Bacterial cells	0.522	0.151	0.223	**<0.001**	0.533	0.297	**0.042**	0.508
Fungal hyphae	0.609	**0.024**	**0.004**	**<0.001**	0.115	**<0.001**	**<0.001**	**0.017**
BG activity	0.884	0.444	**<0.001**	**<0.001**	0.132	0.212	**<0.001**	0.217
CBH activity	0.143	0.406	**<0.001**	**<0.001**	0.107	**0.026**	**0.002**	**0.030**
AG activity	**0.036**	0.070	**<0.001**	**<0.001**	0.072	0.340	0.252	0.281
BX activity	**<0.001**	0.230	**<0.001**	**<0.001**	0.194	**<0.001**	**0.008**	0.113
PPO activity	**0.021**	0.822	**<0.001**	**<0.001**	0.918	**<0.001**	0.059	**<0.001**
PER activity	0.935	0.233	**<0.001**	**<0.001**	0.733	0.415	**0.001**	**0.041**
NAG activity	**<0.001**	0.085	**<0.001**	**<0.001**	**0.028**	0.061	**0.042**	0.170
LAP activity	**<0.001**	**0.017**	0.109	**<0.001**	**0.001**	**0.008**	**0.020**	0.343
N_{df}, D_{df}	1, 72	1, 72	1, 72	3, 72	1, 72	3, 72	3, 72	3, 72

Table 1.2 Carbon fraction content across all treatments presented as grams of dry litter mass at the beginning of the study and in June 2013. Bold values indicate when means under UV-block were significantly different ($p<0.05$, Tukey test) from means of the same litter type under UV-pass. Low lignin samples are L-, and high lignin samples are L+. UV block samples are UV-, and UV pass samples are UV+.

	Lignin (g)		Cellulose (g)		Hemicellulose (g)	
Treatment	Initial	June 2013	Initial	June 2013	Initial	June 2013
L–, UV+	0.139±0.001	0.167±0.011	0.738±0.006	0.587±0.025	0.587±0.005	0.284±0.016
L–, UV–	-	0.181±0.015	-	**0.670±0.035**	-	**0.338±0.012**
L+, UV+	0.244±0.001	0.230±0.013	0.827±0.003	0.678±0.019	0.484±0.004	0.267±0.023
L+, UV–	-	0.263±0.023	-	**0.803±0.028**	-	**0.348±0.018**

Table 1.3 Pearson coefficients and p-values for the correlation between C fraction concentration and potential enzyme activity of the enzyme class that degrades that fraction. Bold text indicates significant correlations between enzyme activity and carbon fraction concentration.

	BG(Cellulose)		CBH(Cellulose)		BX(Hemicell.)		PER(Lignin)		PPO(Lignin)	
Treatment	Corr.	p	Corr.	p	Corr.	p	Corr.	p	Corr.	p
L–, UV+	**+0.475**	**0.011**	**+0.423**	**0.025**	+0.243	0.213	**+0.690**	**<0.001**	**+0.551**	**0.002**
L–, UV–	–0.043	0.823	–0.096	0.612	–0.096	0.612	+0.333	0.083	**+0.517**	**0.005**
L+, UV+	–0.017	0.927	–0.313	0.092	+0.198	0.293	**+0.510**	**0.004**	**+0.484**	**0.007**
L+, UV–	+0.018	0.925	–0.224	0.233	+0.092	0.628	+0.146	0.440	+0.236	0.210

Table 1.4 Pearson coefficients and p-values for the correlation between potential enzyme activity and the change in the carbon content attributable to the compound degraded by that enzyme class. R^2 values for the linear regression of change in C content as a function of potential enzyme activity are shown for significant correlations and indicated by bold text.

	ΔHemicellulose(BX at t-1)			ΔLignin(PER at t-1)			ΔLignin(PPO at t-1)		
Treatment	Corr.	R^2	p	Corr.	R^2	p	Corr.	R^2	p
L–, UV+	**–0.452**	**0.163**	**0.040**	**–0.448**	**0.159**	**0.042**	–0.136	-	0.556
L–, UV–	–0.058	-	0.799	–0.058	-	0.799	–0.127	-	0.574
L+, UV+	–0.227	-	0.287	**–0.497**	**0.213**	**0.013**	**–0.616**	**0.351**	**0.001**
L+, UV–	–0.028	-	0.896	-0.167	-	0.437	-0.237	-	0.265

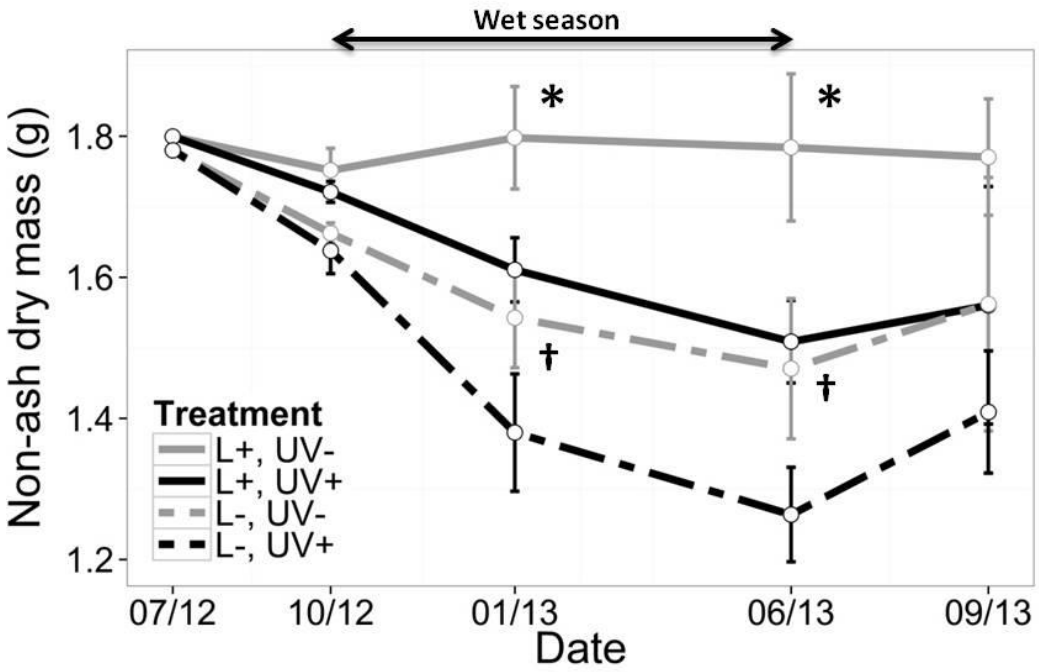

Figure 1.1 Dry mass of the non-ash component of litter, in grams. Significant differences between UV treatments within litter types and sampling dates according to Tukey tests are denoted with asterisks ($p<0.05$) or daggers ($p<0.10$). Low lignin samples (L-) are shown with dotted lines, and high lignin samples (L+) with solid lines. UV pass treatments (UV+) are shown in black, and UV block (UV-) treatments are in grey. Symbols represent means ± SE. The double-headed line above the plot indicates the duration of the wet season .

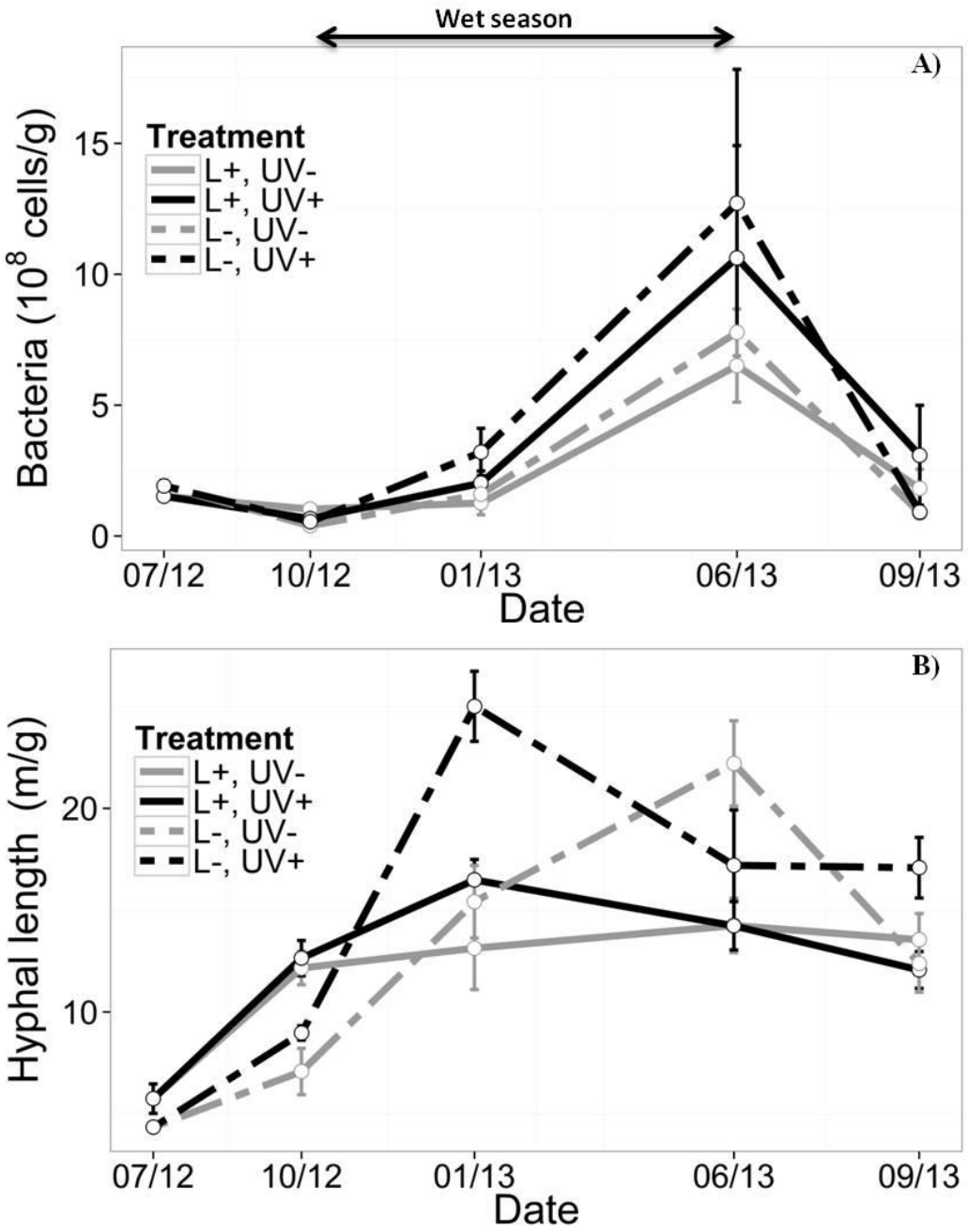

Figure 1.2 Microbial abundance. **A)** Bacterial cell counts measured by flow cytometry, in 10^8 cells per gram of dry litter. Low lignin samples are shown with dotted lines, and high lignin samples with solid lines. UV pass treatments are shown in black, and UV block treatments are in grey. Symbols represent means ± SE. **B)** Length of fungal hyphae in meters per gram dry litter.

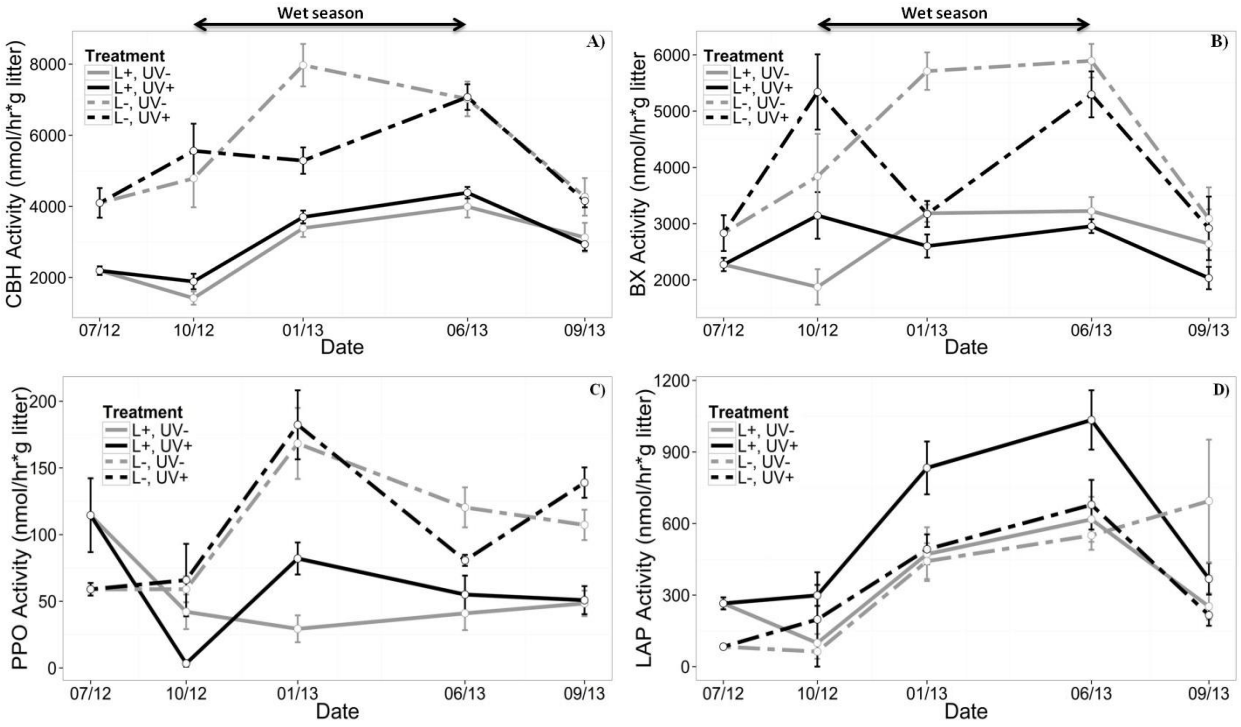

Figure 1.3 Potential extracellular enzyme activities in nanomoles of substrate per hour per gram of dry litter for 4 representative enzymes: **A)** cellobiohydrolase (CBH); **B)** β-xylosidase (BX); **C)** phenol oxidase (PPO); and **D)** leucine-aminopeptidase (LAP).

CHAPTER 2

Extracellular enzyme kinetics and thermodynamics across five biomes in southern California

Introduction

A substantial fraction of ecosystem respiration is generated by microbial decomposers in soils and litter (Raich and Schlesinger, 2002). Many microbes secrete extracellular enzymes (EE) capable of degrading complex biological polymers into bio-available compounds that fuel metabolism and respiration (Burns et al., 2013; Sinsabaugh et al., 1994). Abiotic climate variables such as moisture and temperature affect these microbial processes by altering diffusion, reaction rates, and osmotic potential. In addition to these direct effects, climate indirectly shapes microbial communities by exerting strong control on the composition of plant communities (IPCC, 2014), thereby determining substrate availability for microbial decomposers (Saleska et al., 2002).

