

# *Root ectomycorrhizal status of oak trees symptomatic and asymptomatic for Acute Oak Decline in southern Britain*

Article

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1 **Root ectomycorrhizal status of oak trees symptomatic and asymptomatic for Acute Oak**  
2 **Decline in southern Britain**

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21

22 **Abstract**

23 Acute Oak Decline (AOD) is a decline-disease that has distinctive symptoms and poses a  
24 serious threat to oak. Our understanding of the causal factors of AOD remains poor but it is  
25 likely that multiple biotic and abiotic factors contribute to a deterioration in oak condition.  
26 There is evidence that indications of above-ground tree health status are frequently reflected  
27 below-ground in roots and associated ectomycorrhizal (ECM) fungal communities. However,  
28 no study has yet explored these potential relationships specifically in AOD affected trees. In  
29 this study, we compare the composition and range of functional exploration types of ECM  
30 communities associated with AOD symptomatic oak trees and with AOD asymptomatic trees  
31 in three oak-dominated woodlands in southern England. We additionally assess the abundance  
32 of fine roots tips in surface soils beneath AOD symptomatic and asymptomatic trees and  
33 consider soil physico-chemical effects on ECM communities. The frequency of fine root tips  
34 was found to be significantly higher on asymptomatic compared with symptomatic trees in two  
35 of the three woodlands studied and long-distance ECM exploration types had a weak positive  
36 association with AOD asymptomatic trees. ECM diversity and composition were, however,  
37 unaffected by tree symptom status and were not related to the frequency of fine root tips. ECM  
38 diversity and compositional (but not exploration type) differences were evident only between  
39 the different woodlands and this was related to a small number of soil chemistry variables. This  
40 study revealed a relationship between the above-ground symptoms of AOD (i.e. stem lesions  
41 and *Agilus biguttatus* exit holes) and the frequency of live root tips, providing a potential  
42 additional diagnostic tool of trees in decline and highlighting the importance of considering  
43 belowground rhizosphere microbiome communities.

44 .

45 **Key-words:** Acute Oak Decline; ectomycorrhizal fungi; *Quercus*; exploration types; fine roots

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47

## 48 1. Introduction

49 Oak decline has been reported for more than 250 years in Europe (Thomas et al., 2002, Thomas,  
50 2008). Since the 1980's Acute Oak Decline (AOD) has emerged as a new disorder within the  
51 wider oak decline complex, causing rapid decline in tree condition over three to five years and  
52 with distinct symptoms that have been described among afflicted trees in Britain (Denman et  
53 al., 2014, Brown et al., 2016). The symptoms include weeping stem patches, black exudate  
54 emanating from longitudinal splits between bark plates and frequently, at late stages of decline,  
55 larval galleries and exit holes on the bark formed by the buprestid beetle, *Agrilus biguttatus*  
56 (Denman and Webber, 2009, Denman et al., 2014, Brown et al., 2015, 2016). A deterioration  
57 in crown condition can also occur, although this is not considered to be a reliable symptom of  
58 AOD (Denman et al., 2014). AOD mainly affects mature trees (DBH of 35-80cm) but can also  
59 occur in young oaks and has caused much concern amongst woodland owners due to the threat  
60 to oak tree vigour and survival, particularly among native oaks (i.e. *Quercus robur* and *Q.*  
61 *petraea*) (Denman and Webber, 2009). The incidence of AOD-affected woodland is increasing  
62 and currently occurs in southern and central England, extending also into Wales (Brown et al.,  
63 2018).

64 No single causal agent of AOD has been identified and current thinking is that as a 'decline-  
65 disease', AOD is caused by multiple abiotic and biotic variables that act together in  
66 combination, cumulatively and/or sequentially to weaken the tree (Denman et al., 2018). The  
67 bacterial species associated with AOD weeping stem lesions (i.e. *Gibbsiella quercinecans*,  
68 *Rahnella victoriana* and, particularly, *Brenneria goodwinii*.) are found in much reduced  
69 abundances in apparently healthy trees and so it is unclear whether this is a case of a weakened  
70 tree becoming more susceptible to bacterial infection or bacteria causing AOD (Broberg et al.,  
71 2018, Denman et al., 2018). The role of the *Agrilus* beetle is similarly unclear; i.e. it is not

72 known whether their association with stem bleeds implicates them as a disease vector, or if  
73 they are simply opportunists, taking advantage of weakened trees (Reed et al., 2018). Recent  
74 research indicates that the distribution of trees with AOD correlates significantly with sites in  
75 Britain that have high dry nitrogen (N) deposition, low rainfall, low elevation and warm  
76 temperatures (Brown et al., 2018). However, at the woodland stand scale, oak trees displaying  
77 AOD symptoms have been found to co-occur with apparently healthy ‘asymptomatic’ trees  
78 and in some cases, show clear signs of remission (Brown et al., 2016). The distribution of AOD  
79 symptomatic trees in woodlands has, nevertheless, been found to cluster at small scales (<40m)  
80 in among apparently unaffected trees. This suggests that, as a possible interaction with regional  
81 agents of stress (e.g. high temperatures, high N-loading), there may be a localised cause such  
82 as: (i) a pest or pathogen that mainly afflicts competitively suppressed trees in dense stands  
83 (Zhou et al., 1997, Jones et al., 2003, Mosca et al., 2007, Brown et al., 2016), (ii) genetic factors  
84 influencing susceptibility to AOD (Harper et al., 2016) and/or (iii) highly localised differences  
85 in soil conditions and the wider rhizosphere microbiome that together might predispose  
86 individual or small clusters of trees to AOD.

87 In this study we investigate the belowground rhizosphere microbiome of oak trees and how it  
88 relates to local soil conditions under trees showing signs of AOD compared with nearby AOD  
89 asymptomatic trees (i.e. without detectable lesions). More specifically, we explore how the  
90 presence of lesions on AOD symptomatic trees influences ectomycorrhizal (ECM) fungal  
91 communities colonising oak fine roots. ECMs are ancient fungal-plant mutualisms that play  
92 important roles in tree growth and health. Comprised of hyphae that, glove-like, cover tree  
93 roots, ECMs provide protection against pathogens (Marx, 1972, Sinclair et al., 1982, Duchesne  
94 et al., 1988 a, b, Branzanti et al., 1999, Lambers et al., 2018) and drought (Parke et al., 1983,  
95 Futai et al., 2008, Smith and Read, 2010), whilst also gathering soil nutrients and water for  
96 their tree hosts in exchange for photosynthetic carbon (Read, 1986, Hobbie and Hobbie, 2006).

97 Many ECM species have evolved functional traits that further enhance soil exploration and  
98 resource acquisition capabilities in their host tree. Among these are medium- and long-distance  
99 exploration types that send out filamentous hyphae from tree roots. As these hyphae are thinner,  
100 longer and able to produce a wider array of enzymes than tree fine roots, they greatly increase  
101 the efficiency of resource capture (Hobbie and Agerer, 2010).

102 The position of ECMs at the interface between the soil and tree roots results in a sensitivity not  
103 only to changes in soil chemistry, but also to carbon allocation from the tree host to the ECM.  
104 Any impacts on the ECM community are therefore also likely to have a knock-on impact on  
105 tree performance. Conversely, a tree in poor condition is likely to have an impact on the ECM  
106 community associated with its roots (Kuikka et al., 2003, Swaty et al., 2004, Pena et al., 2010,  
107 Treu et al., 2014), potentially serving as an early warning of host stress (Scattolin et al., 2012).  
108 Factors that are known to significantly affect ECM communities include high levels of N  
109 deposition, low soil pH, available P, K and soil organic deposition rates measured as C:N ratios  
110 (Tedersoo et al, 2014, Maghnia et al., 2017, Lilleskov et al., 2019). In high N environments,  
111 for example, ECM species richness will tend to decline and the functional traits (e.g.  
112 exploration strategies) of species that tolerate high N can differ significantly from those of  
113 ECMs present in low N conditions (Cox et al., 2010, Jarvis et al., 2013, Suz et al., 2014,  
114 Lilleskov et al., 2019). Such trends have been observed over short distances (e.g. 10m), such  
115 as along N gradients from the woodland edge to the woodland interior (Kjøller et al., 2012).

116 Several studies have explored the composition of ECM communities on oak trees that are  
117 displaying clear signs of decline compared with apparently healthy oak trees (Kovacs et al.,  
118 2000, Montecchio et al., 2004, Lancelloti and Franceschini, 2013, Corcobado et al., 2014).  
119 Most of these studies have been conducted along a gradient of tree decline frequently defined  
120 on the basis of levels of ‘crown transparency’, or ‘defoliation’, but tree decline status has also

121 been assigned based on the detection of cancer and the presence of pathogens in the crown,  
122 bark or roots (e.g. Corcobado et al., 2014). The majority of these studies have also relied on  
123 the morphological identification of ECMs, with only a small proportion adopting molecular  
124 genetic techniques as a recognised method that can facilitate the detection and accurate  
125 identification of a wider array of ECM genera and species (Peay et al., 2008). These studies  
126 generally report that healthy trees either have similar or significantly greater proportions of fine  
127 roots colonised by ECMs than trees showing signs of decline (Przybyl and Pukacka, 1995,  
128 Kovacs et al., 2000). ECM species diversity and the abundance of certain ECM species (e.g.  
129 *Lactarius chrysorrheus*, *Cenococcum geophilum*) have also tended to be higher on healthy trees  
130 (Kovacs et al., 2000, Montecchio et al., 2004, Lancelloti and Franceschini, 2013, Corcobado  
131 et al., 2014). Where the abundance of fine roots has additionally been assessed, fine root  
132 abundance has been found to either be significantly higher (Corcobado et al., 2014), or  
133 significantly lower (Bzdyk et al., 2019) in healthy oak trees compared with trees in decline.

