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**

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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

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Data availability statement
Raw DNA sequencing data are available at the NCBI Sequence Read Archive under BioProject PRJNA1108048. All data and R code used for analyses in this manuscript are available at zenodo (https://doi.org/10.5281/zenodo.11108026).

Abstract

1. 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.
2. 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. 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.
3. Both macrofauna and elevated CO_2 enhanced mass loss at late decay stages in the first study, with no interactive effect. Again, mass loss was greater at elevated CO_2 at late decay stages in the second study, 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.
4. *Synthesis:* 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.

26 **Introduction**

27 Litter decomposition is a key process in terrestrial ecosystems, with plant litter being a
28 key carbon (C) source for decomposers that break down organic matter and make nutrients
29 available for sustained plant growth (Swift *et al.*, 1979; Bardgett *et al.*, 2005). Litter
30 decomposition rates, however, differ substantially among and within biomes, with climate,
31 litter chemistry and decomposer assemblages largely governing the breakdown process
32 (Hättenschwiler *et al.*, 2005; García-Palacios *et al.*, 2013). Climate regulates decomposition
33 through effects on vegetation composition, decomposer assemblage structure and biological
34 processes (e.g., metabolism, enzymatic activity, grazer activity; Suseela & Tharayil, 2018),
35 with generally greater rates of litter decomposition in warm and humid ecosystems where
36 biological activity is high (García-Palacios *et al.*, 2013). Plant functional traits have strong
37 effects on decomposability in that litter with high nitrogen (N) and phosphorus (P) content
38 decomposes more rapidly than litter with lower nutrient and high lignin contents (Cornwell *et*
39 *al.*, 2008). Microbes, particularly fungi, are the primary decomposers while soil fauna
40 contribute both directly as decomposers and through comminution, microbial grazing and
41 modifiers of the soil structure and microenvironment (Hättenschwiler *et al.*, 2005; Nielsen *et*
42 *al.*, 2015).

43 While our understanding of the influences of these drivers on litter decomposition
44 dynamics has increased substantially over the past few decades, there are still important
45 knowledge gaps, including how increasing atmospheric carbon dioxide (CO_2) concentrations
46 will interact with these drivers to affect litter decomposition processes and, through this, C
47 dynamics. Elevated CO_2 concentrations (henceforth, eCO_2) can reduce litter decomposition
48 due to reduced litter quality (i.e., reduced N content, increased lignin content) (Norby *et al.*,
49 2001). However, increased rates of mass loss have been observed in some studies indicating
50 that eCO_2 effects are context dependent, potentially moderated by increased saprotroph activity

51 in soil mediated by greater plant water use efficiency (Hall *et al.*, 2006), particularly when
52 water is limiting (e.g., Volk *et al.*, 2000; Blumenthal *et al.*, 2013) and increased root exudation
53 (Phillips *et al.*, 2011). Accordingly, eCO_2 has been shown to increase microbial biomass and
54 the abundance of detritivores (Blankinship et al., 2011), both of which could promote litter
55 decomposition. Adding further complexity, increased abundances of detritivore fauna may also
56 impact litter decomposition due to increased grazing of fungi or through changes in soil
57 decomposer assemblage composition (van der Wal *et al.*, 2013; A'Bear *et al.*, 2014).

58 Besides these effects, eCO_2 may alter herbivory through changes in resource
59 availability and quality to moderate litter decomposition while herbivory itself can also affect
60 leaf chemistry. Several studies have shown that leaf herbivory results in increased leaf N
61 content (Chapman *et al.*, 2003; Hall *et al.*, 2006), which might ameliorate the negative effect
62 of eCO_2 on litter decomposition by counteracting eCO_2-induced reductions in leaf N.
63 Importantly, these factors may interact to moderate eCO_2 impacts, with changes in leaf
64 chemistry expected to slow down decomposition whereas increased soil water content and
65 decomposer activity may enhance decomposition processes (Kuzyakov *et al.*, 2019).

66 Decomposition is a dynamic process, an important point to consider when investigating
67 eCO_2 effects on the drivers of decomposition. The first substantial meta-analysis of eCO_2
68 effects on leaf litter chemistry showed reduced N and increased lignin content in leaves (i.e.,
69 lower quality) but found no consistent associated effects on litter mass loss except for a slight
70 reduction for woody species (Norby *et al.*, 2001). A key finding was that the expected eCO_2-
71 induced reduction in leaf N concentration was substantially lower in leaves following N
72 resorption at senescence, which would ameliorate eCO_2 driven effects on decomposition. The
73 authors further hypothesized that while reduced N might slow down mass loss early in
74 decomposition, impacts on microbial assemblages can increase lignin degradation, resulting in
75 enhanced rates of mass loss at later stages in the decomposition process. Some fungi generally

76 utilize labile carbon sources and available nutrients during the early stages of decomposition
77 while others break down more recalcitrant plant material and translocate nutrients from other
78 sources, particularly later during decomposition (van der Wal *et al.*, 2013).

79 We investigated litter decomposition dynamics at ambient and elevated CO_2 (+150
80 ppm relative to ambient) in the *Eucalyptus* FACE (EucFACE) facility in eastern Australia to
81 provide further insight into the controls on litter decomposition using a standard litter bag
82 approach. Specifically, in two complementary studies, we assessed the independent and
83 interactive effects of eCO_2, macrofauna and herbivory on litter decomposition and associated
84 changes in litter chemistry and fungal decomposer assemblage structure. Previous work at the
85 site has shown increased photosynthesis under eCO_2 but not a concurrent increase in
86 ecosystem productivity or C storage, with higher soil respiration contributing to greater
87 ecosystem C losses under eCO_2 indicating greater soil biological activity (Jiang *et al.*, 2020).
88 Moreover, eCO_2 has been found to increase root litter mass loss at late decay stages, possibly
89 associated with greater relative abundance and activity of saprotrophic fungi (Castañeda-
90 Gómez *et al.*, 2020).

91 Our main hypothesis was that eCO_2 would enhance leaf litter decomposition rates at
92 this site *via* increased activity of saprotrophs and detritivores in soil but that the effect would
93 be moderated by i) whether the litter developed under ambient CO_2 (aCO_2) or eCO_2 given
94 expected influences on litter chemistry, ii) whether macrofauna were excluded given potential
95 changes in densities between aCO_2 and eCO_2, and iii) whether the litter was impacted by
96 herbivores given shifts in leaf chemistry. For the latter, we took advantage of an outbreak of
97 leaf feeding psyllids occurring at the site throughout 2014 (Gherlenda *et al.*, 2016) comparing
98 decomposition of litter collected during the outbreak and with visible signs of herbivory to
99 that of litter collected from canopy that developed after the outbreak, when psyllid
100 populations were much lower. In addition, we used amplicon sequencing to assess fungal

101 assemblages in the litter for each treatment combination at the time point prior to where
102 treatment effects were observed and related this to mass loss at a following time point to
103 better assess whether changes in litter decomposition is related to eCO_2-induced shifts in
104 fungal assemblages, including effects on abundances of saprotrophic fungi.
105

106 **Methods and Materials**

107 *Site description*

108 EucFACE is located in a mature warm-temperate evergreen forest dominated by
109 *Eucalyptus tereticornis*, with minimal human disturbance for at least 90 years (Jiang *et al.*,
110 2020). The understory is dominated by native grasses and shrubs. The facility was established
111 in 2012, exposing three experimental plots (25 m diameter circles) to elevated atmospheric
112 CO_2 concentrations at ~150 ppm above ambient conditions through fumigation. Three control
113 plots were established with similar infrastructure but was fumigated with air without CO_2
114 addition. Treatments commenced in September 2012, but CO_2 concentrations were raised
115 incrementally (~30 ppm increase per month), with full-strength treatment concentrations
116 reached in February 2013 and then maintained throughout the experimental duration when
117 conditions allowed (Ellsworth *et al.*, 2017). The site is characterised by low-fertility alluvial
118 soil of the Clarendon Formation with high sand content (>75%) and low phosphorus content,
119 with growth considered P-limited. The deeper horizons are sandy clay loam with the presence
120 of clay bands that affect site hydrology (Ross *et al.*, 2020). A license or permit was not
121 required for the work as the experiment is on property owned by Western Sydney University.
122

123 *Litter bags*

124 A standard litter bag approach was used to assess decomposition rates through time,
125 including the effect of macrofauna exclusion by contrasting rates for bags with different mesh

126 sizes (see below). Although this approach has been criticised for inducing non-target effects
127 (see Kampichler and Bruckner 2009), more recent assessments indicate that the findings are
128 robust given comparable findings when litter bag studies are contrasted with other means of
129 soil fauna suppression / exclusion (García-Palacios *et al.*, 2013).

