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RESEARCH ARTICLE

Litter microbial respiration and enzymatic resistance to drought stress

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Many ecosystems are experiencing an increase in drought conditions as a consequence of climate warming and changing precipitation patterns. The stress imposed by these environmental changes can affect ecosystem processes such as the extracellular enzymatic degradation of carbon-containing leaf litter by soil microbial communities. However, the magnitude of these impacts may depend on the composition and metabolism of the microbial community. Based on the hypothesis of local adaptation, microbial communities native to warm-dry ecosystems should display a greater capacity to degrade leaf litter polymers with extracellular enzymes following exposure to warm-dry conditions. To test this hypothesis, we performed a microcosm study in which we monitored extracellular enzyme activity and respiration of microbial communities from five ecosystems along a southern California climate gradient, ranging from warmer, drier desert to wetter, cooler subalpine forest. To simulate drought and rewetting, we subjected microcosms to periods of high temperature and low moisture followed by a water pulse. We found that enzyme activity of wet-cool communities generally exceeded that of warm-dry communities across enzyme types for the five sites we considered. Additionally, we observed a significant decrease in respiration for all communities after longer durations of drought exposure. Although these findings did not align with our expectations of local adaptation, they suggest litter-inhabiting microbial communities are able to retain metabolic functioning in environmental conditions different from those of their native ecosystems. These results may imply that factors such as litter chemistry impose greater constraints than climate on community metabolic function. Overall, despite differences in local climates, microbial communities from semiarid regions may be metabolically adapted to maintain functioning in the face of drought.

Keywords: Microbial community; Respiration; Extracellular enzyme; Drought stress; Climate change; Local adaptation

1. Introduction

Ecosystems across the globe face the imminent threat of increasingly warm conditions as a consequence of climate change (Diffenbaugh et al., 2015; Mann and Gleick, 2015). In characteristically xeric regions, however, this warming is accompanied by increased drying and shifts in precipitation regimes (Huang et al., 2017; Cherwin and Knapp, 2012). For example, in southern California over the past two decades, moderately low annual precipitation events co-occurred more frequently with high temperature years, leading to an increase in warmer, drier soil conditions (NNDC, 2014). Over this same time frame, the occurrence of moderate drought events has doubled, meaning that co-occurring increased annual temperature and lower annual precipitation events contribute to longer and more frequent statewide episodes of soil moisture deficiency (Diffenbaugh et al., 2015; Cook et al., 2015). Additionally, climate models predict changes in annual precipitation regimes, with more intense rainfall occurring over shorter time periods (Swain et al., 2018). Increased aridity and precipitation shifts not only disrupt ecosystem processes in semiarid regions like southern California, but they also affect the underlying soil microbiology of these systems.

As key components of ecosystems, soil microorganisms can play an important role in regulating ecosystem processes under climate change (De Vries et al., 2013). When organisms like soil-inhabiting microbes face abiotic constraints in their native ecosystem, individuals may acclimate through physiological changes that affect metabolic processes (DeAngelis et al., 2010; Crowther and Bradford, 2013). Evolutionary adaptation of the traits underlying these metabolic processes within populations is a likely consequence of prolonged environmental change, a concept known as local adaptation (Kawecki and Ebert, 2004; Leimu and Fischer, 2008). Shifts in the abundances of microbial taxa with adaptive traits may allow the community as a whole to perform better in response to changes in temperature and available precipitation,

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resulting in local adaptive responses at the community level (Hoostal et al., 2008; Kraemer and Boynton, 2017; Wallenstein and Hall, 2012).

Ecosystem functioning depends heavily on soil carbon and nutrients, including nitrogen and phosphorus, made available by microbial extracellular enzymatic degradation of complex organic macromolecules (Allison et al., 2010). Such community metabolic activity is constrained by moisture and temperature, and consequently, soil carbon availability within an ecosystem can vary with changes in climate (Davidson et al., 1998; Fierer et al., 2005). Soil microbial communities may display optimized metabolic activity under climate conditions of their local environment, reflecting adaptations accumulated over generations in the same moisture and temperature regime (Evans and Wallenstein, 2012; Wallenstein and Hall, 2012). Therefore, local adaptation of organic matter degradation will likely involve changes in microbial extracellular enzymes and respiration. Extracellular enzymes enable heterotrophic soil microorganisms to externally degrade organic polymers that are otherwise too large for direct uptake (Skujiņš and Burns, 1976). Once broken down, heterotrophic microbes incorporate the enzymatic products into their metabolic pathways and release CO_2 and mineralized nutrients back into the environment (Skujiņš and Burns, 1976; Sinsabaugh and Moorhead, 1994).

