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

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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

some trees may live for millennia, AMF communities may differ in composition and turnover (β-diversity). For instance, it is known that plants in early successional systems have higher AMF β-diversity, perhaps due to environmental heterogeneity (Christensen and Peet 1984). Similarly, plants in late successional systems tend to exhibit lower AMF β-diversity, perhaps due to environmental filtering (Derroire et al. 2016). While the idea of AMF and community convergence has received some attention (Caruso et al. 2012, Maherali and Klironomos 2012), whether this is true for AMF over successional time scales is not known.

Current ideas in community assembly theory suggest that ecological processes may not be as easily inferred from patterns of species co-existence as previously expected (Gerhold et al. 2015). Overdispersion, in which species co-exist less often than expected by chance, is often assumed to represent the process of competitive exclusion: related species with comparable traits will compete more intensely than more distantly related species. Clustering, in which species co-exist more often than would be expected by chance, is often assumed to be caused by niche partitioning, where competition is less important than the suitability of the environment for that suite of organisms. Meta-analysis of competition experiments in plants has shown little evidence to support a direct link between these patterns and processes (Cahill et al. 2008), however, niche partitioning has been demonstrated in some detailed studies of overdispersed communities (e.g. Cavender-Barnes and Pahlich 2009). Importantly, the case can be made that overdispersion patterns can be created by both niche partitioning and competition acting in tandem (Mayfield and Levine 2010), although clustering is less likely to be caused by a myriad of interacting processes.

Here we explore the richness and diversity of AMF communities associated with western redcedar (*Thuja plicata* Donn *ex* D. Don), a long-lived woody host; examining both roots and soil.. We hypothesised that these long-lived plants would exert selective pressures on their AMF symbionts that differ from those produced by herbs, grasses, and other short-lived

hosts, due to differences in the availability of carbon resources, leading to: i) an increase in AMF richness with increasing host age, and ii) succession of distinctive AMF communities among host age classes.

Materials and methods

Field sites

Two study sites were selected within the Interior Cedar-Hemlock (ICH) Biogeographic Zone (Ketcheson *et al.* 1991) of British Columbia, one at the northern range limit (53º45'45.68" N, 121º13'5.93" W) and the other in the southern end of the distribution (49°40'44.47"N, 117°43'5.92"W) within the ICHvk (very wet cool) and ICHdw (dry warm) variants, respectively. Commonly known as the interior temperate rainforest, due to high annual precipitation, these areas can represent continuous stands that have been present for thousands of years, and are host to very large western redcedar trees; potentially 800-1000 years or older in some cases, especially at the northern 'Ancient Forest' site 100 km east of Prince George. The most abundant alternative AMF host species in the northern site were bunchberry (*Cornus canadensis*) and devils club (*Oplopanax horridus*), whereas common snowberry (*Symphoricarpos albus*), Oregon-grape (*Mahonia sp.*), twinflower (*Linnea borealis*), and false box (*Paxistima myrsinites*) were the most abundant in the south.

Fungal community sampling

Trees were selected from three 'age classes' based on their diameter-at-breast-height (dbh): young trees (dbh < 5 cm), mature trees (dbh 19 – 65 cm), and old trees (dbh 150 – 455 cm). These age classes represent estimates of tree life stages, for which dbh is considered a useful proxy, due to the tendency for western redcedar to rot from the centre outwards as it matures, rendering the determination of tree age by coring impossible. Five trees were sampled per age class at each site. From each tree, five fine root samples were obtained by digging along large

roots, thus ensuring that sampled roots belonged to the sample tree. For three of the five trees per age class, three soil cores (2.5 cm diameter) of the top 10 cm organic horizon were sampled adjacent to each root sampled (within approx. 2m of the base of the trunk). All samples were sealed in plastic bags and kept on ice in a cooler for transport, prior to storage at 4 ^{o}C. Processing took place within 3 months of sample collection. Subsamples for both roots and soil were pooled at the level of individual tree for subsequent analyses, for a total of 48 samples (15 root, 9 soil at each site).

Molecular methods

For each sample DNA was extracted from 150 mg of randomly selected root segments (first cleaned in deionized water), and from 250 mg of homogenized soil, using the Powersoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Glomeromycota 28S large sub-unit ribosomal DNA sequences were amplified using FLR3 (forward) and FLR4 (reverse) (Gollotte *et al.*, 2004) primers, linked to 454-sequencing adapters and linkers. PCR was carried out using 20pmol dNTPs, 3.5mM $MgCl_2$, 40µg BSA, 20 pmol of each primer, and 1U GoTaq with supplied buffer (Promega Corporation, WI, USA). Thermocycling conditions were as follows: 95°C for 1 minute, 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 7 minutes and stored at 4°C. PCR products were cleaned and normalized to 1-2 ng/µL using SequelPrep™ Normalization Plate (96) kit (Life Technologies™, NY, USA). The samples were pooled and sequenced on a Genome Sequencer FLX System, using Titanium Series reagents (Roche Applied Science) at the Vancouver Prostate Centre. All sequences and sample information are available online https://dx.doi.org/10.6084/m9.figshare.1451402.v1.

