UC Irvine

UC Irvine Previously Published Works

Title

Microbial decomposers not constrained by climate history along a Mediterranean climate gradient in southern California.

Permalink

<https://escholarship.org/uc/item/38d5q6jq>

Journal

Ecology, 99(6)

ISSN

0012-9658

Authors

Baker, Nameer R
Khalili, Banafshe
Martiny, Jennifer BH
et al.

Publication Date

2018-06-01

DOI

10.1002/ecy.2345

Peer reviewed

**Microbial decomposers not constrained by climate history
along a Mediterranean climate gradient in southern
California**

Journal:	<i>Ecology</i>
Manuscript ID	ECY17-1192.R1
Wiley - Manuscript type:	Articles
Date Submitted by the Author:	n/a
Complete List of Authors:	Baker, Nameer; University of California Berkeley Department of Environmental Science Policy and Management Khalili, Banafshe; University of California, Irvine, Ecology and Evolutionary Biology Martiny, Jennifer; University of California-Irvine, Ecology and Evolutionary Biology Allison, Steven; University of California, Irvine, Ecology and Evolutionary Biology; Earth System Science
Substantive Area:	Decomposition < Ecosystems < Substantive Area, Community Analysis/Structure/Stability < Community Ecology < Substantive Area, Experimental Microcosms/Mesocosms < Community Ecology < Substantive Area, Interspecific Competition < Species Interactions < Community Ecology < Substantive Area, Plant/Fungal/Microbial Interactions < Species Interactions < Community Ecology < Substantive Area, Climate/Microclimate < Ecosystems < Substantive Area, Climate Change < Ecosystems < Substantive Area, Regional Studies < Ecosystems < Substantive Area, Biogeochemistry < Ecosystems < Substantive Area
Organism:	Eubacteria < Prokaryotes, Fungi (specify type in field below), Grasses < Angiosperms < Plants
Habitat:	Terrestrial < Habitat, Prairie/Grasslands < Temperate Zone < Terrestrial < Habitat, Coniferous Forest < Temperate Zone < Terrestrial < Habitat, Mixed Forest < Temperate Zone < Terrestrial < Habitat, Scrubland < Temperate Zone < Terrestrial < Habitat, Desert < Temperate Zone < Terrestrial < Habitat, Chaparral/Sclerophyll/Scrublands < Terrestrial < Habitat, Alpine/Montane < Terrestrial < Habitat
Geographic Area:	Southwest US (AZ, CA, CO, NM, NV, UT) < United States < North America < Geographic Area
Additional Keywords:	Climate gradient, Microbial decomposition, Extracellular enzymes, Temperature sensitivity, Community constraints
Abstract:	Microbial decomposers mediate the return of CO ₂ to the atmosphere by producing extracellular enzymes to degrade complex plant polymers,

	<p>making plant carbon available for metabolism. Determining if and how these decomposer communities are constrained in their ability to degrade plant litter is necessary for predicting how carbon cycling will be affected by future climate change. We analyzed mass loss, litter chemistry, microbial biomass, extracellular enzyme activities, and enzyme temperature sensitivities in grassland litter transplanted along a Mediterranean climate gradient in southern California. Microbial community composition was manipulated by caging litter within bags made of nylon membrane that prevent microbial immigration. To test whether grassland microbes were constrained by climate history, half of the bags were inoculated with local microbial communities native to each gradient site. We determined that temperature and precipitation likely interact to limit microbial decomposition in the extreme sites along our gradient. Despite their unique climate history, grassland microbial communities were not restricted in their ability to decompose litter under different climate conditions across the gradient, although microbial communities across our gradient may be restricted in their ability to degrade different types of litter. We also found some evidence that local microbial communities were optimized based on climate, but local microbial taxa that proliferated after inoculation into litterbags did not enhance litter decomposition. Our results suggest that microbial community composition does not constrain C-cycling rates under climate change in our system, but optimization to particular resource environments may act as more general constraints on microbial communities.</p>

Dear Dr. Yavitt and our anonymous reviewers,

We thank you all very much for your interest in our manuscript, and are very gracious for the opportunity to revise it with the help of your contributions. We appreciate the many thoughtful comments of both reviewers, and have endeavored to address each of their comments and suggestions. We have reworked our results section to relegate as many nuts and bolts to the supplemental as possible, and have reframed our presentation of the results as relative differences in order to better convey their significance to the reader. We did not present as many relevant sources from the literature as we should have in both our introduction and our discussion, and we have attempted to remedy this oversight and better frame our study and put our findings in context. We believe that the result is a significantly improved manuscript that we hope that you and the reviewers will find acceptable for publication in *Ecology*. Thank you very much for your time; please find our specific responses to the reviewers below.

Sincerely,
Dr. Nameer Baker
University of California, Berkeley

Reviewer: 1

Evaluating the effects of microbial community selection on decomposition processes is an interesting and ongoing topic of study. Unfortunately, this paper requires major work before it can be considered for publication. Key methodological information is lacking; the results section lacks a clear presentation of findings; the discussion lacks comparisons with similar studies, synthesis and acknowledgement of study limitations.

We thank the reviewer for their interest, and for their many helpful comments and criticisms. We agree that our results section presented many unnecessary details to the reader, and that our discussion lacked adequate framing of our study and results in context with the literature. We believe we have addressed these concerns, and our specific responses to your comments are below. Thank you once again for your time and thoughts.

Introduction:

What is the rationale for analyzing only bacterial community composition? Fungi are often considered more important actors in litter decomposition.

Previous research at the grassland site indicates that bacterial biomass dominates microbial biomass over the course of the year (see line 427), and we thought this likely to be especially true in our microbial cage litterbags (see line 141-145). We therefore designed the study to focus on the bacterial community, which we now mention in the methods. Our expectation was generally corroborated by the high B:F biomass ratios observed after transplantation in all sites other than the scrubland (see Table S5), and by the fact that bacterial biomass explained a significant amount of the variation in mass loss across the gradient (see Figure 5).

Methods/Results:

What species of grass was used for litter bags? Single species or a mix of species?

The grass litter used was a mix of two Avena species, A. barbata and A. fatua. We now indicate this in the text (line 104).

The composition of inoculum litter is not clear. Was the inoculum composed only of grassland litter from each site or was the inoculum mostly litter associated with the dominant vegetation at each site?

If only grassland litter, did the mix of grass species vary across sites and by how much?

The inoculum consisted of the dominant litter local to each type, whether that be grasses, leaves, or needles. We now indicate this in the text (line 133-4).

Was there a bag effect? The 0.2um mesh undoubtedly introduces artifacts. There are studies, mostly from aquatic systems, that compare mass loss rates and other functional variables in relation to mesh size. In general, metabolism slows as exchange processes get more constrained by decreasing mesh size. How did mass loss rates or EEA or bacteria community composition of confined litter compare to values for unconfined or loosely confined litter at these sites?

Previous work at the grassland site using microbial cage litterbags indicates that mass loss is likely reduced, and we believe that the fungal community in particular may be detrimentally affected by the inability to traverse the membrane and translocate nutrients into or out of the litterbag. This is why we chose to use unsterilized litter, as sterilized litter takes a significant amount of time to develop an active microbial community within these litterbags. This also ties into why we chose to focus on the bacterial rather than the fungal community. We now indicate this in the text, and also draw attention to the caveats this entails (see lines 141-5).

Bacterial biomass was quantified by flow cytometry. Does this method work for filamentous bacteria (Actinobacteria)? How does the estimated biomass compare to values reported in other litter studies?

What did phylogenetic sequencing show about actinobacteria abundance?

We cannot speak with authority as to whether flowcytometry underestimates the abundance of filamentous bacteria such as Actinobacteria. However, we have determined through our 16S results that Actinobacteria made up a small fraction (<2% of reads) of the bacterial community. It is possible that Actinobacteria themselves were more detrimentally affected by our microbial cage litterbags than other bacterial taxa, and we now indicate as much in the text when discussing caveats of our design (see lines 461-465). Estimated microbial biomass in our litterbags is higher than that observed in previous litterbag studies at the grassland site or that observed in local litter at the same sampling date, and we now indicate as much in the text and provide data to corroborate this in the supplemental (Table S4, S5). However, there are not similar studies in the literature for which absolute bacterial biomass was assayed directly from litter – there are a plethora of studies that do so for soil, but these are not comparable to estimates in litter. Litter microbial biomass is often assayed by substrate addition assays and measurements of CO₂ flux, but these provide relative measures rather than absolute measures. As such our results address a significant gap in the literature that we can best place in context by referring to previous or concurrent work done at these sites; we have attempted to do so in the discussion while also admitting the caveats that come with our presentation of the data (see lines 441-467).

Given that fungal biomass was similar to that of bacteria, what is the rationale for excluding fungal community composition?

Please see response above (“Previous research at the...”).

Bacterial and fungal biomass numbers are very low. How do these values compare to those reported in other studies, or to biomass abundance in unconfined litter at your sites?

They are generally comparable to those observed in local litter from these sites, though bacterial biomass, in particular, is significantly enhanced within our litterbags relative to local litter in the subalpine and pine-oak sites. This is likely because the dominant litter types at these two sites are not grasses and instead are more structurally resistant leaves and needles. We have added a sentence

indicating this to the discussion (lines 447-8), and have also added a column to the bacterial biomass and hyphal biomass columns in the supplemental so that readers can compare litterbag values to those observed in local, unconfined litter (Tables S4, S5).

Did mass loss for individual chemical fractions correlate with corresponding EEA?

We did find some significant correlations between C-degrading enzymes and their substrates indicating that higher substrate mass corresponds to greater enzyme activity. However, these correlations were weak and were found between enzyme activity and mass attributable to the substrate, rather than between enzyme activity and concentration of the substrate in the litter, and as such we do not believe they merit presentation.

How do Bacteria/Fungi biomass ratios compare to those reported in other studies.

We now compare observed B:F ratios and fungal biomass to those observed previously at the grassland site and concurrently in local litter in the discussion (see lines 447-448, 458-459).

In general, the results are poorly presented. Each section consists largely of occult statistics rather than empirical data. What were the findings and what were the responses (e.g. effect size, response ratio) to treatment. No one can evaluate your findings or compare them to other studies if you do not present them. The statistical results should be relegated to tables that are briefly referenced in the results text. *We agree with the reviewer and have relegated descriptions of our ordinations to the supplemental so as not to detract from the actual data. We have also attempted to reframe our results section to present our results as relative differences from the grassland or from the control litterbags, rather than presenting the absolute numbers that have minimal context.*

Lines 346-356: No data on bacterial community composition are presented here, or elsewhere in the paper or in the supplemental information. It is difficult to tell from Figure 6, whether in fact the inoculum treatment affected bacterial community composition.

We agree with the reviewer that the figure did not add to our presentation of the results, and have removed it. Instead, we present a table (Table S6) of relative abundances of the most abundant (>1%) bacterial phyla (or classes, in the case of the Proteobacteria), and detail differences in the phylum that was most responsible for explaining the variation in bacterial community composition between sites.

Line 380: Why normalize EEA to bacterial biomass rather than total biomass?

We normalized to bacterial biomass because bacterial biomass generally dominated microbial biomass in our litterbags and because we found that bacterial biomass explained a significant amount of the variation in mass loss rates observed across the gradient (see Figure 5). Doing so also allowed us to make this comparison over multiple timepoints rather than just the final timepoint, while also increasing the number of replicates included in the analysis.

Were apparent K_m and V_{max} values correlated?

V_{max} and K_m values were correlated across the gradient, but given the weak nature of this correlation ($R^2=0.06$) and the comments of the second reviewer indicating that we have overemphasized our enzyme kinetics results, we have decided to remove our K_m results from the main text and have relegated them to the supplemental.

Lines 411-430: No results are presented, only statistics that are of no value to readers. What were the numbers and the effect sizes.

We believe we have addressed this concern throughout our results section by reframing our results as relative differences.

Discussion:

The discussion is almost entirely a restatement of information in the results with little improvement in clarity. Based on the initial hypotheses, mass loss and other parameters within the control bags should diverge from values observed at the grassland site. Did they?

We have reframed our results to be presented as relative differences from the grassland, and have also reworded our introduction and discussion to better establish the climatic framework for the study, and to better relate the results. To do so we have adopted Prescott's (2010) framework of climatic thresholds and discuss our gradient results in that context. We then delve into the general effects of inoculation across the entire gradient, rather than honing in on the effect in individual sites.

