Plant litter chemistry and associated changes in microbial decomposition under drought

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# Introduction

Changes in precipitation regimes are projected to occur with climate change, with increasing drought activity having already been observed in semi-arid ecosystems across the globe such as in California, western South America, and the Mediterranean [(1)](https://www.zotero.org/google-docs/?C3laeI). This is driven by increasing evapotranspiration due to increasing temperatures [(1)](https://www.zotero.org/google-docs/?XfXaNc), causing an increase in the co-occurrence of high temperatures and low precipitation [(2)](https://www.zotero.org/google-docs/?qmFBxH). Furthermore, the number of extreme precipitation events, including events with low precipitation, are also projected to increase [(3)](https://www.zotero.org/google-docs/?jZZRwQ). In addition, changes in precipitation are not uniform at a regional scale [(1)](https://www.zotero.org/google-docs/?64e1VP), with arid and semi-arid ecosystems in southern California experiencing decreasing annual precipitation [(4)](https://www.zotero.org/google-docs/?D03ocj). These changes can have major effects on key ecosystem processes such as decomposition and the organisms driving it, mainly fungi and bacteria. The effects on decomposer microorganisms can be both direct impacts – through changes in abundance of individual taxa, community composition, and traits – and indirect impacts through changes in the litter substrate [(5–7)](https://www.zotero.org/google-docs/?Qi5EdQ).

Microbial community response to drought may not always be consistent and may vary across ecosystems [(8)](https://www.zotero.org/google-docs/?40pUaD) or climate history [(9, 10)](https://www.zotero.org/google-docs/?9uDRpz). Drought in semi-arid ecosystems decreases litter decomposition rates in some systems [(11–13)](https://www.zotero.org/google-docs/?kNNZIN) but not others [(14)](https://www.zotero.org/google-docs/?YV8DzT). Decreases in decomposition have been attributed to decreases in microbial biomass [(13)](https://www.zotero.org/google-docs/?Nd5Lxl) and the efficiency of extracellular enzymes [(15)](https://www.zotero.org/google-docs/?7qxxQh). While drought shifted the overall microbial community composition of an oak forest towards fungal dominance, drought increased both bacterial and fungal abundance in a mixed pine-oak forest such that fungal:bacterial ratios remain unchanged [(14)](https://www.zotero.org/google-docs/?rri22l). Drought can also shift investment in microbial community traits. Drought induced a shift in resources from growth to stress tolerance in an exotic grassland, although this shift was not observed in the shrub ecosystem at the same site [(8)](https://www.zotero.org/google-docs/?4yF2y6).

Litter chemistry had previously been shown to be a major control on decomposition [(16–18)](https://www.zotero.org/google-docs/?KZcGnH), including decomposition in grasslands [(19)](https://www.zotero.org/google-docs/?QJuU2h). Drought had been shown to alter litter chemistry [(13, 20–22)](https://www.zotero.org/google-docs/?T3nMxQ) through changes in plant physiology [(21, 22)](https://www.zotero.org/google-docs/?6B1uq1) and changes in plant community composition [(23, 24)](https://www.zotero.org/google-docs/?N5iJzh). These changes in litter chemistry had, in turn, affected the microbial community, altering microbial community composition [(25)](https://www.zotero.org/google-docs/?XDu8sm) by decreasing bacterial abundance [(13)](https://www.zotero.org/google-docs/?UTYmOh), and decreasing investment in extracellular enzyme activity by decreasing proportions of certain litter substrates [(15)](https://www.zotero.org/google-docs/?kfr885). Therefore, drought can exert complex direct and indirect effects on litter decomposition, and how these effects interact still remain understudied [(7)](https://www.zotero.org/google-docs/?5xILCX).

Here we investigate the direct and indirect effects of a decade-long drought, their interactions, and impacts on the decomposition traits of microbial communities in a field litter bag experiment in a semi-arid climate in southern California. We used four distinct litter types: grass and shrub each under ambient and reduced precipitation for 10 years. We hypothesised that a microbial community’s investment in resource acquisition traits is influenced by the vegetation community and the corresponding litter chemistry. We further hypothesised that shrub litter communities will possess higher abundance of genes for degradation of complex substrates reflecting the higher recalcitrance of shrub compared to grass litter; and similar higher abundance of genes for degradation of complex substrates in litter from drought treatments relative to the control due to drought-induced changes in plant community composition and physiology. We tested these hypotheses using shotgun metagenomics, extracellular enzyme assays, and Fourier Transform Infrared Spectroscopy (FTIR). Based on our hypotheses, drought can indirectly affect resource acquisition traits by altering litter chemistry. Drought can decrease or increase certain resource acquisition trait values if proportions of their putative substrates decrease or increase under drought [(15)](https://www.zotero.org/google-docs/?Dv9rLW).