Decreases in community extracellular enzyme production and respiration have been observed upon exposure to high temperature and low moisture conditions (Baldrian et al., 2013; Schindlbacher et al., 2011; Allison and Treseder, 2008). These studies analyzed microbial communities from cool, wet ecosystems that may not show local adaptation to drought-like conditions, and they represent a current literature bias towards studies considering climate effects on the soil microbiology of mesic ecosystems. We expect that microbial communities from ecosystems with warmer temperatures and drier conditions might show greater extracellular enzyme activity and respiration rates following exposure to drought conditions, as they are more likely to contain thermotolerant or drought tolerant community members, and there are some available studies to support this expectation (Yuste et al., 2013; Evans and Wallenstein, 2012). The current literature has even less consideration of local adaptation in soil microbial communities responding to long stretches of aridity punctuated by periods of intense rainfall, a precipitation regime that is expected to increase in prevalence with climate change.

Microbial community respiration may increase following pulse wetting events that occur after periods of drying. This increased CO_2 flux following wetting is known as the "Birch effect", and may be due to the remobilization of soluble organic matter, available for use by microbes, or increased respiration by plant roots (Birch, 1958). While this effect is typically observed in studies of soil systems that do not contain a significant amount of plant litter, there is support for a similar effect in litter-decomposing soil communities (Hanson et al., 2003; Borken et al., 2006). In these systems, the primary source of CO_2 flux may be heterotrophic microbes stimulated by water to metabolize organic matter that has accumulated over the dry period; under drying, soil systems can see an increase of leaf litter input and dead microbial matter, contributing to organic matter build up (Cisneros-Dozal et al., 2007). This build up may also explain why there are larger pulses of CO_2, in response to wetting, following longer periods of drying. This effect may be more pronounced for microbial communities subjected to intense rainfall events in their native environment such as occurs in the summer for southern Californian deserts (Bachelet et al., 2016; Fiedler et al., 2013). While there is evidence for a Birch-like effect following wetting in litter communities, closer evaluation of this phenomenon is needed to further understand water pulse response in litter-dominated soil communities and whether originally warm-dry communities (such as deserts) will display an adaptive response to shifts in precipitation under climate change.

Building on prior work along a climate gradient in semiarid southern California, we tested for local adaptation of microbial communities to increased warming and drying punctuated by water pulses over time (Baker and Allison, 2017; Glassman et al., 2018). Specifically, extracellular enzyme activities and respiration of CO_2 were used to assess local adaptation at the community level. We hypothesized that microbial communities originating from warm-dry sites would show a greater degree of local adaptation to high temperature incubation and a water pulse following exposure to drought conditions. Relative to communities from warmer, drier sites, there should be a decrease in extracellular enzyme activity and respiration of communities from cooler, wetter sites, and this trend should be retained across time. Additionally, we hypothesized that microbial community respiration following water pulse events should increase with prior drought duration in accordance with a Birch effect, and microbial communities from warmer, drier ecosystems should display greater respiration pulses upon wetting at each time point.

2. Materials and methods

2.1. Community sampling

We sampled bacterial- and fungal-dominated microbial communities from five distinct ecosystems across a southern California climate gradient. These ecosystems included the Colorado desert, pinyon-juniper scrubland, grassland, pine-oak forest, and a subalpine forest. Sites spanned 20°C and 500 mm precipitation, allowing us to investigate local adaptation across a range of temperature and precipitation conditions within the Mediterranean climate regime (**Table 1**).

Microbial communities were collected from leaf litter on October 23 and 24, 2016, at the end of the southern California dry season, from the five sites along the climate gradient. Differences in microbial composition and biomass have been found across the sites, such as lower fungal biomass in the desert site (Baker and Allison, 2017; Glassman et al., 2018). Within each site, 1.0 g of litter was collected at random from each of four different 0.5 m^2 plots within 50 m of each other; collection was performed by carefully scooping up the loose plant litter with a gloved hand (Baker and Allison, 2017). Plot samples

Table 1: Site descriptions for microbial community collection including mean (±SE) annual temperature and precipitation (Baker and Allison, 2017). DOI: https://doi.org/10.1525/elementa.442.t1

Ecosystem	Site Location	Latitude, Longitude	Mean Annual Precipitation (mm)	Mean Annual Temperature (°C)
Desert	Philip L. Boyd Deep Canyon Desert Research Center	33.65, −116.37	100 ± 24	22.8 ± 0.8
Scrubland	Burns Piñon Ridge Reserve	33.61, −116.46	193 ± 33	15.6 ± 0.8
Grassland	Loma Ridge—Limestone Canyon	33.74, −117.70	242 ± 76	16.4 ± 0.3
Pine-Oak	James San Jacinto Mountains Reserve	33.81, −116.77	402 ± 118	12.3 ± 0.6
Subalpine	San Jacinto Mountains Subalpine Forest	33.80, −116.69	~265	10.3 ± 1.8

were combined and mixed to create a representative community sample for each site. Litter from each community sample was ground into an inoculum of fragment lengths <0.5 cm (Baker and Allison, 2015).

