1 Title

2 Plant-fungal interactions in hybrid zones: ectomycorrhizal communities of willows (*Salix* L.)
 3 in an alpine glacier forefield.

5 Authors

6 Arraiano-Castilho, R.^{1,2}, Bidartondo, M.I.^{1,2}, Niskanen, T.³, Zimmermann, S.⁴, Frey B.⁴,
 7 Brunner, I.⁴, Senn-Irlet, B.⁵, Hörandl, E.⁶, Gramlich, S.⁶ & Suz, L.M.¹

9 Authors affiliations

10 1 - Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew TW9 3DS, UK

11 2 - Department of Life Sciences, Imperial College London, London SW7 2AZ, UK

12 3 - Identification and Naming, Royal Botanic Gardens, Kew TW9 3DS, UK

13 4 - Forest Soils and Biogeochemistry, Swiss Federal Institute for Forest, Snow and
 14 Landscape Research WSL, Birmensdorf, Switzerland

15 5 – Biodiversity and Conservation Biology, Swiss Federal Institute for Forest, Snow and
 16 Landscape Research WSL, Birmensdorf, Switzerland

17 6 - Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), Georg
 18 August University Göttingen, Göttingen, Germany

20 Authors for correspondence

21 1 - Arraiano-Castilho R., email: r.arraiano-castilho@kew.org, Tel: +4402083323708

22 2 - Suz, L.M., email: lmartinez-suz@kew.org, Tel: +4402083323704

Abstract

Ectomycorrhizal (EcM) fungi are essential in the establishment of woody perennial plants in the European Alps. From continental to local scales, environmental conditions and plant host characteristics can predict EcM community structure and composition. However, it is unclear whether EcM communities of congeneric host species and their hybrids are differentially structured at local scales. We aimed to i) characterize EcM communities of *Salix helvetica*, *S. purpurea* and their hybrids and ii) elucidate the abiotic and biotic factors affecting EcM communities in hybrid zones. We analyzed willows in a glacier valley by combining molecular identification of fungi from individual ectomycorrhizas and from soil. We detected diverse EcM fungi forming non-modular and unnested networks, but we did not find significant differences in the overall EcM fungal community richness and composition among parental species and hybrids. Nevertheless, preference of individual fungal OTU per host varied. Our results demonstrate that in a sub-alpine hybrid zone with heterogeneous geomorphology, host genotype was not a strong predictor of overall EcM fungal community, but it influenced the occurrence of particular fungal OTUs.

Keywords

Ectomycorrhizal; fungi, host specificity; sub-alpine, hybrids; *Salix helvetica*; *Salix purpurea*; network analysis; Sanger sequencing; high throughput sequencing

Introduction

The ectomycorrhizal (EcM) symbiosis enhances plant nutrient and water uptake in exchange for significant amounts of photosynthates (Smith and Read, 2008). Furthermore, EcM fungi can influence plant diversity, productivity and community composition in terrestrial ecosystems (van der Heijden et al., 2008). To understand and predict these plant-fungal interactions under future ecosystem changes, it is necessary to know what controls them in the environment. Ectomycorrhizal fungal communities are known to respond to abiotic factors like soil pH and nutrient availability across local, regional and continental scales (Cox et al., 2010; Suz et al., 2014; van der Linde et al., 2018) and to temperature and precipitation at global scales (Tedersoo et al., 2014, 2012). In addition, EcM fungal communities can also be affected by biotic factors like plant community composition, diversity and productivity (Bahram et al., 2012; Waldrop et al., 2006). Moreover, plant host genotype and competition with other fungi can play important roles structuring EcM communities (Dickie, 2007; Ishida et al., 2007). In general, plant host influences EcM fungal community structure (Dickie, 2007; Tedersoo et al., 2012, van der Linde et al., 2018) and more closely related hosts share more similar EcM communities (Ishida et al., 2007; van der Linde et al., 2018), but in some cases congeneric hosts may show significant differences in EcM community composition by providing distinct ecological niches for EcM fungi (Morris et al., 2008). However, the relationships among these biotic and abiotic drivers and their influence on EcM fungal communities across host hybridization zones are still poorly understood, especially in harsh environments, such as alpine glacier forefields.

In alpine ecosystems, where many dominant plants like willows are ectomycorrhizal, environmental change may have strong effects on EcM plant hosts and their associated fungal communities (Donhauser and Frey, 2018). At a regional scale, the richness of alpine EcM fungal communities has been shown to be positively correlated with plant species richness,

suggesting that EcM fungal diversity is an important driver of ecosystem functioning (Pellissier et al., 2014, 2013). Several studies have addressed this question at host family level (Ishida et al., 2007; Smith et al., 2009; Tedersoo et al., 2008), but our understanding of the interaction network between closely related plant hosts (i.e. at genus or intraspecific level) and their associated EcM communities is still very limited. Host identity in willows, when different willow species co-occurred in similar environmental conditions, had little influence on soil fungal community composition (Erlandson et al., 2018, 2016). Arctic and alpine willows have also shown low host specificity compared to other co-occurring EcM plants (Botnen et al., 2014; Ryberg et al., 2011, 2009). Consequently, the variability of EcM fungal communities across the distribution of these shrub willows has been linked predominantly to abiotic factors so far. In addition to host species richness and identity, plant-host genetic variability may also affect EcM fungal communities although studies show variable results. For instance, EcM fungal community composition associated with differently drought-tolerant genotypes of *Pinus edulis* was strongly influenced by host plant genetics (Gehring et al., 2017) while host genotype in *Populus* clones had little effect in determining the structure and composition of their fungal symbionts (Karliński et al., 2013). These contradictory and context-dependent results highlight how restricted is our understanding about how plant host genetic structure interacts with the environment to shape EcM fungal communities.

Ectomycorrhizal fungal communities may be influenced by host hybridisation, but this has been rarely tested, even though increasing plant genetic variation and its associated phenotypes may influence species interactions and ecosystems processes (Bailey et al., 2009; Crutsinger et al., 2006). Host genotypic variation can result in different phenotypes that putatively favour specific associated organisms, while in return these organisms feedback differently on the plant host (Whitham et al., 2012). The combination of different species

genotypes in hybrids has the potential to generate much greater genetic variation than that found in the parents (Whitham et al., 1999); in fact, genetically based variation in the phenotype of hybrids (e.g. leaf surface, root structure or density) can influence belowground communities. In a study of cottonwoods and their hybrids, controlling for environmental effects, host genotype played a minor role in mycorrhizal colonization compared to environmental factors (Gehring et al. 2006). Nevertheless, natural hybridisation and introgression create a genetic continuum between the two parental species that is ideal for examining changes in EcM community structure and composition. Thus, studying EcM fungal communities associated with congeneric hosts and their hybrids in alpine systems could provide further insights into host specificity across plant hybrid zones and a deeper understanding of the role of EcM fungi in the resilience of these habitats to environmental change.

Network analysis at the population and genotype levels allows to identify key interactions in plant-fungal mutualistic relationships in an ecological framework. Species may show varying levels of preference and/or specialization, resulting in certain network structures in which some species are more or less frequently connected than expected from random interactions. Nestedness and modularity indices are commonly used to characterize plant-fungal networks (Bahram et al., 2014; Toju et al., 2016, 2015, 2014, 2013). Nestedness measures the tendency of specialist nodes in one level of the network (plants or fungi) to interact with generalist nodes in the other level to infer the generalist-specialist balance in the community. Modularity allows inferring the existence of groups of species that form more closely interacting communities within the entire network and whether interactions within these groups are more common than among groups (Almeida-Neto and Ulrich, 2011; Guimerà and Nunes Amaral, 2005). Thus, network analysis can generate new insights into the structure of complex fungal-host communities complementing and expanding our

knowledge about descriptive measures of alpha and beta diversity (Barberán et al., 2012). Extensively applied to the study of community structure in plant-fungal interactions (Caruso et al., 2012 ; Bennett et al., 2013; Bahram et al., 2014; Toju et al., 2013; Pölme et al., 2018), network analyses could provide information on fungal niche preference and its role on plant settlement in harsh and heterogenous alpine habitats.

The assessment of EcM fungal community diversity and dynamics has been transformed by the advent of new high-throughput sequencing (HTS) techniques targeting the ITS1 or ITS2 regions. Despite generating millions of DNA sequence reads across numerous samples, the use of these data has its own limitations, such as the inability to discern between dead, dormant or active sources of DNA template, which combined with the PCR-based nature of these techniques, can lead to potentially biased observations (Hawksworth and Lücking, 2017; Lindahl et al., 2013; Nguyen et al., 2015; Wutkowska et al., 2019). Moreover, the use of HTS on bulk soil samples does not generate direct evidence of plant-fungus associations. Therefore, comparing direct sequencing of ectomycorrhizas with HTS of soil samples potentially offers robust and in-depth complementary views of EcM diversity and allows to infer host preference for the EcM fungi inoculum available in soil.

