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Supplementary Information for

Decomposition responses to climate depend on microbial community composition

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Supplementary Figures

Figure S1. Soil surface temperature (ºC) from two plots at each site for a year of the experiment with colors representing day (7/8am to 7/8pm) and night (7/8pm to 7/8am) temperatures. The grassland had larger day-night temperature differences between 6 and 12 months than other sites. Subalpine had the most days with freezing soil temperatures (below 0 °C), followed by Pine-Oak, then Scrubland, Grassland; Desert experienced no freezing soil temperatures.

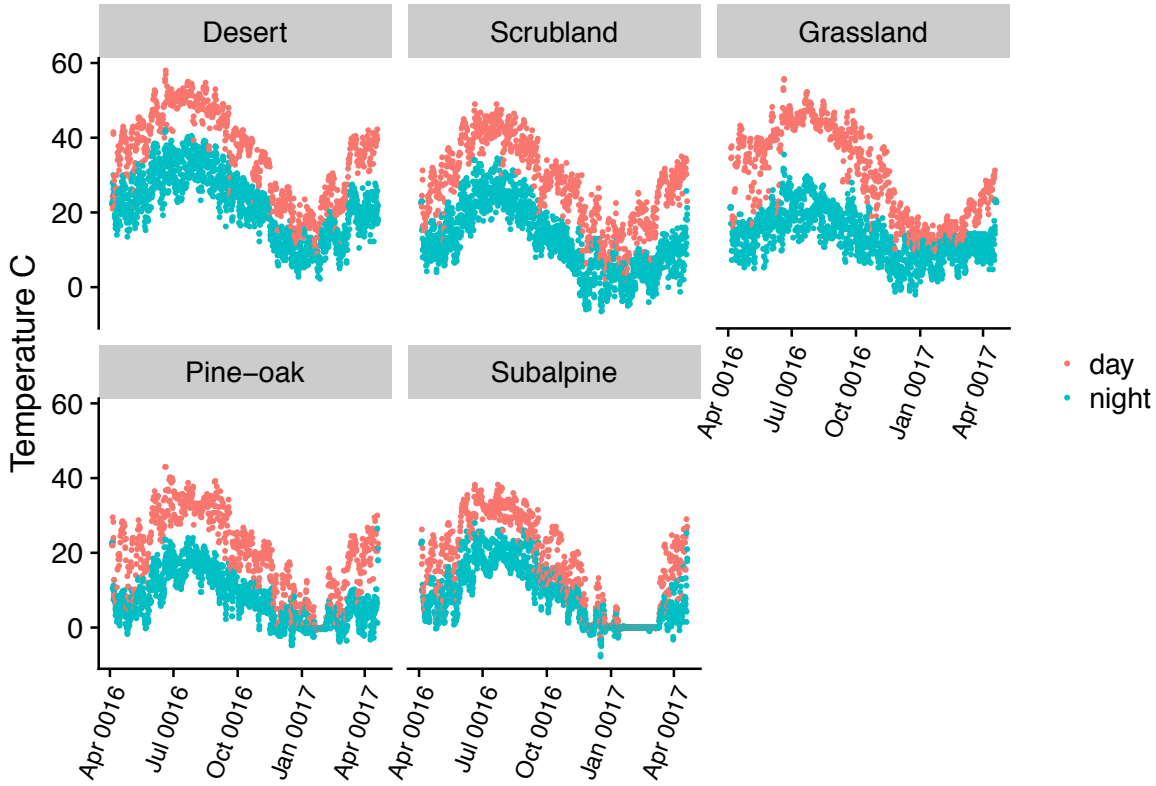
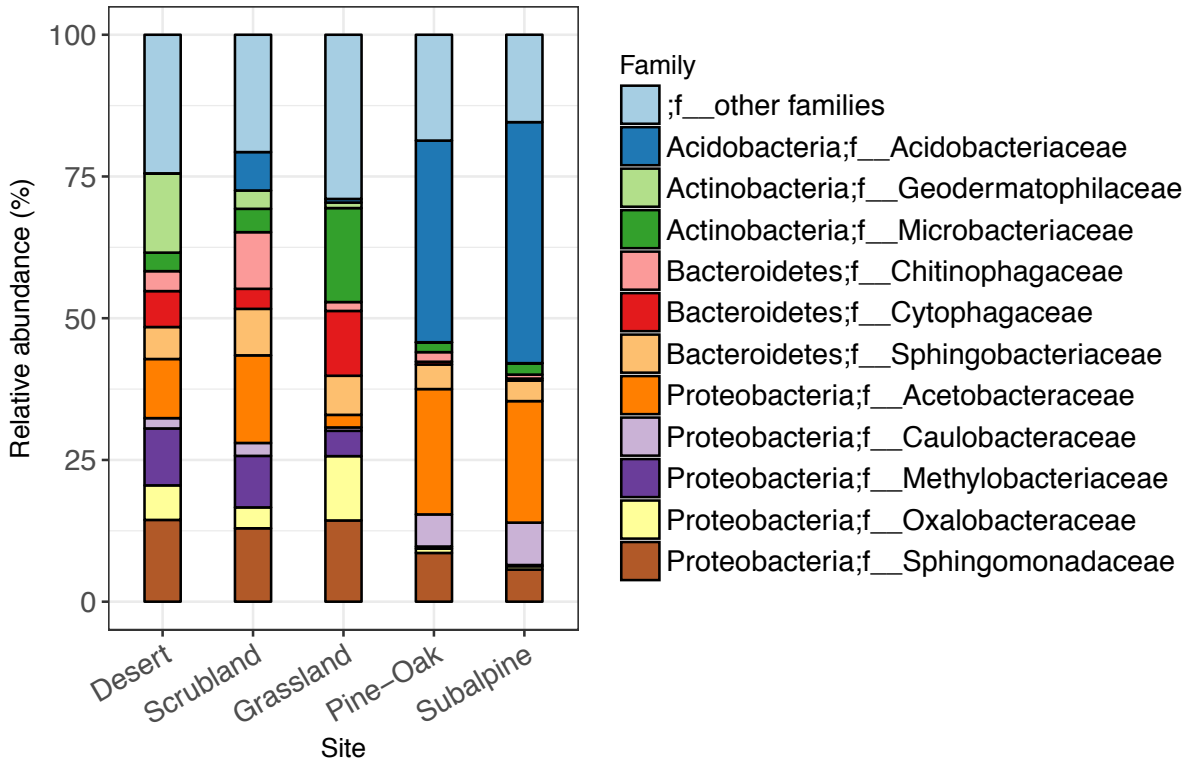


Figure S2. Relative abundance of families (phylum listed in legend) in the inoculum leaf litter for A) bacteria and B) fungi.

A)



B)

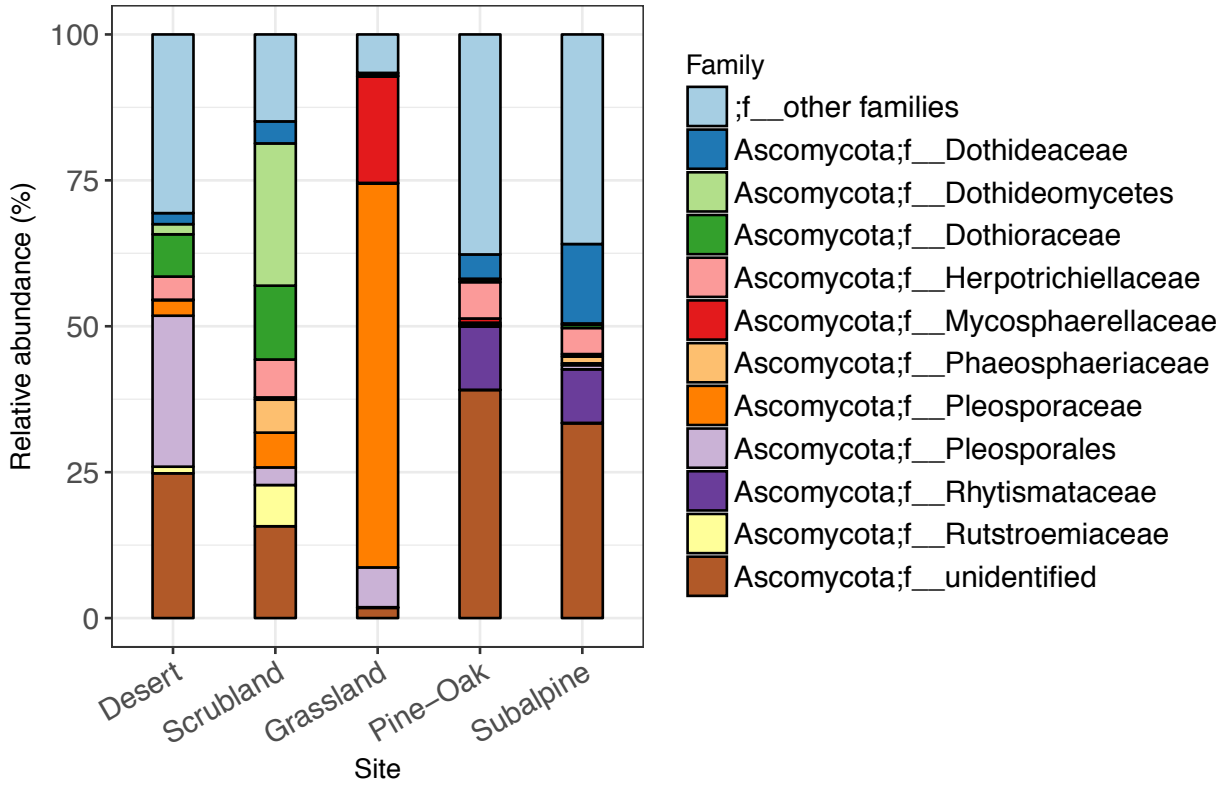


Figure S3. Mean OTU (97%) richness per site for bacteria and fungi in the inoculum leaf litter (after rarefying to a 10,000 sequence reads per sample). Letters indicate Tukey HSD differences.

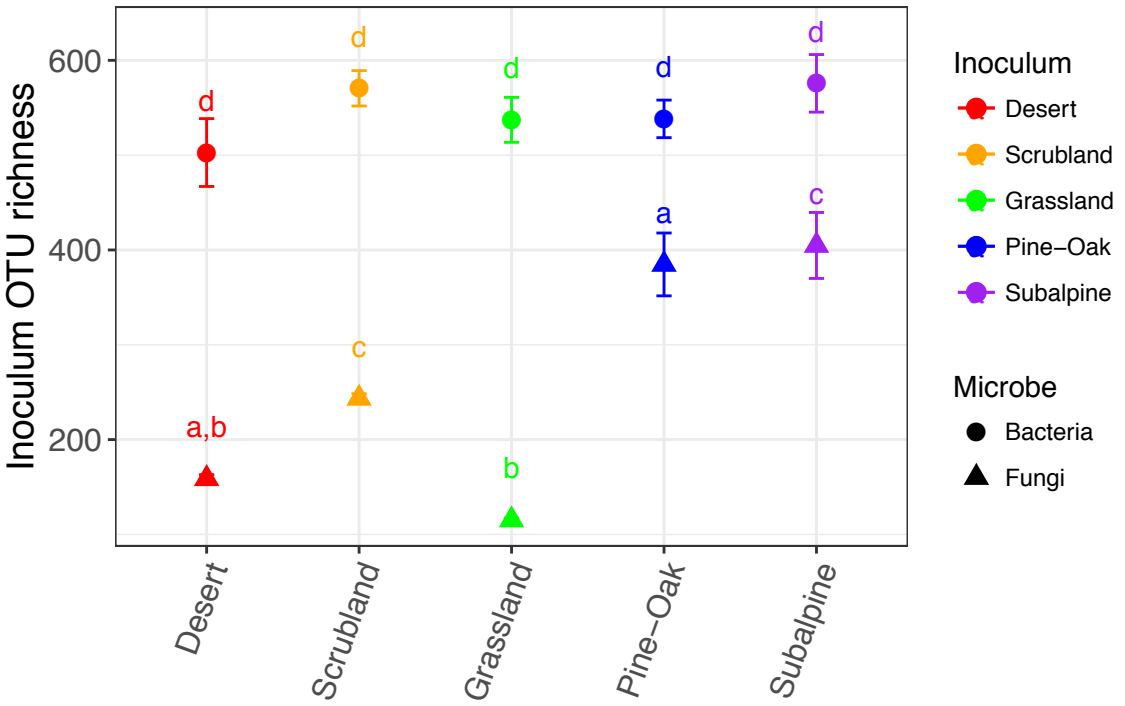


Figure S4. NMDS of Bray-Curtis microbial community composition for leaf litter used for Inoculum (September 2015), and survey of in-situ leaf litter adjacent to each plot at 0, 6, 12, and 18 months after inoculation for A) Bacteria and B) Fungi. Both plots have the same axis scale and legends apply to both plots. Colors represent microbial communities per site and shapes represent microbial communities per time point.

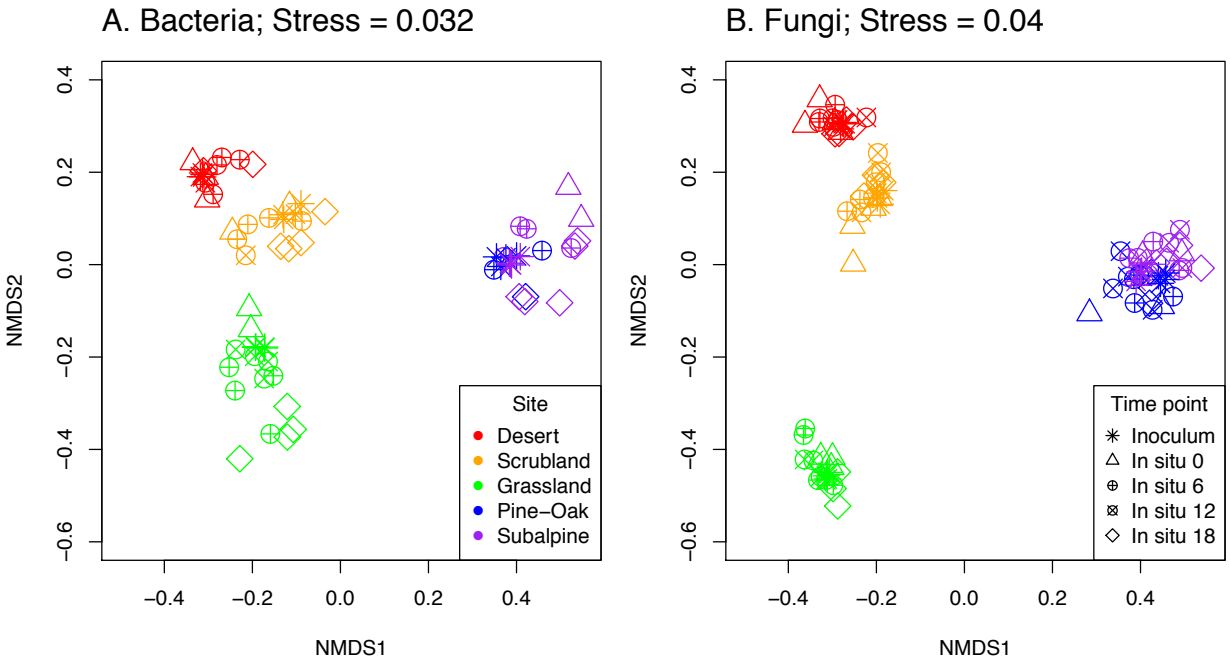


Figure S5. Species accumulation curves for bacteria and fungi from the inoculum leaf litter and transplanted litterbags collected from 6, 12, and 18 months after inoculation. Points are mean number of OTUs and lines represent 95% confidence intervals.

