

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**

3

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12

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14 microbial succession, western redcedar (*Thuja plicata*).

15

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20

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

22

23 **Abstract (232 words)**

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

43 **Introduction**

44 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
45 (Smith & Read 2008). The presence and composition of AMF impacts plant biodiversity (van
46 der Heijden *et al.* 1998), plant response to increases in CO₂ (Terrer *et al.* 2016), nutrient
47 cycling (Phillips *et al.* 2013; Johnson *et al.* 2015) and soil carbon sequestration (Averill *et al.*
48 2014). Given the global influence and distribution of AMF (Davison *et al.* 2015;
49 Soudzilovskaia *et al.* 2015), we know surprisingly little about how their communities are
50 structured in most ecosystems.
51

52 Current knowledge on the drivers of AMF community composition indicates that both
53 dispersal limitation and habitat filtering play significant roles (Öpik *et al.* 2006, 2010; Kivlin *et al.*
54 2011). Recently it has been shown that host identity can exert a greater influence on AMF
55 composition than competition between the fungi themselves (Davison *et al.* 2016). Temporal
56 processes such as plant community succession may also structure AMF communities (Koske &
57 Gemma 1997), with pattern detection requiring that samples be collected in a time-series or
58 across a chronosequence (Dornelas *et al.* 2013). While community studies of AMF are
59 important, the overwhelming research focus has been on easily manipulated, short-lived hosts
60 such as forbs and grasses (Ohsowski *et al.* 2014; Davison *et al.* 2015), thus our understanding
61 of AMF communities may overlook dynamics that occur over long time scales.

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

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 Pahlisch 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

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 °C.
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 MgCl₂, 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

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 $\beta_{.3}$ (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 $\log(\text{host tree dbh})$. 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 *rhapidacantha* 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 composition 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

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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591 **Data Accessibility**

592 DNA sequences: Sequences and supporting information are available at

593 <http://dx.doi.org/10.6084/m9.figshare.1451402>

594

595 **Author contributions**

596 M.A.G. and M.M.H designed the study, collected the data, and contributed to the writing and

597 editing. M.A.G. performed the lab work and bioinformatics, and produced the OTU and MCA

598 tables. B.J.P. analysed the data in R and EstimateS, drafted the manuscript, compiled the data

599 on AMF communities of other AM hosts and contributed to the writing and editing.

600

601 **Supporting Information**

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

603 **Table S1.** Analysis of OTU community sequence abundance data using PERMANOVA (9999

604 permutations, stratified by source) and either: variance stabilising transformations vst and rlog

605 (Euclidean distances) following independent filtering, raw abundance (Bray-Curtis

606 dissimilarity), or rarefied abundance (Bray-Curtis dissimilarity).

607 **Table S2.** Comparison of the observed number of AMF taxa found on the roots of different

608 host species using 454 pyrosequencing and classification using the virtual taxa (VT) or

609 monophyletic clade approach (MCA).

610 **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 β -diversity partitions
623 using host tree dbh as a proxy for host age. (a) Northern site root β_{cc} (c) Northern site root β_{-3}
624 (e) Northern site root β_{rich} (b) Southern site root β_{cc} (d) Southern site root β_{-3} (f) Southern site
625 root β_{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 β -diversity partitions
629 using host tree dbh as a proxy for host age. (a) Northern site root β_{cc} (c) Northern site root β_{-3}
630 (e) Northern site root β_{rich} (b) Southern site root β_{cc} (d) Southern site root β_{-3} (f) Southern site
631 root β_{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

