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Title

Habitat specialization controls ectomycorrhizal fungi above the treeline in the European Alps.

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Summary

- Alpine habitats are one of the most vulnerable ecosystems to environmental change, however, little is known about the drivers of plant-fungal interactions in these ecosystems and their resilience to climate change.
- We investigate the influence of the main drivers of ectomycorrhizal (EM) fungal communities along elevation and environmental gradients in the alpine zone of the European Alps and measured their degree of specialization using network analysis. We sampled ectomycorrhizas of *Dryas octopetala*, *Bistorta vivipara* and *Salix herbacea*, and soil fungal communities at 28 locations across five countries, from the treeline to the nival zone.
- We found that: i) EM fungal community composition, but not richness, changes along elevation, ii) there is no strong evidence of host specialization, however, EM fungal networks in the alpine zone and within these, EM fungi associated with snowbed communities, are more specialized than in other alpine habitats, iii) plant host population structure does not influence EM fungal communities, and iv) most variability in EM fungal communities is explained by fine-scale changes in edaphic properties, like soil pH and total nitrogen.
- The higher specialization and narrower ecological niches of these plant-fungal interactions in snowbed habitats make these habitats particularly vulnerable to environmental change in alpine ecosystems.

Keywords

Alpine ecosystems; *Bistorta vivipara*; *Dryas octopetala*; Ectomycorrhizal fungi; Elevation gradients; Environmental drivers; plant-fungal networks; *Salix herbacea*.

Introduction

Habitat loss due to environmental change is one of the main threats to plant and fungal diversity and distributions; in mountain ecosystems, climate change is already driving species upslope (Steinbauer *et al.*, 2018), favouring communities that are more resilient and well adapted to the new environmental conditions over more specialized ones, such as those from alpine habitats (Gottfried *et al.*, 2012). Across global and continental scales, environment and host are main drivers of ectomycorrhizal (EM) fungal communities (Tedersoo *et al.*, 2012, 2014; van der Linde *et al.*, 2018), but to better understand the impact of environmental change in alpine ecosystems and the resilience of their EM plant-fungal networks, it is essential to know their main biotic drivers across sharp altitudinal and environmental gradients.

Mountains provide opportunities to explore how communities change along environmental gradients in a relatively small geographical area (Körner, 2007). Abrupt shifts in elevation and variation in climatic and edaphic properties accommodate a great diversity of ecological niches spread in a well-defined vertical habitat zonation (Körner, 2003; Nagy & Grabherr, 2009). These elevation gradients strongly affect plant and animal diversity (Körner, 2003; Gobbi *et al.*, 2006), yet little is known about their effects on soil organisms, including fungi (Donhauser & Frey, 2018; Geml, 2017). Kernaghan and Harper (2001) found that EM fruitbody richness and diversity decreased with elevation from sub-alpine forest to the alpine zone at two sites in the Canadian Rockies. In Iran, Bahram *et al.* (2012) found that EM fungal richness declined monotonically with increasing elevation. This declining trend with increasing elevation has been reported from mountains around the globe, from Mt. Fuji (Miyamoto *et al.*, 2014) to the Andes (Nottingham *et al.*, 2018) and White Mountains (Collins *et al.*, 2018). These studies were carried out across multiple habitats and included different plant hosts that covaried with elevation resulting in confounding effects. In contrast, no significant differences in EM communities in beech (Coince *et al.*, 2014) or pine (Jarvis *et al.*, 2015) were found along elevation gradients. Large-scale studies across multiple hosts and mountains zones are needed to understand how elevation and underlying environmental covariates drive EM communities.

Ectomycorrhizal fungal communities are known to respond to abiotic factors (e.g. soil pH, nutrient availability) across local, regional and continental scales (Cox *et al.*, 2010; Suz *et al.*, 2014; van der Linde *et al.*, 2018; Koizumi & Nara, 2020) and to temperature and precipitation at global scales (Tedersoo *et al.*, 2012, 2014). Furthermore, EM fungi are affected by biotic factors like plant community composition, diversity and productivity (Waldrop *et al.*, 2006; Bahram *et al.*, 2012). Pellissier *et al.* (2013) found fungal richness positively correlated with the distribution of high elevation plants, improving the predictive power of alpine plant species distribution models. In Europe's Alps, EM fungi are very diverse locally (Pellissier *et al.*, 2014; Brunner *et al.*, 2017) similarly to other alpine regions (Bjorbækmo *et al.*, 2010; Gao & Yang, 2010; Ryberg *et al.*, 2009; Mundra *et al.*, 2016; Botnen *et al.*, 2019). Ectomycorrhizal fungi are essential for establishment and habitat colonization by alpine plants (Nara & Hogetsu, 2004), but despite their importance, alpine EM fungi are still poorly known and many remain undiscovered. Therefore, we need baseline data on large-scale EM diversity and its environmental drivers in the face of climate change.

Alpine ecosystems have wide ranges of habitats with heterogeneous geomorphology and ecological conditions regarding wind exposure, solar radiation, snow cover and duration, soil depth, nutrient availability and moisture (Nagy & Grabherr, 2009). Alpine plants have therefore high morphological and physiological specializations (Körner, 2003). In the Alps, grasslands and dwarf-shrub heath formations, typically dominated by EM mountain avens (*Dryas octopetala*, Rosaceae) and dwarf willows (*Salix* spp., including *S. herbacea*, Salicaceae), are characteristic habitats above the treeline. In contrast, the alpine bistort (*Bistorta vivipara*, Polygonaceae) is a widespread arctic-alpine EM plant (Wang & Qiu, 2006) occurring from montane to nival zones across the Alps (Delarze *et al.*, 2015). Previous studies have found that these and similar arctic and alpine EM plants seem to associate mostly with generalist fungi, indicating low specialization that might facilitate colonisation of new habitats and favour succession above the treeline (Kernaghan & Harper, 2001; Ryberg *et al.*, 2009, 2011; Botnen *et al.*, 2014; Brunner *et al.*, 2017). Specialization can be influenced by host phylogenetic signal and/or by environment, making it particularly difficult to disentangle both effects.

The architectural properties of ecological networks provide information about coevolutionary adaptation and determine the resilience, persistence and structural stability of communities. Modularity and nestedness are two extensively studied network properties in EM fungi (Bahram *et al.*, 2014; Toju *et al.*, 2014, 2015; Pölme *et al.*, 2018). Due to their qualitative

nature, their sensitivity to first-order network aspects such as dimensions (number of species in higher or lower trophic levels) and asymmetry (marginal abundance distributions or number of observations per species), they may be mere reflections of the sampling properties of the underlying network (Blüthgen *et al.*, 2008; Dormann *et al.*, 2009; Bahram *et al.* 2014). However, network theory can also be used to assess specialization in plant-fungal interactions using quantitative measures based on interaction frequencies that explicitly account for uneven sampling and can be applied regardless of network size and asymmetries. The degree of specialization can be measured at the species level and also among two parties in an entire network, allowing detection of different degrees of specialization within and across networks (Blüthgen *et al.*, 2006).