130 For the first study, we used *E. tereticornis* leaves from a fallen branch found outside
131 the main CO_2 treatment plots in 2013 to reduce the effect of environmental and microbial
132 influences associated with litter already in contact with the ground. The litter is considered
133 green leaf material given that the leaves were still attached to the branches. As such, it was
134 expected to have higher nutrient content than senesced leaf litter as resorption would not have
135 occurred which is likely to affect the litter decomposition process. These litter bags were
136 deployed in June 2013. For the second study, we used *E. tereticornis* leaves collected in litter
137 traps within the respective CO_2 treatment plots to consider the CO_2 concentration at which the
138 leaves developed. We distinguished litter collected between December 2013 and December
139 2014, during the psyllid outbreak (Gherlenda *et al.*, 2016), and litter collected between January
140 2015 and June 2016, after the psyllid outbreak had ended. All leaf litter was dried at 40 ºC to
141 constant weight (Ellsworth *et al.*, 2017) prior to storage in paper bags in an air-conditioned
142 room. We only used litter with lerps (produced by the psyllid) collected during the psyllid
143 outbreak to ensure herbivore effects and only litter without lerps for litter collected after the
144 psyllid outbreak. These litter bags were deployed in August 2017. Approximately 2 grams was
145 added to each bag for both studies.

146 For both studies, litter bags were deployed in each of four 1 m^2 subplots in each plot,
147 with bags pegged to the soil surface in each subplot after gently brushing aside existing
148 vegetation and litter, where necessary, and redistributing after the litter bag had been deposited
149 to best simulate natural conditions. In the first study, we deployed litter in bags with two
150 different mesh sizes, with 2 mm mesh bags collected after approximately 3, 6, 9, 12, 18 and 24

151 months, while 4 mm mesh bags were collected after approximately 3, 6, 12 and 24 months
152 only. Given that only one litter type (i.e. 'green leaf') was used in this study, this resulted in a
153 total of 288 litter bags with 2 mm mesh (2 replicates per subplot × 6 time points × 4 subplots
154 × 3 plots × 2 CO_2 treatments) and 192 with 4 mm mesh (2 replicates per subplot × 4 time points
155 × 4 subplots × 3 plots × 2 CO_2 treatments). In the second study, all litter bags were of the same
156 mesh size (2 mm, same material as in the first study) to limit the number of experimental units.
157 Bags were collected at four time points across the first 16 months (4, 8, 12 and 16 months)
158 where the treatment effects were observed during the first study. We used a full factorial design
159 for CO_2 concentration under which leaves grew prior to senescence ('CO_2 [leaf]'), CO_2
160 concentration during litter decomposition ('CO_2 [litter]'), and psyllid presence during leaf
161 growth ('psyllid') with one bag per subplot and four subplots per CO_2 treatment area, resulting
162 in 384 experimental units (1 replicate per subplot × 4 time points × 4 subplots × 3 plots × 2
163 litter CO_2 treatments × 2 leaf CO_2 treatments × 2 psyllid treatments). Upon collection of bags
164 the remaining litter was dried at 40 °C until constant weight before the litter was weighed to
165 calculate mass loss. All non-litter material, including mineral soil, that had entered during
166 incubation was removed prior to weighing.

167 Soil temperature and moisture were continuously monitored at multiple locations
168 within each plot (but outside of the four subplots) over the course of each study. Soil
169 moisture, as volumetric water content, was monitored using frequency-domain reflectometers
170 (CS650 Soil Water Content Reflectometer, Campbell Scientific, Logan, UT, USA) installed
171 at a depth of 30 cm at eight locations within each plot. Soil temperature was monitored using
172 temperature probes (TH3-s, UMS GmbH, Frankfurt, Germany) installed at a depth of 5 cm at
173 two locations in each plot. Soil temperature conditions were similar between the two studies
174 but soil moisture was much lower in the second study than in the first (Fig. S1), at values less
175 than 5% volumetric water content for much of the second study. We found generally similar

176 levels of soil moisture under both aCO_2 and eCO_2 over the duration of both studies (Fig. S2),
177 which is consistent with previous findings at the site (Pathare *et al.*, 2017; Ginemo *et al.*,
178 2018).
179
180 *Litter chemistry*
181 Litter C and N content was determined using a LECO TruMac CN analyser (Leco
182 Corporation, St Joseph, MI, USA) based on the Dumas method after grinding dried material
183 to a fine powder. Litter P content was determined using an Epsilon 4 Benchtop X-ray
184 fluorescence (XRF) spectrometer (Malvern Panalytical, Malvern, UK). Litter C, N and P
185 concentrations were measured on a sub-set of the litter bags chosen in each study to represent
186 stages prior to and following the initiation of CO_2 effects on decomposition. In the first study,
187 this included litter harvested after 6 and 12 months in the 2 mm mesh bags and after 3 and 6
188 months in the 4 mm mesh bags; the litter from the two bags collected from within a subplot
189 during each harvest were composited prior to chemical analysis. In the second study, this
190 included psyllid-affected litter harvested after 8 and 12 months and psyllid-unaffected litter
191 harvested after 8 and 16 months; there was no compositing of samples here since only one
192 bag of each litter origin was collected from a subplot during each harvest. We also measured
193 C, N and P concentrations on three composite samples of litter that was not deployed in litter
194 bags for each set of initial conditions, of which there was only one in the first study (green
195 leaf litter picked off the fallen branch) and four in the second (relating to previous CO_2
196 condition during growth and collection date relative to the psyllid outbreak). The leaf litter
197 used in the second study had lower concentrations of nitrogen and phosphorus as expected in
198 senesced litter compared with green leaf material used in the first study (Table S1).
199
200 *Fungal assemblages*

201 DNA was extracted from approximately 200 mg of each of the samples that were analysed
202 for litter chemistry. The samples were ground into a fine powder with 5 mm steel beads in a
203 TissueLyser II (Qiagen), then 1 ml of CTAB buffer (0.1 M Tris-HCL, 1.4 M NaCl, 0.02M
204 EDTA, 20 g.l-1 of cetyltrimethyl ammonium bromide with 4% (w/v) polyvinylpyrrolidone)
205 was added to each sample. The samples were digested at 65 °C with mixing at 1000 rpm for 1
206 hour. The samples were then spun for 7 minutes at 16 000 rpm, following which 500 µl of the
207 supernatant was transferred to a new tube and 500 µl of chloroform:isoamyl alcohol (24:1)
208 added. The samples were mixed by inversion for 5 minutes and spun at 16 000 g for 7
209 minutes, after which up to 450 µl of the upper aqueous phase was then transferred to a new
210 tube. The DNA was precipitated by addition of 0.08 volumes of cold 7.5M ammonium
211 acetate and 0.54 volumes of cold isopropanol, followed by 30 minutes at -20 °C. The samples
212 were spun at 16 000 g for 3 minutes and the supernatant removed. The DNA was then
213 washed with 700 µl of 70 % cold ethanol, followed by 700 µl of cold 95% ethanol. The
214 samples were spun at 16 000 g for 1.5 minutes after each wash and the ethanol removed. The
215 samples were dried for 30 minutes at 65 °C and then re-suspended in 100 µl of TE buffer (10
216 mM Tris-HCL, 1 mM EDTA).
217
218 Many of the raw extracts were darkly coloured. To remove polyphenolic compounds,
219 humic/fulvic acids, tannins and other PCR inhibitors from the raw DNA extracts, the samples
220 were treated using the Zymo OneStep™ PCR Inhibitor Removal Kit according the
221 manufacture's protocol. Twenty-four samples were subsequently checked for successful
222 amplification of the ITS gene by PCR following the protocol of Gourmelon *et al.* (2016)
223 using the MyTaq PCR system (Bioline). Three samples did not successfully amplify. These
224 samples remained darkly coloured after inhibitor clean-up. All darkly coloured samples were

