Chapter 14
Archaeorhizomycetes: Patterns of Distribution and Abundance in Soil

Anna Rosling, Ina Timling, and D. Lee Taylor

14.1 Introduction

Archaeorhizomycetes represents one of the most ubiquitous lineages of soil fungi, and its formal description adds a prominent branch to the Taphrinomycotina among the basal Ascomycota (Rosling et al. 2011). Fungi in the class are strongly associated with soil environments containing plant roots. However, experimental analyses suggest that interactions with roots are neither mycorrhizal nor pathogenic. Instead, species in the Archaeorhizomycetes may exist along a continuum from root endophytic to free-living saprophytic life strategies. It is possible that Archaeorhizomycetes are mycoparasitic, but these life strategies have not yet been studied. Among thousands of published environmental sequences belonging to the class, only one was neither from soil nor roots. The sequence (GenBank Acc nr. EF67470) was cloned from samples of particulate organic matter collected in sediment from a freshwater stream (Bärlocher et al. 2008). While this could indicate that Archaeorhizomycetes is not restricted to terrestrial habitats, a more likely explanation is that the sequence originated from terrestrial material, i.e., spores, soil, and organic matter, that were deposited in the stream. Hence, all

A. Rosling (✉)
Department of Ecology and Genetics, EBC, Uppsala University, Uppsala, Sweden
e-mail: Anna.Rosling@ebc.uu.se

I. Timling
Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK, USA
e-mail: itimling@alaska.edu

D.L. Taylor
Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK, USA

Department of Biology, University of New Mexico, Albuquerque, NM, USA
e-mail: ltaylor@iab.alaska.edu; fflt@unm.edu

B.A. Horwitz et al. (eds.), *Genomics of Soil- and Plant-Associated Fungi*, Soil Biology 36, DOI 10.1007/978-3-642-39339-6_14,

available evidence supports the notion that Archaeorhizomycetes is restricted to vegetated terrestrial ecosystems (Porter et al. 2008; Rosling et al. 2011).

Based on similarity to environmental ITS and LSU sequences available in public databases, the class has been estimated to comprise more than 250 putative species (Rosling et al. 2011). Strong biogeographic patterns with significant global association between geographic and phylogenetic distance were detected across the class. Despite these strong biogeographic patterns, a number of putative species, such as *A. finlayi*, have a broad geographic distribution. Habitat specificity towards host species or genus, e.g. Tsuga, Picea, and Pinus, was detected for many putative species, while others have a broader habitat range (Rosling et al. 2011). It is possible that species that show habitat specificity are more closely associated with roots than species with broad distribution. It is important to keep in mind that Archaeorhizomycetes is an ancient class of fungi and may thus encompass diverse life strategies and ecologies.

In this chapter we analyze available ITS, LSU, and SSU sequences and their associated publications to discuss global distribution and abundance of Archaeorhizomycetes. Furthermore we expand our knowledge about Archaeorhizomycetes by analyzing publicly available ITS-LSU sequences from ten studies, seven with sequences in GenBank as well as unpublished sequence data from three studies: the North American Arctic Transect (NAAT) (Timling et al. unpublished), black spruce forest (TKN), and successional gradient in an upland ecosystem (UP) (Taylor et al. 2010).

14.1.1 Global Distribution

Based on new analysis of available environmental sequences of the ITS, LSU, and SSU regions, we demonstrate that species in the class Archaeorhizomycetes occur on all continents, except Antarctica, and in most terrestrial biomes, including tundra, taiga, tropical rainforest, temperate forest, and grasslands (Fig. 14.1). The size of dots in Fig. 14.1 illustrates the number of sites from which sequences of Archaeorhizomycetes have been identified in different regions. The number of observations is strongly biased towards regions where many studies of soil fungal communities are performed using molecular identification methods, i.e., Europe and North America. Ecosystems in these regions are mostly comprised of coniferous forests (Buscardo et al. 2010; Cox et al. 2010; Lindahl et al. 2007; Parrent and Vilgalys 2007; Rincon and Pueyo 2010; Taylor et al. 2008; Urban et al. 2008) and tundra-/shrub-type ecosystems (Bjorbaekmo et al. 2010; Bougoure et al. 2007; Deslippe et al. 2012; Schadt et al. 2003) but also include mixed deciduous forests (Edwards and Zak 2011; Stefani et al. 2009).

Observations of sequences belonging to the Archaeorhizomycetes in the Southern Hemisphere include the following: tropical rain forest in southwestern Costa Rica (Porter et al. 2008); tropical mountain pine forest in North Eastern Australia (Curlevski et al. 2010); dry sclerophyll forest in New South Wales, Australia (Chen

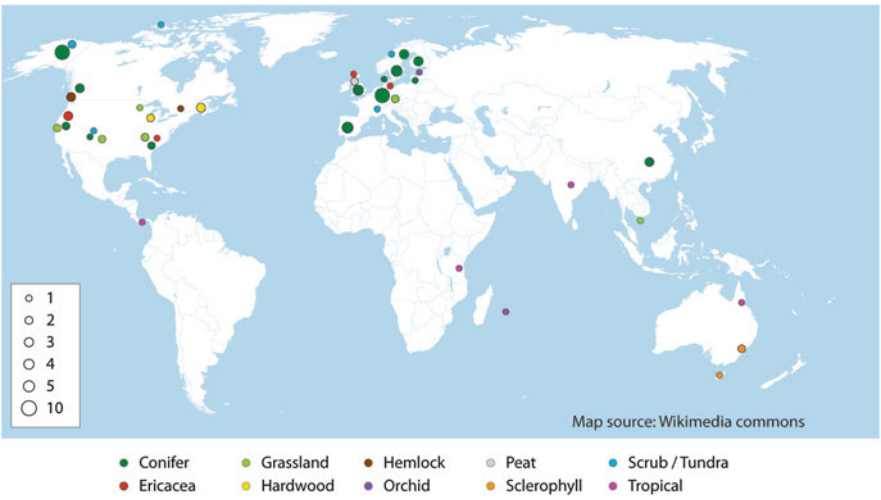

Fig. 14.1 Map of the world illustrating location and ecosystem where Archaeorhizomycetes has been detected using environmental sequencing. Size of *dots* corresponds to the number of sampling occasions (i.e., different sites or different studies from the same site) in which sequences of Archaeorhizomycetes have been detected. Data are compiled using sequences of the ITS, LSU, and SSU rRNA region available in GenBank with associated publications as well as additional sequences from three studies from the North American Boreal Forest and Arctic (Taylor unpublished data)