134 The primary aims of this study were twofold. First, using morphological and molecular genetic  
135 identification techniques, we sought to describe the taxonomic and functional composition of  
136 ECM communities associated specifically with AOD symptomatic oak trees compared with  
137 nearby oak trees showing no symptoms of AOD in three oak dominated woodlands. This  
138 involved (i) exploring the potential to identify ECM species, families and/or functional types  
139 that are indicators of AOD symptomatic trees, (ii) quantifying the relative abundance of live  
140 roots tips available for ECM colonisation on AOD symptomatic and asymptomatic trees and  
141 (iii) utilising the currently accepted defining features of trees with AOD symptoms (i.e. stem  
142 or bark plate bleeds, *Agrilus* adult exit holes), rather than other criteria used to distinguish oak  
143 trees in decline, such as high levels of canopy transparency, or defoliation. Second, we aimed  
144 to assess the responses of ECM communities to any variations in soil chemistry (particularly  
145 soil pH, and levels of soil N, K, P and C:N ratios) within and between the three woodland



146 locations. We predicted that AOD asymptomatic trees would have a higher abundance of fine  
147 roots than AOD symptomatic trees. This would be consistent with numerous studies comparing  
148 fine root responses in declining and healthy trees of various tree species (Bauce and Allen,  
149 1992, Blaschke, 1994, Power and Ashmore, 1996, Nechwatal and Oßwald, 2008, Corcobado  
150 et al., 2014). We expected that a greater availability of colonisable fine roots on AOD  
151 asymptomatic tree fine roots would be reflected by greater ECM species richness, diversity and  
152 abundance compared with AOD symptomatic trees. In addition, we expected AOD  
153 asymptomatic trees to recruit more often ECM exploration types that are thought to have higher  
154 plant carbon demands for mycelial growth (e.g. medium fringe and long-distance exploration  
155 types) (Lilleskov et al., 2019, Veselá et al 2019). This is based on the assumption that  
156 belowground plant C allocation would be greater in AOD asymptomatic trees compared with  
157 symptomatic trees of declining health, leading to altered ECM species composition (Saikkonen  
158 et al., 1999). Furthermore, we predicted that ECM community composition would be sensitive  
159 to levels of soil pH, N, K, P and/or C:N ratios and that any variation in soil chemical properties  
160 between symptomatic and asymptomatic trees would be related to ECM community  
161 composition responses to tree symptom status. For example, we expected lower ECM species  
162 richness and fewer ECMs with long-distance exploration strategies on symptomatic trees  
163 present at woodland locations with comparatively high soil N or low soil pH.

## 164 **2. Material and methods**

### 165 2.1 Study locations

166 Three oak-dominant woodlands known to have cases of AOD were selected for study in  
167 southern England. These were Monks Wood (52°41'N, 0° 23'W), Stratfield Brake (51° 80'N,  
168 1° 28'W) and Writtle Forest (51° 70'N, 0° 35'E). The three woodlands are at similar  
169 developmental stages and experience similar climatic conditions, although at 28m a.s.l.

170 Monks Wood is at a lower elevation than Stratfield Brake (69 m a.s.l.) and Writtle (90 m  
171 a.s.l.) (see Table A.1 in Supplementary material). Soils at Monks Wood are also comparatively  
172 fine-textured with higher silt and clay content than the other two woodlands.

173 At each woodland location, ten trees that showed symptoms of AOD and ten trees that were  
174 asymptomatic for AOD were selected for sampling. The selected symptomatic and  
175 asymptomatic trees were evenly distributed across each of the three woodlands (Figure 1).  
176 AOD symptomatic trees were identified on the basis of the presence of bleeding cracks  
177 between bark plates and in many, but not all trees, *Agrilus biguttatus* exit holes on the tree  
178 bark (Denman et al., 2014). Crown density of all sample trees was additionally assessed in  
179 5% classes where 0% was equivalent to a fully foliated tree crown. AOD symptomatic and  
180 asymptomatic trees selected for sampling were found to have similar average crown density  
181 (Table A.1). Sampled trees at Monks Wood and Stratfield Brake had comparable average  
182 crown density, while sample trees in Writtle had considerably lower average crown density  
183 than the other two woodland locations. We also assessed the average basal area of trees in the  
184 vicinity of sample trees and percentage shrub cover around sample trees; these were both  
185 found to be similar between symptomatic and asymptomatic trees and between the three  
186 woodland locations. With the exception of five trees identified as *Quercus petraea* in Writtle  
187 Forest, the trees selected at all three woodland locations were identified as *Quercus robur*.

188

## 189 2.2 Data collection

### 190 2.2.1 ECM surveys

191 In each of the oak woodlands, four soil cores were collected in the four cardinal directions  
192 around the perimeter of each of the 10 symptomatic and 10 asymptomatic trees to yield 80  
193 soil cores per woodland location. This sampling intensity was adopted based on previous

194 sampling of ECMs in oak forests in southern England (Suz et al., 2014, Spake et al., 2016).  
195 Soil cores were collected using a 2 cm diameter x 25 cm depth soil auger at distances no  
196 greater than 0.5 m from the trunk of each tree. The soil auger was cleaned between each soil  
197 core to avoid cross-sample contamination. Soil cores were moistened slightly with distilled  
198 water to prevent desiccation of roots and transported in sealed plastic bags in cool boxes to  
199 the laboratory where they were stored at 4°C for up to seven days. All soil cores were  
200 collected from each woodland location on a single day. Sampling dates for each woodland  
201 location were 8/10/2018 for Writtle, 18/10/2018 for Monks Wood and 6/11/2018 for  
202 Stratfield Brake.

203 In the laboratory each soil core sample was gently rinsed with water in a clean 0.5 mm sieve  
204 to separate soil particles and organic material from roots. To reduce sample bias and  
205 maximise sample independence, three of the longest live ectomycorrhizal oak fine roots  
206 (<2mm diameter) were removed during a five-minute timed search per sample using a  
207 binocular microscope (10-40X) following Cox et al. (2010). Taking each ECM root in turn, a  
208 single live ECM tip was removed, and its morphology (colour, ramification, shape and  
209 mantle surface) described; photographs were taken for each morphologically distinct  
210 morphotype. The ECM root tip was then placed in ethanol in a labelled Eppendorf tube for  
211 molecular identification. Using the remaining soil core root samples, the number of live oak  
212 root tips (<2mm diameter) were counted using a binocular microscope and their dry weight  
213 recorded. Oak roots were identified based on their morphological characteristics such as  
214 surface structure, colour of the periderm and ramification pattern (Meinen, 2008, Rewald et  
215 al., 2012). Live roots were distinguished from dead roots largely on the basis of their turgidity  
216 and intact appearance. Grass and herb roots were distinguished from tree roots by their  
217 smaller diameter, non-lignified structure and lighter colour.

218 Out of a total of 720 possible samples (three fine roots x four soil cores x a total of 60 trees at  
219 three woodland locations), 33% (234) of the samples had either no visible ECM fungi on  
220 roots (9%) or no roots available for sampling (23%). This occurred most frequently among  
221 the asymptomatic trees at Monks Wood where 37% of samples had no fungi or roots to  
222 sample. This compared with between 12% and 22% of samples with no roots or fungi to  
223 sample among the sample trees at Writtle and Stratfield Brake, respectively (Table 1). There  
224 were only two soil cores that had no roots and fungi to sample; these were a soil core under  
225 an AOD symptomatic tree at Writtle and a soil core under an AOD asymptomatic tree at  
226 Monks Wood.

227

### 228 2.2.2 Molecular identification of ECMs and categorisation into exploration types

229 ECMs were air-dried prior to extraction. Fungal DNA was extracted using the Extract-N-  
230 Amp™ Plant PCR kit (Sigma-Aldrich, St.Louis, USA). ECMs were incubated at 95° C for  
231 10 minutes in 10µl of extraction solution and subsequently diluted in 10µl of dilution solution  
232 (Extract-N-Amp™ Plant PCR Kit, Sigma-Aldrich, St. Louis, MO, USA). 1µl of a 1:10 sterile  
233 distilled water dilution of this mix was used as the DNA template in a 20µl PCR reaction  
234 using primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990).

235 DNA was amplified using the following reaction mixture: 1X NH PCR buffer (pH 8.8, 0.1%  
236 Tween 20, 20mM MgCl<sub>2</sub> (Bioron, Germany), 4µM of each primer, 0.2mM of each dNTP and  
237 0.25U SuperHot Taq DNA polymerase (Bioron, Germany). PCR was carried out using the  
238 following program: initial denaturation of 94° C for 2 minutes, followed by 35 cycles of  
239 denaturation at 94° C for 30 seconds, primer annealing at 53° C for 55 seconds and elongation  
240 at 72° C for 50 seconds. The cycle was finished with a final elongation step of 7 minutes at  
241 72° C. PCR products were checked on a 1.4% agarose gel and samples that produced a band

242 were cleaned up with EXOSAP-IT (Affymetrix Inc., Santa Clara, USA) following the  
243 manufacturer's protocol and then sent for sequencing at Edinburgh Genomics. The sequences  
244 were edited and trimmed using Sequencher v5.4 (Gene Codes Corporation, USA). All  
245 sequence chromatograms were visually checked prior to inclusion in the analysis to ensure  
246 accuracy of calls e.g. bases masked by dye peaks and corrected manually where necessary.  
247 The edited fungal sequences were identified using the Basic Local Alignment Search Tool  
248 (BLAST) against the National Centre for Biotechnology Information (NCBI) GenBank  
249 public sequence database. Fungal sequences ranged from 129 bp – 853 bp in length, with  
250 mean, median and mode lengths of 520 bp, 566 bp and 656 bp, respectively. We assigned  
251 species or genus names to each morphotype where pairwise identity (i.e. the amount of  
252 nucleotide that matches exactly between two different sequences) was equal to or higher than  
253 96%. Most sequences (84%) returned an identical (100%) match. 12% of samples produced a  
254 similarity match of 98-99% and a further 3% of samples had a similarity match of 96-97%.  
255 To confirm the sequencing matches, morphotype characteristics were additionally compared  
256 to reference photos (where these were available) on the DEEMY database (Agerer and  
257 Rambold, 2020) and, to a lesser extent, the Ectomycorrhizae Descriptions Database (BCERN,  
258 2020). A total of 69% of the ECM root tip samples could be identified to species or genus  
259 levels using molecular and morphological identification. The remaining 31% of the ECM  
260 root tip samples could not be identified morphologically, yielded no PCR result or did not  
261 generate sufficiently high-quality sequences to be used to match against library sequences. In  
262 37 cases, the fungi sampled on roots were removed from the dataset because they were found  
263 to be saprotrophic fungi (e.g. Trichocomaceae) or fungi not proven to be ectomycorrhizal  
264 including several members of the Ascomycota such as Eurotiales (1), Helotiales (6),  
265 Leotiomycetes (1), Pezizales (2) and Pezizomycetes (3). Samples of this kind were evenly  
266 distributed across all woodland locations and among symptomatic and asymptomatic trees,

267 making up no more than 19% of ectomycorrhizal root tip samples (Table 1). Once these non-  
268 ECM fungi had been removed from the dataset, only two remained that had a sequence  
269 similarity match of 97% and four had a sequence similarity match of 98%. The identity of  
270 these six morphotypes could be confirmed by morphological identification to species or  
271 genus level.