225 then checked for successful PCR amplification at template concentrations of 100, 20 and 1
226 ng/µl. All samples at a concentration of 1 ng/µl successfully amplified.
227
228 For sequencing of clear DNA samples, concentrated samples were generally diluted to 10
229 ng/µl while samples with concentrations of less than 15 ng/µl were left neat. The darkly
230 colours samples were diluted to 1 ng/µl prior to sequencing. DNA samples were submitted to
231 the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, NSW,
232 Australia). Amplicons were generated using fITS7 (5'-GTGARTCATCGAATCTTTG-3';
233 Ihrmark *et al.* 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White *et al.* 1990). All
234 amplicons were purified using the Agencourt AMpure XP system (Beckman Coulter, Lane
235 Cove, NSW, Australia) and genomic libraries were prepared using the Nextera XT Index Kit
236 (Illumina, San Diego, CA, USA). Paired-end (2 x 251 bases) sequencing was performed on
237 the Illumina MiSeq platform. Raw DNA sequencing data are available at the NCBI Sequence
238 Read Archive under BioProject PRJNA1108048.
239
240 To process the DNA sequencing data, we used the approach described by Bissett *et al.*
241 (2016) with a few modifications. Contigs were generated from paired-end reads using the
242 'fastq_mergepairs' command in VSEARCH (version v2.3.4; Rognes *et al.*, 2016) using a
243 minimum overlap of 30 base pairs. Initial quality filtering removed DNA sequences
244 containing ambiguous bases and/or homopolymers greater than eight bases in length.
245 Sequences were kept for further analysis if they were within 200-470 base pairs in length and
246 contained fewer than 0.5 expected errors. De novo operational taxonomic units (OTUs) at
247 97% sequence similarity were initially picked using numerically dominant sequences
248 (observed at least two times) using the '-cluster_smallmem' command in VSEARCH. All
249 quality-filtered sequences were mapped at 97% sequence similarity against representative

250 sequences of these OTUs using the '-usearch_global' command in VSEARCH. Non-mapped
251 sequences were subjected to a second round of de novo OTU picking, as above but only
252 using sequences observed at least two times. All initially non-mapped sequences were then
253 mapped against these newly picked OTUs, as above. Non-mapped sequences at this step
254 represent singleton OTUs and were excluded from further analysis. Sequence read counts that
255 were less than ten within individual samples were removed to reduce the likelihood of
256 sequence reads being assigned to samples incorrectly. As a result of this, as well as the high
257 level of sequencing depth across all samples (between 12122 and 41536 reads per sample in
258 the first study, between 16619 and 58666 reads per sample in the second study), coverage
259 was estimated to be high (Good's coverage = 100) in all samples. Therefore, we did not rarefy
260 the OTU table before further analysis.
261
262 Putative taxonomic identities for fungal OTUs were generated using BLAST (v.2.6.0,
263 Altschul *et al.*, 1990) to compare representative sequences for each OTU to a reference
264 database of gene sequences and taxonomic annotations (UNITE version 8.3,
265 sh_general_release_dynamic_s_10.05.2021; Abarenkov *et al.*, 2021). Fungal ITS2 sequences
266 were extracted using ITSx (Bengtsson-Palme *et al.*, 2013, v1.1.3) for use during BLAST.
267 Trophic modes and guilds of fungal OTUs that were assigned to taxa were then inferred using
268 FUNGuild (Nguyen *et al.*, 2016).
269
270 *Replication Statement*

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Atmospheric CO_2 concentration	Plot	Three plots for each of two CO_2 levels
Local soil microenvironment	Subplot	Four subplots within each plot
Decay agent (mesh size; experiment 1)	Litter bags	Two bags per mesh treatment within each plot

		for each harvest timepoint (total = 48 per harvest)
Litter origin (CO_2 and psyllid conditions; experiment 2)	Litter bags	One bag per combination of litter conditions within each plot for each harvest timepoint (total = 24 per harvest)

271

272 *Data analyses*

273 All data analyses were performed using R v.4.1.2 (R Core Team, 2021). All data and R code
274 used for analyses in this manuscript are available at zenodo
275 (https://doi.org/10.5281/zenodo.11108026; Powell, 2024). We tested for main and interactive
276 effects of CO_2 treatment during litter decay, mesh size and months of decay (study 1) or CO_2
277 treatment during litter decay, during leaf development, psyllid presence and months of decay
278 (study 2) on litter decomposition using linear mixed effects models, treating 'plot' and
279 'subplot within plot' as random effects ('lme4' package; Bates *et al.*, 2015). Months of decay
280 was treated as an ordered factor in both models. Litter decomposition was represented in the
281 model as the proportion of the original mass that remained following incubation and applying
282 the arcsine-square root transformation (Sokal and Rohlf, 1995) to account for the distribution
283 being bounded at both ends (0 and 1). The same models were used to assess responses of
284 litter nitrogen and phosphorus concentrations, except we only analysed one timepoint so did
285 not include months of decay.

286

287 For relevant interactive effects, we estimated the effect size of CO_2 treatments on
288 decomposition based on log-response ratios (LRRs) calculated using the model prediction of
289 the mean response under each of the two CO_2 treatments, i.e., log($response_{elevated\ CO2}$ /
290 $response_{ambient\ CO2}$). Standard errors for LRRs were also calculated from model predictions
291 according to Hedges *et al.* (1999), i.e., square root(($se_{elevated\ CO2}^2$ / $mean_{elevated\ CO2}^2$) + ($se_{ambient}$
292 $_{CO2}^2$ / $mean_{ambient\ CO2}^2$)). LRRs were considered significant when the confidence interval of the

293 mean (+/- two standard errors) did not overlap zero. Model predictions were obtained using
294 the 'ggemmeans' function from the 'ggeffects' package (Lüdecke, 2018). We also used the
295 'pairs' and 'emmeans' functions from the 'emmeans' package (Lenth, 2024) to obtain P-
296 values associated with effect sizes for CO_2 treatments for relevant interactive effects.
297
298 Variation in fungal assemblages for both studies was visualised using principal coordinates
299 analysis based on Bray-Curtis dissimilarities, after Hellinger-transformation of each OTU
300 table, using functions from the 'vegan' package (Oksanen *et al.*, 2022). We also performed
301 further analysis of fungal assemblages sampled at the time point prior to the observation of
302 CO_2 effects on decomposition in each study. For this, we first performed PerMANOVA to
303 assess the significance and effect size of all of the main effects and interactions associated
304 with each study, again using Bray-Curtis dissimilarities after Hellinger transformation. Then
305 we visualised these patterns using constrained analysis of principal coordinates (CAP),
306 including each main effect in the constraint. Finally, we performed multi-level pattern
307 analysis using the 'indicspecies' package (De Caceres and Legendre, 2009) to identify OTUs
308 indicative of groups within relevant treatments; for this analysis we used a conservative cut-
309 off of $P < 0.01$ to identify indicators so as to identify those taxa that were most strongly
310 associated with each treatment.
311

312 **Results**

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

315 In the first study, both mesh sizes resulted in a median value of less than 10% mass
316 remaining after 12 months (Figure 1a). Mesh size had a significant effect on litter mass loss
317 ($P_{mesh} < 0.001$) with higher loss in the large mesh size bags (Figure 1a), and this varied

318 through time as indicated by the time:mesh interaction ($P_{time:mesh} < 0.001$; Table 1). In
319 addition, mass loss was greater in the eCO_2 treatment at late decay stages as indicated by the
320 time:treatment interaction ($P_{time:treatment} < 0.001$; Table 1) and confidence intervals for LRRs
321 barely overlapping zero at 12 ($P_{treatment} = 0.096$) and 18 ($P_{treatment} = 0.068$) months with 2 mm
322 mesh (Figure 1b), when the median mass remaining in the eCO_2 treatment was between 5 and
323 10%. All other eCO_2 contrasts were clearly nonsignificant ($P_{treatment} > 0.19$). eCO_2 effect sizes
324 were consistent across both mesh sizes ($P_{time:treatment:mesh} = 0.31$; Table 1; Figure 1b). The
325 eCO_2 effect appeared greater in the large mesh size bags (Figure 2) but this was not
326 significant ($P_{treatment:mesh} = 0.41$; Table 1).

327 In the second study, decomposition occurred more slowly and all but one treatment
328 combination resulted in a median value of more than 50% mass remaining after 16 months
329 (Figure 2a). Here, we observed that concurrent CO_2 treatment effects again tended to appear
330 during later decay stages ($P_{time:treatment} = 0.001$), but the timing of this effect was inconsistent
331 depending on whether the litter was impacted by psyllids and the number of months of decay
332 ($P_{time:treatment:psyllid} = 0.02$; Figure 2b). For litter impacted by psyllids, we observed an
333 ephemeral increase, after 12 months, in decomposition for litter derived from leaves that
334 developed under elevated CO_2 relative to those developing under ambient CO_2. For litter
335 unimpacted by psyllids, we observed this same pattern but not until 16 months into the study.

336 Additional effects on litter decomposition were observed in the second study that
337 were dependent on the condition that leaves developed under but were independent of the
338 CO_2 treatment during decay ($P_{time:previousTreatment:psyllid} = 0.02$; Figure 2c). This was due to an
339 ephemeral increase in decomposition after eight months for litter from leaves developing
340 under elevated CO_2, relative to those developing under ambient CO_2, but only in the absence
341 of the psyllid outbreak.

