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

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Summary

- 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/or 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, chemistry, moisture content, and decomposer communities.
- 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 and C:N changes were regulated by initial litter properties and the decomposition environment, while elevated CO_2 acted *via* changes to 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: BIFoR FACE, Decomposition, Drought, Elevated CO_2, Mesofauna, Litter, Microbial, O_3HP

Plain Language Summary

In mature oak woodlands, global change factors like drought and elevated atmospheric CO_2 concentrations can impact litter decompositions, an important ecosystem process, *via* changes to litter properties and the decomposer community. Examining links between litter properties, decomposers and decomposition is therefore critical to understand how both drought and elevated CO_2 will affect nutrient release and cycling of belowground environments.

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 & 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 (mites, collembolans, etc.) 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 those 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 (incubation) environment). Here, the mechanisms, the

direction, and the magnitude of responses to initial litter properties and decomposition environment against elevated CO_2 and drought likely differ substantially.

Drought and elevated atmospheric CO_2 can affect litter decomposition by modifying the 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. Here, 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 C below-ground inputs. 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 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 less C and allocate less below-ground (Hagedorn *et al.*, 2016), although when plants are recovering from drought, they tend to 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 & 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 & Wang, 2025). For elevated CO_2, N dilution within leaves together with C, can alter the C:N ratio (Taub & 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 & 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 require further examination.

Disentangling the effects of litter origin 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 initial litter properties and decomposition environment interactions 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 hypothesized that drought and elevated CO_2 differentially impact due to their contrasting impacts

on soil properties and microclimate. The second pathway involves litter origin, which occurs when litter originating from different treatments (e.g. control vs. drought, or control vs. elevated CO_2) decomposes within the same environment. These effects reflect how plant growth under drought and elevated CO_2 conditions modify litter properties, especially chemistry (e.g. C:N ratio, lignin, tannins), prior to litterfall and subsequent decomposition. We hypothesized that litter originating from treated plots would have long-term (carry over) effects on decomposition stemming from differences in litter properties. Our reciprocal transplant design allowed us to test whether interactive effects between litter origin and decomposition environment influence decomposition rates when subject to global change drivers.

Methods

Overview

Within each facility, we collected control and experimental litter and in January 2020, transplanted the two litter types into both control and treated plots (Supplementary Information Figure S1). We harvested litterbags across four time points and measured litter decomposition, litter chemistry and metabolomics, and microbial (phospholipid fatty acid analysis; PLFA) and animal community structure (the abundance of mites, collembolans and other larger macrofauna) directly associated with the recovered litter. We then constructed structural equation models (SEMs) to test for significant pathways between litter moisture, C:N, and the decomposer community with litter decomposition to determine how these relationships are affected by drought or elevated atmospheric CO_2.

Experimental sites

The reciprocal transplant study used the BIFoR FACE (52.801°N, 2.301°W) experiment in the United Kingdom and the O_3HP (43.965°N, 5.878°E) 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 lateral 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 in 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 (e.g., 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 placing the litterbags on the ground surface and then replaced over the litterbags. Litterbags were then fixed with two galvanizes nail 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 seasons 4 and 5 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). Decomposition duration differed between experimental sites due to variation in decomposition rate, we initially aimed for ~24 months of decomposition, incubation in the drought experiment was extended by an additional year to ensure at least 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.

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 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 functional chemical composition of H3 and H4 litter from both sites using Fourier transform infrared (FTIR) spectroscopy (Bell *et al.*, 2018). Ball-milled litter samples were placed onto a Universal Attenuated Total Reflection (UATR) spectrometer diamond crystal to cover the 2-mm window, and the absorbance (A cm^{-1}) was measured within a 650 to 4000 A cm^{-1} range, at 4 cm^{-1} resolution and 16 scans per sample. We also ran a background correction after every ten samples. We applied a baseline correction for each spectra using the modpolyfit method in the R package 'baseline'. To standardize values across our samples, we Z-transformed each spectra, where values were transformed to a mean of 0 and SD of 1 by subtracting by its own mean and dividing by its own standard deviation (akin to a Standard Normal Variate normalization) to reduce multiplicative scatter effects (Jardine *et al.*, 2017; Bell *et al.*, 2018). We then calculated the area of the curve for each respective functional group using a shoelace formula (Wang *et al.*, 2023). The assignment of wave number regions to leaf litter functional groups was based on Liu *et al*., (2016) who used FTIR to examine functional groups across four deciduous litter origins, including *Quercus robur* (Supplementary Information Table S1). Finally, we also calculated leaf litter moisture content for H3 and H4 litter from the chemical compartment of the litterbag.

