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ECOLOGY

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

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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, Chapparal/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,

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.

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Manuscripts

For Review Only

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

For Review Only

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 Km and Vmax 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 dessert 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 litterbaags.

• 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.

1 Running head: Decomposers and climate history

2

3 **Microbial decomposers not constrained by climate history along a Mediterranean climate**

4 **gradient in southern California**

5

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20 **Abstract**

21 Microbial decomposers mediate the return of CO_2 to the atmosphere by producing
22 extracellular enzymes to degrade complex plant polymers, making plant carbon available for
23 metabolism. Determining if and how these decomposer communities are constrained in their
24 ability to degrade plant litter is necessary for predicting how carbon cycling will be affected by
25 future climate change. We analyzed mass loss, litter chemistry, microbial biomass, extracellular
26 enzyme activities, and enzyme temperature sensitivities in grassland litter transplanted along a
27 Mediterranean climate gradient in southern California. Microbial community composition was
28 manipulated by caging litter within bags made of nylon membrane that prevent microbial
29 immigration. To test whether grassland microbes were constrained by climate history, half of the
30 bags were inoculated with local microbial communities native to each gradient site. We
31 determined that temperature and precipitation likely interact to limit microbial decomposition in
32 the extreme sites along our gradient. Despite their unique climate history, grassland microbial
33 communities were not restricted in their ability to decompose litter under different climate
34 conditions across the gradient, although microbial communities across our gradient may be
35 restricted in their ability to degrade different types of litter. We also found some evidence that
36 local microbial communities were optimized based on climate, but local microbial taxa that
37 proliferated after inoculation into litterbags did not enhance litter decomposition. Our results
38 suggest that microbial community composition does not constrain C-cycling rates under climate
39 change in our system, but optimization to particular resource environments may act as more
40 general constraints on microbial communities.

41

42 **Keywords**

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43 Climate gradient, microbial decomposition, extracellular enzymes, temperature sensitivity,
44 community constraints
45

46 Introduction

47 To predict how carbon (C) cycling will change with climate, it is crucial to determine
48 how microbial communities respond to changes in abiotic conditions (Aerts 1997). In the
49 American Southwest, anthropogenic climate change is causing a shift to hotter and drier
50 conditions (Seager et al. 2007, IPCC 2014), and such changes could alter heterotrophic
51 respiration generated by microbial decomposition of plant litter (Raich and Schlesinger 2002).
52 Global C-cycling models generally assume that respiration rates only depend on chemical and
53 climatic factors. Yet these models might be inaccurate if they fail to account for differences in
54 microbial responses to climate change across ecosystems (Reed and Martiny 2007, Strickland et
55 al. 2009, Keiser et al. 2011).
56 Previous studies have suggested that climate parameters act as ultimate controls on litter
57 decomposition, but cross-site comparisons are challenging this notion. The main influence of
58 climate may instead may be mediated through effects on litter chemistry (Prescott 2010). In
59 addition, biases inherent in many litterbag decomposition studies can lead to overestimation of
60 climate effects and underestimation of microbial community effects at the regional scale
61 (Bradford et al. 2016). Microbial communities responding to climate change may be constrained
62 by their composition if they are dispersal limited and optimized to local conditions in terms of
63 survival and resource acquisition strategies. These constraints could affect the community's
64 functioning and ability to respond to future changes (Reed and Martiny 2007). Evans and
65 Wallenstein (2011) found that long-term exposure to different precipitation regimes resulted in

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66 significant "legacy" effects of precipitation treatment on CO_2 respiration when field communities
67 were subjected to drying and rewetting perturbations in the lab. Similar legacy effects were
68 found following reciprocal transplantation of microbial communities historically exposed to
69 either grass or hardwood substrate inputs (Keiser et al. 2011). These and other similar studies
70 (Strickland et al. 2009, 2015) clearly indicate that the composition of microbial decomposer
71 communities is shaped by long-term climate and litter chemistry and can constrain the
72 communities' responses to perturbation.

73 Microbial decomposition is largely an enzymatic process, as decomposer communities
74 produce extracellular enzymes (EEs) to degrade the complex organic polymers in plant inputs
75 (Sinsabaugh et al. 1994). The characteristics and quantity of different EEs produced by microbial
76 decomposer communities are therefore key functional traits (Allison et al. 2007). In a hotter,
77 drier future, elevated temperatures should accelerate enzymatic reactions because of their
78 inherent temperature sensitivity (Davidson and Janssens 2006), but drying could lead to
79 inhibition of microbial processes (Allison and Treseder 2008), including enzyme synthesis and
80 interactions with substrates. Thus, the net impact on decomposition rates is unclear.

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

86 **1.** Rates of decomposition are controlled by differences in precipitation along the gradient.
87 Plant litter in sites with greater precipitation has greater microbial biomass and enzymatic
88 activity, resulting in faster decomposition (**Figure 1A**).

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89 **2.** Microbial decomposers show optimal functioning under local climate conditions (**Figure**
90 **1B**). Based on this hypothesis, we predict greater microbial biomass, enzymatic activity, and
91 decomposition rates in litter inoculated with microbial communities historically exposed to
92 the local climate versus in litter with microbial communities established under a foreign
93 climate.

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

97

98 **Methods**

99 *Site description*

100 To test how microbial communities and decomposition in the American Southwest will
101 respond to future climate change, we transplanted litter from a coastal grassland to five different
102 sites along a climate gradient in southern California – subalpine forest, pine-oak forest, coastal
103 grassland, pinyon-juniper scrubland, and Colorado desert. We chose to use grassland litter
104 (*Avena barbata* and *A. fatua*) because invasive grasses are prevalent in the scrubland and desert
105 site and dominate the grassland site where we have previously investigated the role of microbial
106 community composition in litter decomposition (Allison et al. 2013, Alster et al. 2013).
107 Temperature and moisture co-vary along the gradient, with colder, wetter sites at high elevations
108 and hotter, drier sites at lower elevations. As such, moving to lower elevations emulates how
109 future climate change is expected to progress in the American Southwest. All five sites are
110 located on granitic parent material and experience Mediterranean precipitation patterns (cool,
111 wet winters; hot, dry summers). The gradient spans a range of 12.5 °C in mean annual

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112 temperature, from 10.3±1.8 °C at the subalpine site to 22.8±0.8 °C at the desert site (**Table S1**).
113 The pine-oak forest (hereafter referred to as "pine-oak") site experienced the greatest mean
114 annual precipitation in the form of rainfall over the five years prior to this study (402.0±118.1
115 mm), and the desert experienced the least (99.7±29.3 mm), though the subalpine forest (hereafter
116 referred to as "subalpine") site likely receives almost half of its precipitation in the form of snow,
117 making it the wettest site (National Centers for Environmental Information 2011). All sites other
118 than the subalpine have eddy covariance towers that collect air temperature, soil temperature,
119 rainfall, and solar radiation data (Goulden et al. 2006). Two iButton temperature sensors (Maxim
120 Integrated) were also installed at each site on January 18, 2015, to collect surface temperature at
121 90 minute intervals until the final sampling date on December 2, 2015.

122 *Litter collection and deployment*

123 We employed one litter type found across the gradient to focus on climate vs. microbial
124 community constraints on decomposition, and we employed "microbial cage" litterbags to
125 restrict microbial community composition and test community origin hypotheses. On October
126 16, 2014, we collected ~800g of grass litter from the grassland site by clipping standing litter at
127 least 20 cm above the soil surface to avoid litter with prior soil contact. Litter was collected from
128 six different 1 m^2 plots located within a 50 m^2 sampling region. This litter was clipped to <5 cm
129 lengths and mixed, and a sub-sample was weighed and oven-dried to determine gravimetric
130 moisture content. The equivalent of 2.1 g dry weight of this litter (including ash content) was
131 used to make each litterbag. ~15 g of chopped grassland litter was ground to use as control
132 inoculum. Local inoculum was also collected from each of the other four sites on October 16,
133 2014. Using gloves, ~15g litter (grasses, leaves, and/or needles) was collected from the soil
134 surface of each site by lightly raking across the surface to collect loose material, using clippers to

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135 detach senescent grass litter from root bundles if necessary. Collected material was ground and
136 used as the inoculum. In addition to the 2.1 g of unsterilized grassland litter in each bag, 50 mg
137 of grassland inoculum was added to half the bags as a control (control, –); the other half received
138 50 mg of local inoculum native to the transplant destination site (inoculated, +) to determine
139 whether access to local microbes would affect microbial activity and litter mass loss over the
140 course of the study. Each litterbag was made of 0.2 μm nylon mesh that creates a "microbial
141 cage" by restricting microbial dispersal into or out of the litterbag. These microbial cages likely
142 reduce overall decomposition and microbial biomass, and may be particularly detrimental to
143 fungal activity, especially when the initial litter is sterilized (Allison et al. 2013, Alster et al.
144 2013). For this reason, we chose to use unsterilized litter and to focus on bacteria for analyses of
145 community composition.

