Title: Elevated CO_2 enhances decomposition and modifies litter-associated fungal assemblages in a natural *Eucalyptus* woodland

Authors:

Uffe N Nielsen*, Dylan Bristol, Michaela Blyton, Brendan Delroy, Jeff R Powell*#

Author affiliations:

Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith 2751, New South Wales, Australia

***Equal contribution, #Corresponding author – jeff.powell@westernsydney.edu.au**

Running title: Elevated CO_2 affects litter decay and fungi

Keywords: EucFACE, herbivory, Illumina MiSeq, nitrogen, phosphorus

ORCID:

Uffe Nielsen: 0000-0003-2400-7453

Dylan Bristol: 0000-0001-7356-3152

Michaela Blyton: 0000-0002-6112-5320

Jeff Powell: 0000-0003-1091-2452

Abstract

Litter decomposition is a key process governing carbon and nutrient cycles in forest ecosystems that is expected to be impacted by increasing atmospheric carbon dioxide (CO_2) concentrations. We conducted two complementary field studies to assess the effects of elevated CO_2 on *Eucalyptus tereticornis* litter decomposition processes. First, we used bags of two different mesh sizes to assess the effect of macrofauna and elevated CO_2 over 24 months on mass loss of litter grown under ambient CO_2. Both macrofauna and elevated CO_2 enhanced mass loss at late decay stages, with no interactive effect. We then assessed the effect of elevated CO_2 during decomposition of litter grown under each combination of (i) ambient CO_2 or elevated CO_2 and (ii) during a psyllid outbreak that triggered significant canopy loss or later in canopy developing when psyllid densities were low. Again, mass loss was greater at elevated CO_2 at late decay stages, particularly for non-psyllid impacted litter grown at elevated CO_2. In both studies, CO_2 concentration during decomposition influenced fungal assemblages and these effects were observed before any effects on decomposition were observed, with some fungi linked to saprotrophic guilds being found with higher frequency under elevated CO_2. CO_2 concentrations under which leaves developed and whether leaves were psyllid-impacted was also important in shaping fungal assemblages. The positive effect on mass loss at late decay stages are contrary to previous findings where elevated CO_2 generally reduce decomposition rates. Our results show that elevated CO_2 effects on decay rates are context specific. Further research is required to establish the mechanisms through which this occurs to better model elevated CO_2 effects on global carbon dynamics.

Introduction

Litter decomposition is a key process in terrestrial ecosystems, with plant litter being a key carbon (C) source for decomposers that break down organic matter and make nutrients available for sustained plant growth (Swift et al., 1979; Bardgett et al., 2005). Litter decomposition rates, however, differs substantially among and within biomes, with climate, litter chemistry and decomposer assemblages largely governing the breakdown process (Hättenschwiler et al., 2005; García-Palacios et al., 2013). Microbes, particularly fungi, are the primary decomposers while soil fauna contribute both directly as decomposers and through comminution, microbial grazing and modifiers of the soil structure and microenvironment (Hättenschwiler et al., 2005; Nielsen et al., 2015). Some fungi generally utilize labile carbon sources and available nutrients during the early stages of decomposition while others break down more recalcitrant plant material and translocate nutrients from other sources, particularly later during decomposition (van der Wal et al., 2013).

Besides decomposer composition, decomposition is also indirectly regulated by environmental conditions and by litter attributes. Climate regulates decomposition through effects on vegetation composition, decomposer assemblage structure and biological processes (e.g., metabolism, enzymatic activity, grazer activity; Suseela & Tharayil, 2018). Litter decomposition is generally faster in warm and humid ecosystems, with slower rates in cold or hot deserts where temperature and low water availability constrain biological decomposition processes (García-Palacios et al., 2013). Plant functional traits have strong effects on decomposability with greater variation in litter decomposition among plants with contrasting traits within a given site than of common substrates across biomes (Cornwell et al., 2008). Specifically, Cornwell and colleagues (2008) showed that litter with high nitrogen (N) and phosphorus (P) content decompose more rapidly than litter with high lignin and water and acid

71 soluble polysaccharide content. Similarly, green leaves with high N content decompose faster
72 than green leaves with high leaf mass per area decompose.
73 Our understanding of the influences on litter decomposition dynamics has increased
74 substantially over the past few decades, but there are still important knowledge gaps, including
75 how the increasing atmospheric carbon dioxide (CO_2) concentrations will affect litter
76 decomposition processes and through this carbon sequestration. Elevated CO_2 concentrations
77 (henceforth, eCO_2) are hypothesized to affect litter decomposition processes via multiple
78 mechanisms including changes in leaf litter chemistry (at the individual level through nutrient
79 uptake and allocation, species level through shifts in plant functional traits and at the
80 community levels through changes in vegetation composition), environmental conditions (e.g.,
81 soil water content) and shifts in belowground assemblages and activity (Nielsen et al., 2015).
82 Importantly, these factors may interact to moderate eCO_2 impacts, with changes in leaf
83 chemistry expected to slow down decomposition whereas increased soil water content and
84 decomposer activity may enhance decomposition processes (Kuzyakov et al., 2019).
85 Accordingly, previous studies show substantial variation in eCO_2 effects on litter
86 decomposition. The first substantial meta-analysis of eCO_2 effects on leaf litter chemistry
87 showed reduced N and increased lignin content in leaves (i.e., lower quality) but found no
88 consistent associated effects on litter mass loss except for a slight reduction for woody species
89 (Norby et al., 2001). A key finding was that the expected eCO_2-induced reduction in leaf N
90 concentration was substantially lower in leaves following N resorption at senescence, which
91 would ameliorate eCO_2 driven effects on decomposition. The authors further hypothesized that
92 while reduced N might slow down mass loss early in decomposition, impacts on microbial
93 assemblages can increase lignin degradation, resulting in enhanced rates of mass loss at later
94 stages in the decomposition process. A more recent meta-analysis corroborates these findings,
95 with limited effects of eCO_2 on litter mass loss although a significant reduction in mass loss (-

96 5.86%) was observed for woody species in forests under field conditions (Wu et al., 2020). In
97 addition, the effects were greater when eCO_2 was >150 ppm above ambient conditions and in
98 field experiments, but no significant effects were observed in free-air CO_2 enrichment (FACE)
99 facilities. However, increased rates of mass loss have been observed in some studies indicating
100 that eCO_2 effects are context dependent, potentially moderated by increased soil biological
101 activity mediated by greater plant water use efficiency (Hall et al., 2006) and increased root
102 exudation (Phillips et al., 2011).

103 The effects of eCO_2 on litter decomposition will be moderated by biota both above-
104 and belowground. For example, eCO_2 has been shown to increase microbial biomass and the
105 abundance of detritivores (Blankinship et al., 2011), both of which could promote litter
106 decomposition. Plants are expected to show increased water use efficiency under eCO_2 which
107 would positively impact soil water content and through this soil biological activity,
108 particularly when water is limiting, although this effect may be negated by increased plant
109 biomass production which can promote soil biological activity through greater belowground
110 carbon allocation (e.g., Volk et al., 2000; Blumenthal et al., 2013). However, increased
111 abundances of detritivore fauna may impact litter decomposition due to increased grazing of
112 fungi or through changes in soil decomposer assemblage composition (A'Bear et al., 2014).
113 In addition, eCO_2 may alter herbivory through changes in resource availability and quality to
114 moderate litter decomposition while herbivory itself can also affect leaf chemistry. Several
115 studies have shown that leaf herbivory result in increased leaf N content (Chapman et al.,
116 2003; Hall et al., 2006) which might ameliorate the negative effect of eCO_2 on litter
117 decomposition by counteracting eCO_2-induced reductions in leaf N. However, few studies
118 have assessed the interactive effects of eCO_2 and herbivory under field conditions.

119 We investigated litter decomposition dynamics at ambient and elevated CO_2 (+150
120 ppm relative to ambient) in the *Eucalyptus* FACE (EucFACE) facility in eastern Australia to

provide further insight into the controls on litter decomposition using a standard litter bag approach. Specifically, in two complementary studies, we assessed the independent and interactive effects of eCO_2, macrofauna and herbivory on litter decomposition and associated changes in litter chemistry and fungal decomposer assemblage structure. Previous work at the site has shown increased photosynthesis under eCO_2 but not a concurrent increase in ecosystem productivity or C storage, with higher soil respiration contributing to greater ecosystem C losses under eCO_2 indicating greater soil biological activity (Jiang et al., 2020). Moreover, eCO_2 has been found to increase root litter mass loss at late decay stages, possibly associated with greater relative abundance and activity of saprotrophic fungi (Castañeda-Gómez et al., 2020).

Our main hypothesis was that eCO_2 would enhance leaf litter decomposition rates at this site via increased soil biological activity but that the effect would be moderated by i) whether the litter developed under ambient CO_2 (aCO_2) or eCO_2 given expected influences on litter chemistry, ii) whether macrofauna were excluded given potential changes in densities between aCO_2 and eCO_2, and iii) whether the litter was impacted by herbivores given shifts in leaf chemistry. For the latter, we took advantage of an outbreak of leaf feeding psyllids occurring at the site throughout 2014 (Gherlenda et al., 2016) comparing decomposition of litter collected during the outbreak and with visible signs of herbivory to that of litter collected from canopy that developed after the outbreak, when psyllid populations were much lower. In addition, we used amplicon sequencing to assess fungal assemblages in the litter for each treatment combination at the time point prior to where treatment effects were observed and related this to mass loss at a following time point to better assess whether changes in litter decomposition is related to eCO_2-induced shifts in fungal assemblages.

Methods and Materials

Site description

EucFACE is located in a mature warm-temperate evergreen forest dominated by *Eucalyptus tereticornis*, with minimal human disturbance for at least 90 years (Jiang et al., 2020). The understory is dominated by native grasses and shrubs. The facility was established in 2012, exposing three experimental plots (25 m diameter circles) to elevated atmospheric CO_2 concentrations at ~150 ppm above ambient conditions through fumigation. Three control plots were established with similar infrastructure but was fumigated with air without CO_2 addition. Treatments commenced in September 2012, but CO_2 concentrations were raised incrementally (~30 ppm increase per month), with full-strength treatment concentrations reached in February 2013 and then maintained throughout the experimental duration when conditions allowed (Ellsworth et al., 2017). The site is characterised by low-fertility alluvial soil of the Clarendon Formation with high sand content (>75%) and low phosphorus content, with growth considered P-limited. The deeper horizons are sandy clay loam with the presence of clay bands that affect site hydrology (Ross et al., 2020).

Litter bags

A standard litter bag approach was used to assess decomposition rates through time, including the effect of macrofauna exclusion by contrasting rates for bags with different mesh sizes. Although this approach has been criticised for inducing non-target effects (see Kampichler and Bruckner 2009), more recent assessments indicate that the findings are robust given comparable findings when litter bag studies are contrasted with other means of soil fauna suppression / exclusion (García-Palacios et al., 2013).

For the first study, we used *E. tereticornis* leaves from a fallen branch found outside the main CO_2 treatment plots in 2013 to reduce the effect of environmental and microbial

171 influences associated with litter already in contact with the ground. The litter is considered
172 green leaf material given that the leaves were still attached to the branches. As such, it was
173 expected to have higher nutrient content than senesced leaf litter as resorption would not have
174 occurred which is likely to affect the litter decomposition process. These litter bags were
175 deployed in June 2013. For the second study, we used *E. tereticornis* leaves collected in litter
176 traps within the respective CO_2 treatment plots to consider the CO_2 concentration at which the
177 leaves developed. We distinguished litter collected between December 2013 and December
178 2014, during the psyllid outbreak (Gherlenda et al., 2016), and litter collected between January
179 2015 and June 2016, after the psyllid outbreak had ended. All leaf litter was dried at 40oC to
180 constant weight (Ellsworth et al. 2017) prior to storage in paper bags in an air-conditioned
181 room. We only used litter with lerps (produced by the psyllid) collected during the psyllid
182 outbreak to ensure herbivore effects and only litter without lerps for litter collected after the
183 psyllid outbreak. These litter bags were deployed in August 2017. Approximately 2 grams was
184 added to each bag for both studies.