Phospholipid fatty acid analysis (PLFA)

We used PLFAs to measure how fungal and bacteria concentrations and the general microbial community were impacted by drought and elevated CO_2 in H3 leaf litter at both sites

(Buyer & Sasser, 2012). We quantified PLFAs with ~0.4-0.5 g of ball milled, freeze-dried litter. Four ml of Bligh-Dyer extractant containing C19:0 of an internal standard was added to each sample to extract lipids. 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) (Liu *et al.*, 2016; Biryol *et al.*, 2024) bacteria, respectively. In addition, we used 14:0, 15:0, 16:0 and 17:0 as general bacteria markers (Wang *et al.*, 2016; Dhaou *et al.*, 2022), and 10Me16:0, 10Me17:0, and 10Me18:0 as markers for actinobacteria (Biryol *et al.*, 2024). The marker 18:2ω6,9 was the sole fungal marker in our experiments (Aupic-Samain *et al.*, 2021; Biryol *et al.*, 2024). 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 community composition was quantified on H3 and H4 litter at both sites and was extracted using Tullgren funnels over seven days into absolute ethanol. 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.

Statistical analysis

All statistical analyses were performed with the program R (version 4.3.0) (R Core Team, 2023) and figures 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 decomposition environments (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), microbial community composition, and animal 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. For microbial and animal 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 structural equation models (SEMs) to construct a global model to analyze the pathways by which drought and elevated CO_2 affect litter decomposition on H3 data. We chose to use data from H3 where mass loss was on average 40-55% from the drought experiment and 60-70% for the elevated CO_2 experiment (Figure 1), ensuring that decomposition was on-going within our study and had not yet plateaued. We constructed three *a priori* models for the drought and elevated CO_2 experiment (Supplementary Information Figure S4) to detect significant pathways ($p < 0.05$) between parameters (Garrido *et al.*, 2022). Here, we tested whether litter properties (remaining litter C:N and moisture content), the decomposer community (animals and microbials) or both litter properties and decomposers best explained litter mass loss in our experiments. Within our SEMs, we included decomposition environment and litter origin as binary variables where elevated CO_2 or drought litter (litter origin) and elevated CO_2 or drought environment (decomposition environment) where coded as 1 (with the respective controls coded as 0) to reduce the number of pathways in our model. As well, we Z-transformed litter mass loss, C:N ratio, litter moisture content, along with animal and microbial abundance, but not PCA axes, to standardize parameters. To include parameters on microbial and animal communities in our SEM that best described litter mass loss, we constructed four candidate models (linear mixed models with experimental plot as random factor) that contained either total animal abundance or animal community PC1 (first axis from PCA), along with total microbial abundance or microbial PC1. We compared models using AIC (Akaike Information Criterion) with maximum likelihood and chose the candidate model with the lowest AIC value to be included in the SEM. To construct SEM models, we used the psem function in the 'piecewiseSEM' package (Lefcheck, 2016) where individual models within the global SEM were fitted with linear mixed models (with plot as random factor) with maximum likelihood, using the lme function from the 'nlme' package

(Pinheiro & Bates, 2000; Pinheiro *et al.*, 2023). To evaluate our SEM models, we used AIC and a goodness of fit test of Fisher's C, where p-values > 0.05 represents acceptable data fit for our model. After comparing our three SEM models, that differed in pathways for litter mass loss (and selecting the best model based on AIC), we re-ran all individual linear mixed models within our SEMs with restricted maximum likelihood (REML) and reported those results. After re-running individual linear models with REML, we checked for collinearity if models had more than one term using variance inflation factor (VIF) scores. All VIF scores were < 3 representing absent or low multicollinearity within models.

Results

Initial litter chemistry

We found strong differences between the C:N ratio of the initial control and droughted litter ($F_{1,8} = 13.92$, $p = 0.006$; Supplementary Information Table S2), 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 S5). 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 S2) or metabolomic profiles ($F_{1,8} = 1.04$, $p = 0.336$; Supplementary Information Figure S5) between litter from ambient and elevated CO_2 treatments.

Elevated CO_2 experiment

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 S3 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 S4 and S5). This finding was also supported by FTIR spectroscopy analysis, which showed significant differences in litter functional chemical composition at H3 between litter origins ($F_{1,56} = 4.33$, $p = 0.03$). Litter functional group 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 S4 and S5).

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 S4, Supplementary Information Figure S6). Likewise, no individual or total animal group abundances, along with entire community structure, 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, but animal abundance composition did not shift with litter origin ($F_{1,48} = 1.24$, $p = 0.28$; Supplementary Information Table S5).