Alpine willows (*Salix* spp.) offer an excellent opportunity to compare the EcM communities in congeneric species and their intermediate individuals in hybrid zones given their genetic and ecological differentiation. In this study, we focus on *Salix helvetica* Vill., a shrub that occurs naturally in the sub-alpine to alpine zone, and *S. purpurea* L., a widespread lowland species, able to colonize higher elevations due to global warming and sub-sequent glacier retreat (Gramlich et al., 2018, 2016). Using plant microsatellite markers, Gramlich et al. (2016) analysed the composition of two hybrid populations of *S. purpurea* and *S. helvetica* in the Swiss Alps and found evidence for a recent origin of the hybrids. The fine-scale environmental variation common in alpine ecosystems offered many unoccupied niches for

hybrid establishment. The hybrids seemed to have a broad ecological amplitude and were able to grow under more extreme conditions, regarding soil pH, moisture and nutrient supply, than either parental species, enabling the coexistence of both parental species and the hybrids in a patchy habitat. Therefore, we designed this study to i) characterize the EcM communities that associate with *S. helvetica*, *S. purpurea* and their hybrids in one of the valleys included in Gramlich et al. (2016) and ii) elucidate the main abiotic and biotic factors that affect the structure and composition of EcM communities in a hybrid zone. Furthermore, given their ability to colonize different niches in a very restricted geographical area, we hypothesized that (1) hybrids and parental willows associate with different EcM fungi, and (2) within the same valley, host genotype explains most EcM community variability.

Materials and Methods

Sampling

The sampling location was in the Rhône glacier valley in southern Switzerland (46°34'03.0'' N, 08°22'12.3''E). Soil geological properties at the valley are mainly dominated by granite and granodiorite parent materials (Oberhänsli et al., 1988), formed from the unconsolidated glacier and colluvial deposits, building eutric dystric regosols (FAO-UNESCO, 2007). The sampling area was *ca* 0.14 km² with a maximum distance between sampling sites of one km (Fig. 1) ranging between 1,775 and 1,800 m a.s.l.. The EcM plant community included other *Salix* spp., *Alnus viridis* (Chaix) DC., *Larix decidua* Mill., *Picea abies* (L.) H. Karst. and *Betula* spp.

Previously genotyped individuals of *Salix helvetica*, *S. purpurea*, and their first-generation hybrids (*S. helvetica* $\times$ *purpurea*) were selected based on Gramlich et al. (2016). In total, roots of 97 individual adult plants (*S. helvetica*: 31, *S. purpurea*: 33, hybrids: 33) distributed across 38 sites were sampled. The hybrid populations were max. 20-30 years old

(Gramlich et al., 2016), with plants about 50-200 cm high, and distributed over the valley in a mosaic-like pattern. *Salix purpurea* grows at the more alkaline, nutrient-rich and warm sites, while *S. helvetica* occupies more acidic, nutrient-poor and colder sites. Their hybrids occur at the most extreme, acidic and nutrient-poor sites (Gramlich et al., 2016). Sites were defined as locations where individual(s) from the same or different host co-occurred within a distance of 8 m.

At least 16 roots from each individual plant were tracked from the stem, where the rocky ground permitted, carefully excavated, and stored in plastic bags at 4°C for up to seven days until further processing. Approximately 100 cm³ of soil was collected with a spade under each individual sampled plant for chemistry and bulk soil EcM community analyses. Soil samples for DNA community analysis were stored at 4°C for up to 7 days, then stored at -80°C, and freeze-dried before processing.

Environmental data

For soil chemistry analyses, we collected soil samples down to 10 cm beneath each plant. Samples were dried at 40–60°C and sieved with a 2 mm mesh. Soil pH was measured potentiometrically in 0.01M CaCl₂. Total carbon (C_{total}) and total nitrogen (N_{total}) contents were measured in ground samples by dry combustion using a C/N analyser NC 2500 (CE Instruments, Italy). All soil chemistry analyses were conducted at the Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf (WSL). Geographic coordinates and ectomycorrhizal plant community composition were recorded within a radius of 10 m from each sampled individual plant.

Mycorrhizal root assessment

200 Root samples were rinsed in water and 16 EcM tips from 16 different roots traced
201 from the stem were selected from each individual plant when possible. The presence of
202 hyphae and/or rhizomorphs was recorded (Agerer 2001; 2006) before DNA extraction.
203 Genomic DNA was extracted from individual ectomycorrhizas using Extract-N-Amp (Sigma-
204 Aldrich, Darmstadt, Germany) and the ITS region of the rDNA was amplified using the
205 fungal-specific primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990).
206 Amplicons were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and sequenced
207 bidirectionally using BigDye v. 3.1 (Applied Biosystems, Foster City, CA, USA) in an ABI
208 3730 (Applied Biosystems).

209 Sequences were first analyzed with KB basecaller v1.1.1 (Applied Biosystems) and
210 bases called with minimum quality value (QV) of 20 (i.e. 99% of base call accuracy).
211 Forward and reverse sequences from each ectomycorrhiza were assembled using *phrap*
212 v1.090518 (de la Bastide and McCombie, 2007). When the assembly of both sequences was
213 not possible due to the poor quality of one of the sequences or to uncertainty determining
214 their overlapping region, a high quality base-pair ($QV \geq 20$) per sequence length ratio was
215 applied and the longest sequence (min. 200 bp) with ratio scores $\geq 85\%$ was selected for
216 further analyses. To avoid misleading results in the DNA sequence identification process and
217 to facilitate direct comparison with the fungal community in the soil, adjacent conserved
218 regions (18S, 5.8S and 28S) were removed using ITSx v1.0.11 (Bengtsson-Palme et al.,
219 2013) and the ITS2 region was selected for further analyses. Sequences were clustered in
220 OTUs at a 97% similarity threshold using the UPARSE algorithm implemented in
221 USEARCH v9.2 (Edgar, 2013), while simultaneously excluding chimeric sequences. The
222 taxonomic affiliation of each OTU was inferred by blasting each OTU centroid sequence to
223 the UNITE fungal ITS sequence database v7.2 as a reference for assignment of a species
224 hypothesis (Kõljalg et al., 2013; Nilsson et al., 2011). For centroids with less than 97% blast

id we used the SINTAX algorithm (Edgar, 2016) with a 0.8 cut-off to predict OTU taxonomy. The ecological functions of each OTU (i.e. trophic level and type of mycorrhizal association) were assigned according to UNITE (Kõljalg et al., 2013) and only EcM fungi were used in downstream analyses. Representative sequences of each EcM OTU were deposited in NCBI under accession numbers MK838121-MK838189.

Soil DNA analysis

To analyse the soil fungal communities, we collected soil adjacent to the roots. Samples were sieved (2 mm mesh) and genomic DNA was extracted from 25 mg of 97 freeze-dried soil samples using the DNeasy PowerSoil Kit (Qiagen, Valencia, CA) and quantified using the Qubit 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplifications of the ITS2 region using the primer set fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) were run in triplicates and pooled for library preparation. The PCR, library preparation and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA) were conducted by Génome Québec Innovation Center at McGill University (Montréal, Canada).

Quality filtering and clustering into OTUs was performed using a customized pipeline based on UPARSE implemented in USEARCH (Edgar, 2013), but with some additional modifications as follows. Paired-end reads were merged using the USEARCH fastq mergepairs algorithm (Edgar and Flyvbjerg, 2015) allowing staggered alignment constructs in order to accommodate potentially short ITS2 amplicons. The PCR primers were detected and trimmed using Cutadapt (Martin, 2011) allowing for one mismatch. Reads not matching the primers or with lengths below 150 bp were discarded. Trimmed reads were quality-filtered using the USEARCH fastq filter function with a maximum expected error threshold of one. Sequences were de-replicated to retrieve information on abundance distribution, and

singleton reads were removed prior to clustering in order to avoid artificial OTU inflation. Sequences were clustered into OTUs at 97% sequence identity using the USEARCH cluster_otu function that includes an ‘on-the-fly’ chimera detection algorithm. The OTU centroid sequences were subjected to an additional round of chimera filtering by running UCHIME against the uchime version of the UNITE database. The remaining centroid sequences were tested for the presence of ribosomal signatures using ITSx (Bengtsson-Palme et al., 2013) and 33 (out of 2708) centroid sequences with the ribosomal origin not sufficiently supported were discarded. Finally, all quality-filtered reads were mapped against the final set of centroid sequences using the usearch global algorithm with the most comprehensive search criteria (maxrejects 0, maxaccepts 0 and top hit only). The taxonomic affiliation of each OTU was inferred by blasting each OTU centroid sequence to the UNITE fungal ITS sequence database v7.2. For centroids with less than 97% blast id we used the SINTAX algorithm (Edgar, 2016) with a 0.8 cutoff to predict OTU taxonomy. Representative sequences of each EcM OTU were deposited in NCBI with accession numbers MK838190-MK838412 as well as raw HTS reads deposited in the Sequence Read Archive under the accession number PRJNA575244.

Alpha and beta diversity of EcM fungi

Analyses were carried out using R version 3.5.1 (R Development Core Team 2009). Sequence and read numbers from both root and soil community matrices were normalized using the cumulative sum scaling (CSS) method as implemented in the *metagenomeSeq* package (<http://www.cbcb.umd.edu/software/metagenomeSeq>). Observed OTU diversity and the abundance-based estimators Chao1 and ACE were calculated using the *estimateR* function of the *vegan* package (Oksanen et al., 2019). Differences between observed and estimated values among hosts were assessed by one-way ANOVA and Tukey’s HSD *post*

hoc tests. The percentage of OTU diversity recovered was calculated taking the proportion of OTUs observed from the number of estimated (ACE) OTUs per host. Normal distribution of the residuals and homogeneity of variance were evaluated by analysing *qqnorm* plots and significant deviations of Shapiro and Levene test routines implemented in R. Data with non-normal distribution were square root or log transformed when necessary.