Bioinformatics

Sequences were analysed using the default settings, unless otherwise noted, in QIIME 1.7.0 (Caporaso et al. 2010). OTUs were picked *de novo* using 97% similarity using the UCLUST algorithm (Edgar 2010). Chimera checking was performed using the usearch61 *de novo* method. Sequence 'denoising' was not performed as it has been shown to alter beta-diversity in AMF studies (Hart et al. 2015). Out of the 48 samples a single barcode was not recovered (associated with an old tree soil sample from the northern site). Taxonomy was assigned using the GenBank® database (http://www.ncbi.nlm.nih.gov) with the BLAST (nucleotide) algorithm. All non-Glomeromycotan sequences (<0.01%) were removed. A retained sequence was considered to match known AMF taxa if its similarity to database sequences was 97% or greater (Hart *et al.* 2015), however closest matches to existing 28S accessions within the Glomeromycota at lower levels were retained and classed as 'unknown'.

Of the 201 020 sequences generated, 70 097 fulfilled the retention criteria and were grouped into 1012 OTUs. The total number of these OTUs matching existing AMF sequences at a similarity level of 97%+ was 876, of which 698 produced a 100% match, 111 at 99%, 40 at 98%, and 27 at a 97% level. The remaining 136 OTUs did not match the database at the typically acceptable level (matches varied from 89-96%). Thus, approximately 13% of the OTUs generated were potentially novel Glomeromycotan sequences. Of the 1012 OTUs 325 were singletons, leaving 687 clusters that contained 2 or more sequences. Rarefying to an even sampling depth retained 581 sequences per root sample (total of 665 OTUs) or 91 sequences per soil sample (total of 316 OTUs).

Due to concerns about diversity inflation resulting from the 454-pyrosequencing we applied a monophyletic clade approach (MCA) to convert the raw OTU clusters into clades within the Glomeromycota (see Lekberg *et al.* 2014). The MCA is an OTU delineation method wherein sequence groups are manually combined based on membership within a monophyletic clade. We followed the methodology of Lekberg *et al*. (2014), where the authors demonstrated that the method is robust and generates community patterns that are comparable to OTU

methods based on percentage similarity thresholds. The strength of the MCA method is that it is grounded in evolutionary theory. This conversion of OTUs using MCA resulted in a total of 91 Glomeromycotan clades. Two of these clades were 'sequence singletons' (contained a single sequence), whereas five clades were 'sample singletons' (occurred in a single sample). Each MCA was identified using the GenBank® database (http://www.ncbi.nlm.nih.gov) with the BLAST (nucleotide) algorithm, which matched sequences within each clade to existing 28S accessions. All of the subsequent community analyses presented below were performed using the MCA data tables with sequence singletons removed. We also analysed the OTU data using the same methods and additionally using variance stabilising transformations (McMurdie & Holmes, 2013). Ecological interpretations were not substantially different between the approaches unless otherwise noted (for further details see Supporting Information).

AM fungal diversity of other host plants

To place western redcedar in the context of existing research into the fungal diversity of AM hosts we compiled AMF community data from previous studies that examined trees, shrubs, woody perennials, palms, grasses, and herbs. Due to the variability among marker genes for AMF (see Thiéry *et al*., 2016), and the lack of data on AMF communities of long-lived hosts, we limited our comparisons to studies that: i) presented data for multiple individuals of a specific host species, ii) used 454 sequencing technology to obtain OTUs, and iii) expressed AMF diversity at the clade-level by using MCA-type approaches (e.g. 'virtual taxa'; Öpik *et al.* 2010). This cladistic approach to AMF diversity was utilized to maximise the relevance of comparisons between host species by removing the noise associated with OTU assignments, particularly in regards to the differences in sensitivity levels between the LSU and SSU regions.

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 $ß_{sim}$ (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 ß-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 ß-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 $ß_{cc}$ (Jaccard's index of dissimilarity), which can be
218 subdivided into $ß_{-3}$ (dissimilarity due to species replacement) and $ß_{rich}$ (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

permutations and progressive Holm's correction for multiple testing. In each case, positive r_M values indicated positive autocorrelation (e.g. greater community similarity).

