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Exploring the symbiont diversity of ancient western redcedars: arbuscular mycorrhizal fungi of long-lived hosts

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1   **Exploring the symbiont diversity of ancient western redcedars: arbuscular**

2   **mycorrhizal fungi of long-lived hosts**

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4   Monika A. Gorzelak*, Brian J. Pickles†, Miranda M. Hart**

5

6   *Department of Forest and Conservation Science, University of British Columbia, Vancouver,

7   BC. V6T 1Z4.

8   †School of Biological Sciences, University of Reading, Harborne Building, Whiteknights,

9   Reading, RG6 8AS, UK.

10   **Department of Biology, University of British Columbia Okanagan, Kelowna, BC V1V 1V7,

11   Canada.

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16   Corresponding author: Miranda Hart

17   *Tel: +1 250 807 9398*

18   *Fax: +1 250 807 8830*

19   *Email: miranda.hart@ubc.ca*

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21   Running title: AMF communities of a long-lived host

22

Abstract (232 words)

Arbuscular mycorrhizal fungi (AMF) are globally distributed, monophyletic root symbionts with ancient origins. Their contribution to carbon cycling and nutrient dynamics is ecologically important, given their obligate association with over 70% of vascular plant species. Current understanding of AMF species richness and community structure is based primarily on studies of grasses, herbs, and agricultural crops, typically in disturbed environments. Few studies have considered AMF interactions with long-lived woody perennial species in undisturbed ecosystems. Here we examined AMF communities associated with roots and soils of young, mature, and old western redcedar (*Thuja plicata*) at two sites in the old-growth temperate rainforests of British Columbia. Due to the unique biology of AMF, community richness and structure were assessed using a conservative, clade-based approach. We found 91 AMF OTUs across all samples, with significantly greater AMF richness in the southern site, but no differences in richness along the host chronosequence at either site. All host age classes harboured AMF communities that were overdispersed (more different to each other than expected by chance), with young tree communities most resembling old tree communities. A comparison with similar clade richness data obtained from the literature indicates that western redcedar AMF communities are as rich as those of grasses, tropical trees, and palms. Our examination of undisturbed temperate old growth rainforests suggests that priority effects, rather than succession, are an important aspect of AMF community assembly in this ecosystem.

Introduction

Arbuscular mycorrhizal fungi (AMF) are an ancient lineage of obligate biotrophs (Redecker *et al.* 2000; Bonfante & Genre 2008), requiring symbiosis with roots to complete their lifecycle (Smith & Read 2008). The presence and composition of AMF impacts plant biodiversity (van der Heijden *et al.* 1998), plant response to increases in CO_2 (Terrer *et al.* 2016), nutrient cycling (Phillips *et al.* 2013; Johnson *et al.* 2015) and soil carbon sequestration (Averill *et al.* 2014). Given the global influence and distribution of AMF (Davison *et al.* 2015; Soudzilovskaia *et al.* 2015), we know surprisingly little about how their communities are structured in most ecosystems.

Current knowledge on the drivers of AMF community composition indicates that both dispersal limitation and habitat filtering play significant roles (Öpik *et al.* 2006, 2010; Kivlin *et al.* 2011). Recently it has been shown that host identity can exert a greater influence on AMF composition than competition between the fungi themselves (Davison *et al.* 2016). Temporal processes such as plant community succession may also structure AMF communities (Koske & Gemma 1997), with pattern detection requiring that samples be collected in a time-series or across a chronosequence (Dornelas *et al.* 2013). While community studies of AMF are important, the overwhelming research focus has been on easily manipulated, short-lived hosts such as forbs and grasses (Ohsowski *et al.* 2014; Davison *et al.* 2015), thus our understanding of AMF communities may overlook dynamics that occur over long time scales.

Investigations of the impact of long-lived hosts on AMF community structure are few (but see Hart *et al.* 2014). Undoubtedly, due to physiological differences, long-lived host trees exert different influences on their AMF compared to hosts with shorter lifespans. Ontogenetic studies suggest that early life stages (seedlings, saplings, and pole) provide less photosynthate to symbionts than mature or old trees, particularly when they are establishing under a closed canopy in the shade (Thomas & Winner 2002). This could cause AMF communities to shift in response to changing carbon supply (Bago et al. 2002, Ijdo et al. 2010). Further, given that

3

69   some trees may live for millennia, AMF communities may differ in composition and turnover

70   (β-diversity). For instance, it is known that plants in early successional systems have higher

71   AMF β-diversity, perhaps due to environmental heterogeneity (Christensen and Peet 1984).

72   Similarly, plants in late successional systems tend to exhibit lower AMF β-diversity, perhaps

73   due to environmental filtering (Derroire et al. 2016). While the idea of AMF and community

74   convergence has received some attention (Caruso et al. 2012, Maherali and Klironomos 2012),

75   whether this is true for AMF over successional time scales is not known.

76   Current ideas in community assembly theory suggest that ecological processes may not

77   be as easily inferred from patterns of species co-existence as previously expected (Gerhold et

78   al. 2015). Overdispersion, in which species co-exist less often than expected by chance, is often

79   assumed to represent the process of competitive exclusion: related species with comparable

80   traits will compete more intensely than more distantly related species. Clustering, in which

81   species co-exist more often than would be expected by chance, is often assumed to be caused

82   by niche partitioning, where competition is less important than the suitability of the

83   environment for that suite of organisms. Meta-analysis of competition experiments in plants

84   has shown little evidence to support a direct link between these patterns and processes (Cahill

85   et al. 2008), however, niche partitioning has been demonstrated in some detailed studies of

86   overdispersed communities (e.g. Cavender-Barnes and Pahlich 2009). Importantly, the case can

87   be made that overdispersion patterns can be created by both niche partitioning and competition

88   acting in tandem (Mayfield and Levine 2010), although clustering is less likely to be caused by

89   a myriad of interacting processes.