# Methods

## Field Experiment Description

The study took place at the Loma Ridge Global Change Experiment (33°44’N, 117°42’W, 365 m elevation) near Irvine, California, USA. The climate is Mediterranean with a cool rainy season from November to April and a warm dry season from May to October of each year. The mean annual temperature is 17°C and the mean annual precipitation is 325 mm [(13)](https://www.zotero.org/google-docs/?f11Jn7). The site has plots located in two vegetation communities – coastal sage scrub (shrub) and an exotic grassland – and each plot is subjected to one of three precipitation treatments – drought, control, and added precipitation – and one of two nitrogen treatments – control and added nitrogen [(24)](https://www.zotero.org/google-docs/?aOcyAR). This study used plots of either vegetation types subjected to only the control nitrogen treatment and only the control and drought precipitation treatments. This leads to four treatment combinations (2 vegetation communities x 2 precipitation treatments). Each treatment combination has four replicate plots, leading to a total of 16 plots being used for this study (4 treatment combinations x 4 replicate plots). Drought plots are covered with clear polyethylene tarps during a subset of winter storms, reducing annual precipitation by ~40% [(13, 24)](https://www.zotero.org/google-docs/?ulkDn8). Grassland plots (6.7 m x 9.3 m) are dominated by exotic annual grasses of the genera *Avena*, *Bromus*, *Festuca*, and *Lolium*, forbs of the genera *Erodium* and *Lupinus*, and the native perennial grass *Stipa pulchra* [(8, 26)](https://www.zotero.org/google-docs/?prSxtz). Shrub plots (18.3 m x 12.2 m) are dominated by *Salvia mellifera*, *Artemisia californica*, and *Malosma laurina* [(8)](https://www.zotero.org/google-docs/?RO5pUX).

## Experimental Design

We performed a litter decomposition experiment at the field site with continued precipitation treatment [(27)](https://www.zotero.org/google-docs/?uugt44). Leaf litter was sampled on August 30, 2017 from all four replicate plots within each treatment combination. We only sampled litter from species that are representative of each community (i.e. only litter from shrub species were sampled from shrub plots of both precipitation treatments and vice versa for grassland litter). Litter from all plots within each treatment combination was combined and mixed by hand while keeping treatment combinations separate from each other. We then made litter bags from 1 mm window mesh and filled each bag with 6 g litter from one treatment combination. Litter bags were deployed on September 12, 2017 and were collected from each plot over four time points. In total, this study deployed 64 litter bags (16 plots x 4 time points), with 16 litter bags (one litter bag from each plot) being collected at each time point for laboratory analysis. We collected litter bags on November 30, 2017 (T1), April 11, 2018 (T2), November 2018 (T3), and February 2019 (T4). An aliquot of the sampled litter was ground in a coffee mixer (a quick whirl for 5 s) to create a coarse powder which was used for subsequent analyses.

## Litter Chemistry

​​ The chemical composition of the plant litter organic matter was measured using Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy. The ground litter samples were gently pressed down on a clean surface of the germanium crystal in an ATR configuration (Smart Orbit; Thermo Fisher Scientific). Infrared light beamed from the interferometer (Nexus 870; Nicolet) was focused onto the interface between the sample and the top surface of the crystal through the lower facet. The sample spectrum was recorded with a spectral resolution of 4 cm-1 over the infrared range (4,000-600 cm-1). Data was sum-normalized before analysis. Compositional differences along the entire spectrum were studied using principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) in R using the vegan package [(28)](https://www.zotero.org/google-docs/?2zf0EI) with visualisations created using ggplot2 [(29)](https://www.zotero.org/google-docs/?k6ESZW). Spectral ranges were assigned to different functional groups for different compound classes quantified as peak area (Table 1).