EE catalysis of complex organic substrate degradation is the rate-limiting step for returning C from soils to the atmosphere (Sinsabaugh and Shah, 2011), though physical protection of C and diffusion constraints can supersede the importance of enzyme catalysis in mineral soils (Schimel and Schaeffer, 2012). In the last few decades, decomposition dynamics have been related to microbial activity and assays of EE potential in a host of studies (Allison et al. 2007; also see refs in Burns et al., 2013 and Sinsabaugh et al., 2008), but investigations of how EE characteristics vary in xeric ecosystems is lacking. In a 2008 global meta-analysis of EE potential in soils, 10% or fewer of the sites were located in

dryland ecosystems (Sinsabaugh et al., 2008), even though drylands make up ~40% of terrestrial ecosystems by land area (MEA, 2005).

This knowledge gap is significant because decomposition models validated in mesic ecosystems and built around temperature, moisture, and litter chemistry consistently underestimate rates of decomposition in more xeric drylands ecosystems, such as semiarid Mediterranean grasslands and arid deserts (Whitford et al., 1981). As such, conclusions drawn from decomposition dynamics and EE potentials observed in mesic ecosystems may not be applicable to more xeric ecosystems. This uncertainty complicates efforts to predict future carbon dynamics, especially given that xeric ecosystems are projected become hotter and drier, particularly in the American Southwest (Garfin et al., 2014; Seager et al., 2007).

EE kinetics can be described by the Michaelis-Menten model, whereby activity (V) of an individual enzyme is described as a saturating function of substrate (S) concentration as follows:

$$V = V_{max}[S]/(K_m + [S])$$

where V_{max} is the enzyme's maximum reaction rate and K_m, the half-saturation constant, is the substrate concentration at which the reaction rate is one-half V_{max}. In assays of EEs from ecological systems, V_{max} and K_m are measured as apparent parameters, ${}^{app}V_{max}$ and ${}^{app}K_m$. ${}^{app}V_{max}$ does not represent the reaction rate of a single enzyme, but instead indicates the overall concentration of enzymes in a sample that degrade a particular suite of substrates (Wallenstein et al., 2011). ${}^{app}K_m$ does not represent a single enzyme's substrate affinity, but instead reflects relative substrate availability because the fluorescently labeled substrates added during EE assays compete for the active sites with

naturally occurring substrates already present in environmental samples (Chróst, 1990). For simplicity, $^{app}V_{max}$ and $^{app}K_m$ are hereafter presented as V_{max} and K_m throughout the text.

Because enzyme concentrations *in situ* are controlled by feedbacks between microbial activity and substrate availability, conditions that are conducive to high EE V_{max} should therefore also be conducive to high K_m. A recent meta-analysis of EE studies showed that V_{max} and K_m of microbial EEs scale consistently with one another globally, though most of those studies took place in aquatic or mesic ecosystems (Sinsabaugh et al., 2014).

While we expect EE kinetic parameters to vary as a function of microbial activity, thermodynamic theory predicts that V_{max} and K_m will also increase with increasing temperature (Davidson and Janssens, 2006), which has potential implications for future C-cycling. Increasing temperature can allow more reactants to attain their activation energy, increasing V_{max}. At the same time, the stability of the substrate-enzyme complex may be reduced, causing decreased substrate affinity and higher observed K_m (Johns and Somero, 2004; Sørensen et al., 2015).

Increases in temperature should have a larger effect on the kinetic parameters of EEs from colder environs (Davidson et al., 2006), especially if enzymes are locally adapted (Belotte 2003). In support of this hypothesis, Koch et al. (2007) found that EE temperature sensitivities increased at lower temperatures in alpine soils assayed across three seasons, and both they and Wallenstein et al. (2009) found that EE temperature sensitivity declined over the growing season in alpine and arctic tundra soils, respectively. However, this hypothesis has never been tested along a regional climate gradient, where microbial communities are unlikely to be dispersal limited (Kivlin et al., 2011), and local climate variation is likely to be a strong filter.

The goal of this study is to use a climate gradient across xeric ecosystems of southern California to determine how microbial EEs might respond to long-term climate change. Along this gradient, temperature and moisture co-vary, such that colder, wetter sites at high elevations contrast with hotter, drier sites at lower elevations. As such, moving to lower elevations mimics the shift to more arid climates expected in the American Southwest. We aimed to quantify the environmental drivers of enzyme kinetic parameters along the climate gradient to advance knowledge of biogeochemical mechanisms in xeric ecosystems. Based on the above theory, we formulated the following hypotheses (**Figure 2.1**):

1. Bacterial abundance, EE $^{app}V_{max}$, and EE $^{app}K_m$ will increase with increasing precipitation, as microbial activity is limited by moisture and substrate availability, and in turn limits EE production.
2. V_{max} and K_m of enzymes from colder, wetter sites will be more temperature sensitive, and enzymes assayed in the wet season will be more temperature sensitive than those assayed in the dry season.

We tested these hypotheses by measuring microbial properties, litter substrates, and enzyme kinetics along a climate gradient spanning 12.5 °C and 300 mm precipitation in southern California.

Methods

Site description

To test how EE kinetic parameters and thermodynamics varied with climate, we assayed plant litter from five sites representing five biomes in southern California –

subalpine forest (lat, long: 33.824, -116.755), montane forest (33.808, -116.772), pinyon-juniper scrubland (33.605, -116.455), coastal grassland (33.737, -117.695), and Colorado desert (33.652, -116.372). All five sites are located on granitic parent material and experience Mediterranean precipitation patterns (cool, wet winters; hot, dry summers). The gradient spans a range of ~12.5 °C in mean annual temperature (MAT), from 10.3±1.8 °C at the subalpine site to 22.8±0.8 °C at the desert site. The montane forest (hereafter referred to as "forest") site experienced the greatest mean annual precipitation in the form of rainfall over the five years prior to this study (402.0±118.1 mm), and the desert experienced the least (99.7±29.3 mm), though the subalpine forest (hereafter referred to as "subalpine") site also receives a significant amount of precipitation in the form of snow (*Personal observation*). All sites other than the subalpine site have eddy covariance towers that collect air temperature, soil temperature, rainfall, and solar radiation data (Goulden et al., 2006). Two iButton temperature sensors (Maxim Integrated) were also installed at each site on January 18, 2015 to collect surface temperature at 90 minute intervals until the final sampling date on December 2, 2015.

Sampling

Local plant litter was collected from each of the five sites on June 7, and December 2, 2015. Using gloves, 5 g litter was collected from the soil surface of each site by lightly raking across the surface to collect loose material and using clippers to detach senescent grass litter from root bundles if necessary. Litter replicates were collected from six 0.5 m^2 plots established within a 50 m^2 sampling area at each site. Collected litter was stored in coolers and transported to UC Irvine, where it was ground into fragments <0.5cm in length

and sub-sampled for EE assays and a bacterial cell count assay. The remainder of the litter was weighed and oven-dried to determine moisture content.

Litter chemistry

Oven-dried litter was sent to Cumberland Valley Analytical Services for near-IR spectroscopy, whereby reflectance spectra of near-infrared wavelengths of light are matched to a verified database of spectra for plant materials with known chemical composition as determined by wet chemistry (Shepherd et al., 2005). Relative amounts of the following organic compounds were determined as proportions of total dried litter mass: lignin, cellulose (acid detergent fiber – lignin), hemicellulose (neutral detergent fiber – acid detergent fiber), structural carbohydrates (non-fiber carbohydrates – starch/sugar), and crude protein. The structural carbohydrate fraction includes plant cell components such as pectins, but also microbial cell wall components such as β-glucans and peptidoglycans (CVAS, *personal communication*).

Extracellular enzyme assays, kinetics, and thermodynamics

For simplicity, ${}^{app}V_{max}$ and ${}^{app}K_m$ at 22 °C are presented as V_{max} and K_m throughout the text, though we recognize that our results are measures of apparent EE kinetics rather than actual kinetics.

Litter was assayed for V_{max}, K_m, and the temperature sensitivities of V_{max} and K_m for seven hydrolytic enzyme classes using fluorescently labeled substrates based on German et al. (2012). 125µL of fluorometric substrate solution was combined with 125µL of litter homogenate in each microplate well. Assays were incubated for 4h at 4, 10, 16, 22, 28, or

34°C. Each enzyme was assayed at a range of substrate concentrations for each temperature (**Table B1**). Negative potential activities were converted to zero values before further analyses.

EE kinetic parameters were calculated for each enzyme class and incubation temperature by fitting observed EE activity at each substrate concentration to the Michaelis-Menten equation. Regressions were performed in the R software environment 3.3.1 (R Development Core Team, 2016) using the nls function. Confidence intervals were determined for V_{max} and K_m values using the nlstools package. Fits of V_{max} with lower bounds or upper bounds less or greater than twice the estimate of V_{max} were discarded; to account for greater variability in calculated fits of K_m, fits with lower bounds or upper bounds less or greater than four times the estimate of K_m were discarded.

Temperature sensitivities of EE kinetic parameters for each enzyme class were determined by linear regression of ln V_{max} or ln K_m against incubation temperature. Regressions were performed using the lm function in R. Regressions with $R^2 < 0.50$ were discarded. Slopes were converted to Q_{10} values as in Wallenstein et al. (2009) using the formula:

$$Q_{10} = \exp(\text{slope} \times 10)$$

Bacterial cell density

Methods for estimating bacterial cell density were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in a phosphate-buffered, 1% glutaraldehyde solution on the day of sample collection to "fix" bacterial cells for storage. Within two weeks, 0.1 M tetrasodium pyrophosphate was added to each sample, and

samples were sonicated to dislodge bacterial cells. Filtered extracts of sonicated litter were stained with 1x SYBR-Green and then analyzed with an Accuri flow cytometer to determine cell counts from fluorescing bacterial cells. Bacterial cell counts from June 2015 samples could not be assayed because of technical issues with the flow cytometer; as such, cell counts from litter collected in identical fashion on October 16, 2014 are presented in the Supplemental Information (**Figure B1**) and were used to determine seasonal effects on bacterial cell density.

Statistical methods

Effects of site and sampling date on bacterial abundance were analyzed using mixed-model ANOVA with the identity of each plot as a random factor. Because litter moisture is known to be a strong control on decomposition processes in Mediterranean ecosystems, the model was run as an ANCOVA with litter moisture content as the covariate. ANCOVA was also used to determine if there was a relationship between bacterial cell density and V_{max} or K_m across sites. Post hoc analysis of pairwise comparisons were done with Tukey contrasts using the lsmeans package in R.

Effects of site and sampling date on ln V_{max}, ln K_m, V_{max} temperature sensitivity, and K_m temperature sensitivity of all EE classes were determined through canonical discriminant analysis (CDA) with litter moisture content as a covariate, using the Wilks Lambda method to calculate the test statistic. Data for both sets of analyses were checked for normality visually and by the Shapiro-Wilk test, and non-normal data were natural log-transformed to improve normality when necessary. Bacterial abundance met assumptions of normality after ln-transformation; ln V_{max} and ln K_m did not but passed visual inspection.

Temperature sensitivities of V_{max} and K_m did not meet assumptions of normality but were visually determined to be approximately normal when in base form, and as such were not transformed prior to statistical analyses.

Recent mean annual air temperature at the subalpine site was extrapolated from a linear regression ($R^2 > 0.8$) of elevation versus air temperature observed by the four eddy covariance towers and a Remote Area Weather Station (RAWS) located at the summit of Mt. San Jacinto (2626 m above sea level). Recent rainfall, solar radiation, and daily temperature range at the subalpine site could not be extrapolated from the RAWS data, but the values observed at Mt. San Jacinto are presented for the subalpine site to provide context given the proximity and likely similarity between the two locations.

CDA indicated that most climate variables measured by the eddy covariance towers were positively correlated. As a result, air temperature was used for climate-related analyses because of our high confidence in extrapolated air temperature at the subalpine site. Linear regression was then used to determine if site air MAT explained a significant amount of the variation in V_{max} and K_m temperature sensitivities observed in either June or December 2015.

Results

Climate gradient

Recent climate observed by the four flux towers followed a similar trend for air MAT, soil MAT, soil moisture, rainfall, and mean daily ranges for air and soil temperature. The desert and forest were at the two extremes for each variable, and the grassland and scrubland experienced similar, intermediate climate relative to the two extremes (**Table**

2.1). Based on flux tower data collected over the last six years, the desert experienced the hottest air and soil temperature, received the least rainfall, and experienced the greatest range of daily air and soil temperatures, on average. Plot-level temperature sensors indicated that from January to December of 2015, the desert site was the hottest, but the grassland was significantly hotter than the scrubland. In addition, over the course of the study, the grassland and scrubland experienced the greatest diurnal temperature ranges, on average, and the subalpine site experienced the least diurnal temperature variation. Extrapolating the subalpine site's recent climate from that observed at lower and higher elevations indicates that it was likely the coldest site.

Litter chemistry

Litter chemistry was significantly affected by site ($p<0.001$, $F_{4,24}=40.3$), season ($p<0.001$, $F_{1,6}=11.2$), and interactions between site and season ($p<0.001$, $F_{4,24}=4.2$)(**Table 2.2**). CDA showed that differences in the relative amounts of structural carbohydrates, protein, and cellulose explained most of the variation in litter chemistry both between sites and between seasons. When comparing differences in litter between sites, the first CDA axis explained 90.4% of the variation, and the absolute values of loading coefficients on this axis were 0.95, 0.93, and 0.85 for fractions of structural carbohydrates, crude protein, and cellulose, respectively.

Across seasons, structural carbohydrate content was highest in the subalpine and forest sites, intermediate in the desert, and lowest in the grassland and scrubland sites, at 32.2±0.4, 22.2±1.2, and 14.1±0.7%, respectively. Crude protein content was highest in the scrubland and grassland, intermediate in the desert, and lowest in the subalpine and forest

sites, at 13.8±0.7, 10.0±0.5, and 2.3±0.3%, respectively. Cellulose content was greatest in the grassland and desert, intermediate in the scrubland, and lowest in the subalpine and forest sites, at 35.3±0.6, 27.9±1.0, and 23.4±0.3%, respectively. Relative fractions of cellulose, hemicellulose, lignin, crude protein, and structural and non-structural carbohydrates are presented in **Table 2.3**.

Bacterial cell density

Bacterial cell density varied by site ($p<0.001$, $F_{4,44}=6.8$), season ($p<0.001$, $F_{1,44}=189.5$), and as a result of an interaction between site and season ($p<0.001$, $F_{4,44}=7.2$) (**Table 2.2**). Cell density was significantly higher in the subalpine, forest, and grassland sites than in the scrubland and desert sites in December 2015, with means of $3.9\pm0.7 \times 10^9$ and $1.4\pm0.2 \times 10^9$ cells/g dry litter, respectively (**Figure 2.2A**). Cell density was significantly higher in the subalpine, forest, and scrubland sites than in the grassland and desert sites in October 2014 (Tukey $p < 0.05$), with mean abundance of $5.3\pm0.7 \times 10^8$ and $1.1\pm0.1 \times 10^8$ cells/g dry litter, respectively (**Figure B1**).

Potential extracellular enzyme activity

There was no significant effect of season on V_{max} of all enzyme classes when analyzed in conjunction. There was a significant effect of site ($p<0.001$, $F_{4,28}=28.2$), and a significant interaction between site and season ($p<0.001$, $F_{4,28}=6.6$)(**Table 2.2**). The first CDA axis explained >60% of the variance in V_{max}, and loading coefficients for all seven EE classes along the first axis were greater than 0.79. Therefore V_{max} results were combined across enzyme classes and sampling dates for pairwise comparisons of site-level

differences. Mean V_{max} across all enzyme classes and seasons was highest in the grassland site, intermediate in the scrubland and desert sites, and lowest in the subalpine and forest sites (Tukey $p<0.001$), with mean values of 15.1±2.7, 7.7±1.9 and 1.5±0.3 $\mu mol \cdot hr^{-1} \cdot g^{-1}$, respectively (**Figure 2.2B**). There was no significant relationship between bacterial cell density and mean V_{max} across or within sites (**Figure B2**).

