Global change factors reshape the links between litter properties, decomposers, and decomposition in mature oak forests

Matthew L. Meehan[1,10*], Mathilde Chomel[1,2], Zhabiz Vilkiji[1], Katy J. Faulkner[3,4], Tancredi Caruso[5], A. Robert MacKenzie[6,7], Virginie Baldy[8], Richard D. Bardgett[1,9], David Johnson[1,9]

[1]Department of Earth and Environmental Sciences, The University of Manchester, Manchester, United Kingdom
[2]FiBL France, Research Institute of Organic Agriculture, Eurre, France
[3]Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, United Kingdom
[4]School of Medicine, University of St. Andrews, North Haugh, KY16 9TF, St. Andrews, United Kingdom
[5]School of Biology and Environmental Science, University College Dublin, Dublin, Ireland.
[6]School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, United Kingdom
[7]Birmingham Institute of Forest Research, University of Birmingham, Birmingham, United Kingdom
[8]IMBE, Aix-Marseille Université, Univ Avignon, CNRS, IRD, Marseille, France
[9]Lancaster Environment Centre, Lancaster University, Bailrigg, LA1 4YQ, Lancaster, United Kingdom
[10]Current address: Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Québec, Canada
*Corresponding author's email: matthew.meehan@nrcan-rncan.gc.ca

ORCID
Matthew L. Meehan: https://orcid.org/0000-0001-5427-8241
Mathilde Chomel: https://orcid.org/0000-0001-5110-2355
Katy J. Faulkner: https://orcid.org/0000-0002-9204-6502
Tancredi Caruso: https://orcid.org/0000-0002-3607-9609
A. Robert MacKenzie: https://orcid.org/0000-0002-8227-742X
Virginie Baldy: https://orcid.org/0000-0002-4048-5132
Richard D. Bardgett: https://orcid.org/0000-0002-5131-0127
David Johnson: https://orcid.org/0000-0003-2299-2525

Abstract

Increasing atmospheric CO_2 concentrations alongside more frequent and severe droughts are key global change factors impacting litter decomposition and global carbon cycles. Yet, we have a poor understanding of how these perturbations impact interactions between initial litter properties and decomposition environment. We tested how drought and elevated atmospheric CO_2 concentrations modify litter decomposition *via* litter properties and decomposition environment using two separate, long-term manipulative drought or elevated CO_2 field experiments in mature oak woodlands. Litterbags were deployed in a reciprocal transplant design within each experiment, where we measured litter mass loss, C-biochemistry, C:N ratios, moisture content, and decomposer communities. We found that litter placed in droughted plots decomposed slower than in control plots and experimental litter derived from elevated CO_2 plots decomposed slower over the first three harvests compared to control litter. Under drought, litter mass loss rates and C:N ratio was regulated by initial litter properties and the decomposition environment, while elevated CO_2 impacted mass loss *via* changes in initial litter properties. We show that drought and elevated atmospheric CO_2 can modify the decomposability of litter prior to litterfall and during the subsequent decomposition, highlighting the need to disentangle their individual and interactive effects to better predict how global change factors influence decomposition.

Keywords: Decomposition, Drought, Elevated CO_2, Mesofauna, Litter, Microbial

Highlights

- Drought impacted litter mass loss more than elevated CO_2 conditions
- Decomposition environment shaped mass loss with drought but not elevated CO_2
- Treatment-effects on litter properties is observable after decomposition begins
- Decomposers and litter properties still impact decomposition at middle-to-late stages

Introduction

Forest ecosystems cover 31% of global land cover (FAO, 2020) and play a critical role in global carbon (C) cycling (Harris et al., 2021) as global forests take up 1.1 ± 0.8 Pg C year^{-1} (Pan et al., 2011). Yet these ecosystems are being reshaped by global change factors, including increasing atmospheric CO_2 concentrations and increased frequency and intensity of extreme weather events such as droughts (IPCC, 2021). Elevated CO_2 and drought independently affect C cycling because forests exhibit cellular- to ecosystem-level responses to both factors (Blaschke et al., 2001; Cech et al., 2003; Lee et al., 2021; Arain et al., 2022; Martínez-Sancho et al., 2022; Norby et al., 2024; Reay et al., 2025). For example, in a mature stand of *Eucalyptus tereticornis*, elevated CO_2 increased C uptake (through gross primary production) by 12%, although net gains were lost due to an increase in soil fluxes, including from respiration (Jiang et al., 2020). In addition, a summer drought in 2022 resulted in nearly half of Europe's soil moisture levels becoming critically low (Toreti et al., 2022), which corresponded with a reduced C uptake of ~60 TgC (van der Woude et al., 2023). Because drought and elevated atmospheric CO_2 concentrations affect ecosystem C exchange, it is critical to understand how these global change factors influence key ecosystem processes connected to C cycling, such as litter decomposition.

Litter decomposition is driven by soil biota, which break down litter into simplified compounds, leading to plant uptake of nutrients (Krishna and Mohan, 2017), the release of by-products into the atmosphere, and incorporation of nutrients into various soil pools (Lavallee et al., 2020). The decomposer community consist of soil animals, including mesofauna like mites and collembolans, and larger macrofauna like isopods, which impact decomposition through fragmentation of litter and grazing of microbes (Seastedt, 1984; Coleman et al., 2018), in addition

to bacteria and fungi that mineralize the organic matter *via* the secretion of enzymes (Romaní et al., 2006). Globally, litter decomposition is also driven by climatic factors, which regulate the activity of decomposers, such as temperature and annual precipitation, along with initial litter properties such as C:N ratio, lignin and tannins (Zhang et al., 2008; Wu et al., 2025). Importantly, both elevated CO_2 and drought can impact litter decomposition (Wu et al., 2020; Liu et al., 2024) namely through modifications to initial litter properties (which we refer to below as litter origin) and by modifying the environment (which we refer to below as the decomposition environment). Here, the mechanisms, the direction, and the magnitude of responses to initial litter properties and decomposition environment against elevated CO_2 and drought may differ substantially.

Drought and elevated atmospheric CO_2 can affect litter decomposition by modifying the litter/soil environment and decomposer community. Drought-induced low moisture content has cascading effects on soil processes and microbial and animal communities. Desiccation has differential effects on particular groups of organisms: for example, fungi are generally more resistant to drought than bacteria (Yuste et al., 2011; de Vries et al., 2012, 2018; Manzoni et al., 2012). As well, Martin et al., (2024) found that reduced precipitation decreased soil fauna abundance by 35%, while the effects of elevated CO_2 on decomposer communities are more subtle and often variable (Blankinship et al., 2011). Although not nearly as intense as drought, elevated CO_2 concentrations can also potentially influence the soil microclimate stemming from changes to the canopy under enriched CO_2 levels (Franzaring et al., 2010). In addition, a further distinction between elevated CO_2 and drought on the decomposition environment is from changes in C allocation below-ground. Elevated CO_2 and drought have contrasting effects on the flux of recent plant photosynthate, which in forests is a major pathway of energy flow to soils (Högberg et al.,

2001). Under elevated CO_2, trees increase below-ground inputs of C. For example, at the Duke Forest FACE experiment, fine root biomass increased by 23%, microbial biomass by 15% in response to sustained elevated CO_2 (Drake et al., 2011). In a mature oak forest, similar increases in below-ground productivity were seen, including in the production and turnover of mycorrhizal mycelium (Reay et al., 2025). By contrast, droughted trees tend to fix and allocate less C below-ground (Hagedorn et al., 2016), until their recovery where plants then allocate more C to roots to facilitate water and nutrient uptake (Joseph et al., 2020; Gao et al., 2024).

Both drought and elevated CO_2 concentrations affect leaf properties, such as the carbon and nitrogen content of leaves and shoots (Yuan and Chen, 2015; Sun et al., 2020). Changes due to drought can stem from increased sugar accumulation, decreasing nitrogen (N) content, and changes to primary and secondary plant metabolites (Chen et al., 2015; Orians et al., 2019; Wang and Wang, 2025). For elevated CO_2, N dilution within leaves together with C can alter the C:N ratio (Taub and Wang, 2008; Welti et al., 2020). These and other changes to litter properties can affect litter quality and subsequently decomposer community composition (Ilieva-Makulec et al., 2006; Gergócs and Hufnagel, 2016). Changes to the decomposer community will subsequently impact the litter decomposition process but understanding how changes to both initial litter properties and decomposition environment impact animals and microbes requires further examination.