Results

Western redcedar AMF clade richness differences

Three-way ANOVA revealed that site was the only significant factor in producing clade richness differences ($F_{1,35} = 10.01$; $P = 0.003$), richness per sample did not differ significantly between age classes ($F_{2,35} = 0.09$; $P = 0.913$) or sample source ($F_{1,35} = 1.57$; $P = 0.219$) and there were no significant interaction terms. Significantly more AMF clades per sample were found in the southern site (mean 31.5 ± 2.66, n=24) than in the northern site (mean 20.2 ± 1.18, n=23) (Tukey's HSD: South > North; $P_{adj} = 0.002$). Overall clade richness tended to be higher in root than in soil samples, however, according to clade-based accumulation curves this difference was only significant in the southern site (Fig. 1). Root sampling clearly approached an asymptote (Fig. 1a), and sampling of both root and soil (Fig. 1b) communities was within the 95% confidence interval of extrapolated clade richness obtained from a hypothetical doubling of sample size. Similar results were obtained when examining the data based on OTUs rather than clades (data not presented).

AMF community composition

Analysis of AMF community composition based on clades, and using a three-way PERMANOVA of Bray-Curtis dissimilarities, revealed significant differences between sites and between host age classes, but not between roots and soil (Table 1). The full model accounted for 28% of the partial variance, and no difference in multivariate dispersion was detected between groups (Table 1). A similar result was observed for the rarefied OTU dataset, whereas analysis with variance stabilising transformations indicated significant differences between root and soil communities (Supporting Information Table S1).

Differences in community composition between sites, age classes, and sample source, are illustrated in Fig. 2. *Acaulospora* spp. were only present in significant numbers in northern samples (Fig. 2a-f). *Acaulospora* and *Rhizophagus* spp. were most abundant in northern roots of old trees (Fig. 2c).. These roots also contained fewer sequences from unknown clades (Fig. 2c), compared to young trees (Fig. 2a). A greater proportion of sequences from *Glomus* clades were found in southern site samples in general (Fig. 2g-l) and in young northern site samples (Fig. 2a,d). Overall, more novel taxa were found in the northern site.

Evidence of community structure related to host age was detected in root (Fig. 3) and soil (Fig. 4) communities, with both study sites exhibiting similar β-diversity patterns. The AMF communities of trees within each age class were overdispersed (e.g. significantly less similar to each other in clade composition; Fig. 3a,b and Fig. 4a,b). Comparison between age classes revealed that AMF communities of younger trees (smallest dbh class) tended to be autocorrelated (e.g. were significantly more similar in clade composition) with those of the oldest trees (largest dbh class) (Fig. 3a and Fig. 4a,b). Partitioning of Jaccard's dissimilarity index (β_{cc}) into its turnover (β_{-3}) and richness (β_{rich}) components, indicated that the AMF community patterns were mostly driven by community turnover, which was overdispersed within age classes (Fig. 3c,d and Fig. 4c). However, soil communities displayed autocorrelation between the AMF communities of young and old trees (Fig. 4c,d). Richness differences between AMF communities of the youngest and oldest trees were autocorrelated, but only significantly so in the northern site (Fig. 3e and Fig. 4e). In all cases, a significant increasing linear trend in r_M values from negative in trees of a similar age to positive between the youngest and oldest trees was observed with increasing host pair age difference (r^2 = 0.69 to 0.83, $P < 0.01$).

Comparison with other AM hosts

In terms of overall AMF clade richness derived from host root sampling, western redcedar was ranked second across all host growth forms (Fig. 5; Supporting Information Table S2), with only the palm *Podococcus barteri* observed to associate with a greater number of clades. When examined from the perspective of AMF clade richness per individual host plant, western redcedar communities were again ranked first , but tied for first place with two long-lived tropical tree species (*Polyalthia suaveolens* and *Santiria trimera*), two palms (*Bactris rhaphidacantha* and *Podococcus barteri*), and two grasses (*Agropyron cristatum* and *Potentilla acaulis*) (Fig. 5; Supporting Information Table S2). Using clade-based approaches to AMF richness, the number of clades encountered on western redcedar was consistently greater than those found on perennial woody and herbaceous plants, and the majority of other tree species for which comparable data exists (much of which is drawn from the work of Davison *et al.* 2015).

Discussion

Western redcedar is a long-lived tree that exhibits high clade richness in its associated AMF communities. Despite significant differences in the community compostion between sites, the autocorrelation patterns were similar at both sites, implying that the cedar host may create the same patterns in distinct locations. In particular, we observed overdispersion in AMF β-diversity within each host age class at both study locations – shifts from overdispersion to neutral structure to autocorrelation took place between pairs of hosts as the difference in host age increased. This study represents one of the first investigations of the symbiotic AMF community of a long-lived gymnosperm host using next-generation sequencing techniques, and presents an important starting point for further examination of these under-explored systems.

Clade richness differences

We had anticipated that older trees would exhibit higher clade richness, accumulating more AMF over their lifetime. However, this was not supported by the data. There is some evidence for higher AMF species richness in mature breadfruit (Hart et al. 2014), but in general there is little support for richness increasing with host age, simply because there is a dearth of studies on long-lived AM host trees. This topic requires much more research.