185 For both studies, litter bags were deployed in each of four 1 m^2 subplots in each plot,
186 with bags pegged to the soil surface in each subplot after gently brushing aside existing
187 vegetation and litter, where necessary, and redistributing after the litter bag had been deposited
188 to best simulate natural conditions. In the first study, we deployed litter in bags with two
189 different mesh sizes, with 2 mm mesh bags collected after approximately 3, 6, 9, 12, 18 and 24
190 months, while 4 mm mesh bags were collected after approximately 3, 6, 12 and 24 months
191 only. Given that only one litter type (i.e. 'green leaf') was used in this study, this resulted in a
192 total of 288 litter bags with 2 mm mesh (2 replicates per subplot × 6 time points × 4 subplots
193 × 3 plots × 2 CO_2 treatments) and 192 with 4 mm mesh (2 replicates per subplot × 4 time points
194 × 4 subplots × 3 plots × 2 CO_2 treatments). In the second study, all litter bags were of the same
195 mesh size (2 mm, same material as in the first study) to limit the number of experimental units.

196 Bags were collected at four time points across the first 16 months (4, 8, 12 and 16 months)
197 where the treatment effects were observed during the first study. We used a full factorial design
198 for CO_2 concentration under which leaves grew prior to senescence ('CO_2 [leaf]'), CO_2
199 concentration during litter decomposition ('CO_2 [litter]'), and psyllid presence during leaf
200 growth ('psyllid') with one bag per subplot and four subplots per CO_2 treatment area, resulting
201 in 384 experimental units (1 replicate per subplot × 4 time points × 4 subplots × 3 plots × 2
202 litter CO_2 treatments × 2 leaf CO_2 treatments × 2 psyllid treatments). Upon collection of bags
203 the remaining litter was dried at 40 °C until constant weight before the litter was weighed to
204 calculate mass loss. All non-litter material, including mineral soil, that had entered during
205 incubation was removed prior to weighing.
206 Soil temperature and moisture were continuously monitored at multiple locations
207 within each ring over the course of each study. Soil moisture, as volumetric water content,
208 was monitored using frequency-domain reflectometers (CS650 Soil Water Content
209 Reflectometer, Campbell Scientific, Logan, UT, USA) installed at a depth of 30 cm at eight
210 locations within each ring. Soil temperature was monitored using temperature probes (TH3-s,
211 UMS GmbH, Frankfurt, Germany) installed at a depth of 5 cm at two locations in each ring.
212 Soil temperature conditions were similar between the two studies but soil moisture was much
213 lower in the second study than in the first (Fig. S1), at values less than 5% volumetric water
214 content for much of the second study. We found generally similar levels of soil moisture
215 under both aCO_2 and eCO_2 over the duration of both studies (Fig. S2), which is consistent
216 with previous findings at the site (Pathare et al., 2017; Ginemo et al., 2018).
217
218 *Litter chemistry*
219 Litter C and N content was determined using a LECO TruMac CN analyser (Leco
220 Corporation, St Joseph, MI, USA) based on the Dumas method after grinding dried material

221 to a fine powder. Litter P content was determined using an Epsilon 4 Benchtop X-ray
222 fluorescence (XRF) spectrometer (Malvern Panalytical, Malvern, UK). Litter C, N and P
223 concentrations were measured on a sub-set of the litter bags chosen in each study to represent
224 stages prior to and following the initiation of CO_2 effects on decomposition. In the first study,
225 this included litter harvested after 6 and 12 months in the 2 mm mesh bags and after 3 and 6
226 months in the 4 mm mesh bags; the litter from the two bags collected from within a subplot
227 during each harvest were composited prior to chemical analysis. In the second study, this
228 included psyllid-affected litter harvested after 8 and 12 months and psyllid-unaffected litter
229 harvested after 8 and 16 months; there was no compositing of samples here since only one
230 bag of each litter origin was collected from a subplot during each harvest. We also measured
231 C, N and P concentrations on three composite samples of litter that was not deployed in litter
232 bags for each set of initial conditions, of which there was only one in the first study (green
233 leaf litter picked off the fallen branch) and four in the second (relating to previous CO_2
234 condition during growth and collection date relative to the psyllid outbreak). The leaf litter
235 used in the second study had lower concentrations of nitrogen and phosphorus as expected in
236 senesced litter compared with green leaf material used in the first study (Table S1).
237
238 *Fungal assemblages*
239 DNA was extracted from approximately 200 mg of each of the samples that were analysed
240 for litter chemistry. The samples were ground into a fine powder with 5 mm steel beads in a
241 TissueLyser II (Qiagen), then 1 ml of CTAB buffer (0.1 M Tris-HCL, 1.4 M NaCl, 0.02M
242 EDTA, 20 g.l-1 of cetyltrimethyl ammonium bromide with 4% (w/v) polyvinylpyrrolidone)
243 was added to each sample. The samples were digested at 65 ℃ with mixing at 1000 rpm for 1
244 hour. The samples were then spun for 7 minutes at 16 000 rpm, following which 500 µl of the
245 supernatant was transferred to a new tube and 500 µl of chloroform:isoamyl alcohol (24:1)

added. The samples were mixed by inversion for 5 minutes and spun at 16 000 g for 7 minutes, after which up to 450 µl of the upper aqueous phase was then transferred to a new tube. The DNA was precipitated by addition of 0.08 volumes of cold 7.5M ammonium acetate and 0.54 volumes of cold isopropanol, followed by 30 minutes at -20 ℃. The samples were spun at 16 000 g for 3 minutes and the supernatant removed. The DNA was then washed with 700 µl of 70 % cold ethanol, followed by 700 µl of cold 95% ethanol. The samples were spun at 16 000 g for 1.5 minutes after each wash and the ethanol removed. The samples were dried for 30 minutes at 65 ℃ and then re-suspended in 100 µl of TE buffer (10 mM Tris-HCL, 1 mM EDTA).

Many of the raw extracts were darkly coloured. To remove polyphenolic compounds, humic/fulvic acids, tannins and other PCR inhibitors from the raw DNA extracts, the samples were treated using the Zymo OneStep™ PCR Inhibitor Removal Kit according the manufacture's protocol. Twenty-four samples were subsequently checked for successful amplification of the ITS gene by PCR following the protocol of Gourmelon et al. (2016) using the MyTaq PCR system (Bioline). Three samples did not successfully amplify. These samples remained darkly coloured after inhibitor clean-up. All darkly coloured samples were then checked for successful PCR amplification at template concentrations of 100, 20 and 1 ng/µl. All samples at a concentration of 1 ng/µl successfully amplified.

For sequencing of clear DNA samples, concentrated samples were generally diluted to 10 ng/µl while samples with concentrations of less than 15 ng/µl were left neat. The darkly colours samples were diluted to 1 ng/µl prior to sequencing. DNA samples were submitted to the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, NSW, Australia). Amplicons were generated using fITS7 (5'-GTGARTCATCGAATCTTTG-3';

271 Ihrmark et al. 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990). All
272 amplicons were purified using the Agencourt AMpure XP system (Beckman Coulter, Lane
273 Cove, NSW, Australia) and genomic libraries were prepared using the Nextera XT Index Kit
274 (Illumina, San Diego, CA, USA). Paired-end (2 x 251 bases) sequencing was performed on
275 the Illumina MiSeq platform.
276
277 To process the DNA sequencing data, we used the approach described by Bissett et al.
278 (2016) with a few modifications. Contigs were generated from paired-end reads using the
279 'fastq_mergepairs' command in VSEARCH (version v2.3.4; Rognes et al., 2016) using a
280 minimum overlap of 30 base pairs. Initial quality filtering removed DNA sequences
281 containing ambiguous bases and/or homopolymers greater than eight bases in length.
282 Sequences were kept for further analysis if they were within 200-470 base pairs in length and
283 contained fewer than 0.5 expected errors. De novo operational taxonomic units (OTUs) at
284 97% sequence similarity were initially picked using numerically dominant sequences
285 (observed at least two times) using the '-cluster_smallmem' command in VSEARCH. All
286 quality-filtered sequences were mapped at 97% sequence similarity against representative
287 sequences of these OTUs using the '-usearch_global' command in VSEARCH. Non-mapped
288 sequences were subjected to a second round of de novo OTU picking, as above but only
289 using sequences observed at least two times. All initially non-mapped sequences were then
290 mapped against these newly picked OTUs, as above. Non-mapped sequences at this step
291 represent singleton OTUs and were excluded from further analysis. Sequence read counts that
292 were less than ten within individual samples were removed to reduce the likelihood of
293 sequence reads being assigned to samples incorrectly. As a result of this, as well as the high
294 level of sequencing depth across all samples (between 12122 and 41536 reads per sample in
295 the first study, between 16619 and 58666 reads per sample in the second study), coverage

was estimated to be high (Good's coverage = 100) in all samples. Therefore, we did not rarefy the OTU table before further analysis.

Putative taxonomic identities for fungal OTUs were generated using BLAST (v.2.6.0, Altschul et al. 1990) to compare representative sequences for each OTU to a reference database of gene sequences and taxonomic annotations (UNITE version 8.3, sh_general_release_dynamic_s_10.05.2021; Abarenkov et al. 2021). Fungal ITS2 sequences were extracted using ITSx (Bengtsson-Palme et al. 2013, v1.1.3) for use during BLAST. Trophic modes and guilds of fungal OTUs that were assigned to taxa were then inferred using FUNGuild (Nguyen et al. 2016).

Data analyses

All data analyses were performed using R v.4.1.2 (R Core Team, 2021). We tested for main and interactive effects of CO_2 treatment during litter decay, mesh size and months of decay (study 1) or CO_2 treatment during litter decay, during leaf development, psyllid presence and months of decay (study 2) on litter decomposition using linear mixed effects models, treating 'ring' and 'plot within ring' as random effects ('lme4' package; Bates et al., 2015). Months of decay was treated as an ordered factor in both models. Litter decomposition was represented in the model as the proportion of the original mass that remained following incubation and applying the arcsine-square root transformation (Sokal and Rohlf, 1995) to account for the distribution being bounded at both ends (0 and 1). The same models were used to assess responses of litter nitrogen and phosphorus concentrations, except we only analysed one timepoint so did not include months of decay.

For relevant interactive effects, we estimated the effect size of CO_2 treatments on decomposition based on log-response ratios (LRRs) calculated from the predictions of the model, i.e., $\log(\text{response}_{\text{elevated CO2}} / \text{response}_{\text{ambient CO2}})$. Standard errors for LRRs were calculated according to Hedges et al. (1999) and LRRs were considered significant when the confidence interval of the mean (+/- two standard errors) did not overlap zero. Model predictions were obtained using the 'ggemmeans' function from the 'ggeffects' package (Lüdecke, 2018).

Variation in fungal assemblages for both studies was visualised using principal coordinates analysis based on Bray-Curtis dissimilarities, after Hellinger-transformation of each OTU table, using functions from the 'vegan' package (Oksanen et al., 2022). We also performed further analysis of fungal assemblages sampled at the timepoint prior to the observation of CO_2 effects on decomposition in each study. For this, we first performed PerMANOVA to assess the significance and effect size of all of the main effects and interactions associated with each study, again using Bray-Curtis dissimilarities after Hellinger transformation. Then we visualised these patterns using constrained analysis of principal coordinates (CAP), including each main effect in the constraint. Finally, we performed multi-level pattern analysis using the 'indicspecies' package (De Caceres and Legendre, 2009) to identify OTUs indicative of groups within relevant treatments; for this analysis we used a conservative cut-off of $P < 0.01$ to identify indicators so as to identify those taxa that were most strongly associated with each treatment.

