Microbial legacies alter decomposition in response to simulated global change

Jennifer B.H. Martiny[1], Adam C. Martiny[1,2], Claudia Weihe[1], Ying Lu[1], Renaud Berlemont[2,3], Eoin L. Brodie[4,5], Michael L. Goulden[2], Kathleen K. Treseder[1], and Steven D. Allison[1,2]

[1]Dept. of Ecology and Evolutionary Biology, 321 Steinhaus Hall, University of California, Irvine, CA, 92697 USA.

[2]Dept. of Earth System Science, 3200 Croul Hall, University of California, Irvine, CA, 92697 USA.

[3]Dept. of Biology, 1250 Bellflower Boulevard, California State University, Long Beach, CA 90840 USA.

[4]Ecology Department, Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720 USA.

[5]Dept. of Environmental Science, Policy and Management, 130 Hilgard Way, University of California, Berkeley, CA 94720 USA.

Corresponding author:

Jennifer Martiny
Dept. of Ecology and Evolutionary Biology
321 Steinhaus Hall
University of California, Irvine
Irvine, CA, 92697 USA
949-824-0487
jmartiny@uci.edu

ABSTRACT

Terrestrial ecosystem models assume that microbial communities respond instantaneously, or are immediately resilient, to environmental change. Here we tested this assumption by quantifying the resilience of a leaf litter community to changes in precipitation or nitrogen availability. By manipulating composition within a global change experiment, we decoupled the legacies of abiotic parameters versus that of the microbial community itself. After one rainy season, more variation in fungal composition could be explained by the original microbial inoculum than the litterbag environment (18% versus 5.5% of total variation). This compositional legacy persisted for three years, when 6% of the variability in fungal composition was still explained by the microbial origin. In contrast, bacterial composition was generally more resilient than fungal composition. Microbial functioning (measured as decomposition rate) was not immediately resilient to the global change manipulations; decomposition depended on both the contemporary environment and rainfall the year prior. Finally, using metagenomic sequencing, we showed that changes in precipitation, but not nitrogen availability, altered the potential for bacterial carbohydrate degradation, suggesting why the functional consequences of the two experiments may have differed. Predictions of how terrestrial ecosystem processes respond to environmental change might thus be improved by considering the legacies of microbial communities.

INTRODUCTION

The resilience of microbial communities – the rate at which they respond to environment change – is key to understanding their role in human, engineered, and natural systems (Allison and Martiny 2008, Costello et al 2012, Lozupone et al 2012, McMahon et al 2007, Shade et al 2012). Human activities are altering the variability and long-term trends in temperature, precipitation, and nitrogen availability, factors known to alter microbial composition in natural ecosystems (Allison and Martiny 2008, Evans and Wallenstein 2012, Rousk et al 2013). However, terrestrial ecosystem models assume that microbial communities respond instantaneously, or are immediately resilient, to environmental change (Hawkes and Keitt 2015, Lawrence et al 2009, Schimel and Gulledge 1998, Treseder et al 2012).

In the face of these shifting conditions, it is useful to consider resilience broadly, without regard to an equilibrium state (Loreau et al 2002). Thus, a highly resilient community responds quickly to environmental change, and its composition will be determined by contemporary conditions. In contrast, a non-resilient community will be a function of historical, as well as contemporary, environmental conditions (Hawkes and Keitt 2015). Indeed, plant and animal species often lag for decades in their response to climate change (Parmesan 2006, Wilczek et al 2014). However, microorganisms are generally expected to be more resilient than larger organisms due to faster growth rates, greater dispersal capabilities, and their potential for phenotypic plasticity and rapid evolution (Shade et al 2012).

If microbial composition is not immediately resilient, a key question is whether such delays matter for ecosystem functioning, which could lead to climate change

44 feedbacks in natural ecosystems (Treseder et al 2012). Prior studies testing for the effects
45 of the historical environment on microbial functioning have yielded mixed results (e.g.,
46 Evans and Wallenstein 2012, Li et al 2015, Rousk et al 2013, Updegraff et al 1998,
47 Waldrop and Firestone 2006). Crucially, however, these studies do not tease apart the
48 legacies of abiotic parameters versus those of the microbial community itself. Hence, it
49 remains unclear whether functional predictions would be improved by incorporating the
50 response of the microbial community itself.

51 The Loma Ridge Global Change Experiment manipulates rainfall and nitrogen,
52 two factors subject to anthropogenic change in southern California (CEC 2003, Fenn et al
53 2003). Previously, we have shown that the leaf litter microbial community is sensitive to
54 simulated drought and nitrogen addition in this grassland (Amend et al 2015, Matulich et
55 al 2015). Bacteria and fungi decompose most plant detritus (litter) in terrestrial
56 ecosystems (Swift 1979), and this process is a key step in the carbon cycle, as it mediates
57 the balance of carbon being respired as CO_2 into the atmosphere or stored in the soil
58 (Adair et al 2008).

59 In this study, we tested the resilience of the microbial community – its
60 composition and functioning – in its natural environment using microbial 'cages' (Allison
61 et al 2013, Reed and Martiny 2007) in the largest and longest experiment of its kind. The
62 cages inhibit the exchange of microorganisms, allowing separate manipulation of the
63 microbial community, litter substrate, and contemporary abiotic environment (Fig. 1A).
64 In earlier studies, we reported some results from the first year of this experiment,
65 including extracellular enzyme potential and litter mass loss (Allison et al 2013, Alster et
66 al 2013). Here, we assayed the taxonomic composition of bacteria and fungi in the

67 litterbags and followed composition and litter mass loss over three years. We then
68 compared these metrics to those of control communities remaining in their original
69 environment to quantify how long the origin of the microbial community, as opposed to
70 its contemporary environment or litter substrate, explained variation in microbial
71 composition or functioning (Fig. 1B).
72 We further investigated whether the resilience of microbial functioning in the
73 litterbags was linked to changes in a community's potential for carbohydrate degradation.
74 Microorganisms carry different suites of glycoside hydrolase (GH) genes that encode
75 enzymes that hydrolyze the glycosidic bond between the carbohydrate moieties of
76 polymers such as cellulose (Berlemont and Martiny 2015). We used metagenomic
77 sequencing to compare GH composition among the transplanted communities.
78
79 **MATERIALS AND METHODS**
80 **Field site.** The Loma Ridge Global Change Experiment was established in February 2007
81 in the foothills of Santa Ana Mountains within the Irvine Ranch National Landmark in
82 Orange County, California (117.704°W, 33.742°N)(Potts et al 2012). Annual mean
83 temperature is 17°C, and mean precipitation is 325 mm, falling primarily during a "wet"
84 season from November to April (Kimball et al 2014). The grassland is dominated by the
85 annual grass genera *Avena*, *Bromus*, and *Lolium*; the annual forb genera *Erodium* and
86 *Lupinus*; and the native perennial grass *Nassella pulchra*.
87 We used a subset of the global change manipulations: two levels of precipitation
88 (ambient and ~40% reduction) and two levels of nitrogen (ambient or 60 kg N ha^{-1} yr^{-1}
89 added as slow-release calcium nitrate) applied to subplots within precipitation treatments