2.2. Microcosm experimental design

Inoculum (0.1 g) was established in sterile 40 ml septum-capped vials containing 1.0 g sterile, ground (<2.0 cm fragments) leaf litter substrate originating from the grassland site. Three sterile control vials were also included that contained sterile litter but no inoculum. Grassland substrate was used because similar non-native annual grass species are present across the climate gradient, contributing to a litter chemistry dominated by cellulose and structural carbohydrates in all sites (Khalili et al., 2016; Baker and Allison, 2017). The grassland substrate, however, did contain a greater proportion of hemicellulose relative to litter from the desert, pine-oak, and subalpine sites which contains a greater proportion of lignin due to inputs from shrubs and trees (Baker and Allison, 2017).

Ultrapure water (5.6 ml) was initially added (time 0) to all vials (including sterile controls) to promote inoculum colonization of the substrate. This water was then allowed to evaporate until water was introduced at subsequent wetting events (week 1, 3, 6, or 9), with vials wetted later experiencing longer durations of drying. Experimental combinations were replicated 3 times for a total of 108 microcosm vials, including sterile controls. At week 1, we wetted 18 vials (5 microbial communities times 3 replicates, plus sterile controls), measured their respiration rates 5.5 hours post wetting, and destructively harvested them 24 hours later for enzyme assays to determine initial metabolic differences across the community types. At each of weeks 3, 6, and 9, we similarly analyzed 30 vials, 15 of which were wetted and 15 of which served as dry controls (5 microbial communities times 3 replicates). Note that dry controls were wetted at time 0 to allow inoculum colonization.

The same water quantity (5.6 ml) was used for all wetting events, mimicking a "pulse" storm-like event. This water quantity was enough to saturate the litter and was selected based on summer precipitation events in the Colorado desert ecosystem, which experiences intense, but brief, thunderstorms (Fiedler et al., 2013). In the summer of 2015, the most intense storm event brought 17.78 mm of rain to Boyd Deep Canyon Desert Research Center, roughly equivalent to 5.6 ml of water per gram of surface litter (WRCC, 2015). All microcosm vials were incubated at 31°C throughout the experiment, a temperature based on averaging monthly low and high values for the three hottest months of the year in the desert site: July, August, and September (WRCC, 2015). Selection of this temperature enabled us to mimic heat stress typical of the desert ecosystem.

2.3. Extracellular enzymes

Potential extracellular enzyme activity was measured for each microcosm with microplate fluorescence assays (German et al., 2011). Wetted vials and their dry controls were separately processed into litter homogenate solutions. Homogenate solutions were made by adding the entire 1.0 g litter contained in each microcosm vial to 150 ml of 6.0 pH maleate buffer and homogenizing the contents for four 30-second intervals with 30-second interspersed rest periods using a Biospec (14 mm) tissue-tearor.

Solutions were continuously mixed with a magnetic stir bar prior to assays in 96-well microplates. In each assay well, we combined 125 µl homogenate solution with 125 µl synthetic fluorogenic substrate solution (**Table 2**). Plate layout for each assay followed procedures adapted from German et al. (2012), and included homogenate controls, substrate controls, fluorimetric standards, and a range of eight substrate concentrations per enzyme (**Table 2**). After homogenate and substrate solutions were added, plates were incubated for four hours at 22°C. Observed enzyme activities were fitted to the Michaels-Menten equation, and nonlinear regression analyses were performed with the use of the nls package in R (R Development Core Team, 2019) to obtain estimates of maximum reaction velocity

Table 2: Microbial extracellular enzymes and their corresponding leaf litter targets of degradation. DOI: https://doi.org/10.1525/elementa.442.t2

Enzyme	Target Substrate	Substrate Concentration Range (μM)
Alpha-glucosidase (AG)	Starch	3.9–500
Beta-xylosidase (BX)	Hemicellulose	7.8–1000
Cellobiohydrolase (CBH)	Cellulose	3.9–500
Beta-glucosidase (BG)	Cellulose	7.8–1000
Acid phosphatase (AP)	Organic Phosphorus	15.6–2000
N-acetyl-glucosaminidase (NAG)	Chitin	7.8–1000
Leucine amino peptidase (LAP)	Peptide	3.9–500

(V_{max}). Estimates of maximum enzyme activity, recorded in μmol g^{-1} h^{-1}, were used as a metric of the concentration of enzyme present in each microcosm.