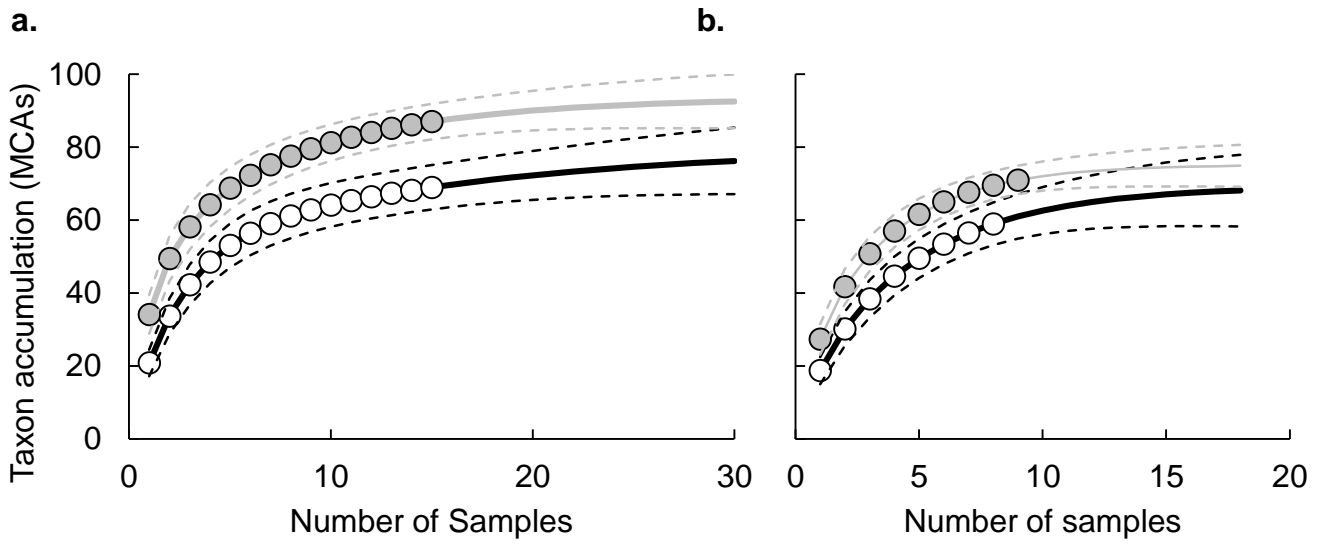
636 circles with solid borders are jointly ranked first for number of clades per individual. Data for
637 individual host species, and the study this data was obtained from, are presented in Supporting
638 Information.

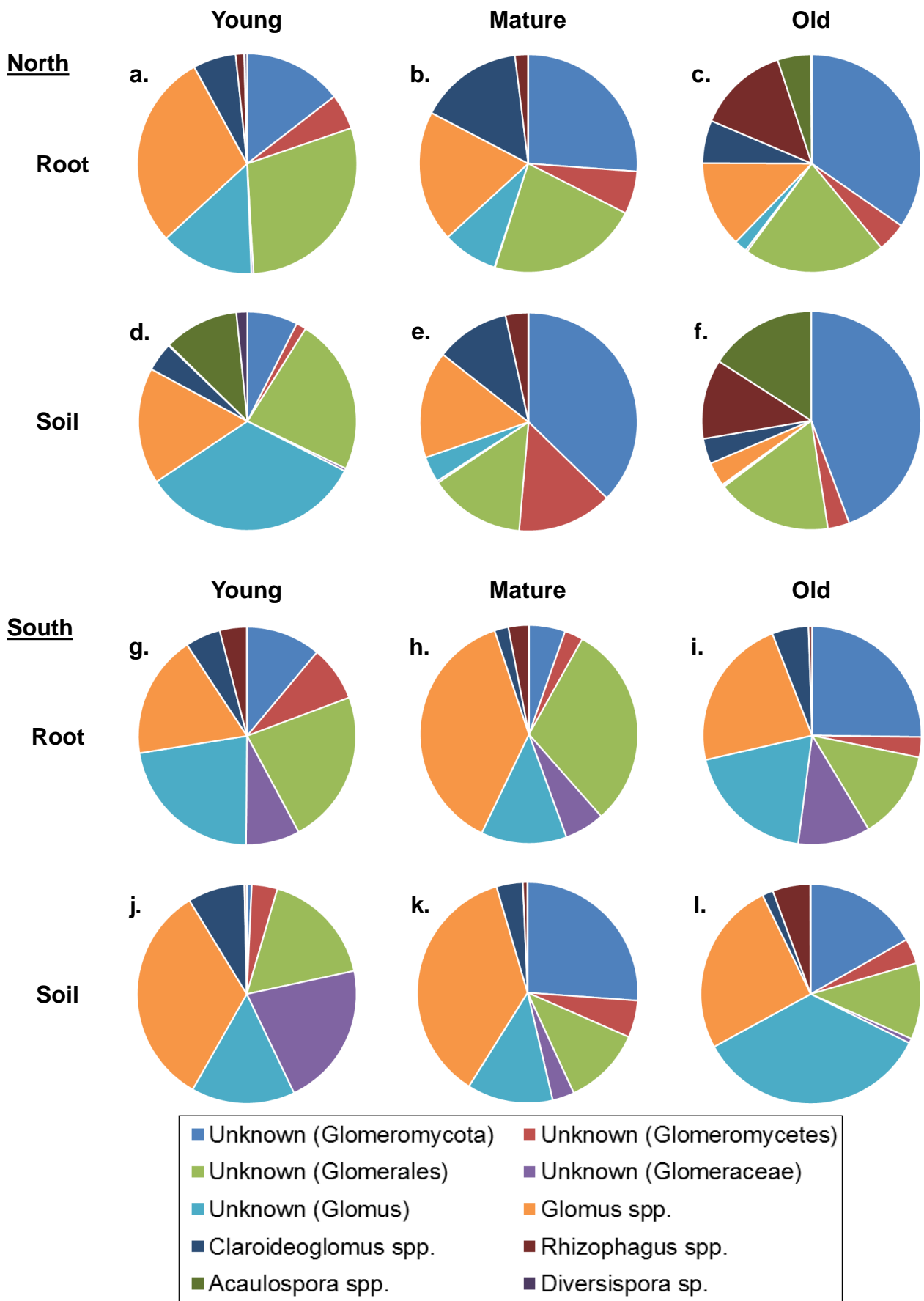
639 **Table 1.** Analysis of rarefied MCA community data with the Bray-Curtis dissimilarity index
 640 using PERMANOVA (9999 permutations, stratified by source) and testing for homogeneity of
 641 multivariate dispersions.