Differences between control and inoculated bags in mass loss, EEA kinetics and bacterial community composition are predicted to increase across gradient as temperature and moisture increasingly diverge from conditions at the control (grassland) site. Did they? This information is the focus of the study and should be clearly summarized, perhaps as effect sizes, in the results section. The key question for each site is the effect size and direction for grassland control relative to site control compared to effect size and direction for control relative to inoculum within each site.

We agree and have attempted to present our results as relative differences from the grassland or from control litterbags when significant differences were observed.

The discussion includes no comparisons of findings with those from other studies, nor any attempt to integrate findings into the literature. There are a good number of decomposition studies that include reciprocal transplants. How do your results compare? There are several studies that compare EEA kinetics and microbial community composition across elevational and latitudinal gradients. How do your results compare? Even straightforward cross study comparisons of biomass concentrations and EEA parameters are lacking.

We agree with the reviewer that we have not cited several studies that are relevant, chief among them Prescott et al. 2010 and Bradford et al. 2016. We have attempted to amend this oversight. However, there is a dearth of litter decomposition studies in semiarid and arid ecosystems, which is part of why this project was carried out. In addition, the purpose of this study is not to establish how enzyme kinetics vary across the gradient, as the reviewer has established – it is to determine how transplantation alters those kinetics, and therefore delving into site-level differences in our enzyme kinetics and their context within the literature distracts from the general trends we are presenting.

The limitations of the study should be addressed here. What is the potential for artifacts with this design. How much are results influenced by fungal effects compared to bacterial effects.

We have adding several sentences addressing caveats of the study both in the methods (see lines 141-5) and in the discussion (lines 451-468)

Reviewer: 2

Baker et al. investigated the influence of climate and microbial decomposer community composition on grass litter decomposition dynamics across a climatic and vegetation gradient in California. Their goal was to address emerging ideas on what controls C cycling and OM degradation in terrestrial ecosystems and how those controls might be influenced by climate change. By reciprocally transplanting one litter type into a variety of vegetation and climate types and then manipulating the colonizing microbial community, the authors were able to test the main hypotheses with an exhaustive array of litter C pools, microbial community metrics, extracellular enzyme activity kinetics, etc. that includes 4 in-manuscript tables, 9 more supplemental tables, 7 in-manuscript figures, and 5 more supplemental figures.

I struggled greatly reviewing this manuscript because I am really interested in this topic and in my opinion, it is a very important research area. The paper creates a compelling introductory framework and very elegantly provides testable and easy to follow hypotheses laid out as graphical-based predictions (just like it should be). My primary issue is that the study-design and results do not live up to the loftiness of the goals or the magnitude and intensity of the data collection. Bradford et al. (2016) summarized much of the debate over litter chemistry, climate, or microbial community controls on litter decomposition. The fact that this study only focused on two and ignored the litter chemistry aspect is hard to reconcile with the overall goals of the study. Within the gradient there are litter types with widely varying C/N ranging from high elevation conifer litter to oak and scrub deciduous litter. Why did they choose the least recalcitrant litter from a life form that grows across the greatest range of environments? Prescott (2010) had a broad discussion of the climate change and litter decomposition and concluded that the biggest climate change impact will be changes in species ranges that alter litter chemistry. The authors here come to much of the same conclusion at the end of the paper, but do not make that point (or cite Prescott) nor make clear that their study was not able to address this aspect because of the single litter type/chemistry.

We thank the reviewer for their thoughtful comments and suggestions. We agree that not citing either of those two papers was a significant oversight on our part, and we have amended our text both in the introduction and the discussion to ameliorate this. However, it should be noted that Bradford et al. determined through their analysis that litter decomposition studies have likely underestimated the potential impact of microbial community constraints on decomposition given that most designs overestimate and confound the effects of climate with other variables. This is likely to be particularly true at the regional scale, making our study a worthy addition to the literature in that context (we now mention this in lines 59-60). In addition, Prescott found that climate thresholds likely interact to drive decomposition and that many observed effects of climate may in fact be driven by litter chemistry (now mentioned in line 58 and lines 400-1). However, there are some notable caveats – the majority of studies referenced by Prescott (and by Bradford et al, for that matter) come from high latitude or temperate forests, not drylands ecosystems like those investigated in our study. In addition, Prescott's findings applied to our system would indicate that precipitation should be the limiting factor across our gradient – yet we find that temperature likely acts as the primary threshold on one end of our gradient. Finally, Prescott points out that evidence of microbial community home-field advantage in litter decomposition points to a knowledge gap regarding the role of the microbial community that our study seeks to address, and in doing so we find support for her litter chemistry paradigm even at the microbial community level.

I realize this all sounds negative and I am down on the study, but that is not the case. This is a valuable study and that produced a ton of good data. However, the first paragraph of the discussion says it all. Despite a lot of methods and results, including all the tables and figures mentioned above, we learn that moisture and temperature controls mass loss and that cold temps can cancel out the moisture effect

and lack of moisture cancels out the temperature effect. You could show much of the same pattern using a common litter in the Weintraub-Moorhead student modeling tutorial I-Mold (<http://imold.utoledo.edu/>). I'm not opposed to that finding as support for longstanding views are good. I am just not sure that all of the rest of the data add that much more. One of the more interesting aspects was the finding that inoculation in the desert ecosystem actually increased mass loss despite low moisture. Nevertheless, I that was not really discussed. A couple of other head scratching issues for me were:

We apologize for the misunderstanding, but we believe the reviewer might be mistaken as we did not present a result whereby inoculation increased mass loss in the desert site – instead, mass loss was generally reduced in inoculated litterbags.

- Recent work has stressed the role of microbial communities that colonize standing litter. If the authors wanted look at the effect of inoculation, why not start with sterilized litter? Blast the litter with gamma rays or expose to ethylene oxide. This seems like an important control.

We have done previous studies (Allison et al. 2013) using sterilized litter in these microbial cage litterbags and have found that it takes a significant amount of time for microbial biomass to build up to levels approaching those found in un-caged litter. We have also found that such treatments are particularly detrimental to the fungal community, and although we did not focus on the fungal community in our study, we did not want to completely remove them as players in litter decomposition. We now mention as much in our methods (see lines 141-144).

- On line 509 it is stated that “Such trends are consistent with EE accumulation over dry periods in the more arid sites and enhanced turnover of EEs in the wetter sites along the gradient”. That being the case, why spend so much time on V-max and Km? Why not just estimate “pools” of standing enzyme using the conventional potential enzyme activity assays compared between sites? Despite all the kinetic data, we do not get actual activity estimates using model substrates.

We agree with the reviewer that we have overemphasized our enzyme kinetics results without discussing them adequately to merit such attention, and as such we have relegated our Km results to the supplemental and focus on Vmax as a measure of standing enzyme pools in our litterbags.

Again, I really do like the study and the level of ambition, but I think it could easily be paired down to meet the normal 20-30 page limit for Ecology. Much of the supplemental data and other pieces do not add much to the story and some cases (e.g. ordinations with loading coefficients (not explained in the methods)) really just distract from the main story. In the end, conclusions are limited by lack of control for litter chemistry, and all of the ordinations and aspects of enzyme kinetics to do not make up for that from the perspective of an ecology journal, even though it might be perfect for a soil biology journal.

We appreciate your very thoughtful comments, and we feel as though we did not do a sufficient job of framing the study as a test of potential responses to future climate change. We also feel as though we did not adequately convey the importance/value of the inoculation treatment, as that was the primary objective of the study. We have rewritten parts of our introduction and discussion to better convey this, as we were not trying to test litter chemistry hypotheses with this design. We have also relegated most of the descriptions of our ordinations to the supplemental so that they detract less from the story we are trying to relate.

Running head: Decomposers and climate history

Microbial decomposers not constrained by climate history along a Mediterranean climate gradient in southern California

Nameer R. Baker^{1,a}, Banafshe Khalili¹, Jennifer B.H. Martiny¹, and Steven D. Allison^{1,2}

¹Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA

²Department of Earth System Science, University of California, Irvine, CA 92697, USA

^aPresent address: Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94709, USA

Correspondence:

Nameer R. Baker

2151 Berkeley Way, Berkeley, CA 94704, USA

nameer@berkeley.edu

Abstract

Microbial decomposers mediate the return of CO₂ to the atmosphere by producing extracellular enzymes to degrade complex plant polymers, making plant carbon available for metabolism. Determining if and how these decomposer communities are constrained in their ability to degrade plant litter is necessary for predicting how carbon cycling will be affected by future climate change. We analyzed mass loss, litter chemistry, microbial biomass, extracellular enzyme activities, and enzyme temperature sensitivities in grassland litter transplanted along a Mediterranean climate gradient in southern California. Microbial community composition was manipulated by caging litter within bags made of nylon membrane that prevent microbial immigration. To test whether grassland microbes were constrained by climate history, half of the bags were inoculated with local microbial communities native to each gradient site. We determined that temperature and precipitation likely interact to limit microbial decomposition in the extreme sites along our gradient. Despite their unique climate history, grassland microbial communities were not restricted in their ability to decompose litter under different climate conditions across the gradient, although microbial communities across our gradient may be restricted in their ability to degrade different types of litter. We also found some evidence that local microbial communities were optimized based on climate, but local microbial taxa that proliferated after inoculation into litterbags did not enhance litter decomposition. Our results suggest that microbial community composition does not constrain C-cycling rates under climate change in our system, but optimization to particular resource environments may act as more general constraints on microbial communities.

Keywords

Climate gradient, microbial decomposition, extracellular enzymes, temperature sensitivity, community constraints

Introduction

To predict how carbon (C) cycling will change with climate, it is crucial to determine how microbial communities respond to changes in abiotic conditions (Aerts 1997). In the American Southwest, anthropogenic climate change is causing a shift to hotter and drier conditions (Seager et al. 2007, IPCC 2014), and such changes could alter heterotrophic respiration generated by microbial decomposition of plant litter (Raich and Schlesinger 2002). Global C-cycling models generally assume that respiration rates only depend on chemical and climatic factors. Yet these models might be inaccurate if they fail to account for differences in microbial responses to climate change across ecosystems (Reed and Martiny 2007, Strickland et al. 2009, Keiser et al. 2011).

Previous studies have suggested that climate parameters act as ultimate controls on litter decomposition, but cross-site comparisons are challenging this notion. The main influence of climate may instead may be mediated through effects on litter chemistry (Prescott 2010). In addition, biases inherent in many litterbag decomposition studies can lead to overestimation of climate effects and underestimation of microbial community effects at the regional scale (Bradford et al. 2016). Microbial communities responding to climate change may be constrained by their composition if they are dispersal limited and optimized to local conditions in terms of survival and resource acquisition strategies. These constraints could affect the community's functioning and 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 CO₂ respiration when field communities were subjected to drying and rewetting perturbations in the lab. Similar legacy effects were found following reciprocal transplantation of microbial communities historically exposed to either grass or hardwood substrate inputs (Keiser et al. 2011). These and other similar studies (Strickland et al. 2009, 2015) clearly indicate that the composition of microbial decomposer communities is shaped by long-term climate and litter chemistry and can constrain the communities’ responses to perturbation.

Microbial decomposition is largely an enzymatic process, as decomposer communities produce extracellular enzymes (EEs) to degrade the complex organic polymers in plant inputs (Sinsabaugh et al. 1994). The characteristics and quantity of different EEs produced by microbial decomposer communities are therefore key functional traits (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 drying could lead to inhibition of microbial processes (Allison and Treseder 2008), including enzyme synthesis and interactions with substrates. Thus, the net impact on decomposition rates is unclear.

We aimed to test whether microbial functioning is constrained by community composition using a microbial transplantation design across a climate gradient spanning 12.5 °C and 300 mm precipitation in southern California. In these semi-arid sites, we expected that biotic activity is constrained by moisture limitation. Based on measurements of microbial community structure and functioning, we tested two main hypotheses:

1. Rates of decomposition are controlled by differences in precipitation along the gradient.

Plant litter in sites with greater precipitation has greater microbial biomass and enzymatic activity, resulting in faster decomposition (**Figure 1A**).

2. Microbial decomposers show optimal functioning under local climate conditions (**Figure 1B**). Based on this hypothesis, we predict greater microbial biomass, enzymatic activity, and decomposition rates in litter inoculated with microbial communities historically exposed to the local climate versus in litter with microbial communities established under a foreign climate.