Potential extracellular enzyme affinity

There was no significant effect of season on K_m for all enzyme classes when analyzed in conjunction. There was a significant effect of site ($p<0.001$, $F_{4,28}=11.0$), and there was a significant interaction between site and season ($p<0.001$, $F_{4,28}=4.2$) (**Table 2.2**). The first CDA axis explained >55% of the cross-site variance in K_m, with cellobiohydrolase (CBH, 0.88), leucine aminopeptidase (LAP, 0.59) and β-glucosidase (BG, 0.52) K_m distinguishing sites the most. The second CDA axis explained >35% of the remaining cross-site variance in K_m, with N-acetyl-glucosaminidase (NAG, 0.91) and BG (0.73) K_m as the main response variables. As such, mean apparent K_m values across seasons for BG, CBH, LAP, and NAG were analyzed for site-level differences using pairwise comparisons.

Mean BG K_m across seasons was lowest in the subalpine site, intermediate in the desert site, and greatest in the forest, grassland, and scrubland sites (Tukey $p<0.01$), with mean values of 57±8, 96±5, and 247±21 μM, respectively (**Figure 2.3A**). Mean CBH K_m across seasons was also lowest in the subalpine site, intermediate in forest, scrubland, and desert sites (Tukey $p<0.001$), and greatest in the grassland site, with mean values of 25±18, 50±4, and 136±11 μM, respectively (**Figure 2.3B**). Mean LAP K_m across seasons was lowest in the grassland, scrubland, and desert sites and highest in the subalpine and forest sites

(Tukey $p<0.001$), with mean values of 91±7 and 278±89 μM, respectively (**Figure 2.3C**). Mean NAG K_m across seasons was lowest in the subalpine, forest, and desert sites, intermediate in the grassland site, and highest in the scrubland site (Tukey $p<0.05$), with mean values of 46±7, 86±7, and 144±37 μM, respectively (**Figure 2.3D**). There was no significant relationship between bacterial cell density and mean K_m across or within sites (**Figure B3**). There was a significant positive relationship between CBH K_m and cellulose content and a negative relationship between LAP K_m and protein content across sites and seasons, but there was no significant relationship between K_m and putative substrate content for AG, BG, or BX (**Table 2.4**).

Temperature sensitivity of potential extracellular enzyme activity

Temperature sensitivity of V_{max} was positive for all EE classes assayed at all sites in both seasons (**Table 2.5**). There were significant effects of site ($p<0.001$, $F_{4,28}=9.3$), season ($p=0.001$, $F_{1,7}=4.4$), and an interaction between site and season ($p=0.007$, $F_{4,28}=2.0$) on observed temperature sensitivity of EE V_{max} for all enzyme classes when analyzed in conjunction (**Table 2.2**). The temperature sensitivity-MAT relationship was significantly negative for six of the seven EE classes assayed in the wet season, but was only significant (and positive) for LAP in the dry season (**Table 2.6**). The weak relationships during the dry season generally resulted from lower EE temperature sensitivities in the coldest sites and higher temperature sensitivities in the scrubland site when compared to the wet season.

Temperature sensitivity of apparent extracellular enzyme substrate affinity

Temperature sensitivity of K_m was positive for all EE classes assayed at all sites across both seasons, with the exception of AG in the subalpine site and LAP in the subalpine and forest sites (**Table 2.7**). There were significant effects of site ($p<0.001$, $F_{4,20}=6.5$) on observed temperature sensitivity of EE K_m of all enzyme classes when analyzed in conjunction, but there was no significant effect of season or interaction between site and season (**Table 2.2**). Temperature sensitivity of K_m exhibited a significant relationship with MAT across seasons for 4 of the EE classes assayed: a positive relationship with LAP, BG, and NAG K_m (in order of increasing slope and significance) and a negative relationship with BX K_m (**Table 2.6**).

Discussion

The gradient presented in this study spans five biomes in southern California along which climate variables covary. Colder, wetter sites transition to hotter, drier sites in a manner that emulates future climate change in the American Southwest. By analyzing litter samples along the gradient, we determined that microbial communities differ in their EE kinetics in a manner consistent with differences in substrate availability and proteolytic activity, but not differences in bacterial abundance or local climate. We also observed that local microbial communities adapted to different litter chemistry and long-term climate exhibit significantly different EE temperature sensitivities. These sensitivities can be predicted in part by local mean annual temperature. As climate becomes more arid in the future, sites at the wetter end of our gradient will initially produce EEs that are relatively sensitive to increases in temperature, but as they transition to more xeric environments

like the grassland, scrubland and desert the microbial communities they support will potentially produce less sensitive EEs. In addition, EE dynamics of microbes undergoing the transition to xeric environments will be more consistent with accumulation of less active EEs that are potentially more dependent on increasingly episodic rainfall events.

Climate and microbial activity

We hypothesized that bacterial cell density in litter would be driven by differences in climate and substrate availability across the gradient, and that we would observe greater cell density in cooler, wetter sites. Our results did not directly support this hypothesis - bacterial abundance in the wet season was high in the two wettest sites, but not greater than in the grassland or desert after accounting for litter moisture (**Figure 2.2A**). Instead, our results may indicate that wetter sites may support greater bacterial abundance during the drier periods of the year, as bacterial abundance in October 2014 was greatest in the wettest sites (**Figure B1**), though an order of magnitude lower across sites when compared to December of 2015.

We expected that bacterial cell density would be related to both the concentration of enzymes (V_{max}) and the availability of substrates (as indicated by K_m) in litter. Our results also failed to support this hypothesis – bacterial cell density did not explain a significant amount of the variation in mean V_{max} or K_m within or across sites at either time point (**Figures B2** and **B3**). Mean V_{max} for the EE classes assayed in this study differed significantly by site (**Table 2.2**), but the differences were not consistent with our initial hypothesis. Mean V_{max} of all EEs in litter from the two highest elevation sites was orders of

magnitude lower than in the grassland, scrubland, and desert sites (**Figure 2.2B**), even though the three lower elevation sites experience less precipitation and have drier soils (**Table 2.1**).

Differences in K_m between sites varied by substrate class (**Figure 2.3**) and were likely driven in part by availability of substrates. We found that the K_m of enzymes involved in the degradation of cellulose exhibited a significant positive relationship with the cellulose content in litter across our gradient, though we did not observe a significant relationship between other C-degrading EE classes and their putative substrates in litter (**Table 2.4**). Counter to our hypothesis, we observed a significant negative relationship between LAP K_m and protein content, such that LAP substrate affinity appears to decrease when substrate is more available (**Table 2.4**). However, it is also possible that samples with high observed LAP K_m contain abundant protein degradation products that compete with artificial substrates for EE active sites (Chróst, 1990). If so, protein turnover may be occurring more rapidly in these samples. The variation in overall V_{max} and crude protein content across our gradient support this hypothesis: mean V_{max} and crude protein content were greatest in sites that had the lowest observed LAP K_m values, and were lowest in the sites that had the highest observed LAP K_m values.

Our results for bacterial abundance and V_{max} are at odds with our understanding of the factors driving microbial decomposer activity and EE potential in more mesic ecosystems, but they mirror those of a prior study performed in the grassland site along our gradient. Alster et al. (2013) found that increases in microbial biomass over the wet season did not correspond with increases in EE V_{max}, and that EE V_{max} remained static from the end of the wet season to the middle of the dry season, despite significant reductions in

microbial biomass. These EE dynamics can be explained in part by the observation that proteolytic EEs were the only enzyme class to decline in V_{max} under drought. Reduced proteolytic activity may allow EEs in arid and semiarid ecosystems to persist long after their production by ephemeral microbial decomposers, a mechanism supported by our LAP K_m and crude protein results at some sites along our gradient.

Our enzyme and protein accumulation results may help explain pulses of CO_2 that are emitted by microbial communities in Mediterranean ecosystems, particularly grasslands, when exposed to rewetting after long dry periods. EEs persisting in microsites may degrade substrate during periods of elevated humidity or at dewpoint, resulting in accumulation of labile substrates during dry periods. After rewetting, these labile compounds combine with built up microbial necromass (Blazewicz et al., 2014) and soil organic matter released from aggregates (Fierer and Schimel, 2003) to produce large pulses of CO_2 (Zhang et al., 2014).

Temperature sensitivity of EE kinetics

The results of our study support a growing consensus that EE kinetic parameters have positive temperature sensitivities (German et al., 2012; Lehmeier et al., 2013; Min et al., 2014; Stone et al., 2011). We show that these results hold true for EEs in litter, a substrate for which such intrinsic responses to temperature have rarely been explored (but see Bárta et al. 2014). Our hypothesis that cold-adapted EEs would show greater V_{max} temperature sensitivity than warm-adapted enzymes was generally supported for EE in the wet season, but not the dry season. Significant relationships between temperature sensitivity of EE V_{max} and MAT in the wet season were driven by a bimodal trend, whereby

the two high elevation, forested sites had higher temperature sensitivities, and the three lower elevation, grass and scrub-dominated sites had significantly lower temperature sensitivities (**Table 2.5**). During the dry season, this bimodal trend disappeared as the two higher elevation sites no longer exhibited higher temperature sensitivity of EE V_{max} in comparison with the lower elevation sites.

Previous studies have shown seasonal variation in EE temperature sensitivity of V_{max} (Brzostek and Finzi, 2012; Fenner et al., 2005; Koch et al., 2007; Trasar-Cepeda et al., 2007; Wallenstein et al., 2009) and others have shown site-to-site variation in temperature sensitivity of V_{max} (German et al., 2012; Khalili et al., 2011; Stone et al., 2011). Some of these studies indicate that EEs from colder biomes or seasons exhibit greater temperature sensitivity (Koch et al., 2007; Wallenstein et al., 2009), but a previous study of EE V_{max} temperature sensitivity in soils across a latitudinal gradient found no relationship between site MAT and temperature sensitivities for all but one EE class (German et al., 2012). However, this study did not control for season when collecting soils, and our results indicate that ignoring seasonality may obscure potentially significant relationships between site MAT and EE thermodynamics.

Our hypothesis that K_m of cold-adapted EEs would show greater temperature sensitivity was not supported by our results. Instead, there are indications that K_m temperature sensitivity exhibits a significant positive relationship with MAT, as BG, LAP, and NAG K_m temperature sensitivities were generally higher in hotter sites. This is surprising, given that both theory and prior evidence indicate that the opposite should occur (Johns and Somero, 2004; Somero, 2004). It is possible that environmental variables other than mean annual temperature play a greater role in determining EE K_m temperature

sensitivities – all major climatic variables were correlated along our gradient, so an apparent response to MAT could also be linked to MAP, solar radiation, or biotic factors that covary with climate. V_{max} temperature sensitivity also exceeded K_m temperature sensitivity for all EEs, at all sites, in both seasons. Therefore, it is possible that temperature effects on V_{max} outweigh those on K_m, both in lab assays and in response to natural selection. To our knowledge, this is the first study of seasonal variation in K_m temperature sensitivity, though we did not observe significant seasonal variation in this EE parameter.

Conclusion

Our study shows that EE kinetics in southern California are not explained by bacterial abundance, but instead appear to be driven by interactions between substrate availability and proteolytic activity. Accumulation of EE relative to substrate in the more arid sites along our gradient may partially explain the pulses of CO_2 emitted during rewetting events after the dry season, as EEs remain present while bacteria decline in abundance over the course of the dry season.

As the American Southwest shifts to a more arid climate in the future, biomes will shift towards the hotter, drier end of our gradient. Our results indicate that litter will become more cellulosic, and litter will contain more protein – likely as a result of increased accumulation of EEs produced by litter-bound microbial communities. These shifts will increase the influence of rewetting events, making resource pulses more episodic and potentially more difficult to predict. The EEs produced by these communities may initially be very sensitive to temperature, particularly in subalpine and montane forests, but our results indicate that over time EEs produced by these communities will likely become less

sensitive to climate as microbes adapted to hotter, drier conditions become more prevalent.

Our study also shows that the temperature sensitivity of EE kinetics varies seasonally, and that large-scale climate indices can explain variation in EE temperature sensitivities in the wet season, but not in the dry season. When taken in conjunction with evidence of extracellular enzyme accumulation across our gradient, our temperature sensitivity results suggest two lines of research for the next generation of carbon-cycling models that explicitly account for enzymatic decomposition (Sihi et al., 2015; Sulman et al., 2014; Wieder et al., 2014). First, if EE potential in arid and semiarid systems indicates enzyme accumulation, not just activity, then we must determine when enzymes are seasonally most active in order to parameterize C-cycling models. Second, we must determine temperature sensitivities of EE kinetics during the seasons when EEs are most active in order to predict how enzyme-driven decomposition will be affected by future changes in temperature.

Table 2.1 Mean (± SE) historic climate parameters for the five sites used in this study, and plot-level temperature over the course of one year encompassing both sampling dates.

Variable	Subalpine	Forest	Grassland	Scrubland	Desert	Period
Air temperature (°C)	*10.3±1.8*	12.3±0.6	16.4±0.3	15.6±0.8	22.8±0.8	2009-14
Air daily temp. range	-	6.2±0.2	8.3±0.3	8.5±0.2	10.8±0.4	2009-14
Soil temperature (°C)	-	9.9±0.3	19.1±0.9	18.4±0.3	28.3±0.3	2008-12
Soil daily temp. range	-	2.6±0.1	4.6±0.8	5.5±0.7	10.9±0.2	2008-12
Plot temperature (°C)	11.5±0.4	13.2±0.4	22.0±0.4	19.5±0.5	29.3±0.4	2015
Plot daily temp. range	13.7±0.4	20.8±0.5	26.2±0.5	24.8±0.4	20.3±0.3	2015
Rainfall (mm)	*~265*	402±118	242±76	193±33	100±24	2009-14
Soil moisture (mL/cm^3)	-	8.6±0.3	7.3±0.8	7.3±0.5	4.6±0.1	2008-12
Solar radiation (KW/m^2)	*>1850*	2640±500	2540±470	2770±520	2620±500	2006-13
Elevation (m)	2250	1710	470	1280	275	

Table 2.2 MANCOVA and ANCOVA results, run with gravimetric litter moisture (H_2O) as a covariate.

Response variable	Site	Season	Site:Season	H_2O	Normality
Vmax	**<0.001**	0.227	**<0.001**	**<0.001**	Log, visual
Km	**<0.001**	0.114	**<0.001**	**0.004**	Log, visual
Vmax TS	**<0.001**	**0.001**	**<0.001**	0.051	Base, visual
Km TS	**<0.001**	0.628	0.104	0.510	Base, visual
Bacterial cells	**<0.001**	**<0.001**	**<0.001**	**0.007**	Log, test
Litter chemistry	**<0.001**	**<0.001**	**<0.001**	0.694	Log, visual

Table 2.3 Mean (± SE) percentage of non-ash dry weight of litter attributed to cellulose, hemicellulose, lignin, crude protein, structural carbohydrates, and non-structural carbohydrates, in all five sites during the dry season (June 2015) and the wet season (December 2015).