Results

Effects of macrofauna exclusion, CO_2 concentration at leaf development, eCO_2 and herbivory on decomposition through time

345 In the first study, both mesh sizes resulted in a median value of less than 10% mass
346 remaining after 12 months (Figure 1a). Mesh size had a significant effect on litter mass loss
347 ($P_{mesh} < 0.001$) with higher loss in the large mesh size bags (Figure 1a), and this varied
348 through time as indicated by the time:mesh interaction ($P_{time:mesh} < 0.001$; Table 1). In
349 addition, mass loss was greater in the eCO_2 treatment at late decay stages (Figure 1b) as
350 indicated by the time:treatment interaction ($P_{time:treatment} < 0.001$; Table 1); these effect sizes
351 were consistent across for both mesh sizes ($P_{time:treatment:mesh} = 0.31$; Table 1; Figure 1b). The
352 eCO_2 effect appeared greater in the large mesh size bags (Figure 2) but this was not
353 significant ($P_{treatment:mesh} = 0.41$; Table 1).
354 In the second study, decomposition occurred more slowly and all but one treatment
355 combination resulted in a median value of more than 50% mass remaining after 16 months
356 (Figure 2a). Here, we observed that concurrent CO_2 treatment effects again tended to appear
357 during later decay stages ($P_{time:treatment} = 0.001$), but the timing of this effect was inconsistent
358 depending on whether the litter was impacted by psyllids and the number of months of decay
359 ($P_{time:treatment:psyllid} = 0.02$; Figure 2b). For litter impacted by psyllids, we observed an
360 ephemeral increase, after 12 months, in decomposition for litter derived from leaves that
361 developed under elevated CO_2 relative to those developing under ambient CO_2. For litter
362 unimpacted by psyllids, we observed this same pattern but not until 16 months into the study.
363 Additional effects on litter decomposition were observed in the second study that
364 were dependent on the condition that leaves developed under but were independent of the
365 CO_2 treatment during decay ($P_{time:previousTreatment:psyllid} = 0.02$; Figure 2c). This was due to an
366 ephemeral increase in decomposition after eight months for litter from leaves developing
367 under elevated CO_2, relative to those developing under ambient CO_2, but only in the absence
368 of the psyllid outbreak.
369

Differences in fungal assemblages, but not litter chemistry, existed prior to observed CO_2 effects on litter decomposition

We observed strong compositional variation in fungal assemblages in the first study when comparing fresh leaves, litter collected prior to the observation of CO_2 effect on decomposition and litter collection after the observation of this effect (Figure S3). When focussing only on assemblages in litter collected prior to that effect (Figure 3; Table S2), we observed significant effects of CO_2 treatment (PerMANOVA, $P < 0.05$; $R^2 = 0.039$) and mesh size ($P = 0.001$; $R^2 = 0.151$) but no interaction between those terms ($P > 0.9$). This is despite our observation that litter N and P concentrations were similar between these treatments at that time ($P_{treatment} > 0.7$ for both; [N] = 2.6 +/- 0.1%, [P] = 0.13 +/- 0.02%; mean +/- SD). Two fungal OTUs had a significantly higher frequency in the elevated CO_2 treatment (Table S3): one with >99% identity with an isolate of *Pilidium anglicum* and one likely belonging to the Xylariales.

In the second study we observed large differences when comparing fresh leaves and litter, but fungal assemblages in litter were highly variable and were difficult to differentiate by the number of months undergoing decay (Figure S4). Again, we observed variation in fungal assemblages associated with the CO_2 treatment (PerMANOVA, $P < 0.01$) during decay even before CO_2 effects on decay were observed (Figure 4; Table S5) and, again, N and P concentrations were similar between these treatments at that time, accounting for variation in initial chemistry among litter from different sources ($P_{treatment} > 0.8$ and $P > 0.06$ for interactions involving 'treatment'; [N] = 1.5 +/- 0.3%, [P] = 0.03 +/- 0.02%; mean +/- SD). We also observed significant effects of the CO_2 condition during leaf development ($P = 0.001$) and whether leaves were psyllid impacted ($P = 0.001$) on fungal assemblages in that same litter, with the psyllid condition having the most important effect (differentiation along

axis 1 of the CAP plots in Figure 4; $R^2 = 0.044$), the CO_2 treatment during litter development being the second-most important (axis 2, y-axis of the plot in Figure 4a; $R^2 = 0.023$) and the CO_2 treatment during litter decay being the third-most important (axis 3, y-axis of the plot in Figure 4b; $R^2 = 0.019$), despite most variation remaining unexplained (residual $R^2 = 0.88$). No significant interactions among terms were observed (all $P > 0.5$). Six fungal OTUs had a significantly higher frequency in the ambient CO_2 treatment, five with >95% identity to genera in the Pleosporales and one with a match to the genus *Cyphellophora* (Table S5). Five fungal OTUs had a significantly higher frequency in the elevated CO_2 treatment, with variable levels of matches to taxa in the Chaetothyriales (two OTUs), Tremellales (one OTU with 100% identity to *Papiliotrema flavescens*), Capnodiales and Pezizales. Several OTUs were significantly associated with conditions during leaf development (13 OTUs for prior CO_2 condition, Table S6, and 19 OTUs for psyllid condition, Table S7).

Discussion

Consistent with our main hypothesis litter mass loss increased at eCO_2 at late decay stages in both studies, which occurred mostly independently of CO_2 concentration under which the leaves were grown, macrofauna exclusion and herbivore presence. Our results differ from the general observation that eCO_2 has a negative albeit slight impact on decomposition of litter from woody species in forests (e.g., Norby et al., 2001; Wu et al., 2020) but align well with those of Hall et al. (2006) who found that long-term leaf litter decomposition was higher at eCO_2, irrespectively of whether it was grown at ambient or elevated CO_2, alongside enhanced accumulation of mineral N indicating greater microbial activity. Hence, eCO_2 may enhance organic matter turnover with potential implications for C sequestration in forests. Other studies have found that eCO_2 contribute to greater soil organic

matter turnover. For example, Carney et al. (2007) found that six-year doubling of CO_2 negatively impacted soil organic matter content offsetting more than half of the C accumulated in the biomass aboveground and coarse roots over the same period although no increase in litter decomposition was observed. The increase in soil organic matter degradation was linked to greater relative abundances of fungi and greater activity of C degrading enzymes. Another study found that increased soil C turnover under eCO_2 negated soil C accrual observed under ambient conditions in aspen-only forests while soil C stocks increased in aspen-maple and aspen-birch forests under both aCO_2 and eCO_2 (Talhelm et al., 2009). Hence, eCO_2 may not result in C sequestration.

It has been shown that eCO_2 increase plant water use efficiency which can have a positive effect on soil water content and through this soil biological activity (Hall et al., 2006; Phillips et al., 2011). We found no consistent effects of eCO_2 on soil water content during either of the two studies, and even a tendency for reduced soil water content, which is consistent with previous findings at the site (Pathare et al., 2017; Ginemo et al., 2018). Hence, it is more likely that the eCO_2 effect is mediated by biological activity through changes in plant carbon inputs and nutrient requirements. We have no direct measurement to confirm this but previous findings have shown seasonal increases in N and P availability and mineralization (Hasegawa et al., 2016), and modification of enzyme activities related to C degradation (starch and cellulose specifically; Ochoa-Hueso et al., 2017) indicating enhanced soil biological activity at eCO_2. Similarly, enhanced soil respiration observed under eCO_2 at the site indicate greater turnover of soil organic C (Jiang et al., 2020). Specifically, increased belowground allocation of photosynthetically-derived C may prime organic matter turnover. While most studies to date have focussed on soil organic C, there is evidence that a similar effect could influence decomposition processes. For example, it has been hypothesized that AM fungi can contribute to enhanced C cycling under eCO_2 by stimulating saprotrophic

445 decomposition of soil organic matter via greater host-derived belowground C allocation
446 (Cheng et al., 2012; Parihar et al., 2020). Accordingly, AM and saprotrophic fungi may
447 interact at eCO_2 to enhance litter decomposition as observed in this study (leaf litter) and in a
448 previous study at the site (root litter; Castañeda-Gómez et al., 2020). However, a laboratory
449 study found that AM fungi and low P-availability protect soil organic matter from
450 saprotrophic decomposition while higher C cycling and microbial biomass at eCO_2 enhance
451 SOM decomposition (Castañeda-Gómez et al., 2022). This mechanism should be tested more
452 rigorously.

453 Another possible explanation for the increase in litter mass loss at eCO_2 is via a shift
454 in understory plant community composition. Specifically, while limited effects on overstory
455 (Jiang et al., 2020) or understory (Collins et al., 2018) productivity has been observed at the
456 study site, eCO_2 has been shown to increase the dominance (Hasegawa et al., 2018) and
457 photosynthetic rates (Pathare et al., 2017) of the dominant C3 grass at the site. C3 grasses are
458 considered to produce higher quality leaf litter than co-occurring C4 grasses. Hence, this shift
459 in composition and greater photosynthetic rates may result in greater inputs of more easily
460 degradable leaf litter which could increase microbial activity with cascading effects on
461 decomposition of the more recalcitrant *Eucalyptus* litter (Hättenschwiler et al., 2005). This is
462 consistent also with the higher rates of nutrient availability observed at some time points
463 (Hasegawa et al., 2016; Ochoa-Hueso et al., 2017).

464 Consistent with previous findings (e.g., Wall et al., 2008; García-Palacios et al.,
465 2013), the exclusion of soil fauna had a significant impact on litter decomposition
466 irrespective of CO_2 treatment although we only excluded macrofauna. The response ratio for
467 eCO_2 when macrofauna were included appeared greater but this was not significant.
468 Interestingly, this occurred despite the observation that eCO_2 result in fewer ground dwelling
469 macrofauna, including the known decomposers Isopoda, at the site (Facey et al., 2017).

Similarly, previous studies suggest that eCO_2 generally have a negative effect on mesofauna (Blankinship et al., 2011; A'bear et al., 2014) although no eCO_2 effects on mite densities or community composition have been observed at this site (Ross et al., 2020). Hence, these findings suggests that the higher litter decomposition rates at eCO_2 are not driven by increased soil fauna densities.

Gherlenda et al. (2016b) found that eCO_2 impacted psyllid performance during the outbreak, with fewer lerps (protective casings) produced by one flush-feeding and two senescence feeding species as well as compensatory feeding by the flush-feeding *Glycaspis* at eCO_2. In turn, the presence of psyllids likely affected leaf chemistry through herbivory-induced changes in plant physiology which have been observed in other studies to increase leaf quality (Chapman et al., 2003; Hall et al., 2006). This may explain the observation that eCO_2 only enhanced litter decomposition of material collected after the psyllid-outbreak had completed. We did examine litter nitrogen and phosphorus concentration on a small number of samples prior to the start of each study (Table S1), but not enough to have confidence in whether differences existed among the different growth conditions.

We cannot demonstrate that altered composition of fungal assemblages was responsible for eCO_2-associated increases in decomposition rates in the two studies, but the fact that compositional differences existed prior to those increases leads us to speculate that could be the case. Some of the fungal taxa associated with eCO_2 are associated with lineages known to have capacity for saprotrophy of recalcitrant carbon sources. *Plectania* has been observed associated with litter at high frequencies during later stages of decomposition (Bani et al., 2019). *Papiliotrema* was observed in association with avocado peels during a stage of rapid decay (Becerra-Lucio et al. 2021), although the best match here was to a yeast known as a facultative pathogen also associating with trees (Serna-Espinosa et al. 2023). Members of the Xylariales have demonstrated an association with decaying litter (Osono et al. 2012) and

a capacity to break down lignocellulose (Nghi et al. 2012). The identity of taxa associated with eCO_2 in each study differed, suggesting that redundancy could exist in terms of the fungi responding positively in an eCO_2 environment and possibly responsible for enhanced decay rates.