These hypotheses are not mutually exclusive; climate could drive mass loss across the gradient while optimization confers an advantage to local microbial communities (**Figure 1C**). If so, both factors would warrant consideration in predictive models of the C cycle.

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 sites along a climate gradient in southern California – subalpine forest, pine-oak forest, coastal grassland, pinyon-juniper scrubland, and Colorado desert. We chose to use grassland litter (*Avena barbata* and *A. fatua*) because invasive grasses are prevalent in the scrubland and desert site and dominate the grassland site where we have previously investigated the role of microbial community composition in litter decomposition (Allison et al. 2013, Alster et al. 2013).

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 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 S1**). The pine-oak forest (hereafter referred to as “pine-oak”) 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 likely receives almost half of its precipitation in the form of snow, making it the wettest site (National Centers for Environmental Information 2011). All sites other than the subalpine 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

We employed one litter type found across the gradient to focus on climate vs. microbial community constraints on decomposition, and we employed “microbial cage” litterbags to restrict microbial community composition and test community origin hypotheses. 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 m² plots located within a 50 m² sampling region. This litter was clipped to <5 cm lengths and mixed, and a sub-sample was weighed and oven-dried to determine gravimetric moisture content. The equivalent of 2.1 g dry weight of this litter (including ash content) was used to make each litterbag. ~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 (grasses, leaves, and/or needles) 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. In addition to the 2.1 g of unsterilized grassland litter in each bag, 50 mg of grassland inoculum was added to half the bags as a control (control, -); the other half received 50 mg of local inoculum native to the transplant destination site (inoculated, +) to determine whether access to local microbes would affect microbial activity and litter mass loss over the course of the study. Each litterbag was made of 0.2 μm nylon mesh that creates a “microbial cage” by restricting microbial dispersal into or out of the litterbag. These microbial cages likely reduce overall decomposition and microbial biomass, and may be particularly detrimental to fungal activity, especially when the initial litter is sterilized (Allison et al. 2013, Alster et al. 2013). For this reason, we chose to use unsterilized litter and to focus on bacteria for analyses of community composition.

Four litterbags of each type (-/+) were deployed into the six plots used to collect initial inocula at each of the five sites on November 20, 2014 ($4 \times 2 \times 6 \times 5 = 240$ total litterbags). One litterbag of each type was removed from each plot for destructive sampling on March 9, June 7, September 11, and December 2, 2015. Litter local to each site was also collected on June 7 and December 2, 2015. Collected litterbags and litter were stored in coolers and transported to UC Irvine, where litter from bags was weighed to determine mass loss before being ground into fragments <0.5 cm in length and sub-sampled for DNA extractions (June 2015 samples only), EE assays, and biomass of bacteria (all samples) and fungi (December 2015 samples only). 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 reaction velocity (V) of an individual enzyme is described as a saturating function of substrate (S) concentration:

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

where $V_{\max}$ is the enzyme's maximum reaction velocity 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), and apparent K_m is a metric of potential substrate availability (Sinsabaugh et al. 2014). We interpret apparent $V_{\max}$ as a measure of the standing enzyme pool in a sample (Alster et al. 2013).

Local inoculum, initial grassland litter, and litterbags collected in 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 based on German et al. (2012). 125 μ L of fluorometric substrate solution was combined with 125 μ L of litter homogenate in each microplate well. Samples were incubated for 4h at 4, 10, 16, 22, 28, or 34°C. For each temperature, each enzyme was assayed at a range of eight substrate concentrations achieved through seven serial two-fold dilutions of the concentrations shown in **Table S2**. Negative potential activities were converted to zero values before further analyses.

Litter chemistry

Oven-dried litter was sent to Cumberland Valley Analytical Services (Hagerstown, MD) for near-IR spectroscopy, whereby reflectance spectra 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*).

Microbial biomass

Litter bacterial cell density was estimated by methods 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.

The length of fungal hyphae in litter was measured by adapting methods used in Allison et al. (2013). 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 17 mm in diameter were made for each litter sample and affixed to a glass slide. Hyphal lengths were measured with a Carl Zeiss photomicroscope at 100X magnification using Axioplan 2 Imaging software. Hyphal lengths were measured in 89 x 67 μm viewing panes using 30 panes per slide (15 per filter). Total hyphal length in all viewing panes for a single sample was converted to estimates of hyphal length in meters per gram of dry litter using a modified procedure of Sylvia (1992).

Bacterial cell density and fungal hyphal lengths were converted to bacterial and fungal biomass (mg C g^{-1} dry litter) and used to calculate bacterial:fungal biomass (B:F ratio) using

methods identical to those in Alster et al. (2013). In brief, bacterial cells were assumed to be spherical with a radius of $0.6\mu\text{m}$ and C density of $2.2 \times 10^{-13} \text{ g } \mu\text{m}^{-3}$ (Bratbak 1985), and fungal hyphae were assumed to have a fresh density of 1.1 g cm^{-3} , 33% dry mass, 40% C in dry mass, and diameter of $5.2 \mu\text{m}$ (Paul and Clark 1996).

Bacterial community composition

To PCR amplify the bacterial 16S rRNA gene, $5 \mu\text{L}$ of a 1:50 dilution of DNA (average $1.43 \pm 0.44 \text{ ng DNA}$) from each extract was added to a cocktail containing: 1 unit per reaction of Hot Start Taq DNA polymerase (BioLabs, Inc), $1 \times$ PCR Rxn Buffer ($-\text{MgCl}_2$) (Invitrogen), $1200 \mu\text{M MgCl}_2$ (Invitrogen), $200 \mu\text{M dNTP}$, $0.2 \mu\text{M}$ Forward primer and $0.2 \mu\text{M}$ Reverse Primer, 200 mM Bovine Serum Albumin Acetylated (PROMEGA), and H_2O to a final volume of $25 \mu\text{L}$. We used the 515 forward primer (GTGYCAGCMGCCGCGGTAA) and 926 reverse primer (CCGYCAATTYMTTTRAGTTT) designed by Caporaso et al. (2012) and Fierer et al. (2012) and modified by Apprill et al. (2015) to target the V4-V5 region of the 16S gene. Following an initial denaturation step at 94°C for 3 min, PCR was cycled 35 times at 94°C for 45 s, 55°C for 30s, 68°C for 20s, with a final extension at 68°C for 10 min. We amplified each subsample in duplicate from the extracted DNA.

All amplified samples were pooled based on gel pictures, with 1.0, 2.0, $3.0 \mu\text{L}$ added for strong, moderate, weak bands respectively, into a low binding tube. After pooling, PCR products were cleaned using the Agencourt AMPure XP PCR Purification Kit (Beckman Coulter Inc., Indianapolis IN, USA), following the standard manufacturer's instructions. We then performed a gel extraction on the pooled and cleaned samples to isolate the target band. Specifically, the cleaned PCR products were run on a TAE agarose gel at 80V for 1 hour. The DNA was then gel extracted and purified using the standard ZymocleanTM Gel DNA recovery Kit protocol (Zymo

Research Corp). PCR products were assessed for quality using a High Sensitivity DNA Assay on an Agilent Bioanalyzer and quantified (10.7 ng/ μ L) on a Qubit at the Genome High-throughput Facility at University of California, Irvine. Products were then sequenced at the University of California, Davis Genome Center at the DNA Technologies Core using multiplexed paired-end Illumina MiSeq platform. Unprocessed sequences are available through NCBI's Sequence Read Archive (submission #: SUB2740524, Bioproject #: PRJNA415979).

Illumina sequence data was processed using the QIIME (version 1.9.1) toolkit (Caporaso et al. 2010). Paired end files were joined and operational taxonomic units (OTUs) were picked at 97% identity level using UCLUST (Edgar 2010) with the nearest neighbor method in QIIME. Taxonomy was assigned using SILVA v119 as the reference database (Quast et al. 2013) using QIIME scripts. For the 60 samples, the number of reads ranged between 8903 and 89,395 with a median of 37,029. Using the OTU-by-sample matrix from QIIME, we generated a rarefied composition distance matrix. We created 100 OTU tables from the original data, randomly drawing the lowest common number of sequences ($n=8,903$) from each sample. To weight rarer taxa more heavily, we transformed each table by taking the square root of each cell value and rounding to the nearest integer. We then calculated a Bray-Curtis distance matrix for each of the 100 OTU tables. Finally, for each pair-wise comparison between samples (i.e., each cell in the distance table), we chose the median Bray-Curtis value among the 100 distance matrices to yield a median, rarefied distance matrix that was used in the remaining analyses.

Statistical methods

Effects of site, sampling date, and inoculation on mass loss and bacterial biomass 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 using the `lme` function in R. Effects of site and inoculation on fungal biomass at the final sampling date were analyzed using the same ANCOVA without sampling date as a factor. ANCOVA was also used to determine any relationship between bacterial biomass and mass loss across sites, sampling dates, and inoculation treatments. Post hoc analysis of pair-wise comparisons was done with Tukey contrasts using the `lsmeans` package in R.

EE kinetic parameters were calculated for each enzyme class and incubation temperature by fitting observed EE activity to the Michaelis-Menten equation. Non-linear regressions were performed in the R software environment 3.3.1 (R Development Core Team 2017) using the `nls` function. Confidence intervals were determined for $V_{\max}$ and K_m values using the `nlstools` package. Fits of $V_{\max}$ with a 95% CI greater than twice the magnitude of $V_{\max}$ were discarded; because of greater variability in calculated fits of K_m , fits with a 95% CI greater than four times the magnitude of K_m were discarded. $V_{\max}$ 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 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:

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

Effects of site, sampling date, and inoculation on litter chemistry and $V_{\max}$ of all EE classes were determined through MANCOVA with litter moisture content as a covariate, using the Wilks Lambda method to calculate the test statistic. Canonical discriminant analysis was used to analyze multivariate litter chemistry, enzyme kinetics, and 16S community composition

at the phylum and class level (**SI, Figure S1-S4**). Given that a significant positive effect of inoculation was found on both bacterial biomass and EE $V_{\max}$ of multiple enzyme classes, $V_{\max}$ values were expressed per unit bacterial biomass and analyzed with a mixed-model ANOVA to determine if there was a significant effect of inoculation on per-biomass $V_{\max}$. For $V_{\max}$ temperature sensitivity, mixed-model ANOVAs were run for each enzyme class individually to allow for post hoc comparisons between litterbags from the December sampling date.

Data for all univariate 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 biomass met assumptions of normality after ln-transformation; $V_{\max}$ per unit bacterial biomass did not but passed visual inspection after ln-transformation. Litter chemistry did not meet assumptions of normality but passed visual inspection in base form. Temperature sensitivities of $V_{\max}$ 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.

Multivariate analysis of bacterial composition was conducted using PRIMER 6.0 and PERMANOVA+ (Clarke and Gorley 2006, Anderson et al. 2008). To test for the effects of the experimental factors on composition, we performed a two-way PERMANOVA using the default settings, including site and inoculum as fixed effects. Non-metric multidimensional scaling (NMDS) ordination was used to visualize patterns in community composition.

Results

Mass loss

Mass loss varied by site ($p < 0.001$, $F_{4,188} = 85.9$), sampling date ($p < 0.001$, $F_{3,188} = 561.9$), and inoculation treatment ($p = 0.001$, $F_{1,188} = 10.9$) (**Table 1**). Litter in the pine-oak site

decomposed 13% faster than litter in the grassland site, whereas litter in the subalpine, scrubland, and desert sites decomposed 52-55% slower. Litterbags containing local inoculum lost $25.3 \pm 1.5\%$ dry mass by December, significantly less than the $27.7 \pm 1.7\%$ mass loss observed in control litterbags containing only grassland inoculum (**Figure 3A**). Though not significant, this trend was also observed in “inoculated” litterbags in the grassland site.

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 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, accounting for 41% and 28% of total mass loss, respectively. Cellulose, hemicellulose, and the combined starch and sugar fractions declined in all sites over the course of the study (**Figure 4**). Crude protein and lignin fractions, however, were only reduced in the grassland and pine-oak sites, which experienced the most mass loss overall. The structural carbohydrate fraction increased an average of 96% (0.10 ± 0.04 g) by December in all litterbags, but this increase was enhanced by 38% in the subalpine, scrubland, and desert sites (0.11 ± 0.01 g) relative to the pine-oak and grassland sites (0.08 ± 0.004 g).