Dry season (June 2015)					
Compound	**Subalpine**	**Forest**	**Grassland**	**Scrubland**	**Desert**
Cellulose	24.1±0.7	24.1±0.4	36.3±1.5	28.0±0.7	35.6±1.4
Hemicellulose	6.1±0.3	5.6±0.2	16.0±2.3	17.1±2.7	0.0
Lignin	13.8±0.5	13.6±0.1	8.0±0.4	8.0±0.7	17.4±0.6
Crude protein	2.9±0.5	1.9±0.2	11.9±0.9	12.8±0.9	9.1±0.8
Struct. Carbs.	34.0±0.3	31.3±0.5	13.3±0.8	17.2±1.7	25.7±0.9
Non-struct. Carbs.	8.9±0.9	12.6±0.5	8.8±1.6	10.5±0.3	5.0±1.1
Wet season (December 2015)					
Compound	**Subalpine**	**Forest**	**Grassland**	**Scrubland**	**Desert**
Cellulose	23.1±0.5	22.2±0.7	36.0±1.0	27.6±4.3	33.1±0.5
Hemicellulose	5.5±1.1	5.3±0.7	8.6±1.9	20.9±0.2	0.9±0.4
Lignin	13.9±0.8	13.1±0.7	14.3±0.7	8.2±0.3	16.2±0.1
Crude protein	2.8±0.7	1.5±0.3	16.5±1.1	14.9±2.6	11.0±0.2
Struct. Carbs.	32.8±0.7	30.6±0.4	12.6±0.9	12.0±0.1	18.7±0.4
Non-struct. Carbs.	11.6±1.3	16.0±0.8	5.1±0.8	11.0±0.7	4.9±0.2

Table 2.4 Pearson coefficients and p-values for the correlation between EE K_m and percentage of litter composed of the fraction degraded by that EE class. R^2 values for the linear regression between the two variables are presented for significant relationships.

Enzyme	Substrate	Correlation	R^2	p
α-glucosidase	Starches	0.12	-	0.383
β-glucosidase	Cellulose	-0.14	-	0.312
β-xylosidase	Hemicellulose	0.18	-	0.193
Cellobiohydrolase	Cellulose	0.60	0.35	**<0.001**
Leucine aminopeptidase	Protein	-0.60	0.35	**<0.001**

Table 2.5 Mean (± SE) Q_{10} of V_{max} for each EE class by site and season. n = number of samples.

		Subalpine		Forest		Grassland		Scrubland		Desert	
Enzyme	Season	Q_{10}	n	Q_{10}	n	Q_{10}	n	Q_{10}	n	Q_{10}	n
AG	Dry	1.78±0.17	6	2.82±0.19	5	2.10±0.06	6	2.88±0.37	4	2.35±0.16	6
	Wet	2.71±0.45	6	3.94±0.94	6	2.07±0.04	6	2.51±0.27	6	2.28±0.06	6
AP	Dry	1.53±0.06	6	1.77±0.04	5	1.64±0.02	6	1.60±0.03	6	1.58±0.03	6
	Wet	1.89±0.03	6	1.94±0.04	6	1.73±0.03	6	1.58±0.02	6	1.64±0.02	6
BG	Dry	1.99±0.06	6	2.26±0.04	5	1.80±0.03	6	2.42±0.12	6	2.04±0.02	6
	Wet	2.33±0.07	6	2.38±0.07	6	1.89±0.04	6	1.95±0.05	6	2.05±0.02	6
BX	Dry	2.16±0.16	6	2.84±0.16	5	1.99±0.03	6	2.42±0.10	6	2.20±0.03	6
	Wet	3.25±0.37	6	3.25±0.23	6	2.10±0.03	6	1.99±0.05	6	2.22±0.02	6
CBH	Dry	2.69±0.12	6	3.21±0.07	5	2.04±0.02	6	3.93±0.32	6	2.55±0.06	6
	Wet	3.29±0.30	6	3.98±0.28	6	2.06±0.09	6	2.36±0.06	6	2.51±0.03	6
LAP	Dry	1.78±0.13	4	2.08±0.08	5	1.99±0.03	6	2.29±0.09	6	2.19±0.06	6
	Wet	2.32±0.72	3	2.28±0.63	4	1.98±0.12	6	2.27±0.08	6	2.09±0.08	6
NAG	Dry	2.19±0.08	6	2.13±0.02	5	1.90±0.05	6	1.74±0.02	6	2.09±0.02	6
	Wet	2.63±0.13	6	2.22±0.03	6	2.06±0.13	6	1.90±0.03	6	2.06±0.08	6

Table 2.6 Slope (± SE), R^2, and p-values for linear regression of EE V_{max} and K_m Q_{10} against site mean annual temperature for each EE class by site and season. Bolded p-values are significant (<0.05).

	Vmax, dry season			Vmax, wet season			Km, both seasons		
Enzyme	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p
AG	0.019±0.025	-	0.450	-0.080±0.040	0.08	0.053	-0.004±0.024	-	0.879
AP	-0.001±0.005	-	0.766	-0.023±0.005	0.41	**<0.001**	-0.005±0.005	-	0.318
BG	-0.005±0.011	-	0.649	-0.028±0.008	0.27	**0.002**	0.039±0.007	0.39	**<0.001**
BX	-0.017±0.016	-	0.279	-0.094±0.025	0.30	**<0.001**	-0.057±0.014	0.27	**<0.001**
CBH	-0.030±0.032	-	0.351	-0.097±0.030	0.24	**0.004**	-0.013±0.014	-	0.368
LAP	0.023±0.010	0.15	**0.028**	-0.018±0.030	-	0.530	0.084±0.014	0.62	**<0.001**
NAG	-0.010±0.008	-	0.261	-0.039±0.012	0.25	**0.003**	0.012±0.005	0.09	**0.013**

Table 2.7 Mean (± SE) Q_{10} of K_m for each EE class by site, across seasons. n = number of samples.

	Subalpine		Forest		Grassland		Scrubland		Desert	
Enzyme	Q_{10}	n	Q_{10}	n	Q_{10}	N	Q_{10}	n	Q_{10}	n
AG	0.66±0.02	2	1.77±0.49	2	1.22±0.02	9	1.49±0.15	4	1.06±0.21	3
AP	1.28±0.05	4	1.28±0.03	11	1.28±0.07	11	1.30±0.03	10	1.22±0.02	8
BG	1.37±0.03	11	1.32±0.02	9	1.89±0.06	12	1.67±0.08	9	1.80±0.04	12
BX	2.07±0.24	7	2.04±0.22	6	1.39±0.07	11	1.29±0.03	8	1.36±0.03	10
CBH	1.98±0.34	6	1.89±0.18	9	2.06±0.05	11	2.11±0.11	8	1.80±0.07	12
LAP	0.52±0.08	7	0.40±0.02	2	1.20±0.06	8	1.29±0.06	3	1.34±0.27	2
NAG	1.43±0.06	10	1.60±0.04	11	1.52±0.04	12	1.40±0.03	11	1.65±0.02	11

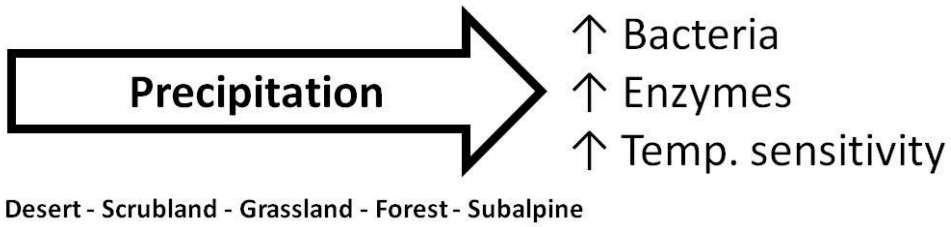

Figure 2.1 Conceptual figure of hypotheses for variation in bacterial abundance, enzyme kinetic parameters, and temperature sensitivity of enzyme kinetics as a function of precipitation differences between each site along the gradient. Arrow indicates increases in precipitation as one goes from the desert to the scrubland, grassland, forest, and subalpine sites.

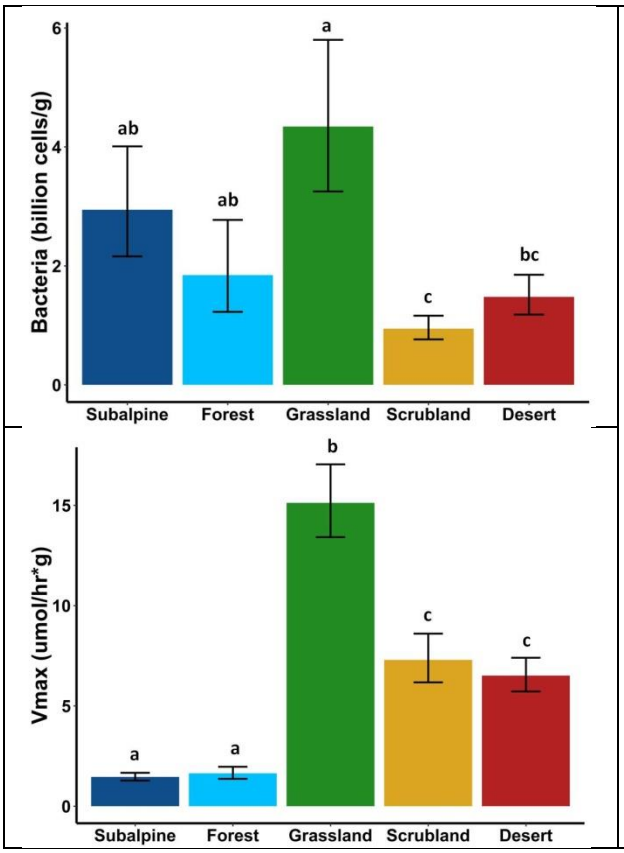

Figure 2.2
A) Mean bacterial abundance in billions of cells per gram of dry litter by site in December 2015 (n=6 for each site). **B)** Mean EE V_{max} across all enzyme classes and both seasons (n=12 for each site). Error bars denote standard error. Depicted means and standard errors are back-transformed from ln values. Means sharing the same letter are not statistically different ($p > 0.05$).

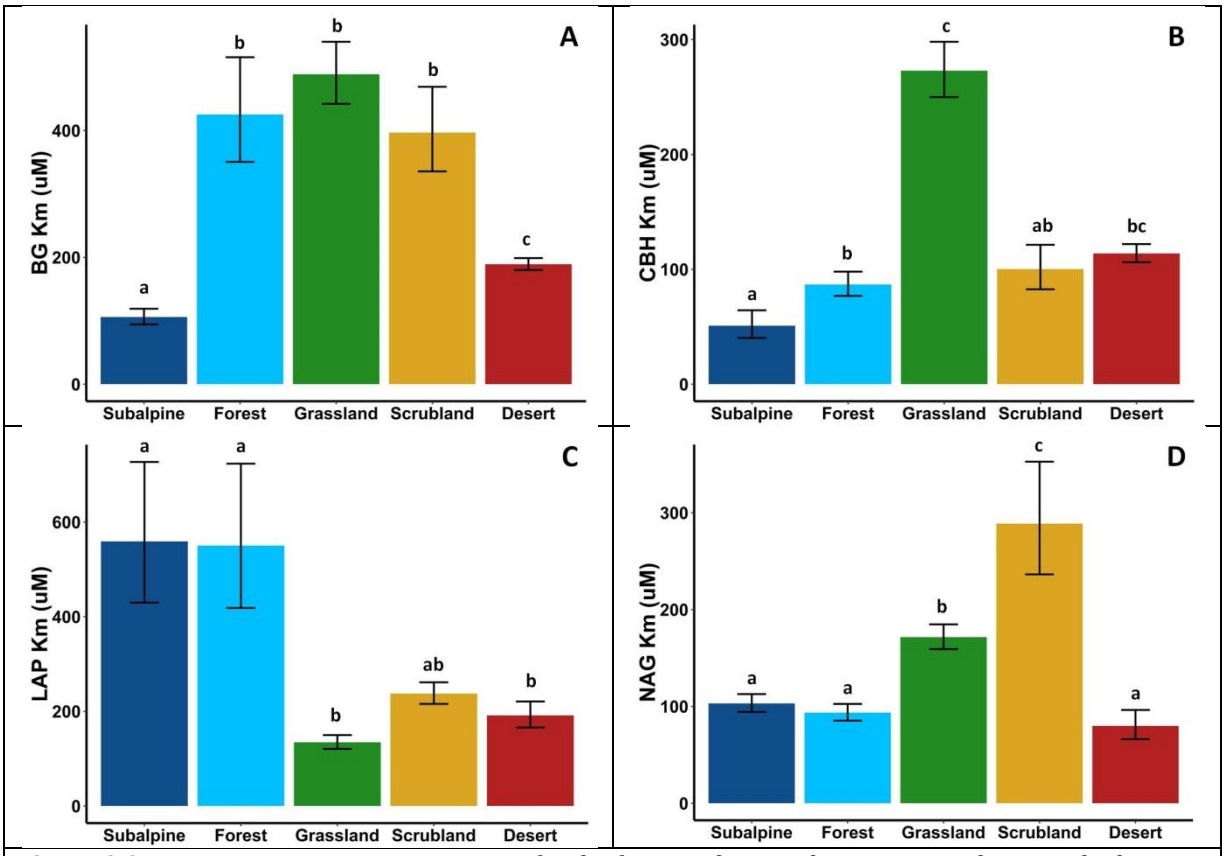

Figure 2.3 Mean EE K_m in µM across seasons for the four EE classes whose K_m most distinguished sites from one another: **A**) BG, **B**) CBH, **C**) LAP, and **D**) NAG (n=12 for each site). Error bars denote standard error. Depicted means and standard errors have been back-transformed from ln K_m values. Means sharing the same letter are not statistically different ($p > 0.05$)

CHAPTER 3

Grassland litter decomposition not constrained by community origin along a regional climate gradient

Introduction

In the American Southwest, anthropogenic climate change is causing a shift to hotter and drier conditions in the future (IPCC, 2014; Seager et al., 2007). This shift could alter heterotrophic respiration generated by microbial decomposition of plant litter in those ecosystems (Raich and Schlesinger, 2002). To predict how microbial contributions to the carbon (C) cycle will change with climate, we must determine how microbial communities respond to changes in abiotic conditions as well as shifts in the chemistry of litter inputs as plant communities change (Aerts, 1997; Keiser et al., 2013). To incorporate these mechanistic changes into global C-cycling models, we must also determine whether different microbial communities are constrained in their responses to climate, or whether their responses can be generalized across regions, continents, or the entire Earth System (Keiser et al., 2011; Reed and Martiny, 2007; Strickland et al., 2009).

Microbial decomposition is at its root an enzymatic process, as decomposer communities produce extracellular enzymes (EEs) to degrade the complex organic polymers in plant litter (Sinsabaugh et al., 1994). The characteristics and quantity of different EEs produced by microbial decomposer communities are therefore key functional traits of those communities (Allison et al., 2007). In a hotter, drier future, elevated temperatures should accelerate enzymatic reactions because of their inherent temperature sensitivity (Davidson and Janssens, 2006), but could also lead to drying and inhibition of

microbial processes (Allison and Treseder, 2008). Reduced moisture should limit microbial activity, but impacts on overall decomposition rates are less clear. This is because of the added importance of other abiotic drivers of decomposition in arid and semiarid ecosystems, such as thermal degradation and ultraviolet photodegradation (Throop and Archer, 2009) as well as hypothesized mechanisms that cause the pulse-like nature of CO_2 efflux following rewetting events in these ecosystems (Zhang et al., 2014).