Disentangling the effects of initial litter properties from those of the decomposition environment on litter mass loss is challenging, especially under field conditions. For instance, Prieto et al., (2019) found strong interactive effects between litter origin and decomposition

environment on litter mass under experimental (i.e., warming and reduced rainfall) conditions. They also found that subsequent linkages between microbial communities, litter moisture, and litter mass loss differed when experimental treated litter was incubated in control plots (both litter moisture and microbial communities were correlated with mass loss) or in treated plots (litter moisture was correlated with microbial community structure and microbes was the sole parameter correlated with mass loss) further highlighting interactions between initial litter properties and decomposition environment during mass loss. Overall, both drought and elevated atmospheric CO_2 have potential to affect litter decomposition *via* alteration of litter properties and the decomposition environment (which includes decomposers and litter moisture content) but how initial changes to litter properties interact with the decomposition alongside changes to the decomposer community *in situ* remains poorly understood. Resolving this uncertainty requires large-scale experimental infrastructure in combination with multi-variate analysis of litter properties and other factors.

We investigated how drought and elevated atmospheric CO_2 independently affect litter decomposition in mature forests by conducting reciprocal transplants within two, large-scale manipulative field experiments: the University of Birmingham Institute of Forest Research Free-Air CO_2 Enrichment (BIFoR FACE) experiment and the Oak Observatory at the Observatoire de Haute-Provence (O_3HP) drought experiment. We hypothesized that drought and elevated CO_2 influences litter decomposition through two distinct pathways. The first involves the decomposition environment, which arise when the same litter type decomposes in contrasting environments (control vs. drought or elevated CO_2 plots). These effects capture how changes in soil moisture, microclimate, and decomposer communities directly regulate decomposition. We

118 hypothesized that drought and elevated CO_2 have contrasting impacts on soil properties and
119 microclimate, notably because drought has strong direct impact on environmental conditions, such
120 as litter and soil moisture. The second pathway involves litter origin, which occurs when different
121 treatments (e.g. control vs. drought, or control vs. elevated CO_2) changes litter properties that affect
122 subsequent decomposition. For example, plant growth under drought and elevated CO_2 conditions
123 may modify litter properties, especially chemistry (e.g. C:N ratio, lignin, tannins), prior to litterfall
124 and subsequent decomposition. We hypothesized that litter originating from treated plots would
125 have long-term (carry over) effects on decomposition stemming from differences in litter
126 properties. Our reciprocal transplant design allowed us to test whether interactive effects between
127 litter origin and decomposition environment influence decomposition rates when subject to global
128 change drivers.
129

130 **Methods**

131 *Overview*

132 Within each experimental facility, we collected control and experimental litter and in
133 January 2020 transplanted the two litter types into control and treated plots (Supplementary
134 Information Figure S1). We harvested litterbags across four time points and measured (across all
135 or a subset of time points) litter mass loss, litter C-biochemistry and metabolomics, and the
136 microbial (phospholipid fatty acid analysis; PLFA) and animal community structure (the
137 abundance of mites, collembolans and other larger macrofauna) directly associated with the
138 recovered litter. Note that we did not transplant litter between the two experiments (i.e., litter from
139 BIFoR FACE was not transplanted at O_3HP and vice versa). We only transplanted litter within

each experimental facility between the control and experimental apparatuses. We will continue to refer to it as a reciprocal transplant experiment below given that our study follows that design.

Experimental sites

The reciprocal transplant study used the BIFoR FACE (52.801°N, 2.301°W) elevated CO2 experiment in the United Kingdom and the O_3HP (43.965°N, 5.878°E) drought experiment in southern France (Supplementary Figure S1). The BIFoR FACE facility started in 2017 on a *Quercus robur* L. forest that was planted around 1850 (Hart et al., 2020; MacKenzie et al., 2021) containing coppice *Corylus avellana* L. and abundant self-set *Acer pseudoplatanus* L. understory. The CO_2 treatment began in April 2017 and comprises six infrastructure plots (elevated CO_2, n = 3, ambient CO_2, n = 3). Each plot is approximately 30 m in diameter and comprises 16 peripheral towers and one central tower that extend ~ 1m above the canopy. In the elevated CO_2 plots, an additional 150 ppm of CO_2 above ambient (i.e. ~36 % increase over ambient) is emitted laterally from the ground to the forest canopy, while ambient CO_2 arrays mimic the treatment but only receive ambient air. The increase of CO_2 concentrations is seen from the soil level infrastructure and across the entire vertical profile of the plot (Hart et al., 2020), meaning litterbags would be incubated in an enriched CO_2 environment. The towers release CO_2 from ~April 1 to October 31 each year, coinciding with oak bud burst and last leaf fall (Supplementary Information Figure S2). The +150 ppm of CO_2 is similar to other large-scale experimental facilities such as EucFACE (e.g., Jiang et al., 2020) and represents the projected mid-century CO_2 concentration, regardless of model scenario (Friedlingstein et al., 2014). It also allows for ecophysiological effects to be detected with both aboveground and belowground measurements (Gardner et al., 2022; Norby et al., 2024;

Reay et al., 2025) and provides an end-member of the small set of forest FACE treatments (Norby, 2025).

The O_3HP drought experiment started in 2012 on *Quercus pubescens* Willd forest, containing *Acer monspessulanum* L. (75% vs. 25% coverage, respectively) and *Cotinus coggygria* Scop understory. A rain exclusion device, measuring 15 m x 20 m excludes ~30% of precipitation at canopy top, relative to the nearby control plot. In our study, the rain exclusion device was operational between May to September and the total precipitation was 40% lower in rain exclusion plots over the course of the experiment (total precipitation, control: 1652 mm, drought: 997 mm; Supplementary Information Figure S3) (Gauquelin et al., 2011; Santonja et al., 2017; Aupic-Samain et al., 2021). There is only a single drought apparatus established at O_3HP, but to create near-independent 'plot-like' conditions relevant to the scale of litterbags, the bags were placed in a clumped distribution under and outside of the rain exclusion devices to create three experimental plots within the treatment, mirroring the set-up at BIFoR FACE.

Litterbag preparation and sampling

Quercus litter was sampled from spatially separated areas of each site in BIFoR FACE and O_3HP in October 2019 and shipped in coolers to The University of Manchester and air dried. We measured the %C, %N, C:N and metabolomic signatures on the initial litter (Quer et al., 2022; see *Metabolomic signature of initial litter* in Methods S1 for more details). Litterbags comprised 1.8 mm nylon mesh with two separate compartments (Supplementary Information Image S1), each receiving 3 g of dried litter. One compartment was used for chemical analysis of litter and assessment of microbial community composition, decomposition, and moisture content. The other

compartment was used solely to characterize soil fauna communities. A total of 480 litterbags were used (i.e. 240 litterbags for BIFoR FACE and 240 for O_3HP). For each experimental site, we had 2 decomposition environments (control and drought or elevated CO_2) x 2 litter origins (control and experimental) x 3 plots x 4-time points x 5 replicates = 240). The experiment began January 2020 where litterbags were placed on the experimental sites. Freshly fallen litter was removed prior to placing the litterbags on the ground surface and then replaced over the litterbags. Litterbags were then fixed with two galvanized nails to prevent movement by animals or wind. Litterbags were harvested across four time points at both experimental sites. At BIFoR FACE, litterbags were harvested September 2020 (8 months; Harvest one; H1), January 2021 (12 months; Harvest two; H2), June 2021 (17 months; Harvest three, H3) and December 2021 (23 months; Harvest four, H4), corresponding to the fourth and fifth year of activation of the elevated CO_2 treatment. Four litter bags could not be retrieved from BIFoR FACE at H4 due to a tree limb falling and disturbing litter bags. At O_3HP, litterbags were harvested October 2020 (10 months; H1), January 2021 (13 months; H2), September 2021 (21 months; H3) and October 2022 (34 months; H4). The duration of decomposition differed between experimental sites due to variation in decomposition rate. We initially aimed for ~24 months of decomposition but incubation in the drought experiment was extended by an additional year to achieve 50% mass loss.