2.4. Respiration rate measurements

Microbial respiration was measured for all dry and wetted vials using an EGM-4 infrared gas analyzer (PP Systems, Amesbury, MA, USA). Prior to taking any sample measurements, the EGM-4 analyzer was flushed of CO_2 for a period of 15 minutes and/or until the analyzer displayed a consistent reading of atmospheric CO_2 concentration (~410 ppm). Septum caps of microcosm vials were sealed gas-tight for ~5.5 h, after which 10 ml headspace air was extracted and injected into the gas analyzer to measure CO_2 concentration (Allison et al., 2009). Post-pulse respiration declines back to ambient levels after 24 hours following water pulse introduction, allowing our accumulation time of 5.5 h to capture a true post-water pulse metabolic response (Waring and Powers, 2016). The ambient CO_2 concentration was taken just before injection of sample gas, and all sample CO_2 concentrations were subsequently corrected for this value. One dry vial for the desert community at the week 9 time point was excluded due to a break in the rubber cap over the accumulation period.

Sample CO_2 concentrations were used to calculate respiration rates in micromoles per gram per hour (μmol g^{-1} h^{-1}) with the following equation adapted from Dossa et al., 2015:

$$R_{CO_2} = \frac{dCO_2}{dt} \bullet \frac{P(V_v)}{RT} \bullet \frac{1}{W_s}$$

Where R_{CO_2} is the rate of CO_2 respired in (μmol g^{-1} h^{-1}), dCO_2/dt is the change in CO_2 concentration (μmol mol^{-1}) over time, P is assumed atmospheric pressure of 1 atm, V_v represents volume of the vial in mL, R is the gas constant in mL × atm μmol^{-1} K^{-1}, T is incubation temperature in Kelvin, and W_s is the dry weight of the sample in grams.

2.5. Statistical analysis

For enzyme activities, we initially ran a mixed model, repeated measures analysis of variance (rmANOVA) on all wetted microcosms to test the effects of drying duration and community type on activity. We ran an additional rmANOVA on all wetted and dry control enzyme microcosms, excluding week 1, with community type, time, and water addition as factors. The separation of these two analyses was necessary because of the unbalanced experimental design which lacked dry control vials at week 1. For both enzyme activities and respiration rates, a one-way ANOVA was run on week 1 microcosms to determine the community effect, followed by Tukey post-hoc analyses. A separate mixed-model rmANOVA was applied to respiration rates from wetted microcosms on weeks 3, 6, and 9 to test effects of drying duration and community type. Dry control microcosms were omitted from this analysis because their respiration rates were very low, and we wanted to focus on time and community effects among the wetted vials. We also excluded week 1 respiration data from this analysis because those data were analyzed separately with the aforementioned one-way ANOVA for the inoculum establishment phase.

All analyses were performed on untransformed activity values unless residuals displayed non- normality as revealed through a Shapiro Wilk's test and visually through a quantile-quantile plot; in these cases, normality was improved by applying the most appropriate and lowest strength transformation necessary, and ANOVA analyses were then performed using these transformed values. These adjustments included inverse transformations (1/X) on CBH values and square root transformations (√x) for BX, CBH, and AP. If ANOVA results showed significant community effects, then pairwise comparisons were performed to evaluate specific differences at a given time point. Analyses were performed with the caveat that data were pseudo-replicated within sites at each time point of water addition.

3. Results

3.1. Extracellular enzyme activity

We found a significant effect of community type on initial activity (just after the week 1 water addition) for each of the seven enzymes ($p < 0.05$; **Figure 1**). While post-hoc results revealed substantial variation in community V_{max} values for the different enzymes, there was a general trend of higher activity from the wet-cool communities (grassland, pine-oak, or subalpine) relative to the scrubland and desert (Dataset S1).