The greatest differences in clade richness were between the northern and southern study locations, with the southern site containing a significantly greater number of clades. This is a typical pattern for biodiversity in general (Hillebrand 2004), and fungal biodiversity in particular (Tedersoo et al. 2014), although we note that ectomycorrhizal fungi are highly diverse in boreal and arctic ecosystems (Timling et al. 2012; Taylor et al. 2014). In our study, samples from the Northern site had a greater number of clades matching *Acaulospora* and *Rhizophagus* spp. However, at least two-thirds of the clades in each sample failed to match existing database sequences at the species or genus level. Although this likely reflects the lack of LSU sequences deposited in the NCBI database for AMF it also opens up the possibility that a large number of currently unknown AMF reside in these communities. We propose that further investigation of western redcedar mycorrhizas may be most profitably targeted at the communities associated with both mature trees and the oldest extant trees. The use of a variety of molecular markers (see Hart *et al.* 2015) will be key to determining how many of their symbionts truly represent previously undiscovered AMF clades.

Clade richness differences between AM hosts

Our compilation of data from the literature on the number of clades per host species and individual host plant, revealed a wide range in the values of these richness measures. Considering the data in terms of rank order alone western redcedar was at the upper end of the richness distribution: 2nd for total number of AMF clades and joint 1st for number of clades per individual sample. We encourage future research using multiple estimators of AMF diversity

(Hart *et al.* 2015) that focuses on old individuals selected from long-lived host tree species, and particularly gymnosperms. While many mycorrhizal studies of gymnosperms exist, they focus almost exclusively on ectomycorrhizal hosts (Chaudhary *et al.* 2016).

AMF clade community composition

Overdispersion of AMF communities within each host age class was not expected. That the same pattern of beta-diversity was observed at both of our study sites suggests a general pattern within inland temperate rainforests, but further studies are required and comparison to coastal western redcedar forests would be informative. Conversely, in northern site roots and in the soils at both sites, the youngest trees hosted AMF communities that were significantly more similar to those of the oldest trees than was expected (autocorrelated). Overdispersion has historically been interpreted as evidence of competitive exclusion structuring a community (see reviews by HilleRisLambers et al., 2012; Gerhold et al. 2015). That is, closely related species exclude each other because their trait similarity leads to more intense competition. Whereas the opposite pattern, increased clustering (co-occurring more often than expected by chance) indicates environmental filtering. In the latter case, competition is less important in structuring a community than environmental conditions. This pattern does not manifest consistently (Cahill et al., 2008), and it has been argued that only clustering is indicative of environmental filtering, whereas the pattern of overdispersion can be caused by many interacting forces, including but not limited to competitive exclusion (Mayfield and Levine 2010; HilleRisLambers et al., 2012; Alexandrou et al., 2015; Gerhold et al. 2015; Li et al., 2015). In our study, overdispersion indicates that, within a cohort, each tree assembled a unique community of AMF. However, autocorrelation increased as the age (dbh) difference between trees increased, resulting in young trees having similar AMF communities to those of the oldest trees. Our interpretation is that this represents a ‘parent tree’ or ‘nurse tree’ effect in which young trees inherit their AMF communities from the old trees that surround them when they

establish. In a temperate rainforest formed by long-lived tree species, in which sudden stochastic events (e.g. fires) are not part of the typical cycle of regeneration, seedlings are likely to collect their first symbionts via hyphal connections from the roots of surrounding trees. If so, the maintenance of overdispersion within a cohort suggests a major role for priority effects in determining the composition of AMF on any given host tree. Although priority effects have not previously been investigated in long-lived hosts, strong AMF priority effects have been observed in lab studies on the roots of an annual legume (*Medicago truncatula*; Werner and Kiers, 2015).

Redcedar ontogeny and AMF community structure

The prevailing theory that old trees become less productive with age (Weiner & Thomas 2001) has recently been challenged by studies on California redwood (*Sequoia sempervirens*) (Sillett *et al.* 2010; Stephenson *et al.* 2014).While detailed work deciphering the ontogeny of western redcedar remains to be performed, much is known about related species (Koch *et al.* 2004) and conifers in general (Meinzner *et al.* 2011). Careful measurements mid-stem and at the crowns indicate that size is the greatest predictor of tree productivity, and indeed, old trees become more productive with age (Sillett *et al.* 2015). If western redcedar similarly become more productive with age, the old redcedars sampled in our study are likely to have been the most photosynthetically active. Hence young trees would be expected to exude the lowest quantities of sugar, mature trees variable levels dependent on their dominance in the canopy, and old trees would exude the most sugar. Yet, despite having the necessary resources to deliver larger quantities of sugar to symbionts, old western redcedars do not appear to influence their communities toward a specific composition.