90 (Allison et al 2013). Drought was imposed by covering the plots only when rainfall was
91 forecast. The covers (polyethylene sheeting over steel arch frames) were removed again
92 soon after rainfall, reducing rainfall by half with negligible effects on temperature or
93 sunlight in the plots (Potts et al 2012). Both treatments altered litter chemistry (Allison et
94 al 2013).
95
96 **Reciprocal transplant.** After the global change treatments were in place for 3.5 years,
97 we set up reciprocal transplants within the larger manipulations. The first year of the
98 transplant experiments are described in Allison et al. (2013), but for this study, we
99 continued the experiments for a total of three years. To manipulate litter origin, we
100 collected senesced plant material from the three types of plots (ambient, drought, and
101 added nitrogen). To keep the litter treatment constant across years, the litter from this
102 collection was stored at dry at room temperature and used for each year of the
103 experiment. Homogenized dry litter (2g) was placed into nylon membrane bags with 0.45
104 μm pores (GE Healthcare Life Sciences, Pittsburgh, PA). The bags allowed water,
105 nutrients, and possibly small bacteria to pass through. We sterilized all bags and their
106 contents with at least 22 kGy gamma irradiation. To manipulate microbe origin, we
107 inoculated the sterile litterbags with 50 mg non-sterile, dried and ground (Wiley mill, 1
108 mm mesh) litter. To manipulate the plot environment, we distributed these litterbags
109 (N=360) into the treatment plots on December 15, 2010. We retrieved the litterbags in
110 batches of 120 (15 treatment combinations x 8 blocks) on March 3, June 14, and
111 November 14, 2011. For the second year, new sterile litterbags were made, inoculated
112 with 50 mg from each of the litterbags collected in November 2011, and placed in the

113 field on December 7, 2011. Litterbags were collected on March 5, June 4, and November
114 19, 2012. For the third year, new sterile litterbags were inoculated with the November
115 2012 samples and placed in the field on December 3, 2012. A final collection was taken
116 on March 4, 2013.

117 Each litterbag was destructively sampled for litter mass, bacterial and fungal
118 abundance, and DNA extraction. Before placing the litterbags in the field, we weighed
119 the fresh litter and dried a subsample to constant mass at 65°C to obtain dry mass. All
120 mass losses are reported as percentage initial dry mass.

121

122 **Microbial abundance and biomass.** Bacterial cell densities were measured by flow
123 cytometry (Allison et al 2013). A 0.1 g subsample of fresh litter was ground and fixed
124 within 8 h of collection. Cells were extracted from litter by adding tetrasodium
125 pyrophosphate to a final concentration of 0.01M and gently sonicated for 30 min at 4°C.
126 The extract was filtered, stained with SYBR Green, and incubated for 15 min at room
127 temperature. Particles in stained extracts and unstained controls were counted by flow
128 cytometry (BD Accuri C6; BD Biosciences, San Jose, CA, USA), and cell densities were
129 calculated as the number of stained counts minus unstained counts per g dry litter. We
130 converted bacterial cell counts to biomass (ug C g^{-1} dry litter) assuming spherical cells
131 with radius 0.6 μm and C density of 2.2 x 10^{-13}g mm^{-3} (Bratbak, 1985).

132 Fungal hyphal lengths were determined by modifying a procedure described
133 previously (Allison et al 2013, 1992). Briefly, another 0.1 g subsample of fresh litter was
134 ground, dried, and dispersed in 10-mL sodium hexametaphosphate solution (0.395%
135 mass/volume) with vigorous stirring. This solution was filtered and stained with acid

136 fuchsin and filtered onto two filters. Both filters were dried and mounted on a microscope
137 slide. Hyphal lengths (m/g dry litter) were determined on phase contrast microscope
138 under 100X magnification using the grid-intercept method (Giovannetti and Mosse 1980)
139 and 50 grids per filter. Hyphal lengths were converted to biomass (μg C g^{-1} dry litter)
140 assuming a fresh density of 1.1 g cm^{-3}, 33% dry mass, 40% C in dry mass, and hyphal
141 diameter of 5.2 μm (Paul and Clark 1996). Hyphal diameter was estimated as described
142 in Alster et al. (2013) and did not vary by treatment or date.
143
144 **Genetic analyses.** From each litterbag, 0.05 g of ground litter was flash frozen using
145 liquid nitrogen and stored at -80°C for DNA extraction. Samples were extracted using a
146 previously described procedure (Matulich et al 2015), and cleaned using an AllPrep Kit
147 (Qiagen, Valencia, CA, USA). To characterize bacterial composition, the V4 region of
148 the 16S rRNA gene and the D1 and D2 region of the 28S rRNA gene was PCR amplified
149 as in (Matulich et al 2015). PCR products were cleaned using the Agencourt AMPure XP
150 PCR Purification System (Beckman Coulter Inc., Indianapolis, IN, USA), quantified
151 using a Quant-iT (Life Technologies, Grand Island, NY, USA) Assay Kit and a Synergy
152 4 microplate reader (BioTek, Winooski, VT, USA), pooled into equimolar concentrations
153 and pyrosequenced at the Duke University ISGP Sequencing Facility on a 454 Life
154 Sciences FLX sequencer using Titanium chemistry (454 Life Sciences, Branford, CT,
155 USA).
156 The amplicon sequences were processed using the QIIME (version 1.6.0) toolkit
157 (Caporaso et al 2010) with the following parameters: quality score >50, sequence length
158 >300 and <700 for fungi and >200 and <550 for bacteria, maximum homopolymer of 6, 6

159 maximum ambiguous bases, and 0 mismatched bases in the primer. Sequences were
160 denoised using Denoiser (Reeder and Knight 2010), and operational taxonomic units
161 (OTUs) were picked at the 97% identity level using UCLUST (Edgar 2010) in QIIME.
162 The taxonomic identity of the OTUs was assigned to bacteria by reference to SILVA 108
163 (Quast et al 2013) and the Greengenes database (DeSantis et al 2006) using the naïve
164 RDP classifier within QIIME (Wang et al 2007). Taxonomic identity of the fungal OTUs
165 were assigned by reference to the 28S LSU RDP database (Cole et al 2013) using
166 BLAST. Nucleotide sequences are available from NCBI SRA (deposition SRP067657).
167 To assay glycoside hydrolase (GH) composition, we created metagenomic
168 libraries from the March 2011 samples. We first pooled equal concentrations of DNA
169 extracts from four litterbags undergoing the same treatment yielding 2 replicates for each
170 of the combinations of factors (microbial origin, plot environment, and litter origin) for
171 the two experiments (drought and added nitrogen). This pooling was necessary to yield
172 enough DNA for some of the samples. We fragmented the DNA of the pooled samples to
173 300bp using a hydroshear (Covaris, MA, USA) and prepared 30 metagenomic libraries
174 using a Truseq library kit (Illumina, San Diego, CA, USA). (The same ambient-ambient-
175 ambient (microbe-litter-plot) samples were used for the two experiments.) The libraries
176 were sequenced with on the Illumina HiSeq 2000 platform (100 bp-paired ends; treated
177 as single reads for downstream analysis). Sequences were uploaded onto the MG-RAST
178 server (Glass and Meyer 2011) (accessions 4514321-31, 4535613-25, 4537557-62). In
179 total, 44.4 Gbp (passed QC) were obtained. Taxonomic annotation, using the M5NR
180 database, at the genus level, was considered for sequences with e-value $\leq 10^{-5}$.