90   Here we explore the richness and diversity of AMF communities associated with

91   western redcedar (*Thuja plicata* Donn *ex* D. Don), a long-lived woody host; examining both

92   roots and soil.. We hypothesised that these long-lived plants would exert selective pressures on

93   their AMF symbionts that differ from those produced by herbs, grasses, and other short-lived

94 hosts, due to differences in the availability of carbon resources, leading to: i) an increase in

95 AMF richness with increasing host age, and ii) succession of distinctive AMF communities

96 among host age classes.

97

98 **Materials and methods**

99 *Field sites*

100 Two study sites were selected within the Interior Cedar-Hemlock (ICH) Biogeographic Zone

101 (Ketcheson *et al.* 1991) of British Columbia, one at the northern range limit (53°45'45.68" N,

102 121°13'5.93" W) and the other in the southern end of the distribution (49°40'44.47"N,

103 117°43'5.92"W) within the ICHvk (very wet cool) and ICHdw (dry warm) variants,

104 respectively. Commonly known as the interior temperate rainforest, due to high annual

105 precipitation, these areas can represent continuous stands that have been present for thousands

106 of years, and are host to very large western redcedar trees; potentially 800-1000 years or older

107 in some cases, especially at the northern 'Ancient Forest' site 100 km east of Prince George.

108 The most abundant alternative AMF host species in the northern site were bunchberry (*Cornus*

109 *canadensis*) and devils club (*Oplopanax horridus*), whereas common snowberry

110 (*Symphoricarpos albus*), Oregon-grape (*Mahonia sp.*), twinflower (*Linnea borealis*), and false

111 box (*Paxistima myrsinites*) were the most abundant in the south.

112

113 *Fungal community sampling*

114 Trees were selected from three 'age classes' based on their diameter-at-breast-height (dbh):

115 young trees (dbh < 5 cm), mature trees (dbh 19 – 65 cm), and old trees (dbh 150 – 455 cm).

116 These age classes represent estimates of tree life stages, for which dbh is considered a useful

117 proxy, due to the tendency for western redcedar to rot from the centre outwards as it matures,

118 rendering the determination of tree age by coring impossible. Five trees were sampled per age

119 class at each site. From each tree, five fine root samples were obtained by digging along large

5

120   roots, thus ensuring that sampled roots belonged to the sample tree. For three of the five trees

121   per age class, three soil cores (2.5 cm diameter) of the top 10 cm organic horizon were sampled

122   adjacent to each root sampled (within approx. 2m of the base of the trunk). All samples were

123   sealed in plastic bags and kept on ice in a cooler for transport, prior to storage at 4 ºC.

124   Processing took place within 3 months of sample collection. Subsamples for both roots and

125   soil were pooled at the level of individual tree for subsequent analyses, for a total of 48

126   samples (15 root, 9 soil at each site).

127

128

129   *Molecular methods*

130   For each sample DNA was extracted from 150 mg of randomly selected root segments (first

131   cleaned in deionized water), and from 250 mg of homogenized soil, using the Powersoil®

132   DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Glomeromycota 28S

133   large sub-unit ribosomal DNA sequences were amplified using FLR3 (forward) and FLR4

134   (reverse) (Gollotte *et al.*, 2004) primers, linked to 454-sequencing adapters and linkers. PCR

135   was carried out using 20pmol dNTPs, 3.5mM $MgCl_2$, 40µg BSA, 20 pmol of each primer, and

136   1U GoTaq with supplied buffer (Promega Corporation, WI, USA). Thermocycling conditions

137   were as follows: 95°C for 1 minute, 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds,

138   72°C for 1 minute, followed by 72°C for 7 minutes and stored at 4°C. PCR products were

139   cleaned and normalized to 1-2 ng/µL using SequelPrep™ Normalization Plate (96) kit (Life

140   Technologies™, NY, USA). The samples were pooled and sequenced on a Genome Sequencer

141   FLX System, using Titanium Series reagents (Roche Applied Science) at the Vancouver

142   Prostate Centre. All sequences and sample information are available online

143   https://dx.doi.org/10.6084/m9.figshare.1451402.v1.

144

145   *Bioinformatics*

146 Sequences were analysed using the default settings, unless otherwise noted, in QIIME 1.7.0

147 (Caporaso et al. 2010). OTUs were picked *de novo* using 97% similarity using the UCLUST

148 algorithm (Edgar 2010). Chimera checking was performed using the usearch61 *de novo*

149 method. Sequence 'denoising' was not performed as it has been shown to alter beta-diversity in

150 AMF studies (Hart et al. 2015). Out of the 48 samples a single barcode was not recovered

151 (associated with an old tree soil sample from the northern site). Taxonomy was assigned using

152 the GenBank® database (http://www.ncbi.nlm.nih.gov) with the BLAST (nucleotide)

153 algorithm. All non-Glomeromycotan sequences (<0.01%) were removed. A retained sequence

154 was considered to match known AMF taxa if its similarity to database sequences was 97% or

155 greater (Hart *et al.* 2015), however closest matches to existing 28S accessions within the

156 Glomeromycota at lower levels were retained and classed as 'unknown'.

157   Of the 201 020 sequences generated, 70 097 fulfilled the retention criteria and were

158 grouped into 1012 OTUs. The total number of these OTUs matching existing AMF sequences

159 at a similarity level of 97%+ was 876, of which 698 produced a 100% match, 111 at 99%, 40 at

160 98%, and 27 at a 97% level. The remaining 136 OTUs did not match the database at the

161 typically acceptable level (matches varied from 89-96%). Thus, approximately 13% of the

162 OTUs generated were potentially novel Glomeromycotan sequences. Of the 1012 OTUs 325

163 were singletons, leaving 687 clusters that contained 2 or more sequences. Rarefying to an even

164 sampling depth retained 581 sequences per root sample (total of 665 OTUs) or 91 sequences

165 per soil sample (total of 316 OTUs).