At each sampling time, litterbags were shipped to The University of Manchester for processing. In order to prevent contamination of litter by soil, remaining litter from the 'chemical analysis compartment' was wiped thoroughly by hand or with a brush when needed. The fresh weight (g) of the litter was taken before litter was frozen at -20 °C overnight and 48 hours of freeze-drying for determination of dry weight. For soil fauna, litter was removed from the

compartment and placed on Tullgren funnels to extract individual animals for seven days into absolute ethanol. After extraction, litter was placed into ovens at 65 °C for two days before weighing to measure litter dry weight. Apart from litter mass loss rates (measured at all dates), litter properties and decomposer communities were measured from H3 onward, when ~20-60% of the original litter remained, to capture drought and elevated CO_2 effects during mid to late stage of decomposition.

Litter decomposition, quality, stoichiometry, and moisture content

Litter decomposition was calculated as litter mass loss using the following equation:

$$Mass\ loss\ (\%) = \frac{Initial\ litter\ (g) - harvested\ litter\ (g)}{Initial\ litter} * 100$$

We also calculated the exponential decomposition rate coefficient (k_t) at each harvest point using the equation from (Olson, 1963):

$$k_t = \frac{-\ln\left(\frac{X_H}{X_I}\right)}{t}$$

Where X_H is the weight (in g) of harvested litter, X_I is the weight of initial litter (in g), and t is time in years since the beginning of the experiment. Litter decomposition and litter moisture was measured from all litterbags across all four time points. We measured the %C, %N and C:N ratio of initial and decomposed litter with an Elementar Vario EL element analyser (Hanau, Germany) on 5 mg of ball-milled litter (Retsch Ball Mill MM 400, Haan, Germany) at both sites.

We also measured litter C-biochemistry (specifically, characterizing C-containing functional groups) of H3 and H4 litter from both sites using Fourier transform infrared (FTIR)

230 spectroscopy (Bell et al., 2018). Ball-milled litter samples were placed onto a Universal
231 Attenuated Total Reflection (UATR) spectrometer diamond crystal to cover the 2-mm window,
232 and the absorbance (A cm^{-1}) was measured within a 650 to 4000 A cm^{-1} range, at 4 cm^{-1} resolution
233 and 16 scans per sample. We also ran a background correction after every ten samples. We applied
234 a baseline correction for each spectra (i.e., sample) using the modpolyfit method in the R package
235 'baseline'. To standardize values across our samples, we Z-transformed each spectra, where
236 values were transformed to a mean of 0 and SD of 1 by subtracting by its own mean and dividing
237 by its own standard deviation (akin to a Standard Normal Variate normalization) to reduce
238 multiplicative scatter effects (Jardine et al., 2017; Bell et al., 2018). We then calculated the area
239 of the curve for each respective functional group using a shoelace formula (Wang et al., 2023).
240 The assignment of wave number regions to leaf litter functional groups was based on Liu et al.,
241 (2016) who used FTIR to examine functional groups across four deciduous litter origins, including
242 *Quercus robur* (Supplementary Information Table S1).
243
244 *Phospholipid fatty acid analysis (PLFA)*
245 We used PLFAs to measure how fungal and bacteria concentrations and the general
246 microbial community were impacted by drought and elevated CO_2 in leaf litter at both sites (Buyer
247 and Sasser, 2012). Due to high litter mass loss rates in the fourth harvest of BIFoR FACE, we did
248 not have enough material remaining to quantify microbial communities through PLFAs. So, we
249 elected to measure PLFAs for both sites only on litterbags collected in the third harvest. We
250 quantified PLFAs with ~0.4-0.5 g of ball milled, freeze-dried litter. Four ml of Bligh-Dyer
251 extractant containing C19:0 of an internal standard was added to each sample to extract lipids.
252 Once extracted, we used a gas chromatograph (Agilent 7890A Gas chromatograph, Santa Clara,

CA, USA) to assign and calculate peak height, where PLFA group concentrations were expressed as nmol g dry weight of litter^{-1}. We used 18 fatty acid markers to characterize microbial communities (Frostegård et al., 1993; Hill et al., 2000) on leaf litter, consisting of 6 and 4 markers for gram positive (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) and gram negative (16:1ω7c, cy17:0, 18:1ω7, and cy19:0) bacteria, respectively. In addition, we used 15:0 and 17:0 as general bacteria markers, and 10Me16:0, 10Me17:0, and 10Me18:0 as markers for actinobacteria. Fatty acids 14:0 and 16:0 were used as general microbial markers in our study, not specific to fungi or bacteria. The marker 18:2ω6,9 was the sole fungal marker in our experiments. With these markers, we analyzed decomposition environment and litter origin effects on total PLFA concentrations (proxy for microbial biomass), F:B ratio (by dividing 18:2ω6,9 abundance by the summed total of bacteria markers) and PLFA community composition across both experiments.

Soil fauna community

Soil fauna was sorted and counted into higher taxonomic or mixed groups, comprising: Collembola, Oribatida, Astigmata, Prostigmata, Mesostigmata, and 'other' fauna, which included Annelida, Isopoda, and Insecta. Soil fauna abundances were standardized by the dry weight of litter (individuals g dwt^{-1}), to account for differences in sample mass prior to analysis. Although found in litterbags, macrofauna counted for less than 5% (on average) of total abundances in our study. Meaning, most individuals captured were mesofauna, like mites and collembolans.

Statistical analysis

All statistical analyses were performed with the program R (version 4.3.1) (R Core Team, 2023) and figures were produced with the package 'ggplot2' (Wickham, 2016). To analyze for

differences in initial litter chemistry between our litter types (litter from control and experimental plots) prior to the experiment beginning, we used one-way Analysis of Variance (ANOVA) tests and Permutation Multivariate ANOVAs (PERMANOVAs) to examine for differences between C%, N% and C:N ratio (ANOVA) and leaf metabolomic profile (PERMANOVA, with Euclidean distance) between our treatments (ambient vs. drought or elevated CO_2). To do this, we used the aov function in base R and the adonis2 function in the package 'vegan' (Oksanen et al., 2022), respectively. Here, we partitioned the data by sites (BIFoR FACE and O_3HP) and ran separate models for both.

We tested the effect of decomposition environment (ambient vs. drought or elevated CO_2) and litter origin (control vs. experimental) on our parameters, analyzing the sites (BIFoR FACE and O_3HP) and harvest time points (H1-4) separately (excluding litter decomposition, for more details see below). For univariate variables, we used linear mixed models where the fixed factors were decomposition environment, litter origin, and their interaction, with a random factor of experimental plot. We used linear mixed models for %C, %N, C:N ratio, total PLFA concentration (nmol g dwt^{-1}), fungi : bacteria ratio, animal total and group abundances (individuals g dwt^{-1}), which includes Collembola, Oribatida, Astigmata, Mesostigmata, Prostigmata, and 'other' fauna. Note, we did not analyze Astigmata abundances in the drought experiment as low abundances were present in samples. For litter decomposition (measured as mass loss) we included all time points together into a single model, where the fixed factors were decomposition environment, litter origin, harvest, and their subsequent interactions with a random factor of experimental plot. To run linear mixed models, we used the lmer function in the package 'lme4' (Bates et al., 2015) where we calculated F-statistics and p-values with Satterthwaite's method using the package

'lmerTest' (Kuznetsova et al., 2017). If model residuals were not normally distributed, we ln or arscine-transformed the dependent variable and re-ran the model. At times, we found singularity within our linear mixed models as the variance within our random factor terms was extremely low (essentially zero) and the model was overfit. When this occurred, we removed the random factor, and we re-ran the model without the random term. We found this had no effect on p-values (because experimental plot explained little variance within the linear mixed model), so we reported the ANOVA results.