Here we were able to take advantage of a rare opportunity arising from two significant circumstances. The establishment and continuing support of EucFACE allowed us to perform, over a period of eight years, two complementary studies under different (but representative for this climate) field conditions and to observe consistency in the responses associated with eCO_2 under those conditions. The support for long-term monitoring of core data streams, here involving monthly monitoring of litter production, and archival of sampled materials allowed us to take advantage of a natural experiment involving a psyllid outbreak and to compare effects associated with eCO_2 to those associated with litter quality. Few examples come to mind where long-term experimental manipulations, continuous monitoring and natural experiments have come together to enhance our general understanding of how ecosystems are likely to respond to environmental change. One such example includes the observation, over the course of almost 20 years, that N mineralisation responded to eCO_2 on different timeframes under C_3 and C_4 plants, controlling their short- and long-term responses (Reich et al. 2018). Examples such as this, and ours, highlight the value of long-term experimental observatories and the need for research organisations and funders to support them (Kuebbing et al. 2018).

Acknowledgements

We thank Steven Wohl, Vinod Kumar, Craig Barton and Craig McNamara for maintaining the EucFACE facility; David Ellsworth, Kristine Crous and Remko Duursma for litter collections; Gillian Powell and Alicia Harb-Akins for making litter bags; and Kylie Brice for

technical support. EucFACE is supported by the Australian Commonwealth government in collaboration with Western Sydney University. This is part of a Terrestrial Ecosystem Research Network SuperSite facility. EucFACE was built as an initiative of the Australian government as part of the Nation-building Economic Stimulus Package. JRP acknowledges funding from the Australian Research Council (DP130102501 and DP160103765).

Data availability statement

Raw DNA sequencing data are available at the NCBI Sequence Read Archive under BioProject XXXXXXXXX. Soil temperature and moisture data are archived on HIEv (). All other data used in this manuscript are available at figshare (DOI: XXXXXXXXX). R code used for data analyses are available at zenodo (DOI: XXXXXXXX). <*The links in this statement will be updated following review.*>

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U. (2021). *UNITE general FASTA release for Fungi 2* [Application/gzip]. UNITE Community. https://doi.org/10.15156/BIO/1280089

A'Bear, A. D., Jones, T. H., & Boddy, L. (2014). Potential impacts of climate change on interactions among saprotrophic cord-forming fungal mycelia and grazing soil invertebrates. *Fungal Ecology*, *10*, 34–43. https://doi.org/10.1016/j.funeco.2013.01.009

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bani, A., Borruso, L., Matthews Nicholass, K. J., Bardelli, T., Polo, A., Pioli, S., Gómez-Brandón, M., Insam, H., Dumbrell, A. J., & Brusetti, L. (2019). Site-Specific Microbial Decomposer Communities Do Not Imply Faster Decomposition: Results from a Litter Transplantation Experiment. *Microorganisms*, *7*(9), Article 9. https://doi.org/10.3390/microorganisms7090349

Bardgett, R., Usher, M., & Hopkins, D. (Eds.). (2005). *Biological Diversity and Function in Soils*. Cambridge University Press. https://doi.org/10.1017/CBO9780511541926

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Becerra-Lucio, P. A., Labrín-Sotomayor, N. Y., Apolinar-Hernández, M. M., Becerra-Lucio, A. A., Sánchez, J. E., & Peña-Ramírez, Y. J. (2021). Degradation activity of fungal communities on avocado peel (Persea americana Mill.) in a solid-state process: Mycobiota successions and trophic guild shifts. *Archives of Microbiology*, *204*(1), 2. https://doi.org/10.1007/s00203-021-02600-3

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., … Nilsson, R. H. (2013). Improved software detection and extraction of ITS1

562 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of
563 environmental sequencing data. *Methods in Ecology and Evolution*, *4*(10), 914–919.
564 https://doi.org/10.1111/2041-210X.12073

565 Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P. M., Reith, F., Dennis, P. G., Breed, M. F.,
566 Brown, B., Brown, M. V., Brugger, J., Byrne, M., Caddy-Retalic, S., Carmody, B., Coates,
567 D. J., Correa, C., Ferrari, B. C., Gupta, V. V. S. R., Hamonts, K., Haslem, A., … Young, A.
568 (2016). Introducing BASE: The Biomes of Australian Soil Environments soil microbial
569 diversity database. *GigaScience*, *5*(1), s13742-016-0126–5. https://doi.org/10.1186/s13742-
570 016-0126-5

571 Blankinship, J. C., Niklaus, P. A., & Hungate, B. A. (2011). A meta-analysis of responses of
572 soil biota to global change. *Oecologia*, *165*(3), 553–565. https://doi.org/10.1007/s00442-011-
573 1909-0

574 Blumenthal, D. M., Resco, V., Morgan, J. A., Williams, D. G., LeCain, D. R., Hardy, E. M.,
575 Pendall, E., & Bladyka, E. (2013). Invasive forb benefits from water savings by native plants
576 and carbon fertilization under elevated CO2 and warming. *New Phytologist*, *200*(4), 1156–
577 1165. https://doi.org/10.1111/nph.12459

578 Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites:
579 Indices and statistical inference. *Ecology*, *90*(12), 3566–3574. https://doi.org/10.1890/08-
580 1823.1

581 Castañeda-Gómez, L., Powell, J. R., Pendall, E., & Carrillo, Y. (2022). Phosphorus
582 availability and arbuscular mycorrhizal fungi limit soil C cycling and influence plant
583 responses to elevated CO2 conditions. *Biogeochemistry*, *160*(1), 69–87.
584 https://doi.org/10.1007/s10533-022-00939-3

585 Castañeda-Gómez, L., Walker, J. K. M., Powell, J. R., Ellsworth, D. S., Pendall, E., &
586 Carrillo, Y. (2020). Impacts of elevated carbon dioxide on carbon gains and losses from soil
587 and associated microbes in a Eucalyptus woodland. *Soil Biology and Biochemistry*, *143*,
588 107734. https://doi.org/10.1016/j.soilbio.2020.107734

589 Chapman, S. K., Hart, S. C., Cobb, N. S., Whitham, T. G., & Koch, G. W. (2003). Insect
590 Herbivory Increases Litter Quality and Decomposition: An Extension of the Acceleration
591 Hypothesis. *Ecology*, *84*(11), 2867–2876. https://doi.org/10.1890/02-0046

592 Cheng, L., Booker, F. L., Tu, C., Burkey, K. O., Zhou, L., Shew, H. D., Rufty, T. W., & Hu,
593 S. (2012). Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under
594 Elevated CO2. *Science*, *337*(6098), 1084–1087. https://doi.org/10.1126/science.1224304

595 Collins, L., Bradstock, R. A., Resco de Dios, V., Duursma, R. A., Velasco, S., & Boer, M. M.
596 (2018). Understorey productivity in temperate grassy woodland responds to soil water
597 availability but not to elevated [CO2]. *Global Change Biology*, *24*(6), 2366–2376.
598 https://doi.org/10.1111/gcb.14038

599 Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy,
600 O., Hobbie, S. E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M.,
601 Santiago, L. S., Wardle, D. A., Wright, I. J., Aerts, R., Allison, S. D., Van Bodegom, P.,
602 Brovkin, V., Chatain, A., … Westoby, M. (2008). Plant species traits are the predominant
603 control on litter decomposition rates within biomes worldwide. *Ecology Letters*, *11*(10),
604 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

605 Ellsworth, D. S., Anderson, I. C., Crous, K. Y., Cooke, J., Drake, J. E., Gherlenda, A. N.,
606 Gimeno, T. E., Macdonald, C. A., Medlyn, B. E., Powell, J. R., Tjoelker, M. G., & Reich, P.
607 B. (2017). Elevated CO2 does not increase eucalypt forest productivity on a low-phosphorus
608 soil. *Nature Climate Change*, *7*(4), Article 4. https://doi.org/10.1038/nclimate3235

609 Facey, S. L., Fidler, D. B., Rowe, R. C., Bromfield, L. M., Nooten, S. S., Staley, J. T.,
610 Ellsworth, D. S., & Johnson, S. N. (2017). Atmospheric change causes declines in woodland
611 arthropods and impacts specific trophic groups. *Agricultural and Forest Entomology*, *19*(1),
612 101–112. https://doi.org/10.1111/afe.12190

613 García-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter
614 quality differently modulate the effects of soil fauna on litter decomposition across biomes.
615 *Ecology Letters*, *16*(8), 1045–1053. https://doi.org/10.1111/ele.12137

616 Gherlenda, A. N., Esveld, J. L., Hall, A. A. G., Duursma, R. A., & Riegler, M. (2016). Boom
617 and bust: Rapid feedback responses between insect outbreak dynamics and canopy leaf area

618 impacted by rainfall and CO2. *Global Change Biology*, *22*(11), 3632–3641.
619 https://doi.org/10.1111/gcb.13334

620 Gimeno, T. E., McVicar, T. R., O'Grady, A. P., Tissue, D. T., & Ellsworth, D. S. (2018).
621 Elevated CO2 did not affect the hydrological balance of a mature native Eucalyptus
622 woodland. *Global Change Biology*, *24*(7), 3010–3024. https://doi.org/10.1111/gcb.14139

623 Hall, M. C., Stiling, P., Moon, D. C., Drake, B. G., & Hunter, M. D. (2006). Elevated CO2
624 increases the long-term decomposition rate of Quercus myrtifolia leaf litter. *Global Change*
625 *Biology*, *12*(3), 568–577. https://doi.org/10.1111/j.1365-2486.2006.01119.x

626 Hasegawa, S., Macdonald, C. A., & Power, S. A. (2016). Elevated carbon dioxide increases
627 soil nitrogen and phosphorus availability in a phosphorus-limited Eucalyptus woodland.
628 *Global Change Biology*, *22*(4), 1628–1643. https://doi.org/10.1111/gcb.13147

629 Hasegawa, S., Piñeiro, J., Ochoa-Hueso, R., Haigh, A. M., Rymer, P. D., Barnett, K. L., &
630 Power, S. A. (2018). Elevated CO2 concentrations reduce C4 cover and decrease diversity of
631 understorey plant community in a Eucalyptus woodland. *Journal of Ecology*, *106*(4), 1483–
632 1494. https://doi.org/10.1111/1365-2745.12943

633 Hättenschwiler, S., Tiunov, A. V., & Scheu, S. (2005). Biodiversity and Litter Decomposition
634 in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1),
635 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

636 Hedges, L. V., Gurevitch, J., & Curtis, P. S. (1999). The Meta-Analysis of Response Ratios
637 in Experimental Ecology. *Ecology*, *80*(4), 1150–1156. https://doi.org/10.1890/0012-
638 9658(1999)080[1150:TMAORR]2.0.CO;2

639 Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J.,
640 Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012).
641 New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial
642 and natural communities. *FEMS Microbiology Ecology*, *82*(3), 666–677.
643 https://doi.org/10.1111/j.1574-6941.2012.01437.x

644 Jiang, M., Medlyn, B. E., Drake, J. E., Duursma, R. A., Anderson, I. C., Barton, C. V. M.,
645 Boer, M. M., Carrillo, Y., Castañeda-Gómez, L., Collins, L., Crous, K. Y., De Kauwe, M. G.,
646 dos Santos, B. M., Emmerson, K. M., Facey, S. L., Gherlenda, A. N., Gimeno, T. E.,

Hasegawa, S., Johnson, S. N., … Ellsworth, D. S. (2020). The fate of carbon in a mature forest under carbon dioxide enrichment. *Nature*, *580*(7802), Article 7802. https://doi.org/10.1038/s41586-020-2128-9