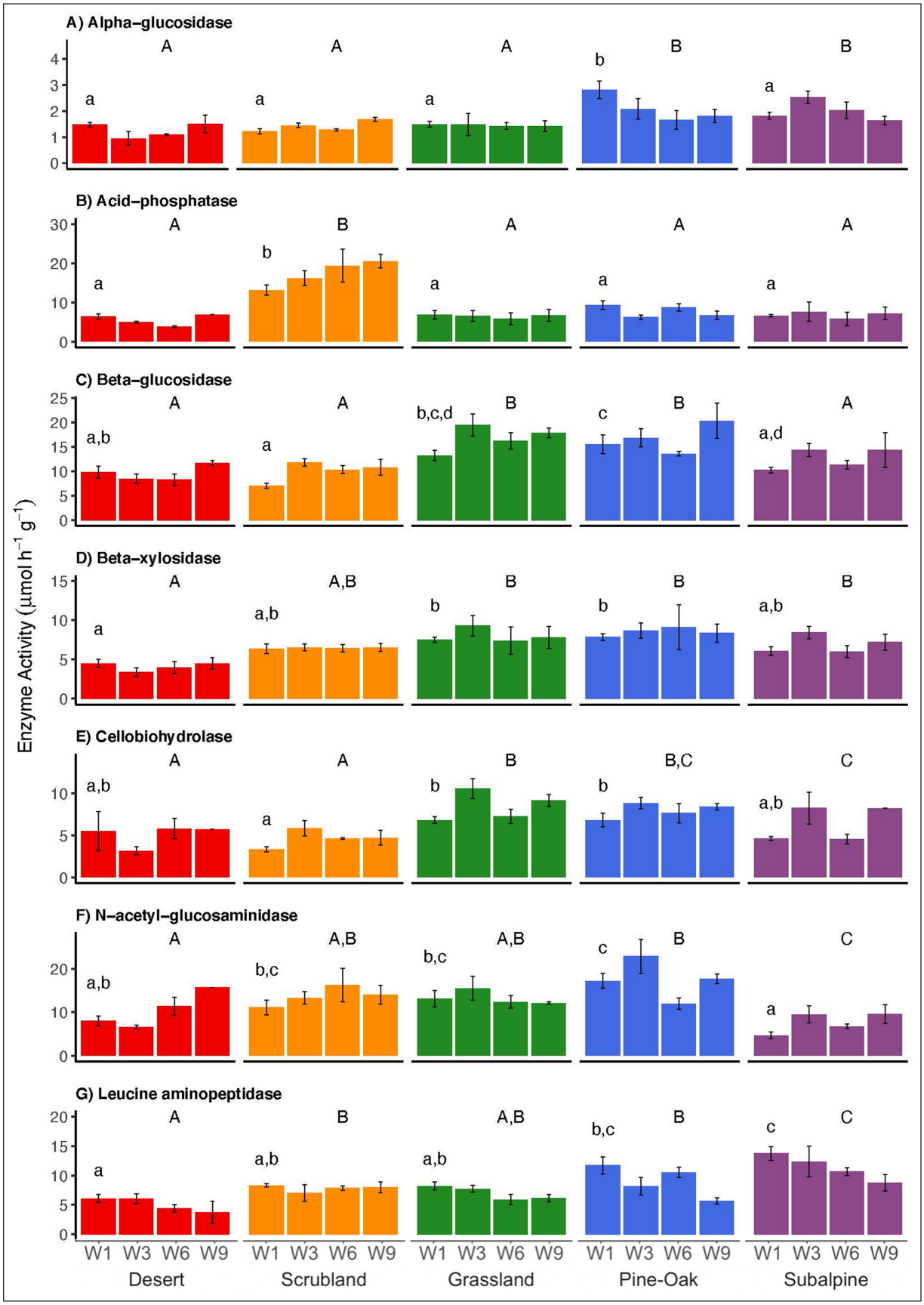

Figure 1: Comparison of extracellular enzyme activities in μmol g^{-1} h^{-1} following wetting at week 1, 3, 6, or 9. Error bars represent mean activity ± SE (n = 3 for each community). Post-hoc results for the community effects on week 1 activities are displayed with lower case letters, while pairwise comparison results for the average community effects across weeks 1, 3, 6, and 9 activities are displayed with capital letters. Communities with the same letter are not significantly different ($p > 0.05$). W1–W9 on the inner x-axis label denotes the wetting time at week 1, 3, 6, or 9, respectively. DOI: https://doi.org/10.1525/elementa.442.f1

We also found significant community effects on enzyme activities in our analysis of all wetted vials (**Table 3**). Since no significant interactive effect between community and time was detected for any enzyme, average activity (across weeks 1, 3, 6, and 9) for each community was compared in pairwise tests (**Figure 1**). Community activities at weeks 3, 6, and 9 were similar to those displayed in week 1 for each enzyme class. Extracellular enzyme activity increased from the warm-dry communities to the wet- cool ones except for AP, for which the scrubland displayed the greatest activity, and NAG, for which the subalpine displayed the lowest activity (**Figure 1**).