342

343 *Differences in fungal assemblages, but not litter chemistry, existed prior to observed CO_2*
344 *effects on litter decomposition*
345 We observed strong compositional variation in fungal assemblages in the first study when
346 comparing fresh leaves, litter collected prior to the observation of CO_2 effect on
347 decomposition and litter collection after the observation of this effect (Figure S3). When
348 focussing only on assemblages in litter collected prior to that effect (Figure 3; Table S2), we
349 observed significant effects of CO_2 treatment (PerMANOVA, $P < 0.05$; $R^2 = 0.039$) and mesh
350 size ($P = 0.001$; $R^2 = 0.151$) but no interaction between those terms ($P > 0.9$). This is despite
351 our observation that litter N and P concentrations were similar between these treatments at
352 that time ($P_{treatment} > 0.7$ for both; [N] = 2.6 +/- 0.1%, [P] = 0.13 +/- 0.02%; mean +/- SD).
353 Two fungal OTUs had a significantly higher frequency in the elevated CO_2 treatment (Table
354 S3): one with >99% identity with an isolate of *Pilidium anglicum* and one likely belonging to
355 the Xylariales.
356
357 In the second study we observed large differences when comparing fresh leaves and litter, but
358 fungal assemblages in litter were highly variable and were difficult to differentiate by the
359 number of months undergoing decay (Figure S4). Again, we observed variation in fungal
360 assemblages associated with the CO_2 treatment (PerMANOVA, $P < 0.01$) during decay even
361 before CO_2 effects on decay were observed (Figure 4; Table S5) and, again, N and P
362 concentrations were similar between these treatments at that time, accounting for variation in
363 initial chemistry among litter from different sources ($P_{treatment} > 0.8$ and $P > 0.06$ for
364 interactions involving 'treatment'; [N] = 1.5 +/- 0.3%, [P] = 0.03 +/- 0.02%; mean +/- SD).
365 We also observed significant effects of the CO_2 condition during leaf development (P =
366 0.001) and whether leaves were psyllid impacted (P = 0.001) on fungal assemblages in that
367 same litter, with the psyllid condition having the most important effect (differentiation along

368 axis 1 of the CAP plots in Figure 4; R^2 = 0.044), the CO_2 treatment during litter development
369 being the second-most important (axis 2, y-axis of the plot in Figure 4a; R^2 = 0.023) and the
370 CO_2 treatment during litter decay being the third-most important (axis 3, y-axis of the plot in
371 Figure 4b; R^2 = 0.019), despite most variation remaining unexplained (residual R^2 = 0.88).
372 No significant interactions among terms were observed (all $P > 0.5$). Six fungal OTUs had a
373 significantly higher frequency in the ambient CO_2 treatment, five with >95% identity to
374 genera in the Pleosporales and one with a match to the genus *Cyphellophora* (Table S5). Five
375 fungal OTUs had a significantly higher frequency in the elevated CO_2 treatment, with
376 variable levels of matches to taxa in the Chaetothyriales (two OTUs), Tremellales (one OTU
377 with 100% identity to *Papiliotrema flavescens*), Capnodiales and Pezizales. Several OTUs
378 were significantly associated with conditions during leaf development (13 OTUs for prior
379 CO_2 condition, Table S6, and 19 OTUs for psyllid condition, Table S7).
380
381
382 **Discussion**
383 Our main hypothesis was that eCO_2 would enhance leaf litter decomposition rates at
384 this site *via* increased activity of saprotrophs and detritivores and that the effect would be
385 moderated by eCO_2 and herbivore effects on leaf chemistry during growth and on whether
386 macrofauna could access litter. Consistent with our main hypothesis, litter mass loss
387 increased at eCO_2 at late decay stages in both studies, but this occurred mostly independently
388 of CO_2 concentration under which the leaves were grown, macrofauna exclusion and
389 herbivore presence. Our results differ from the general observation that eCO_2 has a negative
390 albeit slight impact on decomposition of litter from woody species in forests (e.g., Norby *et*
391 *al.*, 2001; Wu *et al.*, 2020) but align well with those of Hall *et al.* (2006) who found that long-
392 term leaf litter decomposition was higher at eCO_2, irrespectively of whether it was grown at

393 ambient or elevated CO_2, alongside enhanced accumulation of mineral N indicating greater
394 microbial activity. Other studies have found that eCO_2 contribute to greater soil organic
395 matter turnover. For example, Carney *et al.* (2007) found that six-year doubling of CO_2
396 negatively impacted soil organic matter content offsetting more than half of the C
397 accumulated in the biomass aboveground and coarse roots over the same period although no
398 increase in litter decomposition was observed. The increase in soil organic matter degradation
399 was linked to greater relative abundances of fungi and greater activity of C degrading
400 enzymes. Hence, eCO_2 may enhance organic matter turnover with potential implications for
401 C dynamics in forests.

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

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

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

437 Consistent with previous findings (e.g., Wall *et al.*, 2008; García-Palacios *et al.*,
438 2013), the exclusion of soil fauna had a significant impact on litter decomposition
439 irrespective of CO_2 treatment although we only excluded macrofauna. The response ratio for
440 eCO_2 when macrofauna were included appeared greater but this was not significant.
441 Interestingly, this occurred despite the observation that eCO_2 result in fewer ground dwelling
442 macrofauna, including the known decomposers Isopoda, at the site (Facey *et al.*, 2017).

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

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

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

468 and a capacity to break down lignocellulose (Nghi *et al.*, 2012). The identity of taxa
469 associated with eCO_2 in each study differed, suggesting that redundancy could exist in terms
470 of the fungi responding positively in an eCO_2 environment and possibly responsible for
471 enhanced decay rates.
472 Here we were able to take advantage of a rare opportunity arising from two significant
473 circumstances to observe increases in litter decomposition during exposure to eCO_2. These
474 increases may have been due to shifting fungal community composition but also appeared to
475 be independent of eCO_2 and herbivore effects on leaf chemistry during growth and on
476 whether macrofauna could access litter. The establishment and continuing support of
477 EucFACE allowed us to perform, over a period of eight years, two complementary studies
478 under different (but representative for this climate) field conditions and to observe
479 consistency in the responses associated with eCO_2 under those conditions. The support for
480 long-term monitoring of core data streams, here involving monthly monitoring of litter
481 production, and archival of sampled materials allowed us to take advantage of a natural
482 experiment involving a psyllid outbreak and to compare effects associated with eCO_2 to those
483 associated with litter quality. Few examples come to mind where long-term experimental
484 manipulations, continuous monitoring and natural experiments have come together to
485 enhance our general understanding of how ecosystems are likely to respond to environmental
486 change. One such example includes the observation, over the course of almost 20 years, that
487 N mineralisation responded to eCO_2 on different timeframes under C_3 and C_4 plants,
488 controlling their short- and long-term responses (Reich *et al.*, 2018). Examples such as this,
489 and ours, highlight the value of long-term experimental observatories and the need for
490 research organisations and funders to support them (Kuebbing *et al.*, 2018).
491

492 **References**

493 Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U.
494 (2021). *UNITE general FASTA release for Fungi 2* [Application/gzip]. UNITE Community.
495 https://doi.org/10.15156/BIO/1280089

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

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

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

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

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

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

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

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

524 Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P. M., Reith, F., Dennis, P. G., Breed, M. F.,
525 Brown, B., Brown, M. V., Brugger, J., Byrne, M., Caddy-Retalic, S., Carmody, B., Coates,
526 D. J., Correa, C., Ferrari, B. C., Gupta, V. V. S. R., Hamonts, K., Haslem, A., … Young, A.
527 (2016). Introducing BASE: The Biomes of Australian Soil Environments soil microbial
528 diversity database. *GigaScience*, *5*(1), s13742-016-0126–5. https://doi.org/10.1186/s13742-
529 016-0126-5

530 Blankinship, J. C., Niklaus, P. A., & Hungate, B. A. (2011). A meta-analysis of responses of
531 soil biota to global change. *Oecologia*, *165*(3), 553–565. https://doi.org/10.1007/s00442-011-
532 1909-0

533 Blumenthal, D. M., Resco, V., Morgan, J. A., Williams, D. G., LeCain, D. R., Hardy, E. M.,
534 Pendall, E., & Bladyka, E. (2013). Invasive forb benefits from water savings by native plants
535 and carbon fertilization under elevated CO2 and warming. *New Phytologist*, *200*(4), 1156–
536 1165. https://doi.org/10.1111/nph.12459

537 Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites:
538 Indices and statistical inference. *Ecology*, *90*(12), 3566–3574. https://doi.org/10.1890/08-
539 1823.1