Kampichler, C., & Bruckner, A. (2009). The role of microarthropods in terrestrial decomposition: A meta-analysis of 40 years of litterbag studies. *Biological Reviews*, *84*(3), 375–389. https://doi.org/10.1111/j.1469-185X.2009.00078.x

Kuebbing, S. E., Reimer, A. P., Rosenthal, S. A., Feinberg, G., Leiserowitz, A., Lau, J. A., & Bradford, M. A. (2018). Long-term research in ecology and evolution: A survey of challenges and opportunities. *Ecological Monographs*, *88*(2), 245–258. https://doi.org/10.1002/ecm.1289

Kuzyakov, Y., Horwath, W. R., Dorodnikov, M., & Blagodatskaya, E. (2019). Review and synthesis of the effects of elevated atmospheric CO2 on soil processes: No changes in pools, but increased fluxes and accelerated cycles. *Soil Biology and Biochemistry*, *128*, 66–78. https://doi.org/10.1016/j.soilbio.2018.10.005

Lüdecke, D. (2018). ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software*, *3*(26), 772. https://doi.org/10.21105/joss.00772

Nghi, D. H., Bittner, B., Kellner, H., Jehmlich, N., Ullrich, R., Pecyna, M. J., Nousiainen, P., Sipilä, J., Huong, L. M., Hofrichter, M., & Liers, C. (2012). The Wood Rot Ascomycete Xylaria polymorpha Produces a Novel GH78 Glycoside Hydrolase That Exhibits α-l-Rhamnosidase and Feruloyl Esterase Activities and Releases Hydroxycinnamic Acids from Lignocelluloses. *Applied and Environmental Microbiology*, *78*(14), 4893–4901. https://doi.org/10.1128/AEM.07588-11

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Norby, R. J., Cotrufo, M. F., Ineson, P., O'Neill, E. G., & Canadell, J. G. (2001). Elevated CO2, litter chemistry, and decomposition: A synthesis. *Oecologia*, *127*(2), 153–165. https://doi.org/10.1007/s004420000615

Ochoa-Hueso, R., Hughes, J., Delgado-Baquerizo, M., Drake, J. E., Tjoelker, M. G., Piñeiro, J., & Power, S. A. (2017). Rhizosphere-driven increase in nitrogen and phosphorus availability under elevated atmospheric CO2 in a mature Eucalyptus woodland. *Plant and Soil*, *416*(1), 283–295. https://doi.org/10.1007/s11104-017-3212-2

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package* (2.6-4) [Computer software]. https://cran.r-project.org/web/packages/vegan/index.html

Osono, T., Tateno, O., & Masuya, H. (2012). Diversity and ubiquity of xylariaceous endophytes in live and dead leaves of temperate forest trees. *Mycoscience*, *54*(1), 54–61. https://doi.org/10.1016/j.myc.2012.08.003

Parihar, M., Rakshit, A., Meena, V. S., Gupta, V. K., Rana, K., Choudhary, M., Tiwari, G., Mishra, P. K., Pattanayak, A., Bisht, J. K., Jatav, S. S., Khati, P., & Jatav, H. S. (2020). The potential of arbuscular mycorrhizal fungi in C cycling: A review. *Archives of Microbiology*, *202*(7), 1581–1596. https://doi.org/10.1007/s00203-020-01915-x

Pathare, V. S., Crous, K. Y., Cooke, J., Creek, D., Ghannoum, O., & Ellsworth, D. S. (2017). Water availability affects seasonal CO2-induced photosynthetic enhancement in herbaceous species in a periodically dry woodland. *Global Change Biology*, *23*(12), 5164–5178. https://doi.org/10.1111/gcb.13778

Phillips, R. P., Finzi, A. C., & Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. *Ecology Letters*, *14*(2), 187–194. https://doi.org/10.1111/j.1461-0248.2010.01570.x

R Core Team. (2021). *R: The R Project for Statistical Computing* [Computer software]. R Foundation for Statistical Computing. https://www.r-project.org/

Reich, P. B., Hobbie, S. E., Lee, T. D., & Pastore, M. A. (2018). Unexpected reversal of C3 versus C4 grass response to elevated CO2 during a 20-year field experiment. *Science*, *360*(6386), 317–320. https://doi.org/10.1126/science.aas9313

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Serna-Espinosa, B.-N., Forero-Castro, M., Morales-Puentes, M. E., Parra-Giraldo, C. M., Escandón, P., & Sánchez-Quitian, Z. A. (2023). First report of environmental isolation of Cryptococcus and Cryptococcus-like yeasts from Boyacá, Colombia. *Scientific Reports*, *13*(1), Article 1. https://doi.org/10.1038/s41598-023-41994-6

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry*. W. H. Freeman.

Suseela, V., & Tharayil, N. (2018). Decoupling the direct and indirect effects of climate on plant litter decomposition: Accounting for stress-induced modifications in plant chemistry. *Global Change Biology*, *24*(4), 1428–1451. https://doi.org/10.1111/gcb.13923

Swift, M. J., Heal, O. W., Anderson, J. M., & Anderson, J. M. (1979). *Decomposition in Terrestrial Ecosystems*. University of California Press.

Talhelm, A. F., Pregitzer, K. S., & Zak, D. R. (2009). Species-specific responses to atmospheric carbon dioxide and tropospheric ozone mediate changes in soil carbon. *Ecology Letters*, *12*(11), 1219–1228. https://doi.org/10.1111/j.1461-0248.2009.01380.x

van der Wal, A., Geydan, T. D., Kuyper, T. W., & de Boer, W. (2013). A thready affair: Linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, *37*(4), 477–494. https://doi.org/10.1111/1574-6976.12001

Volk, M., Niklaus, P. A., & Körner, C. (2000). Soil moisture effects determine CO2 responses of grassland species. *Oecologia*, *125*(3), 380–388. https://doi.org/10.1007/s004420000454

Wall, D. H., Bradford, M. A., St John, M. G., Trofymow, J. A., Behan-Pelletier, V., Bignell, D. E., Dangerfield, J. M., Parton, W. J., Rusek, J., Voigt, W., Wolters, V., Gardel, H. Z., Ayuke, F. O., Bashford, R., Beljakova, O. I., Bohlen, P. J., Brauman, A., Flamming, S., Henschel, J. R., … Zou, X. (2008). Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology*, *14*(11), 2661–2677. https://doi.org/10.1111/j.1365-2486.2008.01672.x

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications* (Vol. 18, pp. 315–322). San Diego. https://msafungi.org/wp-content/uploads/2019/03/February-2013-Inoculum.pdf

Wu, Q., Yue, K., Wang, X., Ma, Y., & Li, Y. (2020). Differential responses of litter decomposition to warming, elevated CO2, and changed precipitation regime. *Plant and Soil*, *455*(1), 155–169. https://doi.org/10.1007/s11104-020-04675-1

739 Table 1: ANOVA table showing significance of fixed effects and interactions associated with
740 CO_2 treatment, mesh size and duration of decomposition for litter mass remaining at harvest.
741 'Ring' and 'Plot' were included as random effects. Raw data and model predictions are
742 shown in Figure 1.

Term	F	df (num)	df (den)	P-value
Harvest	850.6	5	438	< 0.001
CO_2 treatment (litter)	1	1	4	0.37
Mesh size	87.9	1	438	< 0.001
Harvest:CO_2	6.9	5	438	< 0.001
Harvest:Mesh	7.5	3	438	< 0.001
CO_2:Mesh	0.7	1	438	0.409
Harvest:CO_2:Mesh	1.2	3	438	0.309

743

744 Table 2: ANOVA table showing significance of fixed effects and interactions associated with
745 CO_2 treatment during leaf development ('leaf') and litter decomposition ('litter'), psyllid
746 presence during leaf development and duration of decomposition for litter mass remaining at
747 harvest in the second study. 'Ring' and 'Plot' were included as random effects. Raw data and
748 model predictions are shown in Figure 2.

Term	F	df (num)	df (den)	P-value
Harvest	239.8	3	324	< 0.001
CO_2 treatment (litter)	4.5	1	4	0.1
CO_2 treatment (leaf)	2	1	324	0.157
Psyllid	2.5	1	324	0.113
Harvest:CO_2[litter]	5.3	3	324	0.001
Harvest:CO_2[leaf]	0.3	3	324	0.844
CO_2[litter]:CO_2[leaf]	0.8	1	324	0.36
Harvest:Psyllid	1	3	324	0.374
CO_2[litter]:Psyllid	0.2	1	324	0.693
CO_2[leaf]:Psyllid	4.7	1	324	0.03
Harvest:CO_2[litter]:CO_2[leaf]	1.4	3	324	0.235
Harvest:CO_2[litter]:Psyllid	3.3	3	324	0.021
Harvest:CO_2[leaf]:Psyllid	1.6	3	324	0.192
CO_2[litter]:CO_2[leaf]:Psyllid	0.7	1	324	0.388
Harvest:CO_2[litter]:CO_2[leaf]:Psyllid	1	3	324	0.39

749

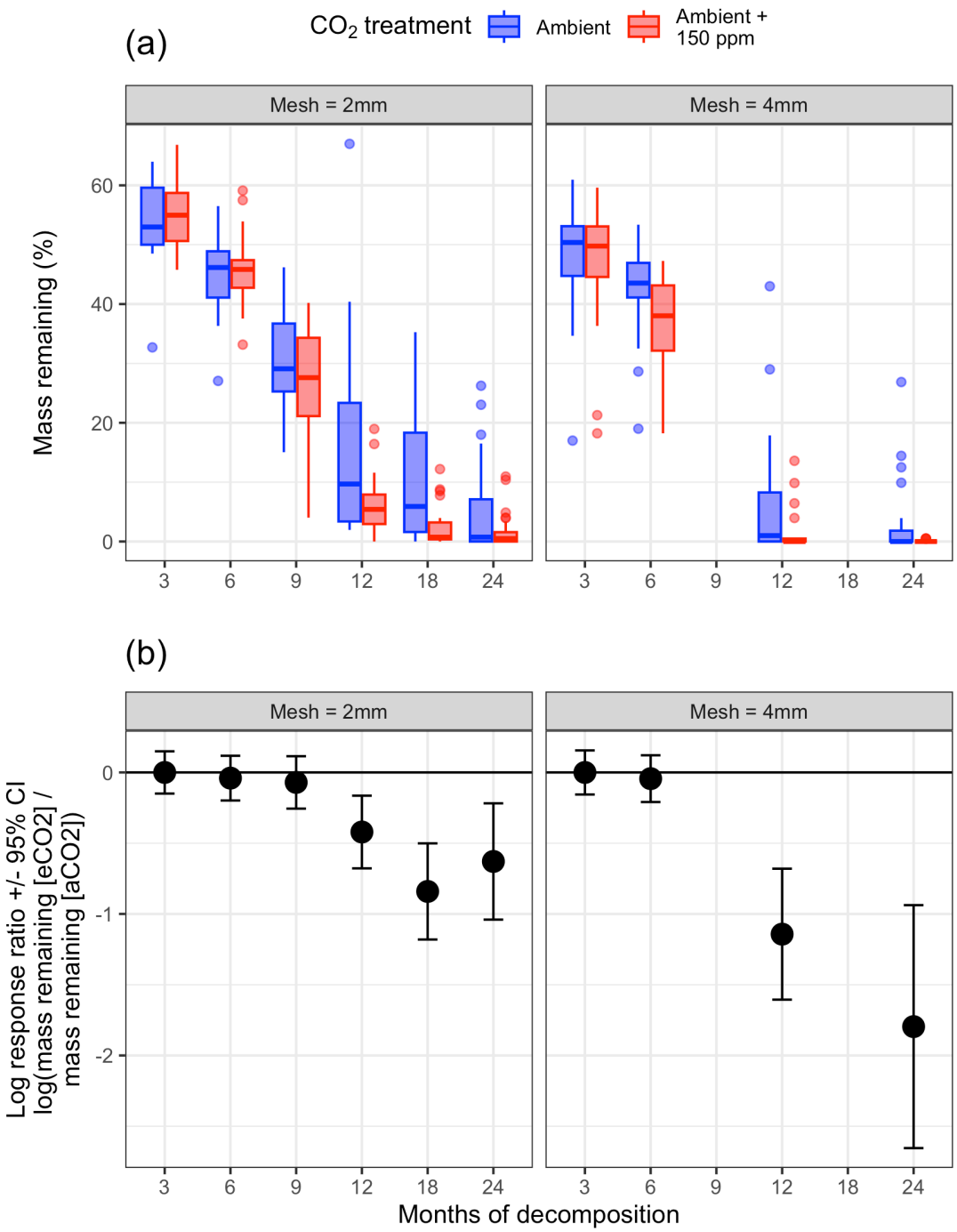
Figure captions

Figure 1. CO_2 effect on litter decomposition in the first study for each timepoint. Litter was contained within bags with two mesh sizes (2mm or 4mm). (a) Box-and-whisker plots showing mass remaining in litterbags over the course of the study under ambient or elevated (+150 ppm) CO_2 conditions. (b) Effect sizes associated with the CO_2 treatment during decomposition for each timepoint and mesh size relative to ambient CO_2. Dots represent the log response ratio calculated from mean values of mass remaining. Negative values indicate greater decomposition of litter under elevated CO_2 ('eCO2') than under ambient CO_2 ('aCO2'). Error bars represent 95% confidence intervals.