Inoculation most affected the change in the mass of the structural carbohydrate and lignin fractions in litterbags over the course of the study. By December, inoculated litterbags accumulated 22% more structural carbohydrate mass than control litterbags (0.11 ± 0.01 g vs. 0.09 ± 0.01 g, **Figure 3B**), and contained 4.3% more lignin than control litterbags by virtue of accumulating rather than losing lignin mass over the course of the study (**Figure 3C**). Mean

mass attributable to each chemical fraction in initial litter and in control and inoculated litterbags collected from each site in June and December 2015 is presented in **Table S3**.

Microbial biomass

Bacterial biomass 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 1**). Bacterial biomass was 95-115% higher in the subalpine and pine-oak sites over the course of the study than it was in the grassland, whereas bacterial biomass was 54-56% lower in the desert and scrubland sites. Bacterial biomass was greatest in most sites in December 2015, though biomass in scrubland litterbags appeared to peak by September (**Table S4**). Bacterial biomass explained a significant amount of the variation in mass loss observed in litterbags across the gradient over the course of the study ($p < 0.001$, $R^2 = 0.38$) – litterbags that contained more bacteria lost more mass (**Figure 5**). This relationship was significant within each individual site across sampling dates, with the exception of the subalpine site. Inoculation significantly increased bacterial biomass over the course of the study from 0.13 ± 0.02 mg C·g⁻¹ dry litter in control litterbags to 0.19 ± 0.03 mg C·g⁻¹ in inoculated litterbags (**Figure 3D**). Inoculation increased bacterial biomass in all sites other than the scrubland, and had the greatest positive effect on biomass in litterbags transplanted to the desert site. Mean bacterial biomass in initial litter, in control and inoculated litterbags collected from each site in June and December 2015, and in local litter collected from each site in December 2015 are presented in **Table S4**.

There was a significant effect of site ($p = 0.034$, $F_{4,43} = 2.9$) on fungal biomass at the final sampling date, but there was no effect of inoculation treatment (**Table 1**). Fungal biomass was only enhanced relative to the grassland in the subalpine site, where it was 50% higher (Tukey $p = 0.098$). There was also a significant effect of site ($p < 0.001$, $F_{4,43} = 14.8$) on B:F ratio at the

final sampling date, but no effect of inoculation treatment (**Table 1**). B:F ratio was 138% higher in the pine-oak site (5.0 ± 1.3) than in the intermediate subalpine, grassland, and desert sites (2.1 ± 0.3), and was 66% lower in the scrubland site (0.7 ± 0.1 , Tukey $p < 0.05$). Mean fungal biomass and the B:F ratio in initial litter, in control and inoculated litterbags collected from each site at the final sampling date in December 2015, and in local litter collected from each site in December 2015 are presented in **Table S5**.

Bacterial community composition

Litterbag bacterial composition, as determined by 16S rRNA sequencing, in the beginning of the dry season in June 2015 was significantly affected by site, and was marginally significantly affected by inoculation treatment as well as the interaction between site and inoculation (**Table 1**). Site-level differences in bacterial community composition at the phylum/class level were driven by variation in the relative abundance of β -proteobacteria, which accounted for >29% of the recovered 16S sequences across the gradient and 19.7% of those observed in grassland litterbags. β -proteobacteria relative abundance was 100% higher in the subalpine site, 50% higher in the pine-oak and scrubland site, and 23% higher in the desert site (relative to the grassland). Across the gradient, most recovered sequences were attributed to Bacteroidetes (>36%), β -proteobacteria, α -proteobacteria (>17%) and γ -proteobacteria (7%), with very few attributed to Cyanobacteria (<3%) Actinobacteria (<2%), Acidobacteria (<1%) and Firmicutes (<1%) (**Table S6**). The effects of inoculation and the site:inoculation interaction were driven by significant differences between inoculated and control litterbags in the desert, scrubland, and pine-oak sites (post-hoc PERMANOVA pair-wise tests; $p = 0.029$, 0.035, and 0.008, respectively). Composition in the grassland and subalpine plots were not altered by

inoculation ($p = 0.671$ and 0.243 , respectively). Differences in bacterial community composition between inoculated and control litterbags could not be distinguished at the phylum/class level.

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 1**). Differences between sites and sampling dates were driven by differences in BX and LAP V_{max} . BX V_{max} decreased over the course of the study in the grassland and pine-oak sites, but not in the subalpine, scrubland or desert sites, resulting in 57% higher observed activity in the latter sites by December (**Figure 6A**). Similar trends (higher observed activity in litterbags from the desert and subalpine sites relative those from the grassland and pine-oak sites) were observed for two other C-degrading EEs, BG and CBH (**Figure S5**). LAP V_{max} increased over the course of the study in all sites, and was only significantly different from the grassland in the pine-oak site, where activity was 41% lower by December (**Figure 6B**).

Differences between inoculated and control litterbags were driven by differences in CBH V_{max} and BG V_{max} . CBH V_{max} was enhanced by 9.5% and BG V_{max} by 6.3% in inoculated litterbags relative to control litterbags over the course of the study. However, expressing CBH and BG V_{max} per unit bacterial biomass at the same sampling date revealed that per-biomass CBH and BG V_{max} were significantly reduced in inoculated litterbags, by 13% and 15%, respectively (**Figure 3E**, **Figure 3F**). The observed significant interaction between inoculation treatment and site on EE V_{max} (**Table 1**) resulted from increased BG and CBH V_{max} in inoculated litterbags in the subalpine and desert sites (**Table S7**). EE K_m results are presented in **Table S8**.

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 1**, **Table S9**). Pair-wise comparisons of $V_{\max}$ temperature sensitivities in December litterbags between sites were used to determine if transplantation to a foreign site resulted in EEs with different temperature sensitivities from those observed in litterbags from the grassland site (**Table 2**). All EEs other than AP exhibited significantly lower $V_{\max}$ temperature sensitivities after transplantation into at least one foreign site, usually the scrubland. EE K_m temperature sensitivities are presented in **Table S10**.

Discussion

Mass loss and bacterial biomass

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 (**Figure 1A**). Prescott (2010) proposed that the aspect of climate most responsible for driving litter decomposition depends on which climatic thresholds are exceeded in a given ecosystem. Under this framework, sites with mean annual temperatures above 10°C are most likely to be limited by moisture because temperatures are warm enough that decomposer activity is less likely to be inhibited. Our results generally supported this hypothesis: decomposer activity was not a linear function of precipitation, and co-varying differences in temperature and precipitation along our gradient likely combine to drive decomposer activity. Even though the subalpine site likely receives the most precipitation once snowfall is taken into account (**Table S1**), litter there decomposed as slowly as in the desert and scrubland (**Figure 2A**). In addition, the pine-oak site receives significantly more rainfall than the grassland, yet

similar mass loss was observed over the course of the study in both sites. Freezing temperatures and snow likely limit the positive effects of increased precipitation in the higher elevation pine-oak and subalpine sites, whereas extreme high temperatures and reduced precipitation limit microbial activity in the scrubland and desert sites (Gliksman et al. 2016). Observed decomposition rates across our gradient are low compared to the 33-40% annual mass loss rates generally observed in grassland ecosystems (Zhang et al. 2008), but are similar to the 20-40% annual mass loss rates observed for grass decomposition in other studies in Mediterranean ecosystems (Steinberger et al. 1990, Vanderbilt et al. 2008, Dirks et al. 2010). Given that, we do not believe that our microbial cage litterbags significantly inhibited litter mass loss.

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 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 microbial residues or necromass contribute to the accumulation of this fraction. Microbial residues can be 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 biomass 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, Baker and Allison 2017). B:F ratios in litterbags collected at the end of the experiment support this interpretation, as bacterial biomass dominated the microbial communities in all sites other than the scrubland (**Table S5**). Our results were in line with our expectations – there was a significant positive log-linear

relationship between bacterial biomass and cumulative mass loss observed in litterbags across the gradient (**Figure 5**). Counter to this observation, litter in the subalpine, scrubland, and desert sites showed similar mass loss despite the presence of significantly more bacterial biomass in subalpine litterbags (**Figure 2B**). It is possible that colder temperatures at the subalpine site inhibit decomposer activity while enhanced moisture availability supports larger bacterial populations than in the much drier scrubland and desert sites, resulting in less efficient degradation of litter per-unit microbial biomass.

It is difficult to determine whether the microbial biomass observed in this study exceeds that found in other studies, as very few studies measure absolute microbial biomass in litter rather than soil (but see Bradford et al. 2017) and this is one of the few studies to have done so in semi-arid ecosystems. Because litter in early stages of decomposition generally supports much larger microbial biomass than does litter that is closer in structure and chemistry to soil organic material (Wardle 1993), litter microbial biomass cannot be compared to soil microbial biomass from separate studies. By the end of the experiment, bacterial and hyphal biomass was significantly higher in litterbags than in local litter in most sites (**Table S4, Table S5**, see also Baker and Allison 2017). We may have observed greater microbial biomass in litterbags due to the differences in litter type and chemistry between grasses and local vegetation. Alternatively, this may be an artifact of our microbial cage design, as total biomass in our litterbags was greater than that found in comparable studies (Austin and Vivanco 2006). It is also surprising that fungal biomass was enhanced in our litterbags relative to local litter, as Alster et al. (2013) and Allison et al. (2013) previously observed that bacterial biomass dominated the microbial community in similar microbial cage litterbags at the grassland site. However, litter in those experiments was pre-sterilized, which in conjunction with the inhibition of microbial dispersal may have

decimated the fungal community to a greater extent than the bacterial community in those studies. Indeed, fungal biomass was at least 5x greater in our litterbags than that observed by Allison et al. (2013). As such, the fungal community may be a more important player in degradation of grass litter in these ecosystems than we had anticipated, and its impact may be overlooked by the design of this study. Given that fungi are potentially more dispersal limited than bacteria (Kivlin et al. 2011), fungal community constraints may also be more restrictive than those of their bacterial counterparts when responding to future climate change. It is also worth noting that filamentous bacteria such as Actinobacteria may have also been detrimentally affected by our microbial cages, given the low relative abundance (<2%) of Actinobacteria observed in our litterbag communities despite their known status as decomposers of plant material (Lee et al. 2011). Taking these caveats into account, however, our results indicate that bacterial biomass in general is indicative of litter mass loss rates across our gradient.

Inoculation effect

Even though mass loss was lower when grassland litter was transplanted to most foreign sites, the results of our inoculation treatment indicate that climate optimization of microbial communities was unlikely to be the cause of this disparity. Inoculated litterbags unexpectedly lost less mass after transplantation than did control litterbags (**Figure 3A**). As such, the degradation abilities of grassland microbial communities do not appear constrained by different climates, at least on grassland litter (**Figure 2A**). Taxa present in grassland microbial communities may be adapted to the range of climates experienced across our regional gradient. Indeed, the grassland site experiences a relatively broad range of daily air and surface temperatures (**Table S1**), and thus, the grassland taxa persist in a wide range of climate conditions. Our 16S results further support this hypothesis – bacterial communities in the control

litterbags transplanted to the five different sites exhibited significant site-level differences by June 2015. The initial grassland microbial community possessed enough variation to diverge into distinct communities after being transplanted into different climates on a relatively short timescale.

Still, it is difficult to explain inoculation effects of increased bacterial biomass (**Figure 3D**) and altered community composition in the pine-oak, scrubland, and desert sites without invoking climate optimization by some members of the microbial community (**Figure 1C**). We speculate that the taxa driving changes in community composition following inoculation may be optimized for local climate conditions (thus the increased biomass) but are not key players in grass litter decomposition (resulting in decreased mass loss). Compared to controls, inoculated litterbags also accumulated lignin (**Figure 3D**) and exhibited greater increases in structural carbohydrates (**Figure 3C**) by the end of the study. These results indicate that the microbes proliferating after inoculation into grassland microbial communities are less effective at degrading grassland litter than the pre-existing members of the grassland microbial community, perhaps because they are optimized to degrade different litter types and chemistries. Prior studies have also found evidence that microbial communities are constrained in their ability to degrade particular litter chemistries (Strickland et al. 2009, Keiser et al. 2011).