146 Four litterbags of each type (-/+) were deployed into the six plots used to collect initial
147 inocula at each of the five sites on November 20, 2014 (4 x 2 x 6 x 5 = 240 total litterbags). One
148 litterbag of each type was removed from each plot for destructive sampling on March 9, June 7,
149 September 11, and December 2, 2015. Litter local to each site was also collected on June 7 and
150 December 2, 2015. Collected litterbags and litter were stored in coolers and transported to UC
151 Irvine, where litter from bags was weighed to determine mass loss before being ground into
152 fragments <0.5 cm in length and sub-sampled for DNA extractions (June 2015 samples only), EE
153 assays, and biomass of bacteria (all samples) and fungi (December 2015 samples only). The
154 remainder of the litter was weighed and oven-dried to determine moisture content.

155 *EE assays, kinetics, and thermodynamics*

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

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158 $$V = V_{max}[S]/(K_m + [S])$$

159 where V_{max} is the enzyme's maximum reaction velocity and K_m (the half-saturation constant) is
160 the substrate concentration at which the reaction rate is one-half V_{max}. In addition,
161 thermodynamic theory predicts that V_{max} and K_m are positively sensitive to temperature
162 (Davidson and Janssens 2006). We note that in ecological systems, observed V_{max} and K_m are
163 apparent kinetic parameters, not actual kinetic parameters (Wallenstein et al. 2011), and apparent
164 K_m is a metric of potential substrate availability (Sinsabaugh et al. 2014). We interpret apparent
165 V_{max} as a measure of the standing enzyme pool in a sample (Alster et al. 2013).

166 Local inoculum, initial grassland litter, and litterbags collected in June and December
167 2015 were assayed for V_{max}, K_m, and the temperature sensitivities of V_{max} and K_m for seven
168 hydrolytic enzyme classes using fluorescently labeled substrates based on German et al. (2012).
169 125μL of fluorometric substrate solution was combined with 125μL of litter homogenate in each
170 microplate well. Samples were incubated for 4h at 4, 10, 16, 22, 28, or 34°C. For each
171 temperature, each enzyme was assayed at a range of eight substrate concentrations achieved
172 through seven serial two-fold dilutions of the concentrations shown in **Table S2**. Negative
173 potential activities were converted to zero values before further analyses.

174 *Litter chemistry*

175 Oven-dried litter was sent to Cumberland Valley Analytical Services (Hagerstown, MD)
176 for near-IR spectroscopy, whereby reflectance spectra of near-infrared wavelengths of light from
177 each sample are matched to a verified database of spectra for plant materials with known
178 chemical composition as determined by wet chemistry (Shepherd et al. 2005). Relative amounts
179 of the following organic compounds were determined as proportions of total non-ash dried litter
180 mass: lignin, cellulose (acid detergent fiber – lignin), hemicellulose (neutral detergent fiber –

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181 acid detergent fiber), structural carbohydrates (non-fiber carbohydrates – starch and sugar), and
182 crude protein. The structural carbohydrate fraction includes plant cell components such as
183 pectins, but also microbial cell wall components such as β-glucans and peptidoglycans (CVAS,
184 *personal communication*).

185 *Microbial biomass*

186 Litter bacterial cell density was estimated by methods identical to those used in Allison et
187 al. (2013). In brief, ground litter was suspended in a phosphate-buffered, 1% glutaraldehyde
188 solution on the day of sample collection to "fix" bacterial cells for storage. Within two weeks,
189 0.1 M tetrasodium pyrophosphate was added to each sample, and samples were sonicated to
190 dislodge bacterial cells. Filtered extracts of sonicated litter were stained with 1x SYBR-Green
191 and then analyzed with an Accuri flow-cytometer to determine cell counts from fluorescing
192 bacterial cells.

193 The length of fungal hyphae in litter was measured by adapting methods used in Allison
194 et al. (2013). Ground litter was suspended in 0.395% (w/V) sodium hexametaphosphate and
195 vigorously stirred before being vacuum-filtered and stained with acid fuchsin. Two filters 17 mm
196 in diameter were made for each litter sample and affixed to a glass slide. Hyphal lengths were
197 measured with a Carl Zeiss photomicroscope at 100X magnification using Axioplan 2 Imaging
198 software. Hyphal lengths were measured in 89 x 67 µm viewing panes using 30 panes per slide
199 (15 per filter). Total hyphal length in all viewing panes for a single sample was converted to
200 estimates of hyphal length in meters per gram of dry litter using a modified procedure of Sylvia
201 (1992).

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

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

208 *Bacterial community composition*

209 To PCR amplify the bacterial 16S rRNA gene, 5 µL of a 1:50 dilution of DNA (average
210 1.43 $\pm$ 0.44 ng DNA) from each extract was added to a cocktail containing: 1 unit per reaction of
211 Hot Start Taq DNA polymerase (BioLabs, Inc), 1 × PCR Rxn Buffer (-$MgCl_2$) (Invitrogen),
212 1200 µM $MgCl_2$ (Invitrogen), 200 µM dNTP, 0.2 µM Forward primer and 0.2 µM Reverse
213 Primer, 200 mM Bovine Serum Albumin Acetylated (PROMEGA), and H_2O to a final volume of
214 25 µL. We used the 515 forward primer (GTGYCAGCMGCCGCGGTAA) and 926 reverse
215 primer (CCGYCAATTYMTTTRAGTTT) designed by Caporaso et al. (2012) and Fierer et al.
216 (2012) and modified by Apprill et al. (2015) to target the V4-V5 region of the 16S gene.
217 Following an initial denaturation step at 94 °C for 3 min, PCR was cycled 35 times at 94 °C for
218 45 s, 55 °C for 30s, 68 °C for 20s, with a final extension at 68 °C for 10 min. We amplified each
219 subsample in duplicate from the extracted DNA.

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

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

233 Illumina sequence data was processed using the QIIME (version 1.9.1) toolkit (Caporaso
234 et al. 2010). Paired end files were joined and operational taxonomic units (OTUs) were picked at
235 97% identity level using UCLUST (Edgar 2010) with the nearest neighbor method in QIIME.
236 Taxonomy was assigned using SILVA v119 as the reference database (Quast et al. 2013) using
237 QIIME scripts. For the 60 samples, the number of reads ranged between 8903 and 89,395 with a
238 median of 37,029. Using the OTU-by-sample matrix from QIIME, we generated a rarefied
239 composition distance matrix. We created 100 OTU tables from the original data, randomly
240 drawing the lowest common number of sequences (n= 8,903) from each sample. To weight rarer
241 taxa more heavily, we transformed each table by taking the square root of each cell value and
242 rounding to the nearest integer. We then calculated a Bray-Curtis distance matrix for each of the
243 100 OTU tables. Finally, for each pair-wise comparison between samples (i.e., each cell in the
244 distance table), we chose the median Bray-Curtis value among the 100 distance matrices to yield
245 a median, rarefied distance matrix that was used in the remaining analyses.

246 *Statistical methods*

247 Effects of site, sampling date, and inoculation on mass loss and bacterial biomass were
248 analyzed using mixed-model ANOVA with the identity of each plot as a random factor. Because
249 litter moisture is known to be a strong control on decomposition processes in Mediterranean

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250 ecosystems, the model was run as an ANCOVA with litter moisture content as the covariate
251 using the lme function in R. Effects of site and inoculation on fungal biomass at the final
252 sampling date were analyzed using the same ANCOVA without sampling date as a factor.
253 ANCOVA was also used to determine any relationship between bacterial biomass and mass loss
254 across sites, sampling dates, and inoculation treatments. Post hoc analysis of pair-wise
255 comparisons was done with Tukey contrasts using the lsmeans package in R.