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

544 Castañeda-Gómez, L., Walker, J. K. M., Powell, J. R., Ellsworth, D. S., Pendall, E., &
545 Carrillo, Y. (2020). Impacts of elevated carbon dioxide on carbon gains and losses from soil
546 and associated microbes in a Eucalyptus woodland. *Soil Biology and Biochemistry*, *143*,
547 107734. https://doi.org/10.1016/j.soilbio.2020.107734

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

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

554 Collins, L., Bradstock, R. A., Resco de Dios, V., Duursma, R. A., Velasco, S., & Boer, M. M.
555 (2018). Understorey productivity in temperate grassy woodland responds to soil water
556 availability but not to elevated [CO2]. *Global Change Biology*, *24*(6), 2366–2376.
557 https://doi.org/10.1111/gcb.14038

558 Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy,
559 O., Hobbie, S. E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M.,
560 Santiago, L. S., Wardle, D. A., Wright, I. J., Aerts, R., Allison, S. D., Van Bodegom, P.,
561 Brovkin, V., Chatain, A., … Westoby, M. (2008). Plant species traits are the predominant
562 control on litter decomposition rates within biomes worldwide. *Ecology Letters*, *11*(10),
563 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

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

568 Facey, S. L., Fidler, D. B., Rowe, R. C., Bromfield, L. M., Nooten, S. S., Staley, J. T.,
569 Ellsworth, D. S., & Johnson, S. N. (2017). Atmospheric change causes declines in woodland
570 arthropods and impacts specific trophic groups. *Agricultural and Forest Entomology*, *19*(1),
571 101–112. https://doi.org/10.1111/afe.12190

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

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

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

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

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

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

588 Hasegawa, S., Piñeiro, J., Ochoa-Hueso, R., Haigh, A. M., Rymer, P. D., Barnett, K. L., &
589 Power, S. A. (2018). Elevated CO2 concentrations reduce C4 cover and decrease diversity of
590 understorey plant community in a Eucalyptus woodland. *Journal of Ecology*, *106*(4), 1483–
591 1494. https://doi.org/10.1111/1365-2745.12943

592 Hättenschwiler, S., Tiunov, A. V., & Scheu, S. (2005). Biodiversity and Litter Decomposition
593 in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1),
594 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

595 Hedges, L. V., Gurevitch, J., & Curtis, P. S. (1999). The Meta-Analysis of Response Ratios
596 in Experimental Ecology. *Ecology*, *80*(4), 1150–1156. https://doi.org/10.1890/0012-
597 9658(1999)080[1150:TMAORR]2.0.CO;2

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

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

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

609 Kampichler, C., & Bruckner, A. (2009). The role of microarthropods in terrestrial
610 decomposition: A meta-analysis of 40 years of litterbag studies. *Biological Reviews*, *84*(3),
611 375–389. https://doi.org/10.1111/j.1469-185X.2009.00078.x

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

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

620 Lenth R (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package
621 version 1.10.0, https://CRAN.R-project.org/package=emmeans

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

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

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

634 Nielsen, U.N., Wall, D.H., & Six, J. (2015). Soil Biodiversity and the Environment. *Annual*
635 *Review of Environment and Resources 40*, 63–90. https://doi.org/10.1146/annurev-environ-
636 102014-021257

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

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

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

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

652 Parihar, M., Rakshit, A., Meena, V. S., Gupta, V. K., Rana, K., Choudhary, M., Tiwari, G.,
653 Mishra, P. K., Pattanayak, A., Bisht, J. K., Jatav, S. S., Khati, P., & Jatav, H. S. (2020). The
654 potential of arbuscular mycorrhizal fungi in C cycling: A review. *Archives of Microbiology*,
655 *202*(7), 1581–1596. https://doi.org/10.1007/s00203-020-01915-x

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

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

663 Powell, J. R. (2024). PowellLab/eucface_litter v1.0.0. *zenodo*.
664 https://doi.org/10.5281/zenodo.11108026

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

667 Reich, P. B., Hobbie, S. E., Lee, T. D., & Pastore, M. A. (2018). Unexpected reversal of C3
668 versus C4 grass response to elevated CO2 during a 20-year field experiment. *Science*,
669 *360*(6386), 317–320. https://doi.org/10.1126/science.aas9313

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

672 Serna-Espinosa, B.-N., Forero-Castro, M., Morales-Puentes, M. E., Parra-Giraldo, C. M.,
673 Escandón, P., & Sánchez-Quitian, Z. A. (2023). First report of environmental isolation of
674 Cryptococcus and Cryptococcus-like yeasts from Boyacá, Colombia. *Scientific Reports*,
675 *13*(1), Article 1. https://doi.org/10.1038/s41598-023-41994-6

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

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

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

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

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

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

691 Wall, D. H., Bradford, M. A., St John, M. G., Trofymow, J. A., Behan-Pelletier, V., Bignell,
692 D. E., Dangerfield, J. M., Parton, W. J., Rusek, J., Voigt, W., Wolters, V., Gardel, H. Z.,
693 Ayuke, F. O., Bashford, R., Beljakova, O. I., Bohlen, P. J., Brauman, A., Flamming, S.,
694 Henschel, J. R., … Zou, X. (2008). Global decomposition experiment shows soil animal
695 impacts on decomposition are climate-dependent. *Global Change Biology*, *14*(11), 2661–
696 2677. https://doi.org/10.1111/j.1365-2486.2008.01672.x

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

701 Wu, Q., Yue, K., Wang, X., Ma, Y., & Li, Y. (2020). Differential responses of litter
702 decomposition to warming, elevated CO2, and changed precipitation regime. *Plant and Soil*,
703 *455*(1), 155–169. https://doi.org/10.1007/s11104-020-04675-1

704

705 Table 1: Analysis of Variance table showing significance of fixed effects and interactions
706 associated with CO_2 treatment (ambient or ambient + 150 ppm), mesh size (2 mm or 4 mm)
707 and duration of decomposition for litter mass remaining at harvest. 'Plot' and 'Sublot' were
708 included as random effects. Raw data and model predictions are shown in Figure 1. 'df' =
709 degrees of freedom.

Term	F-statistic	$df_{numerator}$	$df_{denominator}$	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

710

711 Table 2: Analysis of Variance table showing significance of fixed effects and interactions
712 associated with CO_2 treatment (ambient or ambient + 150 ppm) during leaf development
713 ('leaf') and litter decomposition ('litter'), psyllid presence during leaf development and
714 duration of decomposition for litter mass remaining at harvest in the second study. 'Plot' and
715 'Sublot' were included as random effects. Raw data and model predictions are shown in
716 Figure 2. 'df' = degrees of freedom.

Term	F-statistic	$df_{numerator}$	$df_{denominator}$	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

717

718 **Figure captions**

719

720 Figure 1. CO_2 effect on litter decomposition in the first study for each timepoint. Litter was
721 contained within bags with two mesh sizes (2mm or 4mm). (a) Box-and-whisker plots
722 showing mass remaining in litterbags over the course of the study under ambient or elevated
723 (+150 ppm) CO_2 conditions. (b) Effect sizes associated with the CO_2 treatment during
724 decomposition for each timepoint and mesh size relative to ambient CO_2. Dots represent the
725 log response ratio calculated from mean values of mass remaining. Negative values indicate
726 greater decomposition of litter under elevated CO_2 ('eCO2') than under ambient CO_2
727 ('aCO2'). Error bars represent 95% confidence intervals; note that the error bars for the point
728 on the right extend beyond the y-axis limits (actual minimum = -8.47, actual maximum =
729 4.87)

730

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

740

741 Figure 3. Ordination of fungal assemblages associated with litter collected during the harvest
742 prior to the observation of CO_2 effects on decomposition in the first study (after three months