272 Mycorrhizal exploration types were assigned to each identified taxon following Agerer  
273 (2001, 2006), Suz et al. (2014) and the DEEMY database (Agerer and Rambold, 2020). They  
274 were further classified as low biomass (contact, short- and medium-distance smooth  
275 exploration types) and high biomass (medium-distance fringe, medium distance mat and  
276 long-distance exploration) based on Hobbie and Agerer (2010).

277

### 278 2.2.3 Soil assessments

279 For each of the ten symptomatic and ten asymptomatic trees selected per woodland location,  
280 four soil samples (40-50 g field moist) were collected using a soil auger at a depth of 5-15 cm  
281 from the four cardinal directions at 1.5-2.0 m away from the tree trunk. Soil samples were  
282 subsequently pooled to produce one composite sample per tree. Pooled soil samples were  
283 homogenized by sieving (<2 mm) and then air-dried prior to analysis of soil physico-  
284 chemical properties. Soil particle size distribution was determined using a Malvern  
285 Mastersizer 3000 hydro laser granulometer: i.e. samples were dispersed in 3.3% (m/v)  
286  $\text{Na}_6\text{O}_{18}\text{P}_6$  and 0.7% (m/v)  $\text{Na}_2\text{CO}_3$  and measured in blue and red light and data reported using  
287 a Fraunhofer size distribution model. Soil pH was determined in deionised water (soil: water,  
288 1: 2.5 (m/v)). Total C and N contents were determined on ground samples (Pulverisette 5  
289 Planetary Mill) using a FLASH CN elemental analyser (ThermoFisher Scientific).  
290 Extractable phosphorus was determined by the Olsen method (Olsen et al., 1954). For

291 determination of exchangeable cations ( $K^+$  and  $Al^{3+}$ ), soil samples (2.5 g) were extracted with  
292 3 x 30 ml 0.1M  $BaCl_2$  and pooled extracts (made up to 100 ml) analysed using a Perkin  
293 Elmer 3000 ICP-OES fitted with a cross flow nebuliser (Cools and De Vos, 2016). Technical  
294 replication was included for the analysis as follows: particle size distribution (5), pH (3), total  
295 C and N (2), Olsen P (2) and exchangeable cations (2). In the case of  $BaCl_2$  extractions for  
296  $Al^{3+}$ , a low percentage of samples recorded concentrations in the extract below the detection  
297 limit ( $2.55 \mu g L^{-1}$   $BaCl_2$  extract) for the analysis. Where this was the case, concentrations  
298 were entered into calculations as half the detection limit.

299

### 300 2.3 ECM data preparation

301 Raw data were pooled at two levels: 1) tree AOD symptom status (i.e. ten asymptomatic or  
302 ten symptomatic trees combined at each woodland location) and 2) individual tree level. At  
303 the tree AOD symptom status level, an ECM species occurring on a sample tree was  
304 attributed a score value of 1, with all non-occurring species scoring a zero (0,1 matrix).  
305 Therefore, at the tree symptom status level, the maximum count for any one ECM species  
306 was 10. At the individual tree level, a single ECM species could score a maximum count of  
307 12 occurrences if present in all samples (three roots x four positions around each tree). As  
308 well as establishing tree and tree symptom status level matrices for ECM 'Species', this same  
309 process was repeated considering ECMs grouped by 'Family' or by 'Exploration Type',  
310 resulting in a total of six data matrices.

311

### 312 2.4 Data analysis

313 All analyses were conducted in R version 3.5.1 (R Core Team, 2018), with graphics produced  
314 using ggplot2 in R (Wickham, 2016). To assess how well the sampling intensity captured the

315 diversity of ECM species present, species accumulation curves at the three woodland  
316 locations were estimated using the `specaccum()` function in `vegan()` package in R. The effects  
317 of tree AOD symptom status and woodland location effects on ECM assemblages were  
318 undertaken by fitting multivariate general linear models (GLiMs) to the datasets at the  
319 individual tree level using the R package `mvabund` (Wang et al., 2018), with Poisson errors  
320 and log link function (residual plots confirmed a reasonable fit of this model structure).  
321 Along with the interaction of woodland location and tree symptom status, the following soil  
322 measurements were included in the multivariate GLiMs: pH, C:N ratio, Total N (%), Olsen's  
323 P ( $\text{mg kg}^{-1}$ ), exchangeable K content ( $\text{mg kg}^{-1}$ ) and exchangeable Al content ( $\text{mg kg}^{-1}$ ). Any  
324 ECM species, families or exploration types with only a single occurrence were removed from  
325 the data sets prior to analysis to aid model fit. Analysis of deviance was conducted on each  
326 factor, with pit-trap resampling, 999 iterations and score tests used to determine the  
327 significance of woodland location, tree symptom status and soil measurements.

328 To examine tree-level ECM species/families/exploration type assemblages, a Bray-Curtis  
329 dissimilarity matrix was calculated for each data set, using the `vegan` package in R (Oksanen  
330 et al., 2019). Nonmetric Multidimensional Scaling (NMDS), using 1,000 random starts, were  
331 performed on the Bray-Curtis dissimilarity matrices, with appropriate numbers of dimensions  
332 determined based on stress levels. Stress plots were used to determine goodness-of-fit, and  
333 the first two axes were plotted to visualise the data set. Ordination spider plots were colour-  
334 coded by significant factors, and ordination spiders plotted (using mean ordination points per  
335 woodland location/tree symptom status) to visualise factors.

336 Soil physico-chemical characteristics were compared among symptomatic and asymptomatic  
337 trees at each woodland location and between woodland locations using analysis of variance  
338 with robust standard errors using Box-Cox transformed data. Where analysis of variance  
339 indicated an overall main effect of woodland location on soil physico-chemical properties,



340 Games-Howell post-hoc testing was used for pairwise comparisons between means for  
341 woodland location across tree symptom status. At the tree level, one-way analysis of  
342 variance and Tukey tests were used to test for significant differences in ECM species  
343 richness, diversity (Shannon-Weaver, 1949) and the root characteristics (dry weight, number  
344 of root tips) of AOD symptomatic and asymptomatic trees at each woodland location. Root  
345 datasets were log-transformed prior to analyses.

346

### 347 **3. Results**

#### 348 3.1 ECM species abundance, richness and community composition across woodland locations

349 A total of 90 ECM species belonging to 26 genera and 18 families were identified across all  
350 woodland locations. 45 of the ECM root tip samples were identified to species level and the  
351 remaining 45 to genus level. At all three woodland locations evenness of the ECM  
352 communities was low and species abundance followed a Zipf distribution, indicating that  
353 communities had a few species that were very abundant and a long tail of rare species (See  
354 Figure A.1 in Supplementary material). Species accumulation curves for each woodland  
355 indicate an adequate sampling intensity to capture ECM diversity present (Figure A.2). The  
356 average species richness across all three woodland locations was 33 with the lowest number  
357 of species recovered at Stratfield Brake (17) and the highest number at Writtle (45) (Table 1).  
358 The Russulaceae made up the greatest proportion of ECM species at all three woodland  
359 locations (51% of all ECM samples), within which the genera *Lactarius* (39%) and *Russula*  
360 (11%) dominated. Also present at all woodland locations but in lower abundances were  
361 members of the Boletaceae (12%), Gloniaceae (represented exclusively by *Cenococcum*  
362 *geophilum* - 11% of ECM root samples), Thelephoraceae (10%) and Cortinariaceae (3%)  
363 (Figure A.3). Several ECM families were found only at Writtle (i.e. Amanitaceae,

364 Discinaceae, Elaphomycetaceae, Hydnangiaceae, Paxillaceae, Pezizaceae), or only at Monks  
365 Wood (i.e. Entolomataceae, Inocybaceae, Sclerodermataceae, Tuberaceae), while no ECM  
366 families occurred uniquely at Stratfield Brake. The most abundant species overall were  
367 *Lactarius quietus* (33.8%), *C. geophilum* (10.4%), *Boletus rubellus* (6.2%), *Tomentella*  
368 *sublilacina* (5.2%) and *Lactarius subdulcis* (2.7%); the remainder of species were present in  
369 proportions of <2% of all species (Figure A.4).

370 The multivariate GLiM outputs indicated that ECM communities at the three woodland  
371 locations differed significantly from one another at the species and exploration type levels,  
372 but not at the family level (Table 2a-c).

373

### 374 3.2 ECM communities and AOD symptom status of sample trees

375 At each of the three woodland locations, levels of ECM species richness and diversity were  
376 similar between AOD symptomatic and asymptomatic trees (Table 1). AOD symptomatic and  
377 asymptomatic trees also showed no differences in the composition of ECM species, families,  
378 or exploration types (Table 2). However, when considering ECMs categorised by exploration  
379 type, GLiMs revealed a weak significant interaction effect between woodland location and  
380 the AOD symptom status of sample trees ( $p > 0.01$ ) (Table 2c). ECM species with a long-  
381 distance exploration strategy tended to be associated more often with asymptomatic trees and  
382 ECM species categorised as contact-medium smooth types were associated more often with  
383 AOD symptomatic trees (Figure A.5).

384

### 385 3.3 Soil properties and ECM communities

386 We found that soil pH, P and Al were all significantly ( $p < 0.05$ ) affected by tree symptom  
387 status as a main effect, although effects depended on woodland location for pH and Al. The  
388 effects of tree symptom status on N was marginal ( $p = 0.052$ ). Woodland location exerted a  
389 comparatively strong influence on soil physico-chemical properties. Writtle soils were  
390 composed of significantly higher proportions of sand compared with Monks Wood and  
391 Stratfield Brake, while Monks Wood had comparatively fine textured soils with significantly  
392 higher silt and clay content (silt-clay content  $\sim 70\%$ ) (Table A.2). Monks Wood additionally  
393 had significantly higher soil pH and lower exchangeable P than the other two woodlands,  
394 while the C:N ratio at Writtle was significantly higher than Monks Wood and Stratfield  
395 Brake. Exchangeable K concentrations were higher at Monks Wood than at Writtle, with  
396 intermediate concentrations at Stratfield Brake. Highest concentrations of exchangeable Al  
397 were found at Stratfield Brake (almost two- and three-fold higher than Writtle and Monks  
398 Wood, respectively), with no significant difference in Total N found between the three  
399 woodland locations.