256 EE kinetic parameters were calculated for each enzyme class and incubation temperature
257 by fitting observed EE activity to the Michaelis-Menten equation. Non-linear regressions were
258 performed in the R software environment 3.3.1 (R Development Core Team 2017) using the nls
259 function. Confidence intervals were determined for V_{max} and K_m values using the nlstools
260 package. Fits of V_{max} with a 95% CI greater than twice the magnitude of V_{max} were discarded;
261 because of greater variability in calculated fits of K_m, fits with a 95% CI greater than four times
262 the magnitude of K_m were discarded. V_{max} and K_m parameters calculated from 22°C incubations
263 are hereafter referred to as "V_{max}" and "K_m" in the text.

264 Temperature sensitivities of EE kinetic parameters for each enzyme class were
265 determined by linear regression of ln V_{max} or ln K_m against incubation temperature. Regressions
266 were performed using the lm function in R. Regressions with $R^2 < 0.50$ were discarded. Slopes
267 were converted to Q_{10} values as in Wallenstein et al. (2009) using the formula:
268 $Q_{10} = \exp(\text{slope} \times 10)$.

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

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

279 Data for all univariate analyses were checked for normality visually and by the Shapiro-
280 Wilk test, and non-normal data were natural log-transformed to improve normality when
281 necessary. Bacterial biomass met assumptions of normality after ln-transformation; V_{max} per unit
282 bacterial biomass did not but passed visual inspection after ln-transformation. Litter chemistry
283 did not meet assumptions of normality but passed visual inspection in base form. Temperature
284 sensitivities of V_{max} did not meet assumptions of normality but were visually determined to be
285 most normal when in base form, and as such were not transformed prior to statistical analyses.

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

291

292 **Results**

293 *Mass loss*

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

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296 decomposed 13% faster than litter in the grassland site, whereas litter in the subalpine, scrubland,
297 and desert sites decomposed 52-55% slower. Litterbags containing local inoculum lost
298 25.3±1.5% dry mass by December, significantly less than the 27.7±1.7% mass loss observed in
299 control litterbags containing only grassland inoculum (**Figure 3A**). Though not significant, this
300 trend was also observed in "inoculated" litterbags in the grassland site.
301 *Litter chemistry*
302 Over the course of the study, mass attributable to different chemical fractions in
303 transplanted litter was affected by site ($p<0.001$, $F_{24,326}=13.3$), sampling date ($p<0.001$,
304 $F_{6,93}=20.2$), and inoculation treatment ($p<0.001$, $F_{6,93}=5.5$) (**Table 1**). Total litter mass loss was
305 driven by losses from the cellulose and hemicellulose fractions, which on average lost 0.23±0.01
306 g and 0.15±0.01 g by December, accounting for 41% and 28% of total mass loss, respectively.
307 Cellulose, hemicellulose, and the combined starch and sugar fractions declined in all sites over
308 the course of the study (**Figure 4**). Crude protein and lignin fractions, however, were only
309 reduced in the grassland and pine-oak sites, which experienced the most mass loss overall. The
310 structural carbohydrate fraction increased an average of 96% (0.10±0.04 g) by December in all
311 litterbags, but this increase was enhanced by 38% in the subalpine, scrubland, and desert sites
312 (0.11±0.01 g) relative to the pine-oak and grassland sites (0.08±0.004 g).
313 Inoculation most affected the change in the mass of the structural carbohydrate and lignin
314 fractions in litterbags over the course of the study. By December, inoculated litterbags
315 accumulated 22% more structural carbohydrate mass than control litterbags (0.11±0.01 g vs.
316 0.09±0.01 g, **Figure 3B**), and contained 4.3% more lignin than control litterbags by virtue of
317 accumulating rather than losing lignin mass over the course of the study (**Figure 3C**). Mean

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318 mass attributable to each chemical fraction in initial litter and in control and inoculated litterbags
319 collected from each site in June and December 2015 is presented in **Table S3**.
320 *Microbial biomass*
321 Bacterial biomass varied by site ($p<0.001$, $F_{4,243}=84.5$), sampling date ($p<0.001$,
322 $F_{4,243}=579.1$), and with inoculation treatment ($p<0.001$, $F_{1,243}=27.0$) (**Table 1**). Bacterial biomass
323 was 95-115% higher in the subalpine and pine-oak sites over the course of the study than it was
324 in the grassland, whereas bacterial biomass was 54-56% lower in the desert and scrubland sites.
325 Bacterial biomass was greatest in most sites in December 2015, though biomass in scrubland
326 litterbags appeared to peak by September (**Table S4**). Bacterial biomass explained a significant
327 amount of the variation in mass loss observed in litterbags across the gradient over the course of
328 the study ($p<0.001$, $R^2=0.38$) – litterbags that contained more bacteria lost more mass (**Figure**
329 **5**). This relationship was significant within each individual site across sampling dates, with the
330 exception of the subalpine site. Inoculation significantly increased bacterial biomass over the
331 course of the study from 0.13±0.02 mg $C\cdot g^{-1}$ dry litter in control litterbags to 0.19±0.03 mg $C\cdot g^{-1}$
332 in inoculated litterbags (**Figure 3D**). Inoculation increased bacterial biomass in all sites other
333 than the scrubland, and had the greatest positive effect on biomass in litterbags transplanted to
334 the desert site. Mean bacterial biomass in initial litter, in control and inoculated litterbags
335 collected from each site in June and December 2015, and in local litter collected from each site
336 in December 2015 are presented in **Table S4**.
337 There was a significant effect of site ($p=0.034$, $F_{4,43}=2.9$) on fungal biomass at the final
338 sampling date, but there was no effect of inoculation treatment (**Table 1**). Fungal biomass was
339 only enhanced relative to the grassland in the subalpine site, where it was 50% higher (Tukey
340 $p=0.098$). There was also a significant effect of site ($p<0.001$, $F_{4,43}=14.8$) on B:F ratio at the

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341 final sampling date, but no effect of inoculation treatment (**Table 1**). B:F ratio was 138% higher
342 in the pine-oak site (5.0±1.3) than in the intermediate subalpine, grassland, and desert sites
343 (2.1±0.3), and was 66% lower in the scrubland site (0.7±0.1, Tukey $p<0.05$). Mean fungal
344 biomass and the B:F ratio in initial litter, in control and inoculated litterbags collected from each
345 site at the final sampling date in December 2015, and in local litter collected from each site in
346 December 2015 are presented in **Table S5**.

347 *Bacterial community composition*

348 Litterbag bacterial composition, as determined by 16S rRNA sequencing, in the
349 beginning of the dry season in June 2015 was significantly affected by site, and was marginally
350 significantly affected by inoculation treatment as well as the interaction between site and
351 inoculation (**Table 1**). Site-level differences in bacterial community composition at the
352 phylum/class level were driven by variation in the relative abundance of β-proteobacteria, which
353 accounted for >29% of the recovered 16S sequences across the gradient and 19.7% of those
354 observed in grassland litterbags. β-proteobacteria relative abundance was 100% higher in the
355 subalpine site, 50% higher in the pine-oak and scrubland site, and 23% higher in the desert site
356 (relative to the grassland). Across the gradient, most recovered sequences were attributed to
357 Bacteroidetes (>36%), β-proteobacteria, α-proteobacteria (>17%) and γ-proteobacteria (7%),
358 with very few attributed to Cyanobacteria (<3%) Actinobacteria (<2%), Acidobacteria (<1%)
359 and Firmicutes (<1%) (**Table S6**). The effects of inoculation and the site:inoculation interaction
360 were driven by significant differences between inoculated and control litterbags in the desert,
361 scrubland, and pine-oak sites (post-hoc PERMANOVA pair-wise tests; p= 0.029, 0.035, and
362 0.008, respectively). Composition in the grassland and subalpine plots were not altered by

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

365 *EE potential V_{max}*

366 There were significant effects of site ($p<0.001$, $F_{28,322}=11.1$) and sampling date ($p<0.001$,
367 $F_{7,89}=21.8$) on V_{max} of all enzyme classes when analyzed together, as well as a marginally
368 significant effect of inoculation treatment ($p=0.056$, $F_{7,89}=2.1$) (**Table 1**). Differences between
369 sites and sampling dates were driven by differences in BX and LAP V_{max}. BX V_{max} decreased
370 over the course of the study in the grassland and pine-oak sites, but not in the subalpine,
371 scrubland or desert sites, resulting in 57% higher observed activity in the latter sites by
372 December (**Figure 6A**). Similar trends (higher observed activity in litterbags from the desert and
373 subalpine sites relative those from the grassland and pine-oak sites) were observed for two other
374 C-degrading EEs, BG and CBH (**Figure S5**). LAP V_{max} increased over the course of the study in
375 all sites, and was only significantly different from the grassland in the pine-oak site, where
376 activity was 41% lower by December (**Figure 6B**).