and Cairney 2002); wet sclerophyll forests in Tasmania (Tedersoo et al. 2009); subtropical pine forests in south central China (Huang et al. 2012); and in roots of the terrestrial orchid *Phaius pulchelus* collected on La Reunion, in the Pacific Ocean (Martos et al. 2012). Recent samples from unpublished studies in tropical regions add four more locations for Archaeorhizomycetes to the world map. These include samples collected from bulk soil associated with unhealthy *Axonopus compressus* (Blanket grass) in Singapore (HQ436085), root tips of *Allanblackia stuhlmannii* (a flowering tree indigenous to Tanzania) in the East Usambara Mountains in North Eastern Tanzania (unpublished data, Helena Ström, SLU Sweden), and brinjal (eggplant) rhizosphere soil in India (JQ989336) as well as one more tropical pine forest in Zhenjiang, China (HE814241) (Fig. 14.1). We expect that increased sampling and molecular identification of soil fungi in the Southern Hemisphere will increase the number of observations of Archaeorhizomycetes in tropical and subtropical ecosystems. The significant association between geographic and phylogenetic distance is notable in the global phylogeny of Archaeorhizomycetes where all Alaska sequences are found within the upper 2/3 of the tree among sequences derived mainly from boreal and coniferous ecosystems [Fig. 3 in Rosling et al. (2011)]. We thus expect new lineages, at the level of genus and families, to emerge in the phylogeny of Archaeorhizomycetes as sampling of the Southern Hemisphere proceeds.

Intensified studies of fungi in tropical ecosystems have generally resulted in the recognition of many previously unknown species (Hawksworth 2012). This pattern may become apparent for Archaeorhizomycetes as well, as more sequences are made publicly available from studies in tropical ecosystems. In a comparison of ectomycorrhizal-dominated boreal and tropical forests, McGuire and co-workers studied soil fungal communities by sequencing the ITS and LSU rRNA. The study is still unpublished, but sequences from the study are available in GenBank. While 13 % of the sequences from Delta Junction in Alaska belonged to Archaeorhizomycetes, none of the sequences from their tropical forest sites belonged to this class. This result, as well as the existing global patterns (Fig. 14.1), suggests that fungi in the Archaeorhizomycetes may be more abundant in boreal compared to tropical forest ecosystems.

14.1.2 Abundance in Soil Fungal Communities

Archaeorhizomycetes are also a major component of soil fungal communities from several studies. They were first detected as the novel fungal lineage Cluster 1 in alpine tundra soils (Schadt et al. 2003) at an average abundance of 15.2 % [19 out of 125 clones, see Supplementary material in Porter et al. (2008)]. In this study temporal dynamics of soil fungal communities were studied by sampling soils from below snow cover in winter and into the summer. Schadt and co-workers found that soil fungal community composition was stable from winter to spring but shifted significantly into summer, largely because of the dominance of Archaeorhizomycetes which made up 62 % of the clones sequenced from summer samples (Schadt et al. 2003). A cluster of Archaeorhizomycetes was identified accounting for 7 % of the winter samples, while none were identified in spring samples. Later Porter et al. (2008) examined four ecosystems: two different forests from within the Niwot Ridge Long-Term Ecological Research Site in Colorado (a tree line forest dominated by *Picea engelmannii* and *Pseudotsuga menziesii* and a montane forest with *Pinus contorta*, *Abies lasiocarpa,* and *Picea engelmannii*), one Costa Rican tropical forest on highly weathered P limited soil, and a temperate coniferous forest dominated by *Tsuga canadensis* in eastern Canada. They identified Archaeorhizomycetes, then Soil Clone Group 1, in 6.9–27 % of LSU clones derived from total soil DNA extracts from these ecosystems. Nevertheless, the highest abundance of Archaeorhizomycetes, 73–95 % of clones derived from soil DNA extracts, was detected by Castro et al. (2010) in a study of climate change effects on soil microbial communities associated with a reconstructed old-field plant community. The highest abundance was recorded in the wet treatment, which received 25 mm rain per week, and the lowest in the dry treatment, which received 2 mm per week. Apart from its exceptionally high abundance of Archaeorhizomycetes clones, the study by Castro et al. (2010) stands out in that sampling occurred late in the season (October 2006) which is close to the end of the growing season for Oak Ridge, TN. In addition to previously documented seasonal dynamics associated with plant

growth (Schadt et al. 2003), temporal dynamics of aboveground senescence, decreased carbon allocation to roots, and associated mycorrhizal fungi as well as root decomposition may be major drivers of relative abundance of Archaeorhizomycetes in soil fungal communities.

Assuming that abundance in soil DNA clone libraries reflects actual species abundance in terms of biomass or activity in soil, these studies demonstrate that members of Archaeorhizomycetes are major components of many soil fungal communities. While the internal transcribed spacer region (ITS) is the designated gene to describe fungal communities (Schoch et al. 2012), it has to be noted that Archaeorhizomycetes abundance estimates from studies targeting the ITS region are obscured by two specific mismatches in the binding site of the widely used reverse primer ITS4 (White et al. 1990). This can lead to great underestimation of Archaeorhizomycetes in soils. The three studies discussed above (Schadt et al. 2003; Porter et al. 2008; Castro et al. 2010) targeted the LSU region for amplification and sequencing of fungi from environmental samples by using reverse primers in the LSU region, such as nLSU1221R (Schadt et al. 2003) and TW13 (White et al. 1990), which do not appear to be biased against Archaeorhizomycetes. Therefore, the detected abundance of Archaeorhizomycetes in these studies might reflect their true abundance better than in studies using ITS4.