Here we explore and disentangle the influence of biotic and abiotic factors on alpine zone EM fungal communities and assess their specialization using network analysis along elevation gradients. From the treeline ecotone to the bottom of the nival zone, we analysed EM fungal communities associated with three important EM plants. We hypothesized that: 1) EM fungal richness decreases and composition changes at increasing elevation and harsher environmental conditions; 2) specialization in EM plant-fungal interaction networks changes across hosts and habitats, and 3) host plant population genetic structure determines EM fungal communities.

Materials and Methods

Root, soil and plant tissue sampling

Sampling was carried out across five countries during the summers 2016-2017. Forty-seven sites (10 x 30 m, sub-divided into four 5 x 15 m quadrants) *ca.* 1,500 to 2,600 m above sea level (asl) were sampled from the treeline to the nival zone, covering environmental and climatic gradients along the range of *D. octopetala*, *B. vivipara*, and *S. herbacea* (Table 1). *Dryas octopetala* is a sub-alpine to alpine plant occurring mostly in calcareous open habitats as extensive mats (hereafter referred as *Dryas* mats). *Salix herbacea* occurs in acidic soils (pH < 5) along snowbed communities annually covered by snow up to ten months and with sparse lawns of pioneer and acidophilic plants (Delarze *et al.*, 2015). *Bistorta vivipara* is less restricted by soil conditions and co-occurs with both *D. octopetala* and *S. herbacea*. When possible, sites were selected at least at two different elevations per valley, from the treeline up to the bottom of the nival zone. Geological and lithological soil properties at the sites were diverse (Table 1). At each site, twenty individuals per target species (five per quadrant) were selected maximising the distance among them. Soil blocks *ca.* 10 cm³ were collected underneath plants and including

shoots. Soil and root samples were stored at 4°C up to 10 days until processed. Leaf samples from all plants were preserved in silica gel for genetic analyses. Extra soil samples (*ca.* 250 mg) were collected under each plant and pooled by quadrant for chemical and fungal DNA analysis. Soils for chemical analysis were dried at 40–60°C and stored at room temperature while soils for DNA analysis were sieved through a 2 mm mesh under sterile conditions and freeze-dried.

Environmental data

Mean annual temperature (MAT) and annual precipitation from each site and sampling year were retrieved from CHELSA at 30 arc-sec resolution (Karger *et al.*, 2017). Analyses of soil pH, total carbon (C_{total}) and total nitrogen (N_{total}) content, exchangeable cation content (Ca²⁺, K⁺ and Mg²⁺) and cation exchange capacity (CEC) were performed at WSL, Birmensdorf, details are in Methods S1. Percentage plant cover, co-occurrence of other EM plants and habitat type (treeline ecotone, *Dryas* mats, snowbeds) were recorded at each site.

Mycorrhizal and soil DNA analysis

All roots of each plant were rinsed with water and carefully disentangled from soil and roots of other plants. Twelve roots traced from aboveground structures were selected from each plant and one ectomycorrhiza from each root was collected under a dissecting microscope for analysis. Each individual ectomycorrhiza was visually characterized according to colour and soil exploration type (Agerer, 2001, 2006). Genomic DNA was extracted from individual ectomycorrhizas and the full ITS region of the rDNA amplified and sequenced bidirectionally following Suz *et al.* (2014).

Soil genomic DNA was extracted from 25 mg of each freeze-dried sample pool per quadrant using DNeasy PowerSoil (Qiagen) and quantified using a Qubit 2.0 (Life Technologies). Four DNA extractions per plot were pooled for further analysis. The PCR amplifications of ITS2 with primers fITS7 (Ihrmark *et al.*, 2012) and ITS4, were run in triplicate and pooled for library preparation following Mundra *et al.* (2016b). The PCRs, library preparation and paired-end (250 bp) Illumina MiSeq v3 sequencing (Illumina) were conducted by Génome Québec, Montréal. Details of sequence analyses and OTU clustering are presented in Methods S2. Representative sequences of the centroid of each EM OTU were deposited in NCBI (MT095544-6017 from mycorrhizas, MT095121-543 from soil).

Plant host genotyping

Plant genomic DNA from five plants per site and per species (85 *D. octopetala*, 105 *B. vivipara* and 45 *S. herbacea*) was isolated using the DNeasy Plant Mini Kit (Qiagen) from approx. 20 mg of dried leaf tissue. We used ten nuclear microsatellite loci (SSR) for *D. octopetala* (Vik *et al.*, 2010), ten for *B. vivipara* (Vik *et al.*, 2012) and seven for *S. herbacea* (Stamati *et al.*, 2003) with the amplification methods in the original publications. Further details of the genotype assessment are included in Methods S3.

Statistics

All statistical analyses used R version 3.5.1 (R Development Core Team 2009). Normal distribution of the residuals and homogeneity of variances of each variable were assessed with *qqnorm* plots and significant deviations of Shapiro and Levene test routines. Data with non-normal distribution were square-root or log-transformed. Statistics regarding EM fungal communities used DNA sequences obtained from each individual plant pooled per sampling site. Read numbers from roots and soil DNA were standardized using the Hellinger method with *decostand* in *vegan* (Oksanen *et al.*, 2019).

Alpine EM diversity

The observed number of OTUs per site and the abundance-based estimator (ACE, O'Hara, 2005; Chiu *et al.*, 2014) were calculated with *estimateR* in *vegan*. Differences in observed and estimated values among hosts were assessed by one-way ANOVA and Tukey's HSD *post hoc* tests. Percentage of OTUs recovered per host was calculated as the proportion of the number of observed OTUs and the estimated number of OTUs (ACE). *Bistorta vivipara* co-occurred with the other two hosts in most sites, therefore, we assessed two values of OTU richness for *B. vivipara*, one with *D. octopetala* in *Dryas* mats and another with *S. herbacea* in snowbed communities. To assess the proportion of shared OTUs between roots and soil, we performed local blast using the *blastn* algorithm with soil OTU centroids as references and root OTUs centroids as queries. The OTUs with hits $\geq 97\%$ and congruent taxonomy (i.e. equal UNITE SH number) were considered equivalent. We assessed differences in EM community composition among hosts and habitats using only the root dataset and with two analyses: *i*) we visualized dissimilarities (Bray-Curtis distances) in EM community composition using non-metric multidimensional scaling (NMDS) ordination, and *ii*) we ran permutational multivariate analysis of variance (PERMANOVA) using

adonis in *vegan*. The multivariate homogeneity of group dispersions (variances) among hosts and habitats was assessed using *betadisper* and *permutest* in *vegan* and a multilevel comparison of each PERMANOVA result was used to disentangle pairwise significant differences in EM fungal communities with *pairwise.adonis* (Martinez Arbizu, 2020).

Ectomycorrhizal communities across elevation

All analyses carried out to assess the effect of elevation on EM fungal communities used the root dataset. To determine the effect of elevation on EM communities we tested for differences in estimated OTU richness in each site across the elevation gradient: i) using a linear regression model as a function of elevation; ii) grouping sampling sites in two habitat zones according to their position in the mountain (alpine and treeline) and comparing them using *t*-test statistics in *stats*. Differences in community composition were assessed by PERMANOVA in a two-step approach, i) fitting the pairwise Bray-Curtis distance matrix as a function of elevation (unconstrained), and ii) constraining permutations to mountain zonation (i.e. treeline ($n = 14$) vs alpine ($n = 33$)). However, given the heterogeneity of group dispersions (variances) between alpine and treeline sites, iii) we constrained permutations to habitat type (i.e. treeline ($n = 14$), *Dryas* mats ($n = 17$) and snowbeds ($n = 16$)).