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

385 *Temperature sensitivity of EE V_{max}*

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386 EE V_{max} temperature sensitivities were significantly affected by site ($p<0.001$,
387 $F_{28,304}=5.8$) and sampling date ($p<0.001$, $F_{7,84}=5.8$), but were not significantly affected by
388 inoculation treatment (**Table 1**, **Table S9**). Pair-wise comparisons of V_{max} temperature
389 sensitivities in December litterbags between sites were used to determine if transplantation to a
390 foreign site resulted in EEs with different temperature sensitivities from those observed in
391 litterbags from the grassland site (**Table 2**). All EEs other than AP exhibited significantly lower
392 V_{max} temperature sensitivities after transplantation into at least one foreign site, usually the
393 scrubland. EE K_m temperature sensitivities are presented in **Table S10.**
394

395 **Discussion**

396 *Mass loss and bacterial biomass*

397 We hypothesized that differences in microbial activity and litter mass loss rates in
398 transplanted litterbags would be driven by differences in climate along the gradient, and in
399 particular by differences in precipitation (**Figure 1A**). Prescott (2010) proposed that the aspect of
400 climate most responsible for driving litter decomposition depends on which climatic thresholds
401 are exceeded in a given ecosystem. Under this framework, sites with mean annual temperatures
402 above 10°C are most likely to be limited by moisture because temperatures are warm enough that
403 decomposer activity is less likely to be inhibited. Our results generally supported this hypothesis:
404 decomposer activity was not a linear function of precipitation, and co-varying differences in
405 temperature and precipitation along our gradient likely combine to drive decomposer activity.
406 Even though the subalpine site likely receives the most precipitation once snowfall is taken into
407 account (**Table S1**), litter there decomposed as slowly as in the desert and scrubland (**Figure
408 2A**). In addition, the pine-oak site receives significantly more rainfall than the grassland, yet

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

418 All major chemical fractions of litter declined over the course of the experiment other
419 than structural carbohydrates, which increased in litterbags from all sites (**Figure 4**). Sites that
420 experienced the most mass loss also experienced the greatest declines in cellulose, and the least
421 gains in structural carbohydrates. The structural carbohydrate fraction is composed of pectins, β-
422 glucans, and peptidoglycans, and it is possible that microbial residues or necromass contribute to
423 the accumulation of this fraction. Microbial residues can be more recalcitrant than cellulose or
424 hemicellulose (Grandy and Neff 2008, Miltner et al. 2012), and may represent C that has shifted
425 into slower turnover pools (Khan et al. 2016).

426 We expected that trends in bacterial biomass would mirror mass loss over the course of
427 the study, as bacterial decomposers are responsible for a large majority of microbial activity and
428 biomass in litter from the grassland site (Alster et al. 2013, Baker and Allison 2017). B:F ratios
429 in litterbags collected at the end of the experiment support this interpretation, as bacterial
430 biomass dominated the microbial communities in all sites other than the scrubland (**Table S5**).
431 Our results were in line with our expectations – there was a significant positive log-linear

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

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

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

467 *Inoculation effect*

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

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

482 Still, it is difficult to explain inoculation effects of increased bacterial biomass (**Figure**
483 **3D**) and altered community composition in the pine-oak, scrubland, and desert sites without
484 invoking climate optimization by some members of the microbial community (**Figure 1C**). We
485 speculate that the taxa driving changes in community composition following inoculation may be
486 optimized for local climate conditions (thus the increased biomass) but are not key players in
487 grass litter decomposition (resulting in decreased mass loss). Compared to controls, inoculated
488 litterbags also accumulated lignin (**Figure 3D**) and exhibited greater increases in structural
489 carbohydrates (**Figure 3C**) by the end of the study. These results indicate that the microbes
490 proliferating after inoculation into grassland microbial communities are less effective at
491 degrading grassland litter than the pre-existing members of the grassland microbial community,
492 perhaps because they are optimized to degrade different litter types and chemistries. Prior studies
493 have also found evidence that microbial communities are constrained in their ability to degrade
494 particular litter chemistries (Strickland et al. 2009, Keiser et al. 2011).

495 *Enzyme profiles*

496 We hypothesized that V_{max} of EEs produced by transplanted microbial communities
497 would be enhanced in sites that experienced greater precipitation. Our results did not support this
498 hypothesis. EE V_{max} values differed significantly over time and with transplantation site, but
499 were not generally higher in sites that received more precipitation. C-degrading enzymes
500 generally exhibited the greatest activity in the sites that had the least precipitation (**Figure 6A**),

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

511 Our results supported the hypothesis that inoculation would enhance EE activity. We also
512 found that inoculation shifted EE traits to resemble those observed in sites with lower
513 decomposition rates. Inoculated litterbags exhibited increased activities of C-degrading EE
514 classes such as CBH and BG, much like litterbags transplanted to the low-decomposition
515 subalpine, scrubland, and desert sites. This result suggests that EEs produced by inoculated
516 microbes were less efficient on grassland litter than on their native substrates. Other studies have
517 suggested that microbial optimization to a particular litter chemistry may matter more for litter
518 decomposition rates than optimization to a particular climate (Keiser et al. 2011, Wallenstein et
519 al. 2013). Given that inoculation also increased bacterial biomass, it appears that some of the
520 taxa that proliferated after inoculation may be cheaters – organisms that benefit from EE
521 production without producing EEs themselves (West et al. 2006). Even though BG and CBH
522 V_{max} were higher in inoculated litterbags, BG and CBH activities per bacterial biomass were

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

525 There is also evidence for community optimization to litter chemistry from our EE V_{max}
526 and V_{max} temperature sensitivity results. EE V_{max} and V_{max} temperature sensitivities in
527 transplanted litterbags were similar to one another and did not shift to emulate the EE parameters
528 of local microbial communities (**Figure S3**). This pattern was observed regardless of which site
529 litterbags were transplanted to. EE V_{max} of local microbial communities exhibited greater
530 variance than those observed in transplanted litterbags, which indicates that local microbes
531 decomposing native litter in each site likely produce EEs in different amounts depending on the
532 litter type. EE V_{max} temperature sensitivities were also more varied in microbial communities
533 native to each site than they were in transplanted litterbags, which indicates that microbes on
534 local native litter are also likely producing structurally different EEs from those on grassland
535 litter. This provides a mechanistic explanation as to how microbial communities are optimized to
536 degrade particular litter chemistries.
537
538 **Conclusion**

539 Observed patterns in litter decomposition indicate that precipitation and temperature
540 likely interact to limit decomposition rates at the ends of our gradient. Reduced moisture
541 limitation resulting from higher precipitation is outweighed by temperature constraints at the
542 higher elevation sites, while the effect of warmer temperature is offset by moisture limitation in
543 the hotter, low elevation sites. A future shift to a more arid climate may therefore enhance
544 decomposition rates in subalpine forests as they become warmer montane forests, and may

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545 reduce decomposition rates in grasslands as they experience reduced precipitation and become
546 more similar to scrublands and desert.
547 In the context of a predicted future shift to a more arid climate in the American
548 Southwest, our results indicate that although microbial communities differ both taxonomically
549 and functionally across a wide range of climates, the decomposer function of these communities
550 may not be constrained by climate history (**Figure 1**). Instead, it is more likely to be constrained
551 by litter type and chemistry. This insight potentially simplifies efforts to incorporate microbe-
552 explicit mechanisms of temperature response into global C-cycling models and predict future C
553 dynamics (Allison and Martiny 2008). On the other hand, our findings also suggest that
554 compositional legacies driven by vegetation chemistry could be important in C cycle predictions,
555 as microbial communities adapted to particular litter chemistries appear to produce distinct
556 extracellular enzymes. This is especially true if plant communities and dominant litter
557 chemistries shift with climate change, as has been previously observed by the movement of
558 conifers upslope along this gradient after a period of extended drought (Fellows and Goulden
559 2012).
560

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573

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723

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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 (H_2O) as a covariate. Bolded p-values are significant (<0.05).