14.2 Archaeorhizomycetes in Alaska

The Alaskan boreal forest ecosystem is the best-documented ecosystem with respect to soil fungal communities (Taylor et al. 2010). After removing all singletons, 1,578 OTUs were identified in a dataset of over 52,000 sequenced clones. Among the 30 most common OTUs, only five were non-mycorrhizal and two of these can now be identified as Archaeorhizomycetes (labeled *Candida tepae* because no closer relatives were described at the time) (Taylor et al. 2010). Both these OTUs were found predominantly in the black spruce (*Picea mariana*) habitat. In an earlier study by Taylor et al. (2007), a putative Archaeorhizomycetes sp. (labeled OTU 76) was detected as the most abundant OTU, comprising approximately 25 % of the clones from a pooled DNA extract from humic black spruce soil. Two other OTUs (73 and 78) later identified as belonging to Archaeorhizomycetes were also identified in the study (Taylor et al. 2007). In Alaska, sequences belonging to Archaeorhizomycetes have been identified in ten studies targeting the ITS and LSU region (Table 14.1) as well as in two studies targeting the SSU region (Allison et al. 2008; Allison and Treseder 2008).

Table 14.1 Ten studies identify ITS and LSU sequences belonging to the Archaeorhizomycetes in Alaska

Study	Site	Rel. ab. (%)	# A-OTU	Tot# seq (OTUs)
Allison et al. (2010a)	Delta Junction	1	2	433 (113)
Allison et al. (2010b)	Delta Junction	2	6	327 (110)
Bent et al. (2011)	Bonanza Creek LTER	1[a]	2	152 (71)
Deslippe et al. (2012)	Toolik Lake	1	5	2,293 (777)
McGuire et al. (unpub.)	Delta Junction	13	7	156 (UK)
Taylor et al. (2007)	Bonanza Creek LTER	12	6	588 (148)
Taylor et al. (2008)	Fairbanks	8	6	456 (117)
TK	12 sites[b]	11	31	28,903 (2,537)
UP	9 sites	29	24	23,103 (3,093)
NAAT[c]	7 sites[b]	1	5	7,834 (1,834)

Sites from Interior and northern Alaska are represented. Relative abundance (Rel. ab.) is given as % sequences belonging to Archaeorhizomycetes out of all sequences from the study. Number of OTUs (#OTUs) calculated as described above. The total number of sequences available from each study is given under #sequences with total

[a]Bent et al. (2011) studied roots of spruce and birch. All the other sequences are obtained by cloning from total soil DNA extracts

[b]Archaeorhizomycetes sequences were detected at seven sites in the TKN study and in three sites in the UP study. See Fig. 14.2 for approximate locations of these sites

[c]In the NAAT study, sequence of Archaeorhizomycetes was detected at three sites along the North American Arctic Transect. Two of these are in Alaska (Fig. 14.2), and the third is on Prince Patrick Island in the Canadian High Arctic (Fig. 14.1)

14.2.1 Sequence and Statistical Analysis

One sequence representing each Archaeorhizomycetes OTU found in the ten studies (Table 14.1) was added to an alignment spanning the ITS and LSU rRNA followed by manual editing in Geneious Pro 5.5.5 (Biomatters Ltd.). Reference sequences of *Archaeorhizomyces finlayi* and Archaeorhizomycetes sp. FG15P2b were included in the alignment and AFTOL reference sequences for *Schizosaccharomyces pombe, Protomyces inouyei, Taphrina wiesneri*, *Taphrina deformans,* and *Saitoella complicate* were included as an out-group in the analysis. A maximum likelihood tree was derived from the alignment using RAxML-HPC2 on XSEDE, Cipres (Miller et al. 2010). Bootstrap support values were calculated from 1,000 iterations. Branches were collapsed to represent OTUs clustered in CAP3 at a 97 % similarity across the ITS. Most OTUs represented distinct and well-supported clades in the tree (Fig. 14.2). The exceptions include five cases where additional sequences were clustered within the OTU; these are indicated by + after the OTU number in the tree and one case when two OTUs could not be separated in the tree labeled OTU 7 and 8. Sequences from Allison et al. (2010a, b) comprised the LSU region only and were assigned to OTUs based on clustering within the tree. OTU5+, OTU11+, OTU12+, and OTU18+ all include one or two additional sequences with close to 97 % similarity across the ITS region to the other sequences in the OTU. In these cases sequence dissimilarity is mostly due to ambiguous base calls, i.e., N.