Activities for BG and CBH varied significantly with time, with values for weeks 3 and 9 significantly higher than for weeks 1 and 6, respectively. LAP activities were significantly affected by time as well; however, this result was due to week 6 and 9 activities being significantly lower than in weeks 1 and 3. Additionally, all enzymes, except AG, showed significantly higher activity in rewetted compared to dry vials (Table S1).

3.2. Respiration rates

There was a significant difference in initial respiration rates as determined by one-way ANOVA ($p = 0.023$), with the post-hoc analyses revealing greater respiration from grassland compared to subalpine communities (**Figure 2**). All other communities displayed statistically similar initial respiration rates.

Table 3: P-values for two-way ANOVA on rewetted vial extracellular enzyme activities at weeks 1, 3, 6, and 9. DOI: https://doi.org/10.1525/elementa.442.t3

Enzyme	Community[a]	Time[a]	Community:Time
Alpha-glucosidase	**0.001**	0.584	0.094
Acid-phosphatase	**<0.001**	0.702	0.0530
Beta-glucosidase	**<0.001**	**0.003**	0.593
Beta-xylosidase*	**<0.001**	0.647	0.930
Cellobiohydrolase*	**<0.001**	**0.002**	0.234
N-acetyl-glucosaminidase	**<0.001**	0.717	0.203
Leucine aminopeptidase	**<0.001**	**0.001**	0.288

[a] Bolded p-values are significant (<0.05).
* Tests were performed on square root transformed values.

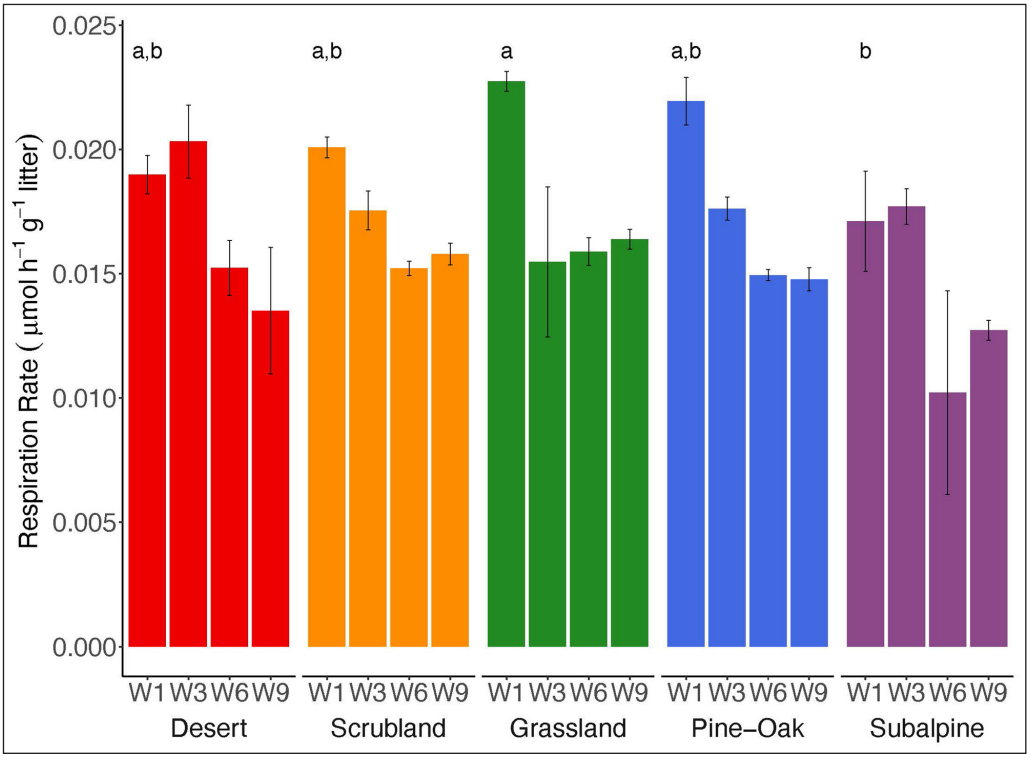

Figure 2: Average respiration rates following wetting at weeks 1, 3, 6, and 9 in µmol g^{-1} h^{-1}. Error bars represent mean rate ± SE (n = 3 for each community). Post-hoc results for initial average community respiration are shown with lower case letters. Means with same letter are not significantly different ($p > 0.05$). DOI: https://doi.org/10.1525/elementa.442.f2