Enzyme profiles

We hypothesized that $V_{\max}$ 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 6A**),

though peptidase activity did increase the most in the sites that received the greatest precipitation (Figure 6B). These opposing trends between peptide degradation and activity of EEs in general were also found in previous studies of native litter communities in these arid and semi-arid ecosystems (Alster et al. 2013, Baker and Allison 2017). Such trends are consistent with EE accumulation over dry periods in the more arid sites and enhanced turnover of EEs in the wetter sites along the gradient. In general, observed EE $V_{\max}$ values were 2-10 times greater than those observed by others in similar semiarid ecosystems (Gallo et al. 2009, Brandt et al. 2010). However, we believe these large enzyme pools are not an artifact of our microbial cage litterbags because comparable EE $V_{\max}$ values were observed across the gradient in unconfined local litter in a previous study (Baker and Allison 2017).

Our results supported the hypothesis that inoculation would enhance EE activity. We also found that inoculation shifted 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. This result suggests that EEs produced by inoculated microbes were less efficient on grassland litter than on their native substrates. Other studies have suggested that microbial optimization to a particular litter chemistry may matter more for litter decomposition rates than optimization to a particular climate (Keiser et al. 2011, Wallenstein et al. 2013). Given that inoculation also increased bacterial biomass, it appears that some of the taxa that proliferated after inoculation may be cheaters – organisms that benefit from EE production without producing EEs themselves (West et al. 2006). Even though BG and CBH $V_{\max}$ were higher in inoculated litterbags, BG and CBH activities per bacterial biomass were

lower (**Figure 3E, F**). This could explain why some inoculated taxa proliferated under their native climate conditions while inhibiting or not affecting decomposition rates.

There is also evidence for community optimization to litter chemistry from our EE $V_{\max}$ and $V_{\max}$ temperature sensitivity results. EE $V_{\max}$ and $V_{\max}$ temperature sensitivities in transplanted litterbags were similar to one another and did not shift to emulate the EE parameters of local microbial communities (**Figure S3**). This pattern was observed regardless of which site litterbags were transplanted to. EE $V_{\max}$ of local microbial communities exhibited greater variance than those observed in transplanted litterbags, which indicates that local microbes decomposing native litter in each site likely produce EEs in different amounts depending on the litter type. EE $V_{\max}$ temperature sensitivities were also more varied in microbial communities native to each site than they were in transplanted litterbags, which indicates that microbes on local native litter are also likely producing structurally different EEs from those on grassland litter. This provides a mechanistic explanation as to how microbial communities are optimized to degrade particular litter chemistries.

Conclusion

Observed patterns in litter decomposition indicate that precipitation and temperature likely interact to limit decomposition rates at the ends of our gradient. Reduced moisture limitation resulting from higher precipitation is outweighed by temperature constraints at the higher elevation sites, while the effect of warmer temperature is 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.

In the context of a predicted future shift to a more arid climate in the American Southwest, our results indicate that although microbial communities differ both taxonomically and functionally across a wide range of climates, the decomposer function of these communities may not be constrained by climate history (**Figure 1**). Instead, it is more likely to be constrained by litter type and chemistry. This insight potentially simplifies efforts to incorporate microbe-explicit mechanisms of temperature response into global C-cycling models and predict future C dynamics (Allison and Martiny 2008). On the other hand, our findings also suggest that compositional legacies driven by vegetation chemistry could be important in C cycle predictions, as microbial communities adapted to particular litter chemistries appear to produce distinct extracellular enzymes. This is especially true if plant communities and dominant litter chemistries shift with climate change, as has been previously observed by the movement of conifers upslope along this gradient after a period of extended drought (Fellows and Goulden 2012).

Acknowledgements

Funding for this project was provided by the U.S. National Science Foundation's Ecosystem Studies Program, a U.C.N.R.S. Mathias Grant, an Ecological Society of America Forrest Shreve Award, a U.C.N.R.S. I.S.E.E.C.I. Graduate Research Fellowship, and a U.C. Public Impact Fellowship. We thank J. Gee and J.W. Laundre of the James Reserve, A. Muth of the Boyd Deep Canyon Desert Research Center, J. Burger of the Irvine Ranch Conservancy, and J. Ladley of the San Bernardino National Forest Service for their support in making use of land under their

management. We thank A.C. Martiny, M. Nelson, and M.K. Goulden for helpful discussion and comments, and C. Weihe for helpful discussion, comments, and elbow grease. We thank D.E. Islip, G.A. Barajas, E. Stogner, M.Y. Farah, and, in particular, D.M. Nisson for their hours of dedicated service on this project, and S.M. Baker for her help deploying litterbags on mountains and in deserts.

References

- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* 79:439–449.
- Allison, S. D., T. B. Gartner, K. Holland, M. Weintraub, and R. L. Sinsabaugh. 2007. Soil enzymes: linking proteomics and ecological process. Pages 704–711 *Manual of Environmental Microbiology*. 3rd edition. ASM Press.
- Allison, S. D., Y. Lu, C. Weihe, M. L. Goulden, A. C. Martiny, K. K. Treseder, and J. B. H. Martiny. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 94:714–25.
- Allison, S. D., and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences* 105:11512–11519.
- Allison, S. D., and K. K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14:2898–2909.
- Alster, C. J., D. P. German, Y. Lu, and S. D. Allison. 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. *Soil Biology and Biochemistry* 64:68–79.
- Anderson, M., R. N. Gorley, and R. K. Clarke. 2008. *Permanova+ for Primer: Guide to Software and Statistical Methods*. Primer-E Limited.
- Apprill, A., S. McNally, R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology* 75:129–137.
- Austin, A. T., and L. Vivanco. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. *Nature* 442:555–558.
- Baker, N. R., and S. D. Allison. 2017. Extracellular enzyme kinetics and thermodynamics along a climate gradient in southern California. *Soil Biology and Biochemistry* 114:82–92.
- Bradford, M. A., B. Berg, D. S. Maynard, W. R. Wieder, and S. A. Wood. 2016. Understanding the dominant controls on litter decomposition. *Journal of Ecology* 104:229–238.
- Bradford, M. A., G. F. Veen, A. Bonis, E. M. Bradford, A. T. Classen, J. H. C. Cornelissen, T. W. Crowther, J. R. De Long, G. T. Freschet, P. Kardol, M. Manrubia-Freixa, D. S. Maynard, G. S. Newman, R. S. P. Logtestijn, M. Viketoft, D. A. Wardle, W. R. Wieder, S. A. Wood, and W. H. van der Putten. 2017. A test of the hierarchical model of litter decomposition. *Nature Ecology & Evolution*.
- Brandt, L. A., J. Y. King, S. E. Hobbie, D. G. Milchunas, and R. L. Sinsabaugh. 2010. The role

- of photodegradation in surface litter decomposition across a grassland ecosystem precipitation gradient. *Ecosystems* 13:765–781.
- Bratbak, G. 1985. Bacterial biovolume and biomass estimations. *Applied and Environmental Microbiology* 49:1488–1493.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. a Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335–336.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, and R. Knight. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* 6:1621–1624.
- Clarke, K. R., and R. N. Gorley. 2006. Primer v6: User Manual/Tutorial. Plymouth Marine Laboratory.
- Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–73.
- Dirks, I., Y. Navon, D. Kanas, R. Dumbur, and J. M. Grünzweig. 2010. Atmospheric water vapor as driver of litter decomposition in Mediterranean shrubland and grassland during rainless seasons. *Global Change Biology* 16:2799–2812.
- Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461.
- Evans, S. E., and M. D. Wallenstein. 2011. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* 109:101–116.
- Fellows, A. W., and M. L. Goulden. 2012. Rapid vegetation redistribution in Southern California during the early 2000s drought. *Journal of Geophysical Research: Biogeosciences* 117.
- Fierer, N., J. W. Leff, B. J. Adams, U. N. Nielsen, S. Thomas, C. L. Lauber, S. Owens, J. A. Gilbert, D. H. Wall, and J. G. Caporaso. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences* 109:21390–21395.
- Gallo, M. E., A. Porras-Alfaro, K. J. Odenbach, and R. L. Sinsabaugh. 2009. Photoacceleration of plant litter decomposition in an arid environment. *Soil Biology & Biochemistry* 41:1433–1441.
- German, D. P., K. R. Marcelo, M. M. Stone, and S. D. Allison. 2012. The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Global Change Biology* 18:1468–1479.
- Gliksman, D., A. Rey, R. Seligmann, R. Dumbur, O. Sperling, Y. Navon, S. Hanel, P. De Angelis, J. A. Arnone, and J. M. Grünzweig. 2016. Biotic degradation at night, abiotic degradation at day: positive feedbacks on litter decomposition in drylands. *Global Change Biology*.
- Goulden, M. L., G. C. Winston, A. M. Mcmillan, M. E. Litvak, E. L. Read, A. V Rocha, and J. R. Elliot. 2006. An eddy covariance mesonet to measure the effect of forest age on land-atmosphere exchange. *Global Change Biology* 12:2146–2162.
- Grandy, a S., and J. C. Neff. 2008. Molecular C dynamics downstream: the biochemical

- decomposition sequence and its impact on soil organic matter structure and function. *The Science of the total environment* 404:297–307.
- IPCC. 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. Geneva, Switzerland.
- Keiser, a. D., M. S. Strickland, N. Fierer, and M. a. Bradford. 2011. The effect of resource history on the functioning of soil microbial communities is maintained across time. *Biogeosciences* 8:1477–1486.
- Khan, K. S., R. Mack, X. Castillo, M. Kaiser, and R. G. Joergensen. 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. *Geoderma* 271:115–123.
- Kivlin, S. N., C. V. Hawkes, and K. K. Treseder. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 43:2294–2303.
- Lee, C. G., T. Watanabe, Y. Sato, J. Murase, S. Asakawa, and M. Kimura. 2011. Bacterial populations assimilating carbon from ¹³C-labeled plant residue in soil: Analysis by a DNA-SIP approach. *Soil Biology and Biochemistry* 43:814–822.
- Miltner, A., P. Bombach, B. Schmidt-Brücken, and M. Kästner. 2012. SOM genesis: Microbial biomass as a significant source. *Biogeochemistry* 111:41–55.
- National Centers for Environmental Information. 2011. 1981–2010 Annual/Seasonal Normals.
- Paul, E. A., and F. E. Clark. 1996. *Components of the Soil Biota*. Page *Soil Microbiology and Biochemistry*. 2nd edition. Academic Press, San Diego.
- Prescott, C. E. 2010. Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry* 101:133–149.
- Quast, C., E. Priesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* 41:590–596.
- R Development Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Vienna, Austria.
- Raich, J. W., and W. H. Schlesinger. 2002. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44:81–99.
- Reed, H. E., and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. *FEMS microbiology ecology* 62:161–70.
- Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.-P. Huang, N. Harnik, A. Leetmaa, N.-C. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. *Science* 316:1181–1184.
- Shepherd, K. D., B. Vanlauwe, C. N. Gachengo, and C. a. Palm. 2005. Decomposition and mineralization of organic residues predicted using near infrared spectroscopy. *Plant and Soil* 277:315–333.
- Sinsabaugh, R. L., J. Belnap, S. G. Findlay, J. J. F. Shah, B. H. Hill, K. A. Kuehn, C. R. Kuske, M. E. Litvak, N. G. Martinez, D. L. Moorhead, and D. D. Warnock. 2014. Extracellular enzyme kinetics scale with resource availability. *Biogeochemistry* 121:287–304.
- Sinsabaugh, R., D. Moorhead, and A. Linkins. 1994. The enzymic basis of plant litter decomposition: emergence of an ecological process. *Applied Soil Ecology* 1:97–111.
- Steinberger, Y., a. Shmida, and W. G. Whitford. 1990. Decomposition along a rainfall gradient in the Judean desert, Israel. *Oecologia* 82:322–324.
- Strickland, M. S., A. D. Keiser, and M. A. Bradford. 2015. Climate history shapes contemporary

- leaf litter decomposition. *Biogeochemistry* 122:165–174.
- Strickland, M. S., C. Lauber, N. Fierer, and M. a Bradford. 2009. Testing the functional significance of microbial community composition. *Ecology* 90:441–51.
- Sylvia, D. M. 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. *Methods in Microbiology* 24:53–65.
- Vanderbilt, K. L., C. S. White, O. Hopkins, and J. A. Craig. 2008. Aboveground decomposition in arid environments: Results of a long-term study in central New Mexico. *Journal of Arid Environments* 72:696–709.
- Wallenstein, M., S. D. Allison, J. Ernakovich, M. J. Steinweg, and R. Sinsabaugh. 2011. Controls on the temperature sensitivity of soil enzymes. Pages 245–258 in G. Shukla and A. Varma, editors. *Soil Enzymology*. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Wallenstein, M. D., M. L. Haddix, E. Ayres, H. Steltzer, K. a. Magrini-Bair, and E. a. Paul. 2013. Litter chemistry changes more rapidly when decomposed at home but converges during decomposition–transformation. *Soil Biology and Biochemistry* 57:311–319.
- Wallenstein, M. D., S. K. McMahon, J. P. Schimel, N. Resource, F. Collins, and S. Barbara. 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology* 15:1631–1639.
- Wardle, D. A. 1993. Changes in the microbial biomass and metabolic quotient during leaf litter succession in some New Zealand forest and scrubland ecosystems. *Functional Ecology* 7:346–355.
- West, S. a, A. S. Griffin, A. Gardner, and S. P. Diggle. 2006. Social evolution theory for microorganisms. *Nature reviews. Microbiology* 4:597–607.
- Zhang, D., D. Hui, Y. Luo, and G. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology* 1:85–93.