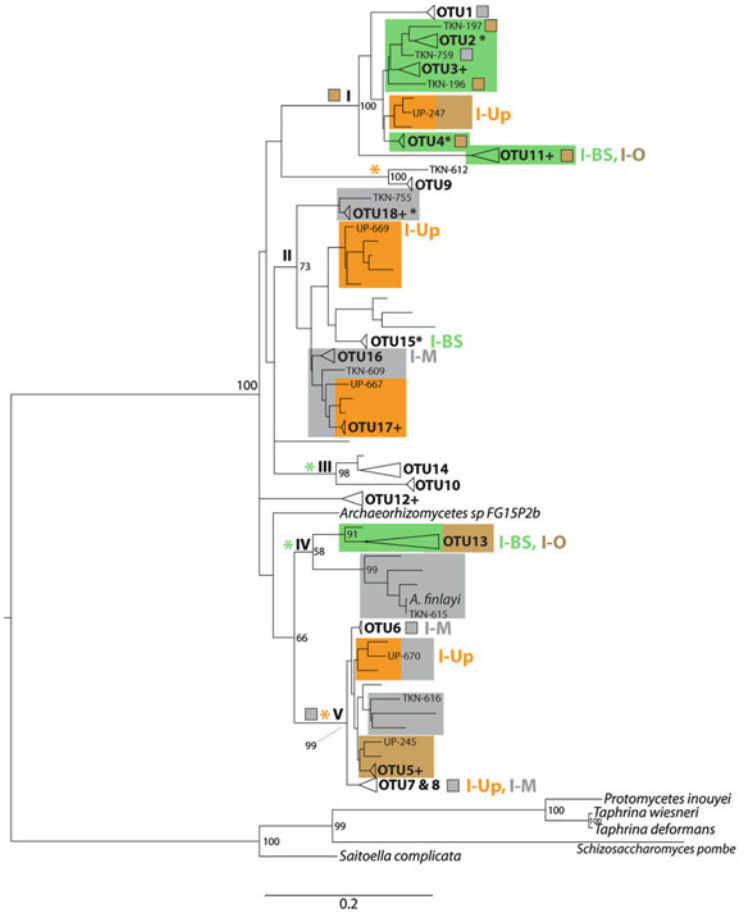

Fig. 14.2 Phylogeny of Archaeorhizomycetes in Alaska illustrated in a maximum likelihood tree derived from an ITS-LSU alignment. Reference sequences of *A. finlayi* and Archaeorhizomycetes sp. FG15P2b were included in the alignment and AFTOL reference sequences for *Schizosaccharomyces pombe, Protomyces inouyei, Taphrina wiesneri*, *Taphrina deformans,* and *Saitoella complicate* were included as an out-group in the analysis. Boot strap support values above 50 are shown in the figure. Putative species containing sequences from the Arctic NAAT study are indicated with *asterisk*. Five well-supported clades containing more than two putative species (I–V) are identified in the tree. Lineage-specific associations at the clade level with ecosystems are indicated with *asterisk* at the highest node, *orange* for mixed upland, and *green* for *black spruce*, and clades associated with soil horizons are indicated with a *filled box* at the highest node, *brown* for organic soil, and *gray* for mineral soil. Within clades significant lineages are shaded, and putative species with significant associations to soil horizon are marked with a *colored box* following the branch name. Indicator species are marked in the tree, for upland (I-Up), *black spruce* (I-BS), mineral soil (I-M), and organic soil (I-O)

Archaeorhizomycetes relative abundances, both as fraction of total sequences and as fraction of total OTUs, were derived from the Alaskan studies (Table 14.1) and analyzed by regression against latitude. Three studies were excluded from this analysis because they used the reverse primer ITS4 that has two mismatches for Archaeorhizomycetes, which may have resulted in an underestimation of abundance in these studies (Bent et al. 2011; Deslippe et al. 2012) and the unpublished study by McGuire and co-workers since we did not have data on total numbers of identified OTUs for this study.

To investigate species-environment relationships for Archaeorhizomycetes in Alaska, we carried out a variety of statistical analyses in PC-ORD 5 (McCune and Mefford 2006) with a combined species X site matrix from the TKN, UP, and NAAT datasets, utilizing abundance-based Bray-Curtis distances after applying a "general relativization" as recommended by McCune et al. (2002) when sample sizes differ among sites. In our first analysis, we tested whether communities differ according to major categorical habitat variables using multiple response permutation procedures (MRPP). We performed ordination of the sites using nonmetric multidimensional scaling (NMS). After 50 randomizations, a 3-dimensional solution was selected by PC-ORD using automatic mode. The best solution had a final stress of 18.86459 and a final instability of 0.00002 after 500 iterations. The relatively high stress indicates that the relationships among the sites were fairly weak, in agreement with the relatively small effect sizes (A) in the MRPP analysis (Table 14.2). Lastly, we carried out indicator species analyses to ascertain whether particular Archaeorhizomycetes taxa have a statistically significant preference for particular site categories. We analyzed only habitat, soil pH, and soil horizon, since those factors had the strongest correlations in the MRPP analyses. Furthermore, the phylogenetic distance of the Archaeorhizomycetes communities associated with (1) soil horizons O and E and (2) with habitats blacks spruce and mixed upland was analyzed separately using UniFrac (Lozupone et al. 2006). Samples from tundra, i.e., the NAAT study, were excluded from the UniFrac analysis because they represented only 20 sequences. Environments were clustered by jackknife analysis with 100 permutations, and lineage-specific analyses were performed to identify lineages with significant affiliation for certain environments. The analyses were performed using sequence abundance data excluding lineages with less than ten descendants and using presence–absences counts excluding lineages with less than four descendants. Using presence–absence data provides a more conservative measurement than abundance data that can be strongly driven by the more abundant species.

14.2.2 Diversity and Distribution of Archaeorhizomycetes in Alaska

Earlier global estimates of Archaeorhizomycetes diversity encompassed ten putative species from Alaska (Rosling et al. 2011). In contrast, the current expanded phylogenetic analysis provides an estimate of 53 putative species of

Table 14.2 Multiple response permutation procedure results

Factor	Chance corrected within-group agreement, *A*	Probability of a smaller or equal delta, *p*
Habitat	0.09521448	0.00000000
Mineral soil pH	0.03604479	0.00000002
Soil horizon	0.03910208	0.00000041
Site moisture	0.03111664	0.00001152
Successional stage	0.02859186	0.00001808
Biome	0.01363238	0.00306595

Archaeorhizomycetes in Alaska. Eighteen of these putative species were detected in more than one study. Forty-six of these putative species were identified among 3,666 Archaeorhizomycetes sequences from the TKN, NAAT, and UP studies. Putative species identified as OTUs, i.e., in more than one study, as well as those represented by more than ten sequences, are named in the tree (Fig. 14.2). A rank abundance curve of the 25 most common Archaeorhizomycetes OTUs detected among the three studies (TKN, UP, and NAAT) demonstrates a classical pattern with a few very common species and numerous rare species (Fig. 14.3). The OTU TKN-615 is among species of average abundance (Fig. 14.3) and was identified as the type species *A. finlayi* (Fig. 14.2). This expands the known distribution of *A. finlayi* beyond its previous identification from Finland, Sweden, and New Hampshire (Rosling et al. 2011). Five well-supported major clades with more than two putative species (I–V) are identified in the tree (Fig. 14.2). The reference sequence of Archaeorhizomycetes sp. FG15P2b did not cluster with any Alaska sequence, supporting earlier indications that this species might be geographically limited to Europe (Rosling et al. 2011).