To assess the proportion of shared OTUs between root and soil datasets, we performed a local blast using the *blastn* algorithm with the soil OTUs centroids as reference and the root OTUs centroids as queries. The OTUs with blast hits superior or equal to 97% and with congruent taxonomy (i.e. equal Species Hypothesis number) were considered equivalent.

The local contribution for beta diversity (LCBD) was calculated to measure the degree of uniqueness of each sample to the variation in community composition following the method described by Legendre & De Cáceres (2013) and implemented in the *microbiomeseq* package, grouping OTUs by their corresponding EcM lineage according to UNITE. Non-metric multidimensional scaling (NMDS) ordination was used to visualize dissimilarities in EcM communities across different plant hosts and in both root and soil datasets using Bray-Curtis dissimilarity distances. Permutation analysis of variance (PERMANOVA) was used to assess differences between groups (3 hosts x 2 datasets, roots or soil) using the *adonis* function and significant differences among pairwise homogeneity of group dispersions (variances) were calculated using *betadisper*.

Plant-fungal network analysis

To explore the interaction network between EcM fungi and their host plants, we computed three ecological network indices (Modularity, Nestedness and C-score) using the *bipartite* package for R (Dormann et al., 2008). Modularity and nestedness were calculated

for both binary and weighted matrices. We computed modularity using the QuaBiMo algorithm (Dormann and Strauss, 2014) to identify aggregated sets of interacting OTUs (i.e. modules). To further investigate the architecture of the bipartite network, we calculated nestedness using “NODF” and “weighted NODF” indices (Almeida-Neto and Ulrich, 2011) for the binary and weighted matrices, respectively. In bipartite networks, nested communities (i.e. with values towards 1) are characterized by specialist nodes in one level of the network (e.g. EcM fungi) that connect to generalist nodes of the other level of the network (e.g. plant hosts) but never with other specialists and *vice versa* (Bascompte et al., 2003). To analyse co-occurrence patterns and determine the randomness of the OTU distribution through the sampling area, we computed the checkerboard score (C-Score, Lewi & Alan, 1990) among all pairs of nodes at the lower level of the bipartite network, i.e. among all fungal OTUs.

To assess the significance of all network indices calculated, we generated 1,000 matrices from a conservative “fixed-fixed” null model constraining row and column marginal sums as implemented in *vegan::nullmodel* and computed *p*-values and standard scores (*z*-scores) over the deviations of the observed values with those predicted by the null models. The C-score was calculated using only the binary matrix. Bipartite networks from both root and soil datasets were visualized using the *igraph* package.

Hierarchical modelling of EcM communities

To assess the dependency of each fungal OTUs on the environmental conditions, we applied a hierarchical joint species distribution model approach (Ovaskainen et al., 2017). This framework allows to assess how much variation in each OTU occurrence is due to environmental filtering, biotic interactions and random processes. Furthermore, this framework assumes that overall OTU responses to environment adhere to a multivariate normal distribution allowing the framework to generate community-level summary statistics

on its response to environmental variability. To model the distribution of EcM fungal OTUs, we used soil chemical properties such as, soil pH, total nitrogen (N_{total}) and total carbon (C_{total}), and to account for other fungal interactions we used Chao1 estimates of soil total fungal community ($S_{fungi.total}$) and soil EcM community ($S_{fungi.EcM}$). As random effects, we used sampling site and plant host identity. We used the *bestNormalize::bestNormalize* function (Peterson, 2017) to determine and apply the ordered quantile normalizing transformation to N_{total} and C_{total} , and the square root to $S_{fungi.EcM}$ covariates. We ran two independent models for each of the datasets (roots and soil) using the binary (presence/absence) or the relative abundance matrix. Model priors were set to default and family distribution to probit for the binary models and lognormal Poisson distribution for the abundance variants. Parameter estimation was achieved by Markov Chain Monte Carlo (MCMC) posterior sampling for 100,000 iterations, 10,000 burning and 10 thinning. Parameter convergence was checked by trace visualization. To assess the level of statistical support for whether the probability of an OTU abundance increased or decreased with the increasing value of a given environmental covariate, we defined the 95% central credible interval by computing the 0.025 and 0.975 quantiles of each parameter. The explanatory power of the models was calculated using the coefficient of discrimination $T_{jur} R^2$ (Tjur, 2009) for each individual fungal OTU and its average at community level.

To assess how much of the variability in fungal OTU occurrence was due to biotic, abiotic or random processes, we partitioned the variance of the explained portion of the model with higher explanatory power by grouping covariates as abiotic (soil pH + N_{total} + C_{total}), biotic ($S_{fungi.total}$ + $S_{fungi.EcM}$) and for each covariate independently using the *variPart* function of the *HMSC* package.

Results

Soil chemistry

Values of soil pH ranged from 3.58 to 5.59, with significantly lower values observed under *S. helvetica* than *S. purpurea* ($P = 0.019$; Fig. 2a). Total soil nitrogen across all sampling sites ranged from 0.028% to 2.4% and soils under *S. purpurea* showed significantly higher values than those under hybrids ($P = 0.012$; Fig. 2b). Total soil carbon values ranged from 0.46% to 42.5% with soils collected under *S. purpurea* showing significantly higher values than hybrids ($P = 0.047$; Fig. 2c).

Alpha and beta diversity of EcM fungi

A total of 1,233 ectomycorrhizas were sampled from the roots of 82 out of 97 individual plants (*S. helvetica*: 27, *S. purpurea*: 27, hybrids: 28) distributed across 37 of the sampling sites. We retrieved 1,076 DNA sequences, from which we kept 1,044 high quality sequences belonging to EcM lineages (*sensu* Tedersoo et al., 2010; Tedersoo & Smith, 2013). After clustering and filtering, we identified 69 OTUs (average per individual 3.42 ± 1.52) across 14 phylogenetic lineages. Approximately 90% of the EcM fungi in roots belonged to Basidiomycota and 10% to Ascomycota. The EcM lineages with higher OTU richness were *Tomentella-Thelephora* (28 OTUs), *Cortinarius* (16 OTUs), *Inocybe* (5 OTUs) and *Hebeloma* (5 OTUs).

From soil samples, a total of 9,036,820 raw DNA reads were generated, from which we retrieved 3,099,112 high quality ones. In total, we identified 2,078 fungal OTUs from which we kept 223 (average per soil sample 9.30 ± 6.32) belonging to 32 EcM fungal lineages after removing OTUs from non-EcM lineages and 22 EcM OTUs known to be specialists of conifers s.l. or *Alnus* spp. (Nilsson et al., 2019; Tedersoo et al., 2009) and that were not detected in the roots. The EcM lineages with higher OTU richness found in the soil

were *Cortinarius* (51 OTUs), *Tomentella-Thelephora* (47 OTUs), *Inocybe* (22 OTUs),
Sebacina (15 OTUs), *Russula-Lactarius* (13 OTUs) and *Laccaria* (12 OTUs).

Fifty-five EcM OTUs were detected in both roots and soil, whilst 14 were found only
in roots and 168 only in soil (Table S1). Across all sites, we observed 40 EcM OTUs in roots
of *S. purpurea*, 44 in roots of hybrids, and 48 in *S. helvetica* roots. In the soil, we found 142
EcM OTUs under *S. purpurea*, 143 under hybrids, and 141 under *S. helvetica*. No significant
differences were observed when comparing observed and estimated (ACE) OTU richness
among hosts (data not shown). The dominant EcM fungal lineages forming ectomycorrhizas
were *Tomentella-Thelephora*, *Cenococcum* and *Cortinarius*, whilst in the soil the most
abundant reads belonged to *Tomentella-Thelephora*, *Cortinarius* and *Russula-Lactarius* (Fig.
3).

Ordination results showed no significant dissimilarities among hosts in the EcM
communities found in roots or in soil (Fig. 4). However, differences in community
composition were observed when comparing root with soil datasets within hosts (Fig. 3).
These results were corroborated by adonis ($F = 3.337$, $R^2 = 0.097$, $P = 0.001$) and by pairwise
betadisper analysis (Table S2).

Plant-fungal network analysis

Using the QuaBiMo algorithm we identified three network compartments (i.e.
modules) both in the soil EcM fungi (Fig. 5a, Soil) and in the roots (Fig. 5b,
ectomycorrhizas) using presence-absence and abundance association matrices (Fig. 5).
Modularity likelihood (Q) observed values in the network between willows and EcM fungi in
roots did not show significant differences from the null models ($Q_{\text{root.bin}} = 0.396$, $P = 0.112$;
 $Q_{\text{root.weighted}} = 0.242$, $P = 0.073$). In the association network between willows and EcM fungi
from soil samples, significant differences from the null models were observed when using the

weighted matrix ($Q_{\text{soil.bin}} = 0.388$, $P = 0.129$; $Q_{\text{soil.weighted}} = 0.329$, $P < 0.001$, $z\text{-score} = 6.167$) (Table S3).

The network nestedness (NODF) values observed in the soil and roots datasets were similar but only the roots x willows network showed significant differences from the null models ($\text{NODF}_{\text{roots}} = 59.2$, $P = 0.032$, $z\text{-score} = -0.061$; $\text{NODF}_{\text{soil}} = 58.5$, $P = 0.420$). Using the weighted matrices, the observed nestedness (WNODF) values were lower and only the soil observed values in the soil fungi x willows was significantly lower than expected by the null models ($\text{WNODF}_{\text{roots}} = 37.7$, $P = 0.315$; $\text{WNODF}_{\text{soil}} = 39.2$, $P < 0.001$, $z\text{-score} = -2.994$).