Differences in EM functional diversity across hosts and between treeline and alpine zones were tested by one-way ANOVA comparing average number of OTUs and number of ectomycorrhizas assigned to each soil exploration type (contact, short distance or medium-distance fringe) per zone and across hosts. Functional trait variation (presence-absence of hyphae and rhizomorphs) between treeline and alpine zones was assessed by *t*-test statistics and one-way ANOVA across hosts. When variances were heterogeneous, a one-way test for equal means (Welch ANOVA) was used in *stats*. Tukey and Games-Howell *post hoc* tests were used after simple ANOVA and Welch ANOVA, respectively, to examine pairwise differences.

The degrees of host and habitat specialization of each OTU along elevation gradients were tested using an ecological network approach (Blüthgen *et al.*, 2006) calculating two quantitative indices: 1) a network-wide specialization index based on standardized two-dimensional Shannon entropy (H_2'), that characterizes specialization in the entire network, and 2) the specialization of elements (i.e. species) within the network using standardized Kullback-Leibler distance (d'). To disentangle host specificity from other habitat effects, we also analysed the specialization of

elements within the network (d') for *B. vivipara* independently when it occurred either in *Dryas* mats or in snowbed habitats. Details are presented in Methods S4.

Environmental drivers of EM communities

To elucidate drivers of EM communities we applied a hierarchical Bayesian framework (Ovaskainen *et al.*, 2017) in *HMSC* v3.0 assessing how much variation in fungal OTU abundances is due to environmental filtering, biotic interactions and random processes. From the root dataset, we selected OTUs present in five or more sites and with counts ≥ 10 ectomycorrhizas. The main environmental drivers of EM communities were selected by fitting all environmental variables collected in the NMDS using *envfit* in *vegan* after 999 permutations. From these, we selected variables significantly correlated (p -value ≤ 0.05) with community dissimilarities and tested their collinearity with *chart.correlation* in *PerformanceAnalytics* (Peterson & Carl, 2018). Pairwise variable combinations with a Pearson correlation coefficient ≥ 0.7 were inspected and one of the pairs removed for posterior analyses. Seven different model variants were assembled grouping environmental variables in biotic (host species + habitat + total plant cover), climatic (MAT + annual precipitation), and edaphic (soil pH + N_{total} + Mg²⁺) components, and all interactions. Model fitting details are in Methods S5. The best model (i.e. highest explanatory power) was used to explore parameter estimates, partition the variance of the explained variability and assess how fungal OTU traits (i.e. presence-absence of hyphae and rhizomorphs) and EM lineage correlate with ecological niches.

Host population structure and EM communities

To test if EM fungal communities were shaped by host population structure, we used a subset of the root dataset based on the best number of clusters (K) defined by STRUCTURE from each host. Only sites from which all individuals shared similar admixture proportions were assigned to a genotype. Clusters included single or multiple sites with the same genotypes. One-way ANOVA was used to infer significant differences on estimated OTU richness across clusters and differences in community composition were assessed by PERMANOVA fitting a Bray-Curtis dissimilarity matrix as a function of host genotype.

Results

Alpine EM diversity

In total, 10,945 ectomycorrhizas were sampled from the roots of 950 individual plants (340 *D. octopetala*, 430 *B. vivipara* and 180 *S. herbacea*) across 47 sampling sites (17 *D. octopetala*, 21 *B. vivipara* and 9 *S. herbacea*). From these, we retrieved 9,393 DNA sequences, of which 8,945 were high quality and belonged to EM lineages (*sensu* Tedersoo *et al.*, 2010a; Tedersoo & Smith, 2013). After clustering and filtering, we identified 423 OTUs across 33 phylogenetic lineages. Approximately 68% of fungi forming ectomycorrhizas were Basidiomycota, dominated by */tomentella-thelephora* and */sebacina* and 32% were Ascomycota, mainly */cenococcum* (Fig. 1A). The lineages with highest richness were */tomentella-thelephora* (134 OTUs), */inocybe* (66 OTUs), */sebacina* (64 OTUs), */cortinarius* (36 OTUs) and */hebeloma-alnicola* (21 OTUs).

A total of 3,792,112 raw DNA reads were generated from soil samples, of which 1,448,522 were high quality. We identified 3,695 fungal OTUs of which 778 (average per soil sample 54.9 ± 29.4) belonged to 32 EM fungal lineages after removing non-EM OTUs, 30 centroid ribosomal sequences not sufficiently supported, and 20 EM OTUs conifer specialists s.l. (Tedersoo *et al.*, 2009; Nilsson *et al.*, 2019) not detected forming ectomycorrhizas in our dataset. Dominants in soil were */inocybe*, */tomentella-thelephora* and */cortinarius* (Fig. 1A). The highest OTU richness in soil was in */inocybe* (205 OTUs), */tomentella-thelephora* (178 OTUs), */sebacina* (80 OTUs), */cortinarius* (74 OTUs) and */tuber-helvella* (25 OTUs).

We detected 302 EM OTUs in both roots and soil, whilst 121 were only detected in roots and 476 only in soil (Table S1). Across all sites, we observed 272 EM OTUs in roots of *D. octopetala*, 341 in *B. vivipara*, and 134 in *S. herbacea*. Per site, we recovered on average 73% of the estimated EM fungal community richness in roots of *D. octopetala* and 81% in *B. vivipara* and *S. herbacea*. In soil, we found 554 EM OTUs under *D. octopetala*, 548 under *B. vivipara*, and 208 under *S. herbacea*. On average, we recovered 93% of the estimated EM diversity in soil under *D. octopetala*, 95% under *B. vivipara* and 96% under *S. herbacea*. Across hosts, we observed significantly lower estimated OTU richness in *S. herbacea* in roots and soil datasets in comparison with the other two hosts ($F_{\text{roots}} = 16.933$, $df = 2$, $p\text{-value} < 0.001$; $F_{\text{soil}} = 5.115$, $df = 2$, $p\text{-value} = 0.010$) (Fig. 1B). On average, the number of EM fungal OTUs associated with *B. vivipara* was higher when it co-occurred with *D. octopetala* than when co-occurring with *S. herbacea* ($F = 5.764$, $df = 3$, $p\text{-value} = 0.002$) (Fig. 1C).

Ordination (NMDS, Fig. 2) and PERMANOVA on the root dataset showed significant differences in EM fungal community composition among hosts, particularly between *D. octopetala* and *S. herbacea* and between *S. herbacea* and *B. vivipara* while communities of *B.*

vivipara and *D. octopetala* were not significantly different from each other. We also observed significant differences in EM fungal community composition across habitat type with pairwise *adonis* supporting significant differences between the snowbeds and treeline habitats, and between snowbeds and *Dryas* mats habitats. No significant differences in EM fungal community composition were found between treeline and *Dryas* mats (Table 2).