Our data suggests that priority effects within the AMF fungi dominate, and two scenarios for this process generating this seem likely: i) the host has no control over AMF assembly, and initial AMF colonists exclude subsequent fungi, or ii) the host rewards

mutualistic behaviour with more resources (Bever et al. 2009, Kiers et al. 2011), which increases their ability to exclude other AMF. In both of these cases priority effects are acting at the AMF community level, but in one case the tree has no control (the 'mycocentric' view (Staddon 2005)), and in the other the tree influences the fungus (the 'phytocentric' view (Johnson & Gehring 2007)). In general the extent to which one partner or the other is in control remains unclear, particularly in natural, diverse AMF communities, although recent evidence suggests that it is beneficial for a tree to maintain a diversity of fungal symbionts throughout its life time (Arguello *et al.* 2016).

In conclusion, we did not find evidence of succession from young to mature to old tree AMF communities nor did we find that old trees harbour unique AMF communities, although we did find evidence of previously undescribed fungi associated with western redcedar roots. AMF communities associated with young trees most closely resembled those with old tree , suggesting that trees may be acquiring their symbionts from adjacent roots.. Mature tree AMF communities did not resemble those associated with old or young trees. These trees may represent a cohort that acquired their symbionts from the previous generation of old trees that have long since died. If so, they are the future source of inoculants for young trees, if they survive to old age.

Our study is the first to consider host age as a structuring force of AMF communities. While we did not find the patterns we expected, our study revealed that community structure may be transferred from old trees to young trees, which is important for understanding the AMF communities of western redcedar. Whether similar patterns are important in other long-lived AMF hosts remains to be tested.

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Data Accessibility

DNA sequences: Sequences and supporting information are available at http://dx.doi.org/10.6084/m9.figshare.1451402

Author contributions

M.A.G. and M.M.H designed the study, collected the data, and contributed to the writing and editing. M.A.G. performed the lab work and bioinformatics, and produced the OTU and MCA tables. B.J.P. analysed the data in R and EstimateS, drafted the manuscript, compiled the data on AMF communities of other AM hosts and contributed to the writing and editing.

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).

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 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

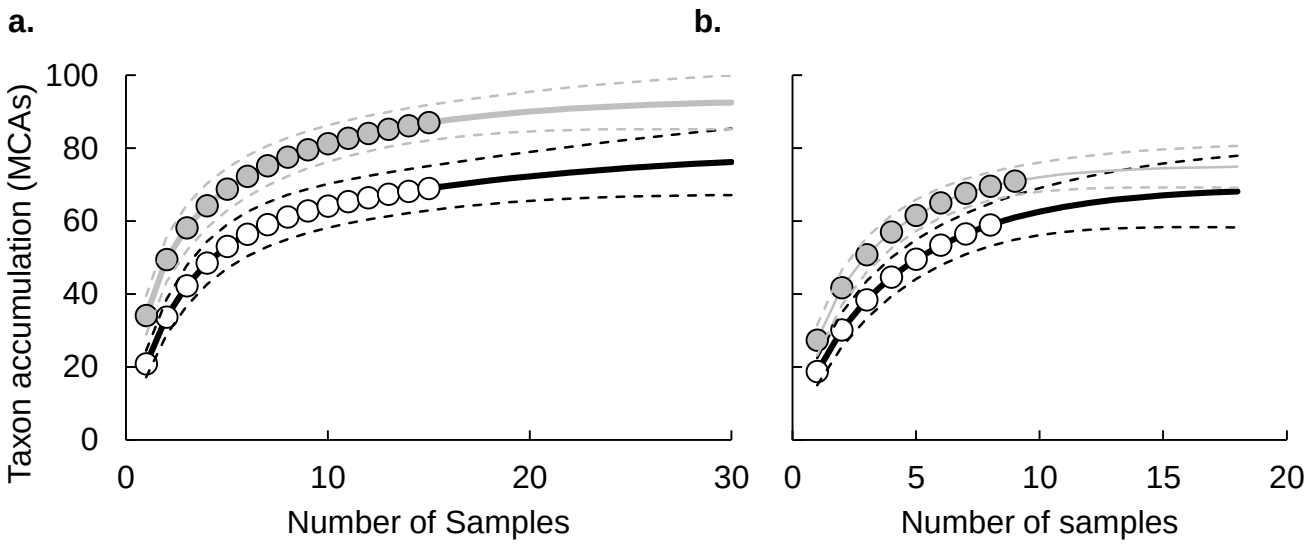
636 circles with solid borders are jointly ranked first for number of clades per individual. Data for
637 individual host species, and the study this data was obtained from, are presented in Supporting
638 Information.