Two-way rmANOVA did not yield any statistically significant differences between the desert community and the other communities at any time point of wetting, and it did not detect a significant interaction between community and time. Respiration rate significantly decreased ($p = 0.002$) from week 3 to week 6 for all communities, except the grassland, and remained similar between weeks 6 and 9 (**Figure 2**). There were also large differences in average respiration rates between dry and rewetted vials at each time point, with values ranging from 9.49×10^{-4} to 1.47×10^{-3} μmol g^{-1} h^{-1} for dry vials and 1.02×10^{-2} to 2.27×10^{-2} μmol g^{-1} h^{-1} for rewetted vials (Dataset S2). Respiration was compared between sterile control vials and all inoculated vials at week 1 to confirm sterility. The average initial respiration rates with standard errors were calculated separately as $3.02 \times 10^{-4} \pm 1.46 \times 10^{-4}$ μmol g^{-1} h^{-1} for sterile control vials and $2.02 \times 10^{-2} \pm 8.0 \times 10^{-4}$ μmol g^{-1} h^{-1} for all non-sterile vials.

4. Discussion

We hypothesized that microbial communities from warmer, drier environments would show a greater degree of local adaptation compared to communities from cooler, wetter environments when exposed to water pulses after periods of drought-like stress exposure. Local adaptation should have resulted in declining extracellular enzyme activities and respiration rates across the climate gradient from desert to subalpine microbial communities. Furthermore, we expected greater respiration upon rewetting after greater duration of drying, with the desert community displaying the highest respiration rate at each time point, in accordance with a Birch effect and local adaptation. Yet overall, our results were not consistent with local adaptation. Instead we found greater V_{max} values in wet-cool compared to warm-dry communities for both initial and subsequent wetted conditions across the majority of enzymes tested. Despite initial differences in respiration, communities from the climate gradient displayed similar respiratory activity under drying and subsequent water pulses. Pulse-driven respiration in all communities declined over time in contrast to the expectation of a Birch effect, and the desert community did not show the greatest respiratory response following water pulses.

4.1. Extracellular enzyme activities

Contrary to our hypothesis, the warm-dry desert and scrubland communities displayed among the lowest extracellular enzyme activities immediately after water pulses (**Figure 1**). Our enzyme activity results are similar to those from incubation studies in which originally wet-cool communities are not largely affected when incubated under increased temperature or precipitation (McDaniel et al., 2013; Bell and Henry, 2011). It is possible that all communities along our gradient, even in wet-cool locations, have enzymatic traits adapted to the regional Mediterranean climate with warm, dry summers every year (Richardson et al., 2012).

Higher activity in the wet-cool communities relative to warm-dry communities, however, may signal local adaptation in response to an abiotic constraint other than climate, such as leaf litter chemistry. Leaf litter across these southern California ecosystems differs in the amount of starch, cellulose, hemicellulose, chitin and protein (Baker and Allison, 2017). Microbes from the wetter, cooler sites typically encounter litter from trees and may produce more extracellular enzymes, such as AG, when colonizing grass litter in our microcosms (Zhou et al., 2012; Sinsabaugh et al., 2009; Baker and Allison, 2017; Pold et al., 2017). In contrast, when microbes from the desert site colonized the grass litter, enzyme activities such as BX tended to decline. This pattern may have resulted from the desert microbes' history with very low hemicellulose (a BX substrate) content in desert litter. This potential role of local microbial adaptation to litter chemistry could be tested in a future experiment in which these microbial communities are inoculated onto the different litter types and analyzed for enzyme activities and respiration.

The enzyme activity patterns in response to high temperature and low moisture stress might be further clarified by distinguishing the contributions of individual microbial taxa. Previous analyses have found that community composition is a major determining factor in leaf litter decomposition, greater even than functional redundancy, and independent of environmental conditions (Glassman et al., 2018; Strickland et al., 2009a). Degradation differences could be further explained by evaluating fungal versus bacterial abundances, with fungi having been found to exerting greater influence over extracellular enzyme production and activity in soil some communities (Romaní et al., 2006; Boer et al., 2005). In regard to our southern California communities, fungal biomass is much lower in the desert than in any of the other communities during the dry season (Baker and Allison, 2017). Lower abundances of fungi may also explain the lower enzyme activities we observed in our desert community. To clarify the impact of community composition on degradation activity and adaptations to drought stress, future studies could analyze the physiological responses and pathways of individual microbial taxa.