Table 1 Analysis of variance results for effects of site, sampling date, inoculation treatment (“Inoc”), and all interactions on litter and microbial properties. All analyses were run with gravimetric litter moisture (H₂O) as a covariate. Bolded p-values are significant (<0.05).

Variable	Site	Date	Inoc	H ₂ O	Site:Date	Site:Inoc	Date:Inoc	S:D:I
Mass loss ¹	<0.001	<0.001	0.001	<0.001	<0.001	0.936	0.529	0.930
Bacterial biomass ¹	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	0.089	<0.001
Fungal biomass ^{1,2}	0.034	–	0.299	0.032	–	0.741	–	–
B:F biomass ^{1,2}	<0.001	–	0.290	0.010	–	0.163	–	–
Litter chemistry ¹	<0.001	<0.001	<0.001	<0.001	<0.001	0.125	0.627	0.639
V _{max} ³	<0.001	<0.001	0.056	<0.001	<0.001	<0.001	<0.001	0.087
V _{max} TS ³	<0.001	<0.001	0.359	0.224	<0.001	0.630	0.641	0.898
16S community ⁴	0.001	–	0.067	–	–	0.052	–	–

¹ANCOVA

²Final sampling date in December 2015 only

³MANCOVA with all enzyme classes

⁴PERMANOVA

Table 2 Mean ($\pm$ 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
Desert	2.17 $\pm$ 0.08	1.57 $\pm$ 0.01	1.85 $\pm$ 0.04	1.90$\pm$0.02	2.15 $\pm$ 0.06	1.88 $\pm$ 0.04	1.95 $\pm$ 0.03
Scrubland	1.95$\pm$0.03	1.51 $\pm$ 0.02	1.68$\pm$0.03	1.84$\pm$0.03	1.93$\pm$0.03	1.81 $\pm$ 0.02	1.79$\pm$0.02
Grassland	2.18 $\pm$ 0.03	1.60 $\pm$ 0.02	1.88 $\pm$ 0.02	2.02 $\pm$ 0.03	2.11 $\pm$ 0.03	1.92 $\pm$ 0.02	2.01 $\pm$ 0.02
Pine-Oak	1.95$\pm$0.03	1.53 $\pm$ 0.02	1.86 $\pm$ 0.02	2.07 $\pm$ 0.03	2.07 $\pm$ 0.03	1.79$\pm$0.02	1.90$\pm$0.02
Subalpine	1.96$\pm$0.02	1.55 $\pm$ 0.02	1.87 $\pm$ 0.02	1.99 $\pm$ 0.02	2.14 $\pm$ 0.03	1.84 $\pm$ 0.03	1.86$\pm$0.02

729

Figure 1. Conceptual figure of hypotheses for transplantation effects on litter mass loss over the course of the study. Mass loss in different sites or in control vs. inoculated litterbags is shown by the differently shaded bars. **A)** Hypothesized litter mass loss assuming that climate (specifically, precipitation), is the main driver of mass loss. **B)** Hypothesized mass loss if microbial communities decompose litter optimally in their native environment and are constrained in their ability to decompose litter in foreign environments. Inoculating transplanted grassland litter with local microbial communities will enhance microbial activity and decomposition. **C)** Hypothesized microbial decomposition if the effects of climate and community optimization both affect mass loss rates across the gradient, such that inoculation with local microbiota (shown by the black arrow) enhances mass loss in conditions that are inherently less favorable for decomposition.

Figure 2. Mean ($\pm$ SE) **A)** percent mass loss from litter and **B)** bacterial biomass (mg C g^{-1} dry litter) in transplanted litterbags over the course of the study, averaged across both control and inoculated litterbags in each site at each sampling date. Depicted means and standard errors are back-transformed from $\ln$ values.

Figure 3. Effect of inoculation with local microbial communities on mean ($\pm$ 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 ($\pm$ SE) **D)** bacterial biomass, **E)** cellobiohydrolase V_{max} per unit bacterial biomass, and **F)** β -glucosidase V_{max} per unit bacterial biomass over the course of the study. All effects shown are significant (Tukey $p < 0.05$).

Figure 4. Mean ($\pm$ SE) mass change in each major fraction of litter by December 2015. Positive values indicate accumulation of that fraction, whereas negative values indicate loss of that fraction.

Figure 5. Mass (g) lost from litter as a function of bacterial biomass ($\ln \text{ mg C g}^{-1} \text{ dry litter}$) at that sampling date, across sites and sampling dates ($p < 0.001$, $R^2 = 0.38$). The shaded region around the line signifies 95% confidence intervals.

Figure 6. Mean ($\pm$ 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.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Supplemental Information

Methods

Canonical discriminant analysis (CDA) summarizes correlated changes in multiple variables within a specified experimental design along one or more axis. Loading coefficients along these axes are attributed to each variable included in the analysis, in this case indicating which variables best partition differences between sites, sampling dates, or inoculated vs. control litterbags. CDA was used to determine that mass of the structural carbohydrate and lignin fractions most distinguished control from inoculated litterbags (**Figure S1**). Absolute values of the loading coefficients on the single CDA axis for structural carbohydrate and lignin mass were 0.68 and 0.67, respectively.

CDA was also used to determine which extracellular enzymes' observed $V_{\max}$ best distinguished litterbags from different sites, from different sampling dates, and between control and inoculated litterbags. 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 (**Figure S2A**). There was only one CDA axis for differences between sampling dates, and the absolute values of the loading coefficients for BX and LAP $V_{\max}$ were 0.57 for both (**Figure S2B**). There was also only one CDA axis for differences between control and inoculated litterbags, and CBH $V_{\max}$ and BG $V_{\max}$ explained the most variation along that axis with loading coefficients with absolute values of 0.77 and 0.61, respectively (**Figure S2C**).

CDA was also used to visually depict how observed extracellular enzyme $V_{\max}$ and $V_{\max} Q_{10}$ values in litterbags generally differed from those observed in local litter at each site (**Figure S3**).

CDA was also used to determine which of the most abundant (relative abundance >2%) phyla and classes of bacterial taxa (Bacteroidetes, β -Proteobacteria, α -Proteobacteria, γ -Proteobacteria,

24 Cyanobacteria, and Actinobacteria) explained the greatest amount of variation in 16S community
25 composition in litterbags collected from the different sites in June 2015. The first CDA axis
26 explained 68.8% of the variation in bacterial community composition at the phylum/class level,
27 and β -proteobacteria was the only bacterial class with a loading coefficient with an absolute
28 value greater than 0.5, at 0.71 (**Figure S4**).

For Review Only

Table S1. Mean ($\pm$ SE) historic climate parameters for the five sites used in this study, and plot-level temperature over the last twelve months (January-December 2015) of the study.

Variable	Desert	Scrubland	Grassland	Pine-Oak	Subalpine	Period
Air temperature ($^{\circ}$ C)	22.8 $\pm$ 0.8	15.6 $\pm$ 0.8	16.4 $\pm$ 0.3	12.3 $\pm$ 0.6	10.3 $\pm$ 1.8	2009-14
Air daily temp. range	10.8 $\pm$ 0.4	8.5 $\pm$ 0.2	8.3 $\pm$ 0.3	6.2 $\pm$ 0.2	-	2009-14
Soil temperature ($^{\circ}$ C)	28.3 $\pm$ 0.3	18.4 $\pm$ 0.3	19.1 $\pm$ 0.9	9.9 $\pm$ 0.3	-	2008-12
Soil daily temp. range	10.9 $\pm$ 0.2	5.5 $\pm$ 0.7	4.6 $\pm$ 0.8	2.6 $\pm$ 0.1	-	2008-12
Plot temperature ($^{\circ}$ C)	29.3 $\pm$ 0.4	19.5 $\pm$ 0.5	22.0 $\pm$ 0.4	13.2 $\pm$ 0.4	11.5 $\pm$ 0.4	2015
Plot daily temp. range	20.3 $\pm$ 0.3	24.8 $\pm$ 0.4	26.2 $\pm$ 0.5	20.8 $\pm$ 0.5	13.7 $\pm$ 0.4	2015
Precipitation (mm)	100 $\pm$ 24	193 $\pm$ 33	242 $\pm$ 76	402 $\pm$ 118	>265	2009-14
Soil moisture (μ L/cm ³)	46 $\pm$ 1	73 $\pm$ 5	73 $\pm$ 8	86 $\pm$ 3	-	2008-12
Solar radiation (W/m ²)	225 $\pm$ 7	234 $\pm$ 7	217 $\pm$ 8	224 $\pm$ 7	~270	2006-13
Elevation (m)	275	1280	470	1710	2250	

Table S2. 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	1.5625-200 μ M
Acid phosphatase (AP)	Organic P	4-MUB Phosphate	6.25-800 μ M
β -glucosidase (BG)	Cellulose degradation products	4-MUB- β -D-glucopyranoside	3.125-400 μ M
β -xylosidase (BX)	Hemicellulose degradation products	4-MUB- β -D-xylopyranoside	3.125-400 μ M
Cellobiohydrolase (CBH)	Cellulose degradation products	4-MUB- β -D-cellobioside	1.5625-200 μ M
Leucine-aminopeptidase (LAP)	Peptide terminals	L-leucine-7-amido-4-methylcoumarin hydrochloride	1.5625-200 μ M
<i>N</i> -acetyl- β -D-glucosaminidase (NAG)	Chitin degradation products	4-MUB- <i>N</i> -acetyl- β -D-glucosaminide	3.125-400 μ M

Table S3. Mean ($\pm$ SE) mass (g) of litter in litterbags attributable to cellulose, hemicellulose, lignin, structural carbohydrate, sugars/starches, and crude protein fractions, in initial litterbags and in control and inoculated litterbags collected from each site in June and December 2015.