The SEM revealed several significant pathways between parameters in response to elevated CO_2 (Figure 5) with strong goodness-of-fit where model 1 (containing both abiotic and biotic factors) best explained mass loss. As with our univariate models, the SEM indicated litter origin affected remaining litter C:N, reflected by experimental litter having slightly higher C:N ratio at H3. Litter moisture content was negatively correlated with microbial community structure. On PC1, microbial composition was driven by fungal abundance (PLFA marker C18:2ω6,9; Supplementary Information Figure S6); the negative association between moisture and microbial community composition suggests that drier litter supported greater fungal abundance. Furthermore, we found a negative correlation between remaining litter C:N and animal abundance, with higher C:N ratio corresponding with lower animal abundances. Animals, microbes and remaining litter C:N explained litter mass loss rates at H3, highlighting the roles of both decomposer communities and litter properties in affecting litter decomposition rate. In accordance with our first hypothesis, decomposition environment had no significant effect in the SEM, further

supporting that elevated CO_2 treatment did not directly affect litter decomposition or associated microbial or animal communities.

Drought experiment

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 over time ($F_{1,220} = 494.76$, $p < 0.001$) and at varying rates (decomposition environment x time: $F_{1,220} = 4.56$, $p = 0.004$; Figure 1; Supplementary Information Table S3). 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 S6 and S7).

Similarly, we observed a strong effect of decomposition environment on leaf litter functional chemical composition (Figure 2C-D). FTIR spectroscopy revealed that drought shifted litter C biochemistry (H3: $F_{1,56} = 6.73$, $p = 0.006$; H4: $F_{1,56} = 15.32$, $p = 0.002$); 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 S6). 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 S7).

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 the control litter in both decomposition environments ($F_{1,52} = 8.43$, $p = 0.005$, 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 (Fig. 4C) with general bacteria 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 S6 and S7). At both H3 and H4, animal communities shifted under drought (H3: $F_{1,56} = 8.07$, $p < 0.001$; H4: $F_{1,56} = 6.70$, $p < 0.001$): 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' fauna 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.

The SEM for the drought experiment yielded several significant pathways and had acceptable goodness-of-fit where model 2 (abiotic factors only) best explained mass loss (Figure 5). Litter origin directly affected mass loss in our SEM while remaining litter C:N was the strongest, direct correlate of mass loss in the drought experiment. The droughted plots lowered litter moisture content which affected microbial communities, as lower levels of moisture corresponded with fungal-rich microbial communities. The complexity of the effect of drought on litter decomposition was reflected by multiple significant pathways in the SEM, including acting *via* litter origin, moisture content and microbial communities.

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 had greater C:N ratio during decomposition and reduced mass loss compared to litter incubated in ambient rainfall conditions. The SEM 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. Both the C:N ratio and the composition of secondary metabolites differed according to the origin of the litter, and both directly and indirectly affected mass loss. Conversely, in the elevated CO_2 experiment, the decomposition environment was not a strong driver of litter decomposition, but litter originating from elevated CO_2 plots had slower decomposition rates. Litter origin affecting 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 at 17 months (elevated CO_2 experiment) and 21 months (drought experiment) after the onset of decomposition. This finding suggests strong carry-over effects of these global change factors on decomposition acting *via* modifications on the chemistry of living leaves. These effects occurred in the elevated CO_2 experiment even though significant differences in initial properties were not detected, suggesting 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 (drought experiment) and animal (elevated CO_2 experiment) communities in litter. Specifically, microbial biomass was higher and the abundance of fungi relative to bacteria (i.e., F:B ratio) lower in experimental litter in both decomposition environments at O_3HP while fewer animals were present in experimental litter in

both incubating environments at BIFoR FACE. Overall, leaves grown under global change factors prior to litterfall continue to impact decomposition and its drivers even when >50% of litter has been decomposed. This result highlights the longevity of impacts of drought and elevated CO_2 on litter decomposition, and thus C cycling within forests.

Litter mass loss in drought and elevated CO_2 experiments was explained by dissimilar pathways. In the drought experiment, both litter origin and decomposition environment affected remaining litter C:N, which was a strong correlate of mass loss rates. By contrast, the elevated CO_2 SEM showed that litter origin but not decomposition environment explained litter mass loss. The lack of a decomposition environment effect at BIFoR FACE is surprising given the plethora of microbially-driven below-ground impacts reported at this site that are known to regulate processes such as decomposition. These effects include increases in abundance and turnover of mycorrhizal hyphae, root exudation, and root traits, such as branching frequency (Rumeau *et al.*, 2025; Kourmouli *et al.*, 2025; Reay *et al.*, 2025). One hypothesis that might explain these findings is that the microorganisms driving the changes noted above are not tightly associated with freshly fallen litter, but with other soil fractions. For example, there is strong evidence for vertical segregation of ectomycorrhizal fungi in organic soils (Dickie *et al.*, 2002; Rosling *et al.*, 2003) and therefore highly probable to find strong impacts of elevated CO_2 on soil properties but perhaps not with recently fallen leaf litter as observed in our study.