For multivariate variables, including leaf litter functional groups (FTIR) and microbial community composition, we ran PERMANOVAs using the adonis2 function in the package 'vegan' (Oksanen et al., 2022). For leaf litter functional groups, we used Euclidean distances within our models, while with microbial communities we used Bray-Curtis dissimilarity as it better suited for compositional data (like the abundances of each PLFA peak). Each model was fitted with the same fixed factors, consisting of decomposition environment, litter origin and their interaction. To visualize leaf litter functional groups and microbial communities we used a Principal Component Analysis (PCA) ordination plot using the rda function in the package 'vegan' (Oksanen et al., 2022). For microbial communities, we Hellinger-transformed datasets using the decostand function in the 'vegan' package prior to the PCA.

Finally, we used additional linear mixed models to detail relationships between the treatments, litter properties, and decomposer communities with litter mass loss at the third and fourth harvest, for each experiment. To do this, we z-transformed (mean = 0, SD = 1) mass loss rates, litter moisture, remaining litter C:N, total PLFAs, F:B, PLFA PC1, PLFA PC2, FTIR PC1,

FTIR PC2, and total animal abundances data for each site at H3 and H4. We then constructed Pearson correlation matrices to provide an initial assessment of the parameters that were correlated with litter mass loss. From there, we selected the significant correlates ($p < 0.05$) and ran linear mixed models where we included those parameters, in addition to litter origin and decomposition (as non-interacting factors) and plot as the random effect using the lmer function from the 'lme4' package (Bates et al., 2015). The selected parameters that were tested alongside litter origin and decomposition environment for each model can be found in Supplementary Tables S2-5. At H3 in both sites, the relative abundance of fungi : bacteria was strongly correlated with PLFA PC1; we elected to retain PLFA PC1 (and remove F:B) in both models as the first principal component analysis strongly explained PLFA composition. After this, we checked for collinearity within our four models and found that all parameters had VIF scores < 5, representing absent or low multicollinearity. With the dual approaches to model litter mass loss (one described here and the other detailed earlier), we examined the overall effect of each treatment on litter mass loss through four harvest points, and then subsequently analyzed the effect of litter properties and decomposer communities on mass loss at specific harvests. Note that mass and C-loss were highly correlated with each other at both sites ($p < 0.0001$), so we elected to model litter mass loss rates for the experiment.

Results

Initial litter chemistry

We found strong differences between the C:N ratio of the initial litter collected at control and droughted plots at O_3HP ($F_{1,8} = 13.92$, $p = 0.006$; Supplementary Information Table S6), with the C:N ratio of control litter exceeding that of the droughted litter. Furthermore, strong

differences were detected between the metabolomic profiles between the initial control and droughted litter ($F_{1,8} = 3.91$, $p = 0.009$; Supplementary Information Figure S4). Control litter from the drought experiment was mainly composed of ellagitannins (from $C_{34}H_{24}O_{22}$ to $C_{56}H_{40}O_{31}$), catechin ($C_{15}H_{14}O_6$), and ellagic hexoside ($C_{20}H_{16}O_{13}$) that were overexpressed within litter. Other metabolites such as quercetin hexosides (or rhamnoside), kaempferol hexosides, and isorhamnetin hexosides were at similar quantities between control and drought treatment. By contrast, there were no differences in C:N ratio ($F_{1,8} = 0.001$, $p = 0.977$; Supplementary Information Table S6) or metabolomic profiles ($F_{1,8} = 1.04$, $p = 0.336$; Supplementary Information Figure S4) between litter collected from the ambient and elevated CO_2 plots at BIFoR FACE.

Elevated CO_2 experiment at BIFoR FACE

Although minimal differences were detected between control and experimental litter when measuring initial litter chemistry, we found that during the first eighteen months of the decomposition experiment, litter from elevated CO_2 plots (i.e., experimental litter) decomposed slower than litter from control plots (litter origin: $F_{1,215.09} = 5.932$, $p = 0.016$) through H1-3 (time: $F_{3,215.46} = 155.97$, $p < 0.001$) until H4 in both decomposition environments. At H4, mass loss varied little between the decomposition environments and litter origins (Figure 1, Supplementary Information Table S7 contains exponential decomposition rate coefficient, k_t, values). Similarly, the C:N of litter was higher in experimental litter compared to controls ($F_{1,56} = 6.03$, $p = 0.02$) at H3, but not at H4 (Supplementary Information Table S8 and S9). This finding was also supported by FTIR spectroscopy analysis, which showed significant differences in leaf litter C-containing functional groups at H3 between litter origins ($F_{1,56} = 4.33$, $p = 0.03$). However, litter C-biochemistry composition between control and experimental litter was no longer significant at H4

($F_{1,52} = 0.26$, $p = 0.81$; Figure 2A-B). Neither decomposition environment nor litter origin affected litter moisture content at either H3 or H4 (Supplementary Information Table S8 and S9).

Decomposition environment and litter origin did not influence PLFA abundance, the fungi : bacteria (F:B) ratio, and microbial community composition at H3 (Supplementary Information Table S8, Supplementary Information Figure S5). Likewise, no individual or total animal group abundances, were affected by decomposition environment or litter origin in H3. In H4, however, the abundance of Mesostigmata ($F_{1,52} = 5.04$, $p = 0.03$) and Prostigmata ($F_{1,52} = 7.30$, $p = 0.009$) was significantly greater in control litter relative to experimental litter in both decomposition environments (Supplementary Information Table S9).

Further modeling of mass loss rates (with litter properties and decomposer communities) yielded strong relationships of mass loss with microbial communities (both PLFA PC1 ($t = -4.871$, $p < 0.001$) and PC2 ($t = 4.219$, $p < 0.001$)), total animal abundances ($t = 2.40$, $p = 0.02$), and litter C-biochemistry (FTIR PC1; $t = 3.30$, $p = 0.002$) for litterbags at the third harvest. Here, larger mesofauna and macrofauna population corresponded with higher mass loss rates. Furthermore, both microbial PC1, driven by fungal abundance (PLFA marker C18:2ω6,9), and microbial PC2, where bacteria markers were strongly separated (Supplementary Information Figure S5), were predictors of litter mass loss rates. In addition, the C-biochemistry of the remaining litter explained mass loss rates, highlighting that chemical differences in litter (stemming from litter origin treatment) impacted mass loss rates, even after more than 50% of material has decomposed. However, by the fourth harvest, only FTIR PC2 was found to significantly explain litter mass loss rates ($t = -3.22$, $p = 0.002$) as litter C-biochemistry was a predictor of mass loss when ~ 20% of

the original leaf material remaining. Differences in the number of significant parameters between the two harvests partly stem from additional measurements detailing microbial communities at H3; however, it is notable that animals were no longer correlated with mass loss by the fourth harvest.

Drought experiment at O_3HP

By contrast to elevated CO_2, there were stronger effects of both decomposition environment and litter origin on measured parameters in response to drought, matching our first and second hypotheses. First, litter decomposition was slower in droughted plots relative to controls (decomposition environment: $F_{1,4} = 147.91$, $p < 0.001$) with remaining litter mass decreasing at varying rates (decomposition environment x time: $F_{1,220} = 4.56$, $p = 0.004$; Figure 1; Supplementary Information Table S7). Remaining litter had lower C:N ratios in control plots (H3: $F_{1,4} = 21.92$, $p = 0.009$; H4: $F_{1,56} = 37.85$, $p < 0.001$) compared to drought plots. In addition, experimental litter (i.e., litter derived from drought plots) had lower C:N ratio, relative to the control litter in both decomposition environments (H3: $F_{1,52} = 32.53$, $p < 0.001$; H4: $F_{1,56} = 9.54$, $p = 0.003$) suggesting both decomposition environment and litter origin affected litter properties during the experiment (C:N but also %C and %N; Figure 3; Supplementary Information Table S10 and S11).