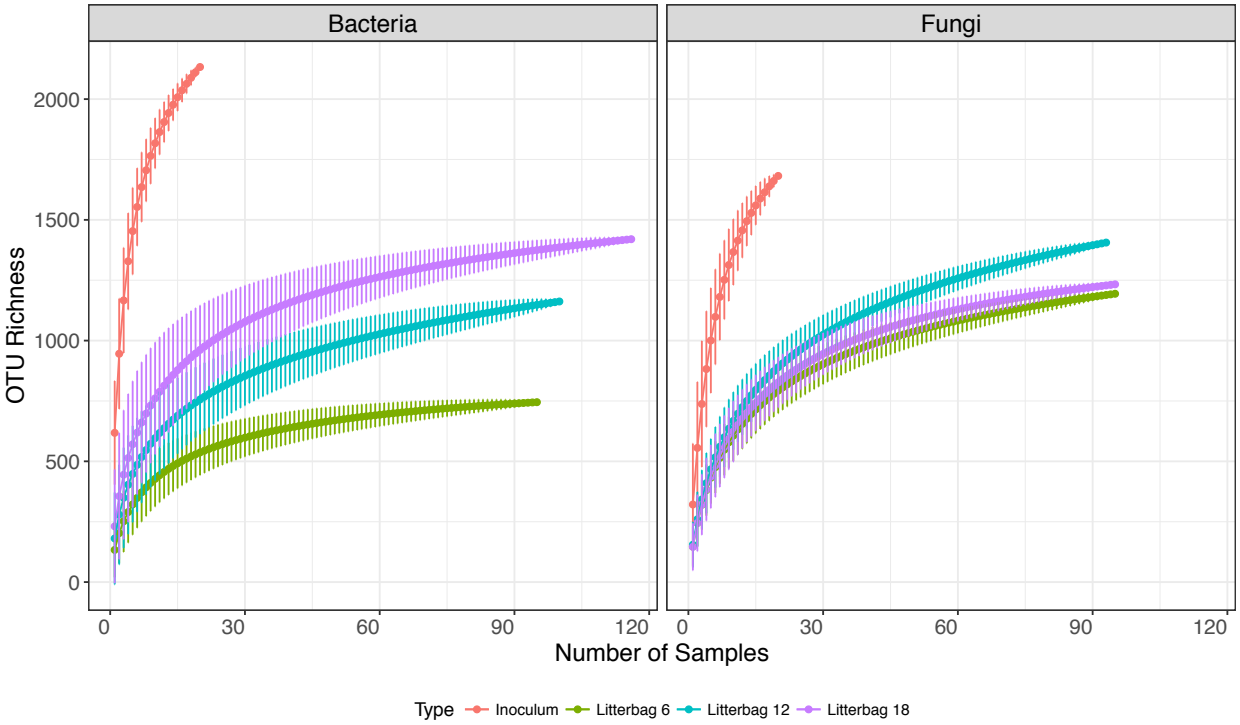
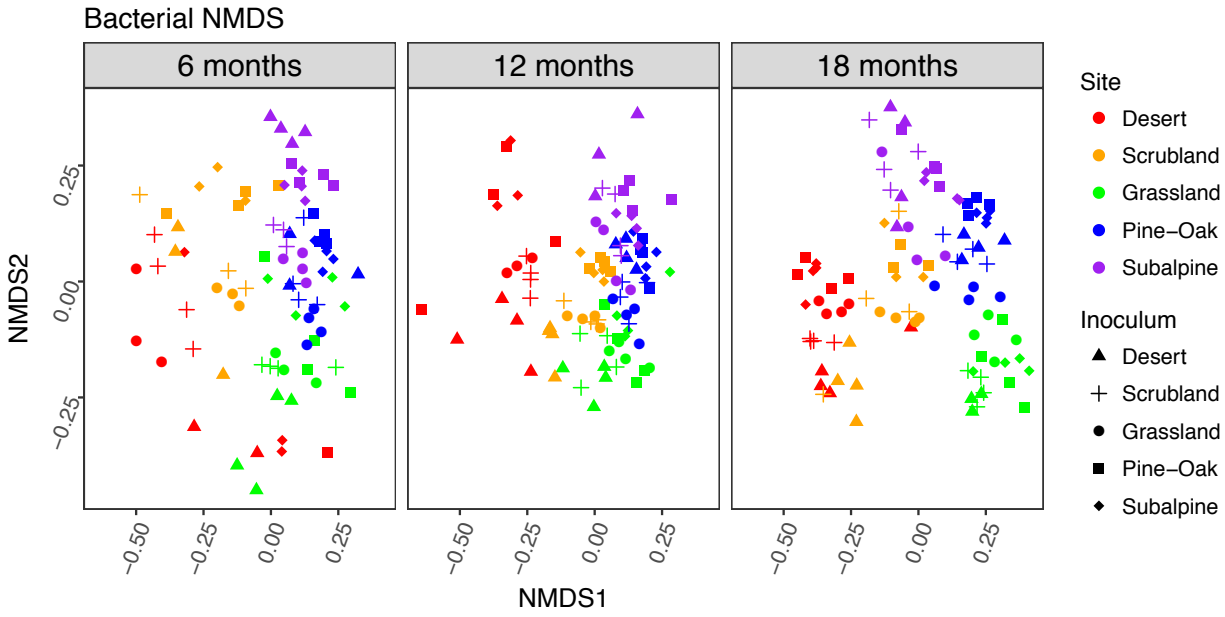


Figure S6. NMDS of 16S bacterial community composition for all three time points for transplant litter bags A) colored by site with shapes by inoculum and B) colored by inoculum with shapes by site. The bacterial communities are more easily differentiated when colored by site than inoculum, even though both effects are significant.

A)



B)

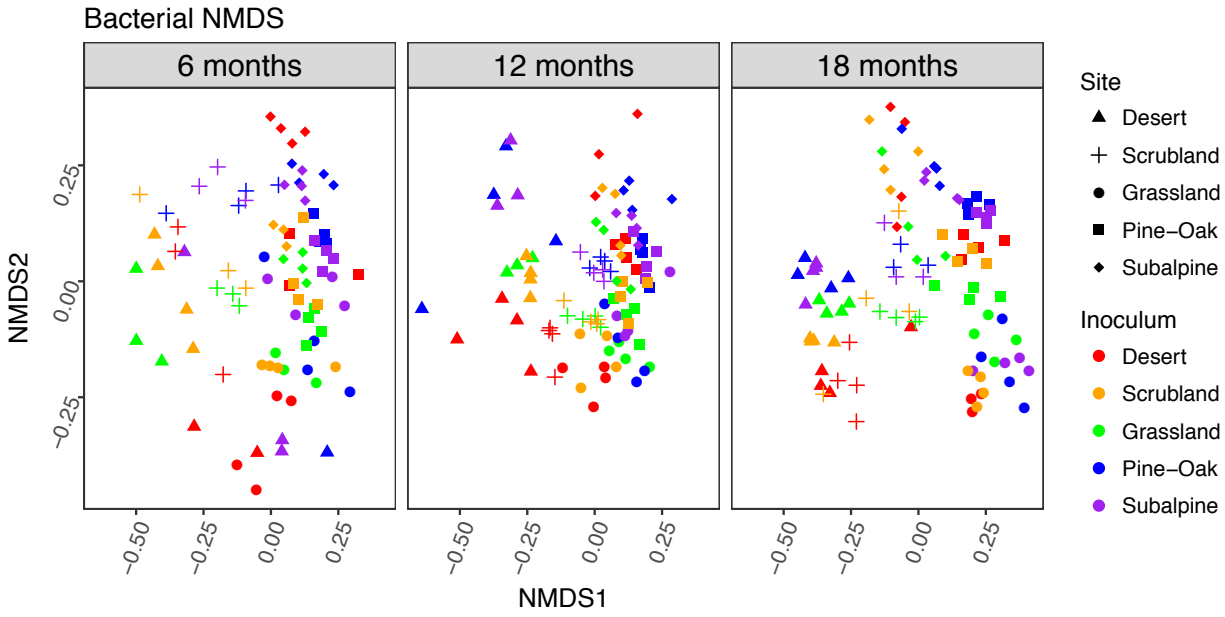
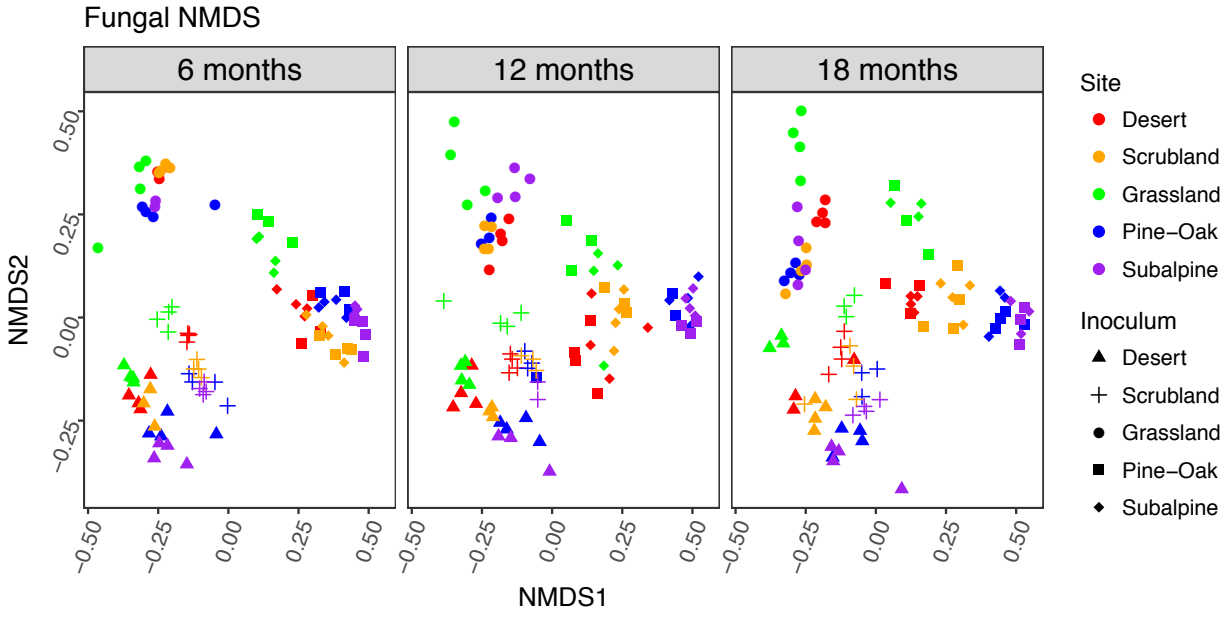


Figure S7. NMDS of ITS2 fungal community composition for all three time points for transplant litter bags A) colored by site with shapes by inoculum and B) colored by inoculum with shapes by site. Compared to the bacterial communities (Figure S7), the fungal communities are more easily differentiated when colored by inoculum than site, even though both effects are significant.

A)



B)

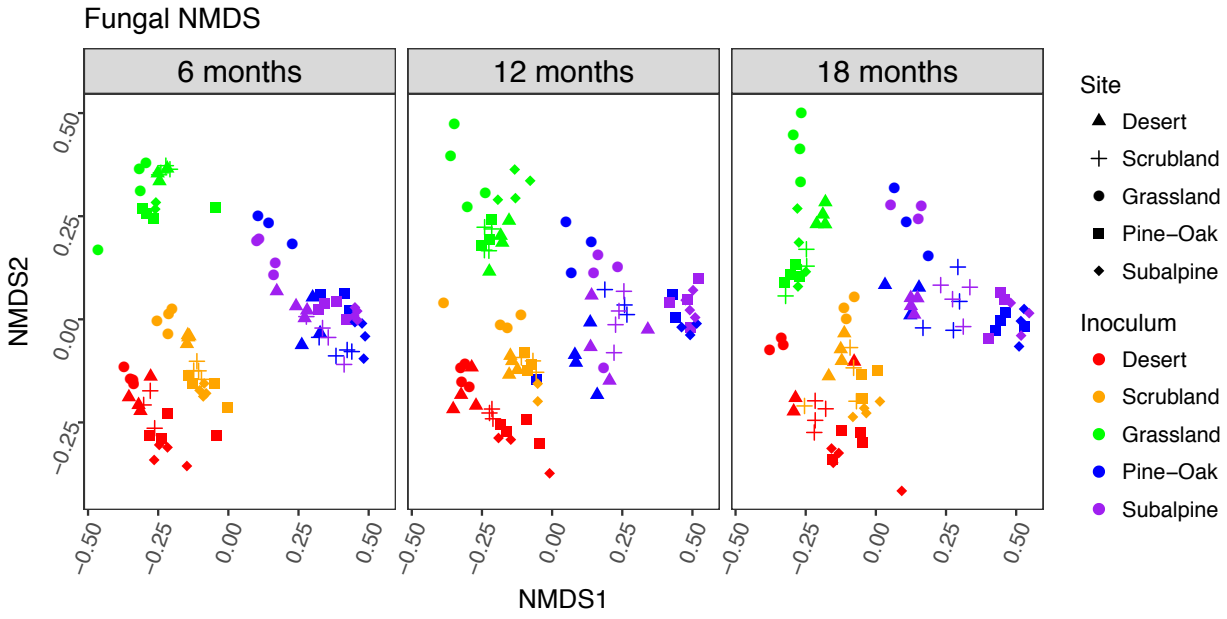
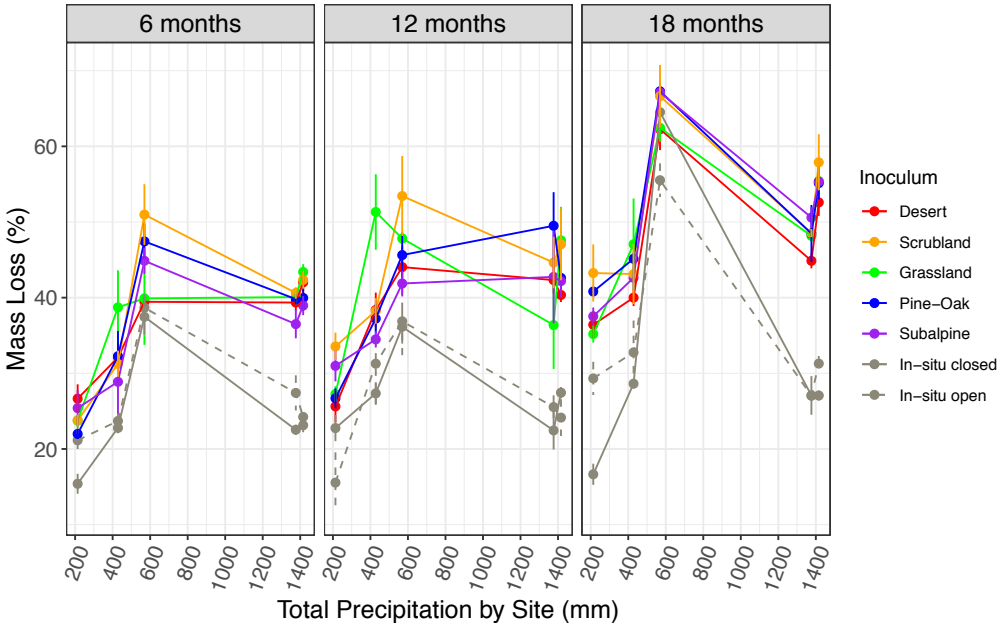


Figure S8. Variation in leaf litter decomposition (mean ± standard error percent mass loss) for the three time points for the full factorial transplant experiment (5 inoculum treatments by 5 sites) against A) total precipitation (mm) at each site and B) mean soil temperature°C at each site. In addition to transplant litterbags, we included open or closed in-situ litterbags for comparison.

A)



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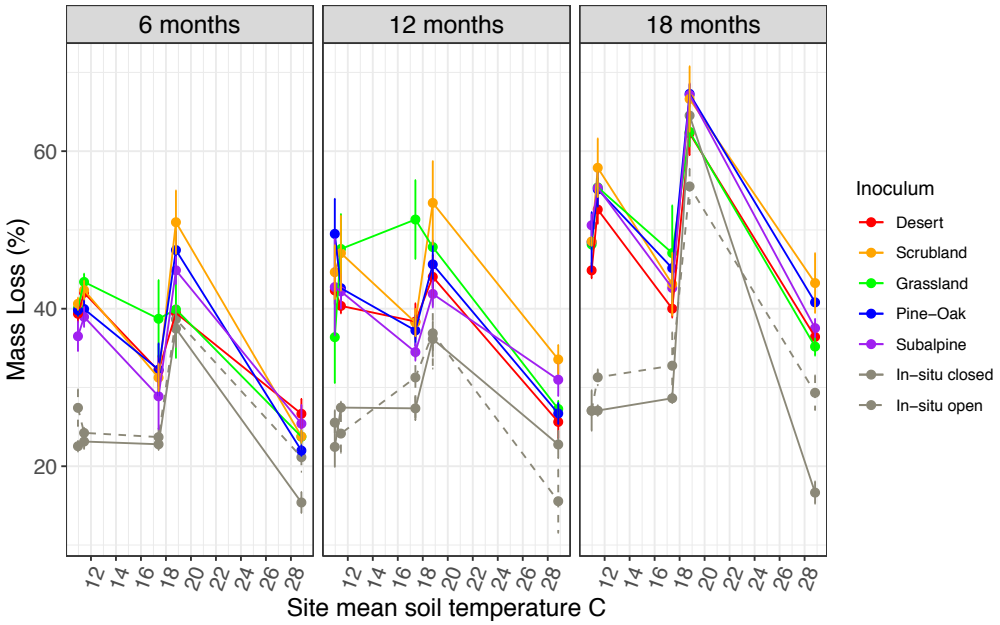


Figure S9. Mean ± standard error leaf litter organic carbon proportions by inoculum for the five sites at 12 months for five non-ash organic compounds: Cellulose, Hemicellulose, Lignin, Crude Protein, Structural Carbohydrates.

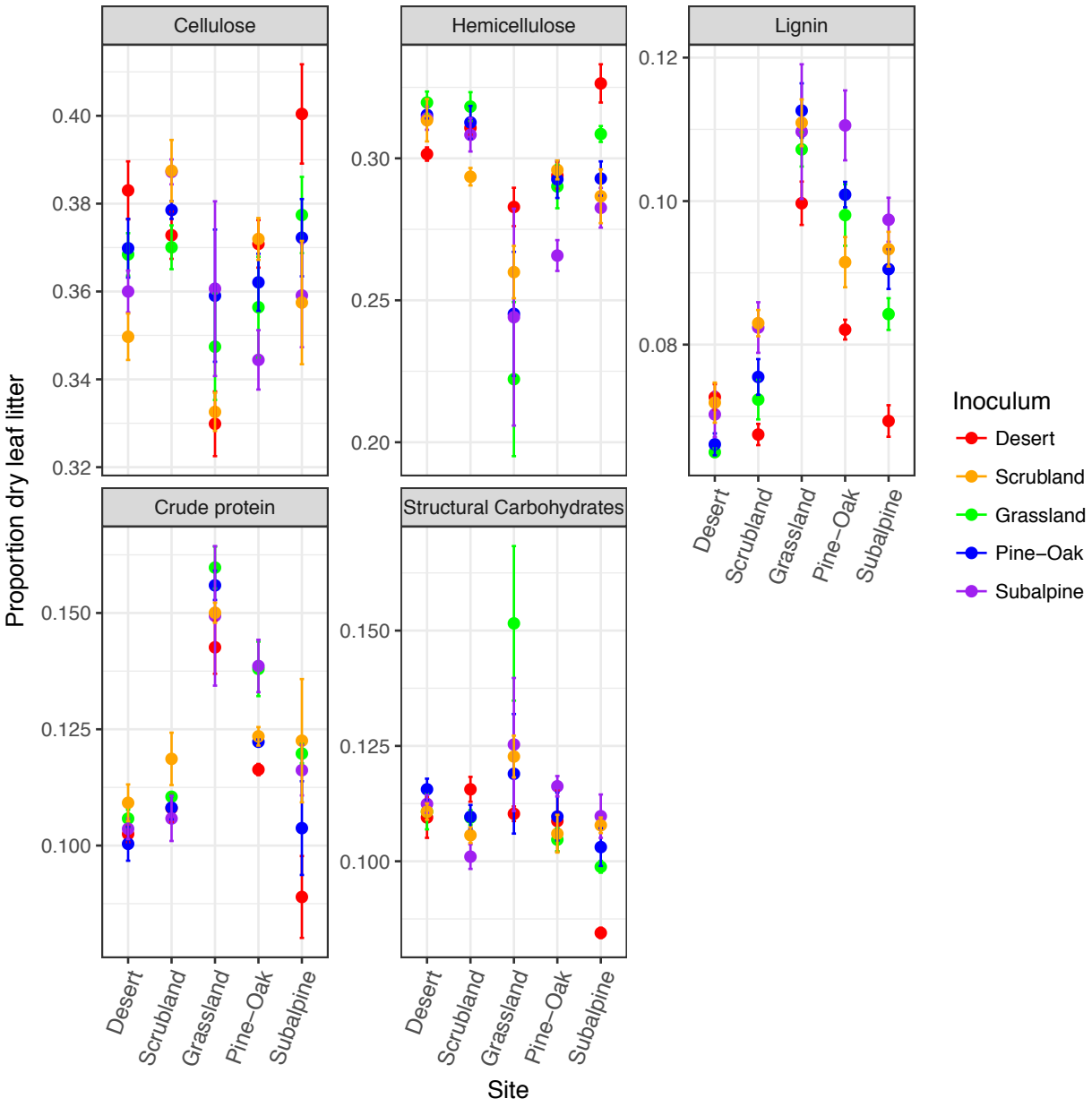


Figure S10. Decomposition as percent mass loss, averaged across inocula replicates at each site, at 6, 12, and 18 months, as a function of mean bacterial (cells/ g dry weight) and mean fungal (cm fungal hyphae/g dry leaf litter) abundance in the leaf litter used to inoculate litterbags from each site. Inoculum origin indicated by shapes and site indicated by colors. Statistics represent Pearson correlations of decomposition at each site against bacterial and fungal abundance in inoculum leaf litter from each site. For clarity, standard error bars not shown.

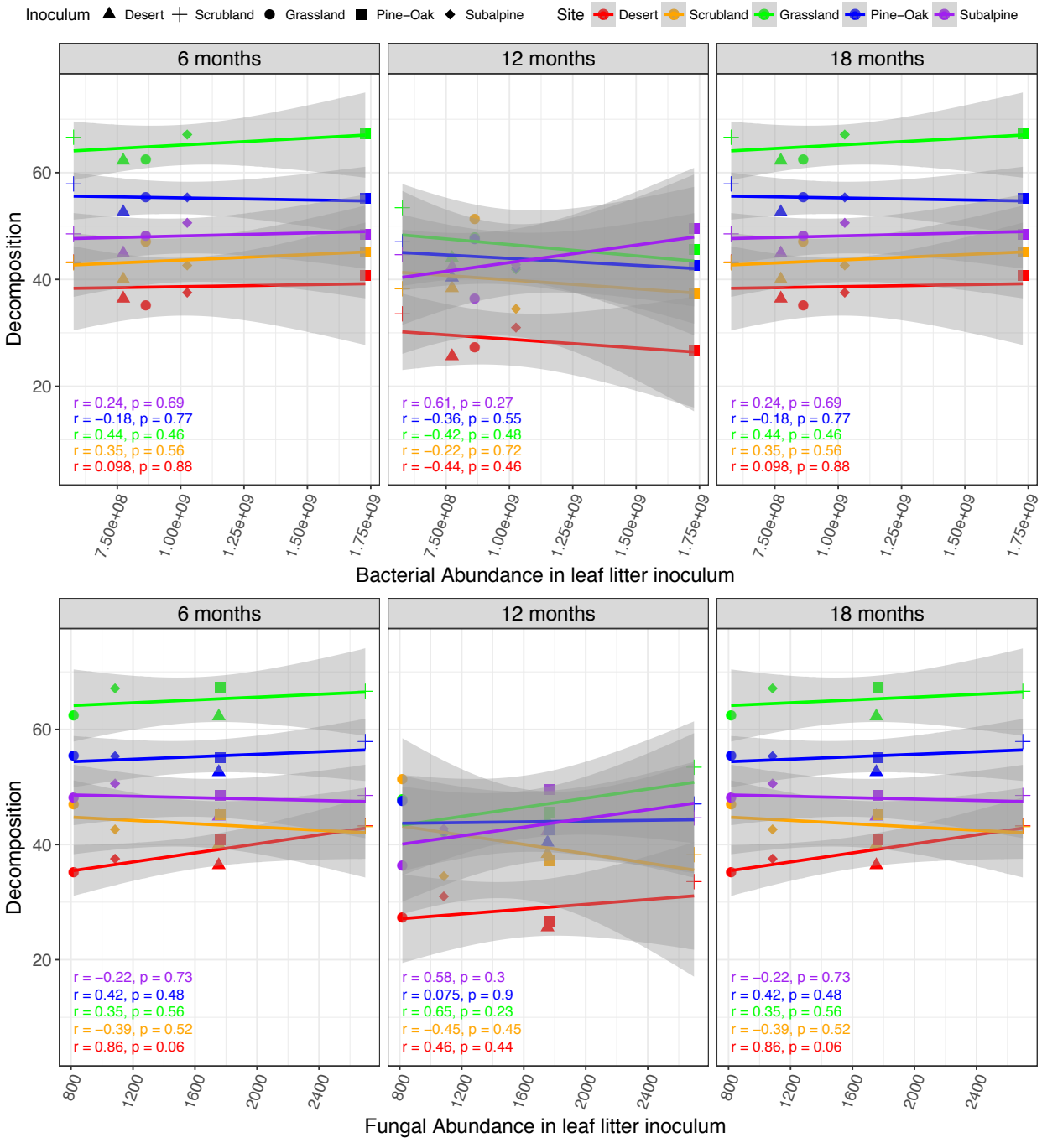
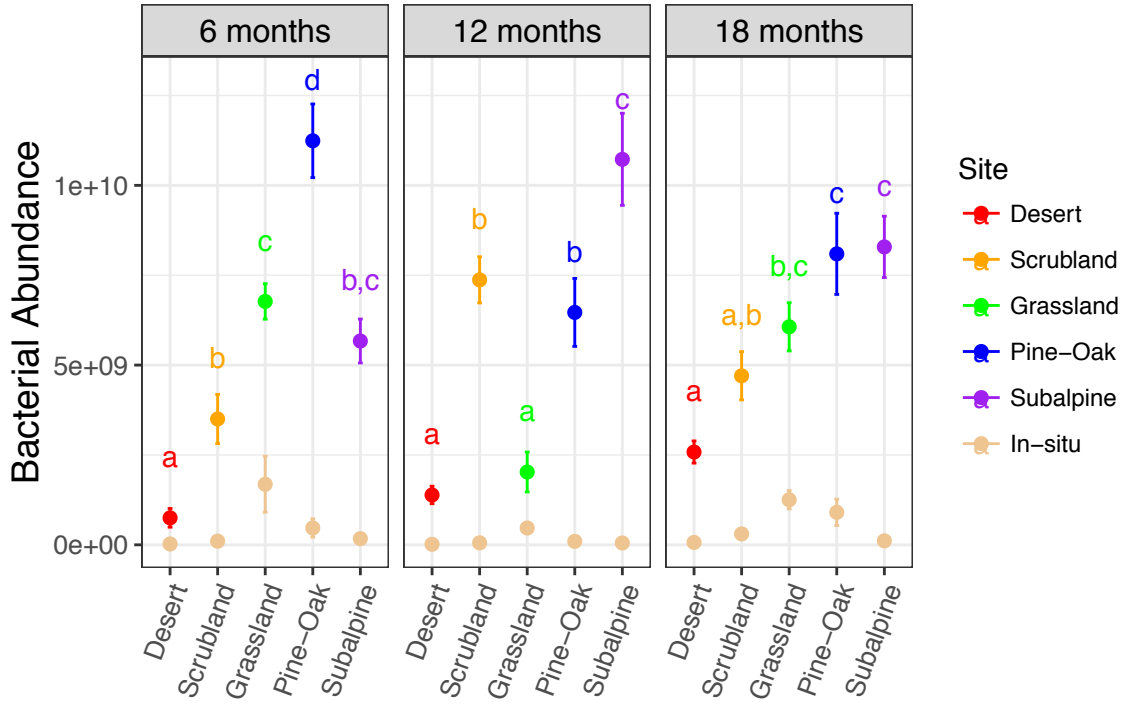
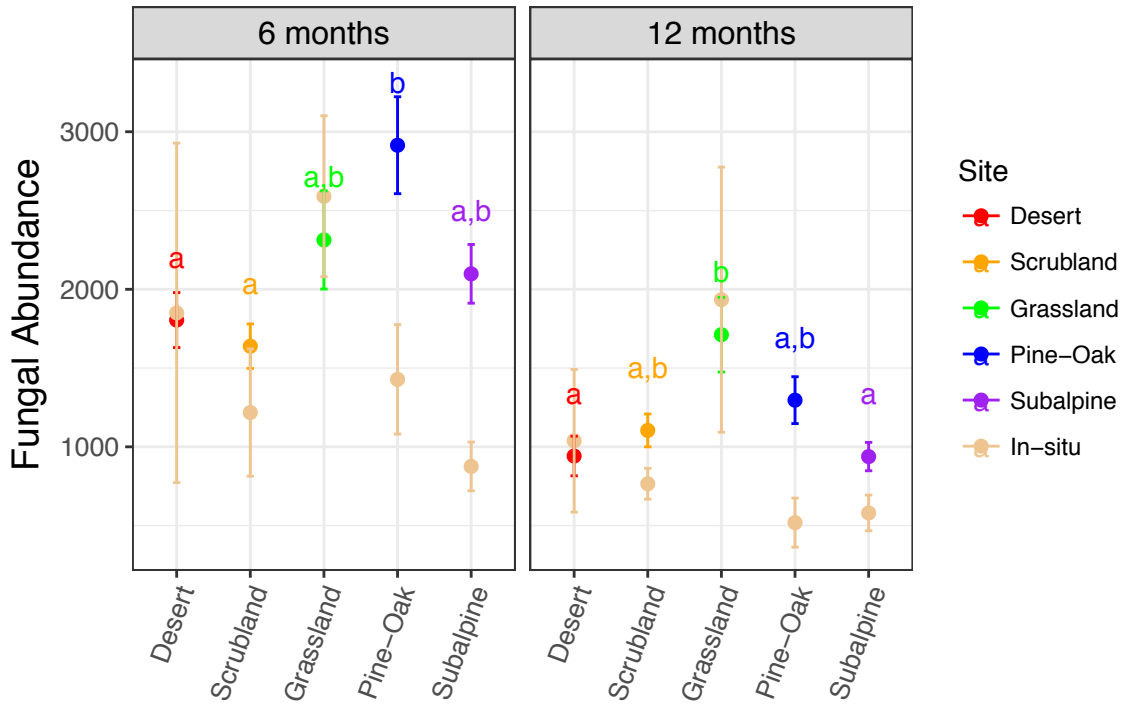


Figure S11. A) Differences in mean ± standard error bacterial abundance (cells/ g dry weight) and B) mean ± standard error fungal abundance (cm fungal hyphae/g dry leaf litter) in transplanted litterbags across the sites averaged across five inocula. Tukey HSD values for significant differences by site for each of the three time points. Mean values of in-situ closed litterbags for comparison.

A.



B.



Supplementary Tables

Table S1. Description of experimental sites along elevation gradient listed in increasing precipitation order (Subalpine precipitation is underestimated due to lack of high elevation precipitation towers). GPS coordinates, Elevation (m), total precipitation (both snow and rain) in mm across the duration of the experiment (from October 1, 2015 to April 30, 2017), and mean, maximum, and minimum soil temperature (°C) (from April 2016 to April 2017).

Site	Latitude	Longitude	Elevation	Total Precipitation	Mean soil temperature	Max soil temperature	Min soil temperature
Desert	33.648	-116.38	275	213.5	26.3	58.0	2.3
Scrubland	33.610	-116.45	1280	428.4	17.4	49.0	-6.5
Grassland	33.737	-117.70	470	569.4	18.8	55.8	-2.0
Pine-Oak	33.683	-116.77	1710	1415.8	11.4	43.0	-4.8
Subalpine	33.823	-116.75	2250	1376.5	11.0	38.3	-7.8

Table S2. Fungal:Bacterial ratios (F:B) or Bacterial:Fungal ratios (B:F) and their standard errors averaged across four samples per site for inoculum leaf litter and five samples per site for transplanted litterbags at 6 and 12 months. Both bacteria and fungi are estimated as g C/g dry leaf litter.

Site	Leaf litter type	F:B	SE	B:F	SE
Desert	Inoculum	0.351	0.128	2.849	1.04
Scrubland	Inoculum	0.724	0.255	1.382	0.49
Grassland	Inoculum	0.147	NA	6.812	NA
Pine-Oak	Inoculum	0.158	0.054	6.330	2.15
Subalpine	Inoculum	0.163	0.025	6.121	0.95
Desert	Litterbags 6 mo	0.372	0.134	2.686	0.96
Scrubland	Litterbags 6 mo	0.072	0.015	13.793	2.95
Grassland	Litterbags 6 mo	0.053	0.008	18.902	2.89
Pine-Oak	Litterbags 6 mo	0.040	0.006	24.889	3.47
Subalpine	Litterbags 6 mo	0.057	0.008	17.462	2.44
Desert	Litterbags 12 mo	0.105	0.023	9.512	2.09
Scrubland	Litterbags 12 mo	0.023	0.003	43.101	5.53
Grassland	Litterbags 12 mo	0.131	0.040	7.643	2.35
Pine-Oak	Litterbags 12 mo	0.031	0.006	32.210	5.98
Subalpine	Litterbags 12 mo	0.012	0.002	83.933	15.40

Table S3. A) PERMANOVA results for the effects of site, date, and site*date interaction on microbial community composition of both the inoculum and survey in-situ leaf litter for A) bacteria and B) fungi. In situ leaf litter was collected adjacent to each plot in September 2015 for initial use as the inoculum for the transplant experiment, and in situ litter collections continued at each sampling point on October 2015 (0 months), April 2016 (6 months), October 2016 (12 months), and April 2017 (18 months) to serve as a survey of the natural microbial community.

A) Bacteria

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	10.5221	2.63053	43.612	0.66	1.00E-04	***
SampleDate	4	0.7527	0.18818	3.12	0.05	4.00E-04	***
Site:SampleDate	13	1.7401	0.13385	2.219	0.11	1.00E-04	***
Residuals	47	2.8349	0.06032		0.18		
Total	68	15.8498			1.00		

B) Fungi

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	19.606	4.9014	39.82	0.58	1.00E-04	***
SampleDate	4	1.27	0.3176	2.58	0.04	3.00E-04	***
Site:SampleDate	16	4.355	0.2722	2.211	0.13	1.00E-04	***
Residuals	68	8.37	0.1231		0.25		
Total	92	33.601			1.00		

Table S4. PERMANOVA results for the effects of site, inoculum, and site*inoculum interaction on bacterial community composition in transplant litterbags at all three time points.

6 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	5.3289	1.33222	17.4236	0.34	1.00E-04	***
Inoculum	4	2.5453	0.63633	8.3224	0.16	1.00E-04	***
Site:Inoculum	16	2.9225	0.18266	2.3889	0.19	1.00E-04	***
Residuals	61	4.6641	0.07646		0.30		
Total	85	15.4608			1.00		

12 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	6.8983	1.72459	19.7618	0.36	1.00E-04	***
Inoculum	4	2.9448	0.7362	8.436	0.15	1.00E-04	***
Site:Inoculum	16	3.216	0.201	2.3032	0.17	1.00E-04	***
Residuals	72	6.2834	0.08727		0.32		
Total	96	19.3425			1.00		

18 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	8.8405	2.21013	28.6693	0.47	1.00E-04	***
Inoculum	4	1.9093	0.47732	6.1916	0.10	1.00E-04	***
Site:Inoculum	16	2.6101	0.16313	2.1161	0.14	1.00E-04	***
Residuals	72	5.5505	0.07709		0.29		
Total	96	18.9104			1.00		

Table S5. PERMANOVA results for the effects of site, inoculum, and site*inoculum interaction on fungal community composition in transplant litterbags at all three time points.

6 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	3.6505	0.9126	16.292	0.14	1.00E-04	***
Inoculum	4	15.1216	3.7804	67.486	0.57	1.00E-04	***
Site:Inoculum	16	3.8354	0.2397	4.279	0.15	1.00E-04	***
Residuals	68	3.8092	0.056		0.14		
Total	92	26.4167			1.00		

12 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	3.8139	0.95347	10.747	0.15	1.00E-04	***
Inoculum	4	11.3907	2.84767	32.096	0.46	1.00E-04	***
Site:Inoculum	16	4.123	0.25769	2.904	0.16	1.00E-04	***
Residuals	64	5.6782	0.08872		0.23		
Total	88	25.0058			1.00		

18 months

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	5.111	1.27775	15.693	0.20	1.00E-04	***
Inoculum	4	10.498	2.62451	32.234	0.41	1.00E-04	***
Site:Inoculum	16	4.5197	0.28248	3.469	0.18	1.00E-04	***
Residuals	65	5.2924	0.08142		0.21		
Total	89	25.4212			1.00		

Table S6. ANOVA results for the effects Site, Inoculum, and Site*Inoculum interaction on leaf litter decomposition as percent mass loss for the three time points. Effect sizes listed as partial eta-squared. We then estimated the total variance explained by multiplying the partial eta-squared by the adjusted R^2 of the model and included that in Figure 3.

6 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	4850	1212.4	52.465	<2e-16	***	0.74	0.51
Inoculum	4	86	21.4	0.925	0.454		0.05	0.03
Site:Inoculum	16	619	38.7	1.675	0.0714	.	0.27	0.19
Residuals	73	1687	23.1					

F-statistic: 10.01 on 24 and 73 DF, p-value: 9.481e-15; Multiple R^2: 0.767; Adjusted R^2: 0.6904

12 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	3929	982.3	24.013	9.34E-13	***	0.57	0.30
Inoculum	4	416	104.1	2.544	0.0465	*	0.12	0.06
Site:Inoculum	16	1273	79.6	1.945	0.029	*	0.30	0.16
Residuals	74	3027	40.9					

F-statistic: 5.723 on 24 and 74 DF, p-value: 3.186e-09; Multiple R^2: 0.6499; Adjusted R^2: 0.5363

18 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	8266	2066.6	86.072	<2e-16	***	0.82	0.64
Inoculum	4	259	64.6	2.692	0.0375	*	0.13	0.10
Site:Inoculum	16	240	15	0.624	0.8548		0.12	0.09
Residuals	73	1753	24					

F-statistic: 15.21 on 24 and 73 DF, p-value: < 2.2e-16; Multiple R^2: 0.8333; Adjusted R^2: 0.7786

Table S7. PERMANOVA results for the effects of site, inoculum, and site*inoculum interaction on organic carbon litter chemistry (Euclidian distance matrix of lignin, cellulose, hemicellulose, crude protein, and structural carbohydrates).

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
Site	4	0.12815	0.032037	36.362	0.53	0.0001	***
Inoculum	4	0.011907	0.002977	3.379	0.05	0.0034	**
Site:Inoculum	16	0.038556	0.00241	2.735	0.16	0.0003	***
Residuals	72	0.063438	0.000881		0.26		
Total	96	0.242051			1.00		

Table S8. Litter chemistry at 12 months site comparisons. SIMPER analysis showing percent contribution of the litter compounds to pairwise site comparisons from the PERMANOVA test (Table S7). P-value is significance of litter chemistry differences between the two sites.

	Hemicellulose	Cellulose	Protein	Lignin	Structural Carbohydrates	Corrected P-value
Desert-Grassland	0.33	0.00	0.23	0.19	0	**0.001**
Desert-Pine-Oak	0.29	0.00	0.20	0.25	0	**0.001**
Desert-Scrubland	0.29	0.25	0.00	0.15	0.16	**0.004**
Desert-Subalpine	0.24	0.22	0.19	0.21	0	**0.001**
Grassland-Pine-Oak	0.32	0.20	0.20	0.00	0	**0.001**
Grassland-Scrubland	0.30	0.22	0.20	0.00	0	**0.001**
Grassland-Subalpine	0.30	0.21	0.23	0.00	0	**0.001**
Pine-Oak-Scrubland	0.25	0.22	0.20	0.23	0	**0.001**
Pine-Oak-Subalpine	0.23	0.26	0.24	0.00	0	**0.008**
Scrubland-Subalpine	0.23	0.23	0.21	0.19	0	0.098

Table S9. ANOVA results for the effects of Site, Inoculum, and Site*Inoculum interactions on A) bacterial cell abundance (cells/g dry leaf litter) across the three time points and B) fungal abundance (cm fungal hyphae/g dry leaf litter**)** in transplant litterbags across two time points (fungal abundance was not collected at 18 months). Bacterial and fungal abundance are square root transformed to improve normality. Effect sizes listed as partial eta-squared. We then estimated the total variance explained by multiplying the partial eta-squared by the adjusted R^2 of the model and included that in Figure 3.

A) Bacteria

6 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	7.08E+10	1.77E+10	46.821	<2e-16	***	0.71	0.47
Inoculum	4	5.32E+09	1.33E+09	3.516	0.011	*	0.16	0.11
Site:Inoculum	16	4.92E+09	3.07E+08	0.813	0.667		0.15	0.10
Residuals	74	2.80E+10	3.78E+08					

F-statistic: 8.931 on 24 and 74 DF, p-value: 1.339e-13; Multiple R^2: 0.7434; Adjusted R^2: 0.6601

12 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	6.55E+10	1.64E+10	36.413	<2e-16	***	0.66	0.40
Inoculum	4	8.17E+08	2.04E+08	0.454	0.769		0.02	0.01
Site:Inoculum	16	1.19E+10	7.42E+08	1.651	0.0766	.	0.26	0.16
Residuals	74	3.33E+10	4.50E+08					

F-statistic: 7.245 on 24 and 74 DF, p-value: 1.954e-11; Multiple R^2: 0.7015; Adjusted R^2: 0.6046

18 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	2.06E+10	5.15E+09	11.184	3.77E-07	***	0.38	0.12
Inoculum	4	3.90E+09	9.76E+08	2.118	0.087	.	0.10	0.03
Site:Inoculum	16	7.99E+09	4.99E+08	1.084	0.385		0.19	0.06
Residuals	74	3.41E+10	4.61E+08					

F-statistic: 2.502 on 24 and 74 DF, p-value: 0.001409; Multiple R^2: 0.448; Adjusted R^2: 0.322

B) Fungi

6 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	1866	466.6	3.743	0.00801	**	0.17	0.01

Inoculum	4	174	43.6	0.35	0.84351	0.02	0.00
Site:Inoculum	16	1450	90.6	0.727	0.75791	0.14	0.01
Residuals	72	8975	124.7				

F-statistic: 1.167 on 24 and 72 DF, p-value: 0.3011; Multiple R^2: 0.28; Adjusted R^2: 0.04

12 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	1132	282.96	4.787	0.00	**	0.26	0.05
Inoculum	4	351	87.74	1.484	0.22		0.10	0.02
Site:Inoculum	16	1151	71.97	1.218	0.28		0.26	0.05
Residuals	56	3310	59.11					

F-statistic: 1.857 on 24 and 56 DF, p-value: 0.02933; Multiple R^2: 0.4432; Adjusted R^2: 0.2045

Table S10. ANOVA results testing decomposition differences in control litterbags. These litterbags contained snipped (coarser than ground) in-situ litter with their natural microbial communities in either open (2mm window screen) or closed (0.22 μm mesh) litterbags. ANOVA tested effect of site, litterbag material (open 2mm window screen vs closed 0.22 μm mesh), and a litterbag material by site interaction on decomposition rates for each of the three timepoints. Effect sizes listed as partial eta-squared. We then estimated the total variance explained by multiplying the partial eta-squared by the adjusted R^2 of the model.

6 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	1.75E+03	4.38E+02	48.068	1.26E-12	***	0.87	0.72
litterbag material	1	7.65E+01	7.65E+01	8.406	0.00693	**	0.22	0.18
litterbag material:site	4	4.40E+01	1.10E+01	1.209	0.32763		0.14	0.12
Residuals	30	2.73E+02	9.10E+00					

F-statistic: 22.84 on 9 and 30 DF, p-value: 4.406e-11; Multiple R^2: 0.8726; Adjusted R^2: 0.8344

12 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	1.22E+03	3.06E+02	8.986	7.56E-05	***	0.55	0.25
litterbag material	1	1.20E+00	1.23E+00	0.036	0.85		0.00	0.00
litterbag material:site	4	1.60E+02	4.01E+01	1.178	0.341		0.14	0.06
Residuals	29	9.87E+02	3.40E+01					

F-statistic: 4.521 on 9 and 29 DF, p-value: 0.0008877; Multiple R^2: 0.5839; Adjusted R^2: 0.4547

18 months

	Df	Sum Sq	Mean Sq	F value	P-value	Sig	partial eta-squared	Estimate of variance explained
Site	4	7.05E+03	1.76E+03	109.936	< 2e-16	***	0.94	0.86
litterbag material	1	5.80E+01	5.76E+01	3.594	0.067646	.	0.11	0.10
litterbag material:site	4	4.95E+02	1.24E+02	7.729	0.000209	***	0.51	0.47
Residuals	30	4.81E+02	1.60E+01					

F-statistic: 52.7 on 9 and 30 DF, p-value: 6.174e-16; Multiple R^2: 0.9405;Adjusted R^2: 0.9227

Supplemental Materials and Methods

Temperature and precipitation: Between April 2016-2017, two iButton temperature sensors (Mouser Electronics, Mansfield, TX, USA) were deployed at each site to record surface temperature at 4 hour intervals. Precipitation data relevant to each site was collected from nearby weather stations (Desert and Scrubland: Campground and Pinyon Crest weather stations, respectively, from this website: http://deepcanyon.ucnrs.org/weather-data/; Grassland: https://www.ess.uci.edu/~california/; Pine-Oak: https://wrcc.dri.edu/cgi-bin/rawMAIN.pl?caucja). Since there was no station directly next to the Subalpine site, the precipitation at this site is likely underestimated, and was averaged from three NOAA weather stations with heated gauges to account for snow (USC00045091; US1CARV0002; USC00044211) from: https://www.ncdc.noaa.gov/cdo-web/datasets#GHCND.

Decomposition and litter chemistry: Decomposition was measured as percent mass loss with mass of litter remaining in each bag converted to dry weight (by multiplying by the ratio of litter dry weight/litter wet weight) and divided by the initial mass (either 5g for transplant or 2g for open and closed in-situ bags). Oven-dried litter from transplant litterbags collected at 12 months was sent to Cumberland Valley Analytical Services (www.foragelab.com; Waynesboro, PA, USA) for near-IR spectroscopy to measure non-ash organic compounds including cellulose (acid detergent fiber – lignin), hemicellulose (neutral detergent fiber – acid detergent fiber), lignin, crude protein, and structural carbohydrates (non-fiber carbohydrates – non structural carbohydrates).

Sample processing, DNA extraction, and genetic analysis: All collected samples (litterbags and in-situ leaf litter) were stored at ambient temperature in an outside shade house on the UC Irvine campus and processed in the laboratory within two days of sampling. Bags were destructively sampled for DNA, mass loss, litter chemistry, and bacterial and fungal abundance. DNA was extracted (from 0.05g litter sample stored at -80 ºC) using the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manual with the modification of adding three freeze/thaw cycles (30s in liquid Nitrogen followed by 3-5 min in 60°C water bath) prior to bead beating step to improve cell lysis. DNA extracts were then frozen (-20 ºC) until processing for genetic analysis as previously described (1). Briefly, bacterial community composition was characterized using the V4 region of the 16S ribosomal RNA (rRNA) gene using the 515F-926R primer pair and fungal composition was characterized using the ITS2 region of the Internal Transcribed Spacer (ITS) using the ITS9f-ITS4 primer combination. Purified libraries were sequenced in six separate Illumina MiSeq PE runs (2 x 250 bp) at the DNA Technologies Core, UC Davis Genome Center, Davis, CA, USA. Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under accession number SRP150375.

Bioinformatics processing: All bioinformatics processing was conducted in UPARSE (2) version 10. Analyses were conducted by defining both 97% OTUs and exact sequence variants (AKA zOTUs), but since the results were nearly identical (1), we only present the analyses using 97% OTUs. Forward and reverse reads were quality checked with FastQC. In UPARSE primers were stripped and reads truncated, then forward and reverse reads were merged, then quality filtered and PhiX removed. OTUs were clustered at 97% sequence similarity removing singletons, and taxonomy was assigned in QIIME 1.9 (3) using the Greengenes database (4) and the UNITE

database (5). For ITS2, only reads mapping to kingdom Fungi were retained, and for 16S, all reads mapping to Chloroplasts, mitochondria, or unclassified were removed.

Microbial Abundance: Bacterial cell densities were measured by flow cytometry and fungal hyphal lengths were measured on a microscope slide, then converted to bacterial and fungal biomass (mg C/g dry litter) for calculation of fungal:bacterial ratios as previously described (6). The formula employed to calculate bacterial biomass C was 2.2×10^{-13} g/μm^3 x 0.90432 μm^3 ($4/3*pi*r^3$ where r = 0.6 μm) x flow cytometry cell count/g litter (7). The formula employed to calculate fungal biomass C was (2.12264 x 10^-7 cm^3) ($pi*r^2$ where r = 0.00026 cm) x 1.1 g/cm^3 x 0.33 x 0.4 x fungal length (cm/g litter)(8).

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