400 The GLiM outputs indicated that ECM communities were significantly influenced at the  
401 species level by soil C:N ratios, Total N and levels of exchangeable Al. At the family level,  
402 ECM communities were significantly influenced only by levels of Al. When categorised by  
403 exploration type, ECMs showed no clear response to any of the measured soil chemistry  
404 variables (Table 2). NMDS ordination spiders illustrate a consistent separation of species  
405 assemblages by woodland location, C:N ratios and levels of exchangeable Al (Figures 2 and  
406 3, respectively), but no clear separation of species assemblages according to tree AOD  
407 symptom status.

#### 408 3.4 Root characteristics

409 No significant root dry weight differences were found between AOD asymptomatic and  
410 symptomatic trees, but significantly more root tips were found per soil core in asymptomatic  
411 compared with symptomatic trees in Stratfield Brake ( $p < 0.05$ ) and Writtle ( $p < 0.01$ ). The dry  
412 weight and frequency of root tips per soil core was noticeably lower at Monks Wood  
413 compared with Stratfield Brake and Writtle (Table 1).

#### 414 **4. Discussion**

415 4.1 Are there significant differences in the number of root tips and composition of ECM  
416 communities on fine roots of AOD symptomatic and asymptomatic oak trees?

417 This study found no difference in the species richness, diversity and composition of ECM  
418 communities on AOD symptomatic and asymptomatic trees. However, there was a weak  
419 interaction between woodland location and the symptom status of trees that resulted in a  
420 positive association between ECMs with a long-distance exploration type and AOD  
421 asymptomatic trees; this positive association was only evident at woodland locations with  
422 coarser sediment texture, lower soil pH and higher soil P and Al (i.e. Stratfield Brake and  
423 Writtle). Soil cores collected at the base of AOD asymptomatic trees were additionally found  
424 to have significantly more root tips per soil core compared with soil cores collected at the  
425 base of symptomatic trees at two out of the three woodland study locations (Stratfield Brake  
426 and Writtle). The third woodland, Monks Wood, had considerably less roots in each soil core,  
427 regardless of tree symptom status, which may have been due to the fine-textured soils at this  
428 woodland location that have a greater potential to become water-logged.

429 These results align well with our prediction that asymptomatic trees will have the advantage  
430 of a greater capability to explore and exploit resources belowground than symptomatic trees  
431 by virtue of a higher number of root tips hosting greater proportions of long-distance ECM  
432 exploration types. It is unclear though whether the higher proportions of long-distance ECM

433 exploration types and higher frequency of root tips found on asymptomatic trees are  
434 reflections of the better condition of these trees, or instead, are due to a more favourable soil  
435 environment under asymptomatic trees compared with symptomatic trees. As discussed  
436 below (section 4.2), we were not able to detect any soil physico-chemical differences,  
437 consistent across forest locations in the surface soils around AOD symptomatic and  
438 asymptomatic trees.

439 With the exception of a much higher abundance of Boletaceae at two of the woodland  
440 locations (Stratfield Brake and Writtle), the ECM fungal communities recorded at the three  
441 study locations were composed of the same dominant genera (i.e. *Russula*, *Lactarius*,  
442 *Cenococcum*, *Tometella*) and had similar ECM richness as previous studies of ECM  
443 communities based on *Q. robur*/*Q. petraea* in England (Suz et al. 2014, Spake et al., 2016)  
444 and further afield in Europe (Van Driessche and Piérart, 1995, Causin et al., 1996, Kovacs et  
445 al., 2000, Mosca et al. 2007, Bzdyk et al., 2019). Nevertheless, some caution is required  
446 when making comparisons between the results of our study and other similar studies  
447 comparing ECM communities on ‘healthy’ and ‘declining’ oak trees. One of the main  
448 difficulties’ rests in the definition of what constitutes a symptomatic tree. In contrast to many  
449 studies, we applied a definition of the symptoms we considered to indicate an AOD  
450 symptomatic tree (i.e. stem lesions - Denman et al., 2014) and did not use crown condition  
451 and levels of defoliation as part of the distinguishing features. Thus, proceeding cautiously  
452 with study comparisons we find that, in terms of root tip abundance, our results concur with  
453 Corcobado et al. (2014) who also found that fine root abundance was significantly higher in  
454 healthy compared with declining oak (*Quercus ilex*) trees (but see opposite results in Bzdyl et  
455 al., 2019). The same finding has been observed in a host of other studies comparing root tip  
456 frequency in declining and healthy trees, although these involved other, non-oak tree species  
457 (Bauce and Allen, 1992, Blaschke, 1994, Power and Ashmore, 1996, Nechwatal and Oßwald,

458 2008). In comparing our findings of ECM richness, diversity and community composition on  
459 AOD symptomatic and asymptomatic trees, our study results align with the findings of  
460 Causin et al. (1996) and Lancellotti and Franceschini (2013). As in our study, Causin et al.  
461 (1996) found no relationship between the health status of sampled *Q. robur* trees sampled and  
462 ECM species richness and community composition. Similarly, Lancellotti and Franceschini  
463 (2013) found no difference in ECM richness and diversity on the fine roots of healthy and  
464 declining *Quercus suber* trees, although they report significant differences in the evenness  
465 and taxonomic distinctness (Clarke and Warwick, 1998) of ECM communities across a tree  
466 decline gradient. In contrast to these results are numerous other studies that have observed  
467 significant differences in the compositions of ECM communities on healthy and declining *Q.*  
468 *robur* /*Q. petraea* (Kovacs et al., 2000, Mosca et al., 2007, Bzdyk et al., 2019) and on healthy  
469 and declining *Q. ilex* (Montecchio et al., 2004, Corcobado et al., 2014, 2015). Significantly  
470 lower species diversity in declining trees is reported by Mosca et al. (2007) and Bzdyk et al.  
471 (2019). Bzdyk et al. (2019) also found a significantly lower diversity of ECM exploration  
472 types in declining compared with apparently healthy oak trees. Most of the other studies  
473 showing significant ECM compositional differences between declining and healthy trees have  
474 reported significantly different proportions of dominant ECM species on declining and  
475 healthy trees (Kovacs et al., 2000, Montecchio et al., 2004, Corcobado et al., 2015). For  
476 example, Kovacs et al. (2000) found that *Amanita rubescens*, *Russula* spp., *Lactarius* spp.  
477 and *C. geophilum*, were significantly more abundant on the fine roots of *Q. robur*/ *Q. petraea*  
478 trees that displayed good health compared with declining trees (i.e. high levels of  
479 defoliation). Similarly, Montecchio et al. (2004) found that *Russula* spp., *C. geophilum* and  
480 the oak specialist ECM species *Lactarius chrysorrhoeus* were more abundant on healthy  
481 compared with declining *Q. ilex* trees (i.e. high levels of defoliation). We found no difference

482 in the relative abundances of any of these dominant ECMs in our AOD symptomatic and  
483 asymptomatic trees.

484

485 4.2 Are ECM communities influenced by any significant differences in soil physico-chemical  
486 conditions under symptomatic and asymptomatic trees and across woodland locations?

487

488 ECM community composition was influenced by significant differences in soil physico-  
489 chemical conditions between woodland locations rather than any detectable soil variable  
490 differences between symptomatic and asymptomatic trees. Woodland locations differed  
491 significantly in terms of soil pH, C:N ratios, P, exchangeable Al and sediment textural  
492 properties with significant ECM community effects observed in relation to a number of these  
493 differences in soil variables. Across the three woodland locations, shifts in ECM community  
494 composition could be related, as in previous studies (e.g. Suz et al 2014, Maghnia et al.,  
495 2017, Bzdyk et al., 2019, Defrenne et al., 2019), to significant differences in C:N ratios, Total  
496 N and exchangeable Al and may explain variations in ECM species richness and diversity  
497 among the woodlands. For example, the comparatively low ECM species richness and  
498 diversity at Stratfield Brake could be associated with the significantly higher levels of  
499 exchangeable Al at Stratfield Brake compared with the other two woodland locations; Al is  
500 known to have a negative impact on many ECMs (Entry et al., 1987, Rühling and  
501 Söderström, 1990, Jongbloed and Borst-Pauwels, 1992). Also noteworthy are the relatively  
502 high C:N ratios at Writtle which are indicative of lower rates of decomposition than at the  
503 other two woodland locations. This may be a reflection of the comparatively high ECM  
504 species richness and diversity at this site contributing to a suppression of saprotrophic fungal  
505 activity and consequently reduced levels of decomposition (i.e. a weakening of the ‘Gadgil  
506 effect’ - Avril and Hawkes, 2016, Fernandez and Kennedy, 2016).

507 As well as compositional differences between woodland locations, we also observed a  
508 number of taxa-specific responses to the significant differences in soil properties between  
509 woodland locations. For example, *C. geophilum* occurred in higher abundances at Writtle  
510 compared with the other two woodland locations. This ubiquitous species has been reported  
511 to be associated with high soil P (Maghnia et al., 2017) and demonstrates a tolerance, and  
512 possibly a preference, for drier soil conditions (Kovacs et al, 2000, Di Pietro et al., 2007,  
513 Corcobado et al, 2015). Writtle had the highest levels of soil P of the three woodland  
514 locations and was likely the driest of the three woodland locations with high proportions of  
515 sand in surface mineral layers. *Tomentella* species also demonstrated a taxa-specific response  
516 to the differences in soil P with higher species richness and abundance of *Tomentella* at the  
517 significantly lower levels of P encountered at Monks Wood. This trend reflects observations  
518 by Maghnia et al. (2017) for *Tomentella*.

519 Contrary to expectations, ECM community compositions were unaffected by the significant  
520 differences in soil pH recorded at Monks Wood (pH 4.7) and Stratfield Brake/ Writtle (at  
521 both woodlands the mean soil pH was 3.6). This is despite evidence from other studies (e.g.  
522 Suz et al., 2014) that ECM species composition and functional exploration types are sensitive  
523 to changes in soil pH. It is possible that the pH ranges across our woodland locations were  
524 not sufficiently great (mean pH of 3.6 to 4.7) to induce a response in ECM communities as in  
525 previous studies where pH gradients studied were much greater (e.g. range of pH3 to pH7 in  
526 Suz et al., 2014).