Enzyme	Site	Initial	June		December	
			Control	Inoc.	Control	Inoc.
Cellulose	Desert		0.74 $\pm$ 0.01	0.76 $\pm$ 0.01	0.70 $\pm$ 0.01	0.68 $\pm$ 0.01
	Scrubland		0.74 $\pm$ 0.02	0.74 $\pm$ 0.01	0.67 $\pm$ 0.02	0.70 $\pm$ 0.01
	Grassland	0.85 $\pm$ 0.01	0.66 $\pm$ 0.02	0.67 $\pm$ 0.02	0.55 $\pm$ 0.03	0.59 $\pm$ 0.05
	Pine-Oak		0.65 $\pm$ 0.01	0.65 $\pm$ 0.01	0.51 $\pm$ 0.01	0.50 $\pm$ 0.01
	Subalpine		0.72 $\pm$ 0.01	0.71 $\pm$ 0.02	0.63 $\pm$ 0.05	0.67 $\pm$ 0.01
Hemicellulose	Desert		0.29 $\pm$ 0.01	0.31 $\pm$ 0.01	0.28 $\pm$ 0.00	0.26 $\pm$ 0.01
	Scrubland		0.30 $\pm$ 0.01	0.28 $\pm$ 0.01	0.24 $\pm$ 0.02	0.28 $\pm$ 0.01
	Grassland	0.38 $\pm$ 0.01	0.22 $\pm$ 0.01	0.24 $\pm$ 0.01	0.22 $\pm$ 0.01	0.23 $\pm$ 0.01
	Pine-Oak		0.26 $\pm$ 0.01	0.25 $\pm$ 0.01	0.20 $\pm$ 0.01	0.20 $\pm$ 0.01
	Subalpine		0.30 $\pm$ 0.02	0.28 $\pm$ 0.01	0.23 $\pm$ 0.02	0.21 $\pm$ 0.02
Lignin	Desert		0.20 $\pm$ 0.00	0.22 $\pm$ 0.00	0.20 $\pm$ 0.00	0.21 $\pm$ 0.00
	Scrubland		0.20 $\pm$ 0.00	0.20 $\pm$ 0.00	0.20 $\pm$ 0.00	0.21 $\pm$ 0.00
	Grassland	0.20 $\pm$ 0.00	0.21 $\pm$ 0.00	0.21 $\pm$ 0.00	0.18 $\pm$ 0.01	0.19 $\pm$ 0.01
	Pine-Oak		0.20 $\pm$ 0.00	0.21 $\pm$ 0.01	0.19 $\pm$ 0.00	0.19 $\pm$ 0.00
	Subalpine		0.20 $\pm$ 0.00	0.21 $\pm$ 0.00	0.20 $\pm$ 0.02	0.23 $\pm$ 0.00
Structural	Desert		0.22 $\pm$ 0.00	0.23 $\pm$ 0.01	0.21 $\pm$ 0.01	0.22 $\pm$ 0.00
carbohydrates	Scrubland		0.22 $\pm$ 0.00	0.23 $\pm$ 0.00	0.21 $\pm$ 0.01	0.19 $\pm$ 0.01

	Grassland	0.10±0.00	0.21±0.01	0.23±0.01	0.17±0.01	0.19±0.01
	Pine-Oak		0.20±0.01	0.22±0.01	0.17±0.01	0.19±0.00
	Subalpine		0.19±0.01	0.23±0.00	0.21±0.03	0.27±0.01
Sugars and starches	Desert		0.05±0.00	0.07±0.00	0.06±0.01	0.06±0.00
	Scrubland		0.06±0.00	0.06±0.00	0.06±0.00	0.07±0.00
	Grassland	0.09±0.01	0.05±0.00	0.04±0.00	0.04±0.00	0.04±0.00
	Pine-Oak		0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00
	Subalpine		0.06±0.01	0.04±0.00	0.03±0.00	0.04±0.00
Protein	Desert		0.22±0.00	0.22±0.01	0.21±0.01	0.21±0.00
	Scrubland		0.21±0.00	0.22±0.00	0.22±0.01	0.20±0.01
	Grassland	0.21±0.01	0.21±0.01	0.21±0.01	0.17±0.01	0.19±0.01
	Pine-Oak		0.18±0.01	0.18±0.01	0.17±0.01	0.18±0.00
	Subalpine		0.18±0.00	0.21±0.00	0.17±0.02	0.22±0.01

Table S4. Mean ($\pm$ SE) bacterial biomass C ($\mu\text{g C}\cdot\text{g}^{-1}$ dry litter) in initial litter, in control and inoculated litterbags collected from each site at each sampling date in 2015, and in local litter collected from each site in June and December 2015.

Site	Initial	March		June		September		December		
		Control	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc	Local
Desert		11±2	50±15	6±1	33±7	110±20	96±12	630±180	520±97	290±70
Scrubland		32±9	38±14	41±13	36±11	250±58	290±89	350±86	310±90	190±40
Grassland	22±3	16±5	29±7	140±20	140±25	170±51	460±150	870±250	570±170	860±290
Pine-Oak		28±8	16±2	320±66	400±63	450±210	1110±360	1090±240	1880±340	370±180
Subalpine		330±140	500±140	40±12	72±11	320±89	380±67	1090±530	1640±490	580±210

33

Table S5. Hyphal biomass C in $\text{mg C} \cdot \text{g}^{-1}$ dry litter and bacterial:fungal biomass ratios in initial litter, in control and inoculated litterbags, and in local litter collected from each site at the final sampling date in December 2015.

Site	Hyphal biomass C				B:F biomass ratio			
	Initial	Control	Inoc.	Local	Initial	Control	Inoc.	Local
Desert		0.31±0.09	0.31±0.05	0.12±0.01		2.05±0.74	1.66±0.55	2.45±0.66
Scrubland		0.48±0.07	0.41±0.04	0.16±0.06		0.74±0.10	0.75±0.24	1.01±0.32
Grassland	0.24±0.02	0.37±0.07	0.36±0.09	0.33±0.05	0.09±0.01	2.34±0.90	1.60±0.90	2.59±1.10
Pine-Oak		0.29±0.06	0.28±0.07	0.18±0.02		3.76±1.11	6.72±2.74	2.10±1.02
Subalpine		0.60±0.16	0.40±0.15	0.09±0.01		1.82±0.78	4.10±1.53	6.40±2.43

34

Table S6. Mean relative abundance ($\% \pm \text{SE}$) of sequences attributable to the most abundant taxa ($>2\%$ of all reads) at the class or phylum level as determined by 16S amplicon sequencing in control and inoculated litterbags collected from each site in June 2015. Total reads recovered from control and inoculated litterbags from each site included for reference

Phylum/Class	Site	Control	Inoc.
Bacteroidetes	Desert	29.3 $\pm$ 3.5	26.1 $\pm$ 1.5
	Scrubland	36.0 $\pm$ 3.5	38.8 $\pm$ 3.0
	Grassland	35.4 $\pm$ 2.5	26.1 $\pm$ 2.0
	Pine-Oak	36.3 $\pm$ 1.2	40.1 $\pm$ 2.1
	Subalpine	39.4 $\pm$ 2.8	39.1 $\pm$ 6.0
β -Proteobacteria	Desert	23.9 $\pm$ 3.8	24.7 $\pm$ 1.9
	Scrubland	30.8 $\pm$ 4.5	26.9 $\pm$ 2.2
	Grassland	18.8 $\pm$ 1.8	20.6 $\pm$ 0.9
	Pine-Oak	30.8 $\pm$ 1.5	29.5 $\pm$ 1.4
	Subalpine	38.8 $\pm$ 3.1	40.4 $\pm$ 4.6
α -Proteobacteria	Desert	19.1 $\pm$ 0.8	21.8 $\pm$ 1.8
	Scrubland	15.0 $\pm$ 0.8	19.1 $\pm$ 1.3
	Grassland	20.1 $\pm$ 2.1	24.2 $\pm$ 1.8
	Pine-Oak	19.5 $\pm$ 1.5	18.9 $\pm$ 0.7
	Subalpine	12.2 $\pm$ 0.8	9.4 $\pm$ 0.8

γ -Proteobacteria	Desert	5.3 $\pm$ 0.9	4.5 $\pm$ 0.9
	Scrubland	6.2 $\pm$ 1.0	6.7 $\pm$ 0.6
	Grassland	15.8 $\pm$ 1.4	17.1 $\pm$ 2.2
	Pine-Oak	6.8 $\pm$ 1.4	4.9 $\pm$ 0.3
	Subalpine	3.1 $\pm$ 0.6	4.1 $\pm$ 0.7
Cyanobacteria	Desert	12.5 $\pm$ 4.0	11.8 $\pm$ 2.4
	Scrubland	3.0 $\pm$ 0.6	1.2 $\pm$ 0.5
	Grassland	1.7 $\pm$ 1.2	1.4 $\pm$ 1.1
	Pine-Oak	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
	Subalpine	0.8 $\pm$ 0.4	0.7 $\pm$ 0.4
Actinobacteria	Desert	3.1 $\pm$ 0.6	3.1 $\pm$ 0.4
	Scrubland	2.5 $\pm$ 0.1	1.9 $\pm$ 0.2
	Grassland	2.3 $\pm$ 0.3	3.9 $\pm$ 0.4
	Pine-Oak	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1
	Subalpine	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1
Total reads	Desert	25.7 $\pm$ 4.7	26.0 $\pm$ 6.9
(1000s)	Scrubland	42.7 $\pm$ 7.2	40.1 $\pm$ 9.8
	Grassland	30.1 $\pm$ 1.4	37.6 $\pm$ 7.4
	Pine-Oak	44.7 $\pm$ 3.5	60.6 $\pm$ 7.6
	Subalpine	45.4 $\pm$ 6.3	50.4 $\pm$ 5.5

Table S7. Extracellular enzyme $V_{\max}$ in $\mu\text{mol}\cdot\text{hr}^{-1}\cdot\text{g}^{-1}$ for seven enzyme classes in initial litter and control and inoculated litterbags collected from each site in June and December 2015.

Enzyme	Site	Initial	June		December	
			Control	Inoc.	Control	Inoc.
α -glucosidase	Desert		2.9±0.2	4.0±0.1	3.5±0.4	3.1±0.1
	Scrubland		3.1±0.3	3.4±0.2	4.8±0.3	3.5±0.2
	Grassland	1.7±0.1	3.2±0.1	3.7±0.6	4.3±0.3	3.9±0.9
	Pine-Oak		3.5±0.3	4.3±0.3	4.1±0.3	4.7±0.4
	Subalpine		3.5±0.1	4.4±0.3	4.2±0.7	4.8±0.3
Acid phosphatase	Desert		34.0±1.2	42.5±1.4	34.5±1.0	32.3±2.6
	Scrubland		32.6±1.5	31.9±2.7	31.0±1.1	23.3±1.9
	Grassland	38.9±1.0	29.8±2.2	35.3±5.1	28.2±1.9	23.5±5.1
	Pine-Oak		36.3±2.1	42.7±2.7	37.5±2.9	31.9±1.6
	Subalpine		34.2±1.3	37.7±3.1	36.1±1.4	41.7±4.8
β -glucosidase	Desert		64.7±4.0	85.8±1.5	77.2±6.5	64.9±4.1
	Scrubland		62.8±4.8	70.5±4.7	76.7±3.4	53.7±2.3
	Grassland	52.3±1.3	54.8±5.0	64.9±4.8	73.0±2.8	62.1±6.2
	Pine-Oak		55.0±3.3	67.4±3.9	56.2±3.0	55.4±2.3
	Subalpine		64.5±1.4	84.7±4.0	71.1±5.3	89.8±11.4
B-xylosidase	Desert		29.9±2.1	38.5±0.9	31.4±5.0	24.1±1.8
	Scrubland		28.2±2.6	28.8±2.9	31.5±2.2	21.6±1.4
	Grassland	29.6±1.2	19.8±2.7	29.0±3.3	21.0±1.7	20.3±3.3

	Pine-Oak		21.8±1.7	27.5±2.0	16.3±0.8	17.0±0.5
	Subalpine		29.0±0.8	34.6±1.7	23.7±2.6	29.4±3.9
Cellobiohydrolase	Desert		37.0±2.6	50.5±1.3	46.2±4.3	38.9±1.9
	Scrubland		35.2±3.0	38.8±2.9	42.6±1.7	32.9±1.6
	Grassland	28.9±1.0	28.8±2.6	36.6±2.4	38.3±0.8	36.7±3.6
	Pine-Oak		30.5±2.9	36.5±2.2	30.1±1.3	30.5±1.2
	Subalpine		34.3±1.1	47.6±2.3	39.2±2.6	45.9±8.3
Leucine- aminopeptidase	Desert		3.5±0.4	2.7±0.1	4.9±0.5	5.9±0.7
	Scrubland		4.3±2.7	3.4±0.4	5.6±0.7	5.2±1.0
	Grassland	1.5±0.0	4.6±0.5	3.6±1.1	6.3±0.6	4.6±1.4
	Pine-Oak		7.0±1.1	8.0±2.2	9.1±0.8	10.6±1.5
	Subalpine		4.0±0.2	6.6±0.7	7.4±3.0	6.7±0.6
<i>N</i> -acetyl- β -D- glucosaminidase (NAG)	Desert		25.3±2.4	34.8±1.3	31.1±3.9	24.5±2.1
	Scrubland		26.0±1.8	28.8±2.4	33.7±1.3	24.6±1.5
	Grassland	15.9±0.5	26.0±2.1	32.6±2.7	31.6±1.7	28.3±3.6
	Pine-Oak		29.1±2.5	34.5±1.9	27.4±1.7	25.5±2.0
	Subalpine		29.2±1.2	34.2±1.8	33.1±4.7	40.2±5.8

Table S8. Extracellular enzyme K_m in μM for seven enzyme classes in initial litter and in control and inoculated litterbags collected from each site in June and December 2015.