The C-score analysis revealed significant deviations from expected by the null models in the soil fungi x willows network, but not in the fungi found in the roots as ectomycorrhizas x willows network ($\text{C-score}_{\text{roots}} = 0.502$, $P = 0.404$; $\text{C-score}_{\text{soil}} = 0.511$, $P = 0.003$, $z\text{-score} = 2.127$).

Hierarchical modelling of EcM communities

The set of variables used in this study were on average better predictors of the EcM fungal OTU occurrences in the soil than in the roots as ectomycorrhizas. The explanatory power (R^2) of the models using the soil EcM community data was on average 0.06 (95% CI: 0.05 - 0.07) and 0.07 (95% CI: 0.04 - 0.10) for the presence-absence and abundance data matrices, respectively. In the case of the root EcM community data, R^2 was on average 0.06 (95% CI: 0.05 - 0.07) and 0.05 (95% CI: 0 - 0.09) for the presence-absence and abundance data matrices, respectively. Due to lack of reliability of the models based on the abundance data, we only considered the results of the presence-absence models.

The partitioning of the overall explained variance in the presence-absence models revealed that sampling site and plant host species (random effects) explained the majority of the variation in both datasets (Fig. 6). In the root dataset, on average, 51% of the variation in

the data was explained by plant host (41%) and site (10%), whilst the abiotic variables explained 35% ($C_{total} = 14\%$, $N_{total} = 13\%$, and soil pH = 8%) and the biotic variables 14% ($S_{fungi, total} = 6\%$ and $S_{fungi, EcM} = 8\%$). In the bulk soil dataset, approx. 46% of the variation in the data was explained by plant host = 32% and site = 14%. The proportion of variance explained on average by fixed effects in this dataset was 36% by abiotic variables ($C_{total} = 15\%$, $N_{total} = 15\%$ and soil pH = 6%) and 18% by biotic variables ($S_{fungi, total} = 13\%$ and $S_{fungi, EcM} = 5\%$).

Discussion

Our findings indicate that in the Rhône glacier valley, host identity did not have a strong influence in richness and composition of the EcM fungal communities associated with *S. helvetica*, *S. purpurea* and their hybrids, despite host habitat/niche preferences. Lack of host specificity among willows has been previously reported beyond the Alps (Botnen et al., 2014; Erlandson et al., 2018, 2016; Ryberg et al., 2009); for instance, Ryberg et al. (2011) found similar EcM communities associated with *Salix polaris* and *S. herbacea* in a Swedish alpine tundra. In a study comparing *Salix viminalis* growing in arable soils versus adjacent natural or naturalized stands in Sweden, Hryniewicz et al. (2012) detected site and host identity effects on EcM root colonisation, but no effects on EcM fungal abundance and diversity. In this study we aimed to investigate how host intra-specific genetics through hybridization could influence EcM fungal communities. Long-term studies of *Pinus edulis* populations have demonstrated an effect of host plant genetics on EcM community composition (Gehring et al., 2014, 2017). Direct or indirect influence of host genotype has also been reported in *Picea abies*, suggesting that individual spruce trees are partly responsible for the high diversity and patchy distribution of EcM communities in boreal forests (Korkama et al., 2006; Velmala et al., 2013). However, despite the ability of hybrid

willows to colonize different ecological niches than their parental types (Gramlich et al., 2016), they do not seem to associate with different EcM communities. This lack of host specificity in arctic and alpine ecosystems might be a mechanism that favours plant hosts to more rapidly and easily colonize newly available habitats, favoured by the establishment of symbiotic relationships with fungi with different physiological attributes (Botnen et al., 2014). Nevertheless, we cannot disregard the overall role EcM fungi may play on the colonization and settlement of the hosts in these habitats.

The high soil heterogeneity characteristic of alpine ecosystems might also influence EcM fungi and mask differential host effects on EcM composition and structure. In our study we observed variation in soil pH, C and N content, leading to the presence of many microhabitats with distinct edaphic characteristics. The same soil heterogeneity was also highlighted by Gramlich et al. (2016) reporting that despite its recent emergence, the hybrid population in the Rhône Glacier forefield occupies ecologically distinct sites with respect to the parental species, scattered over the whole area of the alluvial plains in a mosaic-like spatial pattern. Our results revealed however that the dominant EcM fungi colonising roots were shared among parents and hybrids; these fungi may be better adapted to the extreme environmental characteristics in sub-alpine habitats than host-specialists, thus explaining the lack of plant-fungal specificity or preference observed across willows. Nara & Hogetsu (2004) compared the growth rate of *Salix reinii* seedlings growing next to already established willow shrubs and observed that EcM fungi associated with the latter were essential in facilitating seedling establishment of later-successional plant species. When comparing EcM fungal community composition in roots (ectomycorrhizas) versus fungi in soil, Goldmann et al. (2016) observed that plant host neighbour effects were stabilizers of fungal community composition. They showed less distance decay in root-associated fungal communities compared to the soil fungal communities in a beech-dominated forest, suggesting that host

trees could buffer the effects of changes in microclimatic and environmental conditions that could directly influence fungal community composition in soil. In our study, other EcM hosts, including other willow species, present in the valley may have prevented EcM community differentiation among hosts by being a permanent source of diverse generalist inoculum.

Fungal community richness and composition

On average, we recovered 89% of the estimated EcM diversity (ACE) in willow roots (87% in *S. helvetica*, 90% in hybrids and 91% in *S. purpurea*). Despite the harsh environmental conditions in sub-alpine habitats, these willows harbour diverse EcM fungal communities within a relatively small geographical area. As suggested by previous studies, the high diversity of EcM fungi in alpine and sub-alpine habitats might be linked to the high diversity of EcM plant hosts in these habitats (Krpata et al., 2007; Pellissier et al., 2014), moreover, hybrid zones are considered centers of biodiversity for many organisms (Whitham et al., 1994).

As expected, due to the presence of other EcM hosts in the valley and probably to the methodological bias from using rDNA in bulk soil samples that does not discriminate active, dormant or dead organisms, and intra- versus extra-cellular DNA template sources (Carini et al., 2016; Wutkowska et al., 2019), the EcM OTU richness found in the soil was significantly higher than in ectomycorrhizas (142 OTUs under *S. purpurea*, 143 under hybrids and 141 under *S. helvetica* compared to 40, 44 and 48 in their roots, respectively). Nevertheless, the EcM community richness and composition observed did not differ significantly across hosts, following the same pattern observed in roots. Collecting data from both ectomycorrhizas and from bulk soil allowed testing for fungal specificity or preference of the three hosts from the potential fungal inoculum available. Our results indicate that a small proportion of the full

potential inoculum (approx. 25%) was actually recruited by the plants. Similar rates of recruitment (27%) were also inferred by Goldmann et al. (2016) in a temperate beech forest. In contrast, approximately 80% of the OTUs observed as ectomycorrhizas were detected in the soil.

Soil has been suggested as a good proxy for estimating fungal richness at regional scales (Landeweert et al., 2005); however, the inability to detect in soil some dominant fungi associated with roots (e.g. *Cenococcum geophilum*, ca. 32%) reveals the bias and risk of using only bulk soil data as a source of fungal community information. *Cenococcum geophilum* is an anamorphic complex of species that associates with a vast range of host plants globally, forming abundant black sclerotia in soil (Obase et al., 2017) and increasing host plant drought tolerance (Jany et al., 2003; Pigott, 1982). Studying bulk soil communities without accounting for autecology, potential methodological biases, target limitations of genetic markers, and bioinformatic challenges, can lead to biased views of EcM communities, artificial results and misleading conclusions (Lindahl et al., 2013). In this study, three OTUs of *C. geophilum* were among the most abundant and conspicuous fungi observed in roots (Fig. 3), as in other studies sampling ectomycorrhizas in alpine glacier valleys (Krpata et al., 2007, Mühlmann et al., 2008, Mühlmann & Peintner, 2008). However, our results are also congruent with Pellissier et al. (2014), Rime et al. (2015) and Frey et al. (2016) where *C. geophilum* was not detected in soil. This might be due to primer bias, inability of DNA extraction methods to break thickly melanized cell walls, removing larger sclerotia through sample sieving before DNA extraction, and/or ephemeral or sporadic hyphal growth in soil from a stable population of ectomycorrhizas. To examine whether some of these issues could have contributed to the non-detectability of *C. geophilum* in our soil dataset, we i) manually verified the identity of the binding region of the fITS7 primer in all *C. geophilum* OTUs found in roots and the respective UNITE reference sequences; ii) verified

that no hits of *C. geophilum* were observed using Blast on all centroid sequences from the soil dataset against the UNITE+INSD v8.0 database, and iii) verified the detection of *C. geophilum* in other studies using the same DNA extraction kit and similar analyses in soil (Kirker et al., 2017), roots (Evans et al., 2015), mesh bags (Ning et al., 2019) and cultures (Peter et al., 2016). Thus, our findings may reflect limited extraradical growth by a dominant EcM fungus.

Plant-fungal network structure

Network modularity, which can be directly attributable to partner selectivity, measures how aggregated some sets of interacting species can be in a community (Dormann and Strauss, 2014) while nestedness measures the degree of interaction of specialists in one guild with generalists in the other guild (Bascompte et al., 2003). In the Rhône Glacier valley, plant-fungal networks of willow hosts vs EcM fungi are non-modular and unnested. The networks of soil fungi x willows and root fungi x willows revealed low likelihood values of modularity and no significant deviations from the null models, except when using the weighted matrix of soil fungi x willows (Table S3). The lack of modularity in plant-EcM fungi networks has been previously suggested and inferred to be context-dependent, varying with species identity and potentially with the phylogeny of both partners (Bahram et al., 2014; Pölme et al., 2018). Moreover, modularity indexes are known to be sensitive to network size, as networks with many species and links allow for more possible combinations of species-in-modules, leading to higher values of modularity (Allesina and Pascual, 2009). Similarly to our study, Pölme et al. (2018) also found weak modularity values in EcM fungal networks due to a low number of hosts.

When using the weighted version (i.e. abundance data) of the soil fungi vs willows matrix instead of the binary one (i.e. presence-absence), we observed significantly higher

modularity than expected by the null models. We believe this can be an artefact due to difficulties in the process of generating null models for abundance matrices. We generated our null models constraining matrix row and column sums, but due to the high discrepancies in read numbers (even though CSS-normalized) and to the small number of hosts (i.e. reduced matrix size), the randomization scheme was very constrained, thus increasing the probability of type I errors (Lavender et al., 2016).

In accordance with other studies (Bahram et al., 2014; Toju et al., 2014; Pöhlme et al., 2018), we found that the network of plant hosts vs EcM fungi was unnested. We detected higher values of nestedness when analysing the binary matrices and more significant differences than expected by the null models using the binary matrix from the roots and the weighted matrix from the soil. Despite these differences, none of the indexes were indicative of nestedness in our networks (i.e. values towards 1 indicate nestedness). Similarly to modularity, measuring nestedness in plant-EcM fungal networks is challenging due to specific matrix properties (e.g. matrix size) (Bascompte et al., 2003; Pöhlme et al., 2018).

The C-score analyses did not show significantly higher values than the null models in the ectomycorrhizas, but it did for the EcM fungi detected in soil. The C-score analysis averages the number of checkerboard units (i.e. exclusive occurrences) between all pairs of species in the matrix (Stone and Roberts, 1990). In a competitively structured community, the observed C-score is significantly higher than that generated by null models. Lack of significance suggests that species co-occur randomly, and significant lower C-score than expected from null models indicates species aggregation (Gotelli and Entsminger, 2001). The lack of significance found in ectomycorrhizas suggests that the community is not competitively structured, so species occurrences do not rely on other species co-occurrences. However, for soil EcM fungi, the C-score values suggest a non-random distribution of species and that this community was competitively structured. Non-random distributions of

EcM communities (i.e. higher observed C-scores than expected) were previously described by Koide et al. (2004) who found that some EcM fungal species occurrences were negatively correlated, suggesting that these negative interactions among species at small scales could affect community structure. Using the same C-score analysis, Pickles et al. (2012) found that EcM communities in roots of Scots pine in a forest plantation in Scotland were strongly structured by competitive interactions, or ecological processes generating a similar spatial pattern, rather than neutral processes. We only obtained significantly higher observed C-scores than expected in the bulk soil dataset; however, describing the dynamics of soil fungal communities based on environmental DNA may be biased as it does not discriminate between metabolically active cells, dead biomass, or dormant structures such as spores.

Biotic and abiotic drivers of EcM communities

The joint species distribution modelling revealed that the occurrences of EcM fungi in the Rhône glacier valley were shaped by a complex grid of biotic and abiotic drivers. Despite the number of environmental variables measured, the majority of the variability in EcM fungal community composition remained unexplained. The high soil heterogeneity, the effects of the abiotic factors across fine-scale microhabitats characteristic of alpine ecosystems and the dominance of generalist fungi might partly explain this. In microbial communities, habitat generalists respond mostly to spatial variables rather than to local environmental variability (Luo et al., 2019; Pandit et al., 2009). Thus, given the relatively small scale of the present study and the dominance of EcM fungi that are habitat generalists, the explained variability in EcM fungal community composition should not be overlooked.

We found a weak effect of host on EcM fungal communities associated with the willows across the valley. However, when exploring the factors influencing the explained

variability of the overall EcM fungal community distribution models, host identity emerged as the best predictor of EcM community composition. On average, plant host explained the larger variance on the occurrence of each EcM OTU in both soil and roots, but its influence on the communities might be masked by the different characteristics of the ecological niches in which these plants occur. In contrast, when using relative abundance in the models instead of presence/absence (Fig. S1), site emerged as a better predictor of OTU relative abundance than host, indicating high spatial variation of EcM communities. Using relative abundance data from HTS read counts, Collins et al. (2018) observed that fungal diversity and relative abundance had high spatial variation, overwhelming the predictive power of other abiotic factors. Moreover, Feinstein & Blackwood (2012) found high spatial variation in forest floor fungal communities and little explanatory power of plant traits or plant species identity. Even though using relative abundance data from communities is becoming more frequent than using presence-absence data, without reliable abundance data from HTS technologies, understanding the drivers of fungal community composition and function will remain limited (Friedman and Alm, 2012; Taylor et al., 2016).

Variation partitioning revealed that host and site effects were the main predictors of the explained variation in the overall EcM fungal community composition. However, the presence-absence of some OTUs in particular was mainly explained by soil chemistry and soil fungal community. Individual OTU responses to environmental factors help to better understand EcM fungal community resilience to environmental change, in particular, across the European Alps where complex geomorphology and an array of microclimates contribute to a wide variety of habitats and high levels of biodiversity. For instance, the occurrences of *Russula emetica* (OTU_029) and *Tomentella* sp. 11 (OTU_053) ectomycorrhizas were predominantly but differently explained by soil chemistry and soil fungal community ((Fig.

6b), revealing that the relative importance of environmental and stochastic effects varies sharply among taxa.

Conclusions

Overall, our results indicate that alpine willow EcM communities are highly diverse and dominated by generalist fungi. These communities are non-modular, unnested and they are not competitively structured. Pairing two detection approaches for EcM fungi, we identified their strengths and weaknesses; caution is needed when analysing and extrapolating results from bulk soil DNA that can miss dominant fungi. Hybrids did not associate with significantly different EcM communities than their parental species despite their ability to colonise different ecological niches. When accounting for the effects of environmental variables, we found a differential effect of host on EcM composition, that seems to be masked by the high soil heterogeneity in the valley. Further studies increasing sampling effort to additional valleys along a wider gradient of environmental variables, and including manipulative experiments, are now needed to disentangle the biotic and abiotic factors shaping EcM fungal communities in alpine zones.

Acknowledgments

This research project was funded by Winton Philanthropies towards the Harding Alpine Plant Conservation & Research Programme. We acknowledge Lazlo Csiba (RBG, Kew), Fiachra Sweeney (Imperial College London) and Ross Scambler (IBER, Sofia) for laboratory assistance, Aline Frossard (WSL, Birmensdorf) for NGS data analysis, to the scientists at the McGill University and Génome Québec Innovation Centre, Montréal, Canada, for NGS, to the Genetic Diversity Centre (GDC) of the ETH Zurich for using high-

performance computing to analyse the sequencing data, and to the editor Kabir Peay and two anonymous reviewers whose comments improved the quality of the manuscript.

Author Contribution

R.A.C, M.I.B and L.M.S conceived the study and sampling design. R.A.C, S.G and L.M.S conducted the fieldwork. R.A.C, M.I.B and L.M.S processed and analysed samples. S.Z. perform soil chemistry analysis. R.A.C and L.M.S analysed the data with support of B.F for NGS data and T.N. for OTU clustering pipeline. R.A.C drafted the manuscript, M.I.B. and L.M.S contributed extensively and provided chief contributions.

References

- Agerer, R., 2006. Fungal relationships and structural identity of their ectomycorrhizae. *Mycol. Prog.* 5, 67–107. <https://doi.org/10.1007/s11557-006-0505-x>
- Agerer, R., 2001. Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11, 107–114. <https://doi.org/10.1007/s005720100108>
- Allesina, S., Pascual, M., 2009. Food web models: A plea for groups. *Ecol. Lett.* 12, 652–662. <https://doi.org/10.1111/j.1461-0248.2009.01321.x>
- Almeida-Neto, M., Ulrich, W., 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. *Environ. Model. Softw.* 26, 173–178. <https://doi.org/10.1016/j.envsoft.2010.08.003>
- Bahram, M., Harend, H., Tedersoo, L., 2014. Network perspectives of ectomycorrhizal associations. *Fungal Ecol.* 7, 70–77. <https://doi.org/10.1016/j.funeco.2013.10.003>
- Bahram, M., Pölme, S., Kõljalg, U., Zarre, S., Tedersoo, L., 2012. Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytol.* 193, 465–473. <https://doi.org/10.1111/j.1469-8137.2011.03927.x>
- Bailey, J.K., Schweitzer, J.A., Úbeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D., Rehill, B.J., Bangert, R.K., Fischer, D.G., Allan, G.J., Whitham, T.G., 2009. From genes to ecosystems: A synthesis of the effects of plant genetic factors across levels of organization. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 1607–1616. <https://doi.org/10.1098/rstb.2008.0336>
- Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence

676 patterns in soil microbial communities. *ISME J.* 6, 343–351. <https://doi.org/10.1038/ismej.2011.119>
 677 Bascompte, J., Jordano, P., Melián, C.J., Olesen, J.M., 2003. The nested assembly of plant–animal mutualistic
 678 networks. *Proc. Natl. Acad. Sci.* 100, 9383–9387. <https://doi.org/10.1073/PNAS.1633576100>
 679 Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García,
 680 M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E.,
 681 Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013.
 682 Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and
 683 other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* 4, 914–919.
 684 <https://doi.org/10.1111/2041-210X.12073>
 685 Botnen, S., Vik, U., Carlsen, T., Eidesen, P.B., Davey, M.L., Kauserud, H., 2014. Low host specificity of root-
 686 associated fungi at an Arctic site. *Mol. Ecol.* 23, 975–985. <https://doi.org/10.1111/mec.12646>
 687 Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in
 688 soil and obscures estimates of soil microbial diversity. *Nat. Microbiol.* 2, 16242.
 689 <https://doi.org/10.1038/nmicrobiol.2016.242>
 690 Collins, C.G., Stajich, J.E., Weber, S.E., Pombubpa, N., Diez, J.M., 2018. Shrub range expansion alters
 691 diversity and distribution of soil fungal communities across an alpine elevation gradient. *Mol. Ecol.* 27,
 692 2461–2476. <https://doi.org/10.1111/mec.14694>
 693 Cox, F., Barsoum, N., Lilleskov, E.A., Bidartondo, M.I., 2010. Nitrogen availability is a primary determinant of
 694 conifer mycorrhizas across complex environmental gradients. *Ecol. Lett.* 13, 1103–1113.
 695 <https://doi.org/10.1111/j.1461-0248.2010.01494.x>
 696 Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C., Sanders, N.J., 2006. Plant genotypic
 697 diversity predicts community structure and governs an ecosystem process. *Science* (80-.). 313, 966–968.
 698 <https://doi.org/10.1126/science.1128326>
 699 de la Bastide, M., McCombie, W.R., 2007. Assembling genomic DNA sequences with PHRAP, in: *Current*
 700 *Protocols in Bioinformatics*. John Wiley & Sons, Inc., Hoboken, NJ, USA, p. 11.4.1-11.4.15.
 701 <https://doi.org/10.1002/0471250953.bi1104s17>
 702 Dickie, I.A., 2007. Host preference, niches and fungal diversity. *New Phytol.* 174, 230–233.
 703 <https://doi.org/10.1111/j.1469-8137.2007.02055.x>
 704 Donhauser, J., Frey, B., 2018. Alpine soil microbial ecology in a changing world. *FEMS Microbiol. Ecol.* 94,
 705 99. <https://doi.org/10.1093/femsec/fiy099>

706 Dormann, C., Gruber, B., Fründ, J., 2008. Introducing the bipartite package: Analysing ecological networks. R
 707 News 8, 8–11.

708 Dormann, C.F., Strauss, R., 2014. A method for detecting modules in quantitative bipartite networks. *Methods*
 709 *Ecol. Evol.* 5, 90–98. <https://doi.org/10.1111/2041-210X.12139>

710 Edgar, R., 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *bioRxiv*
 711 074161. <https://doi.org/10.1101/074161>

712 Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*
 713 10, 996–998. <https://doi.org/10.1038/nmeth.2604>

714 Edgar, R.C., Flyvbjerg, H., 2015. Error filtering, pair assembly and error correction for next-generation
 715 sequencing reads. *Bioinformatics* 31, 3476–3482. <https://doi.org/10.1093/bioinformatics/btv401>

716 Erlandson, S., Wei, X., Savage, J., Cavender-Bares, J., Peay, K., 2018. Soil abiotic variables are more important
 717 than Salicaceae phylogeny or habitat specialization in determining soil microbial community structure.
 718 *Mol. Ecol.* 27, 2007–2024. <https://doi.org/10.1111/mec.14576>

719 Erlandson, S.R., Savage, J.A., Cavender-Bares, J.M., Peay, K.G., 2016. Soil moisture and chemistry influence
 720 diversity of ectomycorrhizal fungal communities associating with willow along an hydrologic gradient.
 721 *FEMS Microbiol. Ecol.* 92, fiv148. <https://doi.org/10.1093/femsec/fiv148>

722 Evans, J., Parker, A., Gallagher, F., Krumins, J., 2015. Plant productivity, ectomycorrhizae, and metal
 723 contamination in urban brownfield soils. *Soil Sci.* 180, 1. <https://doi.org/10.1097/SS.000000000000128>

724 FAO-UNESCO, 2007. Soil map of the world. Revised legend with corrections and updates. Wageningen.

725 Feinstein, L.M., Blackwood, C.B., 2012. Taxa-area relationship and neutral dynamics influence the diversity of
 726 fungal communities on senesced tree leaves. *Environ. Microbiol.* 14, 1488–1499.
 727 <https://doi.org/10.1111/j.1462-2920.2012.02737.x>

728 Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M., 2016. Microbial diversity in
 729 European alpine permafrost and active layers. *FEMS Microbiol. Ecol.* 92, fiw018.
 730 <https://doi.org/10.1093/femsec/fiw018>

731 Friedman, J., Alm, E.J., 2012. Inferring Correlation Networks from Genomic Survey Data. *PLoS Comput. Biol.*
 732 8, e1002687. <https://doi.org/10.1371/journal.pcbi.1002687>

733 Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the
 734 identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>

736 Gehring, C., Flores-Rentería, D., Sthultz, C.M., Leonard, T.M., Flores-Rentería, L., Whipple, A. V., Whitham,
 737 T.G., 2014. Plant genetics and interspecific competitive interactions determine ectomycorrhizal fungal
 738 community responses to climate change. *Mol. Ecol.* 23, 1379–1391. <https://doi.org/10.1111/mec.12503>
 739 Gehring, C.A., Mueller, R.C., Whitham, T.G., 2006. Environmental and genetic effects on the formation of
 740 ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. *Oecologia* 149, 158–164.
 741 <https://doi.org/10.1007/s00442-006-0437-9>
 742 Gehring, C.A., Sthultz, C.M., Flores-Rentería, L., Whipple, A. V, Whitham, T.G., 2017. Tree genetics defines
 743 fungal partner communities that may confer drought tolerance. *Proc. Natl. Acad. Sci.* 114, 11169–11174.
 744 <https://doi.org/10.1073/pnas.1704022114>
 745 Goldmann, K., Schröter, K., Pena, R., Schöning, I., Schrumpf, M., Buscot, F., Polle, A., Wubet, T., 2016.
 746 Divergent habitat filtering of root and soil fungal communities in temperate beech forests. *Sci. Rep.* 6,
 747 31439. <https://doi.org/10.1038/srep31439>
 748 Gotelli, N.J., Entsminger, G.L., 2001. EcoSim: Null models software for ecology. URL:
 749 <http://homepages.together.net/~gentsmin/ecosim.htm>.
 750 Gramlich, S., Sagmeister, P., Dullinger, S., Hadacek, F., Hörandl, E., 2016. Evolution in situ: Hybrid origin and
 751 establishment of willows (*Salix* L.) on alpine glacier forefields. *Heredity* (Edinb). 116, 531–541.
 752 <https://doi.org/10.1038/hdy.2016.14>
 753 Gramlich, S., Wagner, N.D., Hörandl, E., 2018. RAD-seq reveals genetic structure of the F2-generation of
 754 natural willow hybrids (*Salix* L.) and a great potential for interspecific introgression. *BMC Plant Biol.* 18,
 755 317. <https://doi.org/10.1186/s12870-018-1552-6>
 756 Guimerà, R., Nunes Amaral, L.A., 2005. Functional cartography of complex metabolic networks. *Nature* 433,
 757 895–900. <https://doi.org/10.1038/nature03288>
 758 Hawksworth, D.L., Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol. Spectr.*
 759 5, 79–95. <https://doi.org/10.1128/microbiolspec.FUNK-0052-2016>
 760 Hryniewicz, K., Toljander, Y.K., Baum, C., Fransson, P.M.A., Taylor, A.F.S., Weih, M., 2012.
 761 Correspondence of ectomycorrhizal diversity and colonisation of willows (*Salix* spp.) grown in short
 762 rotation coppice on arable sites and adjacent natural stands. *Mycorrhiza* 22, 603–613.
 763 <https://doi.org/10.1007/s00572-012-0437-z>
 764 Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J.,
 765 Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal

ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. <https://doi.org/10.1111/j.1574-6941.2012.01437.x>

Ishida, T.A., Nara, K., Hogetsu, T., 2007. Host effects on ectomycorrhizal fungal communities: Insight from eight host species in mixed conifer-broadleaf forests. New Phytol. 174, 430–440. <https://doi.org/10.1111/j.1469-8137.2007.02016.x>

Jany, J.L., Martin, F., Garbaye, J., 2003. Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. Plant Soil 255, 487–494. <https://doi.org/10.1023/A:1026092714340>

Karliński, L., Rudawska, M., Leski, T., 2013. The influence of host genotype and soil conditions on ectomycorrhizal community of poplar clones. Eur. J. Soil Biol. 58, 51–58. <https://doi.org/10.1016/j.ejsobi.2013.05.007>

Kirker, G.T., Bishell, A.B., Jusino, M.A., Palmer, J.M., Hickey, W.J., Lindner, D.L., 2017. Amplicon-based sequencing of soil fungi from wood preservative test sites. Front. Microbiol. 8, 1997. <https://doi.org/10.3389/fmicb.2017.01997>

Koide, R.T., Xu, B., Sharda, J., Lekberg, Y., Ostiguy, N., 2004. Evidence of species interactions within an ectomycorrhizal fungal community. New Phytol. 165, 305–316. <https://doi.org/10.1111/j.1469-8137.2004.01216.x>

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. <https://doi.org/10.1111/mec.12481>

Korkama, T., Pakkanen, A., Pennanen, T., 2006. Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. New Phytol. 171, 815–824. <https://doi.org/10.1111/j.1469-8137.2006.01786.x>

Krpata, D., Mühlmann, O., Kuhnert, R., Ladurner, H., Göbl, F., Peintner, U., 2007. High diversity of ectomycorrhizal fungi associated with *Arctostaphylos uva-ursi* in subalpine and alpine zones: Potential inoculum for afforestation. For. Ecol. Manage. 250, 167–175. <https://doi.org/10.1016/j.foreco.2007.05.012>

Landeweert, R., Leeflang, P., Smit, E., Kuyper, T., 2005. Diversity of an ectomycorrhizal fungal community studied by a root tip and total soil DNA approach. *Mycorrhiza* 15, 1–6. <https://doi.org/10.1007/s00572-003-0284-z>

Lavender, T.M., Schamp, B.S., Lamb, E.G., 2016. The influence of matrix size on statistical properties of co-occurrence and limiting similarity null models. *PLoS One* 11, e0151146. <https://doi.org/10.1371/journal.pone.0151146>

Legendre, P., De Cáceres, M., 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. *Ecol. Lett.* 16, 951–963. <https://doi.org/10.1111/ele.12141>

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjølner, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytol.* 199, 288–299. <https://doi.org/10.1111/nph.12243>

Luo, Z., Liu, J., Zhao, P., Jia, T., Li, C., Chai, B., 2019. Biogeographic patterns and assembly mechanisms of bacterial communities differ between habitat generalists and specialists across elevational gradients. *Front. Microbiol.* 10, 169. <https://doi.org/10.3389/fmicb.2019.00169>

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10–12. <https://doi.org/10.14806/ej.17.1.200>

Morris, M.H., Smith, M.E., Rizzo, D.M., Rejmánek, M., Bledsoe, C.S., 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. *New Phytol.* 178, 167–176. <https://doi.org/10.1111/j.1469-8137.2007.02348.x>

Mühlmann, O., Bacher, M., Peintner, U., 2008. *Polygonum viviparum* mycobionts on an alpine primary successional glacier forefront. *Mycorrhiza* 18, 87–95. <https://doi.org/10.1007/s00572-007-0156-z>

Mühlmann, O., Peintner, U., 2008. Mycobionts of *Salix herbacea* on a glacier forefront in the Austrian Alps. *Mycorrhiza* 18, 171–180. <https://doi.org/10.1007/s00572-008-0169-2>

Nara, K., Hogetsu, T., 2004. Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. *Ecology* 85, 1700–1707. <https://doi.org/10.1890/03-0373>

Nguyen, N.H., Smith, D., Peay, K., Kennedy, P., 2015. Parsing ecological signal from noise in next generation amplicon sequencing. *New Phytol.* 205, 1389–1393. <https://doi.org/10.1111/nph.12923>

Nilsson, R.H., Abarenkov, K., Larsson, K.-H., Kõljalg, U., 2011. Molecular identification of fungi: Rationale, philosophical concerns, and the UNITE database. *Open Appl. Informatics J.* 5, 81–86. <https://doi.org/10.2174/1874136301105010081>

826 Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P.,
 827 Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database
 828 for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic
 829 Acids Res.* 47, D259–D264. <https://doi.org/10.1093/nar/gky1022>
 830 Ning, C., Mueller, G.M., Egerton-Warburton, L.M., Xiang, W., Yan, W., 2019. Host phylogenetic relatedness
 831 and soil nutrients shape ectomycorrhizal community composition in native and exotic pine plantations.
 832 *Forests* 10, 263. <https://doi.org/10.3390/f10030263>
 833 Obase, K., Douhan, G.W., Matsuda, Y., Smith, M.E., 2017. Progress and challenges in understanding the
 834 biology, diversity, and biogeography of *Cenococcum geophilum*, in: Tedersoo, L. (Ed.), *Biogeography of
 835 Mycorrhizal Symbiosis*. Springer, Cham, pp. 299–317. https://doi.org/10.1007/978-3-319-56363-3_14
 836 Oberhänsli, R., Schenker, F., Mercolli, I., 1988. Indications of Variscan nappe tectonics in the Aar Massif.
 837 *Schweizerische Mineral. und Petrogr. Mitteilungen* 68, 509–520.
 838 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B.,
 839 Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community ecology
 840 package*. R package version 2.5-4. <https://cran.r-project.org/package=vegan>.
 841 Ovaskainen, O., Tikhonov, G., Norberg, A., Guillaume Blanchet, F., Duan, L., Dunson, D., Roslin, T., Abrego,
 842 N., 2017. How to make more out of community data? A conceptual framework and its implementation as
 843 models and software. *Ecol. Lett.* 20, 561–576. <https://doi.org/10.1111/ele.12757>
 844 Pandit, S.N., Kolasa, J., Cottenie, K., 2009. Contrasts between habitat generalists and specialists: An empirical
 845 extension to the basic metacommunity framework. *Ecology* 90, 2253–2262. <https://doi.org/10.1890/08-0851.1>
 846
 847 Pellissier, L., Niculita-Hirzel, H., Dubuis, A., Pagni, M., Guex, N., Ndiribe, C., Salamin, N., Xenarios, I.,
 848 Goudet, J., Sanders, I.R., Guisan, A., 2014. Soil fungal communities of grasslands are environmentally
 849 structured at a regional scale in the Alps. *Mol. Ecol.* 23, 4274–4290. <https://doi.org/10.1111/mec.12854>
 850 Pellissier, L., Pinto-Figueroa, E., Niculita-Hirzel, H., Moora, M., Villard, L., Goudet, J., Guex, N., Pagni, M.,
 851 Xenarios, I., Sanders, I., Guisan, A., 2013. Plant species distributions along environmental gradients: do
 852 belowground interactions with fungi matter? *Front. Plant Sci.* 4, 500.
 853 <https://doi.org/10.3389/fpls.2013.00500>
 854 Peter, M., Kohler, A., Ohm, R.A., Kuo, A., Krützmann, J., Morin, E., Arend, M., Barry, K.W., Binder, M.,
 855 Choi, C., Clum, A., Copeland, A., Grisel, N., Haridas, S., Kipfer, T., Labutti, K., Lindquist, E., Lipzen,

856 A., Maire, R., Meier, B., Mihaltcheva, S., Molinier, V., Murat, C., Pöggeler, S., Quandt, C.A., Sperisen,
857 C., Tritt, A., Tisserant, E., Crous, P.W., Henrissat, B., Nehls, U., Egli, S., Spatafora, J.W., Grigoriev, I. V.,
858 Martin, F.M., 2016. Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic
859 fungus *Cenococcum geophilum*. *Nat. Commun.* 7, 12662. <https://doi.org/10.1038/ncomms12662>
860 Peterson, R.A., 2017. Estimating normalization transformations with bestNormalize. URL:
861 <https://github.com/petersonR/bestNormalize>.
862 Pickles, B.J., Genney, D.R., Anderson, I.C., Alexander, I.J., 2012. Spatial analysis of ectomycorrhizal fungi
863 reveals that root tip communities are structured by competitive interactions. *Mol. Ecol.* 21, 5110–5123.
864 <https://doi.org/10.1111/j.1365-294X.2012.05739.x>
865 Pigott, C.D., 1982. Survival of mycorrhiza formed by *Cenococcum geophyllum* FR. in dry soils. *New Phytol.* 92,
866 513–517. <https://doi.org/10.1111/j.1469-8137.1982.tb03409.x>
867 Pölme, S., Bahram, M., Jacquemyn, H., Kennedy, P., Kohout, P., Moora, M., Oja, J., Öpik, M., Pecoraro, L.,
868 Tedersoo, L., 2018. Host preference and network properties in biotrophic plant–fungal associations. *New*
869 *Phytol.* 217, 1230–1239. <https://doi.org/10.1111/nph.14895>
870 Rime, T., Hartmann, M., Brunner, I., Widmer, F., Zeyer, J., Frey, B., 2015. Vertical distribution of the soil
871 microbiota along a successional gradient in a glacier forefield. *Mol. Ecol.* 24, 1091–1108.
872 <https://doi.org/10.1111/mec.13051>
873 Ryberg, M., Andreasen, M., Björk, R.G., 2011. Weak habitat specificity in ectomycorrhizal communities
874 associated with *Salix herbacea* and *Salix polaris* in alpine tundra. *Mycorrhiza* 21, 289–296.
875 <https://doi.org/10.1007/s00572-010-0335-1>
876 Ryberg, M., Larsson, E., Molau, U., 2009. Ectomycorrhizal Diversity on *Dryas octopetala* and *Salix reticulata*
877 in an Alpine Cliff Ecosystem. *Arctic, Antarct. Alp. Res.* 41, 506–514. [https://doi.org/10.1657/1938-4246-](https://doi.org/10.1657/1938-4246-41.4.506)
878 [41.4.506](https://doi.org/10.1657/1938-4246-41.4.506)
879 Smith, M.E., Douhan, G.W., Fremier, A.K., Rizzo, D.M., 2009. Are true multihost fungi the exception or the
880 rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co-occurring *Quercus*
881 species. *New Phytol.* 182, 295–299. <https://doi.org/10.1111/j.1469-8137.2009.02801.x>
882 Smith, S.E., Read, D.J., 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press.
883 Stone, L., Roberts, A., 1990. The checkerboard score and species distributions. *Oecologia* 85, 74–79.
884 <https://doi.org/10.1007/BF00317345>
885 Suz, L.M., Barsoum, N., Benham, S., Dietrich, H.-P., Fetzer, K.D., Fischer, R., García, P., Gehrman, J.,

886 Kristöfel, F., Manninger, M., Neagu, S., Nicolas, M., Oldenburger, J., Raspe, S., Sánchez, G., Schröck,
 887 H.W., Schubert, A., Verheyen, K., Verstraeten, A., Bidartondo, M.I., 2014. Environmental drivers of
 888 ectomycorrhizal communities in Europe's temperate oak forests. *Mol. Ecol.* 23, 5628–5644.
 889 <https://doi.org/10.1111/mec.12947>
 890 Taylor, D.L., Walters, W.A., Lennon, N.J., Bochicchio, J., Krohn, A., Caporaso, J.G., Pennanen, T., 2016.
 891 Accurate estimation of fungal diversity and abundance through improved lineage-specific primers
 892 optimized for Illumina amplicon sequencing. *Appl. Environ. Microbiol.* 82, 7217–7226.
 893 <https://doi.org/10.1128/AEM.02576-16>
 894 Tedersoo, L., Bahram, M., Pölme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios,
 895 A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T.,
 896 Ratkowsky, D., Pritsch, K., Pöldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K.,
 897 Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W.,
 898 Majuakim, L., Lodge, D.J., Lee, S., Larsson, K.H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W.,
 899 Harend, H., Guo, L.D., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan,
 900 R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S.,
 901 Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. *Science* (80-.). 346,
 902 1256688. <https://doi.org/10.1126/science.1256688>
 903 Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A.G., Henkel, T.W., Kjoller, R., Morris, M.H., Nara, K.,
 904 Nouhra, E., Peay, K.G., Pölme, S., Ryberg, M., Smith, M.E., Kõljalg, U., 2012. Towards global patterns
 905 in the diversity and community structure of ectomycorrhizal fungi. *Mol. Ecol.* 21, 4160–4170.
 906 <https://doi.org/10.1111/j.1365-294X.2012.05602.x>
 907 Tedersoo, L., Jairus, T., Horton, B.M., Abarenkov, K., Suvi, T., Saar, I., Kõljalg, U., 2008. Strong host
 908 preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding
 909 and taxon-specific primers. *New Phytol.* 180, 479–490. <https://doi.org/10.1111/j.1469-8137.2008.02561.x>
 910 Tedersoo, L., May, T., Smith, M., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and
 911 evolution of phylogenetic lineages. *Mycorrhiza* 20, 217–263. <https://doi.org/10.1007/s00572-009-0274-x>
 912 Tedersoo, L., Smith, M.E., 2013. Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel
 913 lineages revealed by sequences from belowground. *Fungal Biol. Rev.* 27, 83–99.
 914 <https://doi.org/10.1016/j.fbr.2013.09.001>
 915 Tedersoo, L., Suvi, T., Jairus, T., Ostonen, I., Pölme, S., 2009. Revisiting ectomycorrhizal fungi of the genus

916 *Alnus*: Differential host specificity, diversity and determinants of the fungal community. *New Phytol.* 182,
 917 727–735. <https://doi.org/10.1111/j.1469-8137.2009.02792.x>
 918 Tjur, T., 2009. Coefficients of Determination in Logistic Regression Models—A New Proposal: The Coefficient
 919 of Discrimination. *Am. Stat.* 63, 366–372. <https://doi.org/10.1198/tast.2009.08210>
 920 Toju, H., Guimaraes, P.R., Olesen, J.M., Thompson, J.N., 2015. Below-ground plant-fungus network topology
 921 is not congruent with above-ground plant-animal network topology. *Sci. Adv.* 1, e1500291–e1500291.
 922 <https://doi.org/10.1126/sciadv.1500291>
 923 Toju, H., Guimarães, P.R., Olesen, J.M., Thompson, J.N., 2014. Assembly of complex plant-fungus networks.
 924 *Nat. Commun.* 5, 5273. <https://doi.org/10.1038/ncomms6273>
 925 Toju, H., Kishida, O., Katayama, N., Takagi, K., 2016. Networks depicting the fine-scale co-occurrences of
 926 fungi in soil horizons. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0165987>
 927 Toju, H., Sato, H., Yamamoto, S., Kadowaki, K., Tanabe, A.S., Yazawa, S., Nishimura, O., Agata, K., 2013.
 928 How are plant and fungal communities linked to each other in belowground ecosystems? A massively
 929 parallel pyrosequencing analysis of the association specificity of root-associated fungi and their host
 930 plants. *Ecol. Evol.* 3, 3112–3124. <https://doi.org/10.1002/ece3.706>
 931 van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: Soil microbes as
 932 drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310.
 933 <https://doi.org/10.1111/j.1461-0248.2007.01139.x>
 934 van der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll,
 935 C., Cools, N., De Vos, B., Dietrich, H.P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H.S., Hansen,
 936 K., Jacob, F., Kristöfel, F., Lech, P., Manninger, M., Martin, J., Meessenburg, H., Merilä, P., Nicolas, M.,
 937 Pavlenda, P., Rautio, P., Schaub, M., Schröck, H.W., Seidling, W., Šrámek, V., Thimonier, A., Thomsen,
 938 I.M., Titeux, H., Vanguelova, E., Verstraeten, A., Vesterdal, L., Waldner, P., Wijk, S., Zhang, Y., Žlindra,
 939 D., Bidartondo, M.I., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature*
 940 558, 243–248. <https://doi.org/10.1038/s41586-018-0189-9>
 941 Velmala, S.M., Rajala, T., Haapanen, M., Taylor, A.F.S., Pennanen, T., 2013. Genetic host-tree effects on the
 942 ectomycorrhizal community and root characteristics of Norway spruce. *Mycorrhiza* 23, 21–33.
 943 <https://doi.org/10.1007/s00572-012-0446-y>
 944 Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D., Tilman, D., 2006. Resource availability controls
 945 fungal diversity across a plant diversity gradient. *Ecol. Lett.* 9, 1127–1135. <https://doi.org/10.1111/j.1461->

0248.2006.00965.x

- White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M. (Ed.), PCR Protocols. Academic Press, San Diego, pp. 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Whitham, T.G., Gehring, C.A., Lamit, L.J., Wojtowicz, T., Evans, L.M., Keith, A.R., Smith, D.S., 2012. Community specificity: Life and afterlife effects of genes. Trends Plant Sci. 17, 271–281. <https://doi.org/10.1016/j.tplants.2012.01.005>
- Whitham, T.G., Martinsen, G.D., Floate, K.D., Dungey, H.S., Potts, M., Keim, P., 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. (Hybridization and resistance to parasites). Ecology 80, 1–11. [https://doi.org/10.1890/0012-9658\(1999\)080\[0416:PHZABT\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0416:PHZABT]2.0.CO;2)
- Whitham, T.G., Morrow, P.A., Potts, B.M., 1994. Plant hybrid zones as centers of biodiversity: The herbivore community of two endemic Tasmanian eucalypts. Oecologia 97, 481–490. <https://doi.org/10.1007/BF00325886>
- Wutkowska, M., Vader, A., Mundra, S., Cooper, E.J., Eidesen, P.B., 2019. Dead or alive; or does it really matter? Level of congruency between trophic modes in total and active fungal communities in high arctic soil. Front. Microbiol. 9, 3243. <https://doi.org/10.3389/fmicb.2018.03243>

Figure legends

Figure 1. Sampling sites included in this study, based on Gramlich et al. (2016).

Figure 2. Soil chemistry parameters measured under *Salix helvetica* (n=27), *S. purpurea* (n=27) and their hybrids (n=28) across the sampling area. Each violin plot represents the mean (dot), standard deviation (bars) and the probability density of the data. Different letters indicate significant differences after ANOVA at $P < 0.05$.

Figure 3: Relative abundance and local contribution for beta diversity (LCBD) of the most abundant EcM lineages found as ectomycorrhizas (top) and in the soil (bottom) in each

974 individual willow.

975

976 Figure 4: Nonmetric multidimensional scaling (NMDS) of community dissimilarities using
977 Bray–Curtis distances. Circular points represent EcM communities associated with roots
978 (ectomycorrhizas) and triangular shape points represents EcM communities in soil under each
979 individual plant. Circumferences represent the 95% confidence interval of samples for each
980 plant host. Stress of the ordination was 0.19.

981

982 Figure 5: Visualisation of EcM networks in a) soil EcM fungi vs willows and b)
983 ectomycorrhizas vs willows, based on the Fruchterman Reingold algorithm. Host plants and
984 fungal OTUs are represented by squares and circles, respectively. Host plant abbreviations
985 are: SH – *Salix helvetica*, SX – Hybrids and SP – *S. purpurea*. Network edge thickness on
986 plot b represent the strength of the association. Coloured vertices on b represent the shared
987 OTUs between soil EcM fungi and ectomycorrhizas and derive from their relative position in
988 plot a. White vertices represent OTUs only present as ectomycorrhizas.

989

990 Figure 6: Variance partitioning in EcM fungal communities in a) soil and b) roots
991 (ectomycorrhizas). Variance partitioning was calculated using the fungal OTUs presence-
992 absence in response to, site, plant host, soil pH, total C, total N, estimated total fungal
993 community in the soil and estimated soil EcM community. Only shared OTUs between soil
994 and root datasets are illustrated in a.

995