527

## 528 **5. Conclusions**

529 A key finding of this investigation was the significantly lower number of root tips present in  
530 soil cores collected under AOD symptomatic trees compared with soil cores collected



531 beneath asymptomatic trees at two of the three woodland study locations. This revealed a  
532 relationship between above-ground symptoms used to identify AOD-afflicted trees (i.e. stem  
533 lesions and *Agilus* beetle exit holes) and the abundance of root tips in surface soil layers,  
534 providing a potential additional diagnostic feature of trees in decline. Fewer root tips on  
535 symptomatic trees suggested that there would be a reduced capacity for ECMs to form  
536 mycorrhizal associations compared with the asymptomatic trees, but our results showed no  
537 evidence this. We found no differences in ECM composition, richness or diversity between  
538 symptomatic and asymptomatic trees, although ECMs with a long-distance exploration type  
539 were more commonly found on asymptomatic trees in more free-draining soils. The  
540 composition of ECM communities was nevertheless clearly related to the differing soil  
541 physico-chemical conditions at the tree woodland locations and specifically to differences in  
542 exchangeable Al, Total N and C:N ratios. While we recorded some significant differences in  
543 soil chemistry (soil pH, P and exchangeable Al) between symptomatic and asymptomatic  
544 trees, these differences may have been distilled by the strength of woodland location effects.  
545 A higher replication rate of AOD symptomatic and asymptomatic trees at each woodland  
546 location is recommended for a future study to explore any potential important soil chemistry  
547 differences between symptomatic and asymptomatic trees and related effects on ECM  
548 communities as well as the wider tree-associated soil microbiome. Further work is also  
549 required to assess the cross-regional extent of AOD using a standardised definition; this  
550 would enable more reliable comparison of results between studies than is currently possible.

551 **CRedit authorship contribution statement**

552 Nadia Barsoum: Conceptualization, Investigation, Writing - original draft, Writing - review  
553 & editing. Stuart A'Hara: Investigation, Writing - review & editing. Joan Cottrell: Writing -  
554 review & editing. Jack Forster: Investigation, Writing - review & editing. Liz Shaw: Writing  
555 - review & editing. Karsten Schonrogge: Writing - review & editing. Mateo Garcia: Writing -  
556 review & editing.

557

558 **Declaration of Competing Interest**

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

561

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576

577

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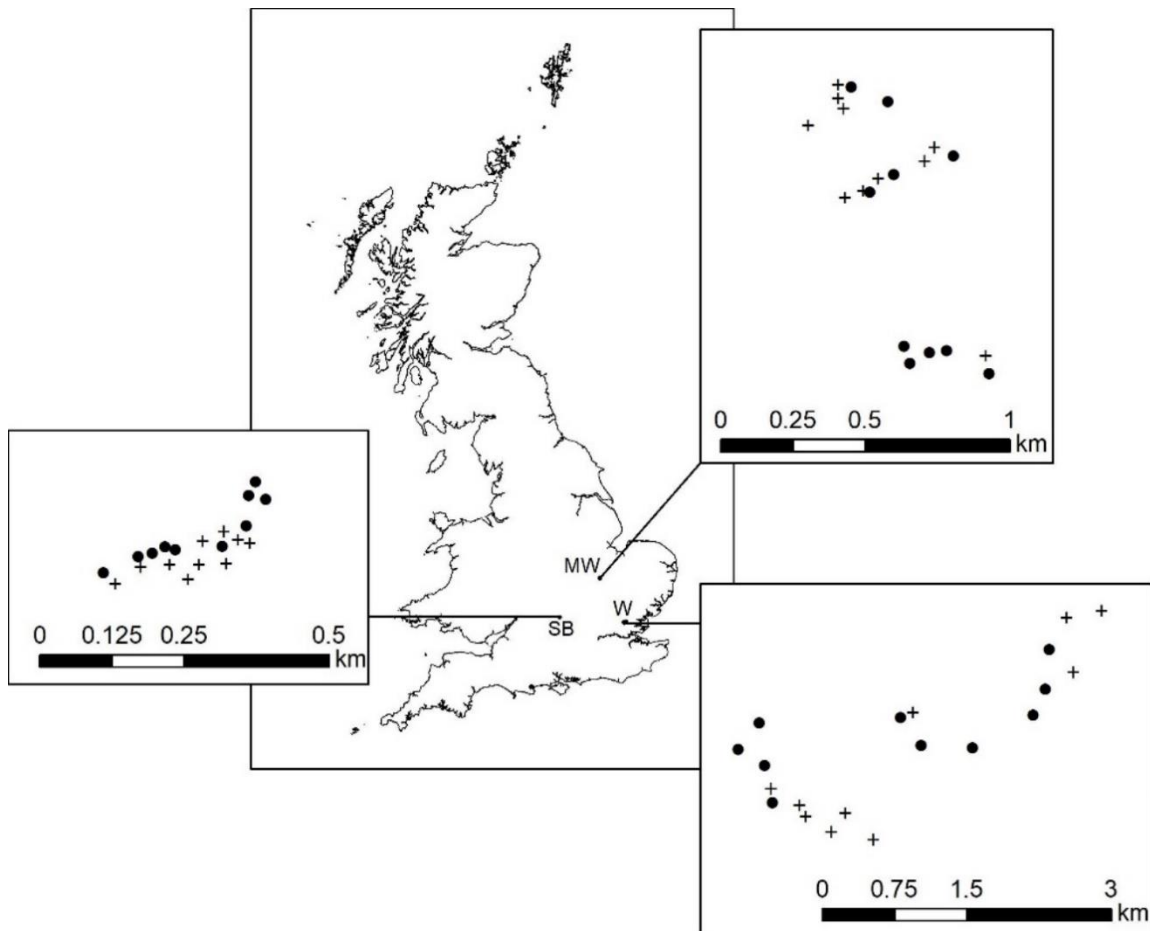
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820 **Figures**

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822 **Fig. 1.** Map showing three woodland sample locations (Monks Wood - MW, Stratfield Brake  
823 – SB and Writtle-W) and the distribution of symptomatic (+) and asymptomatic (●) trees at  
824 each woodland location. Note the differing scales between panels, adjusted to show the  
825 distribution of trees in each woodland.

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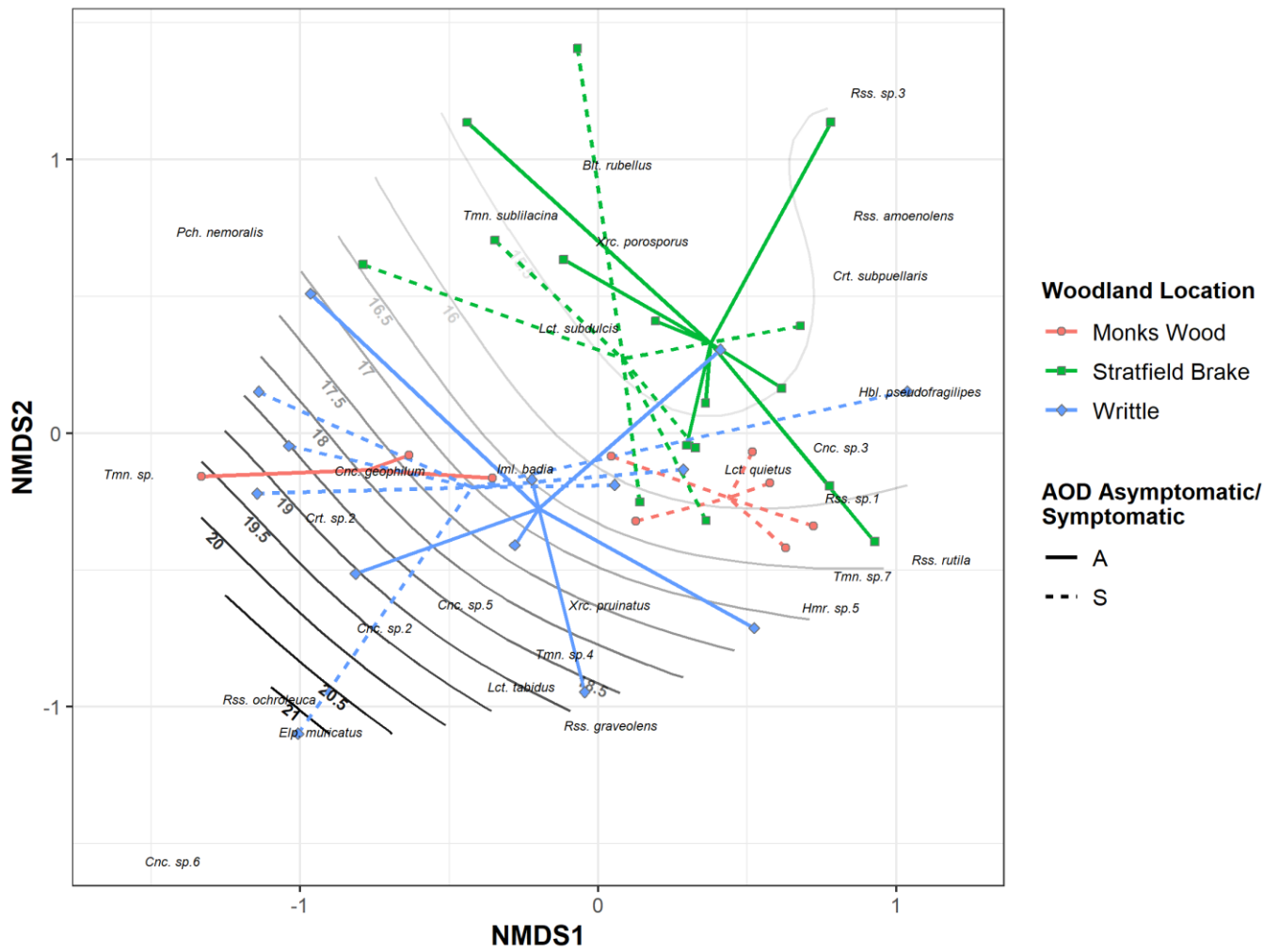
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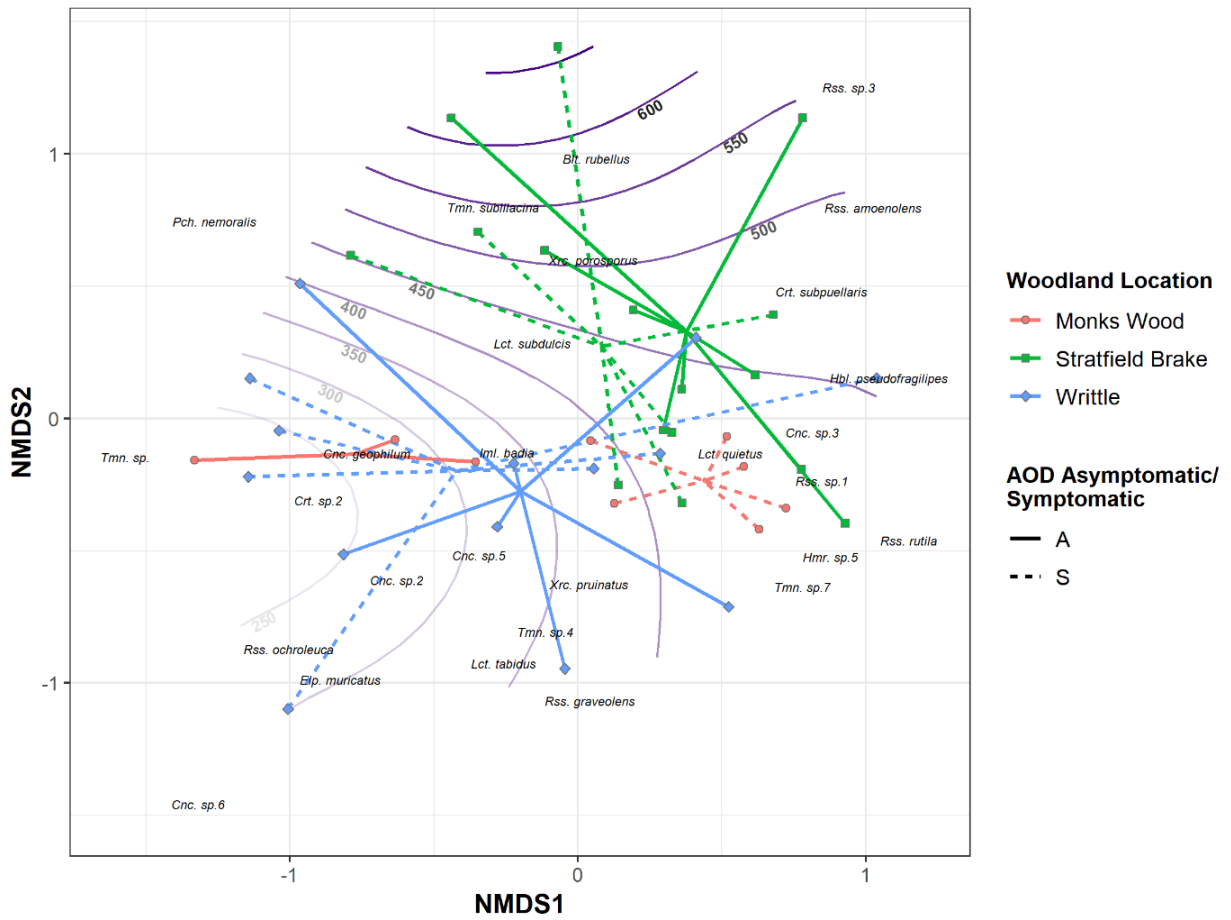
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838 **Figure 2** Non-metric multidimensional scaling (NMDS) ordination of ECM species showing  
839 samples grouped by woodland location and tree symptom status. Surface plot shows C:N  
840 ratios.