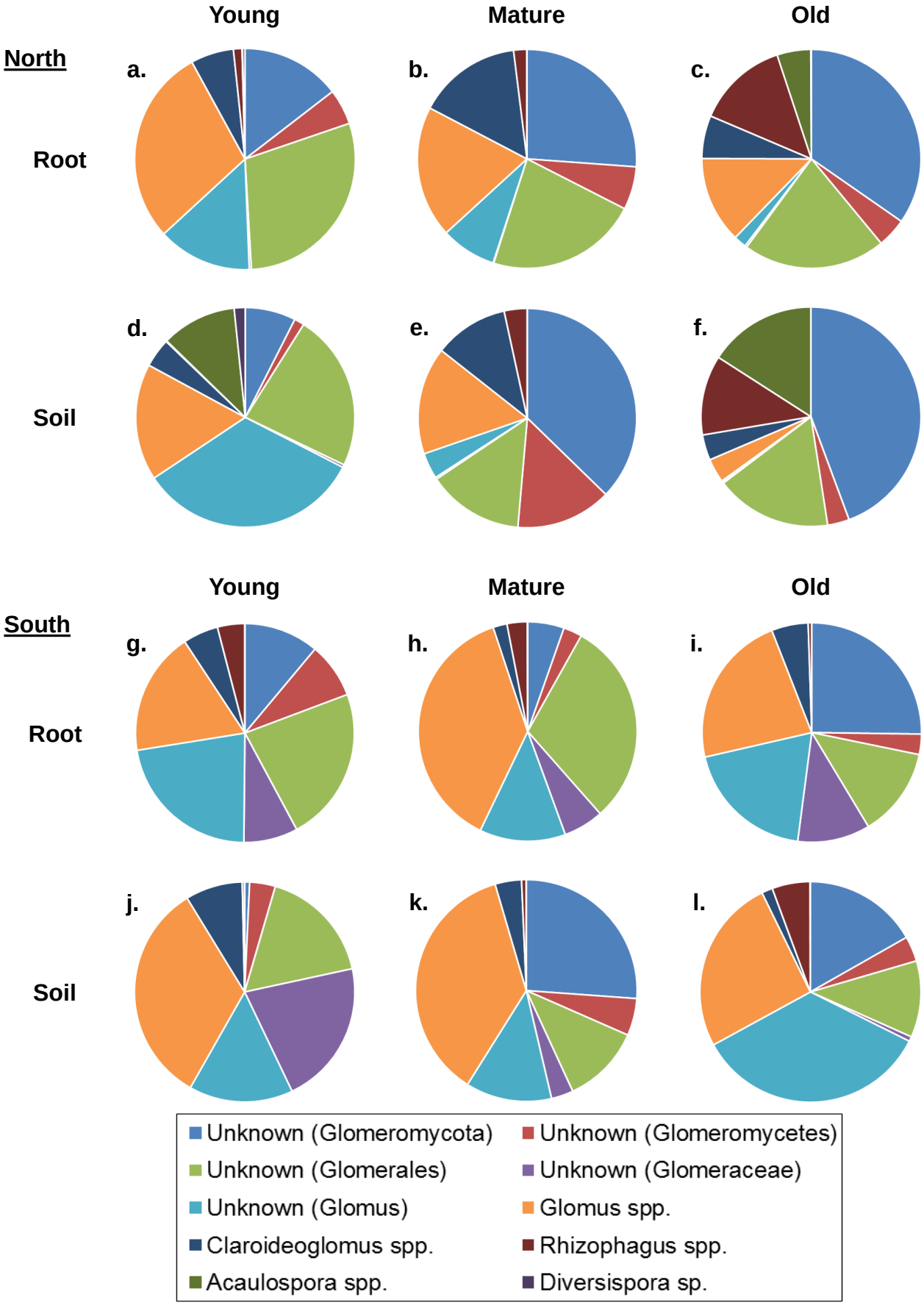
Table 1. Analysis of rarefied MCA community data with the Bray-Curtis dissimilarity index using PERMANOVA (9999 permutations, stratified by source) and testing for homogeneity of multivariate dispersions.