743 for 4 mm mesh and after six months for 2 mm mesh). OTU data were analysed using
744 constrained analysis of principal coordinates (CAP) using Bray-Curtis dissimilarities.
745 Variation along the first CAP axis (percentage of partitioned variation in parentheses) was
746 associated primarily with the size of holes in the litter-containing mesh, while the second was
747 associated primarily with the CO_2 treatment during decomposition. Lines connecting
748 external points for each group are to facilitate visualisation of patterns and are based on
749 concave hulls.
750
751 Figure 4. Ordinations of fungal assemblages associated with litter collected during the harvest
752 prior to the observation of CO_2 effects on decomposition in the second study (after eight
753 months). OTU data were analysed using constrained analysis of principal coordinates (CAP)
754 using Bray-Curtis dissimilarities. Variation along the first CAP axis (percentage of
755 partitioned variation in parentheses) was associated primarily with when the litter was
756 collected (coincident with the occurrence of a psyllid outbreak or in the years following),
757 while the second and third axes were associated primarily with the CO_2 treatment during leaf
758 development (a) or during decomposition (b). Lines connecting external points for each
759 group are to facilitate visualisation of patterns and are based on concave hulls.
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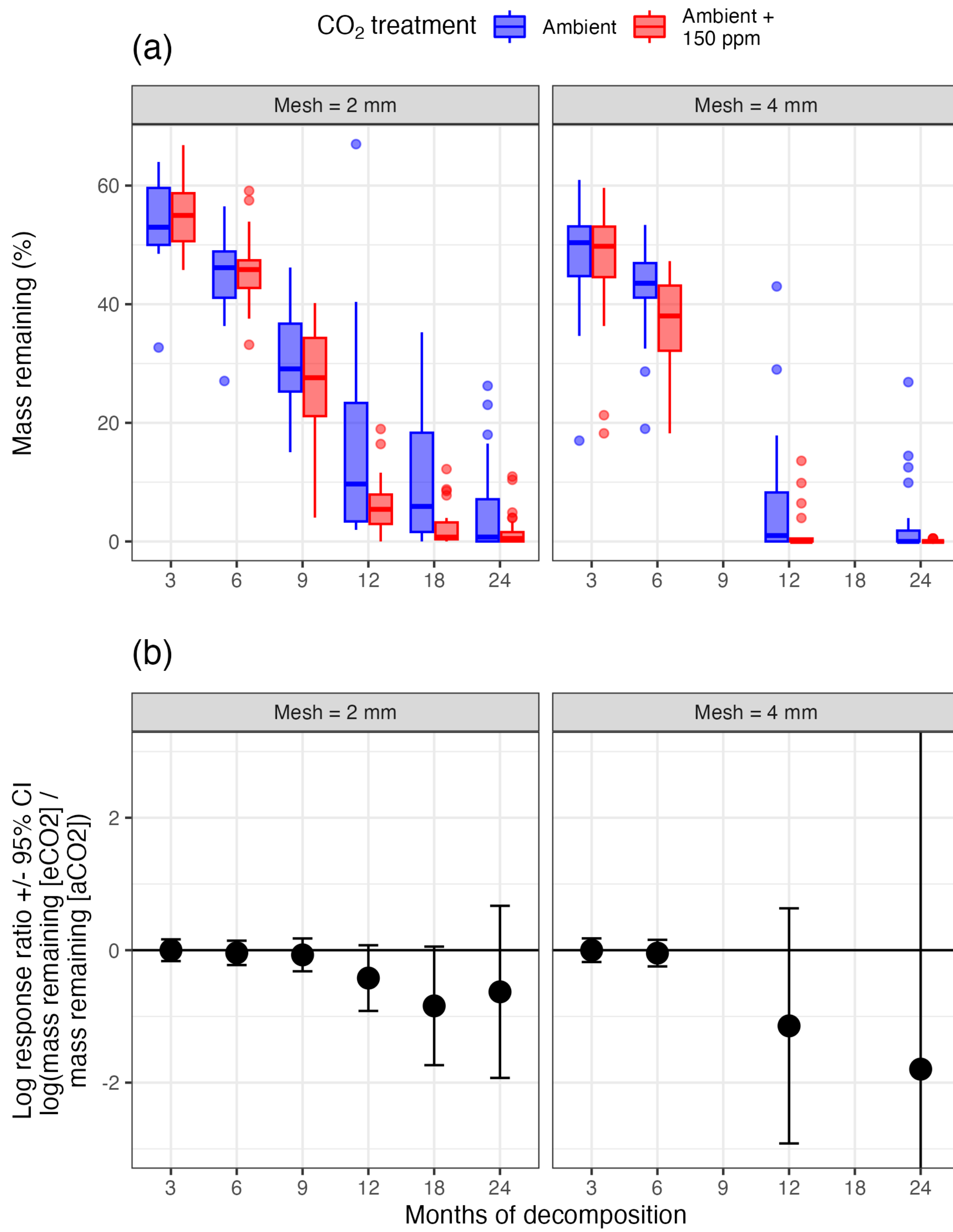
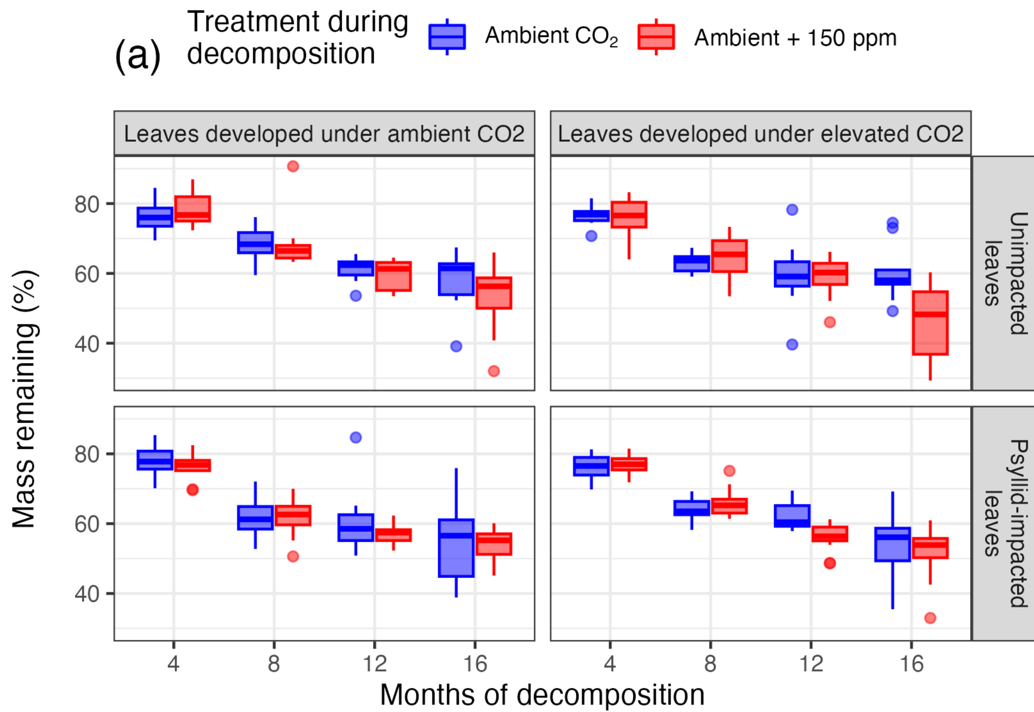
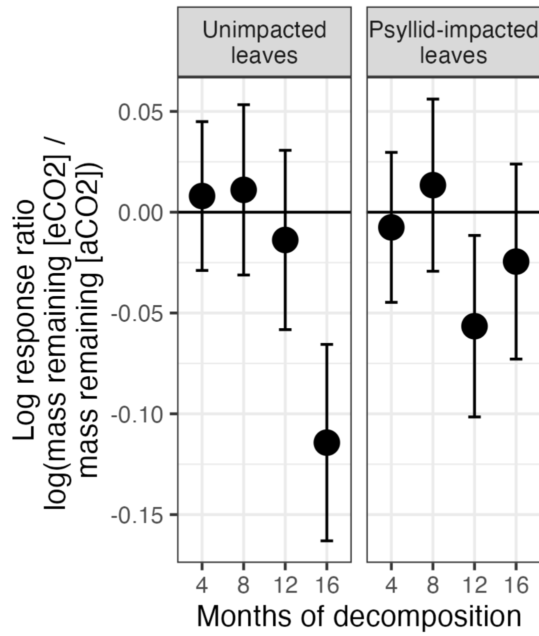