Variable	Site	Date	Inoc	H_2O	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	–	–

724

725 [1]ANCOVA

726 [2]Final sampling date in December 2015 only

727 [3]MANCOVA with all enzyme classes

728 [4]PERMANOVA

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Table 2 Mean (±SE) EE V_{max} Q_{10} from litterbags collected on the final sampling date in December 2015. Bolded values are significantly different ($p<0.05$) from those observed in grassland litterbags.

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

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

741

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

746

747 **Figure 3.** Effect of inoculation with local microbial communities on mean (±SE) **A**) mass loss,
748 **B**) accumulation of structural carbohydrates and **C**) change in the mass of the lignin fraction by
749 the final sampling date, as well as the effect on mean (±SE) **D**) bacterial biomass, **E**)
750 cellobiohydrolase V_{max} per unit bacterial biomass, and **F**) β-glucosidase V_{max} per unit bacterial
751 biomass over the course of the study. All effects shown are significant (Tukey $p<0.05$).

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753 **Figure 4.** Mean (±SE) mass change in each major fraction of litter by December 2015. Positive
754 values indicate accumulation of that fraction, whereas negative values indicate loss of that
755 fraction.
756
757 **Figure 5.** Mass (g) lost from litter as a function of bacterial biomass (ln mg C g^{-1} dry litter) at
758 that sampling date, across sites and sampling dates ($p<0.001$, $R^2=0.38$). The shaded region
759 around the line signifies 95% confidence intervals.
760
761 **Figure 6.** Mean (±SE) EE V_{max} for **A**) β-xylosidase and **B**) leucine aminopeptidase in initial
762 grassland litter and in June and December 2015 litterbags from each site.

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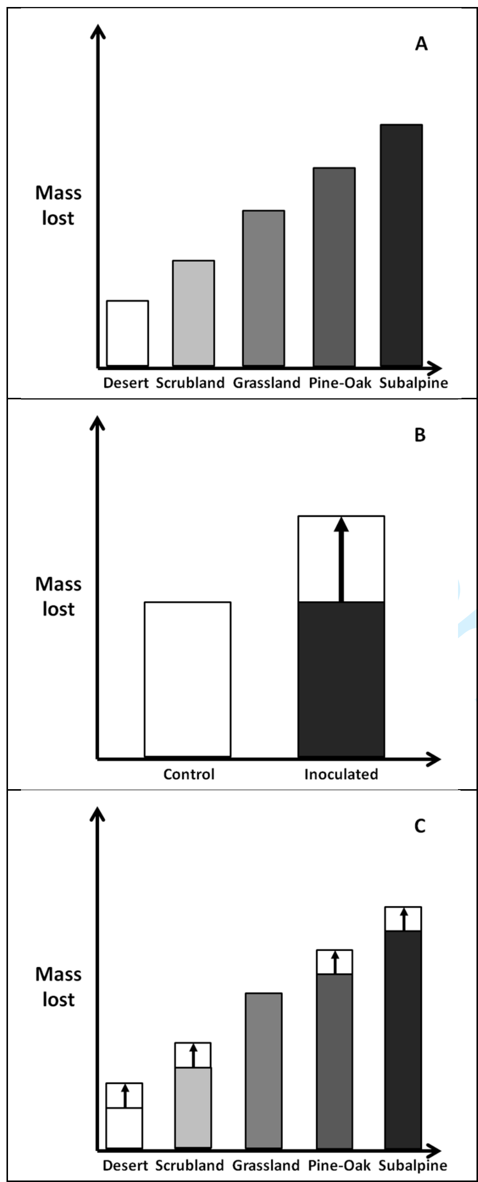

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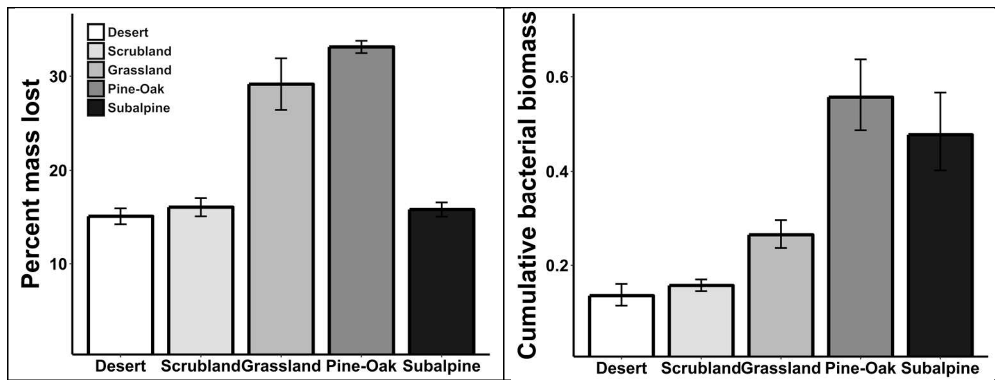
763

764 **Figure 1.**



765

766 **Figure 2.**

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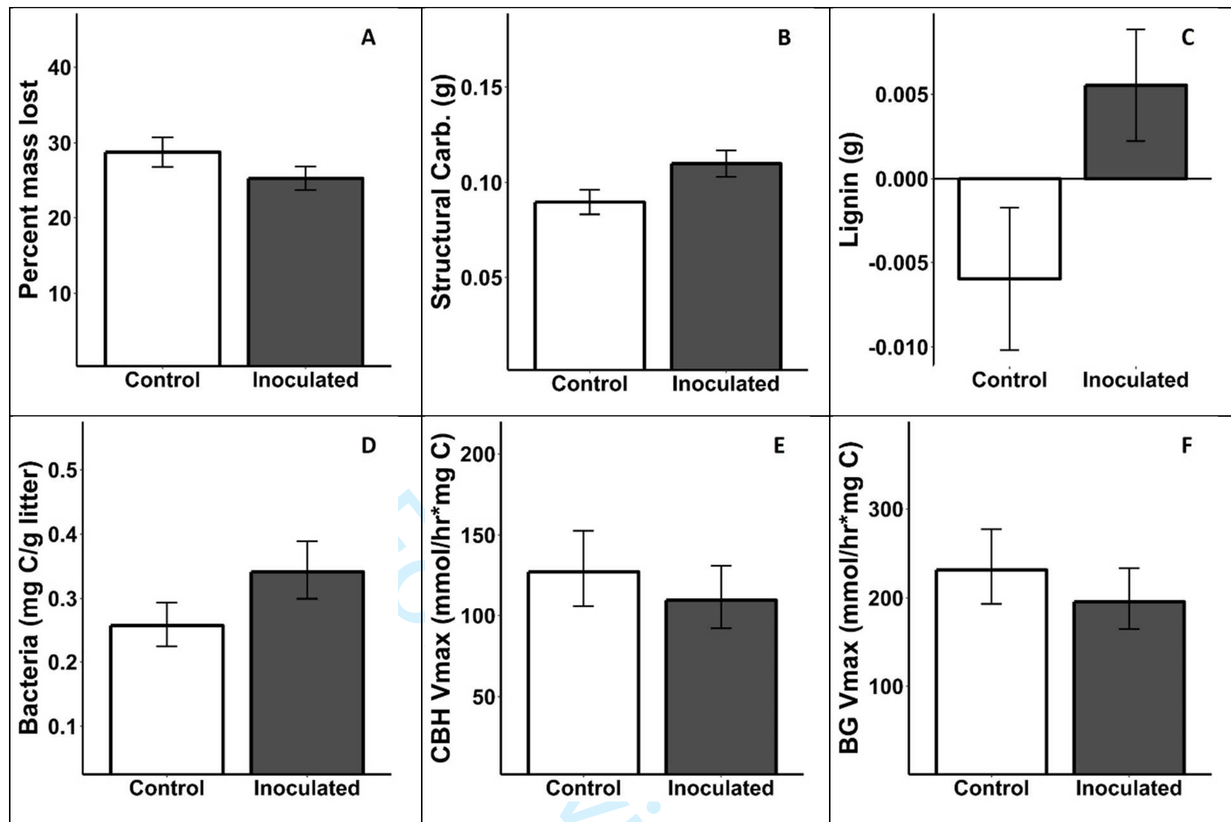

767

768 **Figure 3**.