Across the ten field studies, relative abundance of the Archaeorhizomycetes ranged from 1 % to 29 % of the total fungal community identified by environmental sequencing (Table 14.1). Putative species in the Archaeorhizomycetes were detected from 18 sites in Interior and northern Alaska (Fig. 14.4). In Interior Alaska 9–25 OTUs per site were identified in the TKN study and 7–16 OTUs in the UP study. For the other published studies conducted in Interior Alaska, two to seven OTUs were identified per site (Table 14.1, Fig. 14.4). In the two studies conducted in northern Alaska, two and five OTUs were identified per site. Sampling at Prince Patrick Island in the Canadian Arctic yielded one OTU. Abundance and diversity of Archaeorhizomycetes was found to decrease from boreal to arctic ecosystems as demonstrated by a significant ($P < 0.002$) exponential decrease in relative number of OTUs and in relative abundance with increasing latitude. It is interesting to note that the single OTU from the Canadian Archipelago was identified as OTU4, which is the third most common putative species in Alaska (Fig. 14.3). This putative species also included sequences from the TKN study, a sequence representing the most common OTU (76) detected in Taylor et al. (2007) as well as sequences from Deslippe et al. (2012) and Taylor et al. (2008). Among all the putative

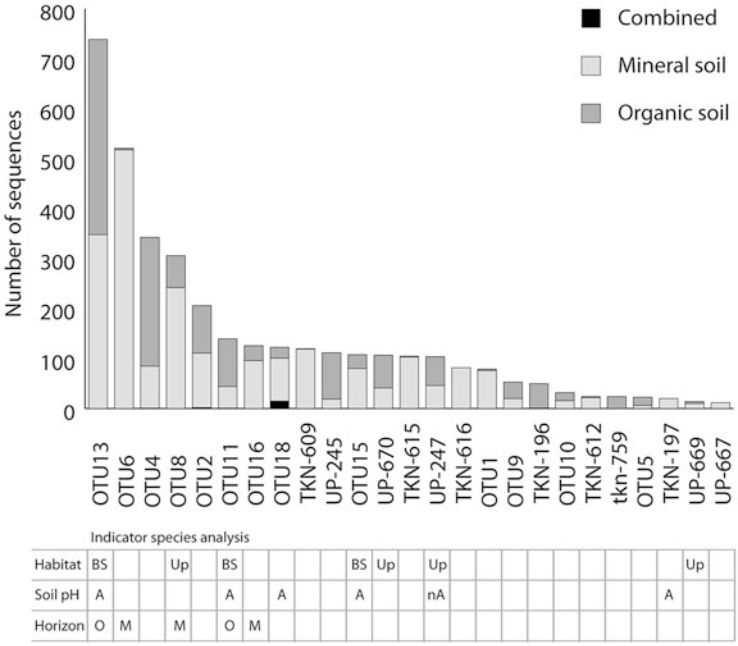

Fig. 14.3 Rank abundance of putative Archaeorhizomycetes species in Alaska, measured as number of sequences assigned to each OTU from the three studies NAAT, UP, and TKN. Sequence origin with respect to soil horizon is illustrated in *dark gray* for organic soil, *light grey* for mineral soil, and *black* for combined samples. Below each OTU results from the indicator species analysis are listed for habitats [*black spruce* (BS) or mixed upland (Up)], soil pH [acid (A) or nonacid (nA)], and soil horizon [organic (O) or mineral (M)]. TKN-207 is identified as an indicator species for acidic soil but does not appear in the figure because it is only represented by ten sequences among all studies

Archaeorhizomycetes species identified here, only one common generalist (OTU4) is detected in among the arctic samples analyzed.

14.2.3 Habitat Specificity

Using MRPP, we found that the composition of the Archaeorhizomycetes community was correlated with several habitat-related environmental variables (Table 14.2). The strongest correlation was with our variable "habitat," by which we distinguished black spruce boreal forest, upland boreal forest, and tundra. We also found highly significant correlations with pH of the mineral soil at these sites (acidic, nonacidic), as well as site moisture (wet, mesic, dry), successional stage (early, middle, late), and biome (boreal forest vs. arctic). These patterns are in accordance with previous observations from these sites where total fungal

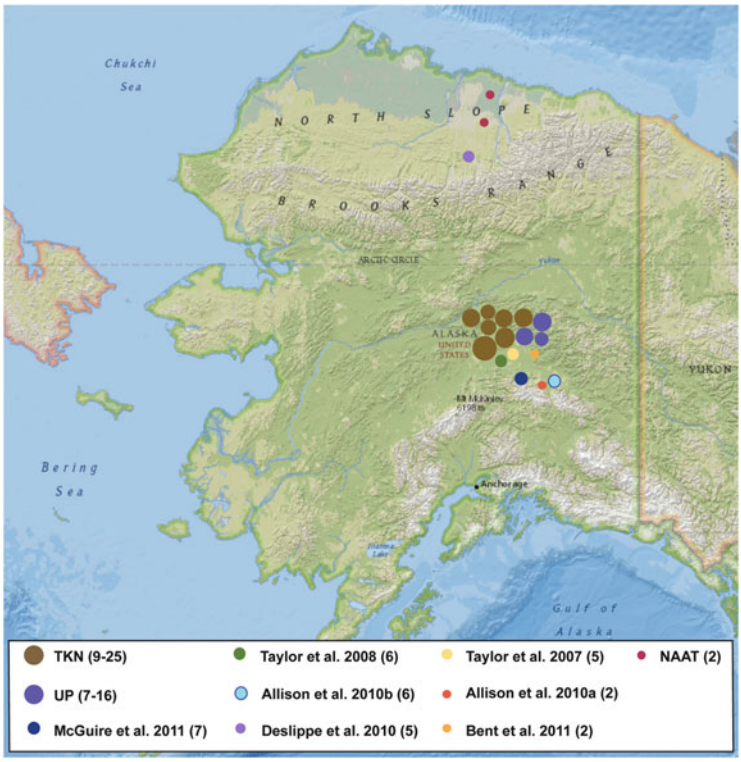

Fig. 14.4 Schematic overview of study sites for ten studies that have identified sequences belonging to the Archaeorhizomycetes in Alaska. Size of *dots* illustrates the number of putative Archaeorhizomycetes species, i.e., OTUs defined at 97 % sequence similarity across the ITS region. Numbers of species per study are also given as values in parenthesis after the study name in the legend

communities were also found to be different among these habitats as well as between the two soil horizons (Taylor et al. 2010). However, it should be noted that these categories are confounded in the dataset. For example, most of the acidic sites are also black spruce forest, and the only early successional sites were in upland boreal forest. Thus, it is most parsimonious to consider habitat (forest type) as the overriding factor, with a set of intercorrelated environmental factors that are associated with habitat. However, because horizon was analyzed independently in both black spruce and upland datasets, the significant correlation with this factor can be interpreted more directly: the composition of the Archaeorhizomycetes community differed between the organic and mineral horizons. In concordance with the MRPP analyses, NMS demonstrated that the environmental factor that best coincided with fungal community variation across the sites was habitat (Fig. 14.5).