Enzyme	Site	Initial	June		December	
			Control	Inoc.	Control	Inoc.
α -glucosidase	Desert		101 $\pm$ 2	119 $\pm$ 7	199 $\pm$ 17	206 $\pm$ 17
	Scrubland		104 $\pm$ 9	104 $\pm$ 2	238 $\pm$ 15	176 $\pm$ 8
	Grassland	267 $\pm$ 12	124 $\pm$ 6	148 $\pm$ 17	262 $\pm$ 13	278 $\pm$ 19
	Pine-Oak		112 $\pm$ 12	113 $\pm$ 7	222 $\pm$ 9	224 $\pm$ 11
	Subalpine		109 $\pm$ 19	117 $\pm$ 6	214 $\pm$ 16	223 $\pm$ 19
Acid phosphatase	Desert		184 $\pm$ 6	222 $\pm$ 4	377 $\pm$ 22	392 $\pm$ 39
	Scrubland		187 $\pm$ 9	175 $\pm$ 9	439 $\pm$ 29	343 $\pm$ 12
	Grassland	480 $\pm$ 22	186 $\pm$ 4	301 $\pm$ 67	380 $\pm$ 33	457 $\pm$ 79
	Pine-Oak		146 $\pm$ 17	182 $\pm$ 7	388 $\pm$ 31	372 $\pm$ 9
	Subalpine		223 $\pm$ 45	187 $\pm$ 3	338 $\pm$ 26	336 $\pm$ 30
β -glucosidase	Desert		258 $\pm$ 12	294 $\pm$ 7	539 $\pm$ 42	517 $\pm$ 49
	Scrubland		251 $\pm$ 22	251 $\pm$ 5	595 $\pm$ 51	407 $\pm$ 29
	Grassland	532 $\pm$ 22	251 $\pm$ 22	253 $\pm$ 12	535 $\pm$ 51	463 $\pm$ 31
	Pine-Oak		209 $\pm$ 19	224 $\pm$ 8	391 $\pm$ 22	469 $\pm$ 62
	Subalpine		231 $\pm$ 6	257 $\pm$ 11	325 $\pm$ 65	335 $\pm$ 68
B-xylosidase	Desert		317 $\pm$ 10	313 $\pm$ 14	603 $\pm$ 65	539 $\pm$ 35
	Scrubland		300 $\pm$ 21	293 $\pm$ 19	656 $\pm$ 41	433 $\pm$ 21
	Grassland	571 $\pm$ 20	264 $\pm$ 16	368 $\pm$ 52	507 $\pm$ 37	576 $\pm$ 77

	Pine-Oak		240±13	263±15	442±6	519±33
	Subalpine		314±8	294±11	449±44	498±24
Cellobiohydrolase	Desert		140±9	170±7	329±29	318±22
	Scrubland		144±9	140±3	321±19	256±13
	Grassland	304±17	138±6	150±8	283±9	307±13
	Pine-Oak		126±11	128±5	256±6	275±8
	Subalpine		121±4	147±5	209±28	189±22
Leucine- aminopeptidase	Desert		73±6	72±3	147±5	170±7
	Scrubland		66±8	78±4	132±7	126±9
	Grassland	84±2	97±7	120±39	157±15	175±16
	Pine-Oak		77±8	81±4	189±8	180±15
	Subalpine		85±3	70±3	139±15	142±10
<i>N</i> -acetyl- β -D- glucosaminidase (NAG)	Desert		135±7	148±6	234±32	222±19
	Scrubland		131±5	128±6	293±12	201±16
	Grassland	164±9	143±10	200±68	274±25	255±13
	Pine-Oak		135±9	121±7	233±14	210±12
	Subalpine		155±6	116±5	178±16	175±21

Table S9. Q₁₀ values for V_{max} of seven extracellular enzyme classes in initial litter and in control and inoculated litterbags collected from each site in June and December 2015.

Enzyme	Site	Initial	June		December	
			Control	Inoc.	Control	Inoc.
α -glucosidase	Desert		2.08±0.12	2.19±0.05	2.16±0.04	2.18±0.15
	Scrubland		2.11±0.02	2.11±0.05	2.00±0.03	1.91±0.03
	Grassland	2.32±0.04	2.14±0.03	2.15±0.12	2.19±0.05	2.17±0.04
	Pine-Oak		1.97±0.08	1.97±0.04	1.90±0.05	2.00±0.03
	Subalpine		1.93±0.12	2.00±0.03	1.98±0.03	1.94±0.03
Acid phosphatase	Desert		1.70±0.13	1.59±0.03	1.58±0.01	1.56±0.03
	Scrubland		1.57±0.03	1.62±0.02	1.52±0.02	1.50±0.03
	Grassland	1.59±0.01	1.60±0.02	1.54±0.05	1.62±0.02	1.58±0.03
	Pine-Oak		1.50±0.04	1.51±0.03	1.51±0.03	1.55±0.01
	Subalpine		1.70±0.14	1.57±0.01	1.55±0.04	1.55±0.01
β -glucosidase	Desert		1.83±0.04	1.81±0.03	1.83±0.08	1.86±0.05
	Scrubland		1.80±0.03	1.81±0.01	1.69±0.07	1.67±0.02
	Grassland	1.96±0.04	1.83±0.02	1.75±0.03	1.88±0.02	1.89±0.04
	Pine-Oak		1.83±0.04	1.82±0.02	1.85±0.04	1.87±0.02
	Subalpine		1.75±0.07	1.75±0.01	1.86±0.03	1.89±0.03
B-xylosidase	Desert		1.99±0.06	1.99±0.02	1.92±0.03	1.89±0.03
	Scrubland		1.98±0.02	1.99±0.03	1.86±0.05	1.82±0.03
	Grassland	1.94±0.02	2.01±0.03	1.93±0.04	2.01±0.03	2.03±0.04
	Pine-Oak		1.96±0.07	1.97±0.03	2.03±0.04	2.10±0.02

	Subalpine		1.95±0.05	1.95±0.03	2.02±0.02	1.95±0.03
Cellobiohydrolase	Desert		2.03±0.06	2.02±0.03	2.13±0.08	2.17±0.10
	Scrubland		1.91±0.05	1.98±0.07	1.98±0.07	1.90±0.02
	Grassland	2.22±0.05	2.02±0.02	1.92±0.04	2.12±0.03	2.10±0.06
	Pine-Oak		2.03±0.07	2.03±0.04	2.02±0.03	2.11±0.03
	Subalpine		1.86±0.07	1.97±0.02	2.13±0.06	2.15±0.03
Leucine- aminopeptidase	Desert		2.06±0.10	1.89±0.08	1.97±0.03	1.83±0.06
	Scrubland		2.03±0.04	1.96±0.02	1.83±0.02	1.79±0.03
	Grassland	2.15±0.01	2.02±0.01	1.97±0.07	1.89±0.02	1.95±0.02
	Pine-Oak		1.73±0.07	1.76±0.04	1.81±0.01	1.76±0.03
	Subalpine		1.91±0.05	1.82±0.02	1.84±0.05	1.85±0.03
<i>N</i> -acetyl- β -D- glucosaminidase (NAG)	Desert		1.98±0.07	1.94±0.06	1.95±0.02	1.95±0.06
	Scrubland		1.94±0.01	1.93±0.03	1.83±0.02	1.76±0.03
	Grassland	1.92±0.01	1.99±0.02	1.90±0.02	2.01±0.02	2.02±0.03
	Pine-Oak		1.86±0.06	1.90±0.03	1.89±0.03	1.91±0.02
	Subalpine		1.84±0.09	1.87±0.02	1.87±0.01	1.84±0.03

Table S10. Q_{10} values for K_m of seven extracellular enzyme classes in initial litter and in control and inoculated litterbags collected from each site in June and December 2015.

Enzyme	Site	Initial	June		December	
			Control	Inoc.	Control	Inoc.
α -glucosidase (AG)	Desert		1.27±0.03	1.31±0.06	1.20±0.03	1.34±0.12
	Scrubland		1.28±0.03	1.24±0.02	1.27±0.01	1.16±0.01
	Grassland	1.29±0.06	1.28±0.12	-	1.21±0.02	1.23±0.07
	Pine-Oak		-	1.20±0.03	1.24±0.03	1.25±0.02
	Subalpine		1.19±0.02	1.17±0.01	1.18±0.02	1.19±0.02
Acid phosphatase (AP)	Desert		1.24±0.04	1.15±0.00	1.25±0.04	1.21±0.03
	Scrubland		1.17±0.02	1.17±0.02	1.14±0.01	1.14±0.01
	Grassland	1.32±0.01	1.20±0.01	-	1.16±0.01	1.17±0.02
	Pine-Oak		1.23±0.00	1.18±0.01	1.20±0.02	1.19±0.03
	Subalpine		1.20±0.05	1.17±0.01	1.18±0.01	1.20±0.05
β -glucosidase (BG)	Desert		1.93±0.03	1.98±0.03	2.05±0.12	2.10±0.06
	Scrubland		1.86±0.05	1.88±0.02	1.71±0.08	1.82±0.02
	Grassland	2.11±0.05	1.83±0.03	1.82±0.03	1.98±0.04	1.99±0.05
	Pine-Oak		1.84±0.05	1.85±0.03	1.90±0.05	1.88±0.02
	Subalpine		1.84±0.07	1.84±0.03	1.94±0.05	2.06±0.05
B-xylosidase (BX)	Desert		1.41±0.04	1.49±0.02	1.32±0.03	1.31±0.03
	Scrubland		1.39±0.04	1.37±0.03	1.34±0.05	1.28±0.03
	Grassland	1.47±0.02	1.38±0.03	1.41±0.01	1.33±0.03	1.36±0.03
	Pine-Oak		1.36±0.04	1.38±0.02	1.41±0.05	1.44±0.03

	Subalpine		1.41±0.02	1.39±0.03	1.34±0.03	1.29±0.04
Cellobiohydrolase (CBH)	Desert		1.98±0.06	2.07±0.05	2.28±0.08	2.27±0.08
	Scrubland		1.75±0.05	1.77±0.07	1.93±0.07	1.89±0.02
	Grassland	2.27±0.10	1.78±0.03	1.74±0.08	2.03±0.05	2.00±0.04
	Pine-Oak		1.76±0.11	1.81±0.04	1.76±0.06	1.86±0.04
	Subalpine		1.66±0.08	1.92±0.05	1.99±0.09	2.16±0.06
Leucine- aminopeptidase (LAP)	Desert		1.37±0.21	1.15±0.02	1.20±0.02	1.13±0.01
	Scrubland		1.13±0.00	1.11±0.01	1.17±0.02	1.12±0.00
	Grassland	1.16±0.02	1.17±0.01	1.20±0.02	1.15±0.02	1.17±0.01
	Pine-Oak		1.16±0.02	1.15±0.01	1.17±0.01	1.18±0.01
	Subalpine		1.16±0.01	1.12±0.01	1.18±0.02	1.15±0.01
<i>N</i> -acetyl- β -D- glucosaminidase (NAG)	Desert		1.69±0.04	1.75±0.04	1.74±0.04	1.69±0.04
	Scrubland		1.70±0.03	1.71±0.03	1.58±0.03	1.59±0.04
	Grassland	1.65±0.02	1.72±0.03	1.73±0.04	1.67±0.03	1.71±0.02
	Pine-Oak		1.64±0.07	1.69±0.02	1.62±0.03	1.70±0.04
	Subalpine		1.67±0.04	1.66±0.03	1.66±0.04	1.58±0.04

Figure S1. Canonical discriminant analysis for variation in litter chemistry fractions between inoculated and control litterbags at the final sampling date in December 2015.

Figure S2. Canonical discriminant analyses for variation in extracellular enzyme V_{max} of all enzyme classes between **A)** sites, **B)** June and December 2015 sampling dates, and **C)** inoculated and control litterbags.

Figure S3. Canonical discriminant ordinations of differences in **A)** $V_{\max}$ and **B)** $V_{\max}$ temperature sensitivities of all enzymes assayed. Plots depict differences between native litter from each site (“Subalpine”, “Pine-Oak”, etc.) and litter in transplanted litterbags from all sites (“Litterbags”) in December 2015. Note that enzyme $V_{\max}$ values and $V_{\max}$ temperature sensitivities observed in transplanted litterbags group together, whereas enzyme traits observed in native litter differ much more widely.

Figure S4. Canonical discriminant analysis for variation in bacterial community composition at the phylum/class level as determined by 16S amplicon sequencing.

Figure S5. 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.