Figure 2. CO_2 effect on litter decomposition in the second study for each timepoint. Leaf litter developed under ambient or elevated CO_2 conditions and during or after a psyllid outbreak at the site. (a) Box-and-whisker plots showing mass remaining in litterbags over the course of the study. (b-c) Effect sizes associated with the CO_2 treatment during decomposition (b) or during leaf development (c) for each timepoint and condition. Dots represent the log response ratio calculated from mean values of mass remaining. Negative values indicate greater decomposition of litter under the elevated CO_2 ('eCO2') condition during litter decay (b) or leaf development (c) than under ambient CO_2 ('aCO2'). Error bars represent 95% confidence intervals.

Figure 3. Ordination of fungal assemblages associated with litter collected during the harvest prior to the observation of CO_2 effects on decomposition in the first study (after three months for 4 mm mesh and after six months for 2 mm mesh). OTU data were analysed using constrained analysis of principal coordinates (CAP) using Bray-Curtis dissimilarities.

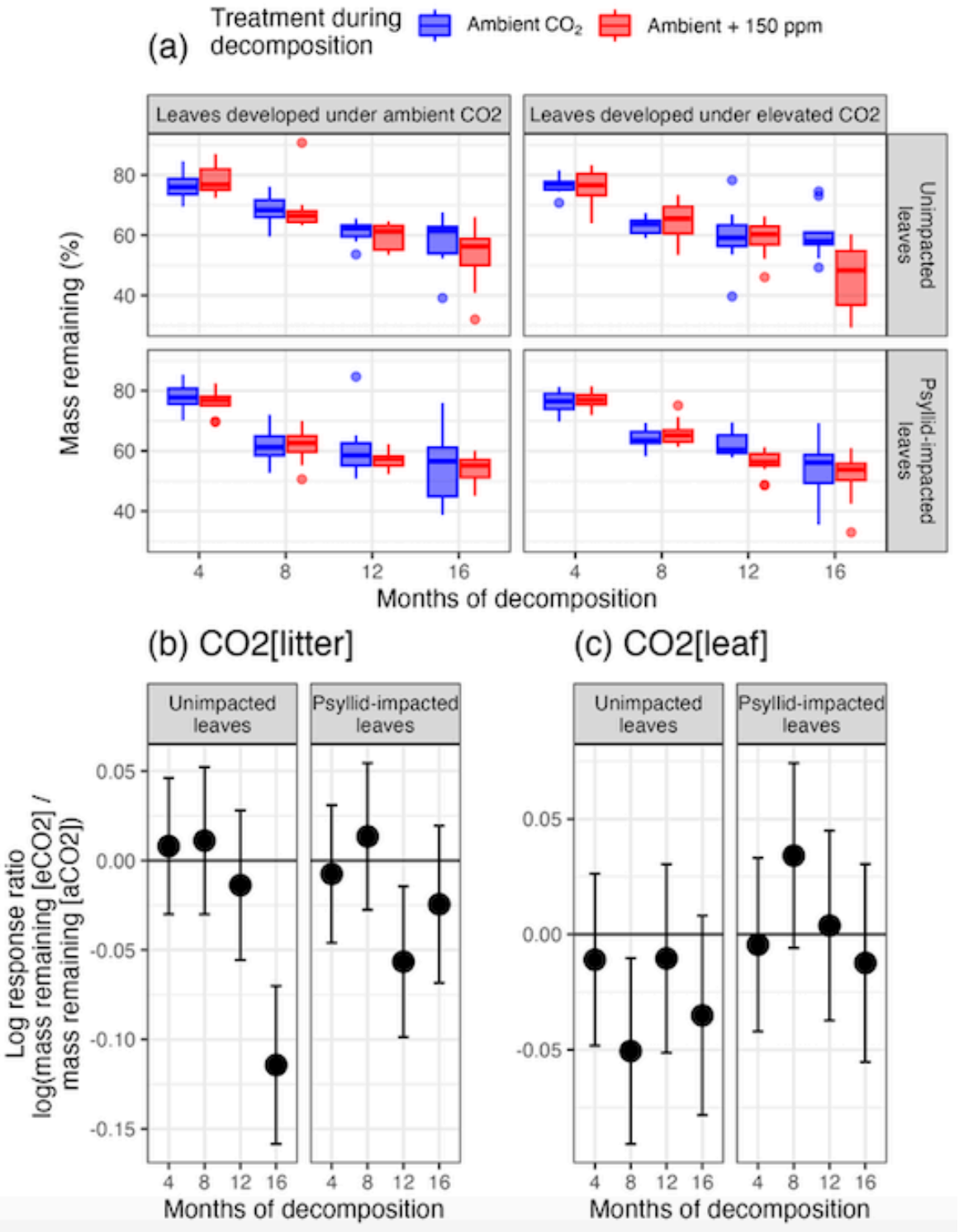
775 Variation along the first CAP axis (percentage of partitioned variation in parentheses) was
776 associated primarily with the size of holes in the litter-containing mesh, while the second was
777 associated primarily with the CO_2 treatment during decomposition. Lines connecting
778 external points for each group are to facilitate visualisation of patterns and are based on
779 concave hulls.
780
781 Figure 4. Ordinations of fungal assemblages associated with litter collected during the harvest
782 prior to the observation of CO_2 effects on decomposition in the second study (after eight
783 months). OTU data were analysed using constrained analysis of principal coordinates (CAP)
784 using Bray-Curtis dissimilarities. Variation along the first CAP axis (percentage of
785 partitioned variation in parentheses) was associated primarily with when the litter was
786 collected (coincident with the occurrence of a psyllid outbreak or in the years following),
787 while the second and third axes were associated primarily with the CO_2 treatment during leaf
788 development (a) or during decomposition (b). Lines connecting external points for each
789 group are to facilitate visualisation of patterns and are based on concave hulls.
790
791
792