Similarly, we observed a strong effect of decomposition environment on leaf litter C biochemistry (H3: $F_{1,56} = 6.73$, $p = 0.006$; H4: $F_{1,56} = 15.32$, $p = 0.002$) (Figure 2C-D); hydroxyl groups (B1) and amides (I and II, B4 and B6) were grouped together along PC1 in drought litter at both H3 and H4 time points. Conversely, carboxylates (B5), sulfones and esters (B10), along

with aromatics (B15) were correlated with ambient litter and both H3 and H4. We found divergent effects of the experimental drought on litter moisture content at O_3HP. At H3, litter moisture content significantly decreased (by around 35%) in drought plots ($F_{1,4}$ = 85.96, $p < 0.001$; Supplementary Information Table S10). However, at H4, when litter moisture content in control plots was already low, there was no significant difference between the control and drought plots ($F_{1,56}$ = 1.086, p = 0.30; Supplementary Information Table S11).

After 21 months of decomposition (H3), experimental litter (i.e., litter from the droughted plots) had greater concentrations of PLFAs in both decomposition environment ($F_{1,52}$ = 9.06, p = 0.004, Figure 4A), but the drought treatment reduced total PLFA concentrations ($F_{1,4}$ = 15.69, p = 0.02) in litter regardless of its origin. The F:B ratio was greater in litter bags from the drought environment across both litter types ($F_{1,4}$ = 19.42, p = 0.01, Figure 4B). Both litter origin ($F_{1,56}$ = 6.64, p = 0.004) and decomposition environment ($F_{1,56}$ = 23.59, $p < 0.001$; Figure 4C) shifted PLFA composition. Microbial composition in response to decomposition environments was largely explained by PC1 whereas microbial composition in response to litter origin was explained more by PC2. These axes reflected distinct PLFAs, with the fungal-related PLFA C18:2ω6,9 driving separation in both PC1 and PC2 (Figure 4C) with general bacteria and microbial markers (14:0, 15:0, 16:0 and 17:0) reflected litter origin differences on PC2. In addition, animal communities responded more to the decomposition environment than litter origin (Supplementary Information Table S10 and S11). Here, mesostigmatid (H3: $F_{1,56}$ = 31.18, $p < 0.001$) and prostigmatid mite (H4: $F_{1,56}$ = 7.65, p = 0.007) abundances significantly decreased, and there was a trend for drought to reduce total animal abundances at H4 ($F_{1,4}$ = 6.04, p = 0.07). Notably, there was a significant interaction of decomposition environment and litter origin on 'other' macrofauna

abundances at H3 (decomposition environment x litter origin: $F_{1,52} = 6.51$, $p = 0.01$) where abundances were greatest in the droughted plots with control litter.

In our expanded models to assess the drivers of litter mass loss at the third and fourth harvest, we found the same significant predictors of decomposition in both models. Here, litter originating from droughted plots (i.e., experimental litter) decreased mass loss rates at both time points (H3: $t = -2.25$, $p = 0.03$; H4: $t = -2.46$, $p = 0.017$). The drought treatment itself was no longer significant in these models, instead, higher C:N ratios in remaining litter in the third ($t = -4.37$ $p < 0.001$) and fourth ($t = -6.89$ $p < 0.001$) corresponded with lower mass loss rates as litter C-biochemistry (FTIR) PC1 was also a predictor of with mass loss in the third and fourth harvest (H3: $t = 3.02$, $p = 0.004$; H4: $t = 4.91$, $p < 0.001$). Together, these models show that litter properties, both initial and after decomposition had begun, were drivers of litter decomposition.

Discussion

The use of two large scale, relatively long-term experimental manipulations of drought and elevated atmospheric CO_2 provide results that address the need for 'ecologically relevant' (Rineau et al., 2019) investigations of the impact global change factors on soil C cycling processes. Our results reveal that long-term *in situ* manipulations of drought and elevated atmospheric CO_2 to mature oak forests have differing impacts on litter decomposition process, thereby supporting our first hypothesis. We found that litter incubated under trees subjected to long-term drought at O_3HP had greater C:N ratio during decomposition and reduced mass loss compared to litter incubated in ambient rainfall conditions. Litter mass loss models containing both treatments and measured parameters also revealed that a main correlate of litter mass loss was the origin of litter, i.e.,

whether leaves were subject to either drought or ambient rainfall prior to litterfall, at both the third and fourth harvest. Both the C:N ratio and the composition of secondary metabolites differed according to the origin of the litter as mass loss was lower in experimental litter. Conversely, in the elevated CO_2 experiment at BIFoR FACE, the decomposition environment was not a strong driver of litter decomposition, but litter originating from elevated CO_2 plots had slower decomposition rates. The effect of litter origin on mass loss in both experiments provides support for our second hypothesis that changes to litter properties stemming from drought and elevated CO_2 has long-term impacts on decomposition rate.

Our analyses indicated that litter origin, capturing treatment-driven changes in initial litter properties, affected mass loss months after the onset of decomposition. This finding suggests strong carry-over effects of these global change factors on decomposition acting *via* modifications on traits of living leaves. These effects occurred in the elevated CO_2 experiment even though significant differences in initial properties were not detected, suggesting that the treatment may have changed important litter traits that we did not measure. For example, it is possible that changes to leaf morphological as thickness, stemming from cell expansion (Pritchard et al., 1999; Gardner et al., 2022) and nutrient concentrations such as phosphorus and potassium (Cha et al., 2017), could have impacted decomposition. Regardless of the exact mechanism, the carry-over effect of litter origin was also reflected by the colonization and establishment of microbial communities (drought experiment) and animal abundances (elevated CO_2 experiment) in litter. Specifically, microbial biomass (measured as total PLFA concentration) was higher in the experimental litter at O_3HP, while fewer animals were present in experimental litter at BIFoR FACE. Overall, leaves grown under global change factors prior to litterfall continue to impact

483 decomposition and its drivers even when >50% of litter has been decomposed. This result
484 highlights the longevity of impacts of drought and elevated CO_2 on litter decomposition, and thus
485 C cycling within forests.
486
487 Litter mass loss in drought and elevated CO_2 experiments was explained by dissimilar
488 pathways. In the drought experiment, both litter origin and decomposition environment affected
489 remaining litter C:N, as litter C-biochemistry also showed strong responses to the droughted
490 decomposition environment, and together were strong correlates of mass loss rates. By contrast,
491 in the elevated CO_2 experiment, litter origin but not decomposition environment explained litter
492 mass loss. The lack of a decomposition environment effect at BIFoR FACE is surprising given
493 the numerous microbially-driven below-ground impacts reported at this site that are known to
494 regulate processes such as decomposition. These effects include increases in abundance and
495 turnover of mycorrhizal hyphae, root exudation, and root traits, such as branching frequency
496 (Kourmouli et al., 2025; Reay et al., 2025; Rumeau et al., 2025). One hypothesis that might explain
497 these findings is that the microorganisms driving the changes noted above are not tightly associated
498 with freshly fallen litter, but with other soil fractions. For example, there is strong evidence for
499 vertical segregation of ectomycorrhizal fungi in organic soils (Dickie et al., 2002; Rosling et al.,
500 2003) and therefore highly probable to find strong impacts of elevated CO_2 in soil fungal
501 communities but not on leaf litter as observed in our study.
502
503 Our experimental design enabled us to test for interactive effects between litter origin and
504 decomposition environment. This analysis is important because litter properties and decomposers
505 interact during decomposition (Prieto et al., 2019) and microbes and animals often show a

consistent ability to accelerate decomposition of 'home' litter relative to 'away' litter (i.e. the home field advantage) (Ayres et al., 2009; Veen et al., 2015). This variation is driven partly by litter quality, where recalcitrant litter is typically subject to stronger home field advantage than more labile litter (Wallenstein et al., 2013; Chomel et al., 2015). Nevertheless, at O_3HP there was no significant interaction between the litter origin and decomposition environment treatments with litter mass loss, suggesting that changes to the decomposer community or initial litter properties under droughted conditions was not strong enough to produce a synergistic or antagonistic response. There was also no interactive response between litter origin and decomposition environment in the elevated CO_2 experiment, although this observation is less surprising given that initial litter properties and microbial and animal communities did not show strong responses to the treatment.