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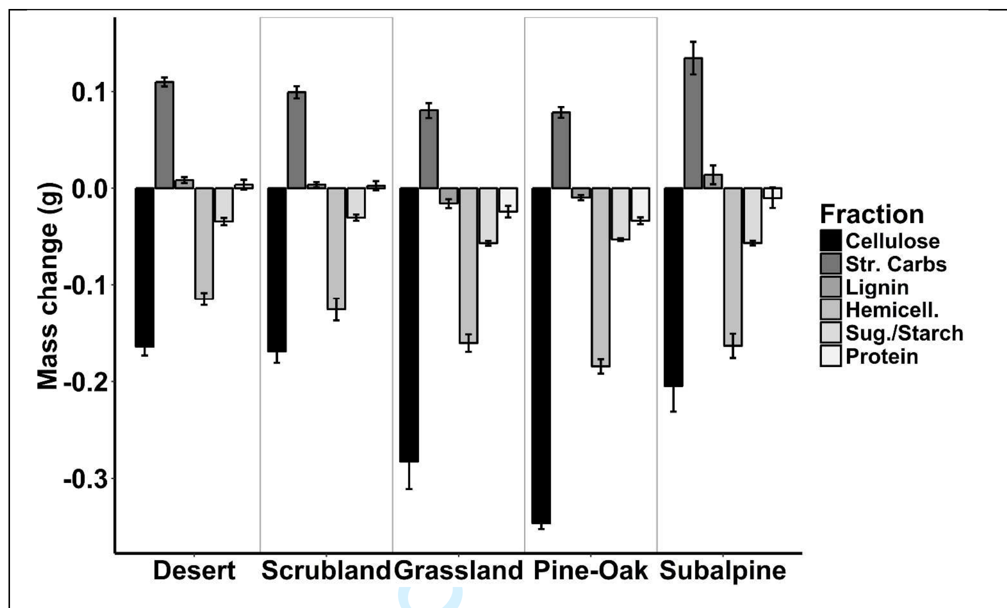

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770 **Figure 4**.

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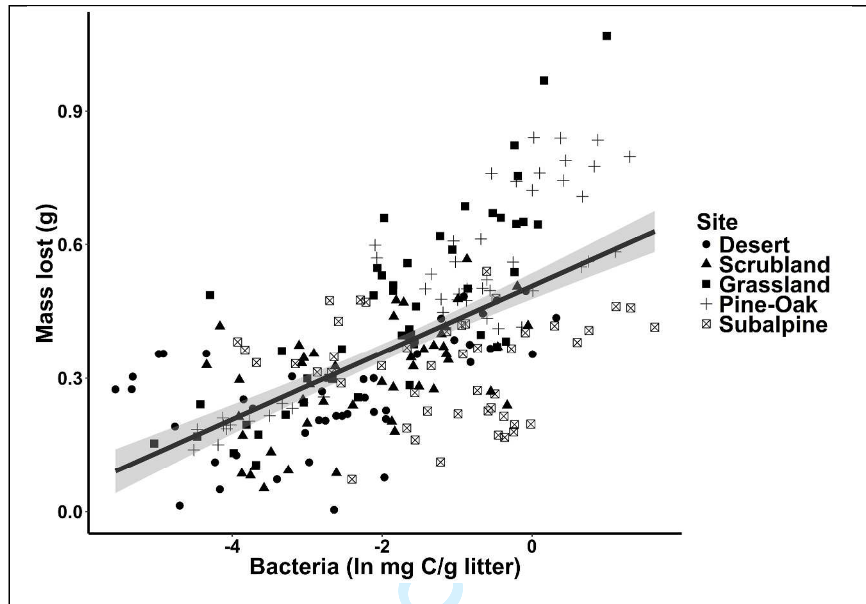

771

772 **Figure 5.**

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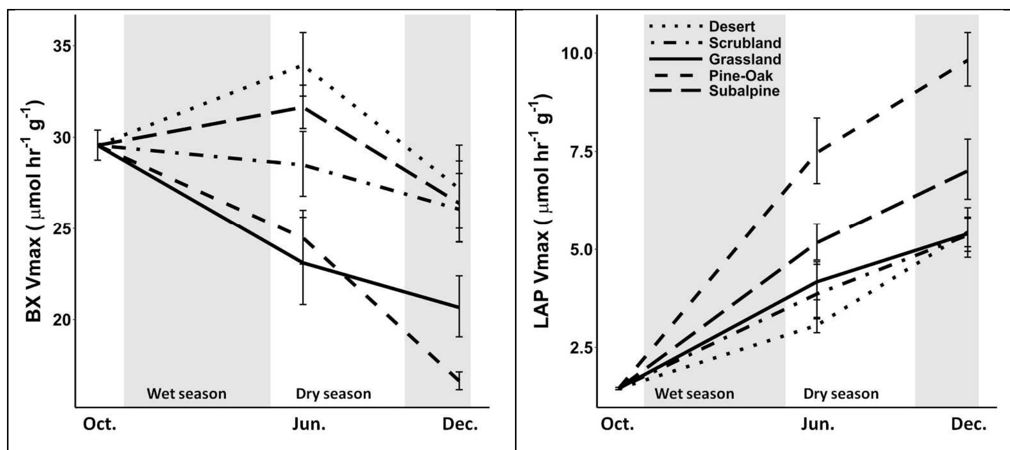

773

774 **Figure 6.**

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1 **Supplemental Information**

2 **Methods**

3 Canonical discriminant analysis (CDA) summarizes correlated changes in multiple variables
4 within a specified experimental design along one or more axis. Loading coefficients along these
5 axes are attributed to each variable included in the analysis, in this case indicating which
6 variables best partition differences between sites, sampling dates, or inoculated vs. control
7 litterbags. CDA was used to determine that mass of the structural carbohydrate and lignin
8 fractions most distinguished control from inoculated litterbags (**Figure S1**). Absolute values of
9 the loading coefficients on the single CDA axis for structural carbohydrate and lignin mass were
10 0.68 and 0.67, respectively.

11 CDA was also used to determine which extracellular enzymes' observed V_{max} best distinguished
12 litterbags from different sites, from different sampling dates, and between control and inoculated
13 litterbags. The first CDA axis accounted for 57.3% of the variation in EE V_{max} between sites, and
14 the absolute values of the loading coefficients for BX and LAP V_{max} were 0.74 and 0.66,
15 respectively (**Figure S2A**). There was only one CDA axis for differences between sampling
16 dates, and the absolute values of the loading coefficients for BX and LAP V_{max} were 0.57 for
17 both (**Figure S2B**). There was also only one CDA axis for differences between control and
18 inoculated litterbags, and CBH V_{max} and BG V_{max} explained the most variation along that axis
19 with loading coefficients with absolute values of 0.77 and 0.61, respectively (**Figure S2C**).

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

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

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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**).