Microbial communities responding to future climate change may be constrained by their taxonomic composition if they are locally adapted and dispersal limited. If microbial communities are locally adapted, then changes in climate should cause shifts in the relative abundances of taxa in a microbial community. These changes could affect the community's functional response as a whole, and may constrain the community's ability to respond to future changes (Reed and Martiny, 2007). Evans and Wallenstein (2011) found that long-term exposure to different precipitation regimes resulted in significant "legacy" effects of precipitation treatment on CO_2 respiration when field communities were subjected to drying and rewetting perturbations in the lab. These legacy effects presumably result from compositional constraints on a community's ability to degrade litter of a particular origin. Similar legacy effects were found for microbial communities historically exposed to either a grass or hardwood environment that were reciprocally transplanted in a lab microcosm study (Keiser et al., 2011). These and other similar studies (Strickland et al., 2015, 2009) clearly indicate that microbial decomposer communities can shift their composition in response to long-term climate and litter chemistry, and that such shifts can constrain a community's future response to perturbation.

We aim to test whether the functional traits of microbial communities are regionally constrained by community composition by observing microbial responses to transplantation along a regional climate gradient. The function we investigated was litter decomposition, and we measured differences in litter mass loss rates, bacterial abundance, EE kinetic parameters, EE temperature sensitivities, and litter chemistry to quantify functional responses. Using a climate gradient spanning 12.5 °C and 300 mm precipitation in southern California, we tested two non-exclusive hypotheses:

1. Rates of decomposition will be controlled by differences in precipitation between sites. Litterbags in sites with greater precipitation will have greater microbial biomass and enzymatic activity, resulting in faster decomposition (**Figure 3.1A**).
2. Microbial decomposers are locally adapted to climate. Litter decomposing in its native climate conditions will have greater microbial biomass and enzymatic activity, resulting in faster decomposition, while litter transplanted to foreign sites will have lower microbial activity and slower decomposition rates. Access to local microbes will enhance microbial activity and decomposition rates (**Figure 3.1B**).

These hypotheses are not mutually exclusive; climate could drive mass loss rates across the gradient while local adaptation confers an advantage to native microbial communities (**Figure 3.1C**).

Methods

Site description

To test how microbial communities and decomposition in the American Southwest will respond to future climate change, we transplanted litter from a coastal grassland to

five different biomes along a climate gradient in southern California – subalpine forest, montane forest, pinyon-juniper scrubland, coastal grassland, and Colorado desert. Temperature and moisture co-vary along the gradient, with colder, wetter sites at high elevations and hotter, drier sites at lower elevations. As such, moving to lower elevations is akin to experiencing more arid climates in a manner that emulates how future climate change is expected to progress in the American Southwest. All five sites are located on granitic parent material and experience Mediterranean precipitation patterns (cool, wet winters; hot, dry summers). The gradient spans a range of ~12.5 °C in mean annual temperature, from 10.3±1.8 °C at the subalpine site to 22.8±0.8 °C at the desert site (**Table C1**). The montane forest (hereafter referred to as "forest") site experienced the greatest mean annual precipitation in the form of rainfall over the five years prior to this study (402.0±118.1 mm), and the desert experienced the least (99.7±29.3 mm), though the subalpine forest (hereafter referred to as "subalpine") site also receives a significant amount of precipitation in the form of snow (*Personal observation*). All sites other than the subalpine site have eddy covariance towers that collect air temperature, soil temperature, rainfall, and solar radiation data (Goulden et al., 2006). Two iButton temperature sensors (Maxim Integrated) were also installed at each site on January 18, 2015 to collect surface temperature at 90 minute intervals until the final sampling date on December 2, 2015.

Litter collection and deployment

On October 16, 2014, we collected ~800g of grass litter from the grassland site by clipping standing litter at least 20 cm above the soil surface to avoid litter with prior soil contact. Litter was collected from six different 1 m^2 plots located within a 50 m^2 sampling

region. This litter was clipped to <5 cm lengths and mixed. A sub-sample was weighed and oven-dried to determine gravimetric moisture content, and the equivalent of 2.1 g dry weight of litter (including ash content) was used to make litterbags. ~15 g of chopped grassland litter was ground to use as control inoculum. Local inoculum was also collected from each of the other four sites on October 16, 2014. Using gloves, ~15g litter was collected from the soil surface of each site by lightly raking across the surface to collect loose material, using clippers to detach senescent grass litter from root bundles if necessary. Collected material was ground and used as the inoculum. To determine the effect of community origin on observed responses to transplantation, 50 mg of grassland inoculum was added to half the bags as a control (control, –); the other half received 50 mg of inoculum native to the transplant destination site (inoculated, +), in addition to the 2.1 g of unsterilized grassland litter in each bag. Each litterbag was made of 0.2 μm nylon mesh that creates a "microbial cage" by preventing microbial dispersal into or out of the litterbag (Allison et al., 2013).

Four of each type of litterbag (-/+) were deployed into the six plots used to collect initial inocula at each of the five sites (4 x 2 x 6 x 5 = 240 total litterbags) on November 20, 2014. One litterbag of each type was removed from each plot for destructive sampling on March 9, June 7, September 11, and December 2, 2015. Collected litterbags were stored in coolers and transported to UC Irvine, where litter was weighed to determine mass loss before being ground into fragments <0.5cm in length and sub-sampled for EE assays and a bacterial cell count assay. The remainder of the litter was weighed and oven-dried to determine moisture content.

EE assays, kinetics, and thermodynamics

EE kinetics can be described by the Michaelis-Menten model, whereby activity (V) of an individual enzyme is described as a saturating function of substrate (S) concentration as follows:

$$V = V_{max}[S]/(K_m + [S])$$

where V_{max} is the enzyme's maximum reaction rate and K_m, the half-saturation constant, is the substrate concentration at which the reaction rate is one-half V_{max}. In addition, thermodynamic theory predicts that V_{max} and K_m are positively sensitive to temperature (Davidson and Janssens, 2006). We note that in ecological systems, observed V_{max} and K_m are apparent kinetic parameters, not actual kinetic parameters (Wallenstein et al., 2011).

Local inoculum, initial grassland litter, and collected litterbags from June and December 2015 were assayed for V_{max}, K_m, and the temperature sensitivities of V_{max} and K_m for seven hydrolytic enzyme classes using fluorescently labeled substrates according to methods detailed in Chapter 2. In brief, assays were incubated for 4h at 4, 10, 16, 22, 28, or 34°C and performed over a range of substrate concentrations for each temperature (**Table C2**).

EE kinetic parameters were calculated for each enzyme class and incubation temperature by fitting observed EE activity at each concentration of substrate to the Michaelis-Menten equation. V_{max} and K_m parameters calculated from 22 °C incubations are hereafter referred to as "V_{max}" and "K_m" in the text. Temperature sensitivities of EE kinetic parameters for each enzyme class were determined by linear regression of ln V_{max} or ln K_m against incubation temperature. Regressions were performed in R using the lm function.

Regressions with $R^2 < 0.50$ were discarded. Slopes were converted to Q_{10} values as in Wallenstein et al. (2009) using the following formula:

$$Q_{10} = \exp(\text{slope} \times 10).$$

Litter chemistry

Oven-dried litter was sent to Cumberland Valley Analytical Services for near-IR spectroscopy, whereby reflectance of near-infrared wavelengths of light from each sample are matched to a verified database of spectra for plant materials with known chemical composition as determined by wet chemistry (Shepherd et al., 2005). Relative amounts of the following organic compounds were determined as proportions of total non-ash dried litter mass: lignin, cellulose (acid detergent fiber – lignin), hemicellulose (neutral detergent fiber – acid detergent fiber), structural carbohydrates (non-fiber carbohydrates – starch and sugar), and crude protein. The structural carbohydrate fraction includes plant cell components such as pectins, but also microbial cell wall components such as β-glucans and peptidoglycans (CVAS, *personal communication*). The proportion of total litter mass attributable to different C compounds will be referred to as content in the text.

Bacterial cell density

Methods for estimating bacterial cell density were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in a phosphate-buffered, 1% glutaraldehyde solution on the day of sample collection to "fix" bacterial cells for storage. Within two weeks, 0.1 M tetrasodium pyrophosphate was added to each sample, and samples were sonicated to dislodge bacterial cells. Filtered extracts of sonicated litter were

stained with 1x SYBR-Green and then analyzed with an Accuri flow cytometer to determine cell counts from fluorescing bacterial cells.

Statistical methods

Effects of site, sampling date, and inoculation on mass loss and bacterial abundance were analyzed using mixed-model ANOVA with the identity of each plot as a random factor. Because litter moisture is known to be a strong control on decomposition processes in Mediterranean ecosystems, the model was run as an ANCOVA with litter moisture content as the covariate. Post hoc analysis of pairwise comparisons was done with Tukey contrasts using the lsmeans package in R.

Effects of site, sampling date, and inoculation on litter chemistry, and ln V_{max} and ln K_m of all EE classes were determined through MANOVA with litter moisture content as a covariate, using the Wilks Lambda method to calculate the test statistic. Canonical discriminant analysis (CDA) indicated that variation in LAP and BX V_{max} explained the most variation in EE V_{max} between both sites and sampling dates. CDA also indicated that K_m values for all enzymes varied similarly across sites and sampling dates, and, as no site:date interaction was found by MANOVA, K_m results are presented as mean K_m across all enzymes assayed in final (December) samples only. For V_{max} and K_m temperature sensitivity, mixed-model ANOVAs were also run for each enzyme class individually to allow for post hoc comparisons between litterbags from the December sampling date.

Data for all three sets of analyses were checked for normality visually and by the Shapiro-Wilk test, and non-normal data were natural log-transformed to improve normality when necessary. Bacterial abundance met assumptions of normality after ln-

transformation; ln V_{max} and ln K_m did not but passed visual inspection. Temperature sensitivities of V_{max} and K_m did not meet assumptions of normality but were visually determined to be most normal when in base form, and as such were not transformed prior to statistical analyses.

Results

Mass loss

Mass loss varied by site ($p<0.001$, $F_{4,188}=55.3$), sampling date ($p<0.001$, $F_{3,188}=179.9$), and with inoculation treatment ($p=0.002$, $F_{1,188}=10.4$)(**Table 1**). Litter in the forest and grassland site lost significantly more mass by December (35.0±1.4% and 21.5±1.2% of dry mass, respectively) than did litter in the subalpine, scrubland, and desert sites. Mass loss occurred between all sampling dates other than from June to September 2015, during which no mass loss was observed in any site (**Figure 3.2A**). Inoculated litterbags lost 25.3±1.5% dry mass by December, significantly less than the 28.8±1.9% mass loss observed in control litterbags (**Figure 3.3A**).

Litter chemistry

Over the course of the study, mass attributable to different chemical fractions in transplanted litter was affected by site ($p<0.001$, $F_{24,326}=13.3$), sampling date ($p<0.001$, $F_{6,93}=20.2$), and inoculation treatment ($p<0.001$, $F_{6,93}=5.5$)(**Table 3.1**). Total litter mass loss was driven by losses from the cellulose and hemicellulose fractions, which on average lost 0.23±0.01 g and 0.15±0.01 g by December, respectively. Cellulose, hemicellulose, and the combined starch and sugar fractions declined in all sites over the course of the study

(**Figure 3.4**). Crude protein and lignin fractions, however, were only reduced in the grassland and forest sites, which experienced the most mass loss overall. The structural carbohydrate fraction increased by an average of 0.10±0.04 g by December in all litterbags, but increased more in the subalpine, scrubland, and desert sites (0.11±0.01 g) than in the forest and grassland sites (0.08±0.00 g).

Based on CDA, which summarizes correlated changes in litter chemical fractions, inoculation most affected the mass of the structural carbohydrate and lignin fractions in litter. Absolute values of the loading coefficients on the CDA axis for structural carbohydrate and lignin mass were 0.74 and 0.67, respectively. By December, inoculated litterbags accumulated 0.11±0.01 g structural carbohydrates compared to 0.09±0.01 g in control litterbags (**Figure 3.3B**), and accumulated 0.006±0.003 g lignin compared to losses of 0.006±0.004 g lignin observed in control litterbags (**Figure 3.3C**).

Bacterial cell density

Bacterial cell density varied by site ($p<0.001$, $F_{4,243}=84.5$), sampling date ($p<0.001$, $F_{4,243}=579.1$), and with inoculation treatment ($p<0.001$, $F_{1,243}=27.0$)(**Table 3.1**). Bacterial cell density was greatest in the forest site over the course of the study, at $3.8\pm0.6\times10^9$ cells per gram dry litter, intermediate and equivalent in the subalpine, grassland, and scrubland sites (mean across sites = $2.1\pm0.3\times10^9$), and lowest in the desert site, at $1.0\pm0.2\times10^9$. Cell density was greatest in December 2015, though there was a peak in scrubland cell density in September (**Figure 3.2B**). Inoculation significantly increased bacterial cell density from $1.9\pm0.3\times10^9$ cells/g in control litterbags to $2.5\pm0.3\times10^9$ cells/g in inoculated litterbags

(**Figure 3.3D**). Inoculation increased cell density in all sites other than the scrubland, and had the greatest positive effect on cell density in litterbags transplanted to the desert site.

EE potential V_{max}

There were significant effects of site ($p<0.001$, $F_{28,322}=11.1$) and sampling date ($p<0.001$, $F_{7,89}=21.8$) on V_{max} of all enzyme classes when analyzed together, as well as a marginally significant effect of inoculation treatment ($p=0.056$, $F_{7,89}=2.1$)(**Table 3.1**). CDA indicated that differences between sites and sampling dates were driven by differences in BX and LAP V_{max}. The first CDA axis accounted for 57.3% of the variation in EE V_{max} between sites, and the absolute values of the loading coefficients for BX and LAP V_{max} were 0.74 and 0.66, respectively. There was only one CDA axis for differences between sampling dates, and the absolute value of the loading coefficients for BX and LAP V_{max} was 0.57 for both. BX V_{max} remained near 29.1±0.6 $\mu mol \cdot hr^{-1} \cdot g^{-1}$ over the course of the experiment in the subalpine, scrubland, and desert sites, but decreased to 18.5±1.0 $\mu mol \cdot hr^{-1} \cdot g^{-1}$ by December in the grassland and forest sites (**Figure 3.5A**). Similar trends (higher observed activity in litterbags from the desert and subalpine relative those from the grassland and forest) were observed for two other C-degrading EEs, BG and CBH (**Figure C1**). LAP V_{max} increased over the course of the study in all sites, and finished significantly higher in the forest, at 9.8±0.7 $\mu mol \cdot hr^{-1} \cdot g^{-1}$, than in the other four sites, which averaged 5.8±0.3 $\mu mol \cdot hr^{-1} \cdot g^{-1}$ in December (**Figure 3.5B**).

According to CDA, differences between inoculated and control litterbags were driven by differences in CBH V_{max} and BG V_{max}, which had loading coefficients on the only CDA axis with absolute values of 0.77 and 0.61, respectively. Inoculation increased mean

CBH V_{max} over the course of the study from 35.7±0.9 μmol·hr^{-1}·g^{-1} in control litterbags to 39.1±1.3 μmol·hr^{-1}·g^{-1} in inoculated litterbags (**Figure 3.3E**), and similarly increased BG V_{max} from 64.9±1.6 μmol·hr^{-1}·g^{-1} in control litterbags to 69.0±2.2 μmol·hr^{-1}·g^{-1} in inoculated litterbags (**Figure 3.3F**).

EE potential K_m

There were significant effects of site ($p<0.001$, $F_{28,322}=6.7$) and sampling date ($p<0.001$, $F_{7,89}= 229.9$) on K_m of all enzyme classes when analyzed together, but no effect of inoculation treatment (**Table 3.1**). CDA indicated that differences in the K_m of individual EE classes did not differentiate sites from one another, and that differences between sampling dates overwhelmed those between sites. Every EE K_m had a loading coefficient with an absolute value greater than 0.65 on the first CDA axis when analyzing variation between sites, and all also had loading coefficients with absolute values greater than 0.75 on the second CDA axis. There was only one CDA axis when analyzing differences between sampling dates, and every EE K_m had a loading coefficient greater than 0.83 on that axis. Therefore, mean EE K_m across all EE classes was combined for comparisons between sites and sampling dates. Mean K_m across all EE classes decreased from 288.1±21.5 μM in initial litter to 157.7±3.7 μM in June, then increased to 292.5±6.7 μM in December (**Table 3.2**). There were no significant differences in mean EE K_m between litterbags collected from different sites in either June or December after accounting for the effects of litter moisture.

Temperature sensitivity of EE V_{max}

EE V_{max} temperature sensitivities were significantly affected by site ($p<0.001$, $F_{28,304}=5.8$) and sampling date ($p<0.001$, $F_{7,84}=5.8$), but were not significantly affected by inoculation treatment (**Table 3.1**). Pairwise comparisons of V_{max} temperature sensitivities in December litterbags between sites were used to determine if transplantation to a foreign site resulted in microbes producing EEs with different temperature sensitivities from those observed in litterbags from the grassland site (**Table 3.3**). All EEs other than AP exhibited different V_{max} temperature sensitivities after transplantation into at least one foreign site, usually the scrubland. EEs in transplanted litter that exhibited significantly different V_{max} temperature sensitivities from those in litterbags from the grassland were always significantly less sensitive to temperature.

Temperature sensitivity of EE K_m

EE K_m temperature sensitivities for BG, BX, CBH, and NAG were significantly affected by site ($p<0.001$, $F_{16,248}=4.5$) and sampling date ($p<0.001$, $F_{4,81}=19.3$), but were not significantly affected by inoculation treatment. K_m temperature sensitivities for AG, AP, and LAP could not be calculated for enough samples to allow them to be included in statistical analyses. Pairwise comparisons of K_m temperature sensitivities in December litterbags between sites were used to determine if transplantation to a foreign site resulted in microbes producing EEs with different temperature sensitivities from those observed in litterbags from the grassland site (**Table 3.4**). Each of the four EEs that we were able to calculate K_m temperature sensitivities for exhibited different K_m temperature sensitivities

after transplantation in at least one site, though there were no discernible patterns as to which EEs or sites exhibited such differences.

Discussion

Mass loss and bacterial abundance

We hypothesized that differences in microbial activity and litter mass loss rates in transplanted litterbags would be driven by differences in climate along the gradient, and in particular by differences in precipitation. Our results supported this hypothesis: though decomposer activity is not a linear function of precipitation, covarying differences in temperature and precipitation along our gradient likely combine to drive decomposer activity. Even though the subalpine site likely receives the most precipitation (**Table C1**), litter there decomposed as slowly as in the desert and scrubland (**Figure 3.2A**). In addition, the forest receives significantly more rainfall than the grassland, yet similar mass loss was observed over the course of the study in both sites. It is likely that temperature and moisture interact to limit decomposer activity across our gradient – sub-zero temperatures and snow limit the positive effects of increased precipitation in the higher elevation forest and subalpine sites, whereas extreme high temperatures and reduced precipitation limit microbial activity in the scrubland and desert sites (Gliksman et al., 2016).

All major chemical fractions of litter declined over the course of the experiment other than structural carbohydrates, which increased in litterbags from all sites (**Figure 3.4**). Sites that experienced the most mass loss also experienced the greatest declines in cellulose, and the least gains in structural carbohydrates. The structural carbohydrate fraction is composed of pectins, β-glucans, and peptidoglycans, and it is possible that a

large proportion of the accumulating structural carbohydrate fraction is composed of microbial residues or necromass. These residues are more recalcitrant than cellulose or hemicellulose (Grandy and Neff, 2008; Miltner et al., 2012), and may represent C that has shifted into slower turnover pools (Khan et al., 2016).

We expected that trends in bacterial cell density would mirror mass loss over the course of the study, as bacterial decomposers are responsible for a large majority of microbial activity and biomass in litter from the grassland site (Alster et al., 2013). However, bacterial cell density in grassland litterbags over the course of the study was similar to that observed in the subalpine and scrubland site, even though grassland litterbags experienced much greater mass loss (**Figure 3.2B**). Litterbags transplanted to the desert also had significantly lower bacterial abundance than in any other site over the course of the study, but experienced mass loss rates akin to those found in the subalpine and scrubland sites. It is possible that in the desert, fungi may contribute more to litter mass loss than do bacteria, given their greater tolerance for drought (Allison et al., 2013; Yuste et al., 2011), or that the contributions of abiotic processes such as photodegradation are enhanced (Austin, 2011). Regardless, our results indicate that bacterial cell density in general is not indicative of litter mass loss rates across our gradient.

Inoculation effect

Even though litter mass loss was lower when litter was transplanted to most foreign sites, the results of our inoculation treatment indicate that local adaptation of microbial communities was likely not the cause of this disparity, as inoculated litterbags unexpectedly lost less mass after transplantation than did control litterbags (**Figure 3.3A**).

Instead, our inoculation results indicate that non-grassland microbes present in our inocula may be less effective at degrading grassland litter than microbes in the pre-existing grassland community. Notably, inoculation effects resembled the effects of exposure to novel climate conditions, as inoculated litter lost less mass, accumulated lignin (**Figure 3.3D**), and exhibited greater increases in structural carbohydrates (**Figure 3.3C**) by the end of the study. These results could indicate that the microbes inoculated into grassland litter communities are less effective at degrading grassland litter than the pre-existing members of the grassland microbial community. Prior studies have found evidence that microbial communities are constrained in their ability to degrade particular litter chemistries, though those studies have also shown that more complex litter chemistry generally results in stronger home-field advantage effects on C mineralization rates (Keiser et al., 2011; Strickland et al., 2009).

Grassland microbial communities themselves do not appear constrained in their ability to decompose grassland litter in different climates, suggesting that the pool of taxa present in grassland microbial communities is adapted to climates experienced across our regional gradient. This is perhaps not that surprising, because the grassland site experiences a relatively broad range of daily air and soil temperatures (**Table C1**), and as such microbial communities in the grassland are likely composed of taxa that are capable of persisting in a wide range of environmental conditions.

Even though inoculation had an effect on mass loss that ran counter to our hypothesis, our hypothesis that inoculation would positively affect bacterial abundance was supported **(Figure 3.3B**). Combined with the negative effect of inoculation on mass loss, this result suggests that microbial communities in inoculated bags had higher C-use

efficiency (CUE), as their increased biomass did not result in greater C-losses from the system. It is possible that these microbes are cheaters – organisms that benefit from EE production without producing EEs themselves (West et al., 2006). This would increase community-wide CUE by increasing the biomass supported by EE production, but would decrease the per-biomass rate of C mineralization relative to a similarly abundant community composed of proportionally fewer cheaters (Allison et al., 2014; Kaiser et al., 2015).

Enzyme profiles

We hypothesized that kinetic properties of EEs produced by transplanted microbial communities would be enhanced in sites that experienced greater precipitation. Our results did not support this hypothesis. EE V_{max} values differed significantly over time and with transplantation site, but were not generally higher in sites that received more precipitation. C-degrading enzymes generally exhibited the greatest activity in the sites that had the least precipitation (**Figure 3.5A**), though peptidase activity did increase the most in the sites that received the greatest precipitation (**Figure 3.5B**). These opposing trends between peptide degradation and activity of EEs in general were also found in previous studies from arid and semi-arid ecosystems (Chapter 2, Alster et al., 2013), and likely indicate EE accumulation over dry periods in the more arid sites along the gradient and more rapid turnover of EEs in the wetter sites along the gradient.

We hypothesized that allowing local microbes to access transplanted litter would either have no effect on EE traits or would enhance EE kinetic parameters after transplantation. Our results did in part support the hypothesis that inoculation would

enhance EE activity, but they were most consistent with inoculation shifting EE traits to resemble those observed in sites with lower decomposition rates. Inoculated litterbags exhibited increased activities of C-degrading EE classes such as CBH and BG, much like litterbags transplanted to the low-decomposition subalpine, scrubland, and desert sites. Given that inoculation also reduced mass loss rates, these results indicate that enzymatic efficiency declined as a result of inoculation. Rather than being locally adapted to climate, microbial communities may be more adapted to litter chemistry (Keiser et al., 2011; Wallenstein et al., 2013), such that EEs produced by inoculated microbes are less efficient on grassland litter than on their native substrates.

There is supporting evidence for community adaptation to litter chemistry from our EE V_{max} and V_{max} temperature sensitivity results. EEs in transplanted litterbags exhibited V_{max} values and V_{max} temperature sensitivities that were more similar to one another than to EE temperature sensitivities of native microbial communities on native litter (**Figure C2**). This signal was observed regardless of the transplant destination site. The greater variation in EE V_{max} values across native microbial communities relative to transplanted microbial communities indicates that microbes decomposing native litter in each site produce EEs in different amounts depending on the litter type. The greater variation in EE V_{max} temperature sensitivities across native communities relative to transplanted microbial communities indicates that microbes decomposing native litter in each site are also likely producing EEs distinct from those produced by communities decomposing grassland litter.

Conclusion

The capability of grassland decomposer communities to degrade grass litter after transplantation along our regional climate gradient was not primarily due to differences in precipitation between sites. Litterbags in the site that received the most precipitation lost mass at the same rate as in the grassland, and litterbags in the desert and subalpine site lost similar mass over the course of the study despite large differences in precipitation between the two sites. Instead, climate variables such as precipitation and temperature likely interact to limit decomposition rates at the ends of our gradient, such that the benefits of higher precipitation are outweighed by temperature constraints at the higher elevation sites, while warmer temperature effects are offset by moisture limitation in the hotter low elevation sites. A future shift to a more arid climate may therefore enhance decomposition rates in subalpine forests as they become warmer montane forests, and may reduce decomposition rates in grasslands as they experience reduced precipitation and become more similar to scrublands and desert.

Although grassland microbes in general decomposed grass litter more slowly in foreign sites, the results of our inoculation treatment indicate that access to locally adapted microbes does not increase mass loss rates. Inoculation of litterbags with local microbiota prior to transplantation was instead consistent with the introduction and proliferation of cheaters. It may be that cheater taxa proliferate in the grassland community after inoculation because active decomposers in the inocula community are best adapted to degrade litter chemistries that differ from those found in the grassland. Our EE V_{max} and V_{max} temperature sensitivity results support this hypothesis, as they indicate that microbes

on native litter are likely producing different EEs at different rates from microbes observed in litterbags or in grassland litter *in situ*.

In the context of a predicted future shift to a more arid climate in the American Southwest, our results indicate that microbial communities may not be constrained in their ability to respond to regional climate change, which potentially simplifies efforts to incorporate microbe-explicit mechanisms into global C-cycling models and predict future C dynamics (Allison and Martiny, 2008). However, our study only establishes that grassland microbial communities are not impaired relative to native microbial communities when decomposing grass litter in a wide variety of environments. Previous investigations of decomposer home-field advantage found stronger effects of community origin on decomposition of more complex litter substrates than on the decomposition of grass litter (Strickland et al., 2009), so it may be that transplantation of more complex substrates across a gradient such as ours might reveal stronger historical contingencies of community origin on decomposition dynamics.

Table 3.1 ANCOVA results for effects of site, sampling date, inoculation treatment, and all interactions on mass loss, bacterial cell density, and litter chemistry, and MANCOVA effects of the same factors for V_{max}, K_m, and V_{max} and K_m temperature sensitivity for all enzyme classes (with the exception of AG and AP K_m temperature sensitivity). All analyses were run with gravimetric litter moisture (H_2O) as a covariate. Bolded p-values are significant (<0.05).

Variable	Site	Date	Inoculation	H_2O	Site:Date	Site:Inoc	Date:Inoc	S:D:I
Mass loss	**<0.001**	**<0.001**	**0.002**	**0.009**	**<0.001**	0.978	0.636	0.619
Bacterial density	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.014**	0.089	**<0.001**
Litter chemistry	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.125	0.627	0.639
EE V_{max}	**<0.001**	**<0.001**	0.056	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.087
EE K_m	**<0.001**	**<0.001**	0.331	**<0.001**	0.246	**<0.001**	0.110	0.122
EE V_{max} TS	**<0.001**	**<0.001**	0.359	0.224	**<0.001**	0.630	0.641	0.898
EE K_m TS	**<0.001**	**<0.001**	0.414	**0.004**	**<0.001**	**0.038**	0.546	0.933

Table 3.2 Mean (±SE) EE Km (in μM) across all EE classes in initial litter as well as in litterbags collected from each site in June and December 2015. Mean EE K_m across all sites at each sampling date is presented because EE K_m did not differ between sites at either sampling date after accounting for the effect of litter moisture.

Date	Mean	Subalpine	Forest	Grassland	Scrubland	Desert
Initial	288.1±21.5					
June	157.7±3.7	157.1±8.5	144.0±6.5	177.9±9.7	151.6±8.6	162.9±9.0
December	292.5±6.8	247.3±13.0	293.7±12.2	325.9±15.9	293.3±16.9	309.7±17.6

Table 3.3 Mean (±SE) EE V_{max} Q_{10} from litterbags collected on the final sampling date in December 2015. Bolded values are significantly different ($p<0.05$) from those observed in grassland litterbags.

	AG	AP	BG	BX	CBH	LAP	NAG
Subalpine	**1.96±0.02**	1.55±0.02	1.87±0.02	1.99±0.02	2.14±0.03	1.84±0.03	**1.86±0.02**
Forest	**1.95±0.03**	1.53±0.02	1.86±0.02	2.07±0.03	2.07±0.03	**1.79±0.02**	**1.90±0.02**
Grassland	2.18±0.03	1.60±0.02	1.88±0.02	2.02±0.03	2.11±0.03	1.92±0.02	2.01±0.02
Scrubland	**1.95±0.03**	1.51±0.02	**1.68±0.03**	**1.84±0.03**	**1.93±0.03**	1.81±0.02	**1.79±0.02**
Desert	2.17±0.08	1.57±0.01	1.85±0.04	**1.90±0.02**	2.15±0.06	1.88±0.04	1.95±0.03

Table 3.4 Mean (±SE) enzyme K_m Q_{10} from litterbags collected on the final sampling date in December 2015. Bolded values are significantly different ($p<0.05$) from those observed in grassland litterbags.

	BG	BX	CBH	NAG
Subalpine	2.00±0.04	1.32±0.02	2.07±0.06	1.62±0.03
Forest	1.89±0.03	**1.43±0.03**	**1.81±0.04**	1.66±0.03
Grassland	1.99±0.03	1.34±0.02	2.02±0.03	1.69±0.02
Scrubland	**1.76±0.04**	1.30±0.03	1.91±0.03	**1.59±0.02**
Desert	2.09±0.05	1.31±0.02	**2.27±0.05**	1.70±0.02

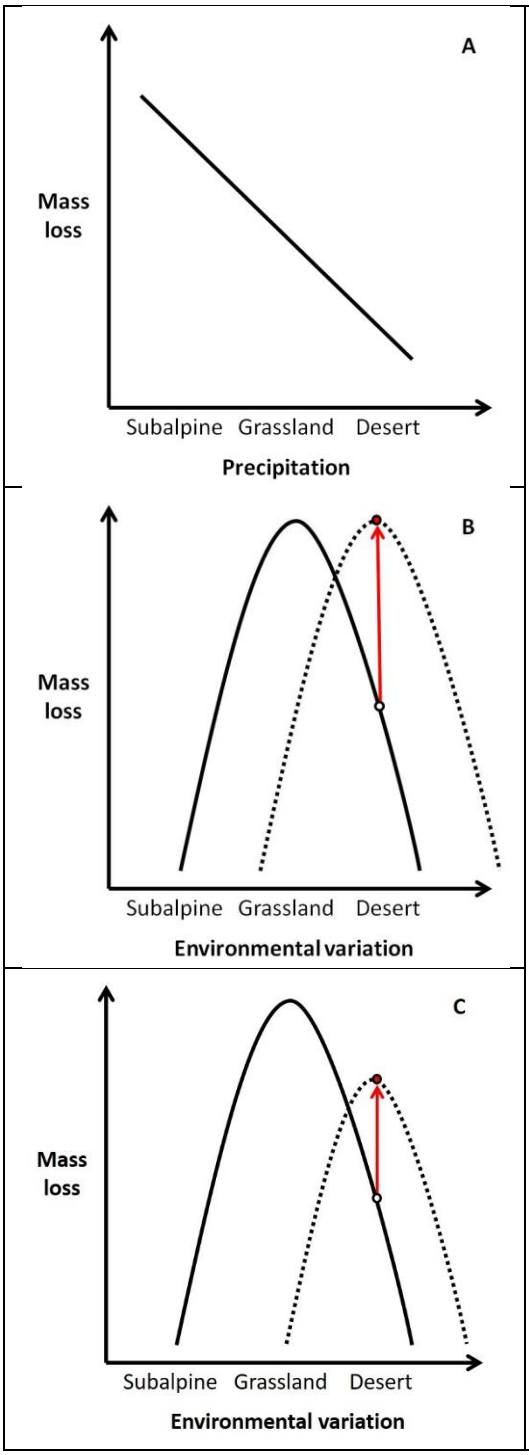

Figure 3.1 Conceptual figure of hypotheses for transplantation effects on decomposition rates.
A) Hypothesized microbial decomposition assuming that climate (specifically, precipitation), is the main driver of mass loss.
B) Hypothesized microbial decomposition assuming that decomposer communities are most adapted to their native environment and constrained in their ability to decompose litter in foreign environments. The solid line shows decomposition by grassland microbial communities, and the dotted line shows decomposition by desert microbial communities. In this scenario, access to local microbial communities will positively affect decomposition after transplantation, as shown by movement along the red arrow.
C) Hypothesized microbial decomposition assuming that the effects of climate and local adaptation combine to drive mass loss rates across our gradient, such that local microbiota at the extremes of our gradient are adapted to conditions that are inherently less favorable for decomposition.

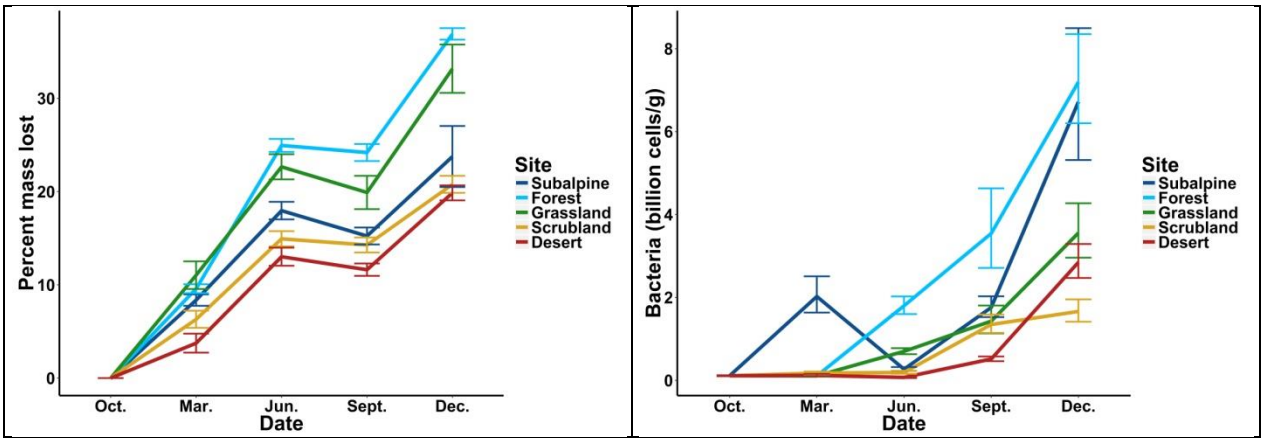

Figure 3.2 Mean (±SE) **A**) mass loss and **B**) bacterial cell density in transplanted litterbags over the course of the study, averaged across both grassland and inoculated litterbags in each site at each sampling date. Depicted means and standard errors are back-transformed from ln values.

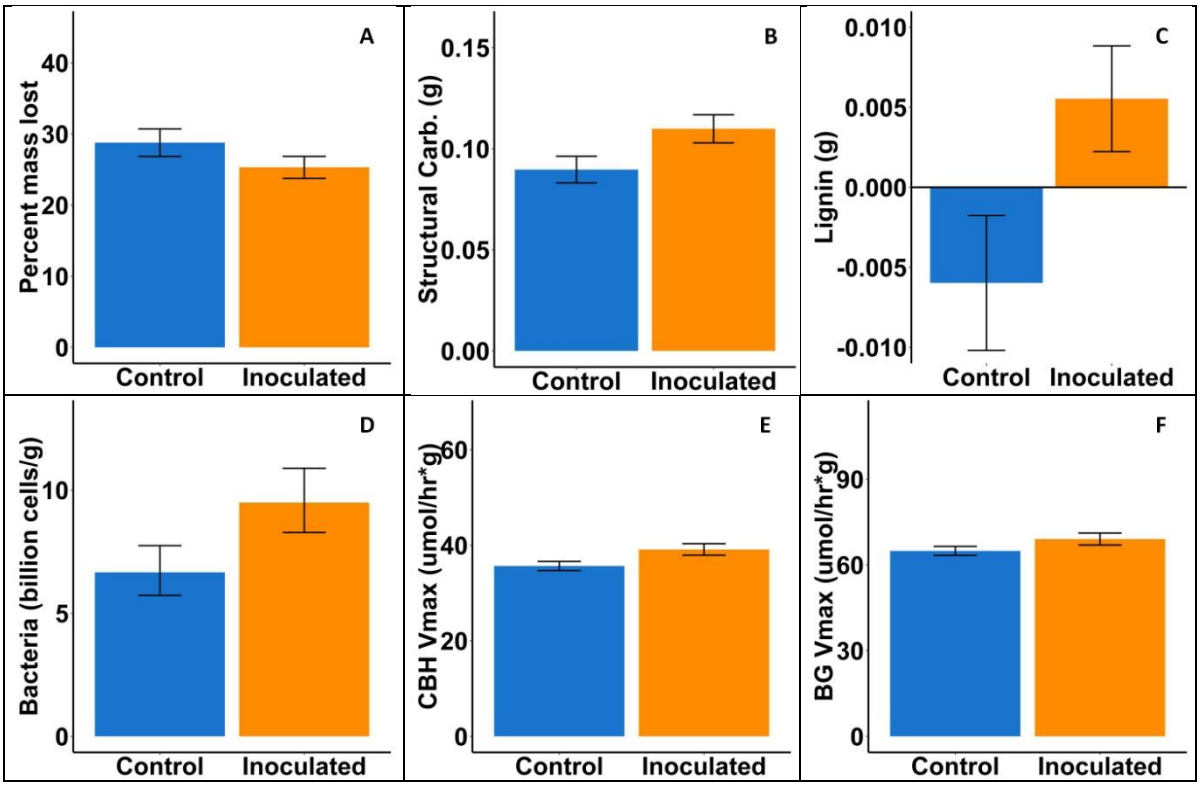

Figure 3.3 Effect of inoculation with local microbial communities on mean (±SE) **A**) mass loss, **B**) accumulation of structural carbohydrates and **C**) change in the mass of the lignin fraction by the final sampling date, as well as the effect on mean (±SE) **D**) bacterial cell density, **E**) cellobiohydrolase V_{max}, and **F**) β-glucosidase V_{max} over the course of the study. All effects shown are significant (Tukey $p<0.05$).

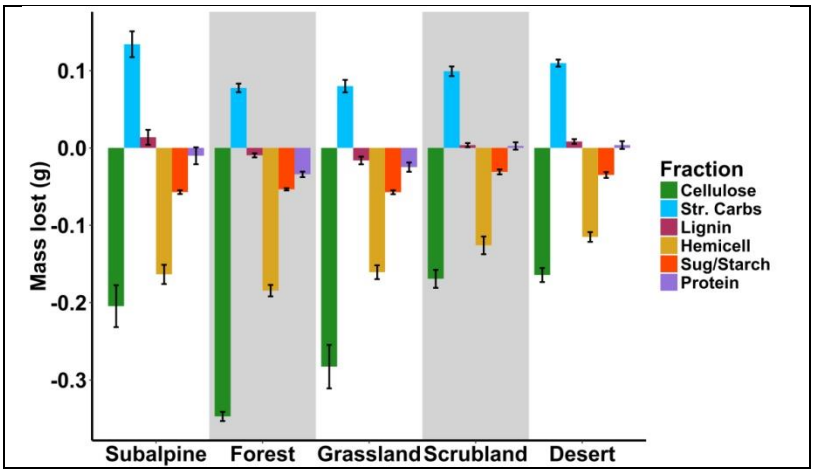

Figure 3.4 Mean (±SE) mass loss from each major component of litter by December 2015. Positive values indicate accumulation of that fraction.

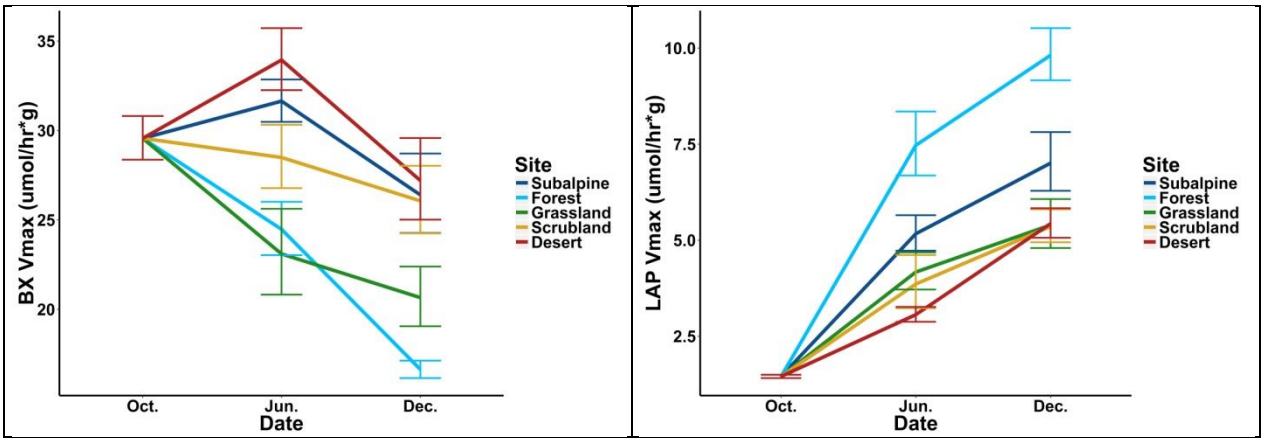

Figure 3.5 Mean (±SE) EE V_{max} for **A**) β-xylosidase and **B**) Leucine aminopeptidase, in initial grassland litter and in June and December 2015 litterbags from each site.

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APPENDIX A

Chapter 1 Supplemental

Table A1. P-values from ANOVA for litter moisture with respect to UV treatment, litter type, time of sampling, and all possible interactions. Significant ($p<0.05$) p-values are bolded. N_{df} and D_{df} are the degrees of freedom for the numerator and denominator of the F-statistic, respectively.

	UV	Litter (L)	Time (T)	UV:L	UV:T	L:T	UV:L:T
Litter moisture	**0.040**	**<0.001**	**<0.001**	0.645	0.555	0.392	0.963
N_{df}, D_{df}	1, 73	1, 73	3, 73	1, 73	3, 73	3, 73	3, 73

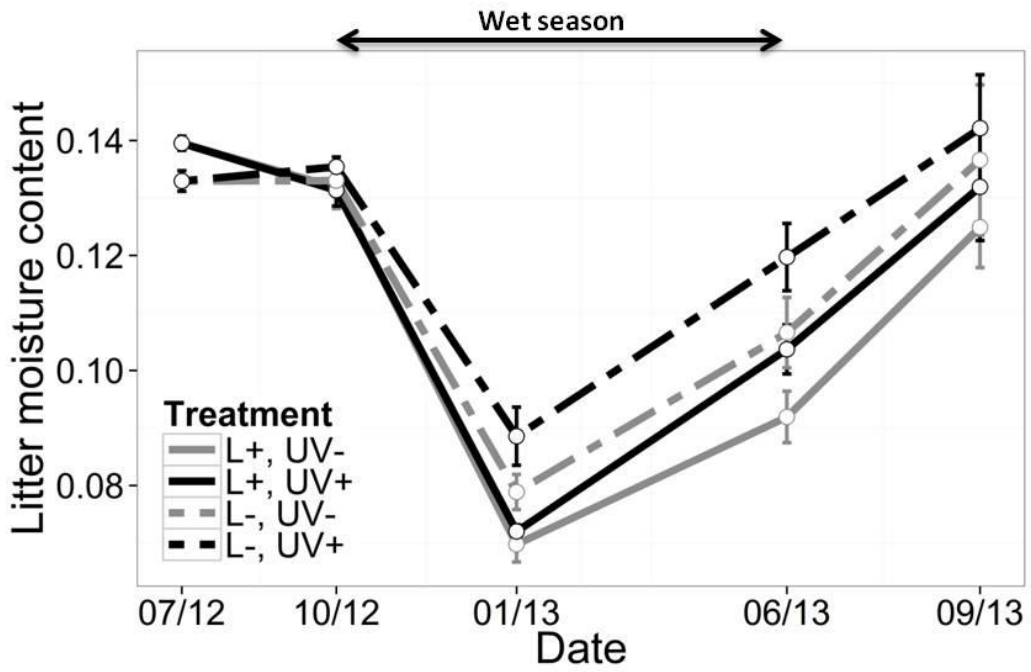

Figure A1. Litter moisture content as fraction of total litter mass. Low lignin samples (L-) are shown with dotted lines, and high lignin samples (L+) with solid lines. UV pass treatments (UV+) are shown in black, UV block treatments (UV-) are in grey. Symbols represent means ± SE. The double-headed line above the plot indicates the duration of the wet season.

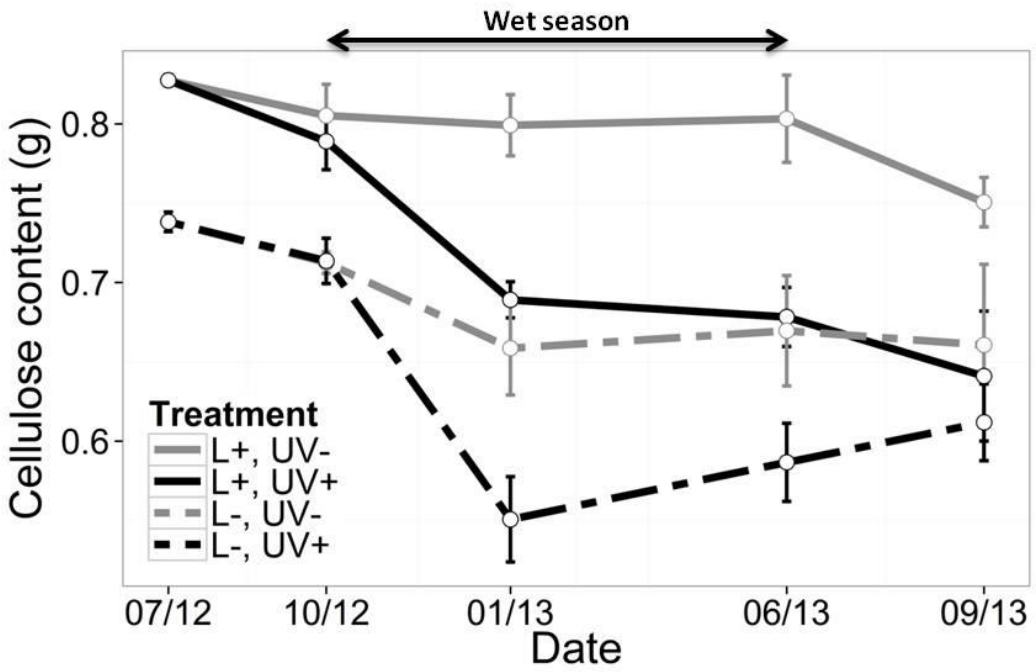

Figure A2. Litter cellulose content in grams at each sampling point.

APPENDIX B

Chapter 2 Supplemental

Table B1. Enzymes, substrates, and substrate concentrations used in this study.

Enzyme	Putative substrate	Synthetic substrate	[Substrate]
α-glucosidase (AG)	Starch degradation products	4-MUB-*α*-D-glucopyranoside	1000 µM
Acid phosphatase (AP)	Organic P	4-MUB Phosphate	4000 µM
β-glucosidase (BG)	Cellulose degradation products	4-MUB-*β*-D-glucopyranoside	2000 µM
β-xylosidase (BX)	Hemicellulose degradation products	4-MUB-*β*-D-xylopyranoside	2000 µM
Cellobiohydrolase (CBH)	Cellulose degradation products	4-MUB-*β*-D-cellobioside	1000 µM
Leucine-aminopeptidase (LAP)	Peptide terminals	L-leucine-7-amido-4-methylcoumarin hydrochloride	1000 µM
N-acetyl-*β*-D-glucosaminidase (NAG)	Chitin degradation products	4-MUB-*N*-acetyl-*β*-D-glucosaminide	2000 µM

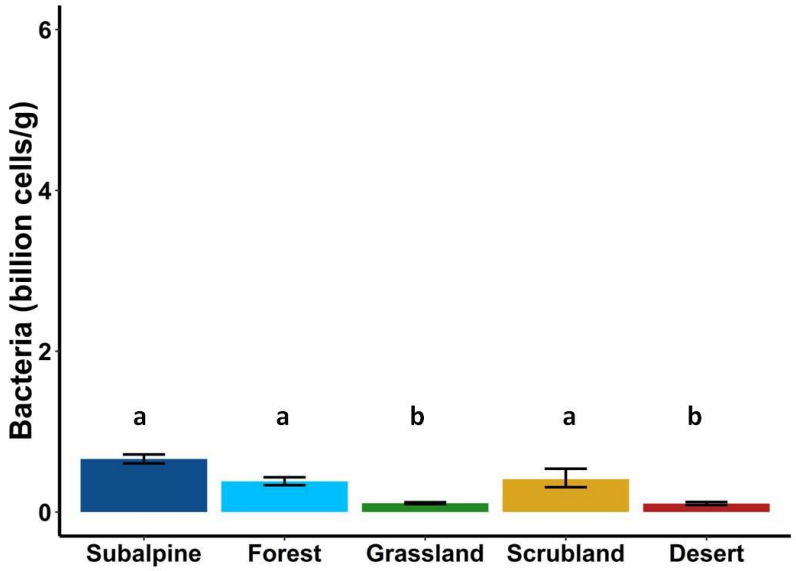

Figure B1. Mean bacterial cell density (billions of cells/g dry litter) in October 2014. Error bars denote standard error. Depicted means and standard errors are back-transformed from ln values. Means sharing the same letter are not statistically different ($p > 0.05$).

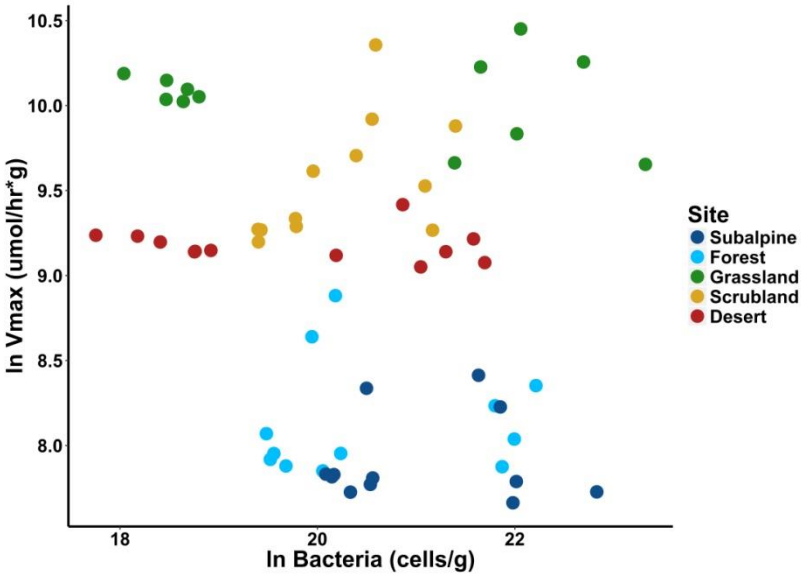

Figure B2. Mean EE V_{max} (μmol·hr^{-1}·g^{-1}) as a function of bacterial cell density (cells/g dry litter). October 2014 and December 2015 samples are depicted due to lack of bacterial cell density data for June 2015 samples.

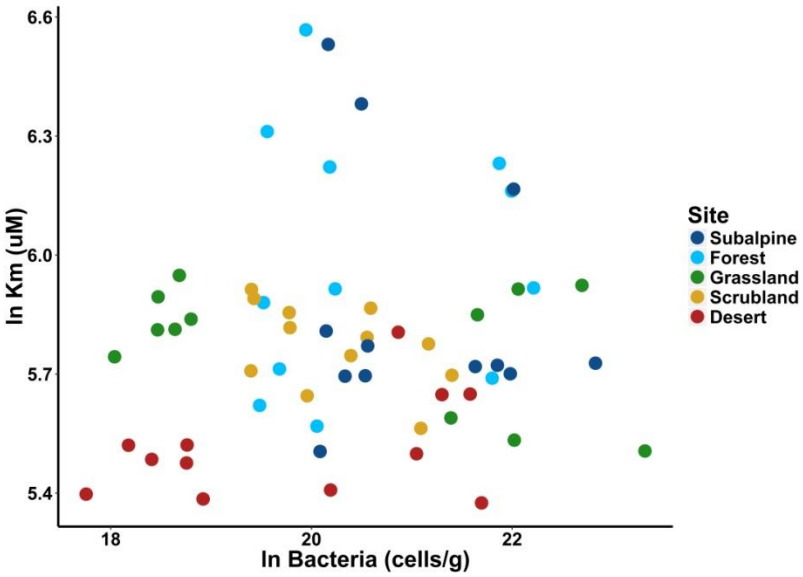

Figure B3. Mean EE K_m (µM) as a function of bacterial cell density (cells/g dry litter). October 2014 and December 2015 samples are depicted due to lack of bacterial abundance data for June 2015 samples.

APPENDIX C

Chapter 3 Supplemental

Table C1. Mean (± SE) historic climate parameters for the five sites used in this study, and plot-level temperature over the course of one year encompassing both sampling dates.

Variable	Subalpine	Forest	Grassland	Scrubland	Desert	Period
Air temperature (°C)	*10.3±1.8*	12.3±0.6	16.4±0.3	15.6±0.8	22.8±0.8	2009-14
Air daily temp. range	-	6.2±0.2	8.3±0.3	8.5±0.2	10.8±0.4	2009-14
Soil temperature (°C)	-	9.9±0.3	19.1±0.9	18.4±0.3	28.3±0.3	2008-12
Soil daily temp. range	-	2.6±0.1	4.6±0.8	5.5±0.7	10.9±0.2	2008-12
Plot temperature (°C)	11.5±0.4	13.2±0.4	22.0±0.4	19.5±0.5	29.3±0.4	2015
Plot daily temp. range	13.7±0.4	20.8±0.5	26.2±0.5	24.8±0.4	20.3±0.3	2015
Rainfall (mm)	*~265*	402±118	242±76	193±33	100±24	2009-14
Soil moisture (mL/cm^3)	-	8.6±0.3	7.3±0.8	7.3±0.5	4.6±0.1	2008-12
Solar radiation (KW/m^2)	*>1850*	2640±500	2540±470	2770±520	2620±500	2006-13
Elevation (m)	2250	1710	470	1280	275	

Table C2. Enzymes, substrates, and substrate concentrations used in this study.

Enzyme	Putative substrate	Synthetic substrate	[Substrate]
α-glucosidase (AG)	Starch degradation products	4-MUB-α-D-glucopyranoside	1000 µM
Acid phosphatase (AP)	Organic P	4-MUB Phosphate	4000 µM
β-glucosidase (BG)	Cellulose degradation products	4-MUB-β-D-glucopyranoside	2000 µM
β-xylosidase (BX)	Hemicellulose degradation products	4-MUB-β-D-xylopyranoside	2000 µM
Cellobiohydrolase (CBH)	Cellulose degradation products	4-MUB-β-D-cellobioside	1000 µM
Leucine-aminopeptidase (LAP)	Peptide terminals	L-leucine-7-amido-4-methylcoumarin hydrochloride	1000 µM
N-acetyl-β-D-glucosaminidase (NAG)	Chitin degradation products	4-MUB-*N*-acetyl-β-D-glucosaminide	2000 µM

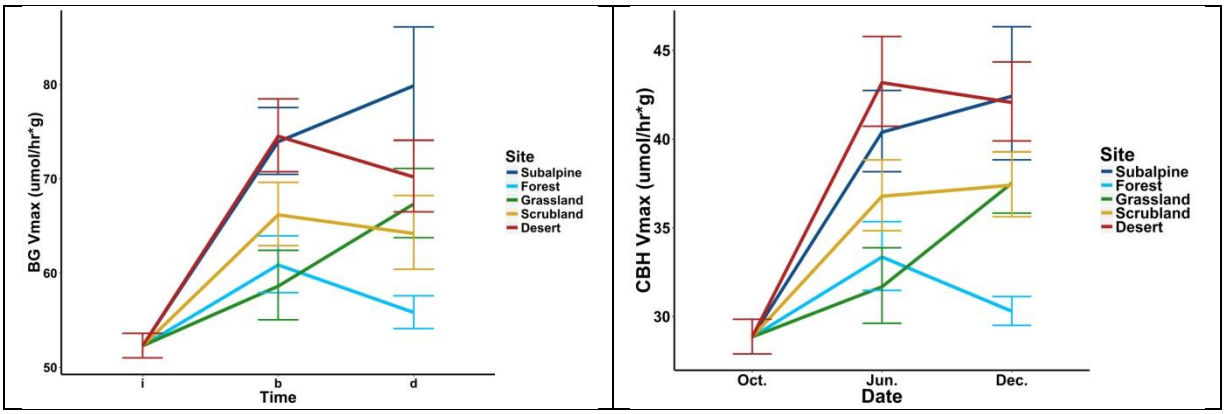

Figure C1. Mean V_{max} of **A**) β-glucosidase and **B**) cellobiohydrolase in initial litter and litterbags collected in June and December 2015. Error bars denote standard error. Depicted means and standard errors have been back-transformed from ln values.

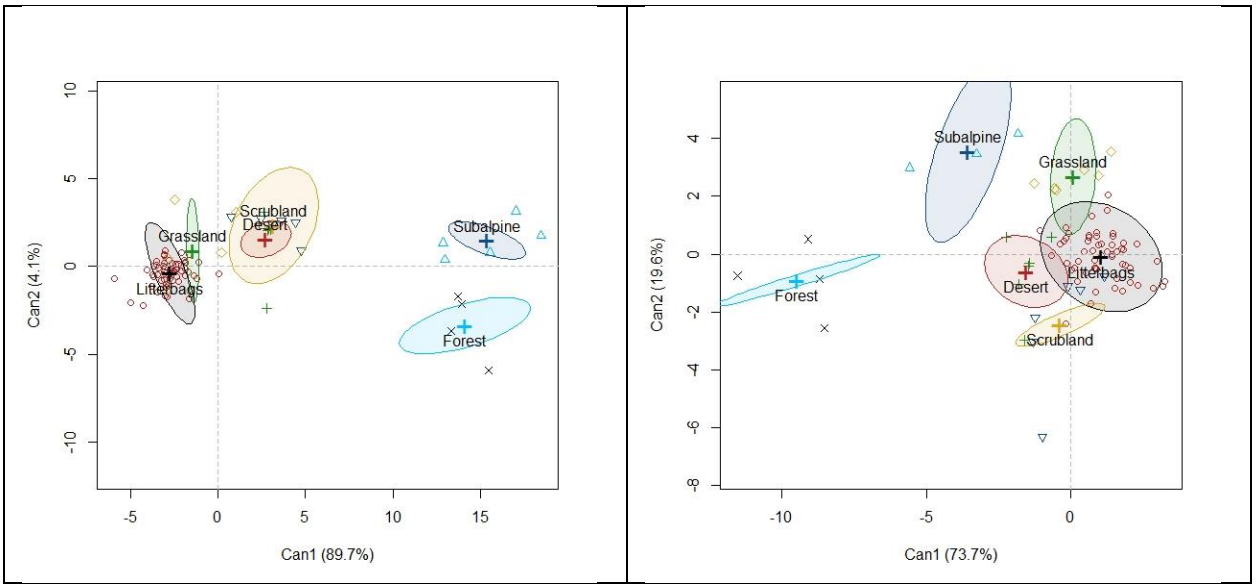

Figure C2. Canonical discriminant ordinations of differences in **A**)EE V_{max} and **B**)EE V_{max} temperature sensitivities of all EEs assayed. Plots depict differences between native litter from each site ("Subalpine", "Forest", etc.) and litter in transplanted litterbags from all sites ("Litterbags") in December 2015. Note that EE V_{max} values and V_{max} temperature sensitivities observed in transplanted litterbags group together, whereas EE traits observed in native litter differ much more widely.