Our experimental design enabled us to test for interactive effects between litter origin and decomposition environment. This analysis is important because litter properties and decomposers interact during decomposition (e.g. 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 statistical interaction between litter origin and decomposition environment 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 (specifically PC1) were strongly correlated with remaining litter C:N, but this link was not present in the elevated CO_2 SEM. 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, like lignin, relative to bacteria (Romaní *et al.*, 2006; Wang & Kuzyakov, 2024; Leifheit *et al.*, 2024). Microbial community composition rapidly changes during decomposition (Voříšková & 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 & 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. For example, at BIFoR FACE, differences in FTIR-functional groups between control and experimental litter converged between the third and fourth harvest. 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, irrespective of litter origin. 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 (Aupic-Samain *et al.*, 2021; Santonja *et al.,* 2017).

Despite modest changes in animal abundances in litter, the SEM from BIFoR FACE showed that both 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 C:N 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 is strongly climate dependent (Wall *et al.*, 2008) which may have contributed to the differing significant pathways in our SEM models. Second, the decomposer community was strongly correlated to mass loss at later stages of decomposition when around half of the original litter mass remained. Potentially, the semi-advanced state of the decomposed litter led to direct significant linkages to both litter properties and decomposers. Because 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

In our study, 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. Our findings reveal how separate *in situ* manipulation of elevated CO_2 and drought impact litter decomposition through distinct pathways involving changes in litter origin and decomposition environment. Consistent with our hypotheses, drought exerted the strongest control on decomposition, primarily through environmental constraints such as reduced soil moisture and altered microclimatic conditions that can limit decomposer activity. 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 results provide clear evidence for the impacts of drought and elevated CO_2 global change drivers on the life stage of leaves 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. While 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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Conflict of Interest Statements

The authors have no conflict of interest to declare.

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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The following Supporting Information is available for this article:

Fig. S1 Overview of the reciprocal transplant experimental design

Fig. S2 Monthly average temperature and total precipitation at BIFoR FACE

Fig. S3 Monthly average temperature and total precipitation at O_3HP

Fig. S4 *A priori* conceptual models for structural equation models (SEMs)

Fig. S5 Principal component analysis (PCA) of initial litter metabolomic profile

Fig. S6 Phospholipid fatty acid analysis (PLFA) for BIFoR FACE

Image S1 Example of harvested litterbag

Table S1 Band numbers and regions for Fourier transform infrared (FTIR) spectroscopy

Table S2 Summary of initial litter %C, %N, and C:N used in the study

Table S3 Summary table of mean (± SE) of the exponential decomposition rate coefficient (k)

Table S4 Summary table of mean (± SE) of results from BIFoR FACE for harvest three

Table S5 Summary table of mean (± SE) of results from BIFoR FACE for harvest four

Table S6 Summary table of mean (± SE) of results from O_3HP for harvest three

Table S7 Summary table of mean (± SE) of results from O_3HP for harvest four

Table S8 AIC (Akaike Information Criterion) and Fisher's C of SEM models

Methods S1 Metabolomic signature of initial litter

Fig. S7 Example output comparing the metabolomics profile of initial litter from BIFoR FACE (within **Methods S1**)

Fig.S8 Example output comparing the metabolomics profile of initial litter from O_3HP (within **Methods S1**)