In the drought experiment, microbial communities were strongly correlated with remaining litter C:N (shown with the Pearson correlation matrix), but this was not found in the elevated CO_2 experiment. This difference may be explained by the overall higher C:N ratios at O_3HP compared to BIFoR FACE. Fungal abundance is typically greater on recalcitrant litter because fungi possess enzymes that breakdown larger carbon-rich molecules and polymers, such as lignin, relative to bacteria (Romaní et al., 2006; Leifheit et al., 2024; Wang and Kuzyakov, 2024). Microbial community composition rapidly changes during decomposition (Voříšková and Baldrian, 2013; Gołębiewski et al., 2019) but litter C:N is strongly related to microbial communities, including F:B ratio, in litter layer material (Kohl et al., 2021). When comparing the two experiments, we found that the F:B was higher in the drought experiment, which had more recalcitrant litter, but lower in the elevated CO_2 experiment, which had more labile litter. By the third harvest, the

advanced state of decomposition in the elevated CO_2 experiment may have weakened the linkage between microbial communities to litter C:N, despite both being correlated with litter mass loss rate. Interestingly, remaining litter C:N was linked to animal abundances in the elevated CO_2 at the third harvest, as evidenced by litter with higher C:N (~25:1) containing fewer animals than litter with lower C:N (~18:1). This finding is supported by other work, albeit in different contexts. For instance, Holmstrup et al. (2017) found two years of elevated CO_2 to a heathland changed litter C:N that subsequently affected soil animal diversity, and Hasegawa and Takeda (1996) found that animal abundance increased with declining litter C:N during the first two years of decomposition in a secondary suburban forest in Kyoto. While the specific mechanisms linking litter C:N to animals remains to be identified, our findings show that linkages exist between resource quality and decomposers and that on-going decomposition will affect pathways between animals, microbes, and litter chemical properties, such as litter C:N. Further research should document how these pathways change over time under these and other global change factors.

Analysis of litter properties using FTIR provides a more detailed perspective of how decomposition shapes C biochemistry. Such detail is important to move away from a simplistic perspective of soil C and better understand how decomposition may influence the formation of specific C pools with different rates of turnover, which underpins recent concepts of soil C cycling (e.g., Lavallee et al., 2020). We found that FTIR signatures generally reflected mass loss rates as in our expanded litter mass models (containing both treatments and measurements), principal component axes from FTIR measurements were correlated with litter mass loss in both experiments. In addition, at BIFoR FACE, the composition of FTIR-functional groups between control and experimental litter converged between the third and fourth harvest as litter origin

effects on mass loss was minimal after two years of decomposition. Conversely, at O_3HP, decomposition environment still shaped FTIR functional groups of litter in the fourth harvest because decomposition was slower in the droughted plots. Here, we found matching responses between litter properties and mass loss between our two experiments. Whether litter properties converge (Moore et al., 2011) or diverge (Wickings et al., 2012) during mass loss will likely depend on interactions between time decomposed, litter properties, decomposer community, and environmental conditions. Overall, we found that FTIR functional groups in litter and mass loss responded similarly to decomposition environment and litter origin within each experiment.

We found that experimental litter from the elevated CO_2 experiment was populated with fewer mesostigmatid and prostigmatid mites at H4 than the control litter. Most mesostigmatid mites, and some prostigmatid mite species, are predators, feeding on arthropods and nematodes (Lindquist et al., 2009; Walter et al., 2009). Lower abundance of these mites suggests that changes to prey composition (but likely not group abundances in our experiments) hindered predators in our litterbags. Changing mesofauna and macrofauna predator composition can indirectly influence litter decomposition (Lang et al., 2014; Koltz et al., 2018) and similar effects may explain decomposition in our study. For example, predators can reduce competition amongst lower trophic levels resulting in higher litter decomposition (Melguizo-Ruiz et al., 2020), highlighting the complexity of these trophic interactions on litter decomposition. Although not statistically significant, there was a trend for oribatid mite abundance to be lower under drought than control conditions, which aligns with past studies (Santonja et al., 2017; Aupic-Samain et al., 2021).

Despite modest changes in animal abundances in litter, the expanded models of litter mass loss for BIFoR FACE showed that animal abundances (in addition to microbial community structure) were correlated with mass loss in the third harvest, supporting past work (Hättenschwiler et al., 2005; Rouifed et al., 2010; Handa et al., 2014; Tan et al., 2021; Njoroge et al., 2022). By contrast, only litter properties, litter C:N and litter C-biochemistry, best explained mass loss in the drought experiment, reflecting the lack of effects of animals in this treatment. We suggest two potential reasons for the contrasting influence of animals between the two experiments: First, there are notable climate differences between the two sites; BIFoR FACE is in a cool, moist temperate-maritime climate (MacKenzie et al., 2021), while O_3HP is in a seasonally hot and drier Mediterranean climate (Gauquelin et al., 2011) (Figures S2 and S3). Joly et al. (2023) highlighted the importance of macroclimate (i.e., temperature and precipitation) in litter decomposition, as animal contributions to decomposition can be strongly climate dependent (Wall et al., 2008) which may have contributed to the differing significant pathways between these models. Second, the decomposer community was strongly correlated to mass loss at later stages of decomposition; potentially, the semi-advanced state of the decomposed litter led to significant linkages to both litter properties and decomposers at BIFoR FACE, and not at O_3HP where decomposition rates were slower. By the fourth harvest, when only ~20% of litter remained, animal abundances were no longer correlated with litter mass loss rate. Because of the relative influence of litter properties, biota, and climate on decomposition will shift over time (Currie et al., 2010; García-Palacios et al., 2016; Canessa et al., 2021), future research should recognize that climate x temporal interactions may be critical to disentangling the contributions of litter properties and local environmental conditions on biota and subsequently litter decomposition.

Conclusions

We investigated how drought and elevated atmospheric CO_2 independently affect litter decomposition in mature forests by conducting reciprocal transplants within two large-scale manipulative field experiments, BIFoR FACE (elevated CO_2) and O_3HP (drought). Our findings reveal how separate *in situ* manipulation of elevated CO_2 or drought impact litter decomposition through distinct pathways involving changes in either litter properties prior to decomposition or the decomposition environment itself. Consistent with our hypotheses, drought exerted the strongest control on decomposition, primarily through environmental constraints such as reduced soil moisture and altered microclimatic conditions. In addition, both drought and elevated CO_2 impacted litter mass loss through modifications of litter properties prior to the onset of decomposition. This litter-origin effect provides clear evidence that the impact of drought and elevated CO_2 leads to strong carry-over effects on decomposition, confirming the importance of pre-litterfall changes in litter properties alongside *in situ* environmental modification. Future climate scenarios of increased atmospheric CO_2 concentrations will alter litter decomposition through alterations to litter properties, which may include C:N ratio and underlying litter chemical functional groups. Reduced seasonal rainfall will both strongly shape litter properties prior to litterfall and during decomposition, alongside the associated decomposer communities. Overall, these findings provide a more complete understanding of how atmospheric global change factors shape C cycling in forests ecosystems and highlight a need to disentangle their individual and interactive effects on litter inputs, properties and decomposition dynamics.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

Data will be submitted to the Natural Environment Research Council (NERC) data repository (Environmental Information Data Center) upon acceptance of the manuscript.

Author Contributions

DJ and MC conceptualized the study, and DJ, MC, VB, RDB, ARMK obtained funding. MLM, MC, ZV and KJF collected data. ARMK and VB help designed the elevated CO_2 and drought sites, respectively, and coordinated work there. MLM and MC curated data, and MLM performed the data analysis with support from TC. MLM wrote the first version of the manuscript and MC,

KJF, TC, ARMK, VB, RDB, and DJ provided feedback and contributed to finalizing the manuscript.

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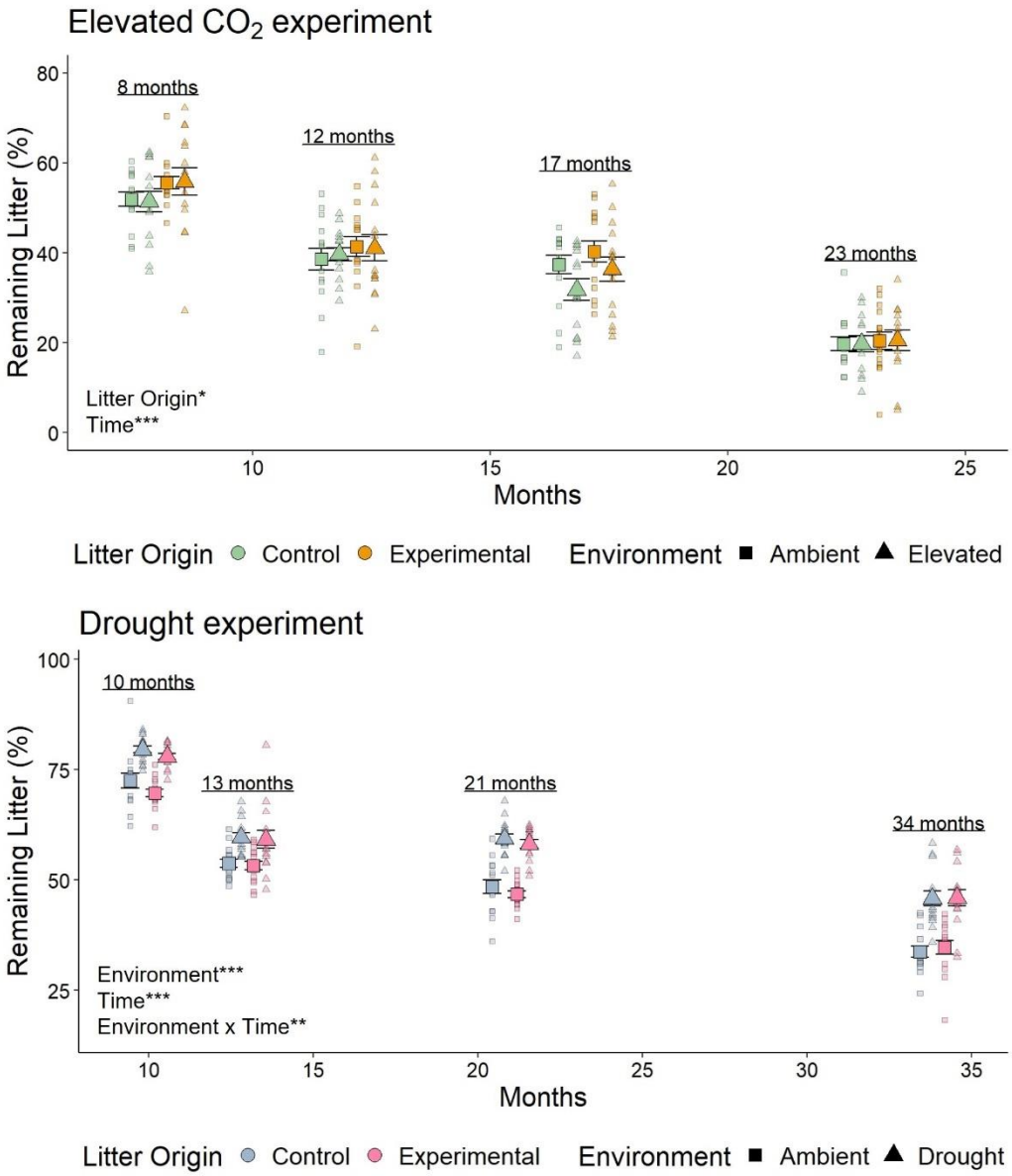

Figure 1. Litter decomposition (shown as % remaining litter) for the elevated CO_2 experiment (top) and drought experiment (bottom). The x-axis indicates the number of months since initiation of the experiment and differs for upper and lower panels. Larger points represent mean (± SE) while smaller points are the raw data values. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided p-values of factors that were statistically significant. Litter bags in the elevated CO_2 experiment were harvested after 8 (H1), 12 (H2), 17 (H3) and 23 (H4) months, while litter bags in the drought experiment were harvested after 10 (H1), 13 (H2), 21 (H3), and 34 (H4) months. To improve plot interpretation, points were positioned with greater spacing on the x-axis. Note the differences in y axis-es between the two experiments if comparing results with one another.

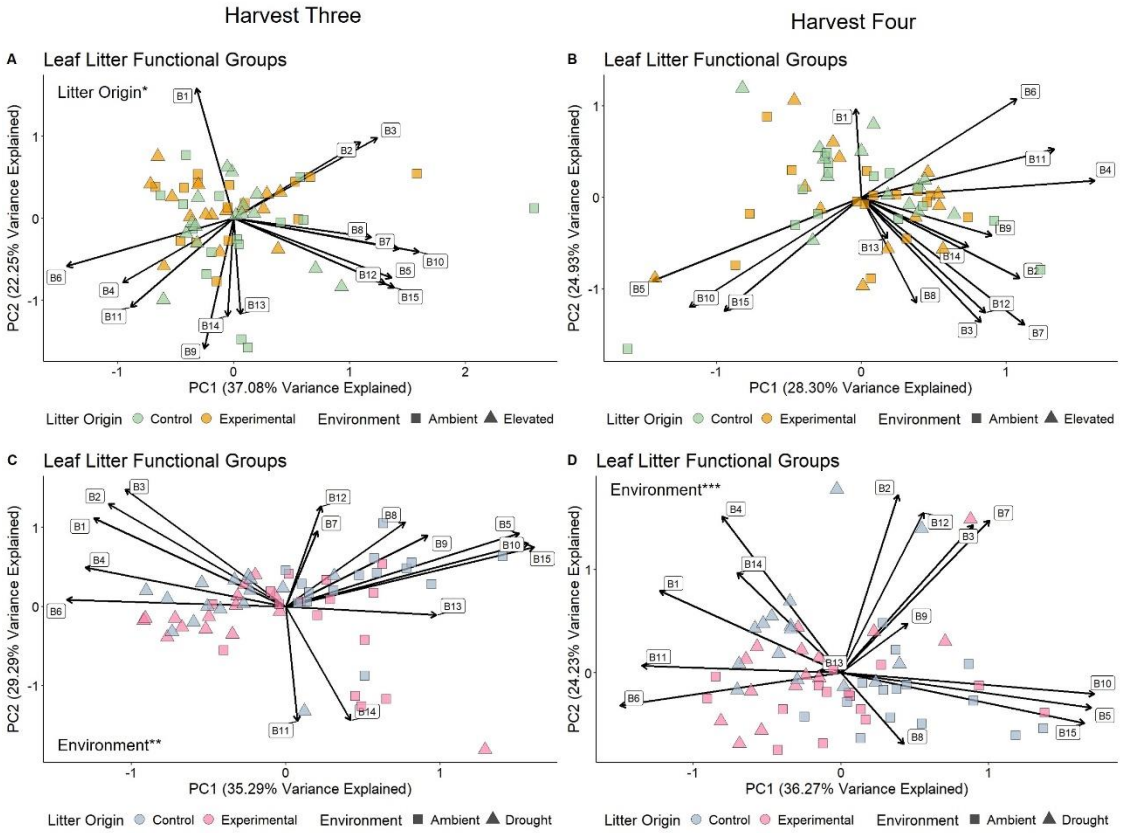

Figure 2. Principal component analysis (PCA) ordinations of leaf litter functional groups measured by Fourier transform infrared (FTIR) spectroscopy for the elevated CO_2 experiment (top) and drought experiment (bottom) at harvest three (A and C, left) and four (B and D, right). Corresponding functional groups for band numbers (B1, B2, B3, etc.) can be found in Supplementary Information Table S1. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided p-values of factors that were statistically significant.

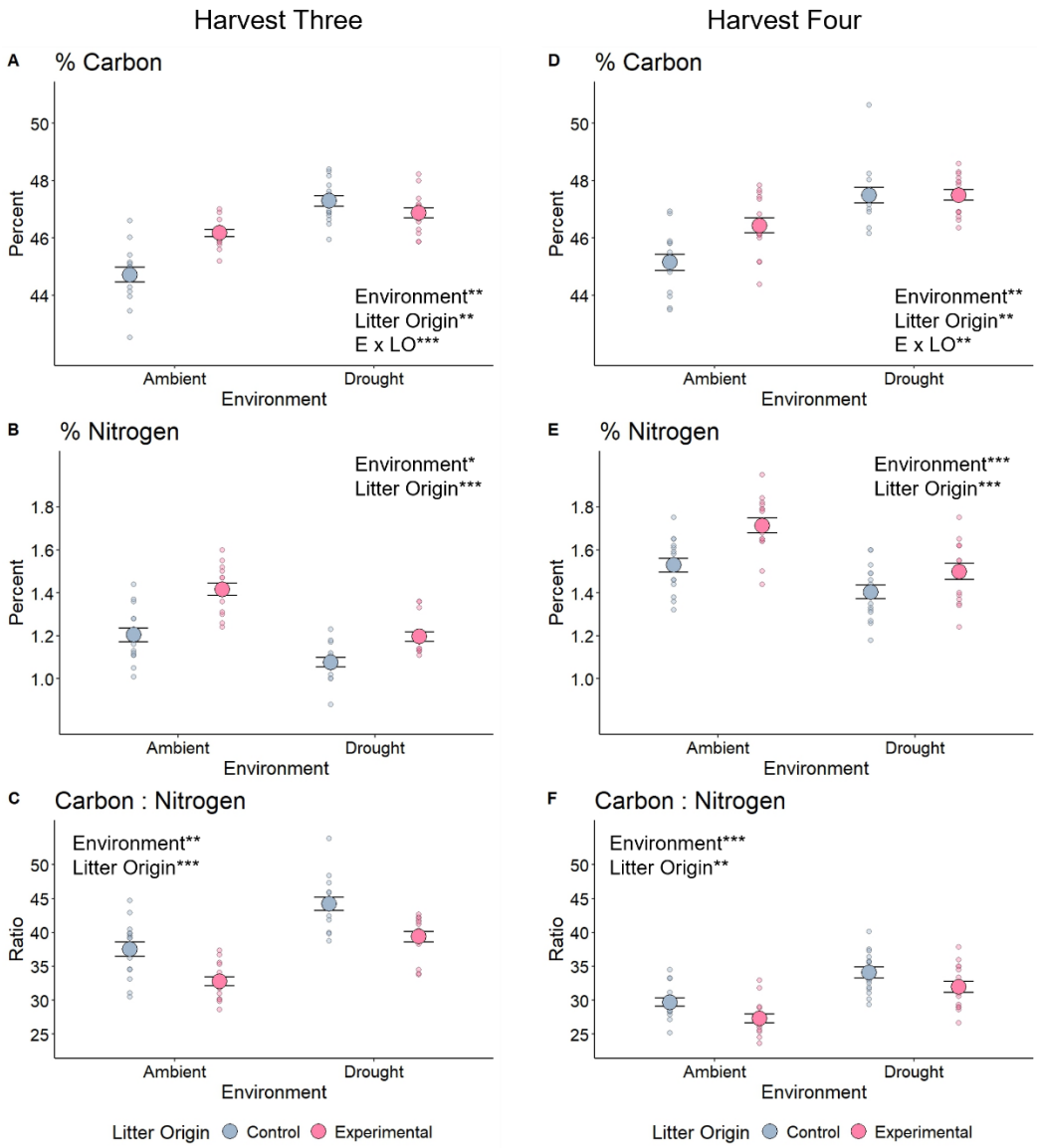

Figure 3. % Carbon (%C; A and D), % Nitrogen (%N; B and E) and C:N (C and F) of drought experiment litter sampled at the at third (left) and fourth harvest (right). Larger points represent mean (± SE) while smaller points are the raw data values. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided p-values of factors that were statistically significant. E = Environment and LO = litter origin.

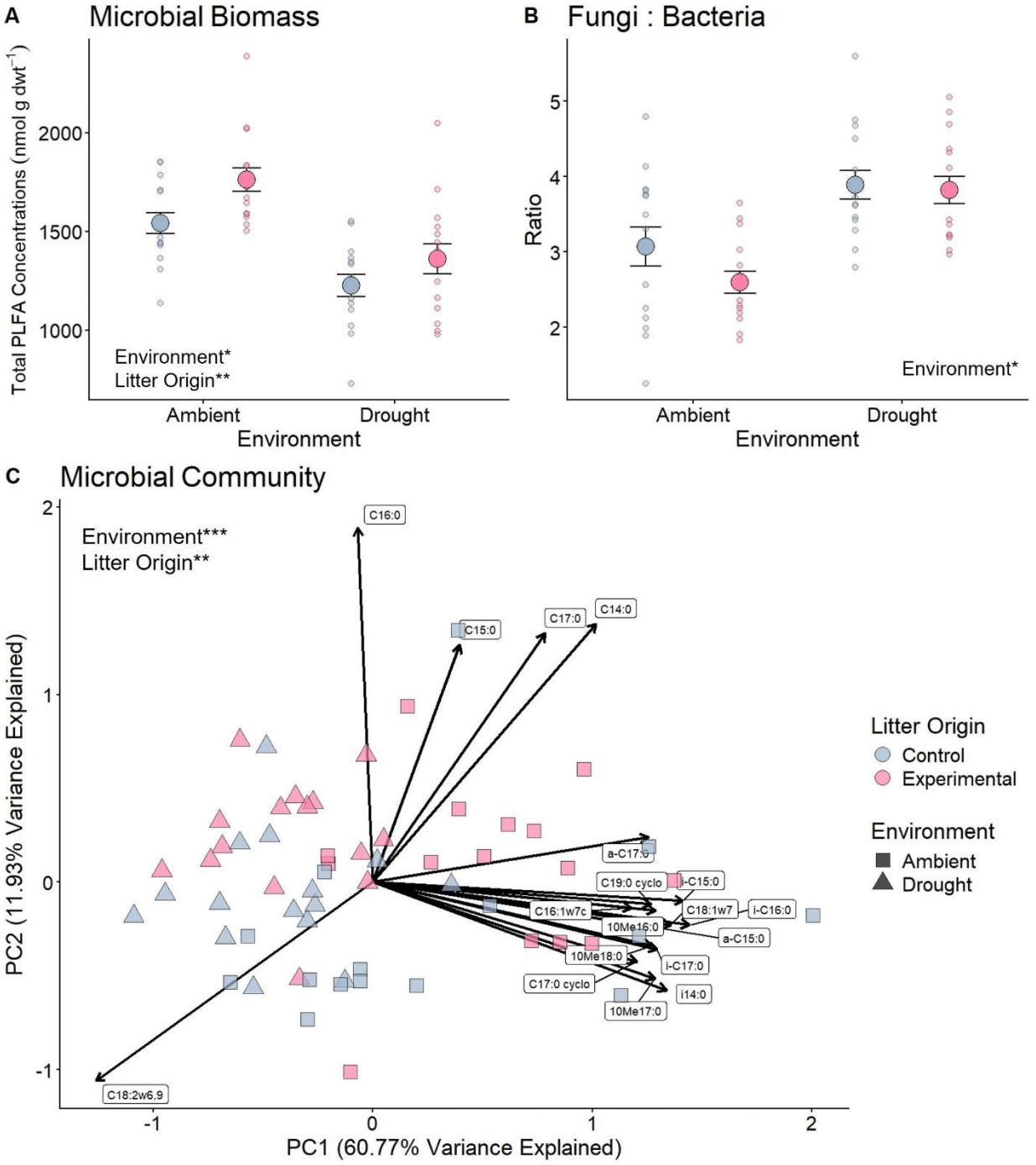

Figure 4. Microbial community indices as measured by phospholipid fatty acid analysis (PLFA) in litter for the drought experiment after 21 months (harvest 3). For A) (total PLFA) and B) (fungi : bacteria) larger points represent mean (± SE) while smaller points are the raw data values. C) Principal component analysis (PCA) ordination of microbial community structure. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided p-values of factors that were statistically significant.