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			

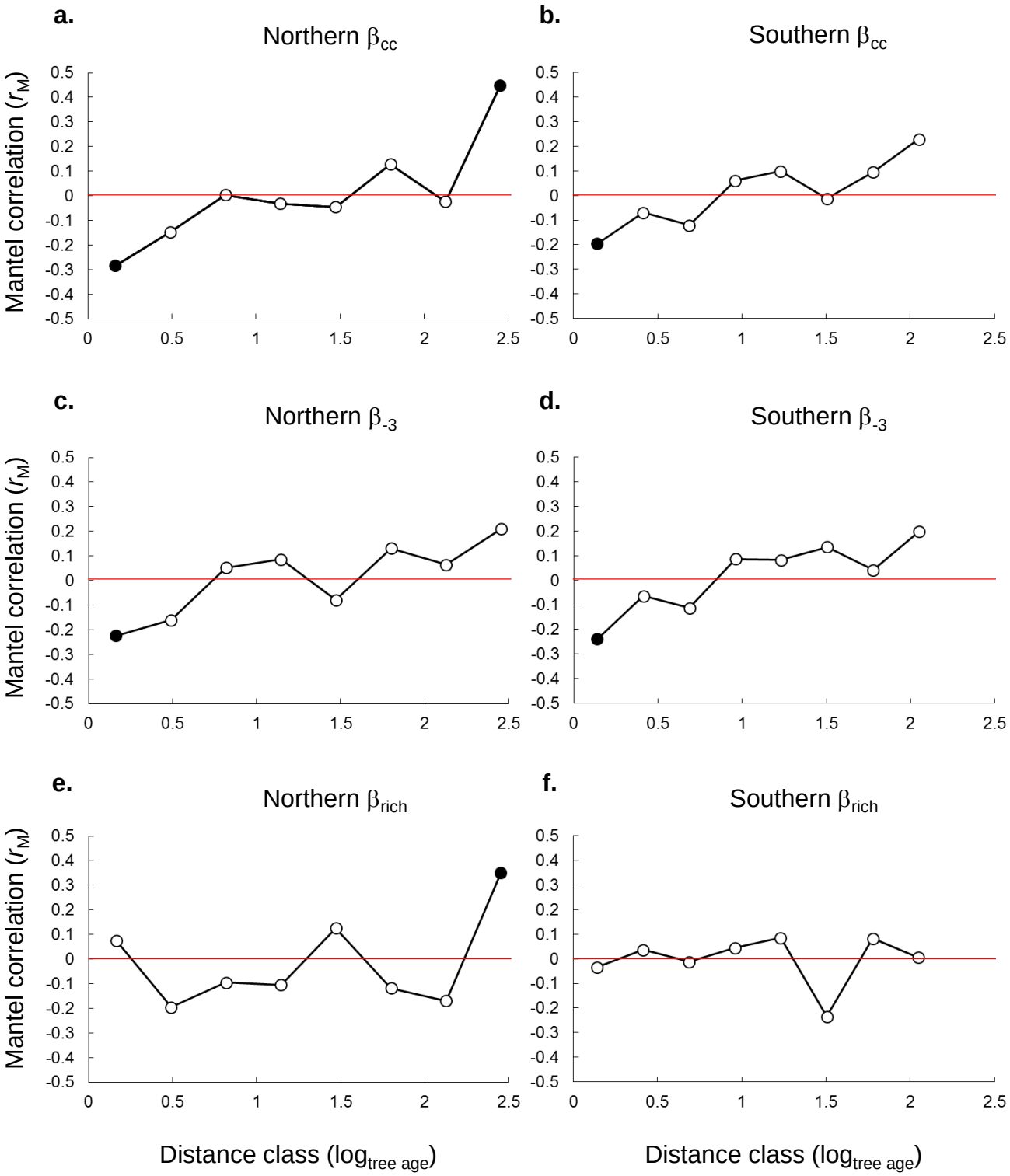
Values in bold are significant at the $\alpha \leq 0.05$ level.