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855 **Figure 3** Non-metric multidimensional scaling (NMDS) ordination of ECM species showing  
 856 samples grouped by woodland location and tree symptom status. Surface plot shows  
 857 exchangeable Al concentrations ( $\text{mg kg}^{-1}$ ).



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872 **TABLES**

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874 **Table 1** Summary of differences in ECM richness, diversity ( $H'$ ) and root characteristics between woodland locations and for asymptomatic (A)  
 875 and symptomatic (S) trees at each woodland location. The values expressed for roots (< 2 mm diameter) are the average dry weight or number of  
 876 root tips per soil core (i.e. per 80cm<sup>3</sup> of soil sampled). One-way ANOVAs tested for significant differences between A and S trees in terms of  
 877 ECM richness, Shannon-Weaver diversity and root characteristics. The root data were log-transformed prior to analysis and back-transformed  
 878 for presentation. There were no significant differences in ECM richness, diversity or root dry weights between A and S trees. ECM richness and  
 879  $H'$  values shown combine all sample trees per woodland location. \* and \*\* indicate that S trees have significantly fewer root tips per soil core  
 880 than A trees at  $p < 0.05$  and  $p < 0.01$ , respectively. Percentage of soil core samples with no roots/fungi and percentage with positive ECM  
 881 identifications are also given for each woodland location.

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	ECM species richness		ECM family richness		ECM species diversity		Root dry weight (g)		Number of root tips		Sample failure <sup>1</sup> (%)	Samples with positive ECM ID <sup>2</sup> (%)
	Total	A or S	Total	A or S	Total	A or S	Total	A or S	Total	A or S		
Monks Wood	38	A 20	12	A 9	2.81	A 2.46	0.18	A 0.15	469	A 535	36.7	81.7
		S 19		S 9		S 2.39		S 0.21		S 403	10.8	84.5
Stratfield Brake	17	A 12	6	A 6	1.9	A 1.89	0.38	A 0.32	1060	A 1060	15.8	92.1
		S 9		S 5		S 1.61		S 0.44		S 890*	21.7	90.4
Writtle	45	A 28	14	A 12	2.63	A 2.83	0.35	A 0.33	1084	A 1320	11.7	89.6
		S 27		S 10		S 2.68		S 0.36		S 848**	17.5	82.2

<sup>1</sup> Percentage of samples without roots and fungi out of 120 possible samples (three fine root samples x four soil cores x ten A or S trees).

<sup>2</sup> Percentage of samples with fungi that have a positive ECM ID. Rejected if not an ECM fungi or could not be identified.

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884 **Table 2** Effect of tree symptom status, woodland location and soil variables on the  
 885 composition of ECM (a) species, (b) families and (c) exploration types assessed by  
 886 multivariate GLiMs. Analysis of deviance was conducted on each factor, with pit-trap  
 887 resampling, 999 iterations and score tests, used to determine the significance of tree symptom  
 888 status and woodland location. Significant *P*-values ( $\leq 0.01$ ) are shown in bold.

889 (a) Effect on ECM species

	<i>Res.Df</i>	<i>Df.diff</i>	<i>Deviance</i>	<i>P</i> -value
Tree symptom status	56	1	47.26	0.402
C:N ratio	55	1	97.20	<b>0.002</b>
Total N	54	1	69.73	<b>0.004</b>
Exchangeable Al	53	1	119.85	<b>0.001</b>
Woodland location	51	2	100.56	<b>0.002</b>

890

891 (b) Effect on ECM families

	<i>Res.Df</i>	<i>Df.diff</i>	<i>Deviance</i>	<i>P</i> -value
Tree symptom status	53	1	23.78	0.548
Exchangeable Al	52	1	54.68	<b>0.006</b>
Woodland location	50	2	56.53	0.097

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893 (c) Effect on ECM exploration type

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	<i>Res.Df</i>	<i>Df.diff</i>	<i>Deviance</i>	<i>P</i> -value
Tree symptom status	55	1	2.07	0.561
Woodland location	53	2	9.62	0.145
Tree symptom status x Woodland location	51	2	9.33	<b>0.010</b>

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902 **Appendix A: Supplementary material**

903 Additional Supplementary tables and figures associated with this article are listed below.

904

905 **Tables:**

906

907 **Table A.1** Climate, soil characteristics and dimensions of asymptomatic (A) and  
908 symptomatic (S) trees sampled at each woodland location. DBH is the diameter at breast  
909 height (1.3m above ground level).

910

911 **Table A.2:** Physico-chemical characteristics of soil samples collected from a depth of 5-15  
912 cm around the 20 sampled trees (10 symptomatic, 10 asymptomatic) at each woodland  
913 location. Means ( $\pm$  SD) are provided for each soil characteristic. Means sharing a letter in  
914 common are not significantly different ( $p < 0.05$ ) among woodland sites, according to Games-  
915 Howell Pairwise Comparisons. Soil pH, P and Al were all significantly ( $p < 0.05$ ) affected by  
916 tree symptom status as a main effect, although effects depended on woodland location for pH  
917 and Al. The effects of tree symptom status on N was marginal ( $p = 0.052$ ).

918

919 **Figure A.1:** Ranked abundance Zipf distributions of ECM species in the three woodland  
920 locations. Abundance values represent the number of trees that ECM species were associated  
921 with. Rank abundance curves were constructed using the `radfit()` function of the ‘vegan’  
922 package using the Zipf-Mandelbrot distribution (Oksanen et al., 2013).

923

924 **Figures:**

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926 **Figure A.2:** Species accumulation curves at the three woodland locations estimated using the  
927 `specaccum()` function in `vegan()` package in R. Method = “exact” (finds the expected (mean)  
928 species richness), permutations = 9999.

929

930 **Figure A.3:** Matrix plot of ECM families recovered by each sample tree, with fill colour  
931 (white to dark purple) indicating presence and abundance of families (maximum count = 12).

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933 **Figure A.4:** Matrix plot of ECM species recovered by each sample tree. Colour indicates  
934 species presence/ abundance (maximum count = 12).

935

936 **Figure A.5.** Matrix plot of ECM exploration types recovered beside asymptomatic (A) and  
937 symptomatic (S) trees at each woodland location, with fill colour (white to green) indicating  
938 presence and abundance of different exploration types (maximum possible count = 10).

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941 **Table A.1** Climate, soil characteristics and dimensions of asymptomatic (A) and symptomatic (S) trees sampled at each woodland location.  
 942 DBH is the diameter at breast height (1.3m above ground level).

Woodland characteristics	Monks Wood		Stratfield Brake		Writtle Forest	
Mean annual precipitation (mm)*	586		660		592	
Max. annual temperature (°C)*	14.4		14.6		14.6	
Min. annual temperature (°C)*	5.9		6.9		6	
Soil type**	Lime-rich loamy and clayey soils with impeded drainage		Slowly permeable seasonally wet base-rich loamy and clayey soils		Slowly permeable seasonally wet acid loamy and clayey soils	
	A	S	A	S	A	S
Mean crown density <sup>+</sup>	40.5	44.0	45.5	58.0	8.5	24.5
Shrub cover (%) <sup>++</sup>	33.4	30.7	25.4	29.5	20.1	24.3
Mean DBH of sample trees (cm)	49.3	51.1	55.0	59.1	58.5	64.4
Mean tree basal area (m <sup>2</sup> ) <sup>+++</sup>	33.4	30.7	25.4	29.5	20.1	24.3
Mean sample tree height <sup>++++</sup> (m)	18.5	18.2	19.6	19.0	16.1	15.5

943 \* Met Office averages 1981-2010 taken at Monks Wood, Oxford and Stratfield Brake weather stations.

944 \*\* Soil descriptions from soil maps at <http://www.landis.org.uk/soilscapes/>

945 <sup>+</sup> The absolute crown density was recorded in 5% classes where 0% = fully foliated crown and 100% = no leaves present.

946 <sup>++</sup> Shrub cover was the estimated average percentage cover of woody shrubs and tree sapling in 2m x2m quadrats placed at two random positions  
 947 within 5m of each sample tree.

948 <sup>+++</sup> Average tree basal area is based on the average basal area of all trees along four 15m transects running in the four cardinal directions away  
 949 from each sample tree.

950 <sup>++++</sup> Tree height was assessed with a clinometer to 0.1m.

951 **Table A.2:** Physico-chemical characteristics of soil samples collected from a depth of 5-15  
 952 cm around the 20 sampled trees (10 symptomatic, 10 asymptomatic) at each woodland  
 953 location. Means ( $\pm$  SD) are provided for each soil characteristic. Means sharing a letter in  
 954 common are not significantly different ( $p < 0.05$ ) among woodland sites, according to Games-  
 955 Howell Pairwise Comparisons. Soil pH, P and Al were all significantly ( $p < 0.05$ ) affected by  
 956 tree symptom status as a main effect, although effects depended on woodland location for pH  
 957 and Al. The effects of tree symptom status on N was marginal ( $p = 0.052$ ).

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Soil characteristics	Monks Wood	Stratfield Brake	Writtle Forest
Clay (%)	$1.6 \pm 0.8^A$	$0.6 \pm 0.3^B$	$0.4 \pm 0.5^B$
Silt (%)	$68.7 \pm 7.8^A$	$56.2 \pm 5.3^B$	$47.6 \pm 10.1^C$
Sand (%)	$30.0 \pm 7.9^C$	$43.1 \pm 5.6^B$	$52.0 \pm 10.7^A$
pH (H <sub>2</sub> O)	$4.7 \pm 0.7^A$	$3.6 \pm 0.2^B$	$3.6 \pm 0.3^B$
C:N ratio	$13.2 \pm 1.5^C$	$14.6 \pm 0.9^B$	$20.0 \pm 2.9^A$
Total N (%)	$0.47 \pm 0.1^A$	$0.52 \pm 0.1^A$	$0.42 \pm 0.2^A$
Olsen P (mg kg <sup>-1</sup> )	$9.4 \pm 5.6^B$	$20.9 \pm 12.5^A$	$24.8 \pm 22.6^A$
Exchangeable K (mg kg <sup>-1</sup> )	$310.7 \pm 90.0^A$	$289.4 \pm 69.0^{AB}$	$227.7 \pm 143.7^B$
Exchangeable Al (mg kg <sup>-1</sup> )	$190.2 \pm 174.2^B$	$531.8 \pm 141.3^A$	$306.7 \pm 116.6^B$

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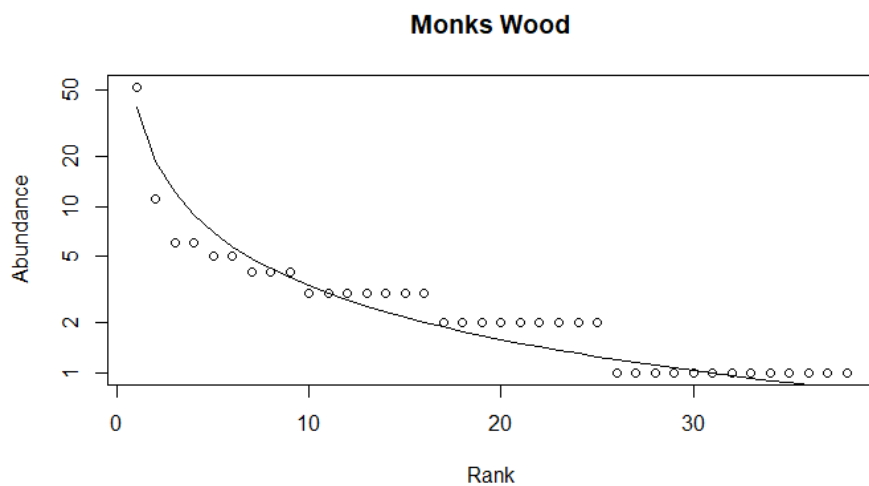
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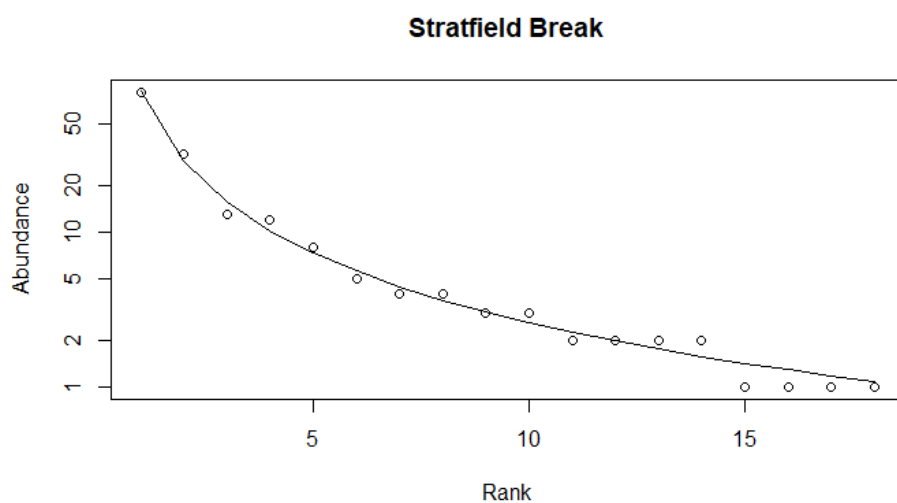
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972 **Figure A.1:** Ranked abundance Zipf distributions of ECM species in the three woodland  
973 locations. Abundance values represent the number of trees that ECM species were associated  
974 with. Rank abundance curves were constructed using the radfit() function of the ‘vegan’  
975 package using the Zipf-Mandelbrot distribution (Oksanen et al., 2013).

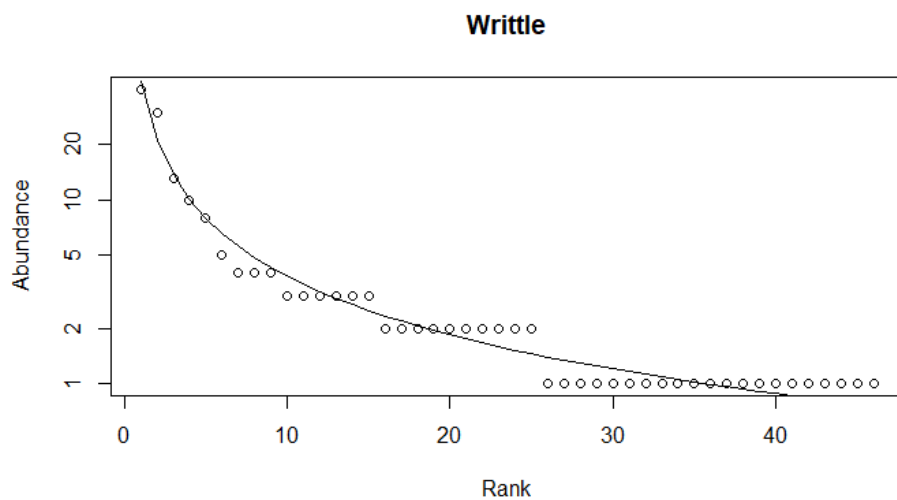
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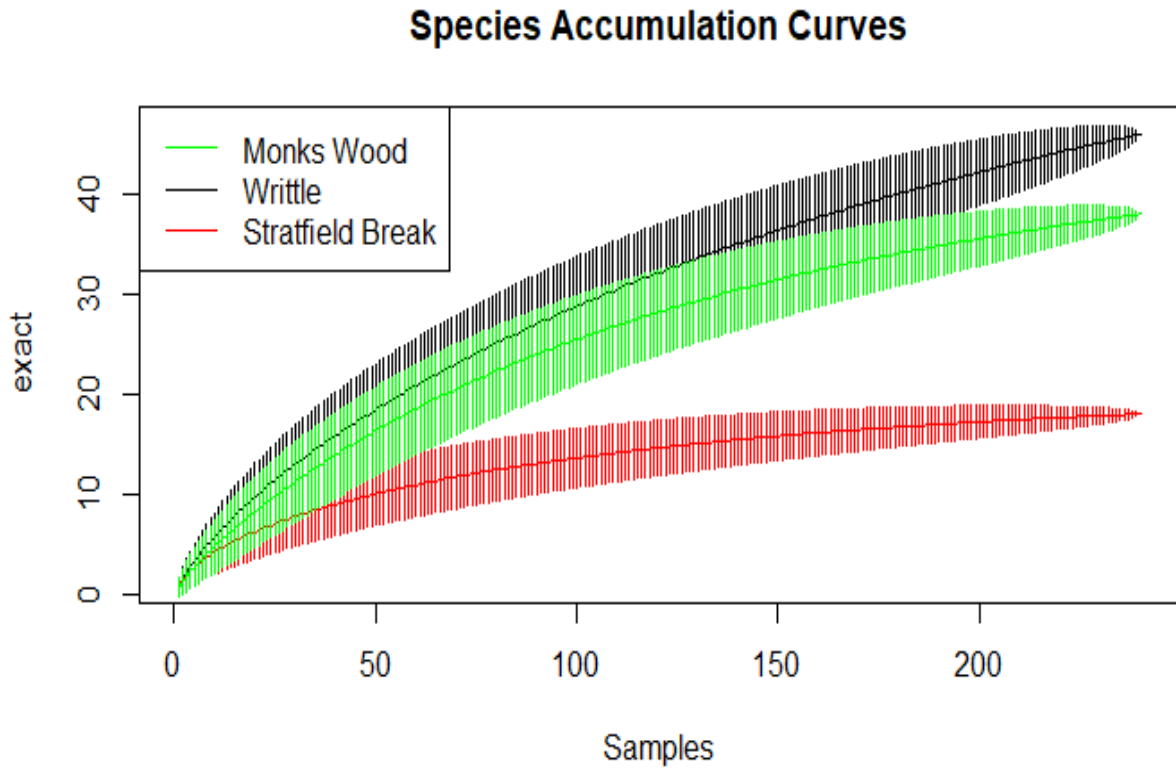
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982 specaccum() function in vegan() package in R. Method = “exact” (finds the expected (mean)  
983 species richness), permutations = 9999.