We used a custom bioinformatics pipeline to detect and assign GHs to particular families and in the case of the GH8 family, further discriminated between functions (Berlemont et al 2014, Berlemont and Martiny 2015). GH families were assigned to substrate target categories according to the substrate specificities of characterized enzymes, as stated in the CAZy database (Lombard et al 2014). In addition, biosynthetic cellulases (BcsZ) from GH8 were separated from hydrolytic enzymes (Berlemont and Martiny 2013).

Statistics. Multivariate analysis was conducted using PRIMER 6.0 and PERMANOVA+ (Anderson MJ 2008, Clarke and Gorley 2006). Prior to these tests, samples with poor quality data were excluded, after which 332 bacterial and 343 fungal samples remained. For both the bacteria and fungi, we then generated a rarefied distance matrix among the remaining samples. We created 100 OTU tables from the original data, randomly drawing the lowest common number of sequences from each of the remaining samples (1265 sequences for Bacteria; 2527 for Fungi). To weight the rarer taxa more heavily, we transformed each table by taking the square root of each cell value and rounding to the nearest integer. We then calculated a Bray-Curtis distance matrix for each of the 100 OTU tables. Finally, for each pairwise comparison between samples (i.e., each cell in the distance table), we chose the median Bray-Curtis value among the 100 distance matrices.

To test for the effects of the experimental factors on bacterial and fungal composition, we performed three-way PERMANOVA using the default settings, including all three factors as fixed effects. Except in one case, there were no significant interactions between the factors on microbial composition. Therefore, to improve the

204 estimates of the variation explained by the significant factors, we reran the tests with only
205 the main factors. In the one case where the plot-by-microbe interaction was significant
206 (for the fungi in the drought experiment in 2011; $p = 0.038$), we included this variation in
207 the calculations. To identify the taxa contributing to significant treatment differences, we
208 used similarity percentage (SIMPER) analysis. Non-metric multimensional scaling
209 (MDS) ordination was used to visualize patterns in community composition.
210 We estimated the effect size – the relative importance of the three manipulated
211 factors – on microbial composition using the estimates of the components of variation
212 from the PERMANOVA. We divided each factor's estimate by the sum of the estimates
213 for all significant factors plus the residual variation (Anderson MJ 2008), then multiplied
214 by 100 to report this value as a percentage. This approach is analogous to the unbiased
215 univariate ANOVA estimators of variance components (Searle et al 1992). We estimated
216 the effect size of the factors on mass loss by using a standard three-way ANOVA (Gotelli
217 and Ellison 2004). To parallel the composition analyses, an ANOVA was performed for
218 each year (averaged over the sampling dates). Although variance partitioning approaches
219 have limitations, they are especially useful for comparing effects within an experiment, as
220 we do here (Olejnik and Algina 2003).
221 To test the effects of global change on GH gene composition, we performed a 3-
222 way PERMANOVA on the relative abundance of bacterial GH genes in the metagenomic
223 libraries, lumping the genes into 11 substrate target categories. Similar results were
224 obtained when the genes were categorized into finer categories, defined by the GH family
225 or by GH family and genus-level taxonomy. Given the minimal replication of the
226 metagenomic libraries, we limited our analysis to tests of the three main terms (i.e., we

227 did not test for interactions between the terms).

228 Finally, we analyzed percentage mass loss and microbial abundances using a
229 factorial mixed-model ANOVA with repeated measures, transforming the data when
230 necessary to improve normality and homogeneity of variances. The model included
231 microbe origin, litter origin, plot environment, and date as fixed effects (along with their
232 interactions) and two random effects: block and subject nested within block. We defined
233 subject as the batch of litterbags with the same block, plot, litter origin, and microbe
234 origin.

235

236 **RESULTS AND DISCUSSION**

237 The taxonomic composition and diversity in the litterbags indicated that the cages
238 allowed a natural grassland litter community to persist while preventing microbial
239 immigration. The dominant taxa (defined by 97% sequence similarity of 16S and 28S
240 rRNA genes) were also abundant outside the litterbags at this site (Matulich et al 2015).
241 Fourteen of the 20 most abundant bacterial taxa (phyla Actinobacteria, Bacteriodetes, and
242 Proteobacteria) were shared in both the litterbags and in naturally occurring leaf litter,
243 and their relative frequencies were highly correlated (r=0.72; p=0.004; Fig. S1). Similar
244 patterns were observed for fungi, with the dominant taxa belonging to the Ascomycota
245 and Basidiomycota phyla (r=0.77, p=0.002; Fig. S1). At the same time, bacterial and
246 fungal richness was highest in the inoculum and declined over time in the litterbags (Fig.
247 S2). Such a decline is expected, as the initial inoculum would comprise litter specialists
248 as well as transients from nearby habitats (e.g., soil and live plants) that would be
249 outcompeted on leaf litter.

To test for a legacy of microbial composition, we compared the effect size (estimated variance explained in a PERMANOVA model)(Anderson 2001) of microbial origin (the initial inoculum in the litterbag) over time (Fig. 1B). This comparison reveals how long a legacy of initial composition was apparent after being transferred to a new litter or plot environment. To illustrate this analysis, we first plot the results of the fungi in the drought experiment using nonmetric multidimensional scaling (Fig. 2). In 2011, just three months after the initial inoculation of the litterbags, all three factors significantly altered fungal composition ($p<0.001$; Table 1). However, most (18%) of the variation in fungal composition could be explained by whether the inoculum originated from an ambient or drought plot (Fig. 2A) rather than the origin of the litter (4%) or the plot environment (5.5%) of the litterbag (Fig. 2B; Table 1). By 2013, the microbial origin effect was much less apparent (6%), while the effects of litter origin and plot environment were slightly clearer (Figs. 2C and 2D). Plotting the effect sizes over time (orange line in Fig. 3A) reveals that fungal composition was in part determined by the original inoculum even after three years.

In the nitrogen experiment, fungal composition was also still affected by the legacy of the microbial community after three years (Fig. 3B; Table 1). In contrast, bacterial composition was more resilient than fungal composition. In the drought experiment, microbial origin did not affect bacterial composition even after the first rainy season (Fig. 3C). In the nitrogen experiment, microbial origin explained a significant amount (12.5%) of bacterial composition during the first year, but was an insignificant factor by the second year (Fig. 3D; Table 1).

Notably, the legacies of the microbial origin were driven by changes in a relatively small number of taxa. Just ten fungal taxa explained more than 45% of the compositional variation between the ambient and treatment communities (Table S1). Similarly, ten bacterial taxa explained more than 20% of the compositional variation in the N experiment (bacterial composition was resilient by the first sampling point in drought experiment)(Table S2). The slower response of fungi versus bacteria could be due to slower growth and turnover rates of fungal spores and hyphae than bacterial cells. Indeed, while bacterial density fluctuated by orders of magnitude between sampling points, fungal density was less dynamic (Fig. S3).

A potential methodological reason for the differential responses of the bacterial and fungal communities could be that very small bacteria are immigrating into the litterbags; however, we do not think this possibility explains this result for two reasons. First, even if a few bacteria (and perhaps even fungal hyphae) immigrate through the litterbag mesh, they would make up a small fraction of the total community. Given that all but the rarest taxa are likely already present in the inoculum to all litterbags, this contamination would have a negligible effect on the abundance of any particular taxon and therefore community composition. Further, if unwanted immigration is much higher than expected, size selection should result in different litterbag communities than those observed outside the bags. In fact, the communities inside and outside of the litterbags were highly correlated (Fig. S1).

In contrast to the dissipating effect of the microbial origin over time, plot environment and litter origin had a relatively consistent effect on microbial composition over the three years (blue and green lines in Fig. 3A-D). This result was expected as the

295 plot and litter treatments were maintained over the course of the experiment (new litter
296 from a single collection was added yearly). Precipitation treatment was a particularly
297 strong determinant of bacterial composition (explaining between 12-28% of composition
298 variation across the three years). The reduced effect in 2012 (Fig. 3C) is likely due the
299 timing of the precipitation manipulations that year; both ambient and drought plots
300 received the same rainfall during the six weeks prior to sampling (Fig. S4).
301 Microbial functioning was also not immediately resilient to the global change
302 manipulations. In the drought experiment, litterbags inoculated with microbial
303 communities from the drought treatment experienced 16.5% slower decomposition by the
304 end of the first rainy season (Fig. 4A; microbe origin first year: p=0.009 (Allison et al
305 2013); microbial origin all 3 years: p=0.056; microbe x year: p=0.032; Table S3). The
306 strength of this microbial origin effect was similar to, or even stronger than, that of the
307 plot environment (Fig. 4B). However, by the second rainy season, microbial origin no
308 longer influenced litter mass loss in the drought experiment (Figs. 3E and 4). In the
309 nitrogen experiment, microbial origin only weakly affected mass loss (Fig. S5); the high
310 nitrogen community increased mass loss by an average of 6% over the three years, but
311 this effect was not significant in the *post hoc* individual year tests (Fig. 3F). Thus, the
312 compositional differences between the ambient and added nitrogen communities
313 appeared nearly functionally redundant, at least in terms of the decomposition rate.
314 What explains the lag in the decomposition response? Because inoculum
315 communities were collected within tens of meters of one another, we assume that the
316 same taxa were present in all litterbags even though immigration was restricted. Also, the
317 differences between the drought and ambient communities were caused by moderate

318 changes in the abundance of common taxa, rather than major shifts in the identity of
319 common taxa (Figure S1)(Matulich et al 2015). We therefore hypothesize that the lag in
320 microbial composition and its functioning was due to slow growth and turnover of
321 individual taxa. However, we cannot rule out the possibility that the convergence in the
322 community response was also partly due to *de novo* point mutations and horizontal gene
323 transfer among cells during the experiment.

324 We also examined the possibility that decomposition rates were altered by
325 changes in microbial abundance rather than microbial composition. Bacterial abundance
326 was measured by flow cytometry and fungal abundance by microscopy. We then
327 converted these metrics to biomass to compare the groups. Drought directly reduced
328 bacterial mass by 30% (p=0.0001; Table S3; Fig. S3), which was accompanied by lower
329 mass loss. However, bacterial mass was also 58% lower when growing on litter from the
330 drought environment (Fig. S3), and this reduction was not accompanied by a change in
331 mass loss (Fig. 3E). Across the entire experiment, bacterial abundance (the vast majority
332 of microbial biomass) had only a marginal effect on litter mass loss by the next sampling
333 date (p=0.054) and no significant interaction between bacterial mass and sampling date
334 (p=0.73)(overall ANCOVA model: $F_{3,472} = 60.07$, adjusted $R^2 = 0.272$). Together, these
335 results suggest that microbial composition, not abundance, was primarily responsible for
336 the community influence on decomposition rate (Fig. 4A).

337 To investigate why microbial functioning might be more resilient to changes in
338 nitrogen than to changes in rainfall, we characterized how the global change treatments
339 altered the potential for carbohydrate degradation. Metagenomic sequencing revealed that
340 the litter communities carried an abundant and diverse collection of GH genes. Most

341 (>98%) of the more than 710,000 identified GH sequences were bacterial, and GH
342 sequences accounted for 0.31% of all annotated bacterial sequences. GH genes can be
343 further classified into different GH families that target different substrates (Berlemont
344 and Martiny 2015, Lombard et al 2014). The sequences fell into 67 different GH families
345 that could be divided into 5805 unique types based on both GH family and genus-level
346 taxonomy. Many of the GH genes target oligosaccharides, but others revealed the
347 potential for structural polysaccharide deconstruction, including cellulose, xylan, and
348 chitin (Fig. S6).
349 The composition of bacterial GH genes in the litterbags was significantly altered
350 by the plot environment (PERMANOVA: $p<0.001$, variance explained: 27.8%) and
351 specifically, by changes in precipitation (Fig. 5). Drought selected for an increased
352 abundance of GH genes that targeted starch, xylan, mixed polysaccharides, and "other
353 animal" (e.g., non-chitin) polysaccharides, and a decreased abundance of those targeting
354 oligosaccharides and "other plant" (e.g., non- cellulose or xylan) polysaccharides (Table
355 S4). In contrast, added nitrogen did not alter GH composition (Fig. 5), in agreement with
356 previous work at this site (Berlemont et al 2014). This result suggests a mechanistic
357 reason for why microbial functioning was more resilient to changes in nitrogen than to
358 changes in rainfall. Drought altered bacterial taxonomic composition in such a way that
359 carbohydrate degradation traits were also altered, leading to a lower decomposition rate.
360 In contrast, taxonomic shifts in response to nitrogen addition did not alter carbohydrate
361 degradation traits, and the functional consequences were barely detectable. This
362 difference highlights the correlation (or lack thereof) between effect and response traits,
363 where an organism's effect traits affect ecosystem functioning and its response traits

influence how a taxon responds to environmental change (Lavorel and Garnier 2002, Martiny et al 2015). In this litter community, traits that affect litter decomposition appear to be correlated with the response to changing rainfall, but not to changing nitrogen availability.

Two additional considerations will be important to understand the consequences of microbial legacies for predictions of ecosystem processes. First, we only investigated the effect on decomposition, but the same legacies might simultaneously alter additional, unmeasured processes. Second, given that ecosystems are constantly being disturbed, it will be important to investigate how even a one-year lag time might compound over time. Nevertheless, terrestrial microbial communities should not broadly be expected to be instantaneously resilient.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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REFERENCES

Adair EC, Parton WJ, Del Grosso SJ, Silver WL, Harmon ME, Hall SA *et al*. (2008). Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. *Global Change Biol* **14:** 2636-2660.

Allison SD, Martiny JBH. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci USA* **105:** 11512-11519.

Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK *et al*. (2013). Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* **94:** 714-725.

Alster CJ, German DP, Lu Y, Allison SD. (2013). Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. *Soil Biology & Biochemistry* **64:** 68-79.

Amend AS, Martiny AC, Allison SD, Berlemont R, Goulden ML, Lu Y *et al*. (2015). Microbial response to simulated global change is phylogenetically conserved and linked with functional potential. *ISME J*.

Anderson MJ. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26:** 32-46.

Anderson MJ GR, Clarke KR. (2008). *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E Ltd: Plymouth, UK.

Berlemont R, Martiny AC. (2013). Phylogenetic distribution of potential cellulases in bacteria. *Applied and Environmental Microbiology* **79:** 1545-1554.

Berlemont R, Allison SD, Weihe C, Lu Y, Brodie EL, Martiny JBH *et al*. (2014). Cellulolytic potential under environmental changes in microbial communities from grassland litter. *Frontiers in Microbiology* **5:** 639.

Berlemont R, Martiny AC. (2015). Genomic potential for polysaccharide deconstruction in bacteria. *Applied and Environmental Microbiology* **81:** 1513-1519.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al*. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7:** 335-336.

CEC. (2003). Global Climate Change and California: Potential Implications for Ecosystems, Health, and the Economy—Consultant Report. California Energy Commission: USA.

Clarke K, Gorley R. (2006). *Primer v6: User Manual/Tutorial*. PRIMER-E: Plymouth.

Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y *et al*. (2013). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research*.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science* **336:** 1255-1262.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K *et al*. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied and Environmental Microbiology* **72:** 5069-5072.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460-2461.

Evans SE, Wallenstein MD. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* **109:** 101-116.

Fenn ME, Poth MA, Bytnerowicz A, Sickman JO, Takemoto BK. (2003). Effects of ozone, nitrogen deposition, and other stressors on montane ecosystems in the Sierra Nevada. *Developments in Environmental Science* **2:** 111-155.

Giovannetti M, Mosse B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84:** 489-500.

Glass EM, Meyer F. (2011). The metagenomics RAST server: a public resource for the automatic phylogenetic and functional analysis of metagenomes. *Handbook of Molecular Microbial Ecology I: Metagenomics and Complementary Approaches*. Wiley: Hoboken, NJ. pp 325-331.

Gotelli NJ, Ellison AM. (2004). *A Primer of Ecological Statistics*. Sinauer: Sunderland, MA.

Hawkes CV, Keitt TH. (2015). Resilience vs. historical contingency in microbial responses to environmental change. *Ecology Letters* **18:** 612-625.

Kimball S, Goulden ML, Suding KN, Parker S. (2014). Altered water and nitrogen input shifts succession in a southern California coastal sage community. *Ecological Applications* **24:** 1390–1404.

Lavorel S, Garnier E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* **16:** 545-556.

Lawrence CR, Neff JC, Schimel JP. (2009). Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. *Soil Biology & Biochemistry* **41:** 1923-1934.

Li Q, Yang Y, Bao XL, Liu F, Liang WJ, Zhu JG *et al*. (2015). Legacy effects of elevated ozone on soil biota and plant growth. *Soil Biology & Biochemistry* **91:** 50-57.

Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research* **42:** D490-D495.

Loreau M, Downing A, Emmerson M, Gonzalez A, Hughes J, Inchausti P *et al*. (2002). A new look at the relationship between diversity and stability. In: Loreau M, Naeem S, Inchausti P (eds). *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. Oxford University Press: New York. pp 79-91.

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature* **489:** 220-230.

Martiny JBH, Jones SE, Lennon JT, Martiny AC. (2015). Microbiomes in light of traits: a phylogenetic perspective. *Science* **350:** aac9323.

Matulich K, Weihe C, Allison SD, Amend A, Berlemont R, Goulden ML *et al*. (2015). Temporal variation overshadows the response of leaf litter microbial communities to simulated global change. *ISME Journal***:** doi: 10.1038/ismej.2015.1058.

McMahon KD, Martin HG, Hugenholtz P. (2007). Integrating ecology into biotechnology. *Curr Opin Biotech* **18:** 287-292.

Olejnik S, Algina J. (2003). Generalized eta and omega squared statistics: Measures of effect size for some common research designs. *Psychol Methods* **8:** 434-447.

Parmesan C. (2006). Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol S* **37:** 637-669.

Paul EA, Clark FE. (1996). *Soil microbiology and biochemistry*, 2nd edn. Academic Press: San Diego, CA.

Potts DL, Suding KN, Winston GC, Rocha AV, Goulden ML. (2012). Ecological effects of experimental drought and prescribed fire in a southern California coastal grassland. *J Arid Environ* **81:** 59-66.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P *et al*. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41:** D590-D596.

Reed HE, Martiny JBH. (2007). Testing the functional significance of microbial composition in natural communities. *Fems Microbiology Ecology* **62:** 161-170.

Reeder J, Knight R. (2010). Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nature Methods* **7:** 668-669.

Rousk J, Smith AR, Jones DL. (2013). Investigating the long-term legacy of drought and warming on the soil microbial community across five European shrubland ecosystems. *Global Change Biol* **19:** 3872-3884.

Schimel JP, Gulledge J. (1998). Microbial community structure and global trace gases. *Global Change Biol* **4:** 745-758.

Searle SR, Casella G, McCulloch CE. (1992). *Variance components*. Wiley: New York.

Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H *et al*. (2012). Fundamentals of microbial community resistance and resilience. *Frontiers in microbiology* **3:** 417.

Swift MJ, O. W. Heal, and J. M. Anderson. (1979). *Decomposition in terrestrial ecosystems*. Blackwell Scientific Publications: Oxford, UK.

Sylvia DM. (1992). Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. In: Norris JR, Read D, Varma AK (eds). *Techniques for Mycorrhizal Research*. Academic Press: London. pp 513-525.

Treseder KK, Balser TC, Bradford MA, Brodie EL, Dubinsky EA, Eviner VT *et al*. (2012). Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry* **109:** 7-18.

Updegraff K, Bridgham SD, Pastor J, Weishampel P. (1998). Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. *Biogeochemistry* **43:** 253-272.

Waldrop MP, Firestone MK. (2006). Response of microbial community composition and function to soil climate change. *Microbial Ecology* **52:** 716-724.

Wang Q, Garrity GM, Tiedje JM, Cole JR. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73:** 5261-5267.

Wilczek AM, Cooper MD, Korves TM, Schmitt J. (2014). Lagging adaptation to warming climate in Arabidopsis thaliana. *Proc Natl Acad Sci USA* **111:** 7906-7913.

568 **FIGURE LEGENDS**

569

570 **Fig. 1.** (**A**) Experimental design of the drought reciprocal transplant within the global
571 change experiment. A parallel design was carried out for the nitrogen reciprocal
572 transplant. Global change manipulations were initiated in February 2007. In December
573 2010, microbial cage litterbags were filled with sterilized leaf litter, collected from either
574 ambient rainfall or drought treatment plots (litter origin). Each litterbag was then
575 inoculated with a microbial inoculum collected from ambient or drought plots (microbial
576 origin). Finally, sealed litterbags were placed back into ambient or drought plots (plot
577 environment). One bag from each of eight replicate blocks was destructively sampled at
578 the end of each rainy season (March) for three years. (**B**) To assay resilience of the
579 microbial community, we quantified how well microbial origin explained either
580 microbial composition or functioning (litter mass loss) at each time point. A steep decline
581 in variance explained over time indicates high resilience; a slow decline over time
582 indicates strong legacy effects (dashed gray arrow).

583

584 **Fig. 2:** Non-metric multidimensional scaling plots of the fungal communities in the
585 drought experiment litterbags in 2011 (**A** and **B**) and 2013 (**C** and **D**). Within a year, the
586 samples are color coded on the left-hand column by the origin of the initial microbial
587 inoculum (dark blue and orange) or on the right-hand column by the plot environment in
588 which the litterbags were placed (light blue and red). The different symbols denote the
589 particular combination of the three experimental factors. In order of plot environment,
590 litter origin, and microbial origin: ■ is AAA, ● is AAD, ▯ is ADA, ✻ is ADD, ▵ is

591 DAA, ▿is DAD, □ is DDD, and ▼ is DDA, where A is ambient and D is drought. Stress

592 values indicate goodness of fit in two dimensions.

593

594 **Fig. 3:** Percent estimated variance explained by plot environment (blue), litter origin

595 (green), and microbe origin (orange) on microbial composition and litter mass loss each

596 year. As in Figure 1B, a steep decline in the variance explained by microbial origin over

597 time indicates high resilience. Top panels are fungal composition, middle are bacterial

598 composition, and bottom are mass loss. Panels on the left-hand side are for the drought

599 experiment and on the right-hand side, for the nitrogen experiment. For fungal and

600 bacterial composition, estimated variance is plotted at zero if the factor was not

601 significant ($p>0.05$, except one case where $p=0.055$) in the PERMANOVA (Table 1). For

602 litter mass loss, all positive estimates are plotted and marked by 'ns' if not significant.

603

604 **Fig. 4:** Effect of (**A**) microbial origin, (**B**) plot environment, and (**C**) litter origin on

605 percentage mass loss in the drought experiment. N=32 for each treatment at each date.

606 Error bars are $\pm$ 1 SEM. See Table S3 and Fig. 3E for significance and effect sizes,

607 respectively.

608

609 **Fig. 5:** Non-metric multidimensional scaling plots of bacterial glycoside hydrolase

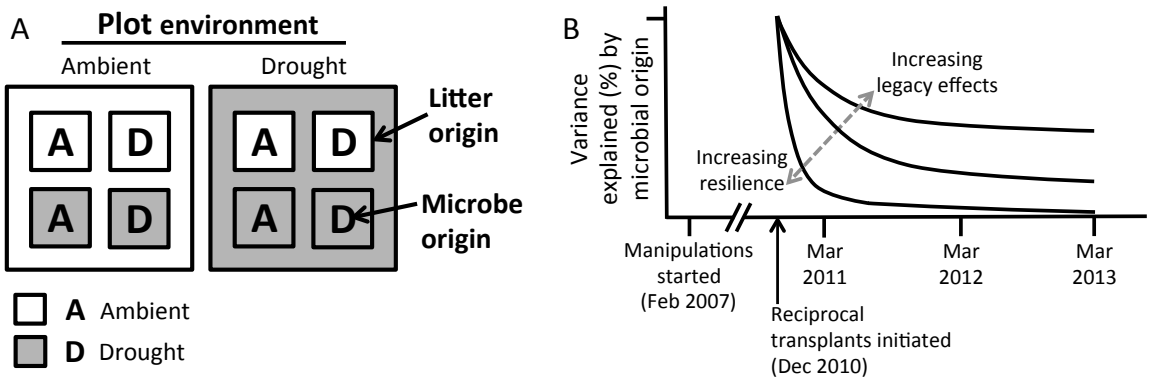
610 composition classified by substrate targeted in the litterbags after the first rainy season.

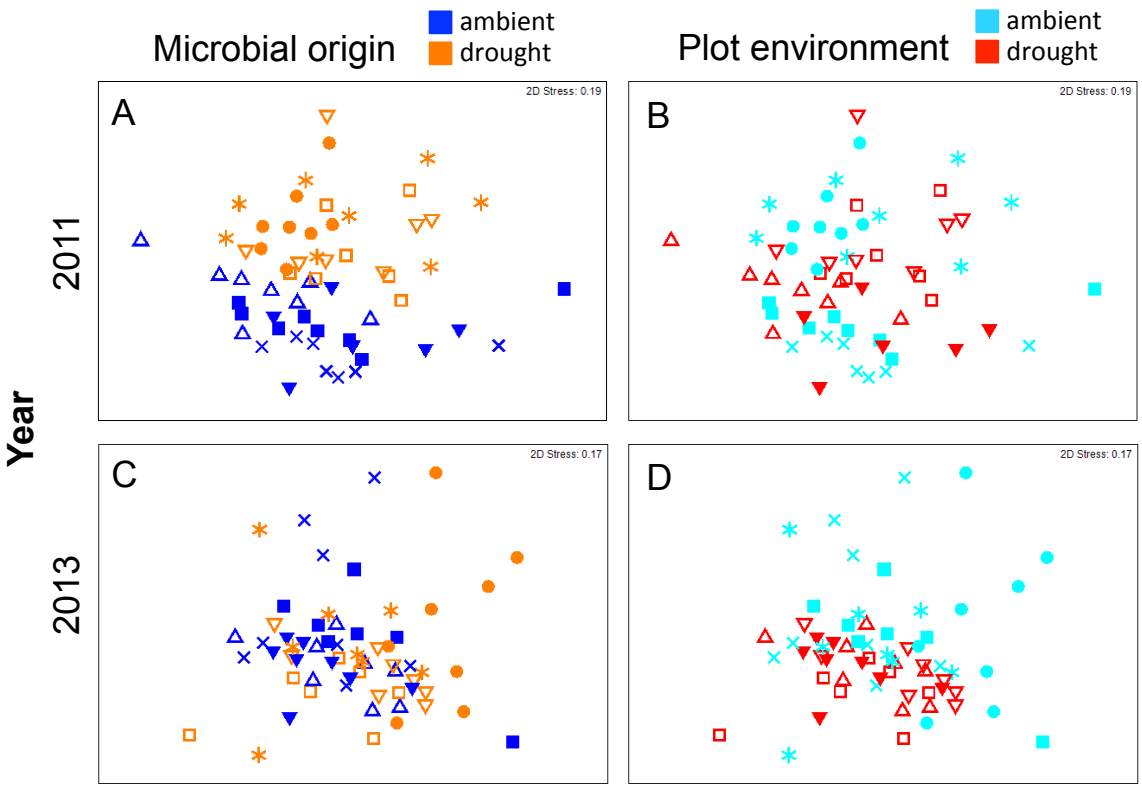
611 Plot environment [ambient (circles), drought (squares), added nitrogen (triangles)]

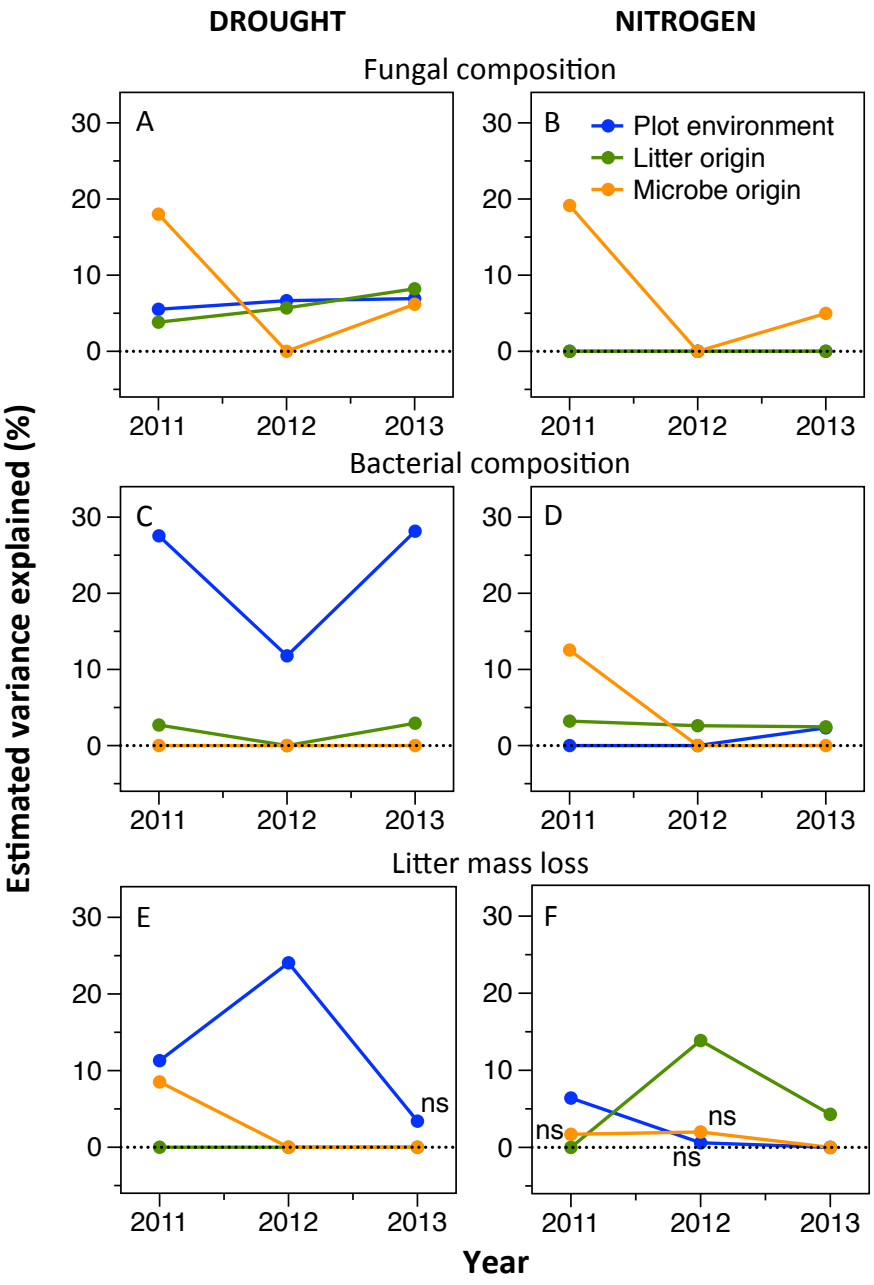
612 significantly altered GH composition ($p<0.001$), but litter origin and microbial origin did