793

794 *Figure 1.*

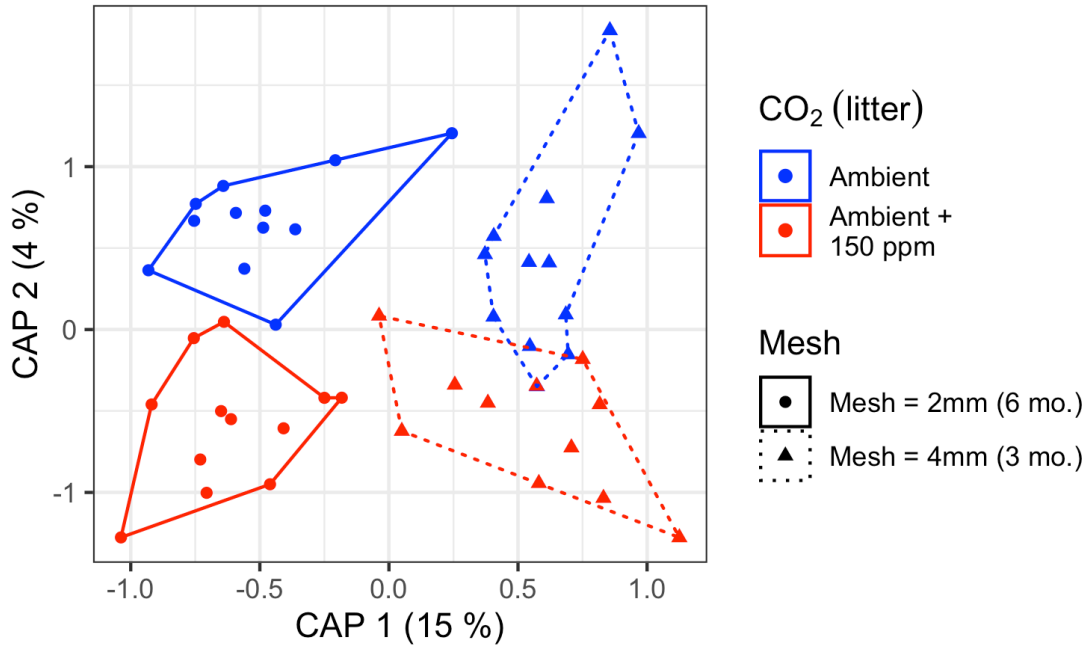
795



796

797 *Figure 2.*

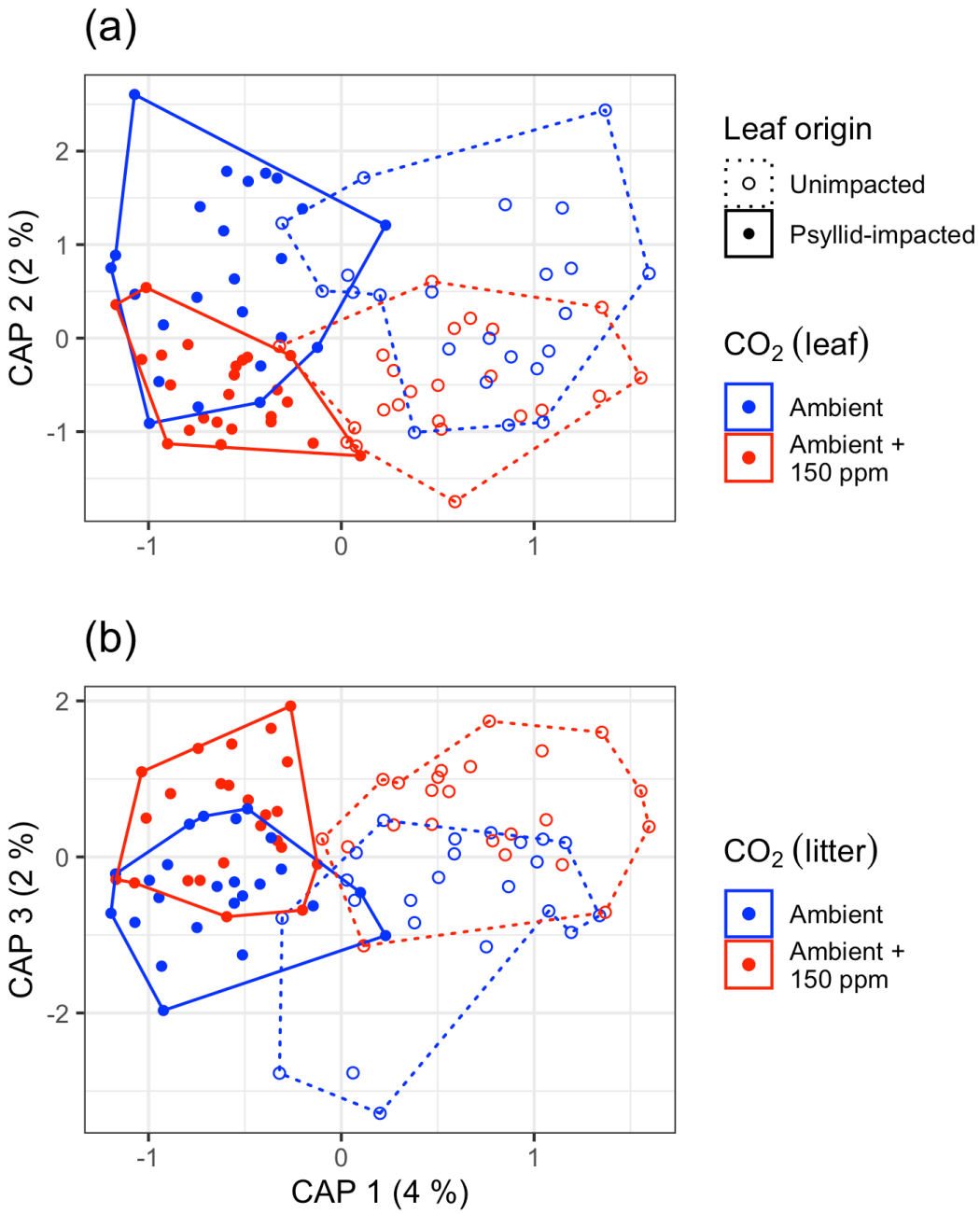
798



799

800 *Figure 3.*

801



802

803 *Figure 4.*

804

Supplementary Materials: Elevated CO_2 enhances decomposition and modifies litter-associated fungal assemblages in a natural *Eucalyptus* woodland

Uffe N Nielsen, Dylan Bristol, Michaela Blyton, Brendan Delroy, Jeff R Powell

Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith 2751, New South Wales, Australia

Table S1. Initial nitrogen and phosphorus concentrations of litter used in each decomposition study and, for the second study, each condition for leaf development prior to litter collection.

Study	Previous CO2 condition	Psyllid exposure	Samples	Mean %N	SD %N	Mean %P	SD %P
1	ambient	-	3	1.8	0.1	0.11	0.03
2	ambient	unimpacted	3	1.1	0.1	0.01	0.01
		Psyllid-impacted	3	1.3	0.6	0.02	0
	elevated	unimpacted	2	1.3	0.4	0.03	0.02
		Psyllid-impacted	3	1.2	0.2	0.04	0.04

Table S2. PerMANOVA analysis of fungal assemblages in the first study (Trt = CO_2 treatment during decomposition, Mesh = hole size in litter-containing mesh). OTU tables were analysed using Bray-Curtis dissimilarities.

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = mat ~ Trt * Mesh, data = temp)
##          Df SumOfSqs      R2      F Pr(>F)
## Trt       1   0.1945 0.03885 2.0880  0.012 *
## Mesh      1   0.7572 0.15119 8.1268  0.001 ***
## Trt:Mesh  1   0.0501 0.00999 0.5372  0.956
## Residual 43   4.0064 0.79997
## Total    46   5.0081 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table S3. Indicator OTUs associated with ambient or elevated CO_2 conditions during litter decomposition in the first study.

OTU ID	Litter condition	A	B	Score	P-value	Phylum	Order	Genus	Species	Guild	Confidence ranking
ITSall_OTUa_3984	eCO2	0.75	0.74	0.75	0.003	Ascomycota	Helotiales	Pilidium	Pilidium_anglicum	Plant Pathogen	Probable
ITSall_OTUg_25	eCO2	0.9	0.3	0.52	0.007	Ascomycota	Xylariales	NA	NA	NA	NA

Table S4. PerMANOVA analysis of fungal assemblages in the second study (Trt = CO_2 treatment during litter decomposition, prevTrt = CO_2 treatment during leaf development, psyllid = whether litter was impacted by a psyllid outbreak during leaf development). OTU tables were analysed using Bray-Curtis dissimilarities.

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = mat ~ Trt * prevTrt * psyllid, data = temp)
##                     Df SumOfSqs      R2      F Pr(>F)
## Trt                  1   0.2630 0.01861 1.8161  0.011 *
## prevTrt              1   0.3200 0.02263 2.2092  0.004 **
## psyllid              1   0.6262 0.04429 4.3232  0.001 ***
## Trt:prevTrt          1   0.1050 0.00743 0.7249  0.880
## Trt:psyllid          1   0.1356 0.00960 0.9365  0.527
## prevTrt:psyllid      1   0.1233 0.00872 0.8511  0.677
## Trt:prevTrt:psyllid  1   0.1074 0.00759 0.7412  0.865
## Residual            86  12.4562 0.88113
## Total               93  14.1366 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table S5. Indicator OTUs associated with ambient or elevated CO_2 conditions during litter decomposition in the second study.

OTU ID	Litter condition	A	B	Score	P-value	Phylum	Order	Genus	Species	Guild	Confidence ranking
ITSall_OTUb_91	aCO2	0.79	0.45	0.6	0.001	Ascomycota	Pleosporales	Neptunomyces	NA	Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotroph	Probable
ITSall_OTUa_1601	aCO2	0.81	0.38	0.56	0.003	Ascomycota	Pleosporales	Shiraia	NA	NA	NA
ITSall_OTUa_2354	aCO2	0.85	0.34	0.54	0.004	Ascomycota	Pleosporales	NA	NA	Fungal Parasite-Plant Pathogen-Plant Saprotroph	Probable
ITSall_OTUa_2071	aCO2	0.86	0.23	0.45	0.01	Ascomycota	Pleosporales	Didymocyrtis	Didymocyrtis_cladoniicola	Lichen Parasite	Highly Probable
ITSall_OTUa_1097	aCO2	0.94	0.19	0.42	0.007	Ascomycota	Chaetothyriales	Cyphellophora	NA	Animal Pathogen-Undefined Saprotroph	Probable
ITSall_OTUa_1554	eCO2	0.95	0.57	0.74	0.001	Ascomycota	Chaetothyriales	NA	NA	NA	NA
ITSall_OTUa_241	eCO2	0.82	0.3	0.49	0.006	Basidiomycota	Tremellales	Papiliotrema	Papiliotrema_flavescens	NA	NA
ITSall_OTUa_598	eCO2	1	0.23	0.48	0.001	Ascomycota	Pezizales	Plectania	NA	Undefined Saprotroph	Probable
ITSall_OTUg_304	eCO2	0.88	0.23	0.45	0.006	Ascomycota	Capnodiales	Cladosporium	NA	NA	NA
ITSall_OTUi_3676	eCO2	1	0.17	0.41	0.004	Ascomycota	Chaetothyriales	NA	NA	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Probable

Table S6. Indicator OTUs associated with ambient or elevated CO_2 conditions during leaf development in the second study.

OTU ID	Leaf condition	A	B	Score	P-value	Phylum	Order	Genus	Species	Guild	Confidence ranking
ITSall_OTUa_310	aCO2	0.79	0.64	0.71	0.001	Ascomycota	Chaetosphaeriales	Dictyochaeta	NA	Undefined Saprotroph	Probable
ITSall_OTUa_2280	aCO2	0.88	0.53	0.68	0.001	Basidiomycota	Sporidiobolales	Rhodotorula	NA	Animal Endosymbiont-Animal Pathogen-Endophyte-Plant Pathogen-Undefined Saprotroph	Probable
ITSall_OTUc_2779	aCO2	0.85	0.47	0.63	0.001	Ascomycota	Chaetosphaeriales	Dictyochaeta	NA	Undefined Saprotroph	Probable
ITSall_OTUa_15	aCO2	0.81	0.49	0.63	0.001	Basidiomycota	Tremellales	Saitozyma	Saitozyma_podzolica	NA	NA
ITSall_OTUb_196	aCO2	0.79	0.4	0.57	0.008	Ascomycota	Eurotiales	Talaromyces	NA	Undefined Saprotroph	Probable
ITSall_OTUa_43	aCO2	0.76	0.4	0.55	0.01	Ascomycota	Eurotiales	Talaromyces	NA	Undefined Saprotroph	Probable
ITSall_OTUa_956	aCO2	1	0.26	0.51	0.001	Ascomycota	Helotiales	Articulospora	NA	Undefined Saprotroph	Probable
ITSall_OTUi_794	aCO2	0.84	0.3	0.5	0.005	Ascomycota	Dothideales	Aureobasidium	NA	Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph	Possible
ITSall_OTUa_241	aCO2	0.81	0.3	0.49	0.005	Basidiomycota	Tremellales	Papiliotrema	Papiliotrema_flavescens	NA	NA
ITSall_OTUg_304	aCO2	0.91	0.26	0.48	0.004	Ascomycota	Capnodiales	Cladosporium	NA	NA	NA
ITSall_OTUe_3884	aCO2	0.93	0.21	0.45	0.003	Ascomycota	Eurotiales	Penicillium	NA	NA	NA
ITSall_OTUa_5159	eCO2	0.77	0.55	0.65	0.001	Basidiomycota	Septobasidiales	NA	NA	NA	NA
ITSall_OTUa_6378	eCO2	0.8	0.53	0.65	0.001	Basidiomycota	Microbotryomycetes_ord_Incertae_sedis	NA	NA	NA	NA
ITSall_OTUa_4331	eCO2	0.78	0.38	0.55	0.004	Basidiomycota	Microbotryomycetes_ord_Incertae_sedis	NA	NA	NA	NA

Table S7. Indicator OTUs associated with the timing of litter collection prior to initiation of the second study.

OTU ID	Leaf condition	A	B	Score	P-value	Phylum	Order	Genus	Species	Guild	Confidence ranking
ITSall_OTUa_768	psyllid-impacted	0.93	0.27	0.5	0.005	Ascomycota	Eurotiales	Talaromyces	NA	Undefined Saprotroph	Probable
ITSall_OTUa_3266	unimpacted	0.94	0.76	0.85	0.005	Ascomycota	Capnodiales	NA	NA	NA	NA
ITSall_OTUa_4152	unimpacted	0.95	0.74	0.84	0.005	Ascomycota	Capnodiales	Myrtapenidiella	NA	NA	NA
ITSall_OTUa_6576	unimpacted	0.97	0.65	0.8	0.005	Ascomycota	Capnodiales	Austroafricana	NA	NA	NA
ITSall_OTUa_5973	unimpacted	0.93	0.67	0.79	0.005	Ascomycota	Capnodiales	Austroafricana	Austroafricana_associata	NA	NA
ITSall_OTUa_7029	unimpacted	0.78	0.72	0.75	0.005	Basidiomycota	NA	NA	NA	NA	NA
ITSall_OTUa_4902	unimpacted	0.8	0.67	0.73	0.005	Basidiomycota	Tremellales	NA	NA	NA	NA
ITSall_OTUa_3166	unimpacted	0.95	0.54	0.72	0.005	Ascomycota	Capnodiales	Readeriella	NA	Plant Pathogen	Probable
ITSall_OTUb_2856	unimpacted	0.96	0.48	0.68	0.005	Ascomycota	Venturiales	Sympoventuria	NA	Undefined Saprotroph	Probable
ITSall_OTUc_2437	unimpacted	1	0.41	0.64	0.005	Ascomycota	Capnodiales	NA	NA	NA	NA
ITSall_OTUa_5744	unimpacted	0.86	0.48	0.64	0.005	Basidiomycota	NA	NA	NA	NA	NA
ITSall_OTUa_5382	unimpacted	0.9	0.41	0.61	0.005	Ascomycota	Chaetothyriales	NA	NA	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Probable
ITSall_OTUi_1663	unimpacted	0.87	0.41	0.6	0.005	Basidiomycota	Tremellales	NA	NA	NA	NA
ITSall_OTUa_3856	unimpacted	1	0.35	0.59	0.005	Ascomycota	Capnodiales	Teratosphaeria	Teratosphaeria_mexicana	NA	NA
ITSall_OTUa_9675	unimpacted	0.76	0.46	0.59	0.005	Basidiomycota	NA	NA	NA	NA	NA
ITSall_OTUa_5209	unimpacted	1	0.33	0.57	0.005	Ascomycota	Capnodiales	NA	NA	NA	NA
ITSall_OTUb_2874	unimpacted	1	0.28	0.53	0.005	Ascomycota	Capnodiales	NA	NA	NA	NA
ITSall_OTUg_315	unimpacted	0.92	0.2	0.42	0.005	Ascomycota	Chaetothyriales	NA	NA	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Probable
ITSall_OTUa_7092	unimpacted	1	0.17	0.42	0.005	Basidiomycota	NA	NA	NA	NA	NA
ITSall_OTUa_1829	unimpacted	0.82	0.17	0.38	0.01	Ascomycota	Xylariales	Gyrothrix	Gyrothrix_eucalypti	NA	NA