166   Due to concerns about diversity inflation resulting from the 454-pyrosequencing we

167 applied a monophyletic clade approach (MCA) to convert the raw OTU clusters into clades

168 within the Glomeromycota (see Lekberg *et al.* 2014). The MCA is an OTU delineation method

169 wherein sequence groups are manually combined based on membership within a monophyletic

170 clade. We followed the methodology of Lekberg *et al*. (2014), where the authors demonstrated

171 that the method is robust and generates community patterns that are comparable to OTU

7

172   methods based on percentage similarity thresholds. The strength of the MCA method is that it

173   is grounded in evolutionary theory. This conversion of OTUs using MCA resulted in a total of

174   91 Glomeromycotan clades. Two of these clades were 'sequence singletons' (contained a

175   single sequence), whereas five clades were 'sample singletons' (occurred in a single sample).

176   Each MCA was identified using the GenBank® database (http://www.ncbi.nlm.nih.gov) with

177   the BLAST (nucleotide) algorithm, which matched sequences within each clade to existing 28S

178   accessions. All of the subsequent community analyses presented below were performed using

179   the MCA data tables with sequence singletons removed. We also analysed the OTU data using

180   the same methods and additionally using variance stabilising transformations (McMurdie &

181   Holmes, 2013). Ecological interpretations were not substantially different between the

182   approaches unless otherwise noted (for further details see Supporting Information).

183

184   *AM fungal diversity of other host plants*

185   To place western redcedar in the context of existing research into the fungal diversity of AM

186   hosts we compiled AMF community data from previous studies that examined trees, shrubs,

187   woody perennials, palms, grasses, and herbs. Due to the variability among marker genes for

188   AMF (see Thiéry *et al*., 2016), and the lack of data on AMF communities of long-lived hosts,

189   we limited our comparisons to studies that: i) presented data for multiple individuals of a

190   specific host species, ii) used 454 sequencing technology to obtain OTUs, and iii) expressed

191   AMF diversity at the clade-level by using MCA-type approaches (e.g. 'virtual taxa'; Öpik *et al*.

192   2010). This cladistic approach to AMF diversity was utilized to maximise the relevance of

193   comparisons between host species by removing the noise associated with OTU assignments,

194   particularly in regards to the differences in sensitivity levels between the LSU and SSU

195   regions.

196

197   *Statistical analyses*

198   Sample-based rarefaction curves were calculated using 1000 permutations of random

199   subsampling without replacement for each site's root and soil MCA communities in EstimateS

200   version 9.1.0 (Colwell 2013). Clade richness differences were examined in a three-way

201   ANOVA in R (R-project Core Team, 2015) using the model: Richness ~ Site * Age * Source.

202   Tukey's HSD test was applied post-hoc to determine which groups differed significantly.

203   AMF community differences were analysed using PERMANOVA by converting MCA

204   data into dissimilarity indices using Bray-Curtis (Sørensen) and β_{sim} (Koleff et $al.$ 2003) in R-

205   package 'vegan' (Oksanen et $al.$ 2013), with the function 'anosim'. Multivariate dispersion

206   was assessed using the function 'betadisper'. Interpretation did not differ between indices, so

207   only the results of Bray-Curtis (Sørensen) index are presented.

208   AMF community β-diversity partitioning was also applied to examine evidence of

209   structural changes between host age classes, analysing soil and root communities separately,

210   and using Mantel correlograms to test for autocorrelation in AMF community composition

211   within each site (see Borcard & Legendre 2012). Three dissimilarity matrices were constructed

212   for each site using the β-diversity partitioning of Jaccard's index (Baselga 2010; Carvalho et $al.$

213   2013; Ensing & Pither 2015) in R-package 'betapart' (Baselga et $al.$ 2013) modified using the

214   additional R-code supplied in Ensing & Pither (2015). Note that Jaccard's index is based on

215   binary presence/absence data and as such was conducted on the MCA dataset with sequence

216   singletons removed, considering each study site's root and soil samples separately. The

217   primary measure of dissimilarity was β_{cc} (Jaccard's index of dissimilarity), which can be

218   subdivided into β_{-3} (dissimilarity due to species replacement) and β_{rich} (dissimilarity due to

219   richness differences). Dissimilarity matrices were standardized using the hellinger

220   transformation to achieve the requirement of second-order stationarity. For each analysis a

221   predictor distance matrix of age classes was obtained from log(host tree dbh). Mantel statistics

222   (r_M) were calculated for age classes and tested for significance using 10,000 Monte-Carlo

223   permutations and progressive Holm's correction for multiple testing. In each case, positive r_M

224   values indicated positive autocorrelation (e.g. greater community similarity).