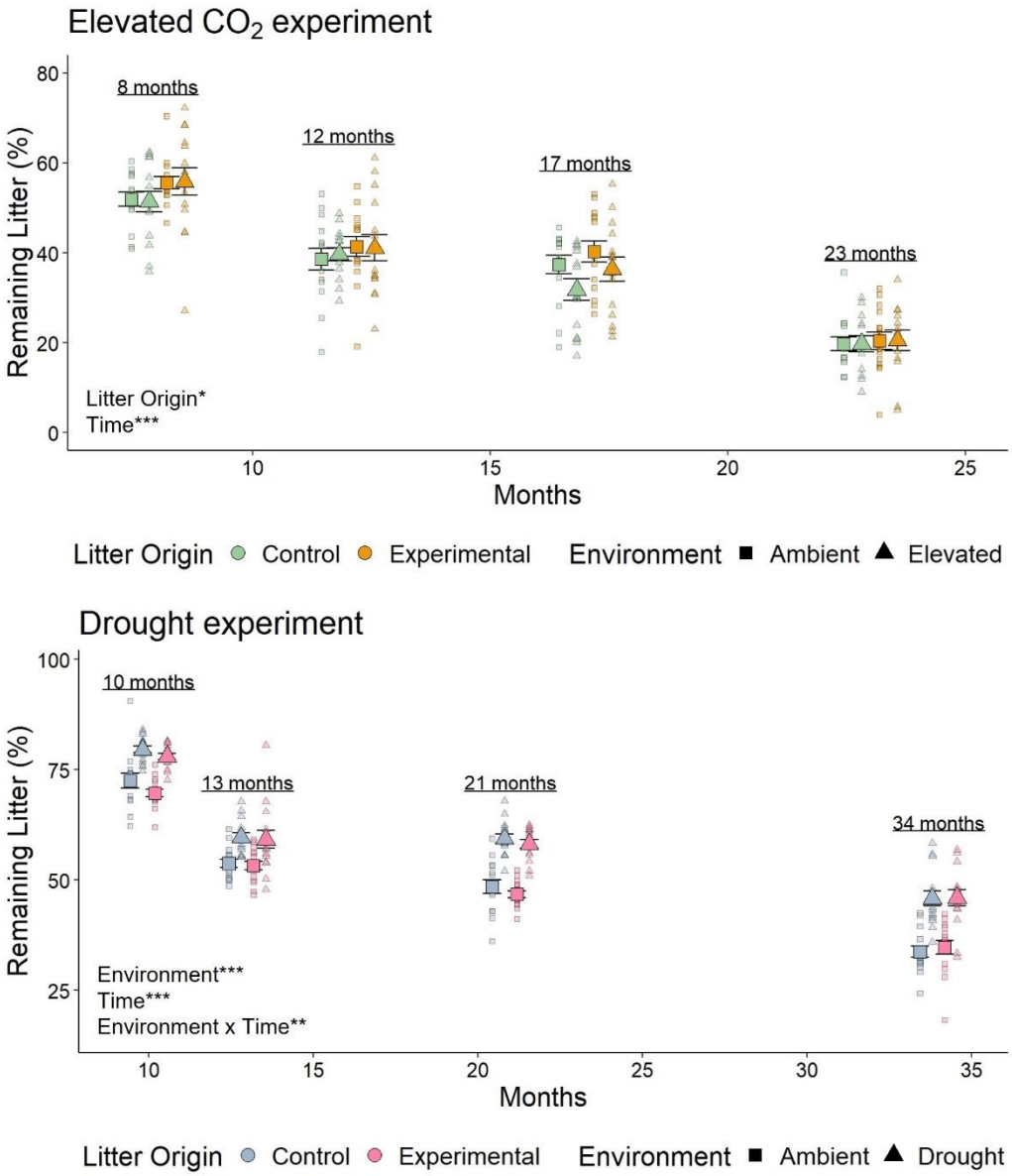

Figure 1. Litter decomposition (shown as % remaining litter) for the elevated CO_2 experiment (top) and drought experiment (bottom). The x-axis indicates the number of months since initiation of the experiment and differs for upper and lower panels. Larger points represent mean (± SE) while smaller points 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.