EM communities along elevation gradients

No significant differences in EM OTU richness were observed along elevation or between treeline and alpine zones. Significant differences in community composition were observed between alpine and treeline zones using constrained and unconstrained permutations. However, due to significant multivariate dispersion caused by heterogeneity of variances between treeline and alpine sites (betadisper: $F = 8.736$, $df = 1$, p -value = 0.008), PERMANOVA results could not be directly interpreted. Therefore, we assessed the effect of elevation in EM fungal community composition by constraining PERMANOVA permutations to habitat type (betadisper: $F = 0.826$, $df = 2$, p -value = 0.425). We detected significant changes in EM fungal community composition within habitats along elevation. Moreover, we observed that these differences among habitats and along the elevation gradient were not just constrained by taxonomic composition (i.e. at the OTU/species level) but also by phylogenetic signal (EM lineage) with some lineages such as */sebacina* more frequent at lower elevations while */cortinarius* and */russula-lactarius* were more frequent at higher elevations (Table 2, Fig. S1).

Across sites, the average number of EM taxa (OTUs) forming mycorrhizas with short distance soil exploration was highest (treeline: 29 ± 7 ; alpine: 22 ± 8), followed by contact (treeline: 7 ± 3 ; alpine: 8 ± 5) and medium-distance fringe (treeline: 3 ± 2 ; alpine: 6 ± 3) (Fig. 3A). We observed more short-distance soil explorers in the treeline ($t_{(28)} = -3.088$, p -value < 0.001) and more medium-distance fringe taxa in the alpine zone ($t_{(44)} = 3.66$, p -value < 0.001) (Fig. 3B). Across the three hosts, the most abundant soil exploration type was also short distance (Fig. 3C), with significantly less OTUs ($F = 27.912$, $df = 2$, p -value < 0.001) and average colonised roots in *S. herbacea* than in other hosts (Fig. 3D). We found more medium-distance fringe ectomycorrhizas per site in *S. herbacea* ($F = 4.190$, $df = 2$, p -value = 0.021; Fig. S2D).

The network specialization index (H_2') indicated that only treeline and snowbed habitat networks were more specialized than expected at random with the snowbed network showing

higher specialization. Across hosts, we observed more specialization in *S. herbacea* followed by *D. octopetala*, and *B. vivipara*. However, when analysing *B. vivipara* occurring in each alpine habitat independently (*Dryas* mats and snowbeds) we observed more specialization than expected when it occurred in snowbed habitats (Table 3).

Environmental drivers of EM communities

Across sites, twelve environmental variables contributed significantly to the dissimilarity of root EM fungal communities: elevation, MAT, annual precipitation, total soil C, total soil N, soil pH, Ca^{2+} , K^{+} , Mg^{2+} , percentage plant cover, plant host species and habitat zonation (treeline vs alpine) (Fig. 2, Table S2). For further analyses, we discarded elevation, total soil C, Ca^{2+} and K^{+} due to high collinearity with other variables (Pearson's $r \geq 0.7$). The model incorporating all biotic, edaphic and climatic variables had the highest explanatory power for EM community data (ca. 23%, Table 4). The explained variance partitioning of the best model fit showed that across OTUs the mean contribution of biotic factors was 12.4%, followed by edaphic variables (7.1%), climatic variables (2.6%), and site (random effect) (0.9%) (Fig. 4). On average, 12% of the niche preference of EM OTUs was determined by soil exploration type while no significant link between niche preference and OTU phylogeny was found ($\rho = 0.52$, with a 95%CI of 0.23 to 0.72). Many OTUs had significant responses to environmental covariates (posterior support > 0.95). Most fungal OTUs responded positively to increasing soil pH (45 OTUs) and total soil N (23 OTUs) and negatively to increasing annual precipitation (20 OTUs). We also observed a generalised negative response of *Cortinarius* OTUs to increasing Mg^{2+} .

Host population structure and EM fungal communities

We estimated the effect of host genotype on EM fungal communities for *D. octopetala* and *B. vivipara*; scoring genetic markers in *S. herbacea* was difficult due to lack of allelic polymorphism and no population structure was found. The optimum number of clusters in *D. octopetala* was four and in *B. vivipara* six (Fig. S3), but we only selected the four most distinct from the latter for further analyses due to unclear population subdivision. Pairwise F_{st} values among clusters (Table S3) ranged from 0.018 (cluster 3 vs cluster 4) to 0.063 (1 vs 2) in *D. octopetala* and 0.017 (3 vs 4) to 0.084 (1 vs 2) in *B. vivipara*. Analysis of molecular variance revealed no significant differentiation among *D. octopetala* or *B. vivipara* clusters (Table S4). Analysis of variance for the mean estimated EM fungal OTU richness per site and PERMANOVA

showed no significant differences among genotype clusters of *D. octopetala* or *B. vivipara* in community richness and composition (Table S5).

Discussion

To the best of our knowledge, this is the most extensive sampling of ectomycorrhizas across the Alps testing how fungal communities above treeline and up to the nival zone are shaped along altitudinal and environmental gradients. We detected over 400 EM fungal OTUs in a variety of ecological niches and influenced by complex biotic and abiotic interactions. We found differences in EM fungal community composition between treeline and alpine habitats where snowbed EM networks were the most specialized. This can have significant implications for mountain biodiversity, and the ecosystem functions supported by plants and fungi, because alpine ecosystems are expected to experience relatively rapid rates of environmental change compared to lower elevations (Winkler et al. 2019), thus placing highly specialized communities at risk.

Alpine EM diversity

In mountains generally, from treeline to nival zone, soils are nutrient deficient in inorganic N and P (Kielland, 1994), and there is high wind exposure and solar irradiation, low temperatures and lengthy snow cover (Nagy & Grabherr, 2009). Despite their small size and harsh environment, *D. octopetala*, *B. vivipara* and *S. herbacea* supported 423 EM fungal OTUs (87% assigned to genus or species level) across the alpine zone of the Alps. Previous studies at smaller scales also found abundant EM fungal species with these plants in the Alps (Mühlmann *et al.*, 2008; Mühlmann & Peintner, 2008; Pellissier *et al.*, 2014; Brunner *et al.*, 2017) and other alpine areas (Ryberg *et al.*, 2009; Bjorbækmo *et al.*, 2010; Gao & Yang, 2010; Mundra *et al.*, 2016; Botnen *et al.*, 2019). The EM fungal lineages */tomentella-thelephora*, */cenococcum* and */sebacina* dominated as in other belowground studies of alpine meadows (Ryberg *et al.*, 2011; Gao & Yang, 2016), alpine glacier valleys (Mühlmann *et al.*, 2008; Mühlmann & Peintner, 2008) and alpine cliff edges (Ryberg *et al.*, 2009). Dominant species of *Cenococcum*, *Tomentella* and *Sebacina*, that form sclerotia or crust-like fruitbodies, do not correspond with dominant aboveground fruiters, i.e. mushroom-forming *Cortinarius*, *Inocybe*, *Hebeloma* and *Russula* (Favre, 1955; Senn-Irlet, 1988; Graf, 1994; Jamoni, 2008). We also detected many hypogeous fungi usually overlooked in fruitbody inventories, such as *Genabea*, *Hydnobolites*, *Hymenogaster*, *Melanogaster*, *Elaphomyces* and *Gautieria*.