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

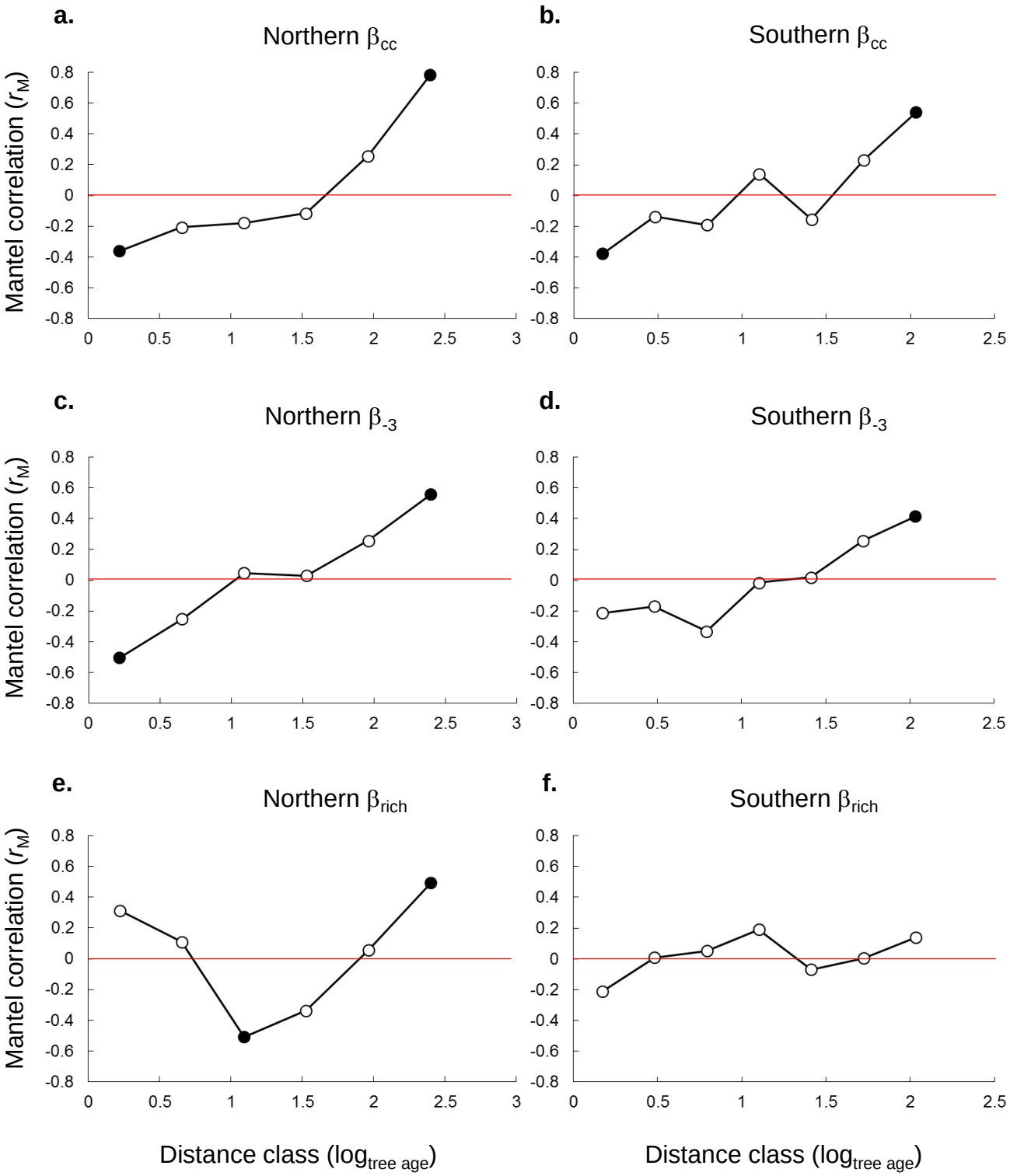

a.
b.
Taxon accumulation (MCAs)
100
80
60
40
20
0
0
10
20
30
Number of Samples
0
5
10
15
20
Number of samples

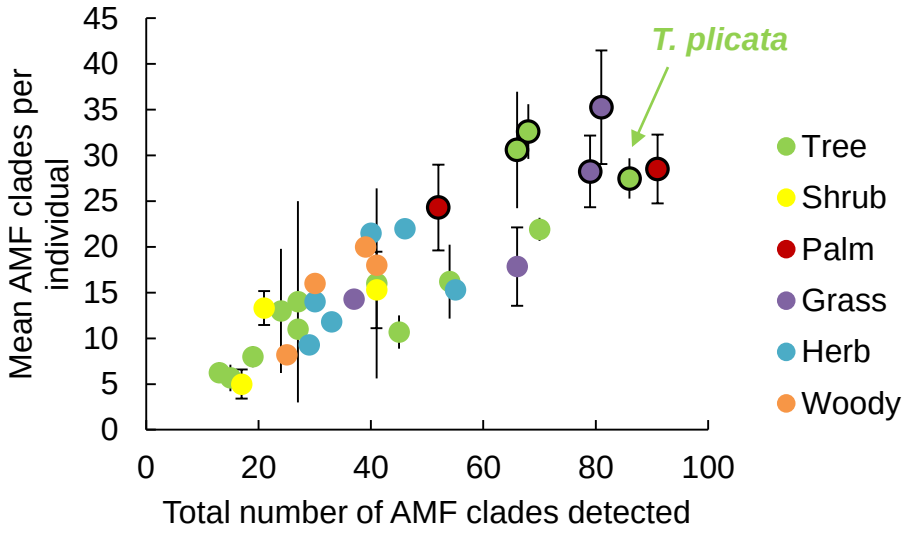

Young
Mature
Old
North
Root
Soil
a.
b.
c.
d.
e.
f.
South
g.
h.
i.
j.
k.
l.
Unknown (Glomeromycota)
Unknown (Glomeromycetes)
Unknown (Glomerales)
Unknown (Glomeraceae)
Unknown (Glomus)
Glomus spp.
Claroideoglomus spp.
Rhizophagus spp.
Acaulospora spp.
Diversispora sp.

AM fungi root communities



AM fungi soil communities




T. plicata
Mean AMF clades per individual
Total number of AMF clades detected
0
5
10
15
20
25
30
35
40
45
0
20
40
60
80
100
Tree
Shrub
Palm
Grass
Herb
Woody