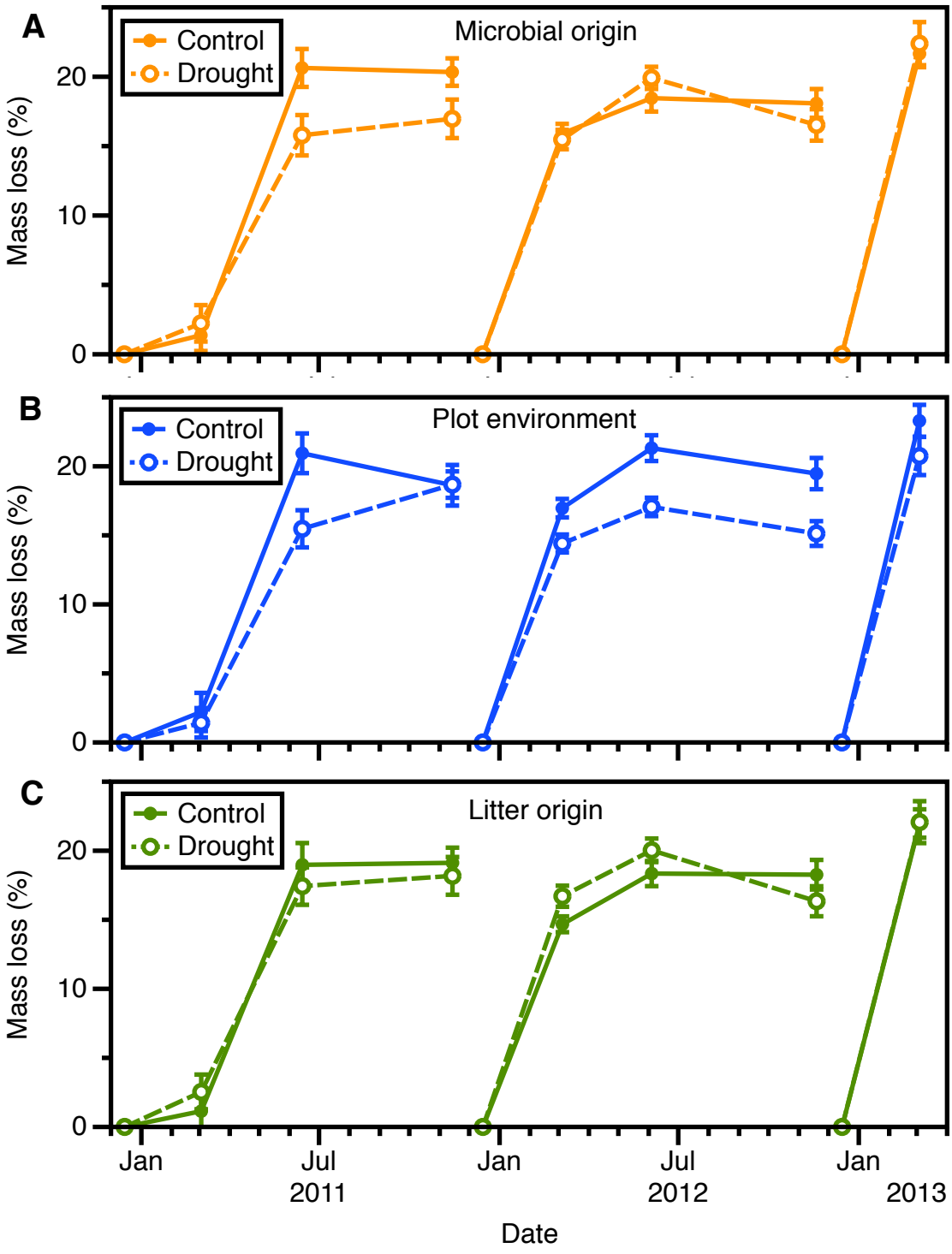
613 not ($p>0.05$). Similar results were observed when the analysis was performed based on

614 unique GH types or GH families.


A
Plot environment
Ambient
Drought
A
D
A
D
A
D
A
D
Litter origin
Microbe origin
A Ambient
D Drought
B
Variance explained (%) by microbial origin
Increasing legacy effects
Increasing resilience
Manipulations started (Feb 2007)
Mar 2011
Mar 2012
Mar 2013
Reciprocal transplants initiated (Dec 2010)


Microbial origin
ambient
drought
Plot environment
ambient
drought
Year
2011
2013
A
B
C
D
2D Stress: 0.19
2D Stress: 0.19
2D Stress: 0.17
2D Stress: 0.17


DROUGHT
NITROGEN
Fungal composition
Bacterial composition
Litter mass loss
A
B
C
D
E
F
Plot environment
Litter origin
Microbe origin
Estimated variance explained (%)
Year
30
20
10
0
2011
2012
2013
ns


A
Microbial origin
Control
Drought
Mass loss (%)
0
10
20
B
Plot environment
Control
Drought
Mass loss (%)
0
10
20
C
Litter origin
Control
Drought
Mass loss (%)
0
10
20
Jan
2011
Jul
Jan
2012
Jul
Jan
2013
Date

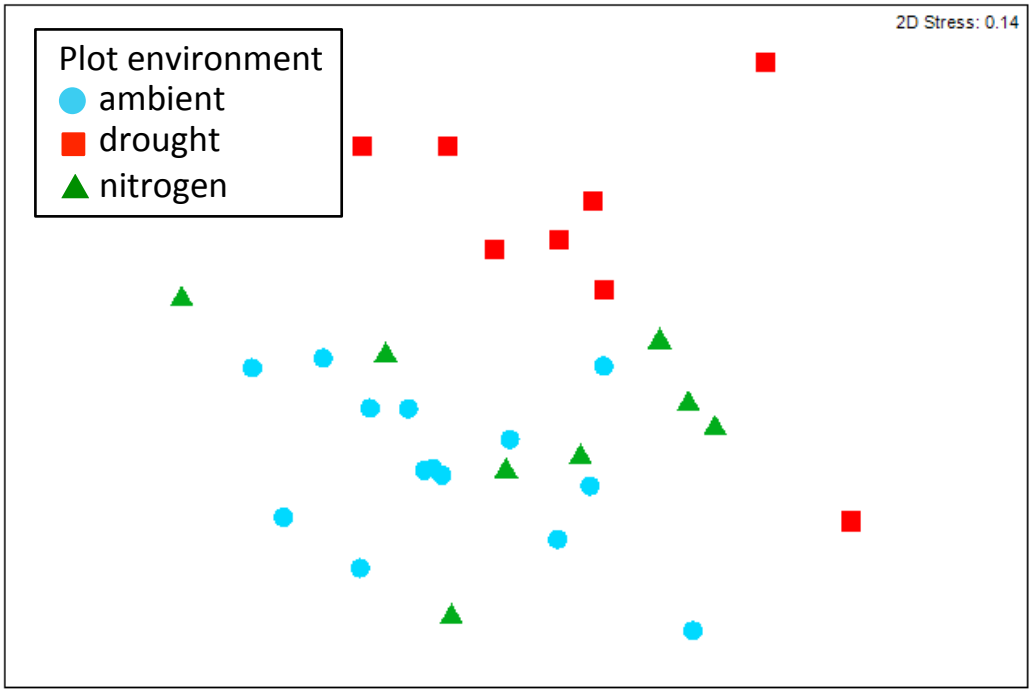

2D Stress: 0.14
Plot environment
ambient
drought
nitrogen

Table 1: The effect of plot environment, litter origin, and microbe origin on microbial composition. The results of PERMANOVA tests for bacterial or fungal composition for each year in each experiment (drought or added nitrogen). Values are p values and bolded when <0.05.

		FACTOR	2011	2012	2013
DROUGHT	**Bacteria**	Plot environment	**0.001**	**0.001**	**0.001**
		Litter origin	**0.011**	0.170	**0.008**
		Microbe origin	0.158	0.158	0.078
	Fungi	Plot environment	**0.002**	**0.001**	**0.001**
		Litter origin	**0.009**	**0.001**	**0.001**
		Microbe origin	**0.002**	0.072	**0.005**
NITROGEN	**Bacteria**	Plot environment	0.569	0.570	**0.038**
		Litter origin	**0.001**	0.055	**0.033**
		Microbe origin	**0.001**	0.462	0.171
	Fungi	Plot environment	0.229	0.292	0.314
		Litter origin	0.117	0.161	0.323
		Microbe origin	**0.001**	0.180	**0.013**