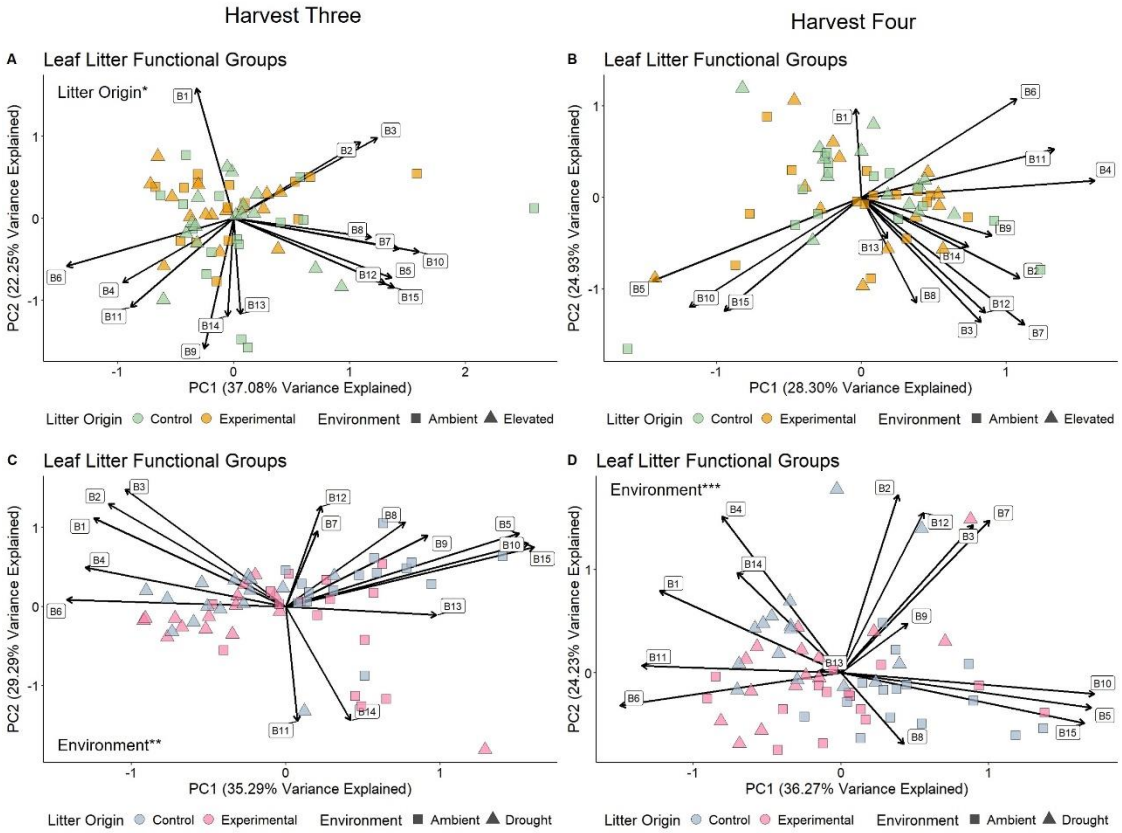

Figure 2. Principal component analysis (PCA) ordinations of leaf litter functional groups measured by Fourier transform infrared (FTIR) spectroscopy for the elevated CO_2 experiment (top) and drought experiment (bottom) at harvest three (A and C, left) and four (B and D, right). Corresponding functional groups for band numbers (B1, B2, B3, etc.) can be found in Supplementary Information Table S2. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided p-values of factors that were statistically significant.