The indicator species analyses (Table 14.3, Figs. 14.2, and 14.3) suggest that 12 taxa are specialists. For example, TKN204 in OTU13 is an indicator of acidic

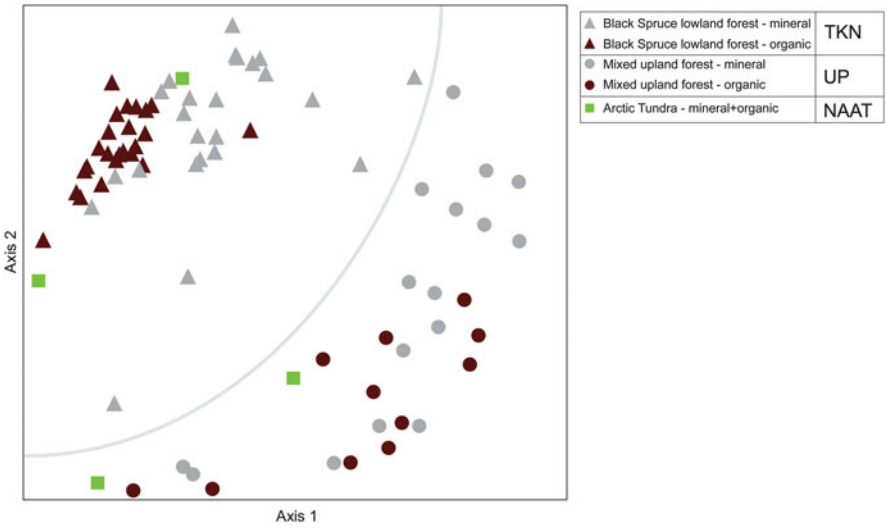

Fig. 14.5 NMS ordination of Archaeorhizomycetes communities from three studies in Alaska (TKN, UP, and NAAT). *Triangles* indicate *black spruce* forest sites, *circles* indicate upland boreal forest sites, and *squares* indicate tundra sites. *Brown symbols* specify organic horizon, and *gray symbols* designate mineral horizons. Notice the strong grouping of *black spruce* vs. upland boreal forest sites

soil in black spruce forests and prefers the organic horizon. In contrast, UP247 is an indicator of nonacidic soils and upland forests, but did not reveal a horizon preference. As with the MRPP analyses, it should be noted that many of these environmental factors covary across sites, meaning that it is impossible to infer which factor drives the observed correlation. For example, it may be that UP247 has a preference for particular tree species and that the apparent preference for nonacidic soils is simply due to the fact that most of the nonacidic sites are also upland forest, where these tree species occur.

Jackknife analysis in UniFrac demonstrates that the environment soil horizons O vs. E as well as ecosystems black spruce vs. mixed upland harbor phylogenetically distinct communities of Archaeorhizomycetes (100 % node support for both presence–absence and sequence abundance data). Lineage specificity towards ecosystems, which were based on presences–absences, is highlighted in green for black spruce and orange for mixed upland (Fig. 14.2). Highlighted clusters are not only significantly associated ($P < 0.01$) but exclusively associated with their ecosystem. Taking sequence abundance into account, significant lineages are identified at higher order as illustrated by colored asterisks at the highest significant node (Fig. 14.2). Based on the latter, Clade III and IV are significantly associated with the black spruce ecosystem, while Clade V and a clade with the two putative species OTU 9 and TKN-612 are significantly associated with the mixed upland ecosystem. Ecosystem specificity among lineages is a strong driver for the observed differences in Archaeorhizomycetes

Table 14.3 Results of indicator species analysis for the three categories: habitat, soil pH, and soil horizon

Taxon	Observed indicator value (IV)	Mean	Standard deviation	*p*	Factor	Preference
OTU 13 TKN204	72.9	23.9	7.45	0.0002	Habitat	Black spruce
UP247	55.2	15.2	6.85	0.0008	Habitat	Upland
OTU 11 TKN198	47.9	18.4	7.36	0.0064	Habitat	Black spruce
OTU 7 and 8 TKN206-UP242-UP244	37.7	20.9	7.67	0.0378	Habitat	Upland
OTU 15 TKN202	31.2	14.6	7.17	0.0396	Habitat	Black spruce
UP669	20.7	8.7	5.21	0.0402	Habitat	Upland
UP670	24.1	9.7	5.78	0.0446	Habitat	Upland
TKN203	41.6	16.5	4.67	0.0002	Soil pH	Acidic
OTU13 TKN204	52.9	26.6	4.53	0.0006	Soil pH	Acidic
OTU15 TKN202	33.7	13.7	3.89	0.0008	Soil pH	Acidic
UP247	28.6	14.4	4.09	0.007	Soil pH	Nonacidic
OTU 11 TKN198	31.8	18.9	4.17	0.0116	Soil pH	Acidic
TKN207	11.1	4.5	1.94	0.029	Soil pH	Acidic
TKN197	11.1	4.4	1.99	0.0332	Soil pH	Acidic
TKN205-UP979	37.5	16.2	7.37	0.0188	Soil horizon	Mineral
TKN204	44.5	23.8	7.39	0.022	Soil horizon	Organic
TKN198	37.6	18.3	7.34	0.0278	Soil horizon	Organic
TKN206-2	38.9	20.8	7.81	0.0352	Soil horizon	Mineral
TKN200-U	32.4	16.7	7.18	0.0442	Soil horizon	Mineral

community composition that we observe in the Alaska dataset. This pattern is most likely driven by host specificity as previously demonstrated for several putative species in the class (Rosling et al. 2011). OTU13 is significantly associated with the black spruce ecosystem (Fig. 14.2), and this putative species shared reference sequences with the previously listed OTU3 specifically associated with spruce root (Rosling et al. 2011). The same is true for OTU2 that was previously listed as the spruce-specific OTU13 (Rosling et al. 2011). The tundra habitat is only represented by four putative species, OTU2, 4, 15, and 18+, which are all associated with the black spruce habitat.

For the environmental variable soil horizon, lineage-specific analysis, based on presence–absence, identified Clade V as significantly associated with mineral soil ($P = 0.0094$) and Clade I as significantly associated with organic soil ($P = 0.0064$). However, the association with soil horizon is not exclusive to the same extent as the association with ecosystems discussed above. Taxa and lineages with significant associations with organic horizons are also identified in mineral soils, while at least eight putative species appear to be restricted to mineral horizons (Fig. 14.3). Thus, sequence abundance data, as opposed

to presence–absence, provide a more informative image of lineage significance towards soil horizon. Significant lineages are highlighted in gray for mineral soil and brown for organic soil (Fig. 14.2). Putative species with significant associated with a soil horizon that is found within a clade without significant associated to the same horizon are indicated by a colored box following the taxa name (Fig. 14.2). Three identified indicator species for mineral soil (I-M), OTU6, OTU8, and OTU16, were all found in lineages with a significant association with mineral soil (Fig. 14.2). The same is true for OTU 11+ and OTU13 which were identified as indicator species for organic soil (I-O) and found in lineages with significant association with organic soil.

Clade IV was significantly associated with the black spruce ecosystem when considering sequence abundance data but not when using the presence counts. The two sequences in this clade were derived from the mixed upland ecosystem, dominated by white spruce (*Picea glauca*), indicating a strong association with spruce for this clade. Clade IV is split into two well-supported clades with contrasting soil horizon preferences. OTU13 is the most abundant taxon in the current dataset. It is an indicator species for organic soil in the black spruce ecosystem, and the lineage is significantly associated with organic soil (Fig. 14.2). Yet OTU13 is still frequently detected in mineral soil (Fig. 14.3). The sister clade which encompasses *A. finlayi*, on the other hand, is significantly associated with mineral soil, and 94 % of all sequences in this clade were derived from mineral soil. This observation is supported by the initial isolation of the *A. finlayi*-type culture from a coniferous root collected in mineral soil at the interface between illuvial and eluvial soil horizons in a podzol soil profile (Rosling et al. 2003).

Clade I was significantly associated with organic soil. Within this clade, contrasting soil horizon specificity was detected among sister species, i.e., TKN-197 and TKN-196 associated with organic soil vs. TKN-759 associated with mineral soil (Fig. 14.2). Similar to patterns of vertical partitioning observed for sister species of Rhizopogon (Beiler et al. 2012), patterns of differential distribution between soil horizon may well be the result of ongoing substrate competition among closely related species.

Overall, the relative abundance of Archaeorhizomycetes was higher in mineral soils, close to 9 % of all clones, compared to roughly 6 % in organic soil across the three studies from Alaska discussed here. Higher relative abundance in mineral soil may reflect lower abundance of other taxa rather than an absolute increase in Archaeorhizomycetes in mineral soil. However, lineage specificity towards mineral soil was common within clades II, IV, and V, suggesting that a large proportion of the diverse class is well adapted to conditions in mineral soil. This observation is supported by previous findings from Lindahl et al. (2007) where fine separation of horizons from a boreal forest floor followed by fungal community characterization using T-RFLP identified six putative Archaeorhizomycetes (then Ascomycete group G) occuring in all soil layers except for the layer of new litter. Putative Archaeorhizomycetes species had different but overlapping patterns of occurrence with most taxa being identified in the upper humus layer. Two putative species,

C2y_8.2 (JN032481) and A2z_5.11 (JN032482) became increasingly common in mineral soil horizons. The latter formed part of OTU9 (Rosling et al. 2011), which represents the putative species OTU18+ in the current analysis.

14.3 Life Strategies in Archaeorhizomycetes

A shift in community composition towards dominance by Archaeorhizomycetes in summer samples was interpreted as an indication that the class depends on carbon derived from root exudation (Schadt et al. 2003). Furthermore, the two cultured representatives of Archaeorhizomycetes, *A. finlayi* and Archaeorhizomycetes sp. FG15P2b, were both isolated from surface-sterilized root tips, further suggesting that species in the class are intimately associated with roots. There are, however, indications that the class is not dependent on living roots for their carbon supply. Five and 14 days after severing of roots in a pine forest, the relative abundance of Archaeorhizomycetes (then Clone Group 1) remained close to that in the control samples with an average abundance of 14 % of total fluorescence as quantified by T-RFLP (Lindahl et al. 2010). That study analyzed abundance in soil DNA extracts, and targeting the active community might give a different representation of Archaeorhizomycetes. Furthermore, the study by Castro et al. (2010) stands out with its exceptionally high relative abundance of Archaeorhizomycetes clones (up to 95 %) of the soil fungal community at the end of the growing season. Possible temporal dynamics of Archaeorhizomycetes associated with aboveground senescence decreased carbon allocation to roots, and associated mycorrhizal fungi as well as root decomposition may be well worth studying in the future.

Species in Archaeorhizomycetes have the ability to grow inside roots as well as on pure carbon sources of varying complexity (Rosling et al. 2011). Sequences of Archaeorhizomycetes have also been detected in decaying wood. Rajala et al. (2011) studied the active fungal community of decaying spruce logs using a combination of DGGE and sequencing from environmental rDNA and rRNA extracts. A sequence representing Archaeorhizomycetes was obtained from strongly decayed spruce logs. These observations in combination with the shear abundance of Archaeorhizomycetes in many soils suggest that these fungi play an important role in the cycling of carbon derived from living or dead roots in soil. Neither mycorrhizal nor pathogenic interactions have yet been documented for the type species *A. finlayi*. Whether Archaeorhizomycetes are directly associated with roots along a trophic continuum from symbiotic–endophytic–saphrotrophic–pathogenic interactions or are secondarily associated through interactions with other root-associated fungi remains unknown. Taking into account that Archaeorhizomycetes is an ancient class of fungi, there is good reason to acknowledge that different life strategies may be represented among species in the class and that no single ecological role may be assigned to the class.