225

226   **Results**

227   *Western redcedar AMF clade richness differences*

228   Three-way ANOVA revealed that site was the only significant factor in producing clade

229   richness differences ($F_{1,35} = 10.01$; $P = 0.003$), richness per sample did not differ significantly

230   between age classes ($F_{2,35} = 0.09$; $P = 0.913$) or sample source ($F_{1,35} = 1.57$; $P = 0.219$) and

231   there were no significant interaction terms. Significantly more AMF clades per sample were

232   found in the southern site (mean 31.5 ± 2.66, n=24) than in the northern site (mean 20.2 ± 1.18,

233   n=23) (Tukey's HSD: South > North; $P_{adj} = 0.002$). Overall clade richness tended to be higher

234   in root than in soil samples, however, according to clade-based accumulation curves this

235   difference was only significant in the southern site (Fig. 1). Root sampling clearly approached

236   an asymptote (Fig. 1a), and sampling of both root and soil (Fig. 1b) communities was within

237   the 95% confidence interval of extrapolated clade richness obtained from a hypothetical

238   doubling of sample size. Similar results were obtained when examining the data based on

239   OTUs rather than clades (data not presented).

240

241   *AMF community composition*

242   Analysis of AMF community composition based on clades, and using a three-way

243   PERMANOVA of Bray-Curtis dissimilarities, revealed significant differences between sites

244   and between host age classes, but not between roots and soil (Table 1). The full model

245   accounted for 28% of the partial variance, and no difference in multivariate dispersion was

246   detected between groups (Table 1). A similar result was observed for the rarefied OTU

247   dataset, whereas analysis with variance stabilising transformations indicated significant

248   differences between root and soil communities (Supporting Information Table S1).

249   Differences in community composition between sites, age classes, and sample source,

250   are illustrated in Fig. 2. *Acaulospora* spp. were only present in significant numbers in northern

251   samples (Fig. 2a-f).   *Acaulospora* and *Rhizophagus* spp. were most abundant in northern roots

252   of old trees (Fig. 2c).. These roots also contained fewer sequences from unknown clades (Fig.

253   2c), compared to young trees (Fig. 2a). A greater proportion of sequences from *Glomus* clades

254   were found in southern site samples in general (Fig. 2g-l) and in young northern site samples

255   (Fig. 2a,d). Overall, more novel taxa were found in the northern site.

256   Evidence of community structure related to host age was detected in root (Fig. 3) and

257   soil (Fig. 4) communities, with both study sites exhibiting similar β-diversity patterns. The

258   AMF communities of trees within each age class were overdispersed (e.g. significantly less

259   similar to each other in clade composition; Fig. 3a,b and Fig. 4a,b). Comparison between age

260   classes revealed that AMF communities of younger trees (smallest dbh class) tended to be

261   autocorrelated (e.g. were significantly more similar in clade composition) with those of the

262   oldest trees (largest dbh class) (Fig. 3a and Fig. 4a,b). Partitioning of Jaccard's dissimilarity

263   index (β_{cc}) into its turnover (β_{-3}) and richness (β_{rich}) components, indicated that the AMF

264   community patterns were mostly driven by community turnover, which was overdispersed

265   within age classes (Fig. 3c,d and Fig. 4c). However, soil communities displayed

266   autocorrelation between the AMF communities of young and old trees (Fig. 4c,d). Richness

267   differences between AMF communities of the youngest and oldest trees were autocorrelated,

268   but only significantly so in the northern site (Fig. 3e and Fig. 4e). In all cases, a significant

269   increasing linear trend in r_M values from negative in trees of a similar age to positive between

270   the youngest and oldest trees was observed with increasing host pair age difference ($r^2 = 0.69$

271   to 0.83, $P < 0.01$).

272

273   *Comparison with other AM hosts*

274   In terms of overall AMF clade richness derived from host root sampling, western redcedar was

275   ranked second across all host growth forms (Fig. 5; Supporting Information Table S2), with

276   only the palm *Podococcus barteri* observed to associate with a greater number of clades.

277   When examined from the perspective of AMF clade richness per individual host plant, western

278   redcedar communities were again ranked first , but tied for first place with two long-lived

279   tropical tree species (*Polyalthia suaveolens* and *Santiria trimera*), two palms (*Bactris*

280   *rhaphidacantha* and *Podococcus barteri*), and two grasses (*Agropyron cristatum* and *Potentilla*

281   *acaulis*) (Fig. 5; Supporting Information Table S2). Using clade-based approaches to AMF

282   richness, the number of clades encountered on western redcedar was consistently greater than

283   those found on perennial woody and herbaceous plants, and the majority of other tree species

284   for which comparable data exists (much of which is drawn from the work of Davison *et al.*

285   2015).

286

287   **Discussion**

288   Western redcedar is a long-lived tree that exhibits high clade richness in its associated AMF

289   communities. Despite significant differences in the community compostion between sites, the

290   autocorrelation patterns were similar at both sites, implying that the cedar host may create the

291   same patterns in distinct locations. In particular, we observed overdispersion in AMF β-

292   diversity within each host age class at both study locations – shifts from overdispersion to

293   neutral structure to autocorrelation took place between pairs of hosts as the difference in host

294   age increased. This study represents one of the first investigations of the symbiotic AMF

295   community of a long-lived gymnosperm host using next-generation sequencing techniques, and

296   presents an important starting point for further examination of these under-explored systems.

297

298   *Clade richness differences*

299 We had anticipated that older trees would exhibit higher clade richness, accumulating more

300 AMF over their lifetime. However, this was not supported by the data. There is some evidence

301 for higher AMF species richness in mature breadfruit (Hart et al. 2014), but in general there is

302 little support for richness increasing with host age, simply because there is a dearth of studies

303 on long-lived AM host trees. This topic requires much more research.

304 The greatest differences in clade richness were between the northern and southern study

305 locations, with the southern site containing a significantly greater number of clades. This is a

306 typical pattern for biodiversity in general (Hillebrand 2004), and fungal biodiversity in

307 particular (Tedersoo et al. 2014), although we note that ectomycorrhizal fungi are highly

308 diverse in boreal and arctic ecosystems (Timling et al. 2012; Taylor et al. 2014). In our study,

309 samples from the Northern site had a greater number of clades matching *Acaulospora* and

310 *Rhizophagus* spp. However, at least two-thirds of the clades in each sample failed to match

311 existing database sequences at the species or genus level. Although this likely reflects the lack

312 of LSU sequences deposited in the NCBI database for AMF it also opens up the possibility that

313 a large number of currently unknown AMF reside in these communities. We propose that

314 further investigation of western redcedar mycorrhizas may be most profitably targeted at the

315 communities associated with both mature trees and the oldest extant trees. The use of a variety

316 of molecular markers (see Hart *et al.* 2015) will be key to determining how many of their

317 symbionts truly represent previously undiscovered AMF clades.

318

319 *Clade richness differences between AM hosts*

320 Our compilation of data from the literature on the number of clades per host species and

321 individual host plant, revealed a wide range in the values of these richness measures.

322 Considering the data in terms of rank order alone western redcedar was at the upper end of the

323 richness distribution: 2nd for total number of AMF clades and joint 1st for number of clades per

324 individual sample. We encourage future research using multiple estimators of AMF diversity

325   (Hart *et al.* 2015) that focuses on old individuals selected from long-lived host tree species, and

326   particularly gymnosperms. While many mycorrhizal studies of gymnosperms exist, they focus

327   almost exclusively on ectomycorrhizal hosts (Chaudhary *et al.* 2016).

328

329   *AMF clade community composition*

330   Overdispersion of AMF communities within each host age class was not expected. That the

331   same pattern of beta-diversity was observed at both of our study sites suggests a general pattern

332   within inland temperate rainforests, but further studies are required and comparison to coastal

333   western redcedar forests would be informative. Conversely, in northern site roots and in the

334   soils at both sites, the youngest trees hosted AMF communities that were significantly more

335   similar to those of the oldest trees than was expected (autocorrelated). Overdispersion has

336   historically been interpreted as evidence of competitive exclusion structuring a community (see

337   reviews by HilleRisLambers et al., 2012; Gerhold et al. 2015). That is, closely related species

338   exclude each other because their trait similarity leads to more intense competition. Whereas the

339   opposite pattern, increased clustering (co-occurring more often than expected by chance)

340   indicates environmental filtering. In the latter case, competition is less important in structuring

341   a community than environmental conditions. This pattern does not manifest consistently

342   (Cahill et al., 2008), and it has been argued that only clustering is indicative of environmental

343   filtering, whereas the pattern of overdispersion can be caused by many interacting forces,

344   including but not limited to competitive exclusion (Mayfield and Levine 2010;

345   HilleRisLambers et al., 2012; Alexandrou et al., 2015; Gerhold et al. 2015; Li et al., 2015). In

346   our study, overdispersion indicates that, within a cohort, each tree assembled a unique

347   community of AMF. However, autocorrelation increased as the age (dbh) difference between

348   trees increased, resulting in young trees having similar AMF communities to those of the oldest

349   trees. Our interpretation is that this represents a 'parent tree' or 'nurse tree' effect in which

350   young trees inherit their AMF communities from the old trees that surround them when they

14

351   establish. In a temperate rainforest formed by long-lived tree species, in which sudden

352   stochastic events (e.g. fires) are not part of the typical cycle of regeneration, seedlings are

353   likely to collect their first symbionts via hyphal connections from the roots of surrounding

354   trees. If so, the maintenance of overdispersion within a cohort suggests a major role for priority

355   effects in determining the composition of AMF on any given host tree. Although priority

356   effects have not previously been investigated in long-lived hosts, strong AMF priority effects

357   have been observed in lab studies on the roots of an annual legume (*Medicago truncatula*;

358   Werner and Kiers, 2015).

359

360   *Redcedar ontogeny and AMF community structure*

361   The prevailing theory that old trees become less productive with age (Weiner &

362   Thomas 2001) has recently been challenged by studies on California redwood (*Sequoia*

363   *sempervirens*) (Sillett *et al.* 2010; Stephenson *et al.* 2014).While detailed work deciphering the

364   ontogeny of western redcedar remains to be performed, much is known about related species

365   (Koch *et al.* 2004) and conifers in general (Meinzner *et al.* 2011). Careful measurements mid-

366   stem and at the crowns indicate that size is the greatest predictor of tree productivity, and

367   indeed, old trees become more productive with age (Sillett *et al.* 2015). If western redcedar

368   similarly become more productive with age, the old redcedars sampled in our study are likely

369   to have been the most photosynthetically active. Hence young trees would be expected to

370   exude the lowest quantities of sugar, mature trees variable levels dependent on their dominance

371   in the canopy, and old trees would exude the most sugar. Yet, despite having the necessary

372   resources to deliver larger quantities of sugar to symbionts, old western redcedars do not

373   appear to influence their communities toward a specific composition.

374   Our data suggests that priority effects within the AMF fungi dominate, and two

375   scenarios for this process generating this seem likely: i) the host has no control over AMF

376   assembly, and initial AMF colonists exclude subsequent fungi, or ii) the host rewards

377 mutualistic behaviour with more resources (Bever et al. 2009, Kiers et al. 2011), which

378 increases their ability to exclude other AMF. In both of these cases priority effects are acting

379 at the AMF community level, but in one case the tree has no control (the 'mycocentric' view

380 (Staddon 2005)), and in the other the tree influences the fungus (the 'phytocentric' view

381 (Johnson & Gehring 2007)). In general the extent to which one partner or the other is in control

382 remains unclear, particularly in natural, diverse AMF communities, although recent evidence

383 suggests that it is beneficial for a tree to maintain a diversity of fungal symbionts throughout its

384 life time (Arguello *et al.* 2016).

385   In conclusion, we did not find evidence of succession from young to mature to old tree

386 AMF communities nor did we find that old trees harbour unique AMF communities, although

387 we did find evidence of previously undescribed fungi associated with western redcedar roots.

388 AMF communities associated with young trees most closely resembled those with old tree ,

389 suggesting that trees may be acquiring their symbionts from adjacent roots.. Mature tree AMF

390 communities did not resemble those associated with old or young trees. These trees may

391 represent a cohort that acquired their symbionts from the previous generation of old trees that

392 have long since died. If so, they are the future source of inoculants for young trees, if they

393 survive to old age.

394   Our study is the first to consider host age as a structuring force of AMF communities.

395 While we did not find the patterns we expected, our study revealed that community structure

396 may be transferred from old trees to young trees, which is important for understanding the

397 AMF communities of western redcedar. Whether similar patterns are important in other long-

398 lived AMF hosts remains to be tested.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1. Analysis of OTU community sequence abundance data using PERMANOVA (9999 permutations, stratified by source) and either: variance stabilising transformations vst and rlog (Euclidean distances) following independent filtering, raw abundance (Bray-Curtis dissimilarity), or rarefied abundance (Bray-Curtis dissimilarity).

Table S2. Comparison of the observed number of AMF taxa found on the roots of different host species using 454 pyrosequencing and classification using the virtual taxa (VT) or monophyletic clade approach (MCA).

Figure legends

611 **Figure 1.** Taxonomic accumulation curves and extrapolated community richness based on

612 MCA data for a. roots and b. soils. White circles and black lines represent Northern samples.

613 Grey circles and grey lines represent Southern samples. Solid lines with and without circles

614 indicate estimated mean richness and extrapolated mean richness, respectively. Dashed lines

615 indicate 95% confidence intervals.

616 **Figure 2.** Proportional composition of AMF root and soil communities based on mean

617 sequence abundance per host at the clade level associated with western redcedar of increasing

618 age at each study site. Panels represent proportional sequence abundance at: Northern site, (**a**)

619 young root (**b**) mature root (**c**) old root (**d**) young soil (**e**) mature soil (**f**) old soil; and Southern

620 site, (**g**) young root (**h**) mature root (**i**) old root (**j**) young soil (**k**) mature soil (**l**) old soil.

621 Legend indicates phylogenetic level to which AMF clade could be identified.

622 **Figure 3.** Mantel correlogram analysis of AMF root MCA community ß-diversity partitions

623 using host tree dbh as a proxy for host age. (**a**) Northern site root ß $_{cc}$ (**c**) Northern site root ß $_{-3}$

624 (**e**) Northern site root ß $_{rich}$ (**b**) Southern site root ß $_{cc}$ (**d**) Southern site root ß $_{-3}$ (**f**) Southern site

625 root ß $_{rich}$. Positive values of r_M represent positive autocorrelation; solid symbols represent

626 significant values following 10 000 Monte Carlo randomizations and sequential Holm's

627 correction for multiple testing.

628 **Figure 4.** Mantel correlogram analysis of AMF soil MCA community ß-diversity partitions

629 using host tree dbh as a proxy for host age. (**a**) Northern site root ß $_{cc}$ (**c**) Northern site root ß $_{-3}$

630 (**e**) Northern site root ß $_{rich}$ (**b**) Southern site root ß $_{cc}$ (**d**) Southern site root ß $_{-3}$ (**f**) Southern site

631 root ß $_{rich}$. Positive values of r_M represent positive autocorrelation; solid symbols represent

632 significant values following 10 000 Monte Carlo randomizations and sequential Holm's

633 correction for multiple testing.

634 **Figure 5.** Comparison of AMF clade richness detected on the roots of different host species