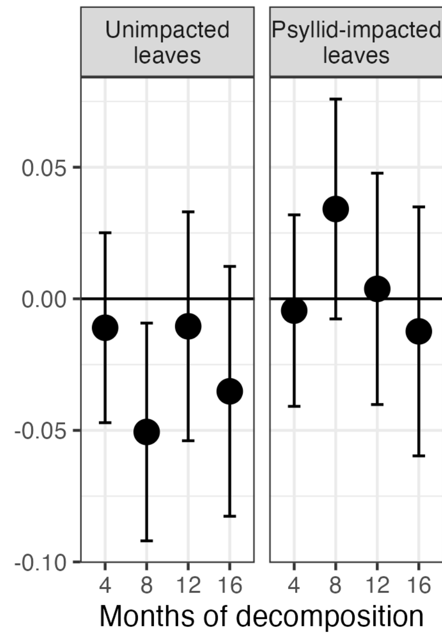
763

764 *Figure 1.*

765







766

767 *Figure 2.*

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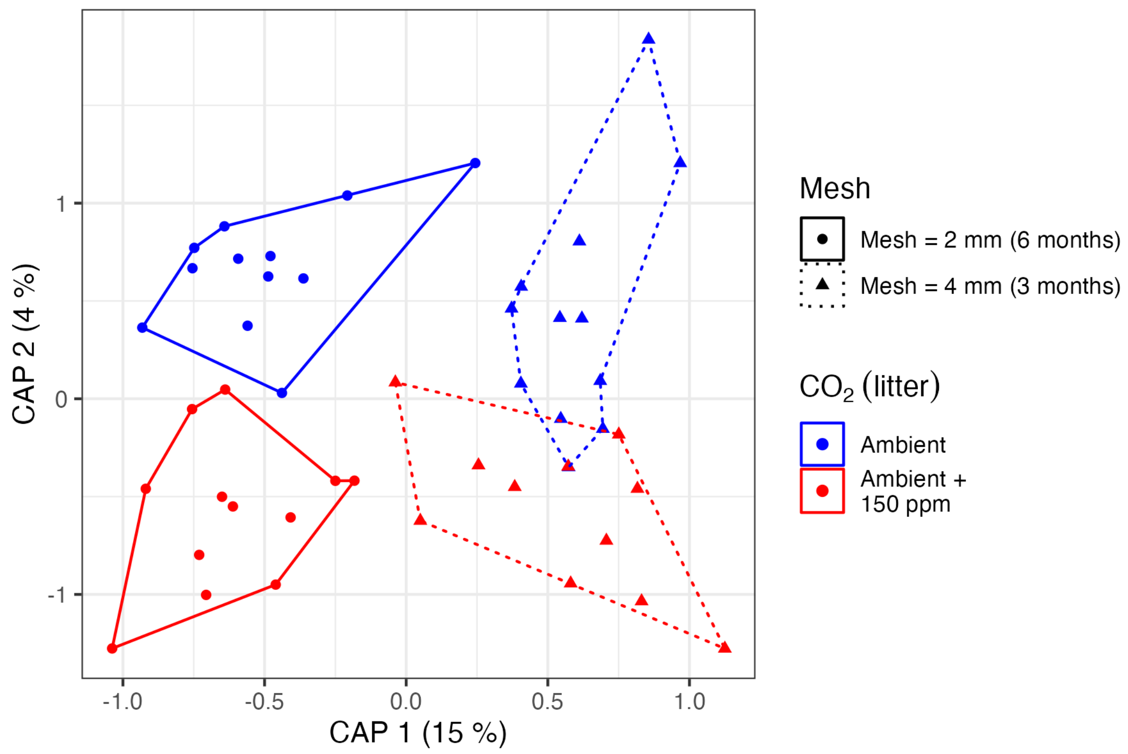

770

771 *Figure 3.*

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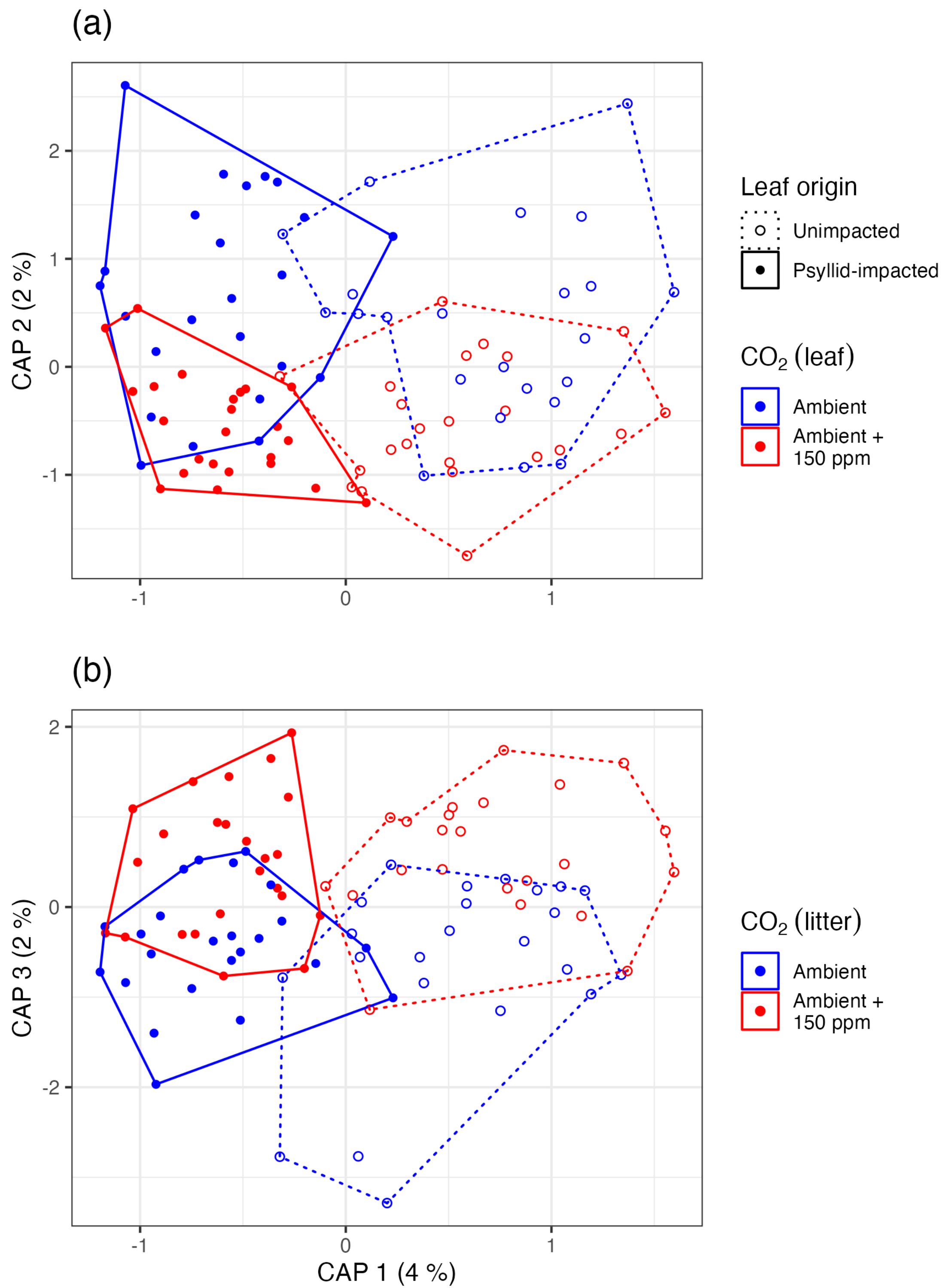