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Table S1. Mean (± 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 (°C)	22.8±0.8	15.6±0.8	16.4±0.3	12.3±0.6	*10.3±1.8*	2009-14
Air daily temp. range	10.8±0.4	8.5±0.2	8.3±0.3	6.2±0.2	-	2009-14
Soil temperature (°C)	28.3±0.3	18.4±0.3	19.1±0.9	9.9±0.3	-	2008-12
Soil daily temp. range	10.9±0.2	5.5±0.7	4.6±0.8	2.6±0.1	-	2008-12
Plot temperature (°C)	29.3±0.4	19.5±0.5	22.0±0.4	13.2±0.4	11.5±0.4	2015
Plot daily temp. range	20.3±0.3	24.8±0.4	26.2±0.5	20.8±0.5	13.7±0.4	2015
Precipitation (mm)	100±24	193±33	242±76	402±118	*>265*	2009-14
Soil moisture (µL/cm^3)	46±1	73±5	73±8	86±3	-	2008-12
Solar radiation (W/m^2)	225±7	234±7	217±8	224±7	*~270*	2006-13
Elevation (m)	275	1280	470	1710	2250	

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

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Table S3. Mean (± 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±0.01	0.76±0.01	0.70±0.01	0.68±0.01
	Scrubland		0.74±0.02	0.74±0.01	0.67±0.02	0.70±0.01
	Grassland	0.85±0.01	0.66±0.02	0.67±0.02	0.55±0.03	0.59±0.05
	Pine-Oak		0.65±0.01	0.65±0.01	0.51±0.01	0.50±0.01
	Subalpine		0.72±0.01	0.71±0.02	0.63±0.05	0.67±0.01
Hemicellulose	Desert		0.29±0.01	0.31±.01	0.28±0.00	0.26±0.01
	Scrubland		0.30±0.01	0.28±0.01	0.24±0.02	0.28±0.01
	Grassland	0.38±0.01	0.22±0.01	0.24±0.01	0.22±0.01	0.23±0.01
	Pine-Oak		0.26±0.01	0.25±0.01	0.20±0.01	0.20±0.01
	Subalpine		0.30±0.02	0.28±0.01	0.23±0.02	0.21±0.02
Lignin	Desert		0.20±0.00	0.22±0.00	0.20±0.00	0.21±0.00
	Scrubland		0.20±0.00	0.20±0.00	0.20±0.00	0.21±0.00
	Grassland	0.20±0.00	0.21±0.00	0.21±0.00	0.18±0.01	0.19±0.01
	Pine-Oak		0.20±0.00	0.21±0.01	0.19±0.00	0.19±0.00
	Subalpine		0.20±0.00	0.21±0.00	0.20±0.02	0.23±0.00
Structural	Desert		0.22±0.00	0.23±0.01	0.21±0.01	0.22±0.00
carbohydrates	Scrubland		0.22±0.00	0.23±0.00	0.21±0.01	0.19±0.01

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	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

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Table S4. Mean (± SE) bacterial biomass C (μg C·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

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Table S5. Hyphal biomass C in mg C·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.

	Hyphal biomass C				B:F biomass ratio			
Site	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

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Table S6. Mean relative abundance (% ± 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±3.5	26.1±1.5
	Scrubland	36.0±3.5	38.8±3.0
	Grassland	35.4±2.5	26.1±2.0
	Pine-Oak	36.3±1.2	40.1±2.1
	Subalpine	39.4±2.8	39.1±6.0
β-Proteobacteria	Desert	23.9±3.8	24.7±1.9
	Scrubland	30.8±4.5	26.9±2.2
	Grassland	18.8±1.8	20.6±0.9
	Pine-Oak	30.8±1.5	29.5±1.4
	Subalpine	38.8±3.1	40.4±4.6
α-Proteobacteria	Desert	19.1±0.8	21.8±1.8
	Scrubland	15.0±0.8	19.1±1.3
	Grassland	20.1±2.1	24.2±1.8
	Pine-Oak	19.5±1.5	18.9±0.7
	Subalpine	12.2±0.8	9.4±0.8

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γ-Proteobacteria	Desert	5.3±0.9	4.5±0.9
	Scrubland	6.2±1.0	6.7±0.6
	Grassland	15.8±1.4	17.1±2.2
	Pine-Oak	6.8±1.4	4.9±0.3
	Subalpine	3.1±0.6	4.1±0.7
Cyanobacteria	Desert	12.5±4.0	11.8±2.4
	Scrubland	3.0±0.6	1.2±0.5
	Grassland	1.7±1.2	1.4±1.1
	Pine-Oak	0.2±0.1	0.3±0.1
	Subalpine	0.8±0.4	0.7±0.4
Actinobacteria	Desert	3.1±0.6	3.1±0.4
	Scrubland	2.5±0.1	1.9±0.2
	Grassland	2.3±0.3	3.9±0.4
	Pine-Oak	0.9±0.1	1.0±0.1
	Subalpine	0.9±0.1	1.0±0.1
Total reads	Desert	25.7±4.7	26.0±6.9
(1000s)	Scrubland	42.7±7.2	40.1±9.8
	Grassland	30.1±1/4	37.6±7.4
	Pine-Oak	44.7±3.5	60.6±7.6
	Subalpine	45.4±6.3	50.4±5.5

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Table S7. Extracellular enzyme V_{max} in umol·hr^{-1}·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

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	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
N-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

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

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	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
N-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

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Table S9. Q_{10} 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

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	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-	Desert		2.06±0.10	1.89±0.08	1.97±0.03	1.83±0.06
aminopeptidase	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
N-acetyl-*β*-D-	Desert		1.98±0.07	1.94±0.06	1.95±0.02	1.95±0.06
glucosaminidase	Scrubland		1.94±0.01	1.93±0.03	1.83±0.02	1.76±0.03
(NAG)	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

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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	Desert		1.27±0.03	1.31±0.06	1.20±0.03	1.34±0.12
(AG)	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±.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	Desert		1.24±0.04	1.15±0.00	1.25±0.04	1.21±0.03
(AP)	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	Desert		1.93±0.03	1.98±0.03	2.05±0.12	2.10±0.06
(BG)	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	Desert		1.41±0.04	1.49±0.02	1.32±0.03	1.31±0.03
(BX)	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

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	Subalpine		1.41±0.02	1.39±0.03	1.34±0.03	1.29±0.04
Cellobiohydrolase	Desert		1.98±0.06	2.07±0.05	2.28±0.08	2.27±0.08
(CBH)	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-	Desert		1.37±0.21	1.15±0.02	1.20±0.02	1.13±0.01
aminopeptidase	Scrubland		1.13±0.00	1.11±0.01	1.17±0.02	1.12±0.00
(LAP)	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
N-acetyl-*β*-D-	Desert		1.69±0.04	1.75±0.04	1.74±0.04	1.69±0.04
glucosaminidase	Scrubland		1.70±0.03	1.71±0.03	1.58±0.03	1.59±0.04
(NAG)	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

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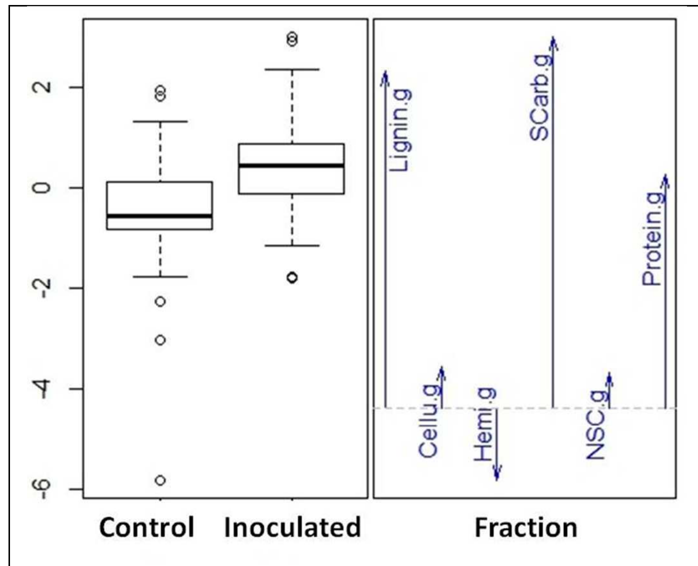

Figure S1. Canonical discriminant analysis for variation in litter chemistry fractions between inoculated and control litterbags at the final sampling date in December 2015.