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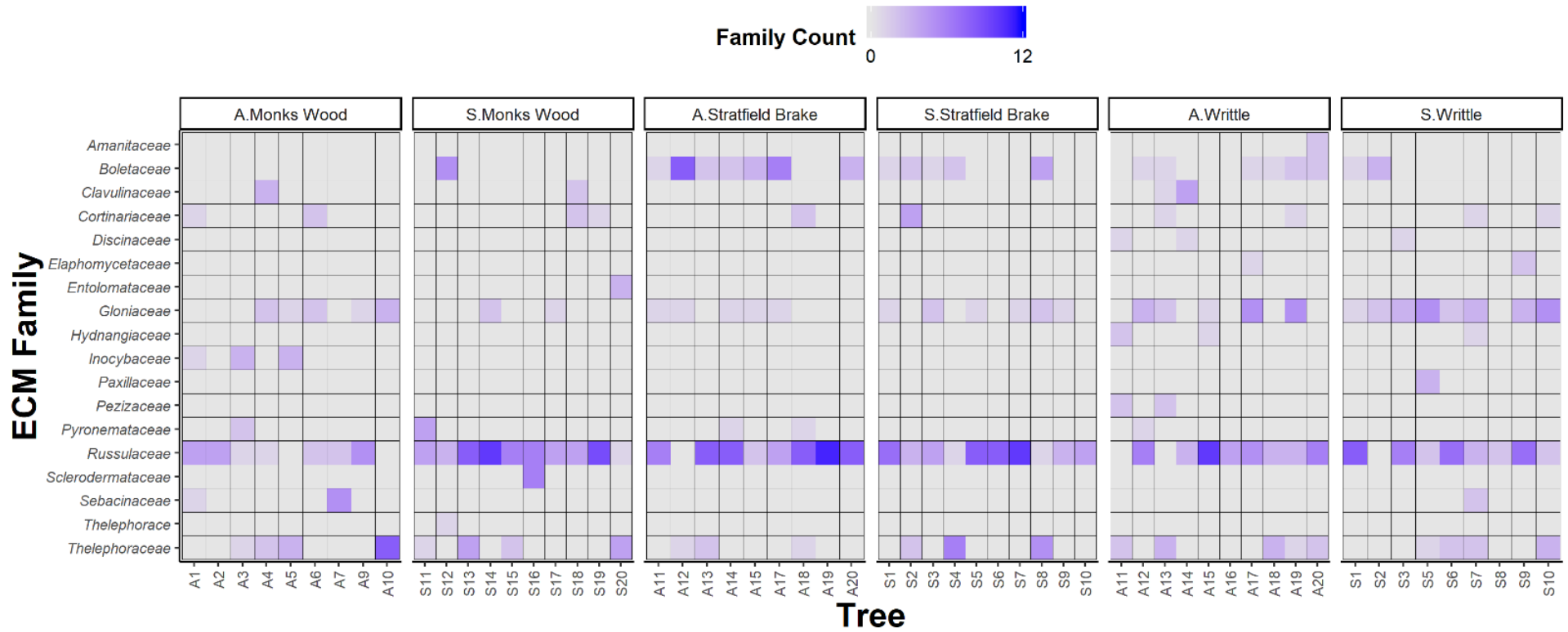
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996 **Figure A.3:** Matrix plot of ECM families recovered by each sample tree, with fill colour (white to dark purple) indicating presence and  
 997 abundance of families (maximum count = 12).



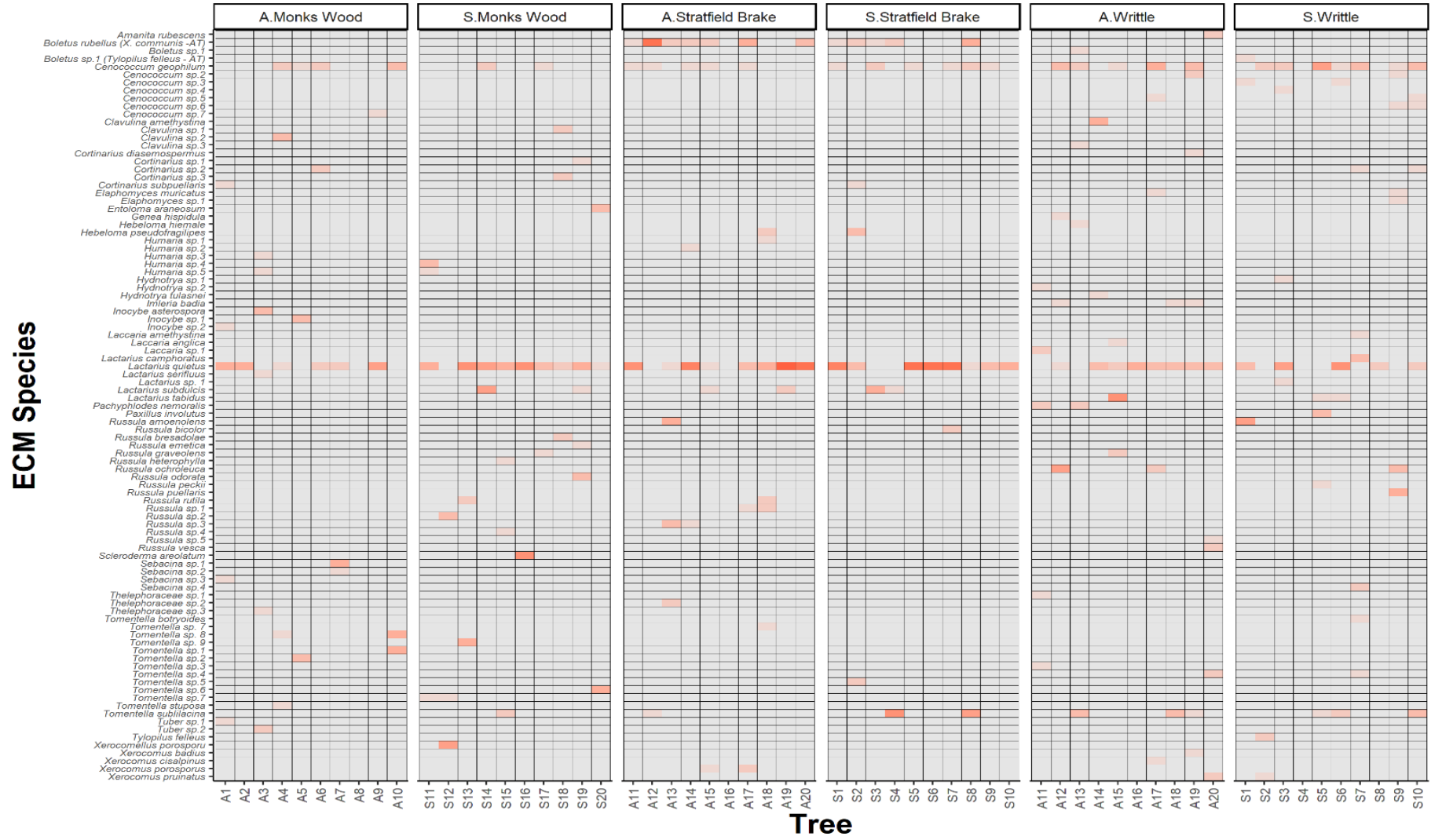
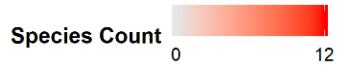
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1001 **Figure A.4:** Matrix plot of ECM species recovered by each sample tree. Colour indicates species presence/ abundance (maximum count = 12).

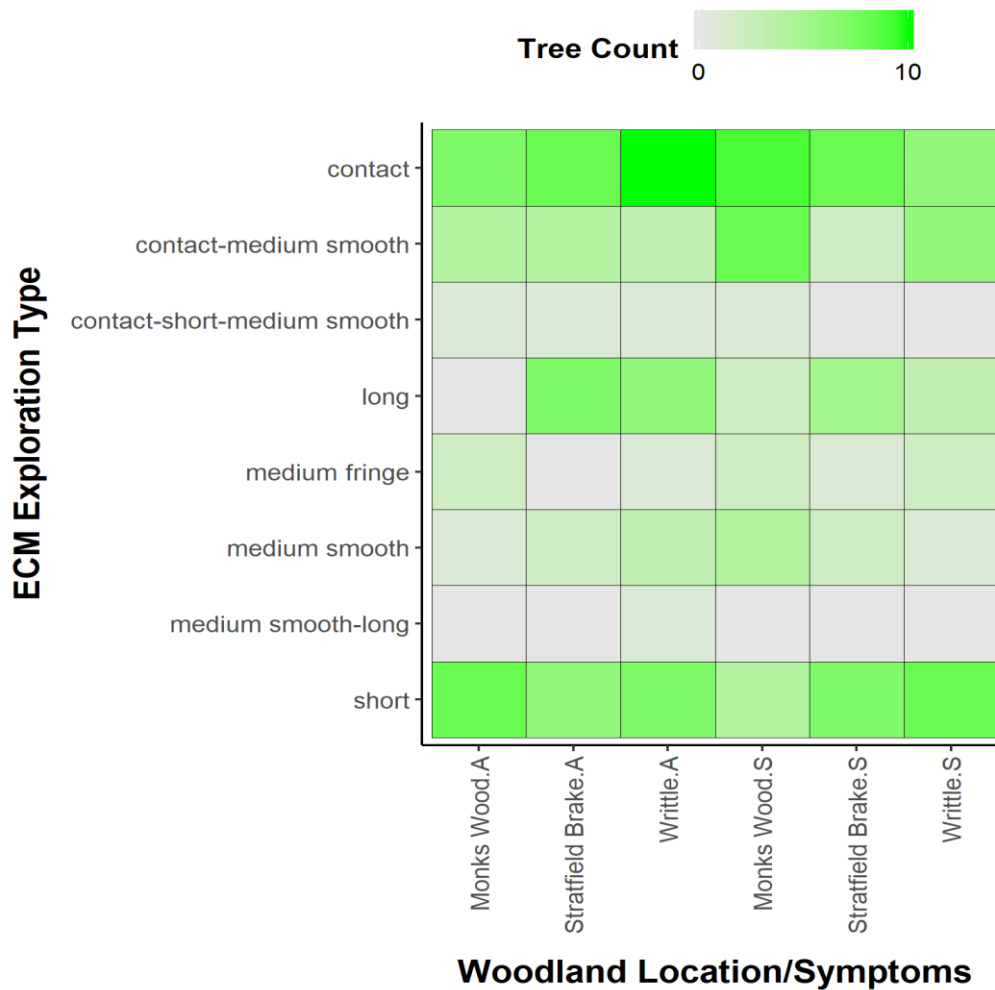
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1005 **Figure A.5.** Matrix plot of ECM exploration types recovered beside asymptomatic (A) and  
1006 symptomatic (S) trees at each woodland location, with fill colour (white to green) indicating  
1007 presence and abundance of different exploration types (maximum possible count = 10).



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