## Metagenomics

DNA was extracted from a 50-mg aliquot of ground litter from all 64 samples using ZymoBiomics DNA isolations kits (Zymo Research, Irvine, CA, USA) following manufacturer instructions. Sample homogenization was performed by bead beating for 5 min at the maximum speed of 6.0 m/s (FastPrep-24 High Speed Homogenizer, MP Biomedicals, Irvine, CA, USA). Gel electrophoresis, a Qubit fluorometer (LifeTechnologies, Carlsbad, CA, USA), and a Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA) were used to assess the purity and concentration of extracted DNA. Library preparation and metagenomic sequencing were carried out at the University of California Davis Genome Center. We used NovaSeq (Illumina, San Diego, CA, USA) with PE150 sequencing and the default insert size of 250-400 bp. Taxonomic classification up to genus level was performed using a reads-based assessment with RefSeq database (maximum e-value cut-off of 10-5, minimum identity cut-off of 60% and minimum length of sequence alignment of 15 nucleotides) on Metagenomics Rapid Annotation using Subsystems Technology (MG-RAST) server version 4.0.3 [(30)](https://www.zotero.org/google-docs/?yVN84m).

We used Metagenome Orchestra (MAGO) (version V2.2b; 2020-03-08) [(31)](https://www.zotero.org/google-docs/?GVP6TH) to produce metaSPAdes (version 3.13.0) [(32, 33)](https://www.zotero.org/google-docs/?Ze3olJ) assemblies for individual samples. Within MAGO, the quality control of the paired-end reads was carried out with fastp (version v0.20.0) [(34)](https://www.zotero.org/google-docs/?KSPRQv) to keep a Q30 read quality while carrying out adapter trimming. seqtk (version 1.3-r106) [(35)](https://www.zotero.org/google-docs/?tEuUFs) was used to remove contigs shorter than 1,000 bp from the metaSPAdes assemblies. Contig-level data was used to assess community-aggregated functional differences across treatments. Prodigal (version 2.6.3) [(36)](https://www.zotero.org/google-docs/?pOaNSY) was used to carry out gene-calling of metagenomic contigs from the individual sample assemblies which was then queried against the carbohydrate active enzymes (CAZy) database using dbCAN2 (version 2.0.11) (Huang et al., 2018). PROKKA (version 1.13.7) [(37)](https://www.zotero.org/google-docs/?TVvauO) was run in metagenome mode over the assemblies to generate respective annotations. To produce a community gene abundance table across the treatments, each dataset of quality-controlled paired-end reads was aligned against its respective assembly using BWA (version 0.7.17-r1188) (Li, 2017). SAMtools (version 1.9) [(38)](https://www.zotero.org/google-docs/?W9AoBY) was used to convert the alignments to binary format as well as to sort them. HTSeq (version 0.11.2) [(39)](https://www.zotero.org/google-docs/?HYgRaJ) was employed to count the number of reads aligned to the annotated features by PROKKA across each sample. CAZy gene abundances were normalised by total protein-coding genes predicted using Prodigal. Normalisation accounts for variation in sequencing depth and assembly bias to provide absolute count data. CAZyme genes for specific substrates (cellulose, hemicellulose, polysaccharides, lignin, starch, oligosaccharides, peptidoglycan and chitin) were summed to obtain the total gene abundances linked to degradation of the substrates [(40)](https://www.zotero.org/google-docs/?1L03qA). Visualisations were made using ggplot2 [(29)](https://www.zotero.org/google-docs/?hUlcO0).

## Extracellular Enzyme Assays

We performed extracellular enzyme assays on hydrolytic enzymes (Table 2) using previously reported fluorometric protocols [(41, 42)](https://www.zotero.org/google-docs/?alA2Xp). Litter from each collected litter bag was homogenized in 25 mM maleate buffer with pH 6. The resulting homogenate was plated in 96-well opaque microplates with standards, controls, and serial dilutions of their respective substrates. Microplates were incubated at room temperature for four hours, and fluorescence was then measured in a plate reader. Enzyme activity was then calculated from fluorescence data [(42)](https://www.zotero.org/google-docs/?mBtaeL) and divided by the dry weight of the litter that was homogenized. The resulting enzyme activity was then plotted against substrate concentration in scatterplots using *matplotlib* (version 3.3.2) in Python. The scatterplots were manually inspected for the artifact of substrate inhibition, in which enzyme activity decreases at high substrate concentrations instead of approaching Vmax due to the substrate now acting as an inhibitor [(43, 44)](https://www.zotero.org/google-docs/?OmejkL). Leaving these data points in can underestimate Vmax [(44)](https://www.zotero.org/google-docs/?7N82Bl). These data points were removed, and the resulting enzyme activity was fitted to the Michaelis-Menten equation using the *curve\_fit()* function in *scipy* (version 1.5.2) to produce Vmax in units of µM/g dry litter/hr. Vmax values from this curve-fitting were then subjected to further statistical analysis. Vmax had traditionally been used as a measure of the size of an enzyme pool in an environmental substrate [(45)](https://www.zotero.org/google-docs/?jFzNfq).