References

Allison SD, Treseder KK (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob Chang Biol 14:2898–2909

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Chang Biol 14:1–13

Allison SD, Gartner TB, Mack MC, McGuire K, Treseder KK (2010a) Nitrogen alter carbon dynamics during early succession in boreal forest. Soil Biol Biochem 42:1157–1164

Allison SD, McGuire KL, Treseder KK (2010b) Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol Biochem 42:1872–1878

Bärlocher F, Seena S, Wilson KP, Williams DD (2008) Raised water temperature lowers diversity of hyporheic aquatic Hyphomycetes. Freshw Biol 53:368–379

Beiler KJ, Simard SW, Lemay V, Durall DM (2012) Vertical partitioning between sister species of Rhizopogon fungi on mesic and xeric sites in an interior Douglas-fir forest. Mol Ecol 21:6163–6174

Bent E, Kiekel P, Brenton R, Taylor DL (2011) Root-associated ectomycorrhizal fungi shared by various Boreal forest seedlings naturally regenerating after a fire in interior Alaska and correlation of different fungi with host growth responses. Appl Environ Microbiol 77:3351–3359

Bjorbaekmo MFM, Carlsen T, Brysting A, Vrålstad T, Hoiland K, Ugland KI, Geml J, Schumacher T, Kauserud H (2010) High diversity of root associated fungi in both alpine and arctic Dryas octopetala. BMC Plant Biol 10:244

Bougoure DS, Parkin PI, Cairney JWG, Alexander IJ, Anderson IA (2007) Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. Mol Ecol 16:4626–4636

Buscardo E, Rodriquez-Echeverria S, Martin MP, de Anfelis P, Pereira JS, Freitas H (2010) Impact of wildfire return interval on ectomycorrhizal resistant propagules communities of a Mediterranean open forest. Fungal Biol 114:628–636

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil microbial community responses to multiple experimental climate change drivers. Appl Environ Microbiol 76:999–1007

Chen DM, Cairney JWG (2002) Investigation of the influence of prescribed burning on ITS profiles of ectomycorrhizal and other soil fungi at three Australian sclerophyll forest sites. Mycol Res 106:532–540

Cox F, Barsoum N, Lilleskov EA, Bidartondo MI (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecol Lett 13:1103–1113

Curlevski NJA, Xu ZH, Anderson IC, Cairney JWG (2010) Diversity of soil and rhizosphere fungi under Araucaria bidwillii (Bunya pine) at an Australian tropical montane rainforest site. Fungal Divers 40:12–22

Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. FEMS Microbiol Ecol 82:30–315

Edwards IP, Zak DR (2011) Fungal community composition and function after long- term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. Glob Chang Biol 17:2184–2195

Hawksworth DL (2012) Global species number of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? Biodivers Conserv 21:2425–2433

Huang J, Nara K, Lian C, Zong K, Peng K, Xue S, Shen Z (2012) Ectomycorrhizal fungal communities associated with Masson pine (*Pinus massoniana* Lamb.) in Pb–Zn mine sites of central south China. Mycorrhiza 22:589–602

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173:611–620

Lindahl BD, de Boer W, Finlay RD (2010) Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. ISME J 4:872–881

Lozupone C, Hamady M, Knight R (2006) UniFrac - an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7:371

Martos F, Munoz F, Pailler T, Kottke I, Gonneau C, Selosse MA (2012) The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Mol Ecol 21:5098–5109

McCune B, Mefford MJ (2006) PC-ORD. Multivariate analysis of ecological data. Version 5. MjM Software, Gleneden Beach, OR

McCune B, Grace JB, Urban DL (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE), New Orleans, LA, pp 1–8, 14 Nov 2010

Parrent JL, Vilgalys R (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. New Phytol 176:164–174

Porter TM, Schadt CW, Rizvi L, Martin AP, Schmidt SK, Scott-Senton L, Vilgalys R, Moncalvo JM (2008) Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. Mol Phylogenet Evol 46:635–644

Rajala T, Peltoniemi M, Hantula J, Mäkipää R, Pennanen T (2011) RNA reveals a succession of active fungi during the decay of Norway spruce logs. Fungal Ecol 4:437–448

Rincon A, Pueyo JJ (2010) Effect of fire severity and site slope on diversity and structure of the ectomycorrhizal fungal community associated with post-fire regenerated *Pinus pinaster* Ait. Seedlings. For Ecol Manage 260:361–369

Rosling A, Landeweert R, Lindahl BD, Larsson K-H, Kuyper TW, Taylor AFS, Finlay RD (2003) Vertical distribution of ectomycorrhizal fungi in a podzol profile. New Phytol 159:775–783

Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet G-A, Lindahl BD, Menkis A, James TY (2011) Archaeorhizomycetes - a new class of ancient, widespread soil fungi. Science 333:876–879

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359–1361

Schoch CL, Seifert KA, Hubndorf S, Robert V, Spouge JL, Levesque A, Chen W, Fungal barcoding consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. Proc Natl Acad Sci USA 109:6241–6246

Stefani FOP, Moncalvo J-M, Séguin A, Bérubé FA, Hamelin RC (2009) Impact of an 8-year-old transgenic poplar plantation on the ectomycorrhizal fungal community. Appl Environ Microbiol 75:7527–7536

Taylor DL, Herriott IC, Long J, O'Neill K (2007) TOPO TA is A-OK: a test of phylogenetic bias in fungal environmental clone library construction. Environ Microbiol 9:1329–1334

Taylor DL, Booth MG, McFarland JW, Herriott IC, Lennon NJ, Nusbaum C, Marr TG (2008) Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. Mol Ecol Res 8:742–752

Taylor DL, Herriott IC, Stone KE, McFarland JW, Booth MG, Leigh MB (2010) Structure and resilience of fungal communities in Alaskan boreal forest soils. Can J Forest Res 40:1288–1301

Tedersoo L, Pärtel K, Jairus T, Gates G, Poldmaa K, Tamm H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. Environ Microbiol 11:3166–3178

Urban A, Puschenreiter M, Strauss J, Gorfer M (2008) Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil. Mycorrhiza 18:339–354

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfland DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, CA, pp 315–322