635 using 454 pyrosequencing. Circles represent individual host species, bars represent s.e.m., and
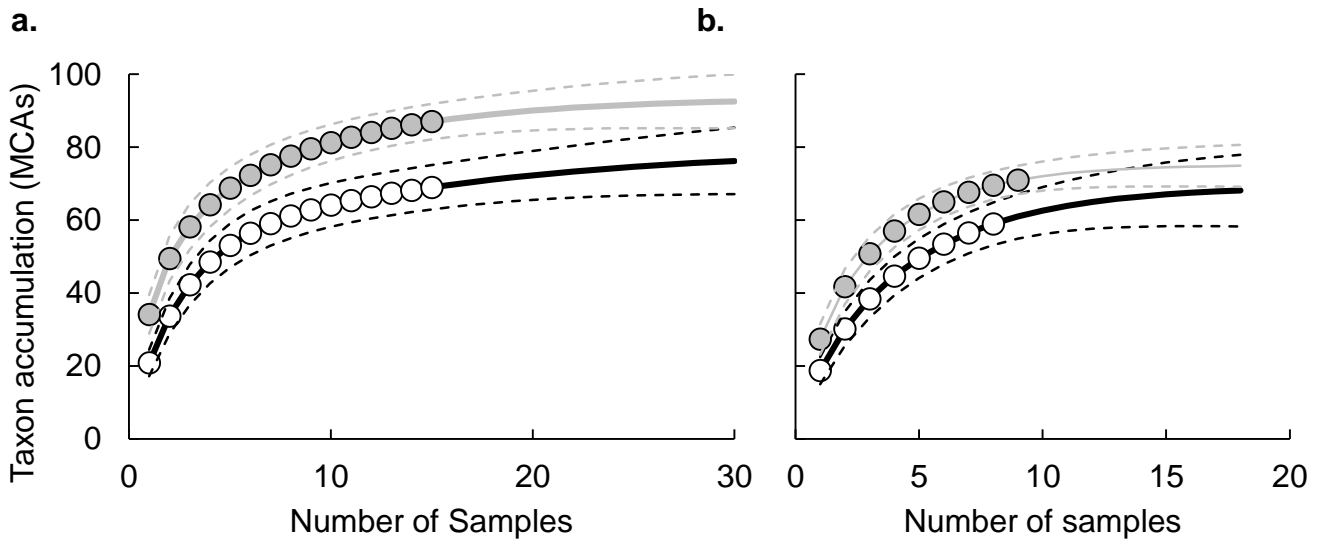
636 circles with solid borders are jointly ranked first for number of clades per individual. Data for

637 individual host species, and the study this data was obtained from, are presented in Supporting
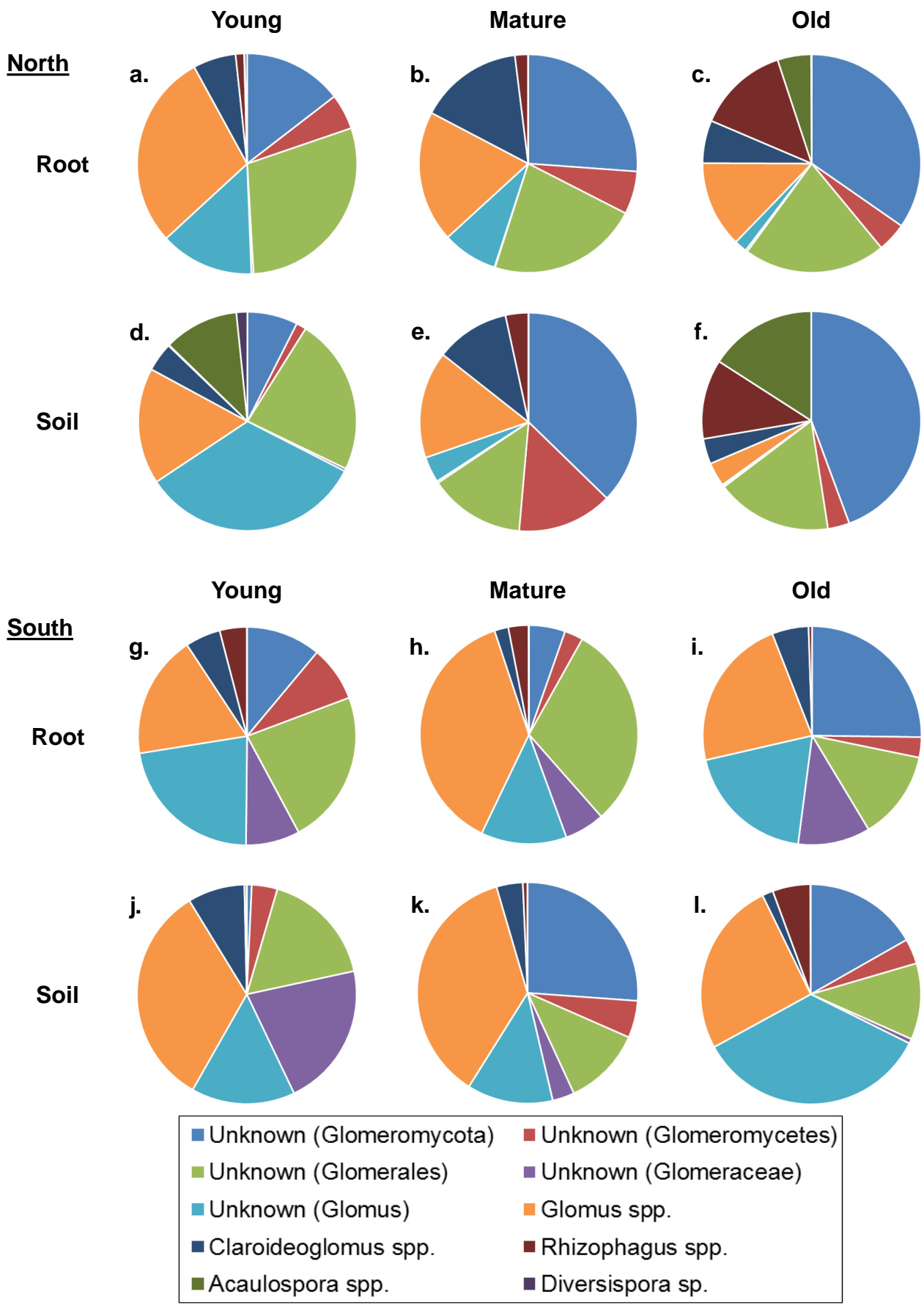
638 Information.

639 **Table 1.** Analysis of rarefied MCA community data with the Bray-Curtis dissimilarity index

640 using PERMANOVA (9999 permutations, stratified by source) and testing for homogeneity of

641 multivariate dispersions.

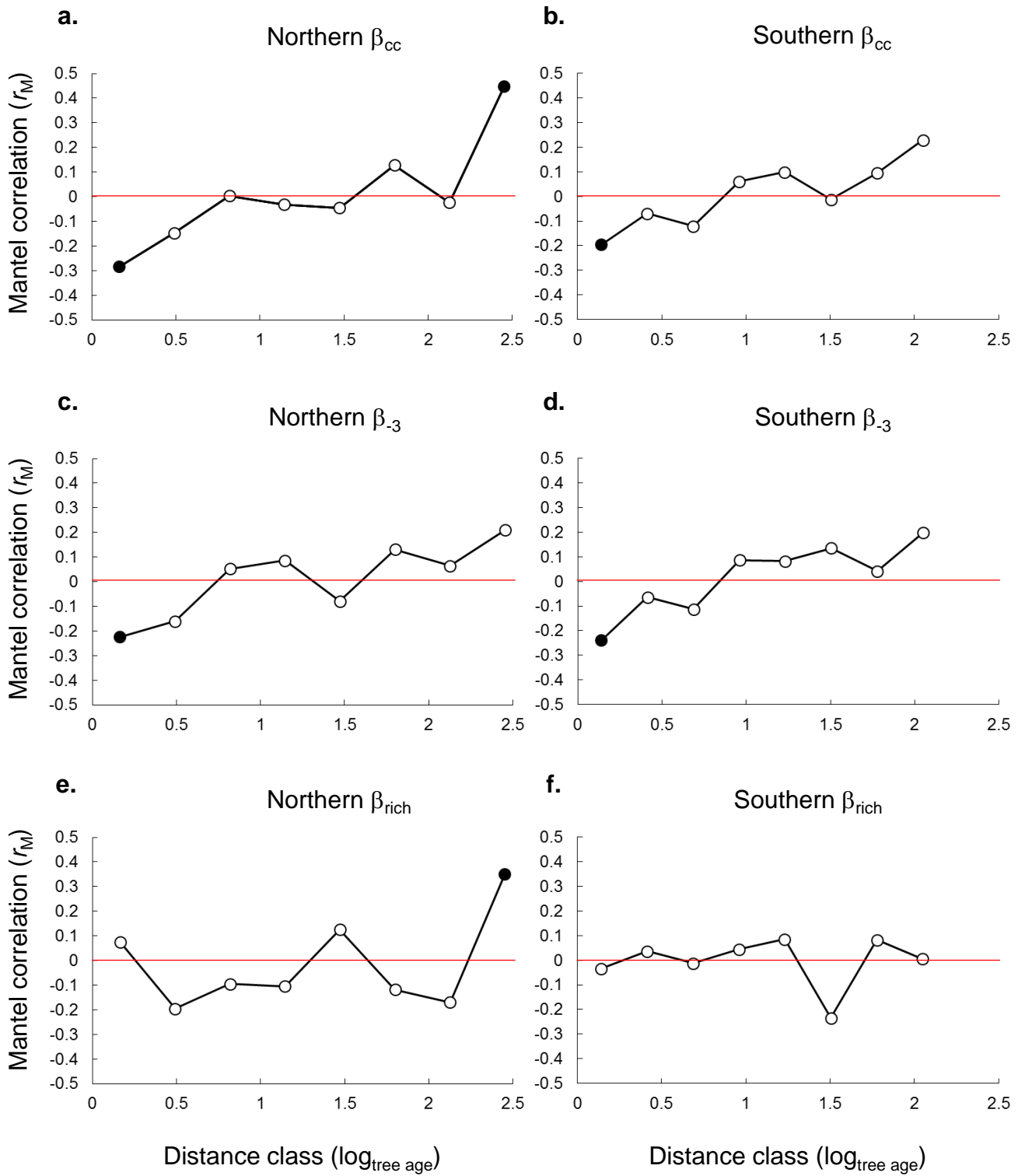| Factor	DF	F	partial r^2	P
Site (Si)	1	**2.17**	0.05	**0.022**
Age (A)	2	**1.68**	0.07	**0.035**
Source (So)	1	0.74	0.02	0.403
Si x A	2	1.39	0.06	0.114
Si x So	1	0.73	0.02	0.691
A x So	2	0.73	0.03	0.788
Si x A x So	2	0.61	0.03	0.906
Residuals	35		0.72	
Multivariate dispersion				
Factors	11	0.95		0.511
Residuals	35			

642 Values in bold are significant at the $\alpha \leq 0.05$ level.

643 Site = north, south; Age = young, mature, old; Source = root, soil

a.

b.

	Young	Mature	Old
North			
Root	a.	b.	c.
Soil	d.	e.	f.
South	Young	Mature	Old
Root	g.	h.	i.
Soil	j.	k.	l.

Legend:
- ■ Unknown (Glomeromycota)
- ■ Unknown (Glomeromycetes)
- ■ Unknown (Glomerales)
- ■ Unknown (Glomeraceae)
- ■ Unknown (Glomus)
- ■ Glomus spp.
- ■ Claroideoglomus spp.
- ■ Rhizophagus spp.
- ■ Acaulospora spp.
- ■ Diversispora sp.

AM fungi root communities

AM fungi soil communities