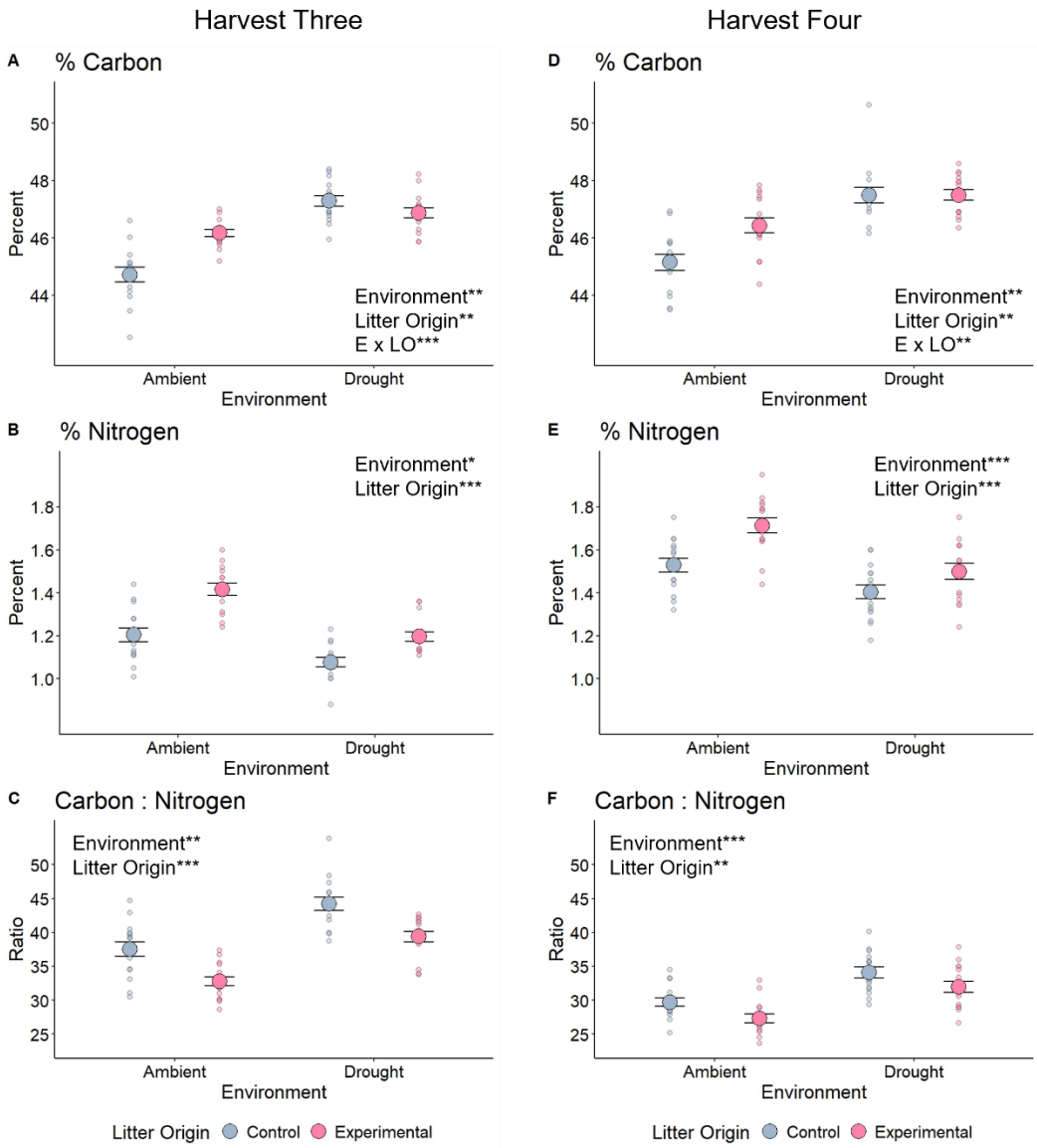

Figure 3. % Carbon (%C; A and D), % Nitrogen (%N; B and E) and C:N (C and F) of drought experiment litter sampled at the at third (left) and fourth harvest (right). Larger points represent mean (± SE) while smaller points are the raw data values. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.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

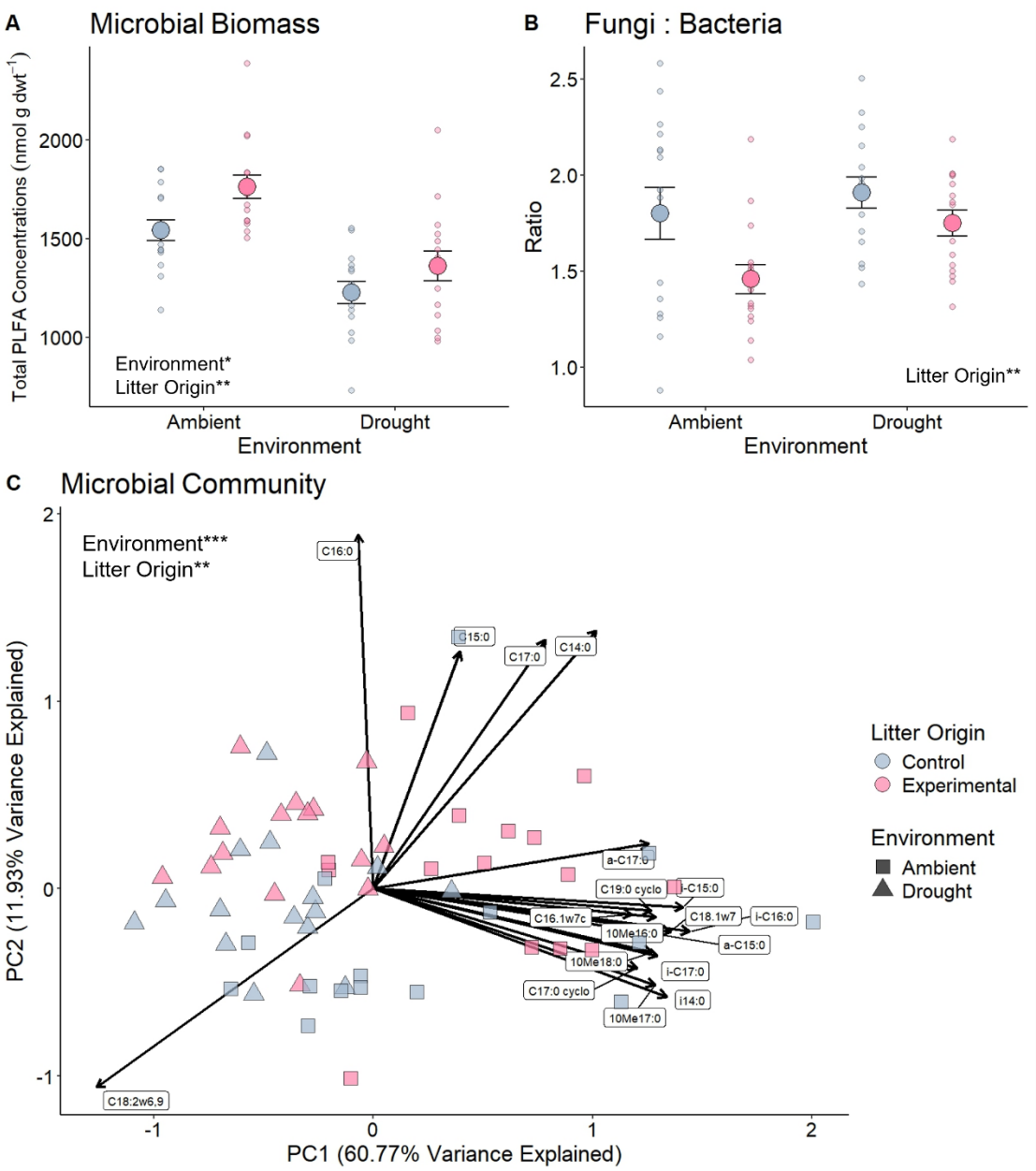

Figure 4. Microbial community indices as measured by phospholipid fatty acid analysis (PLFA) in litter for the drought experiment after 21 months (harvest 3). For A) (total PLFA) and B) (fungi : bacteria) larger points represent mean (± SE) while smaller points are the raw data values. C) Principal component 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.

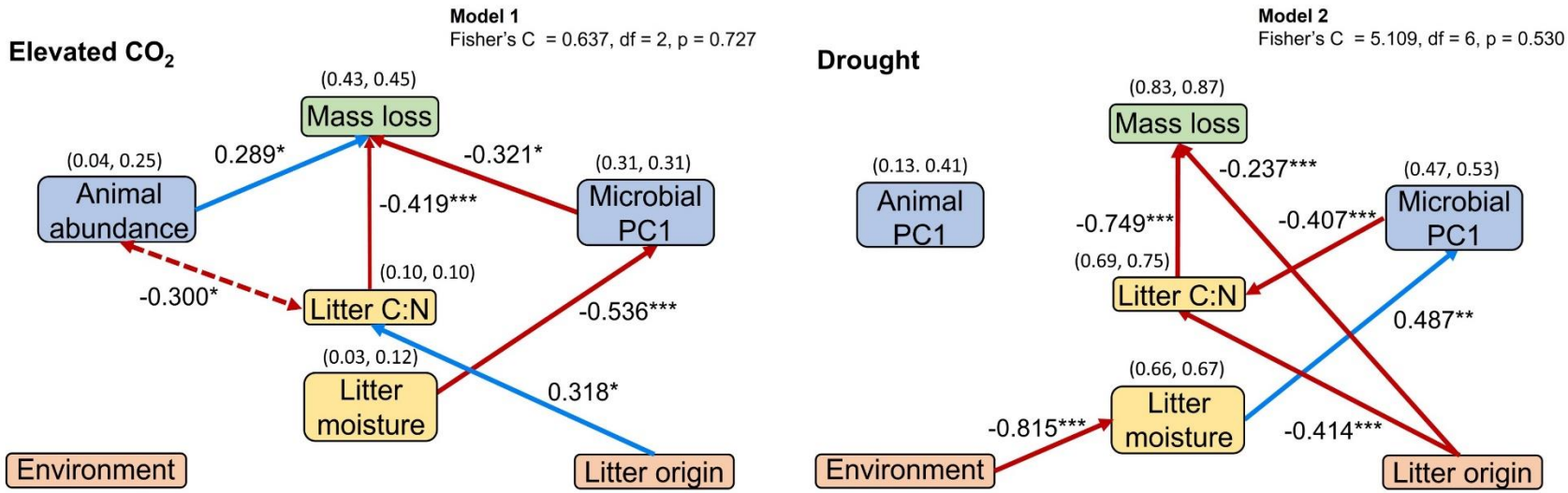

Figure 5. Structural equation models (SEMs) showing significant pathways between parameters from the elevated CO_2 experiment (n = 56, left) and drought experiment (n = 60, right). Data to parameterize models were derived solely from the third harvest in both experiments. Four litterbags were removed from analysis from the elevated CO_2 model as microbial community structure was not measured. Blue arrows indicate positive, significant pathways, while red pathways indicate negative, significant pathways where the standardized path estimates are provided. Solid arrows represent pathways used in our SEM model, with dotted arrows specifically represent correlations between parameters. Parameters are coloured by categories: experiment manipulations (decomposition environment and litter origin, light red), litter properties (litter moisture and C:N, yellow), decomposers (animals and microbes, blue) and mass loss (green). Asterisks indicate significance of path, * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. We have only provided parameter p-values that were statistically significant. Numbers above individual parameters indicate marginal and conditional R-square of models. In the top-right corner of each figure shows the goodness-of-fit test of Fisher's C. Please see Supplementary Information Table S8 for AIC values and goodness-of-fit metrics for all models tested with maximum likelihood for individual linear mixed models.