We found that our three plant species recruit approximately 39% of EM fungi detected in soil. This relatively small percentage can be partly due to sampling challenges when selecting ectomycorrhizas and difficulty detecting rare OTUs (rarely saturating species accumulation curves), the presence of other EM hosts harbouring additional fungi and/or partly to analysing communities from soil DNA using HTS technologies where read numbers hold limited biological meaning and do not discriminate between active, dormant or dead organisms, or intra- *versus* extra-cellular sources (Carini *et al.*, 2016; Wutkowska *et al.*, 2019). For instance, we did not detect DNA of *Cenococcum geophilum* OTUs in soil, whereas these were dominant ectomycorrhizas. Kauserud *et al.* (2012) reported methodological bias in detecting *C. geophilum* during library preparation when comparing pyrosequencing with cloning and Sanger sequencing of root communities in *B. vivipara*, with relatively low overlap in fungal OTUs observed between methods, even among the most abundant taxa. Both HTS and Sanger do not seem to result in significant differences at high taxonomic levels, like order (Amend *et al.*, 2010), and both techniques yield roughly similar phylogenetic community structure (Tedersoo *et al.*, 2010b). However, in this study and at species level, some dominant OTUs in roots were not detected in soil, calling for caution when analysing and extrapolating results from soil mycobiomes. 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 DNA; thus, if this is not a methodological issue, it could be explained by temporal dynamics of mycelial growth or limited extraradical growth and/or reproduction of this dominant EM fungus.

The influence of host on EM fungal community structure and composition has been demonstrated (Ishida *et al.*, 2007; Tedersoo *et al.*, 2013; van der Linde *et al.*, 2018), but the extent of this influence remains poorly understood, mainly due to problems disentangling confounding interactions among hosts, microbes, edaphic and environmental variables (Aponte *et al.*, 2010; Peay *et al.*, 2015). We found that *S. herbacea* associates with the most specialized set of EM fungi, followed by *D. octopetala*, while *B. vivipara* had the least specialised communities. However, we observed significant levels of specificity in EM fungal communities with *B. vivipara* when it co-occurred with *S. herbacea* in snowbeds. These results suggest that alpine EM fungal communities have a high specificity to particular habitat conditions rather than to host. *Salix herbacea* occurred exclusively in snowbed habitats while *B. vivipara* can grow in a wider environmental range and can co-occur with the other two plant species. Snowbed habitats harbour plant communities adapted to extreme edaphic conditions (e.g. *S. herbacea*) with more acidic

soils, which might explain the less species-rich EM fungal communities adapted to these harsher conditions. In contrast, *Dryas* mats and the treeline ecotone with more alkaline soils can harbour plant communities with wider ecological niches. To help disentangle the host specificity effect from other confounding environmental factors like soil pH, we analysed EM fungal communities associated with *B. vivipara* when it co-occurred either with *S. herbacea* in snowbed habitats or with *D. octopetala* in *Dryas* mats. We found *B. vivipara* harboured very similar communities to the corresponding co-occurring host, but contrasting communities and contrasting levels of specialization in different habitat conditions, allowing us to disentangle host from habitat effects. Fujimura and Egger (2012) also detected highly specialized EM communities with *Salix arctica*, and both host identity and environment explained similar proportions of EM community variability. Botnen *et al.* (2014) detected a lack of host fungal specificity in EM communities of *B. vivipara*, *D. octopetala* and *S. polaris* suggesting that: i) associating with fungi with as many different physiological attributes as possible may be favourable for the host to access a broader range of nutrient pools, and ii) association with more generalist fungi may allow plants to more rapidly and easily colonize new habitats. Fungal host specialization could provide greater physiological compatibility with the targeted host, resulting in greater competitive ability and access to its resources (Bruns *et al.*, 2002), but lack of host specificity may facilitate dispersal across the complex mosaic of alpine habitats with highly heterogeneous edaphic properties and a limited number of potential EM plant hosts. The small window of opportunity provided by the alpine snow-free season may disadvantage specialized fungi if their hosts do not achieve optimal metabolic conditions, resulting in less C availability for fungi. Our results indicate that in the alpine zone of the European Alps, plants and EM fungi tend to be generalists in their interactions, but their distribution range is limited by ecological niche and the complex and heterogeneous environmental characteristics.

EM communities along elevation gradients

Disentangling the effects of elevation in mountains is difficult due to underlying co-varying factors, such as temperature, precipitation and soil nutrient content. Across host plants, EM fungal community composition in the alpine zone shifted along the elevation gradient up to the nival zone, but no significant differences were detected in richness. Strong negative effects of elevation on EM fungal richness and changes in community composition have been observed in several mountain ecosystems worldwide at local scales. Bahram *et al.* (2012) found EM fungal

richness declined monotonically along increasing elevation in old-growth Iranian forests due to lower soil resource availability at higher elevations reducing the competitive ability of rare species and having a negative effect on EM richness. However, most studies attempting to characterize EM fungal communities along elevation gradients in mountains were not restricted to the alpine zone, therefore significant differences in richness may have been found due to cross-zone gradients. Kernaghan and Harper (2001) observed changes in EM fungal community composition and richness with elevation in the Canadian Rockies by sampling ectomycorrhizas and fruitbodies across a sub-alpine montane forest of *Picea* and *Abies* spp., a treeline ecotone with scattered willows and ericaceous shrubs, and an alpine zone dominated by *Dryas* spp. Changes were only significant between sub-alpine forest and alpine zone, not between treeline ecotone and alpine zone. Similarly, Collins *et al.* (2018) found that overall soil fungal richness and diversity declined with elevation, but differences were more pronounced between sub-alpine and alpine sites and were not significant within alpine sites. Adamczyk *et al.* (2019) however, analysed nival zone fungal communities in the same valleys in Switzerland sampled in this study and did not detect correlation between fungal richness and elevation, while pH was the strongest predictor of fungal beta diversity. We observed a declining trend in EM fungal community richness with elevation, but it was not significant (Table S6). On a global scale, EM fungal community richness is primarily influenced by the relative proportion and richness of host plants, soil pH, MAT and precipitation (Tedersoo *et al.*, 2012, 2014). In mountains, climatic variables (Nottingham *et al.*, 2018) and plant host distributions (Pellissier *et al.*, 2014; Geml *et al.*, 2017) explain most of the variation in EM fungal community richness. The complex collinearity among climatic, edaphic and topographic predictors in mountain ecosystems makes it difficult to address the isolated effect of elevation across regional scales. We addressed this issue by sampling along multiple elevation gradients across a wide environmental gradient. Our results suggest that, at regional scale, habitat zonation defined by specific climatic and edaphic conditions rather than elevation influence EM fungal community composition turnover along the elevation gradient.

Some EM fungal lineages like */hebeloma-alnicola* or */inocybe*, are dominant in Arctic ecosystems and have differential tolerance to temperature (Tibbett *et al.*, 1998; Geml *et al.*, 2012; Timling *et al.*, 2012; Blaallid *et al.*, 2014). In our extensive study, we found that mostly */cortinari* OTUs were influenced by environmental factors, more frequently found in alpine sites, and in particular in the snowbed habitats than in sites closer to the treeline. Similar results were found by Yao *et al.* (2013) and by Koizumi and Nara (2020) highlighting a temperature

dependency of *Cortinarius* at the treeline, with more frequent records at lower mean summer temperatures.

We observed that variation in EM functional traits (presence of hyphae and rhizomorphs) was more related to alpine ecosystem characteristics (e.g. edaphic properties and short snow-free periods) than to elevation or host specificity. Moreover, despite the high EM taxonomic diversity in the Alps, there is a high degree of functional redundancy with similar traits shared by many different fungi; we found EM fungal communities dominated by short distance explorers. In forest ecosystems, short explorers thrive at higher soil nutrient availability (Lilleskov *et al.*, 2011; Jarvis *et al.*, 2013), in colder climates where hosts have lower photosynthetic capacity (Fernandez *et al.*, 2017), or in colder and drier environments (Castaño *et al.*, 2018; Botnen *et al.*, 2019; Defrenne *et al.*, 2019). Alpine EM plants are adapted to harsh conditions, at least partly through reliance on short and contact explorers (Fig. 3A). A high carbon demand from medium and long explorers (Lilleskov *et al.*, 2011) could be disadvantageous in harsh environments and potentially these fungi are only cost effective at even lower nutrient conditions. This was shown by the higher abundance of medium-distance fringe EM fungi with *S. herbacea* in alpine sites (Fig. S2 D), probably driven by preferential association of *Cortinarius* with *S. herbacea* and the particular ecological niche where they occur. In those nutrient-depleted acidic soils, medium-distance fringe explorers' ability to mine organic sources of nitrogen appears critical (Lindahl & Tunlid, 2015; Kyaschenko *et al.*, 2017).

Environmental drivers of EM fungal communities

Hierarchical modelling indicated that the occurrences of EM fungi across the European Alps are shaped by a complex grid of biotic and abiotic drivers. Alpine communities seemed to be mostly influenced by host species (6.8%), soil Mg^{2+} (4.1%), mountain zone (treeline vs alpine, 3.3%) and soil pH (2.5%). However, we were able to disentangle host from confounding effects of habitat properties by using network analyses. Across European forests, EM fungal communities respond to host and environment (van der Linde *et al.*, 2018) with soil nutrient availability among the main drivers of EM fungal community richness and composition (Cox *et al.*, 2010; Suz *et al.*, 2014). In alpine and arctic ecosystems, *B. vivipara* root-associated fungal community composition correlates with soil pH, C, N and P (Blaalid *et al.*, 2012; Yao *et al.*, 2013). The higher pH characteristic of calcareous and dolomite soils, usually linked to rich plant communities, and the high content of Mg as $CaMg(CO_3)_2$ in the latter, might partly explain the emergence of both

variables as drivers of communities in these habitats. In contrast to studies where climatic variables were drivers of EM fungal communities globally (Tedersoo *et al.*, 2012, 2014) and regionally (Bahram *et al.*, 2012; Botnen *et al.*, 2019), we found precipitation and temperature to be the weakest predictors of EM fungal community composition across the Alps. This may be explained by lower variability in precipitation and temperature at regional scales in mountains, where host identity, distance from the treeline and soil chemistry become more important, explaining altogether *ca.* 23% of EM fungal community variability.

We also observed that environmental filtering leads to variable occurrence of EM fungi across the Alps, but only *Cortinarius* seems to show phylogenetic signal, with higher number of OTUs at higher elevations where soil N is lower. Fungi with different soil exploration types respond differently to soil N concentrations (Lilleskov *et al.*, 2011; Suz *et al.*, 2014), and specialized organic N miners with proteolytic abilities, such as *Cortinarius*, are inhibited by high levels of soil inorganic N, particularly from atmospheric deposition (Cox *et al.*, 2010; Lilleskov *et al.*, 2011; Suz *et al.*, 2014; van der Linde *et al.*, 2018). Moreover, we found that in the alpine zone, only 12% of the niche preference of OTUs was explained by EM soil exploration type. Therefore, isolating environmental filtering from other non-random processes that could affect EM fungal community structure and composition remains difficult. Nevertheless, the responses of individual fungal taxa to environmental factors help to understand EM fungal community resilience to environmental change across the Alps. Despite the weak effect of climate on these communities, habitat loss due to climate change (e.g. via upward expansion of sub-alpine forests) can have major consequences for the most specialised EM fungal communities (Pauli *et al.*, 2012; Wipf *et al.*, 2013). Experiments are now necessary to assess resilience of alpine ecosystems to future climate change scenarios. Extending analysis to other mountain habitats (e.g. grasslands) and zones, hosts and seasons will reveal spatiotemporal variability in alpine zone communities.

Host population structure and EM fungal communities

Empirical studies show that coevolving traits, such as mycorrhizal colonization, exhibit substantial heritable variation within plant and fungal species and are influenced by interactions between plant and fungal genotypes (Hoeksema, 2010). Non-neutral processes such as local adaptation to biotic interactions are important for plants since they associate with specific microbial symbionts that affect their fitness (Friesen *et al.*, 2011; Rúa *et al.*, 2016). Genetic differences associated with plant traits such as resistance and susceptibility to herbivory (Sthultz *et*

al., 2009) or drought tolerance (Gehring *et al.*, 2017) can influence EM fungal community structure and composition. In a sub-alpine hybrid zone of *Salix helvetica* and *S. purpurea*, plant host genotype was not a strong predictor of overall EM fungal community, but it influenced the occurrence of particular fungi (Arraiano-Castilho *et al.*, 2020). Links between plant genotype and EM fungal communities may have implications for fungal diversity, host-symbiont interactions and aboveground-belowground linkages in ecological and evolutionary contexts (Lamit *et al.*, 2016) but these still remain an open question, in particular when plants and fungi occur in harsh environmental conditions. Given that *D. octopetala* and *S. herbacea* reproduce sexually by airborne seeds, and form extensive ground carpets, while *B. vivipara* is apomictic, we expected these differences and the Alps' complex geomorphology and habitat variability to affect plant population structure and therefore their EM fungal communities. Coupled to the lack of host specificity, we did not find any influence of *D. octopetala* genotype on its associated EM fungal communities, potentially due to confounding variables with stronger effects shaping these fungal communities, such as environmental variability and habitat/niche specificity. Lack of allele polymorphism for *S. herbacea* and high ploidy in *B. vivipara* made it difficult to assign genotypes to each population. Future studies involving phenotypically-expressed plant traits (e.g. drought tolerance) and controlling for environmental variability may help uncover host genotype effects on EM fungal communities.

Conclusions

Despite harsh environmental conditions, alpine EM fungal communities are highly diverse and richness does not decrease with elevation, but fungal composition varies from the treeline to the alpine zone. In complex habitat mosaics with diverse climatic and edaphic conditions, mountain zonation (alpine vs treeline), soil pH and soil total nitrogen concentration explain most variation in EM fungal communities of above-treeline plants across the Alps. Our analyses of plant-fungal interaction networks highlight the uniqueness and vulnerability of EM fungal communities in the characteristic snow-patch and snowbed communities of dwarf willows.

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Author Contribution

R.A.-C, M.I.B, T.N., J.J.C. and L.M.S conceived the study and sampling design. R.A.-C, T.N, I.B, S.Z, B.S.-I, U.P, T.M, and L.M.S conducted the fieldwork. R.A.-C, M.I.B and L.M.S processed and analysed samples. S.Z. performed soil chemistry analysis. R.A.-C and L.M.S analysed data with support of B.F for HTS data and T.N. for OTU clustering pipeline. R.A.-C and J.J.C. did the plant population genetic analysis, R.A.-C drafted the manuscript, all authors provided significant contributions to the manuscript, and M.I.B. and L.M.S provided chief contributions.

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Supporting information

Figure S1 Ectomycorrhizal fungi community composition across habitats and elevation.

Figure S2 Comparison of the number of ectomycorrhizas showing different soil exploration types.

Figure S3 STRUCTURE plots with population sub-division in *Dryas octopetala* and *Bistorta vivipara*.

Methods S1 Soil chemistry analysis.

Methods S2 DNA sequence analyses and OTU clustering from mycorrhizas and soil.

Methods S3 Plant host genotype assessment.

Methods S4 Network analysis.

Methods S5 Hierarchical modelling of species communities.

Table S1 Ectomycorrhizal OTUs in the roots and in soil presented in this study.

Table S2 Envfit results for the environmental variables used in NMDS ordination.

Table S3 Population differentiation (F_{st}) values among clusters identified in STRUCTURE analysis.

Table S4 AMOVA results among clusters identified in STRUCTURE analysis.

Table S5 Summary table of the effect of host genotype in ectomycorrhizal fungal communities.

Table S6 Summary table of the effect of elevation in ectomycorrhizal fungal communities.

Table 1. The twenty-eight sampling locations included in this study and their main topographic and edaphic characteristics.

Location	Country	Long	Lat	Host species	Elevation (m)	Habitat	Corine Land Cover	MAT (°C)	Ann. precip. (mm/year)	Soil pH
LAUTAR_01	France	45.063547	6.402991	<i>S. herbacea</i> , <i>B. vivipara</i>	2,592	Snowbeds	333_Sparsely vegetated areas	-0.61	1003	4.79
LAUTAR_02	France	45.062923	6.404146	<i>S. herbacea</i>	2,579	Snowbeds	333_Sparsely vegetated areas	-0.61	1003	6.42
LAUTAR_03	France	45.053431	6.404901	<i>D. octopetala</i> , <i>B. vivipara</i>	2,429	<i>Dryas</i> mats	333_Sparsely vegetated areas	0.86	912	6.77
LAUTAR_04	France	45.037795	6.400640	<i>D. octopetala</i> , <i>B. vivipara</i>	2,114	<i>Dryas</i> mats	322_Moors and heathland	2.51	822	6.72
LAUTAR_05	France	45.052905	6.388098	<i>S. herbacea</i> , <i>B. vivipara</i>	2,359	Snowbeds	333_Sparsely vegetated areas	0.34	984	4.58
ZERNEZ_01	Switzerland	46.628144	10.287766	<i>D. octopetala</i>	2,450	<i>Dryas</i> mats	332_Bare rocks	-0.48	906	7.40
ZERNEZ_02	Switzerland	46.632964	10.284639	<i>D. octopetala</i> , <i>B. vivipara</i>	2,288	Treeline	321_Natural grasslands	-0.25	895	6.24
ZERNEZ_03	Switzerland	46.682419	10.238911	<i>D. octopetala</i> , <i>B. vivipara</i>	2,396	<i>Dryas</i> mats	333_Sparsely vegetated areas	0.20	836	7.29
ZERNEZ_04	Switzerland	46.737765	9.963139	<i>S. herbacea</i>	2,449	Snowbeds	333_Sparsely vegetated areas	-1.06	939	3.81
ZERNEZ_05	Switzerland	46.743415	9.980309	<i>S. herbacea</i>	2,188	Snowbeds	321_Natural grasslands	0.16	817	3.67
ZERNEZ_06	Switzerland	46.641510	10.237138	<i>D. octopetala</i>	2,380	<i>Dryas</i> mats	333_Sparsely vegetated areas	-0.24	884	7.29
SCHARL_01	Switzerland	46.678148	10.374443	<i>D. octopetala</i>	2,300	<i>Dryas</i> mats	321_Natural grasslands	0.05	813	7.34
SCHARL_02	Switzerland	46.671996	10.361511	<i>B. vivipara</i>	2,346	Snowbeds	321_Natural grasslands	0.86	651	4.21
SCHARL_03	Switzerland	46.682399	10.364270	<i>D. octopetala</i> , <i>B. vivipara</i>	2,127	Treeline	231_Pastures	0.75	692	7.09
SCHARL_04	Switzerland	46.692224	10.347199	<i>D. octopetala</i> , <i>B. vivipara</i>	2,329	<i>Dryas</i> mats	321_Natural grasslands	0.21	722	5.45
SCHARL_05	Switzerland	46.682380	10.365192	<i>D. octopetala</i> , <i>B. vivipara</i>	2,137	Treeline	321_Natural grasslands	0.75	692	7.03
GARFUL_01	Austria	47.174157	9.920619	<i>D. octopetala</i> , <i>B. vivipara</i>	1,830	<i>Dryas</i> mats	321_Natural grasslands	1.88	1971	7.08
GARFUL_02	Austria	47.180725	9.921334	<i>D. octopetala</i> , <i>B. vivipara</i>	1,478	Treeline	321_Natural grasslands	3.58	1863	6.71
OBERGU_01	Austria	46.828354	11.043108	<i>S. herbacea</i> , <i>B. vivipara</i>	2,398	Snowbeds	333_Sparsely vegetated areas	-1.34	863	7.55

OBERGU_02	Austria	46.840277	11.032048	<i>S. herbacea, B. vivipara</i>	2,296	Snowbeds	333_Sparsely vegetated areas	-0.88	884	6.67
OBERGU_03	Austria	46.845662	11.020627	<i>B. vivipara</i>	2,266	Snowbeds	411_Inland marshes	-0.15	815	4.36
INNSBR_01	Austria	47.313880	11.396169	<i>D. octopetala, B. vivipara</i>	2,201	<i>Dryas</i> mats	333_Sparsely vegetated areas	0.61	1283	5.9
INNSBR_02	Austria	47.317318	11.391506	<i>D. octopetala, B. vivipara</i>	2,021	Treeline	333_Sparsely vegetated areas	1.63	1386	6.38
VIZZE_01	Italy	46.992634	11.667176	<i>S. herbacea</i>	2,388	Snowbeds	333_Sparsely vegetated areas	0.02	921	3.86
VIZZE_02	Italy	46.993388	11.674160	<i>S. herbacea, B. vivipara</i>	2,492	Snowbeds	332_Bare rocks	-0.56	963	4.04
VRSIC_01	Slovenia	46.427458	13.750879	<i>D. octopetala, B. vivipara</i>	1,727	Treeline	333_Sparsely vegetated areas	3.44	2279	7.32
VRSIC_02	Slovenia	46.437767	13.738510	<i>D. octopetala, B. vivipara</i>	1,987	<i>Dryas</i> mats	332_Bare rocks	1.46	2473	7.29
KRVAVE_01	Slovenia	46.309117	14.542299	<i>D. octopetala, B. vivipara</i>	1,946	Treeline	333_Sparsely vegetated areas	2.63	1814	6.31

French sites are a mixture of limestone, marl and gypsum. In Switzerland, dolomite dominates at sites with *ca.* pH 7 while migmatites and siliceous moraines predominate at other sites. In Austria, sites were characterised by carbonate sedimentary rocks (INNSBR), limestone (GARFUL), gneiss (VIZZE) or rocks rich in quartz and feldspar like amphibolite and phyllite (OBERGU). In Slovenia, limestone and limestone-dolomite characterised sites KRAVEC and VRSIC, respectively.

Table 2. PERMANOVA results of ectomycorrhizal (EM) fungal community composition across plant host, habitat type and along elevation.

EM fungal community composition across:	<i>F</i>	<i>R</i> ²	<i>df</i>	<i>p</i> -value
Plant host				
Three hosts	2.505	0.102	2	0.001
<i>Dryas octopetala</i> vs <i>Bistorta vivipara</i>	1.538	0.041	1	0.087
<i>D. octopetala</i> vs <i>Salix herbacea</i>	4.192	0.149	1	0.003
<i>B. vivipara</i> vs <i>S. herbacea</i>	2.374	0.078	1	0.003
Habitat type				
Three habitats	3.706	0.144	2	0.001
Treeline vs <i>Dryas</i> mats	1.457	0.048	1	0.177
Treeline vs snowbeds	4.371	0.135	1	0.003
<i>Dryas</i> mats vs snowbeds	5.109	0.141	1	0.003
Elevation (habitat type)				
Three habitats (OTU level)	3.869	0.080	1	0.001
Three habitats (EM lineage level)	10.105	0.183	1	0.001

Significant differences (p -value ≤ 0.05) are presented in bold.

Table 3. Results of ecological network analysis at habitat (H_2') and species level (d').

Index	Network	obs	<i>p</i> -value	z-score	L 95% CI	U 95% CI
H_2'	Snowbeds	0.469	<0.001	4.915	0.245	0.250
	<i>Dryas</i> mats	0.340	0.075	1.459	0.370	0.372
	Treeline	0.305	0.007	3.277	0.221	0.225
	<i>Salix herbacea</i>	0.571	<0.001	9.158	0.348	0.351
d'	<i>Dryas octopetala</i>	0.409	0.001	4.833	0.306	0.309
	<i>Bistorta vivipara</i>	0.261	0.960	-1.566	0.293	0.295

<i>B. vivipara</i> in snowbeds	0.606	<0.001	4.944	0.445	0.449
<i>B. vivipara</i> in <i>Dryas</i> mats	0.321	0.926	-1.383	0.349	0.352

Significant deviations from the null models (p -value ≤ 0.05) are presented in bold. Obs: observed value; L and U 95% CI: Null models lower and upper limits of the 95% confidence interval respectively.

Table 4. Hierarchical model structure ranked by increasing explanatory power.

Model description	Covariates	Tjur R^2	AUC
Y ~ Biotic	Host + Habitat + %Plant cover	0.1249	0.8308
Y ~ Climatic	MAT + Annual precipitation	0.1344	0.7905
Y ~ Biotic & Climatic	Host + Habitat + %Plant cover + MAT + Annual precipitation	0.1566	0.8326
Y ~ Edaphic	Soil pH + N _{total} + Mg _{Soil}	0.1722	0.8351
Y ~ Climatic & Edaphic	MAT + Annual precipitation + Soil pH + N _{total} + Mg _{Soil}	0.1900	0.8396
Y ~ Biotic & Edaphic	Host + Habitat + %Plant cover + Soil pH + N _{total} + Mg _{Soil}	0.2003	0.8563
Y ~ Biotic & Climatic & Edaphic	Host + Habitat + %Plant cover + MAT + Annual precipitation + Soil pH + N _{total} + Mg _{Soil}	0.2294	0.87980

All models were fitted with the abundance matrix of ectomycorrhizal (EM) fungal OTUs (Y) as a function of each covariate combinations. MAT: mean annual temperature, N_{total}: soil total nitrogen, Mg_{soil}: exchangeable magnesium content in soil (Mg²⁺).

Figure legends

Figure 1. A) Relative abundance of each ectomycorrhizal (EM) fungal lineage per site across plant hosts as ectomycorrhizas in the roots (left) and in the soil (right). B) Observed and estimated (ACE) number of EM fungal OTUs in roots and soil across hosts. C) Observed and estimated (ACE) number of EM fungal OTUs in roots across hosts, separating *Bistorta vivipara* when co-occurring either with *Dryas octopetala* or with *Salix herbacea*. The distribution of the data is illustrated by each box-plot, vertical lines represent the range between the minimum and the maximum observed value, while the box represents the first and the third quartile and the horizontal line denotes the median. Outlier observations are plotted as individual points. Significant differences (p -value ≤ 0.05) are represented by different letters.

Figure 2. Non-metric multidimensional scaling (NMDS) ordination of the Bray-Curtis dissimilarity matrix of ectomycorrhizal (EM) fungal communities associated with the roots of *Dryas octopetala* (squares), *Bistorta vivipara* (circles) and *Salix herbacea* (triangles) when they occurred at the treeline ecotone (blue), *Dryas* mats (red) and at the snowbed communities (green). Arrows show the direction and strength of correlation of the environmental variables with significant contribution (p -value ≤ 0.05) to community dissimilarities. Environmental variable labels represent edaphic properties (brown), host and habitat (green) and elevation and climate (black). DO: *Dryas octopetala*, BV: *Bistorta vivipara*, SH: *Salix herbacea*. Precip: annual precipitation; MAT: mean annual temperature; N_{total}: soil total nitrogen; C_{total}: soil total carbon; Mg²⁺, Ca²⁺, K⁺: exchangeable magnesium, calcium and potassium content in soil.

Figure 3. Comparison of the number of ectomycorrhizal (EM) fungal OTUs showing different soil exploration types: A) within the treeline and alpine zones, B) in alpine versus treeline zones, C) within hosts, and D) among hosts. The distribution of the data is illustrated by each box-plot, vertical lines represent the range between the minimum and the maximum observed value, while the box represents the first and the third quartile and the horizontal line denotes the median. Significant differences (p -value ≤ 0.05) are represented by letters and asterisks; ns: non-significant.

Figure 4. Posterior support values (≥ 0.95) for ectomycorrhizal fungal OTU environmental responses. Horizontal bar plots represent variance partitioning in the abundance of each individual

OTU with covariates contributions grouped by biotic (host species + habitat + total plant cover), climatic (mean annual temperature + annual precipitation), edaphic (soil pH + soil total nitrogen (N_{total}) + exchangeable magnesium content in soil (Mg^{2+}_{soil})) and random effects (site). DO: *Dryas octopetala*, SH: *Salix herbacea*.

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