## Statistical Analysis

Statistical analysis was conducted in Python (version 3.8.5). Linear mixed effect models – conducted using the package *statsmodels* (version 0.12.0) – were performed on percent FTIR spectral areas of specific bands, CAZyme gene abundance, and Vmax values with vegetation, precipitation, and their interaction as fixed effects and the collection time point – in days since deployment – and plot as random effects. Residuals were checked for normality after each model fit using the Shapiro-Wilk test from *scipy*, and the dependent variable was transformed by log10, reciprocal, or square root transformations and refitted until the model with the most normal residuals – having the largest Shapiro-Wilk p-value – was produced. The square root transformation was dropped as it often did not produce the model with the most normal residuals. Tukey’s pairwise comparisons were performed as a post-hoc test on levels of main effects and combinations of their interactions that were statistically significant in linear mixed effects model (p < 0.05). Cohen’s D was calculated as a measure of effect size for statistically significant main effects (Table S1).

# Results

## Litter chemistry

Litter chemistry was different between the vegetation types (Figure 1, Table S1). Because hemicellulose and pectins are carbohydrates that contain ester groups [(46)](https://www.zotero.org/google-docs/?pbGyQ8), the carbohydrate ester spectral areas (Table 1, Figure 1h, 1i) are likely associated with hemicellulose and pectins. While shrub litter had higher spectral area of carbohydrate C-O stretches (Figure 1e), grassland litter had higher spectral areas of other ranges associated with carbohydrates (Figure 1d, 1h, 1i), indicating that grassland litter had higher overall carbohydrate content than shrub litter. Shrub has higher spectral area associated with C-H methyl and methylene deformation (Figure 1b) and lipid C=O stretching (Figure 1c). The spectral range 1450-1475 cm-1 had been associated with lignin [(47)](https://www.zotero.org/google-docs/?3sO9Wz). The spectral range 1700-1750 cm-1 had been associated with C=O stretching in ketones and carboxylic acids [(48)](https://www.zotero.org/google-docs/?Z5usTj), indicating that this range might be associated with lipids. Shrub litter likely had higher proportions of more recalcitrant compounds, namely lignin and lipids (Figure 1b, 1c), than grassland litter, indicating that shrub litter is more recalcitrant than grassland litter. This is consistent with our hypothesized difference in recalcitrance between these two litter types. Differences in lignin and carbohydrates between both vegetation types are also consistent with other studies that compared one or two litter species from each of the same vegetation types [(49, 50)](https://www.zotero.org/google-docs/?cqgVEM).

While drought significantly affected litter chemistry of both litter types as shown by an overall precipitation effect over some spectral bands (Table S1), drought had much stronger effects on grassland litter than shrub litter (Figure 1). Significant interactions between vegetation and precipitation were present for carbohydrate glycosidic bonds and one carbohydrate ester spectral range (Figure 1d, 1i, Table S1), with drought only lowering spectral areas in these two ranges in grassland litter (Figure 1d, 1i). The effects of drought on carbohydrates in grassland litter were consistent with decreases in cellulose and hemicellulose that had previously been found in the grassland drought plots of this field experiment [(13)](https://www.zotero.org/google-docs/?cJ6l7c). Drought did not affect lignin in either litter type (Figure 1b, Table S1), indicating that drought did not increase the recalcitrance of either litter types. This is inconsistent with the effects of drought predicted by our hypothesis.

Amide spectral ranges, which are indicative of proteins, increased over time (Figure 1f, 1g), consistent with increases of protein in litter over time in other systems [(51)](https://www.zotero.org/google-docs/?DWLz7b). Drought increased protein concentration in litter of both types, although the effects were stronger for grassland litter than shrub litter (Figure 1f, 1g, Table S1). Increases in nitrogen under drought had previously been observed elsewhere [(20, 21)](https://www.zotero.org/google-docs/?bZ6S3s), including a previous study in the grassland system in this field experiment [(13)](https://www.zotero.org/google-docs/?H8r2im).

## Community composition and CAZyme genes

The abundance of CAZyme genes for metabolising certain carbohydrates – hemicellulose and oligosaccharides – tended to be higher in grassland litter than shrub litter (Figure 2d, 2h) while lignin CAZyme genes were more abundant in shrub litter (Figure 2g). These differences in CAZyme gene abundance, when comparing broadly between two ecosystems, are inconsistent with our predictions. The results with precipitation treatments also do not support the indirect effects of drought that we hypothesized. CAZyme genes for carbohydrates – cellulose, hemicellulose, starch, polysaccharides, and oligosaccharides – were not affected by drought in either ecosystem (Figure 2c-f, 2i, Table S1) whether drought decreased carbohydrate fractions – as in grassland litter – or not – as in shrub litter (Figure 1d, 1h, 1i, Table S1). Lignin-related genes decreased in abundance under drought across both systems (Figure 2g) despite drought not affecting lignin fractions in litter (Figure 1b, Table S1). While these results do not necessarily preclude indirect effects of drought on lignin genes predicted by the litter chemistry hypothesis, they more strongly support the direct effects of drought on lignin genes. CAZyme gene abundances related to cellulose, polysaccharides, and oligosaccharides decreased over time (Figure 2c, 2f, 2h) while CAZyme genes related to abundance of hemicellulose and starch increased over time, indicating a succession of the decomposition of different substrates. This was also linked to microbial community composition changes over time across both ecosystems with grassland litter experiencing stronger changes in composition (Figure 3). Taxonomic diversity increased over time between both systems (Figure 3a) while fungal:bacterial ratios decreased over time in both systems (Figure 3b). These changes in composition also corresponded with temporal trends in CAZyme genes involved in microbial cell wall metabolism. Gene abundance for peptidoglycan metabolism increased over time in ambient grassland plots and shrub plots (Figure 2a) while chitin genes decreased over time in both ecosystems (Figure 2b), corresponding with decreases over time in fungal:bacterial ratios (Figure 3b).

## Extracellular enzyme activity

Enzyme activity tended to be higher in grassland than shrub (Figure 4), with statistically significant differences for the enzymes CBH and NAG (Figure 4d, 4f, Table S1) and insignificant differences for AG, BG, and BX (Figure 4a-c, Table S1). Similar to CAZyme gene abundance, these broad differences between ecosystems are inconsistent with our predictions, as the higher carbohydrate content of grassland litter (Figure 1d, 1h, 1i) corresponded with larger pools of extracellular enzymes that target carbohydrates (Figure 4a-d).

Drought had no statistically significant effect on any enzymes, either as a main effect or as an interaction with vegetation (Figure 4, Table S1); there was also very high variability across replicates. This does not support the indirect effect of drought predicted by the litter chemistry hypothesis, as activity of carbohydrate enzymes remained unchanged under drought (Figure 4a-d, 4g) whether carbohydrate fractions decreased – as in grassland litter – or remain unchanged – as in shrub litter (Figure 1d, 1h, 1i).

# Discussion

While resource acquisition trait values and litter chemistry differed between both systems as predicted by our hypothesis, the broad differences between both litter types were not consistent with our specific prediction. While we hypothesized that shrub litter, being the more recalcitrant litter type due to its higher lignin content (Figure 1b), should have higher CAZyme gene abundance and enzyme activity than grassland litter, we observed higher CAZyme gene abundance and carbohydrate-degrading enzyme activity in grassland litter instead (Figures 2d, 2h, 4a-d, 4g). Because grassland litter tended to have higher carbohydrate content than shrub litter (Figure 1d, 1h, 1i), these results are consistent with the induction of enzymes by their substrates [(52, 53)](https://www.zotero.org/google-docs/?LcsxyX) and positive associations between CAZyme genes and their substrates that had been observed elsewhere [(54, 55)](https://www.zotero.org/google-docs/?LLMP4i). In addition, grassland litter has a lower lignin fraction than shrub litter (Figure 1b). Enzyme Vmax had previously been found to be negatively associated with lignin [(56)](https://www.zotero.org/google-docs/?eA1eMB). Microbes that specialize on lignin degradation are associated with genes that function in cell signaling pathways rather than hydrolytic enzymes [(57)](https://www.zotero.org/google-docs/?pZEYGi), corresponding with the grassland microbial community having higher abundance of hemicellulose and oligosaccharide CAZyme genes (Figure 2d, 2h) and higher carbohydrate-degrading enzyme Vmax than the shrub microbial community (Figure 4a-d, 4g). Lignin also adsorbs hydrolases [(58, 59)](https://www.zotero.org/google-docs/?2KnfLU), likely decreasing enzymatic breakdown which reduces concentrations of substrates and intermediate degradation products that induce enzyme production [(52)](https://www.zotero.org/google-docs/?6LRxUF), further contributing to the gaps in Vmax between the shrub and grassland litter microbial communities. The higher enzyme activity and CAZyme genes likely explained the faster decomposition rates of grassland litter that had been observed in this site [(27)](https://www.zotero.org/google-docs/?NoN7I0).

Along with broad differences in functioning between vegetation communities, we also observed changes in functioning that correspond with successional changes in the microbial communities. Fungal-bacterial ratios decreased with time (Figure 1b), corresponding with increasing peptidoglycan gene abundance (Figure 2a) and decreasing chitin gene abundance (Figure 2b) with time, indicating successional changes as bacterial dominance increases and microbial communities become less reliant on fungal necromass with time. Bacteria express some of the peptidoglycan genes in our data to recycle their cell walls in the process of cell growth [(60)](https://www.zotero.org/google-docs/?rQbDhc). Decreasing chitin gene abundance is consistent with decreasing abundance of bacteria that decompose fungal cell walls [(61)](https://www.zotero.org/google-docs/?H6f8ws) as well as decreasing fungal abundance. Decreasing fungal abundance also corresponded with decreasing trends over time of BG, BX, and CBH Vmax (Figure 4b-d), and these trends had been observed in a temperate oak forest [(61, 62)](https://www.zotero.org/google-docs/?nzR62v). Some studies show that fungi are primary producers of extracellular enzymes [(63, 64)](https://www.zotero.org/google-docs/?F1XG8J), and a previous study in our grassland system found that the most abundant fungal taxa explained more variation in extracellular enzyme activity than the most abundant bacterial taxa [(65)](https://www.zotero.org/google-docs/?I9xTUB). However, overall microbial community composition did not influence extracellular enzyme activity in our grassland system [(65)](https://www.zotero.org/google-docs/?a7GA4L).

We also did not observe support for indirect effects of drought that are predicted by our hypothesis. In contrast to our predictions, drought did not increase the recalcitrance of either litter type as lignin remained unchanged under drought (Figure 1b). The effects of drought on lignin in the literature had been mixed. While some studies found that litter that originated from drought environments had higher lignin than litter from ambient environments [(13, 66)](https://www.zotero.org/google-docs/?Xx1f2H), other studies showed that drought decreased lignin in litter of some, but not all, plant species [(67)](https://www.zotero.org/google-docs/?JaVEYB). Also in contrast to our predictions, resource acquisition trait values generally did not change (Figures 2, 4, Table S1) whether litter chemistry changed under drought – as in grassland litter – or was unaffected by drought – as in shrub litter (Figure 1, Table S1). Previous studies have shown negative correlations between lignin fractions and decomposition rates [(16, 18, 19)](https://www.zotero.org/google-docs/?rMsRZ0), and lignin had also been shown to decrease decomposition rates of specific litter fractions such as cellulose and hemicellulose [(56, 68)](https://www.zotero.org/google-docs/?YInXpN). The lack of change in lignin under drought likely contributed to a lack of change in substrate availability that explained the lack of response of resource acquisition traits to changes in litter chemistry under drought. In addition, drought did not have major effects on carbohydrates. While drought decreased the spectral area associated with glycosidic bonds in grassland, grassland drought litter still had more spectral area associated with glycosidic bonds than shrub litter (Figure 1d). Drought also had no effect on the carbohydrate ester band 1015-970 cm-1 (Figure 1h, Table S1), suggesting that drought did not decrease carbohydrate fractions in grassland litter enough to influence substrate availability. Substrate availability in soil is limited by substrate diffusivity while substrate availability in litter likely is not [(69)](https://www.zotero.org/google-docs/?PqAmqT), making it plausible that substrate availability in litter remains high in low moisture conditions [(70, 71)](https://www.zotero.org/google-docs/?7wZ3b8). Our results suggest that grassland litter chemistry might not have changed enough under drought to decrease substrate availability and investment in resource acquisition traits, while the lack of change of shrub litter chemistry under drought made it even less likely for substrate availability to change in shrub litter.

Drought changed community composition in the grassland microbial community as a direct effect and as an indirect effect through changes in litter chemistry [(13, 15, 25, 26)](https://www.zotero.org/google-docs/?pcB9tO). The lack of a relationship between drought-induced changes in litter chemistry and resource acquisition traits despite changes in microbial community composition might indicate functional redundancy [(72, 73)](https://www.zotero.org/google-docs/?WW6Ky0). Functional redundancy had been observed in soil [(74)](https://www.zotero.org/google-docs/?j7cxRa) and in litter [(65, 75, 76)](https://www.zotero.org/google-docs/?kgPYNA). Microbial community functioning tends to respond less to environmental perturbations in microbial communities with prior exposure to these perturbations [(9, 77, 78)](https://www.zotero.org/google-docs/?GhULdO). This resistance can be attributed to changes in community composition, such as increases in relative abundance of taxa that are less sensitive to drought [(77–80)](https://www.zotero.org/google-docs/?LagoUA) that can maintain the same function [(27)](https://www.zotero.org/google-docs/?fpJQ5N). While bacterial populations that were enriched under drought in this same field experiment generally showed a tradeoff between drought tolerance and CAZyme genes such that the number of CAZyme genes decreased under drought, some populations enriched under drought continued to maintain high numbers of CAZyme genes [(27)](https://www.zotero.org/google-docs/?gipySG). Compensatory growth of functionally redundant taxa allows for microbial communities to maintain function in the face of environmental perturbations [(81)](https://www.zotero.org/google-docs/?WpJdqv). Because CAZyme gene abundance for most substrates remained unchanged under drought (Figure 2, Table S1) despite changes in community composition under drought in this field experiment [(13, 15, 25, 26)](https://www.zotero.org/google-docs/?jaAItO), compensatory growth might have occurred as taxa that are resistant to either the direct effects of drought or drought-induced changes in litter chemistry increased in abundance to maintain functioning.

Alternatively, our data indicated that the effects of drought on community composition were fairly small (Figure 1). This is consistent with previous studies that found significant, but small, effect sizes of drought on community composition [(26, 65)](https://www.zotero.org/google-docs/?vKtHtx), including a study that found a small effect of drought-derived litter on bacterial composition [(25)](https://www.zotero.org/google-docs/?hpZVMu). Changes in community composition might have been too small to affect resource acquisition traits, indicating functional resistance [(72)](https://www.zotero.org/google-docs/?uuM4t8) towards either direct effects of drought or drought-induced changes in litter chemistry. Functional resistance to precipitation manipulations had previously been observed in a grassland [(82)](https://www.zotero.org/google-docs/?1bHAVW) and a tropical rainforest [(9, 79)](https://www.zotero.org/google-docs/?7RB9sT). Repeated exposure to drought, similar to the long-term drought treatment in our study, might have conditioned drought sensitive taxa to become more resistant [(79)](https://www.zotero.org/google-docs/?Tnmt92). Our results likely reflect physiological acclimation to dry conditions either in semi-arid or arid ecosystems or long-term climate manipulations [(8, 9, 83, 84)](https://www.zotero.org/google-docs/?SJ2Gh3).

The lack of a relationship between drought-induced changes in litter chemistry and microbial composition and functioning might also be attributed to our study design. Because we specifically sampled for litter of plant species that are characteristic of each plant community rather than species that are characteristic of each plot, our litter chemistry data might only be indicative of changes in plant physiology under drought [(20–22, 66, 67)](https://www.zotero.org/google-docs/?tWF0H7) and did not adequately account for changes in plant community composition [(23, 24)](https://www.zotero.org/google-docs/?7SsvTK). As a result, drought-induced changes in the chemistry of the specific litter we collected might have been too small to influence microbial community composition and resource acquisition traits. Furthermore, while our litter chemistry results are broadly consistent with litter chemistry results in other studies [(13, 20, 21, 49–51)](https://www.zotero.org/google-docs/?2oX76Q), it is difficult to tease apart certain litter fractions and their responses to drought with our litter chemistry data. For example, while grassland litter had higher carbohydrate ester content than shrub litter (Figure 1h, 1i), it is uncertain whether grassland litter had higher proportions of hemicellulose, pectins, or both. While overall carbohydrate content, as indicated by glycosidic bond spectral area, decreased under drought in grassland (Figure 1d), it was uncertain which specific carbohydrate (e.g. cellulose, hemicellulose, starch) decreased. This uncertainty obscured possible relationships between decomposition traits and indirect effects of drought through litter chemistry.

Consistent with our study, studies of litter decomposition in Mediterranean ecosystems so far indicate that drought-induced changes in litter chemistry either do not influence decomposition rates [(13, 25)](https://www.zotero.org/google-docs/?TvDQwV) or do not influence decomposition rates as much as direct effects of drought [(85)](https://www.zotero.org/google-docs/?8wuCCE), although we note that Prieto et al, similar to this study, does not account for changes in plant community composition due to drought [(85)](https://www.zotero.org/google-docs/?cO3tEA). Similarly, the effects of drought on grassland plant community composition in this field experiment were statistically significant but small [(26)](https://www.zotero.org/google-docs/?SipHTD), indicating that the lack of a relationship between drought-derived grassland litter and decomposition rates [(13, 25)](https://www.zotero.org/google-docs/?QHgbg3) can partially be attributed to the relative lack of change in grassland plant community composition under drought. Drought was shown to change plant community composition in observational studies over time [(86–88)](https://www.zotero.org/google-docs/?ohaldC) as well as in field experiments [(23, 24)](https://www.zotero.org/google-docs/?fVaMQP), with drought being a factor that drives vegetation type conversion from chaparral ecosystems to exotic grasslands in California [(24, 87, 88)](https://www.zotero.org/google-docs/?cMacl1). Vegetation type conversions will, in turn, change the litter that microbes decompose, affecting microbial communities and decomposition rates [(89)](https://www.zotero.org/google-docs/?BQCVML). The plant composition of the litter can influence how decomposition rates respond to drought [(11, 12)](https://www.zotero.org/google-docs/?spR1GX), microbial parameters such as biomass, diversity, and composition [(14, 90)](https://www.zotero.org/google-docs/?UzxXCx), and how microbial traits respond to drought [(8, 27)](https://www.zotero.org/google-docs/?C9y0eU). Since our study indicates that microbial decomposition traits are fairly resistant to drought-induced changes in litter chemistry from changes in plant physiology, microbial decomposition traits might be more likely to change if drought also changes plant community composition, especially if plant communities undergo type conversion.

# Tables

| Spectral range (cm-1) | Band assignment | Likely litter substrate |
| --- | --- | --- |
| 1015 - 970 | C-O stretching of esters | Carbohydrate |
| 1080 - 1015 | C-O deformation of glycosidic bonds | Carbohydrate |
| 1160 - 1100 | C-O stretching of esters | Carbohydrate |
| 1230 - 1160 | C-O stretches | Carbohydrate |
| 1475 - 1450 | C-H deformation in methyl and methylene | Lignin |
| 1645 - 1620 | Amide | Protein |
| 1600 - 1545 | Amide | Protein |
| 1750 - 1700 | C=O stretching | Lipid |

Table 1. FTIR band assignments and indicative components of leaf litter chemistryTable 2. Enzymes under analysis [(41, 42)](https://www.zotero.org/google-docs/?2wW64f). Note that the concentration values describe actual concentrations inside 96-well microplates, not concentrations of substrate/standard solutions prior to being pipetted into microplates.

| **Enzyme** | **Substrate** | **EEA substrate** | **Maximum concentration in plates** |
| --- | --- | --- | --- |
| ɑ-glucosidase (AG) | Starch | 4-MUB-ɑ-D-glucoside | 500 µM |
| (acid) phosphatase (AP) | Organic phosphorus | 4-MUB-phosphate | 2000 µM |
| Β-glucosidase (BG) | Cellulose | 4-MUB-β-D-glucoside | 500 µM |
| β-xylosidase (BX) | Hemicellulose | 4-MUB-β-D-xyloside | 500 µM |
| Cellobiohydrolase (CBH) | Cellulose | 4-MUB-β-D-cellobioside | 250 µM |
| Leucine aminopeptidase (LAP) | Protein | L-Leucine-AMC | 500 µM |
| N-acetyl-β-D-  Glucosaminidase (NAG) | Chitin, peptidoglycan | 4-MUB-N-acetyl-β-D-  glucosaminide | 1000 µM |
| Standard (AMC) |  | 7-amino-4-methylcoumarin | 31.25 µM |
| Standard (MUB) |  | 4-methylumbelliferone | 12.5 µM |

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