774

775 *Figure 4.*

776

Supplementary Materials: 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

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

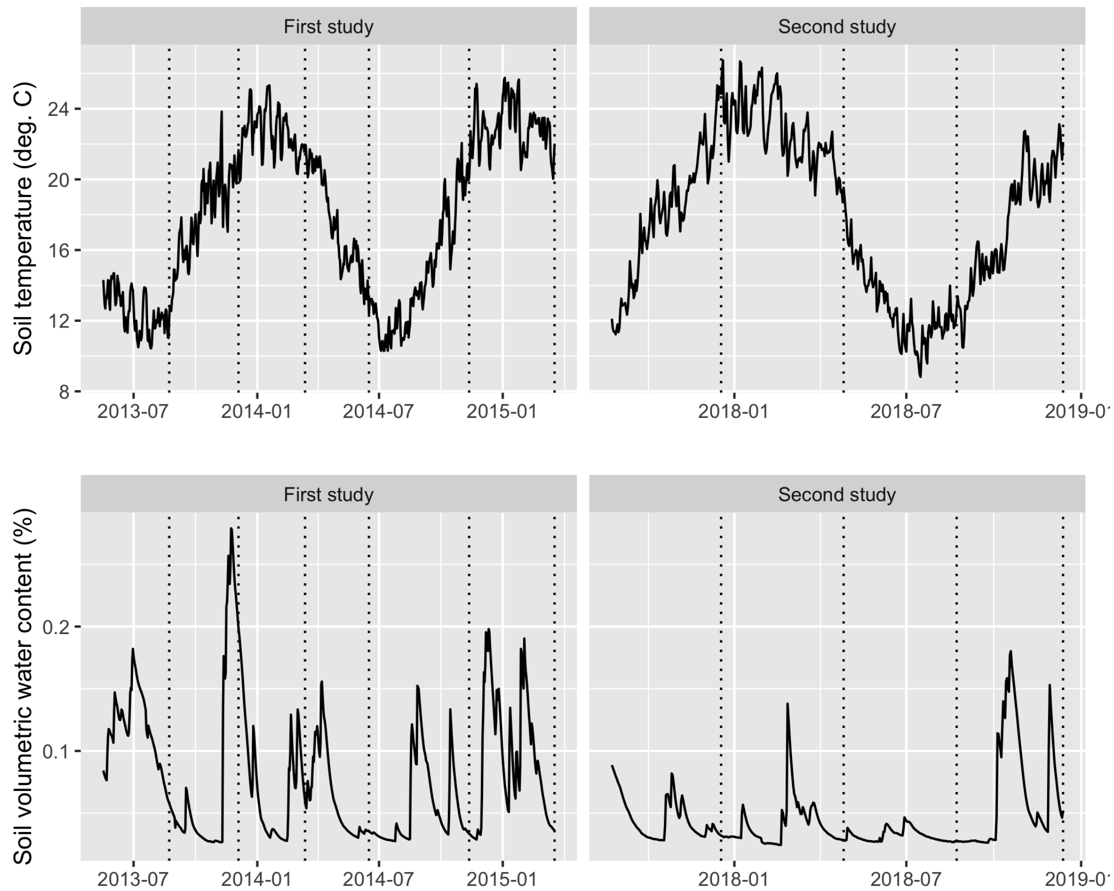

Figure S1. Soil temperature and moisture conditions in the top 10 cm of soil during the two studies. Lines represent average values across all sensors (48 for soil moisture, 16 for soil temperature). Dashed vertical lines indicate sampling dates.

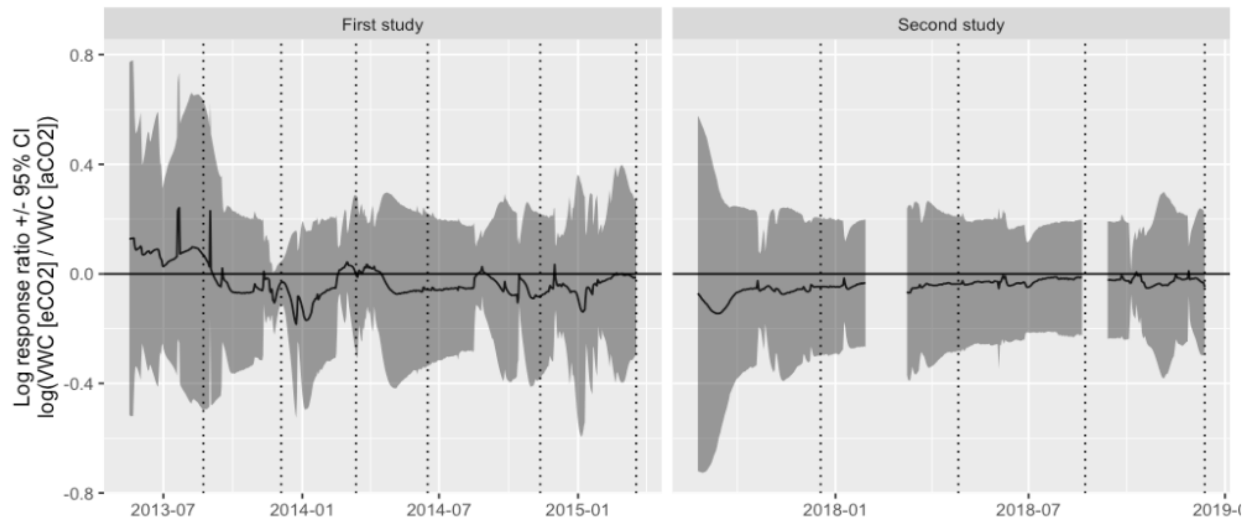

Figure S2. Effect sizes associated with elevated CO2 on soil volumetric water content (VWC) were within the margin of error for almost the entire duration of both studies, with only one short period in the first study where soil moisture was lower in the elevated CO2 treatment relative to ambient conditions. 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 plots.

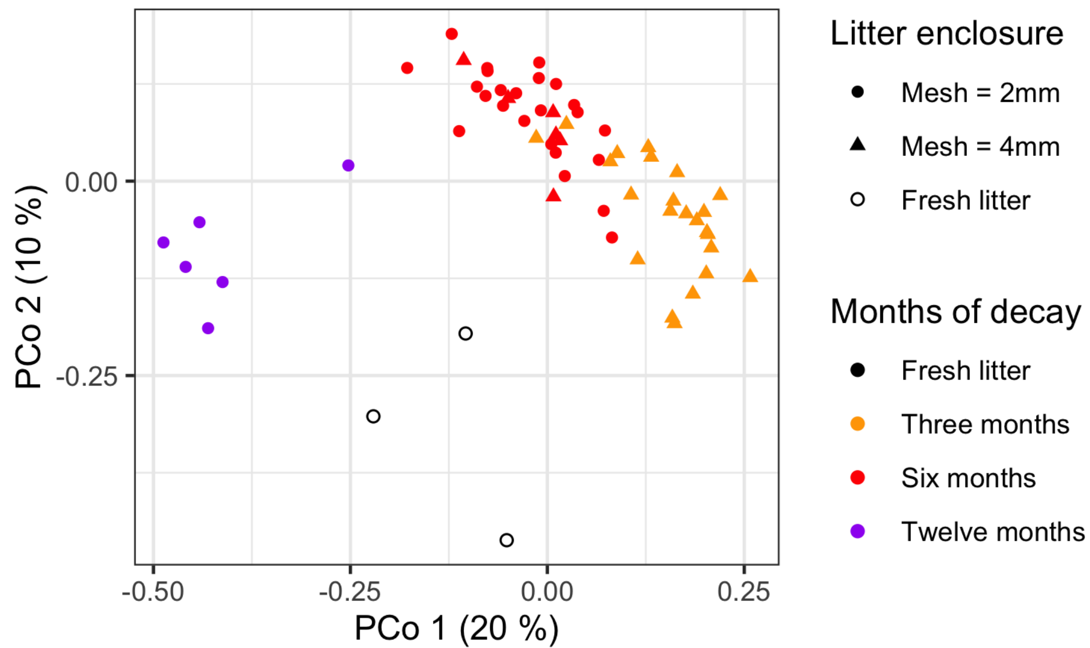

Figure S3. Ordination of litter fungal communities across all characterised samples in the first study. OTU data were analysed using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities.

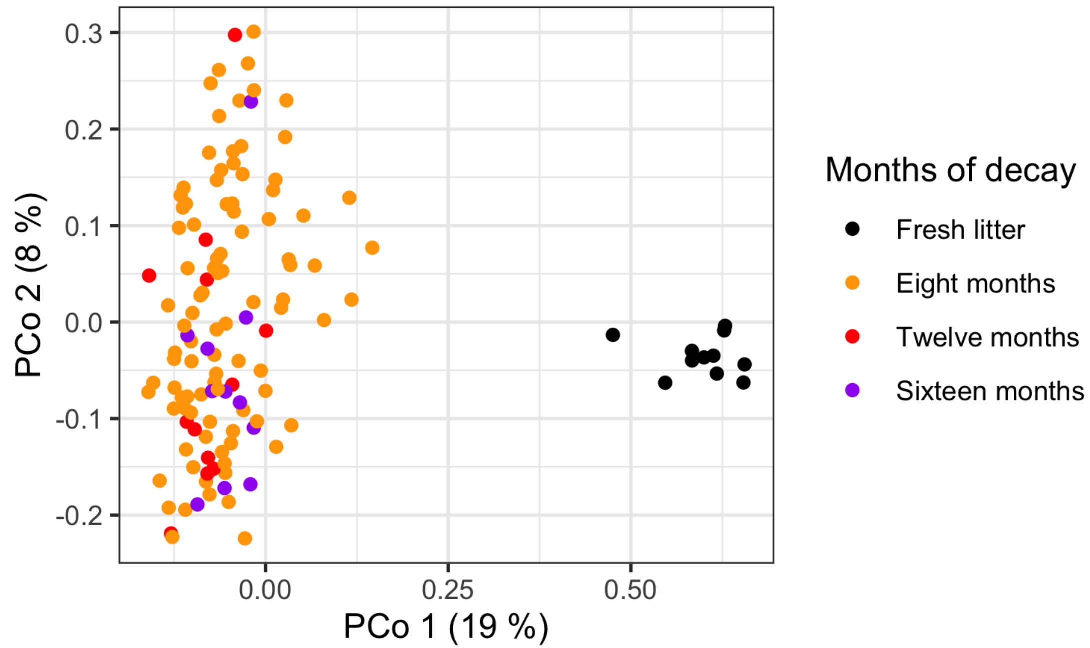

Figure S4. Ordination of litter fungal communities across all characterised samples in the second study. OTU data were analysed using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities.