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RESEARCH ARTICLE

Microbial extracellular enzyme activity with simulated climate change

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It is critical to understand the consequences of environmental change for the microbial regulation of carbon and nutrient cycling. Specifically, understanding microbial community traits, such as extracellular enzyme activity, can help inform nutrient cycling models and address knowledge gaps. We analyzed data on extracellular enzyme activities and litter decomposition from an 18-month experiment in which microbial communities were reciprocally transplanted along a climate gradient in Southern California. Communities were from desert, scrubland, grassland, pine–oak, and subalpine ecosystems. We aimed to test how enzyme activities responded to climate change following transplantation and how those responses related to decomposition rates. We hypothesized that microbial communities would specialize on their native climate conditions, resulting in higher enzyme activities when transplanted back into their native climate. We investigated the relationship between extracellular enzyme V_{max} values, substrate mass loss, and microbial biomass as well as variation in these variables across the climate gradient. We found little evidence for climate specialization, and there was rarely a reduction in enzyme functioning after microbial communities were transplanted into new climate conditions. Moreover, observed differences in decomposition were not related to changes in extracellular enzyme potential, although there were significant differences in enzyme activities and decomposition rates across sites. These results suggest that direct, physiological impacts of climate are likely to be important for enzyme-mediated decomposition, but climate specialization will not constrain the microbial response to climate change in our system.

Keywords: Extracellular enzymes, Climate specialization, Reciprocal transplant, Climate gradient

1. Introduction

Understanding the implications of global warming for decomposition and subsequently, carbon and nutrient cycling is an urgent need (Cavicchioli et al., 2019). Microbial decomposition depends on climate variables, such as temperature and precipitation, which directly affect the abiotic environment (Swift et al., 1979; Allison, 2006; Chapin et al., 2012). Climate may also have indirect effects on decomposition by altering substrate inputs (Hobbie, 1992). Therefore, investigating the impacts of climate change on microbial decomposition is critical for predicting carbon and nutrient cycling.

There is increasing evidence that decomposition responses to climate change may depend not only on abiotic conditions but also on microbial community composition. For example, some studies have found that plant litter from a given site is decomposed more rapidly by microbial communities from that same site (Gholz et al., 2000; Ayres et al., 2009). Still, the mechanisms underlying such community effects remain unclear, making it difficult to predict how soil carbon and nutrient cycling will respond to climate change across ecosystems.

One of the key community-level traits relevant to decomposition is the production of extracellular enzymes. Microbes produce extracellular enzymes to break down complex polymers into soluble carbon and nutrients that can be taken up and used to fuel microbial metabolism (Sinsabaugh and Moorhead, 1994; Burns et al., 2013). The costs of enzyme production may result in specialization to produce only enzymes that degrade the specific litter compounds in the local environment (Allison et al., 2010). In a new environment with different litter chemistry, those specialized enzymes might be less effective, leading to a "home field advantage" for the native microbial community (Gholz et al., 2000; Ayres et al., 2009).

Although there is evidence that microbial communities and their extracellular enzymes specialize on different litter substrates, it is less clear whether communities and enzymes also specialize on different climate conditions. In previous studies with reciprocal litter transplants, both climate and litter substrates varied across sites, making it difficult to disentangle these potential drivers of community specialization (Gholz et al., 2000; Ayres et al., 2009). Like substrate, climate variables could select for production of extracellular enzymes with site-specific

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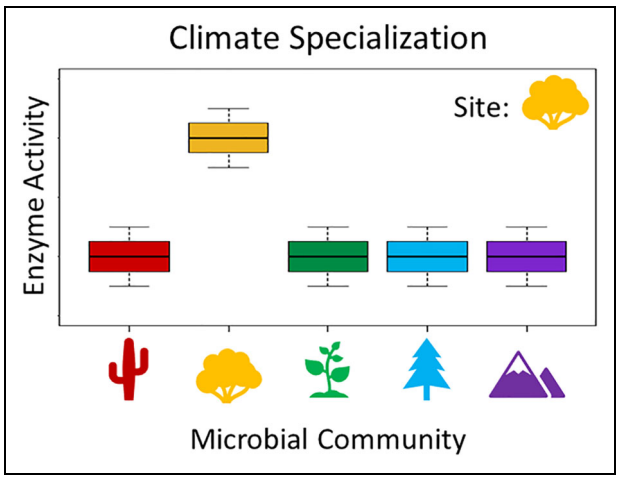

Figure 1. Hypothetical example of climate specialization in which the scrubland microbial community shows the highest extracellular enzyme activity in the scrubland site. DOI: https://doi.org/10.1525/elementa.2021.00076.f1

properties that affect decomposition activity. For example, extracellular enzyme temperature or moisture sensitivity might be tuned to the local climate in a way that alters enzyme kinetic properties (Alster et al., 2020).

Testing for climate specialization requires holding litter substrate constant and measuring the performance of microbial communities across different climate conditions. Recently, Glassman et al. (2018) conducted such a test using a reciprocal transplant design along a climate gradient in Southern California with desert, scrubland, grassland, and forest ecosystems. Although there was no evidence for climate specialization with overall decomposition (i.e., home field advantage based on climate), there were microbial community effects on decomposition rates (Glassman et al., 2018). The microbial community from the grassland ecosystem decomposed a common litter substrate fastest in the scrubland ecosystem and vice versa.

Building on the Glassman et al. (2018) study of decomposition rates and microbial composition, our goal was to test whether microbial enzyme activities showed climate specialization. Using Glassman et al.'s reciprocal transplant design, we simulated climate change effects on microbial communities while holding litter substrate constant. We tested the climate specialization hypothesis, which predicts that extracellular enzyme activities of native microbial communities should be greater than those of non-native microbial communities (**Figure 1**). Although Glassman et al. found no evidence for climate specialization with overall litter decomposition, we tested whether the decay rates of specific litter chemical compounds were specialized to climate and related to specific extracellular enzyme classes or microbial biomass.

2. Materials and methods

2.1. Climate gradient

Our climate gradient includes 5 sites in desert, scrubland, grassland, pine–oak, and subalpine ecosystems (**Figure 2**; Table S1; Baker and Allison, 2017). Temperature and precipitation vary inversely along the gradient: The desert site shows the hottest (22.8°C $\pm$ 0.8°C) and driest climate (100 $\pm$ 24 mm mean annual precipitation), and the subalpine is the coldest site (10.3°C $\pm$ 1.8°C) with the most precipitation (approximately 265 mm; Table S1).

Microbial communities were reciprocally transplanted across 5 sites: desert (D), scrubland (Sc), grassland (G), pine–oak (P), and subalpine (S). These sites vary inversely with temperature and precipitation (Table S1). Litter was collected from each site and individually inoculated onto γ-irradiated grassland leaf litter substrate. Nylon membrane bags containing the inoculum and the substrate were placed in cages and randomly distributed within sites. The bags remained in the field for 18 months and were destructively sampled every 6 months.

2.2. Reciprocal transplant design

Microbial communities from each site along the climate gradient were transplanted into all sites to simulate climate change (**Figure 2**). A microbial community inoculum was created by collecting 4 samples from each of the sites on September 11, 2015, and homogenizing the samples within each site. Fifty milligrams of the site inoculum was added to 5 g irradiated, homogenized grassland leaf litter in sterilized litter bags. To keep the experiment manageable, we only used senesced grass as the litter type because grasses were present at all climate gradient sites. The grassland leaf litter was homogenized with coffee grinders. The litter bags were nylon membrane bags with 0.22 µm pores (cat. no. SPEC17970; Tisch Scientific, Cleves, OH, USA) that allowed nutrients and water to travel freely though the bag. However, the bags did not allow for the movement of microbes and leaf litter.

On October 19, 2015, 300 bags were deployed along the gradient (4 replicates $\times$ 5 sites $\times$ 5 microbial community inocula $\times$ 3 timepoints $=$ 300 bags). This design and level of replication had the statistical power to detect significant site by inoculum interactions that explained as little as 16% of the variation in mass loss within a timepoint (Glassman et al., 2018). The bags were placed in four 1 $\times$ 1 m plots in each ecosystem and destructively sampled every 6 months over an 18-month period. At each of the 3 timepoints, 100 bags were collected (Timepoint 1: April 5, 2016; Timepoint 2: October 24, 2016; Timepoint 3: April 18, 2017). For information on microbial community taxonomic composition and shifts in litter chemistry, see Glassman et al. (2018).

2.3. Substrate mass loss

We reanalyzed litter chemistry data collected previously by calculating the ash-free mass and concentrations of cellulose, hemicellulose, lignin, crude protein, and starch (Baker and Allison, 2017; Glassman et al., 2018). For individual substrate mass loss, we subtracted the Timepoint 2 substrate mass from the initial mass of each substrate. Litter chemistry data were only collected for the initial litter and Timepoint 2. Statistical tests were conducted on the mass change, not the percentage loss. Final substrate mass values are available on Github: https://github.com/stevenallison/UCIClimateExperiment/.

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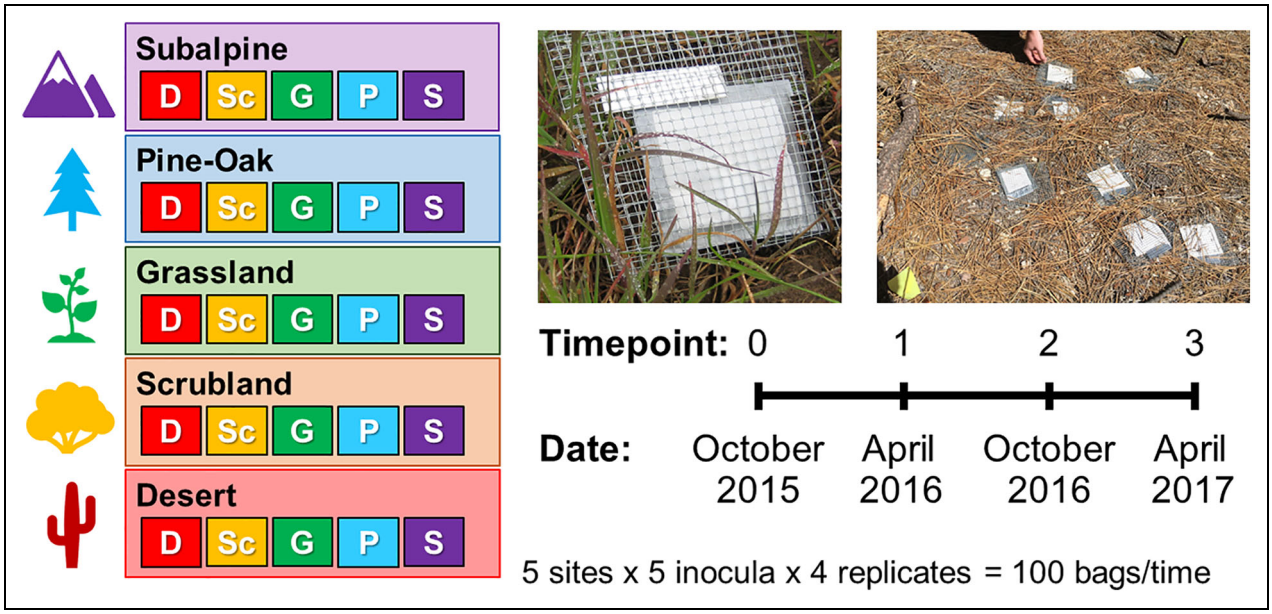

Figure 2. Climate gradient and experimental setup. DOI: https://doi.org/10.1525/elementa.2021.00076.f2

Table 1. Enzyme classes, the substrates they act upon, and the synthetic substrates used in this study. DOI: https://doi.org/10.1525/elementa.2021.00076.t1

Enzyme Class	Substrate	Synthetic Substrate	[Substrate](μM)[a]
α-glucosidase	Starch	4-MUB-α-D-glucopyranoside	1.5625–200
β-glucosidase	Cellulose	4-MUB-β-D-glucopyranoside	3.125–400
β-xylosidase	Hemicellulose	4-MUB-β-D-xylopyranoside	3.125–400
Cellobiohydrolase	Cellulose	4-MUB-β-D-cellobioside	1.5625–200
Leucine aminopeptidase	Protein	L-leucine-7-amido-4-methylcoumarin hydrochloride	1.5625–200
Polyphenol oxidase	Lignin	Pyrogallol	7.8125–1,000

[a] The substrate concentration refers to the concentrations of synthetic substrate. There were 8 concentrations: the maximum concentration and 7 serial dilutions.

2.4. Microbial biomass

Fungal hyphal lengths were measured with microscopy, and bacterial cell counts were measured using flow cytometry (Glassman et al., 2018). Subsequently, both hyphal lengths and bacterial counts were converted to carbon content per gram litter (g C/g) using previously established methods in the literature described by Baker et al. (2017) and Alster et al. (2013). Bacterial cells were considered to have C density of 2.2×10^{-13} g μm^{-3} and treated as spherical with a radius of 0.6 μm (Bratbak, 1985). We assumed fungal hyphae had a diameter of 5.2 μm, 40% C in dry mass, 33% dry mass, and a density of 1.1 g cm^{-3} of hyphae (Paul and Clark, 1996). Microbial biomass is reported as the sum of both bacterial and fungal biomass. Fungal hyphal lengths were not measured at Timepoint 3, so fungal and total microbial biomass are only reported for Timepoints 1 and 2. Fungal and bacterial abundance data are also available on Github: https://github.com/stevenallison/UCIClimateExperiment/.

2.5. Extracellular enzyme assays

Fluorometric and oxidative assays measuring the extracellular enzyme activities of α-glucosidase (AG), β-glucosidase (BG), β-xylosidase (BX), cellobiohydrolase (CBH), leucine aminopeptidase (LAP), and polyphenol oxidase (PPO) were conducted using previously published methods (German et al., 2011; Baker and Allison, 2017). Litter samples (0.4 g) stored at –80°C were thawed and combined with 150 mL maleate buffer (2 5 mM) at pH 6.0 to create a homogenate. Next, 125 μL of litter homogenate was combined with 125 μL of fluorometric substrate solution in microplate wells. Standards and substrate solution were made in maleate buffer. The assays were incubated at 4°C, 16°C, 22°C, 28°C, or 34°C for 4 h. Enzymes were also assayed at a range of 8 substrate concentrations at each of the temperatures (**Table 1**). A plate reader was then used to measure substrate fluorescence or absorbance values. We calculated activity as μmol h^{-1} g^{-1} dry litter based on standard calculations (German et al., 2011).

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Table 2. Multivariate analysis of variance (MANOVA) and analysis of variances (ANOVAs) *P* values of natural log transformed microbial extracellular enzyme V_{max} at 22°C. DOI: https://doi.org/10.1525/elementa.2021.00076.t2

Variables	MANOVA	AG	BG	BX	CBH	LAP	PPO
Inoculum	**<0.001**	**<0.001**	**0.010**	**<0.001**	**0.003**	**0.001**	**0.001**
Site	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Timepoint	**<0.001**	**<0.001**	**0.001**	0.308	**<0.001**	**<0.001**	**<0.001**
Inoculum × Site	**<0.001**	**<0.001**	**<0.001**	**0.020**	**<0.001**	**<0.001**	0.407
Inoculum × Timepoint	**0.025**	0.257	0.226	0.251	0.081	**0.038**	0.553
Site × Timepoint	**<0.001**	**<0.001**	0.056	**0.002**	0.052	**<0.001**	**<0.001**
Inoculum × Site × Timepoint	**0.002**	0.090	0.832	0.802	0.413	**<0.001**	0.265

Only statistically significant *P* values are shown in bold font. AG = α-glucosidase; BG = β-glucosidase; BX = β-xylosidase; CBH = cellobiohydrolase; LAP = leucine aminopeptidase; PPO = polyphenol oxidase.

Activities from the assays were fit to the Michaelis–Menten model using the "nls" R package to obtain maximum reaction velocities, V_{max} (German et al., 2011; Baty et al., 2015). The Michaelis–Menten model describes enzyme reaction velocity as a function of substrate concentration. We fit natural log-transformed V_{max} values at all the temperatures to a linear model and extracted the natural log V_{max} at 22°C from the model for further analysis.

2.6. Statistical analyses

All statistical analyses were completed with R Version 4.1.0 (R Core Team, 2021). First, we performed multivariate analysis of variance (MANOVA) on natural log-transformed potential extracellular enzyme V_{max} to test the fixed effects of site, inoculum, and time on overall enzyme activities. Analysis of variance (ANOVA) was then applied to the individual extracellular enzyme V_{max} values using the "car" package in R (Fox and Weisberg, 2019) to test for site, inoculum, and time effects. Also using the R "car" package, Type III tests were applied to enzymes that showed a significant 3-way interaction, and Type II tests were applied to those that did not (Fox and Weisberg, 2019). This sequential approach was used to avoid Type-1 errors in hypothesis testing. Post hoc ANOVAs on individual enzymes were only run to determine the enzymes driving significant results from the MANOVA.

A significant interaction effect ($P < 0.05$) between site and inoculum may indicate support for climate specialization. However, climate specialization also requires that the native community V_{max} is significantly higher than the non-native communities' V_{max} values. Most often, the extracellular enzymes did not have a 3-way interaction effect, so we averaged the extracellular enzyme potential activity across timepoints for statistical analyses (Figures S1–S3). LAP was the only enzyme with a significant 3-way interaction effect, so we also analyzed LAP activity without averaging over time (Figure S4). We applied pairwise comparisons, specifically Tukey post hoc analyses, to test for significant differences among communities within a site. We performed the Tukey post hoc analyses with "multcomp" package, using the "glht" (general linear hypotheses) and "cld" (compact letter display) functions (Hothorn et al., 2008). Again, to avoid Type-1 errors, post hoc Tukey comparisons were only run if the enzyme ANOVA showed a significant site by inoculum interaction.

To further test for mechanistic relationships between extracellular enzymes and substrate mass changes, we performed Pearson's product-moment correlation tests between natural log-transformed extracellular enzyme V_{max} values and their associated substrate mass losses. Similarly, we applied correlation tests to examine the relationship between natural log-transformed microbial biomass and substrate mass loss. We used the base R "stats" package with the "cor.test" function for the correlation tests (R Core Team, 2021).

3. Results

3.1. Potential extracellular enzyme activities

MANOVA results indicated that site, inoculum, and time had significant ($P < 0.001$) effects on the collective, natural log-transformed extracellular enzyme V_{max} values (**Table 2**). Furthermore, there were significant ($P < 0.05$) interactions between the fixed factors. ANOVA results indicated that inoculum, site, and timepoint each had significant effects on activity for all enzymes except BX, which did not show a significant timepoint effect (**Table 2**). There was a significant site by timepoint interaction for all extracellular enzymes, except those that act on cellulose, BG, and CBH (**Table 2**). All enzymes, except PPO, showed a significant inoculum by site interaction (**Table 2**). Further, Tukey post hoc tests only supported climate specialization in the scrubland site for BX and in the subalpine site for BG, LAP, and PPO (Figures S1–S3). LAP was the only EE with a significant Timepoint × Inoculum × Site interaction effect ($P < 0.05$; **Table 2**). For LAP, potential activities at all sites and timepoints revealed only 2 potential instances of climate specialization: the subalpine site at Timepoint 1 and the desert site at Timepoint 3 (Figure S4).

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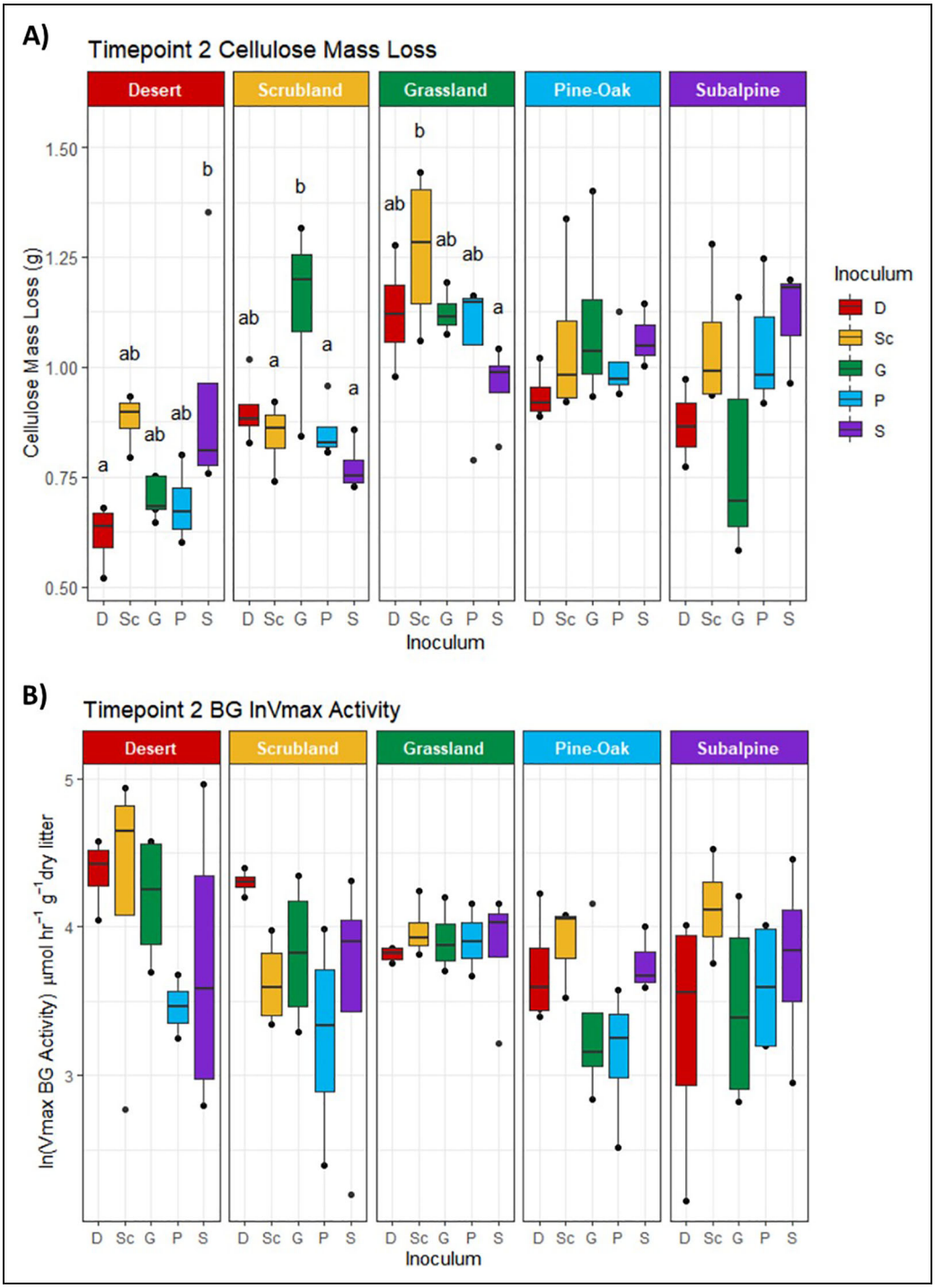

Figure 3. Extracellular enzyme activity and mass loss at Timepoint 2. A) Cellulose-specific mass loss at Timepoint 2. B) Log-transformed V_{max} of BG (β-glucosidase), a carbohydrate degrading extracellular enzyme, at Timepoint 2. Boxplots represent median values with the upper and lower quartiles. Whiskers cover the data range, excluding outlying data points. Within a site, inocula sharing the same Tukey letters above the boxplots are not significantly different from one another. Panels without Tukey letters did not show any significant differences. Extracellular enzyme activity did not reflect climate specialization since there were no significant differences in BG activity within sites. Additionally, BG activity did not have a significant correlation with cellulose mass loss (Table S2) and did not reflect substrate mass loss patterns. DOI: https://doi.org/10.1525/elementa.2021.00076.f3

3.2. Extracellular enzyme activities and mass loss

Compared to other substrates, cellulose had the highest mass loss, similar to the total mass loss patterns (**Figure 3A**; Glassman et al., 2018). At Timepoint 2, there were no significant differences in BG V_{max} values across the transplanted microbial communities within each site that would explain differences in cellulose decomposition (**Figure 3B**). Furthermore, at Timepoints 1 and 2, the enzymes and their respective substrate mass changes did not exhibit any significant ($P < 0.05$) correlations

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Table 3. Pearson correlations of substrate mass loss at Timepoint 2 and natural log transformed biomass. DOI: https://doi.org/10.1525/elementa.2021.00076.t3

Substrate[a]	Cellulose		Hemicellulose		Lignin		Protein		Starch	
Timepoint[b]	1	2	1	2	1	2	1	2	1	2
Bacterial[c]	**0.494***** [d]	N.S.	**0.581*****	N.S.	**−0.269****	**0.285****	N.S.	**0.436*****	**0.329****	N.S.
Fungal[c]	N.S.	N.S.	N.S.	**0.296***	N.S.	**−0.305****	N.S.	N.S.	N.S.	**0.352****
Microbial[c]	**0.501*****	N.S.	**0.589*****	N.S.	**−0.282****	**0.251***	N.S.	**0.442*****	**0.313****	N.S.

N.S. = not significant.

[a] Substrate mass loss at only Timepoint 2.

[b] Timepoint that the natural log-transformed biomass was collected and tested against the substrate mass loss.

[c] Bacterial indicates only bacterial biomass, fungal indicates only fungal biomass, and microbial indicates the sum of fungal and bacterial biomass.

[d] Only statistically significant correlation coefficients (*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$) are shown in bold font.

(Table S2). Only AG V_{max} at Timepoint 1 showed a positive correlation with starch decomposition.

3.3. Microbial biomass and substrate mass loss

Overall, the strongest positive correlation with individual substrate mass loss was observed with bacterial biomass (**Table 3**). Most often, the strongest positive correlations occurred between the bacterial biomass at Timepoint 1 and substrate mass loss at Timepoint 2 (**Table 3**). Protein mass loss had the strongest positive correlation with bacterial biomass at Timepoint 2. In contrast, lignin mass loss at Timepoint 2 had a negative correlation with bacterial biomass at Timepoint 1 (**Table 3**). A negative correlation means there is a higher bacterial biomass with lower lignin mass loss, suggesting that the microbes present in the early stages of decay, mainly bacteria, are not lignin-degraders. Later, at Timepoint 2, there was a positive correlation with the bacterial biomass and lignin mass loss. The total microbial biomass showed mass loss relationships similar to bacterial biomass, but not fungal biomass (**Table 3**). Even when the fungal biomass exhibited a significant correlation—as seen with hemicellulose, lignin, and starch at Timepoint 2—a similar correlation was not observed in the total microbial biomass correlations (**Table 3**).

4. Discussion

Understanding the microbial community functional response to climate change is especially important for predicting future carbon and nutrient cycling. We hypothesized that microbial communities specialize on climate conditions, meaning that their extracellular enzyme activities should be greatest in their native climate. Instead, we found minimal evidence for climate specialization and no relationship between enzymes and substrate mass loss (**Figure 3**). Surprisingly, non-native communities often met or exceeded the V_{max} values of native communities within a site. To the best of our knowledge, this result is the first field test of climate specialization with microbial extracellular enzymes. A previous study along our climate gradient found that access to native microbes did not increase litter decomposition or enzyme V_{max}, but that study's design could not be used to test for climate specialization (Baker et al., 2018).

There are several potential explanations for a lack of climate specialization in our study. Rather than being climate specialists, microbes and their enzymes may have broad climate tolerances given the high climatic variability within sites along our gradient. It is also possible that there is climate specialization of some microbes and traits, but not enzyme activities (Chase et al., 2021). Notably, other studies have also found a lack of support for microbial community specialization on litter chemistry. John et al. (2011) rejected the home field advantage hypothesis in a grassland–forest reciprocal transplant. Furthermore, home field advantage was not observed for leaf litter decomposition in the Atlantic rainforests of Brazil (Gießelmann et al., 2011).

For all enzymes, there was statistical evidence for activity differences across inoculated communities; in most cases, there were also significant Inoculum × Site interactions (**Table 2**). A few of these interactions were consistent with climate specialization, such as slightly higher BG activity in the subalpine community in the subalpine site. More frequently, though, non-native communities matched or exceeded the V_{max} values of native communities—in the scrubland site, for example, the desert community showed the highest BG activity (Figure S2). It is possible that these differences were driven by stochastic variation in community assembly or priority effects as the inoculated communities established on the grassland litter (Zhou and Ning, 2017; Albright et al., 2019). Seeing as we only measured decomposition of grassland litter, different patterns might emerge on different litter types. A forest litter type, for example, would have selected for a different set of communities that might have exhibited a different degree of climate specialization. Additional community transplants across climate gradients with other litter types would be needed to know for sure.

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As with extracellular enzymes, we found no evidence for climate specialization in decomposition rates of specific chemical substrates. Furthermore, there was almost no relationship between extracellular enzyme activity and decomposition, suggesting that other factors mediate variation in decomposition rates (Graham et al., 2016). For cellulose degradation, which involves a complex multienzyme system, it is possible that endoglucanases control the rate-limiting degradation step, whereas the CBH and BG enzymes we measured are active further downstream (Xie et al., 2007; Singh et al., 2016). Another possibility is that the abundance of specific microbial taxa controls decomposition rates.

Overall, our microbial biomass results suggest that bacteria promote the decomposition of nonlignin litter compounds (Wohl et al., 2004). We found positive correlations between bacterial biomass at Timepoint 1 and cellulose, hemicellulose, and starch mass loss, whereas lignin showed a negative correlation under the same conditions (**Table 3**). Protein mass loss at Timepoint 2 correlated positively with bacterial biomass at the second timepoint. In general, there were no strong correlations between fungal biomass and substrate decomposition, although we did find significant positive relationships with hemicellulose and starch loss and a negative relationship with lignin loss (**Table 3**). These results support Glassman et al.'s (2018) finding that for grassland litter types, bacterial communities seem to have a stronger effect than fungal communities. Furthermore, a more detailed analysis of microbial functional composition could help explain why some enzyme potentials and decomposition rates varied across communities.

Taken together, our enzyme and decomposition results indicate that microbial communities vary in their enzymatic efficiency, defined as substrate mass loss per unit of extracellular enzyme activity (Alster et al., 2013). For instance, BG efficiency of cellulose degradation was lower for microbial communities in the desert site relative to other sites at Timepoint 2 (**Figure 3**). Interactions between microbial community members could explain variation in enzymatic efficiency and support enzymatic functioning outside the native climate. Some microbes "cheat" or benefit from extracellular enzymes without incurring the costs of enzyme production, which could limit enzyme efficiency, even in native communities (Allison, 2005). Differences in production strategies or spatial structure across communities may result in different in situ decomposition rates despite similar lab-measured extracellular enzyme potentials (Burns, 2013). Additionally, resource acquisition traits and life history strategies vary between microbial communities, which could affect their overall efficiency of substrate turnover (Malik et al., 2020).

5. Conclusions

In summary, we found little support for climate specialization of extracellular enzymes. Correspondingly, we did not find much evidence for microbial community specialization to climate, and even if it did occur, there was no associated reduction in enzyme functioning with climate change. Likewise, there was no evidence for climate specialization in litter substrate decomposition, and the differences in decomposition that Glassman et al. (2018) observed across microbial communities were not driven by changes in extracellular enzyme potential. There were significant differences in enzyme V_{max} values and decomposition rates across sites, suggesting that direct, physiological impacts of climate change are likely to be more important than indirect effects from community compositional change. The microbial communities in our system appear to have a high degree of metabolic flexibility, meaning that climate specialization is not likely to constrain decomposition as climate changes.

Data accessibility statement

Enzyme activity and initial nutrient data are available as online supporting information. Mass loss, microbial biomass, and Timepoint 2 nutrient data are available online on Github: https://github.com/stevenallison/UCIClimateExperiment/.

Supplemental files

The supplemental files for this article can be found as follows:

Figures S1–S4. Tables S1–S2. docx
Enzyme Activity. cvs
Initial Nutrient Data. xlsx

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Competing interests

Steven D. Allison is the Editor-in-Chief for Elementa's Ecology and Earth Systems Domain. He played no role in the editorial handling or review of this manuscript.

Author contributions

- Contributed to conception and design: BS, SDA.
- Contributed to acquisition of data: SDA.
- Contributed to analysis and interpretation of data: BS, SDA.
- Drafted and/or revised the article: BS, SDA.
- Approved the submitted version for publication: BS, SDA.

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