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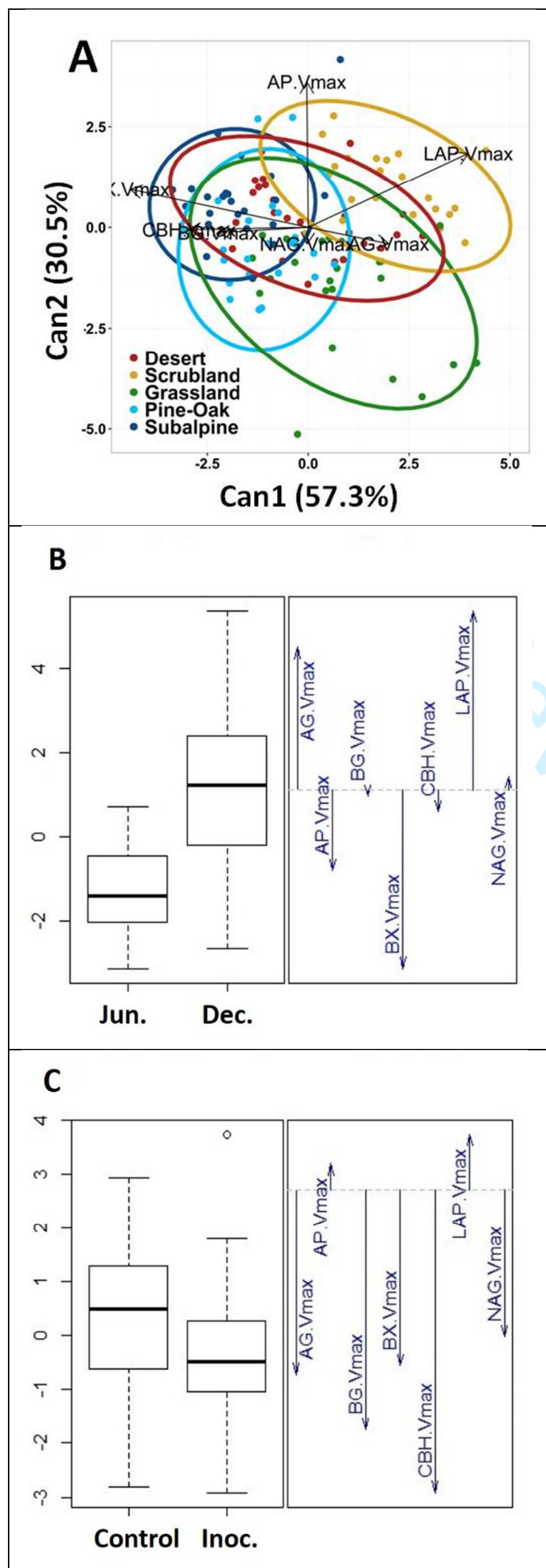

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Figure S2. Canonical discriminant analyses for variation in extracellular enzyme V_{max} of all enzyme classes between **A)** sites, **B)** June and December 2015 sampling dates, and **C)** inoculated and control litterbags.

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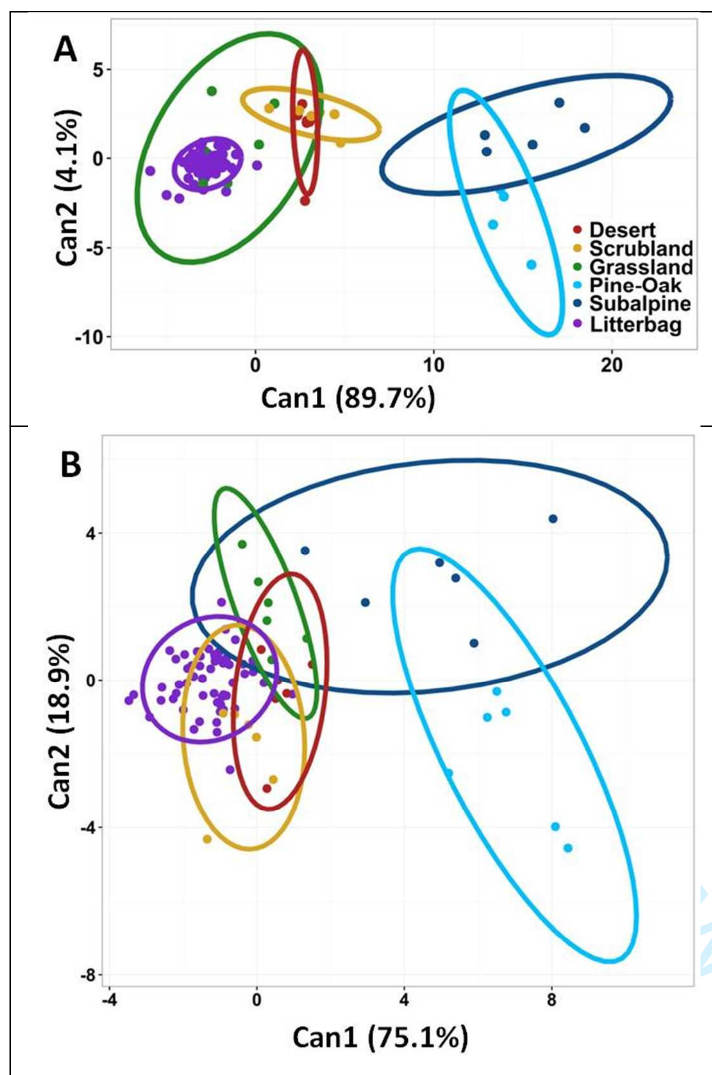

Figure S3. Canonical discriminant ordinations of differences in **A**) V_{max} and **B**) V_{max} temperature sensitivities of all enzymes assayed. Plots depict differences between native litter from each site ("Subalpine", "Pine-Oak", etc.) and litter in transplanted litterbags from all sites ("Litterbags") 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.

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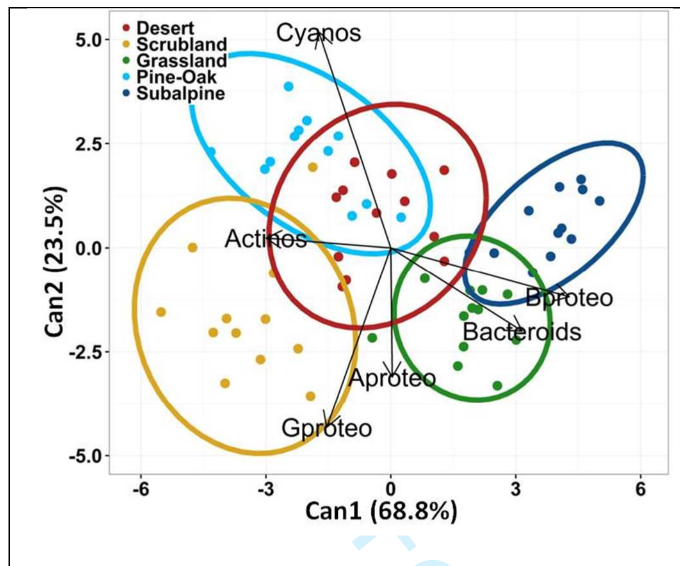

Figure S4. Canonical discriminant analysis for variation in bacterial community composition at the phylum/class level as determined by 16S amplicon sequencing.

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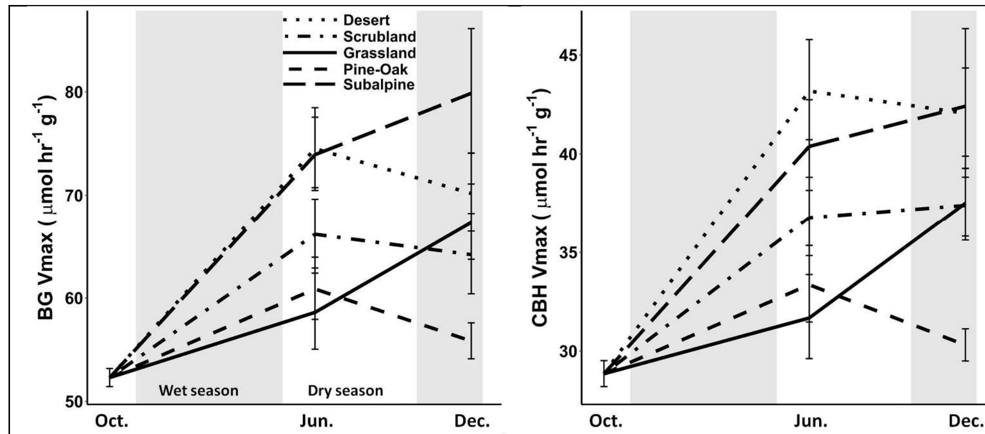

Figure S5. Mean V_{max} of **A**) β-glucosidase and **B**) cellobiohydrolase in initial litter and litterbags collected in June and December 2015. Error bars denote standard error. Depicted means and standard errors have been back-transformed from ln values.

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