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			

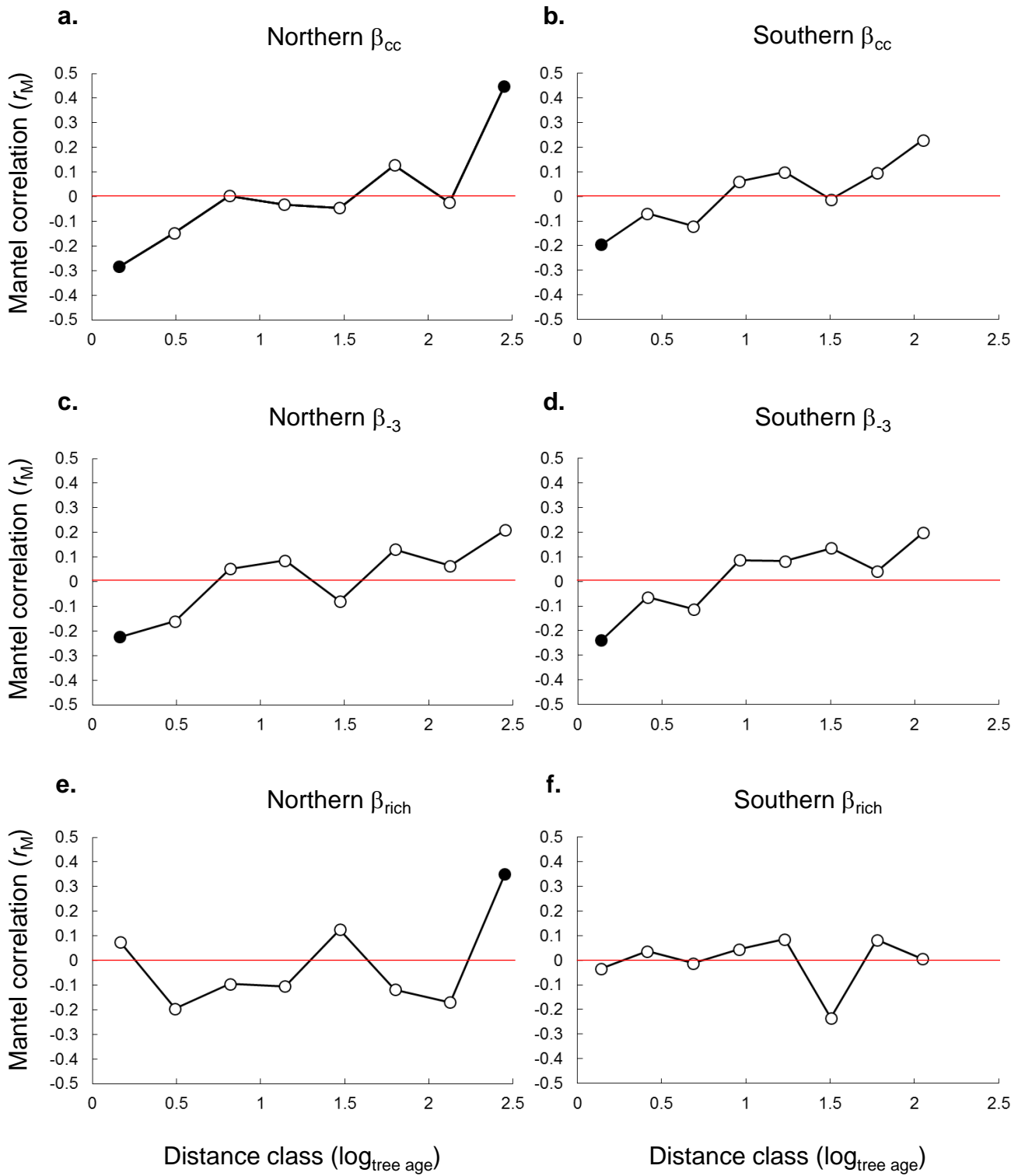
642 Values in bold are significant at the $\alpha \leq 0.05$ level.

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





AM fungi root communities



AM fungi soil communities