4.2. Respiration

In contrast to our prediction that warm-dry communities would have greater respiration rates after each water pulse, we did not see a significant difference in respiration rates across communities with the exception of week 1 (**Figure 2**). However, respiration rates did decline significantly for all communities, except the grassland, by week 6 (**Figure 2**). The significant difference in week 1 respiration rates between the grassland and subalpine communities suggests initial differences in the ability to establish on the grassland substrate. A higher initial respiration rate and a lack of temporal decline in respiration from the grassland community may be due to a type of local adaptive response known as home field advantage, in which a microbial community is optimized to degrade its native litter, similar to what may be reflected in our enzyme activity results (Gholz et al., 2000; Strickland et al., 2009b). Initial differences in community respiration rates and extracellular enzyme activities could also reflect a response to transfer from microbes' native field environment to laboratory microcosms (Awong et al.,

1990). Different patterns in respiration versus enzyme activity could be due to more microorganisms dying or entering dormancy in the vials exposed to greater periods of stress along with the surviving community members becoming less capable of substrate consumption and respiration (Bradford et al., 2010). In this case, upward trends in enzyme activities could result from accumulated extracellular enzymes (Alster et al., 2013). Regardless of the mechanism underlying the declining respiration response, there was no evidence of an adaptive advantage for warm-dry communities.

We did observe higher respiration rates in rewetted vials versus their dry duplicates, suggesting metabolic activity of all communities was negatively affected by high temperature and limited moisture. Our results failed to support a Birch-like effect, however, as most communities decreased their respiration following longer periods of drought stress exposure; for enzymes, declining activity with time was only observed for LAP. This may be due the absence of soil in our microcosm design, suggesting a difference in respiratory response for microorganisms on soil (or soil + litter) versus solely litter substrate. Studies that observed a Birch effect in predominately litter-decomposing communities either find that there is a greater input of leaf litter to the system following long periods of aridity or manually input new leaf litter substrate; no such accumulation or addition occurred in our microcosms (Lopez-Sangil et al., 2018; Edgerley, 2016). Dead microbial matter may accumulate in microcosm vials, but if such accumulation occurred in our study, it was not enough to support a Birch effect. Most natural soil systems include input of dead plant material to some degree, but the solubilization of available organic molecules in soil may contribute to large respiratory pulses instead of, or in addition to, leaf litter or dead microbial accumulation (Joly et al., 2017). Labile organic compounds present in litter may be re-mobilized upon wetting, and this may explain why we see higher respiration rates following the week 3 water pulse. Microbes in vials receiving a water pulse at later times (week 6 or week 9), would have a longer period to decompose labile components in the litter, and as a result, the majority of substrate remaining for decomposition would require greater energy expenditure. In this case, water availability may not be as important as litter C availability in generating a post water-pulse respiratory response; this may also explain why the desert displayed no local adaptive advantage to water pulses.

5. Conclusions

Our results support the potential for microbial communities from wetter, cooler ecosystems to maintain metabolic function despite exposure to high temperatures and drying over a timespan of nine weeks. However, a community-independent temporal decline in respiration may signal that all communities, regardless of ecosystem origin, are negatively affected by increasing duration of drought stress. Regarding microbial performance under climate change, our results suggest metabolic stability in leaf-litter degrading communities, regardless of ecosystem origin, over weekly to monthly timescales. As warming conditions persist, however, we may see a decline in microbial activity across southern California ecosystems, while degradative activities of some extracellular enzymes may persist (Steinweg et al., 2013). To further increase confidence in predictions of ecosystem response to climate warming and subsequent drying, future studies should consider potential effects of enzymatic adaptation, substrate composition, and community taxonomic composition, in addition to monitoring holistic community metabolic responses. These future studies would additionally benefit from monitoring local adaptation *in situ*, providing a unique view into microbial response to climate perturbations under field conditions.

Data Accessibility Statement

Enzyme activity and respiration data are available as online supporting information.

Supplemental files

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

- **Table S1.** P-values for three-way ANOVA on rewetted and dry vial extracellular enzyme activities excluding week 1. DOI: https://doi.org/10.1525/elementa.442.s1
- **Dataset S1.** Average extracellular enzyme activities (±SE) for each community and condition across weeks 1, 3, 6, and 9. DOI: https://doi.org/10.1525/elementa.442.s1
- **Dataset S2.** Average respiration rates (±SE) for each for each community and condition across weeks 1, 3, 6, and 9. DOI: https://doi.org/10.1525/elementa.442.s1
- **Dataset S3.** Extracellular enzyme activities for each microcosm in the experiment. DOI: https://doi.org/10.1525/elementa.442.s1
- **Dataset S4.** Respiration rates for each microcosm in the experiment. DOI: https://doi.org/10.1525/elementa.442.s1

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

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