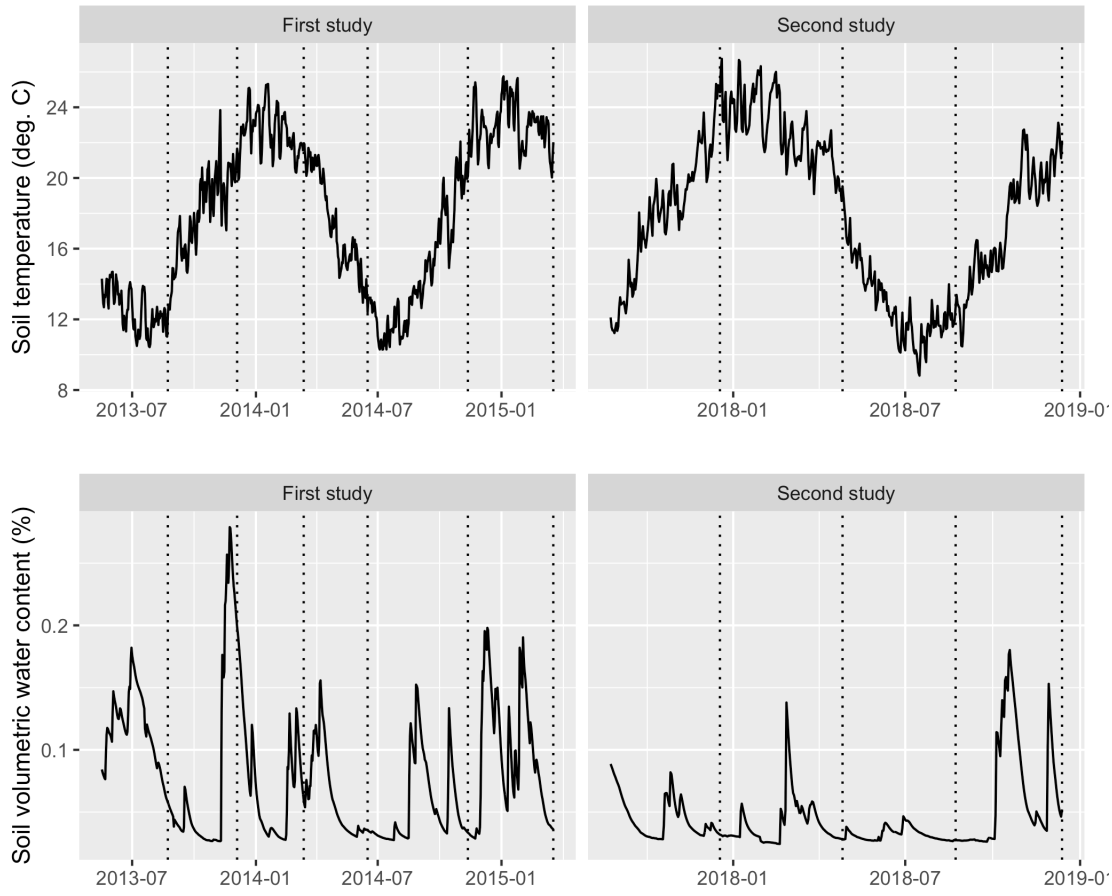


Figure S1. Soil temperature and moisture conditions in the top 10cm of soil during the two studies. Lines represent average values across all sensors (48 for soil moisture, 16 for soil temperature). Dashed vertical lines indicate sampling dates.

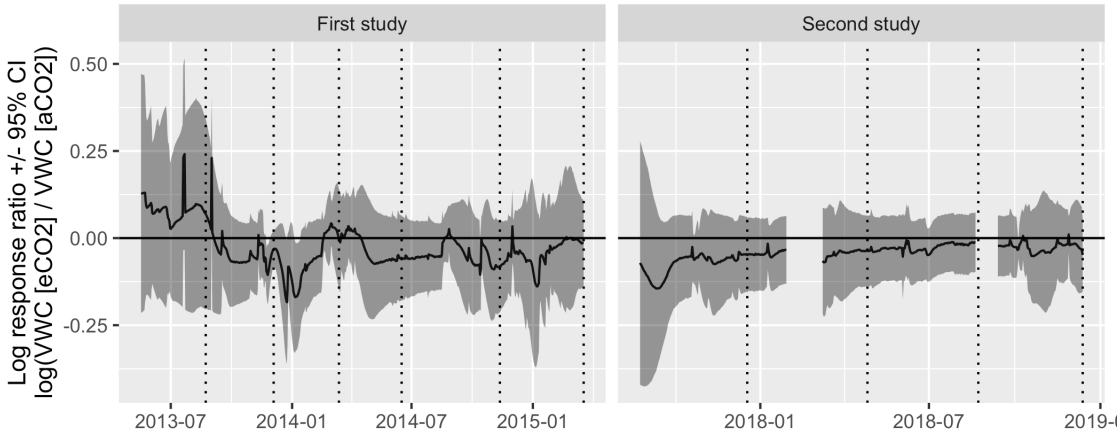


Figure S2. Effect sizes associated with elevated CO2 on soil volumetric water content (VWC) were within the margin of error for almost the entire duration of both studies, with only two short periods in the first study where soil moisture was lower in the elevated CO2 treatment relative to ambient conditions. Therefore, it is unlikely that differences in soil microclimate were responsible for treatment effects on decomposition rates. Solid lines represent mean effect sizes (negative values indicate drier soil moisture conditions under elevated CO2) and ribbons represent 95% confidence intervals. Dashed vertical lines indicate sampling dates. Gaps in the data are during periods where sensor data were not recorded for one or more rings.

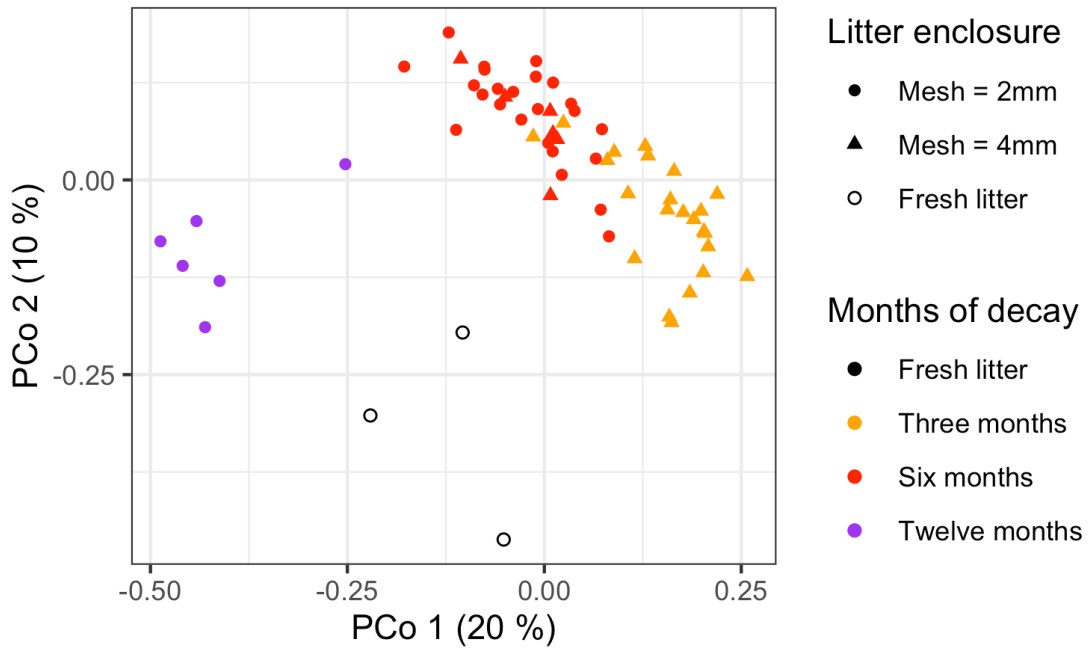


Figure S3. Ordination of litter fungal communities across all characterised samples in the first study. OTU data were analysed using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities.

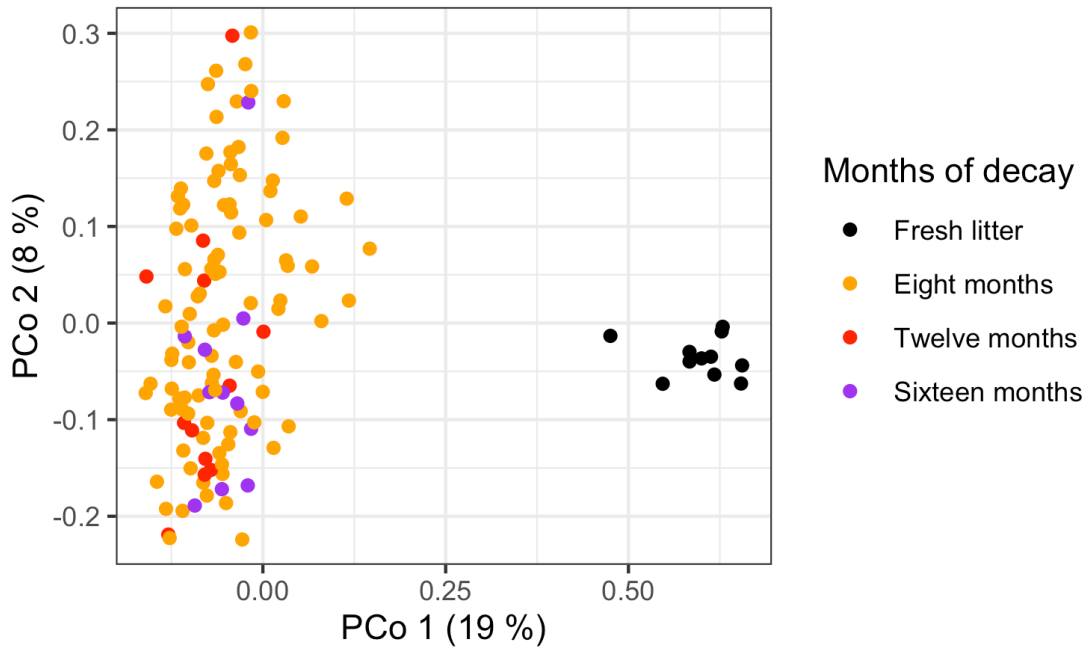


Figure S4. Ordination of litter fungal communities across all characterised samples in the second study. OTU data were analysed using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities.