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RESEARCH ARTICLE

Abiotic conditions along altitude shape plant-fungal associations by influencing both fungal availability and association strength

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Abstract

1. Plant-fungal associations are shaped by both abiotic and biotic variables, but how abiotic variables influence the pool of available fungal species has been much more studied than how the abiotic environment structures the complex and dynamic associations between a host plant and its associated fungi. The question of whether plant-associated fungal communities are primarily driven by fungal species availability or by the effects of the abiotic conditions on the type and strength of the association remains unclear.
2. Here, a large-scale field survey in Fennoscandia investigated how the selected fungal communities within different *Bistorta vivipara* plant compartments (bulbils, leaves and roots) varied with altitude, relative to the available fungal species pool in the soil and surrounding plants.
3. Our results revealed that while the selected fungal species pool was largely determined by the available fungal species pool, the occurrence probability of some fungal species increased at high or low altitudes relative to the respective available fungal species pool. Furthermore, while we found that plant compartment was a major selective force shaping plant-fungal associations, environment-driven selection influenced the type and strength of plant-fungal associations.
4. While the fungi present in the environment largely determined which fungi associated with the focal plants, the type and strength of fungal species associations changed depending on the altitude-related abiotic stressors imposed on the plant, such as lower temperatures, shorter growing seasons and high winds.
5. *Synthesis*. Whether plants associate with fungal species just from the available fungal pool or differentially depending on the environmental stressors imposed on the plant, is not well understood, yet this is central for understanding both plant and microbial assembly. We conclude that plant-fungal associations at different altitudes are not exclusively shaped by passive responses to fungal species

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availability but rather by more strategic responses by intensifying the associations with fungal species that may offer ecological advantages, such as improved productivity and health. These findings deepen our understanding of how plants adapt to environmental stress through selective symbiosis, with broader implications for plant-fungal community assembly and ecosystem dynamics.

KEYWORDS
arctic, biotic selection, environmental selection, joint species distribution model, metabarcoding, plant holobiont, plant microbiota, plant-fungal interactions, Plant–soil (below-ground) interactions

1 | INTRODUCTION

The diverse communities of microorganisms that plants associate with exert key ecosystem functions by promoting the productivity and health of plants (Tedersoo et al., 2020; Trivedi et al., 2020; Van Der Heijden et al., 2008). Among the mutualistic plant-associated microbiota, fungi play pivotal roles in plant fitness by assisting in nutrient and water acquisition and providing protection against herbivores and pathogens (Rodriguez et al., 2009; Smith & Read, 2010). Mutualistic fungi interact with plants in different ways. Endophytic fungi (living within plant tissues) and mycorrhizal fungi (forming root symbioses) establish direct associations, while saprotrophs (decomposing dead organic matter) and epiphytes (living on plant surfaces) interact indirectly. Besides mutualistic fungi, plants also associate with fungal species that do not provide any benefit, including both antagonistic and commensal fungal species. Many pathogenic fungi persist in healthy plant tissues in a latent stage, showing no symptoms until conditions favour their growth (Saikkonen et al., 1998). While it is well-recognized that plant-fungal associations are shaped by both abiotic and biotic factors, oftentimes the focus is on how abiotic variables directly influence either the plant or fungal diversity, rather than how the abiotic environment structures the complex association systems between a host plant and its symbiotic fungi. Abiotic variation related to nutrient and water availability (Lagueux et al., 2021) or climatic conditions (Abrego et al., 2020; Cobian et al., 2019; Jarvis et al., 2015), along with biotic factors related to the host plant's identity (Abrego et al., 2020) and genotype (Johnson et al., 2012; Qian et al., 2018), influence the composition of plant-associated fungal communities. However, overlooking that plant-fungal association types are dynamic depending not only on the available species pool but also on the effects of the abiotic environment on the type and strength of the association may misrepresent a key aspect of community assembly and ecosystem functioning.

While many studies examine plant-fungal associations across scales (Bahram et al., 2022; David et al., 2016; Jarvis et al., 2015; Wagner et al., 2016), few address how environmental context influences plant-driven selection of fungal species. Yet, growing evidence suggests that fungi may enhance plant fitness under harsher environmental conditions (Burg et al., 2024), potentially due to plants selecting fungal species that alleviate environmental stress. Observational studies indicate that symbiotic fungal communities can facilitate plant establishment beyond their typical ecological niches, such as in high-stress environments at high altitudes (Lynn et al., 2019; Pellissier et al., 2013; Rodriguez et al., 2008). Laboratory experiments further support this, showing that abiotic conditions can influence the fungal species with which plants associate (Hoeksema et al., 2010). One greenhouse experiment demonstrated that specific fungal species improve plant fitness in stressful environments (Fitzpatrick et al., 2019). Additionally, recent findings from a translocation experiment between high- and low-altitude populations of a focal plant suggest that specific fungi enhance plant tolerance to altitudinal stressors, including colder temperatures and reduced nutrient availability (Burg et al., 2024). Overall, this body of research suggests that plants may actively modify and recruit beneficial microbial communities to improve survival in harsh environments.

While under stress, plants change their root exudates, which potentially modifies the interacting species pool (Ali et al., 2022). In fact, abiotic conditions can affect which fungal species plants associate with (Hoeksema et al., 2010). However, the strategies that plants employ to select the most beneficial fungal species remain to be fully comprehended (Vandenkoornhuyse et al., 2015). While some studies have found that plants allocate the same amount of carbon to the associated fungal species regardless of how much nitrogen they provide (Valtanen et al., 2014), others have found that plants employ defence mechanisms against less valuable symbiotic fungi (Hortal et al., 2017) or that more carbon is allocated to fungal species providing more nitrogen to the plant (Bogar et al., 2019). Additionally, host physiochemical factors dictate which microbes are selected in different plant compartments (Bernard et al., 2021). Fungal communities in different plant compartments have been found to be as distinct as those between different host plant species, a pattern which indicates the lack of transmission of fungi across plant compartments (Fang et al., 2019; Guevara-Araya et al., 2020; Jin et al., 2015; Peršoh, 2013; Purahong et al., 2019; Wearn et al., 2012). However, some studies have found evidence for compositional nestedness, that is, that the above-ground fungal communities are a subset of the fungal communities from below-ground (Bahram et al., 2022; Bernard et al., 2021; de Souza et al., 2016).

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A key issue that remains largely unanswered is the degree to which the available fungal species pool shapes plant-fungal associations as compared to the effects of the abiotic conditions on the type and strength of the association. In this study, we carried out a large-scale field survey to investigate how the selected fungal communities within different compartments of the arctic-alpine *Bistorta vivipara* plant varied along altitude, relative to the available fungal species pool in the soil and in the surrounding plants (Figure 1). *B. vivipara* is a common, long-living perennial plant in alpine and arctic environments of the northern hemisphere which reproduces through vegetative bulbils (Blaalid et al., 2014; Gardes & Dahlberg, 1996; Kardol et al., 2014; Yao et al., 2013). *B.* vivipara establishes ectomycorrhizal associations as well as many other types of associations with fungi, including endophytes and arbuscular mycorrhizae (Abrego et al., 2020; Mundra et al., 2016; Rasmussen et al., 2022). Importantly, given the aim of the study, the fungal communities within *B. vivipara* roots change along altitude (Abrego et al., 2020) and with soil edaphic factors (Blaalid et al., 2014; Mundra et al., 2016), such changes being key to the plant's growth at higher altitudes (Burg et al., 2024). While previous studies on the fungal communities associated with *B. vivipara* have primarily focused on its root-associated fungi (Abrego et al., 2020; Blaalid et al., 2014; Burg et al., 2024; Mundra et al., 2016; but see Kardol et al., 2014), in the present study we also compare the fungal communities in different plant compartments (roots, leaves and bulbils) to those in the surrounding soil and plants (which were assumed to form the available species pool) to examine both abiotic and biotic factors that influence plant-fungal associations. We collected individuals from 10 populations of *B. vivipara* and the surrounding soil and plant leaves at both low and high-altitude locations in Fennoscandia (Finland, Sweden and Norway) and characterised the fungal communities by applying ITS2-based DNA barcoding on the plant tissue and soil samples (Figure 1).

We expected both the available and selected fungal communities to consistently vary with altitude, but the selected species pool to be only a fraction of the available species pool. Within plants, we expected the plant compartment to explain the most variation in the selected fungal communities, but to also find a degree of overlap, reflecting compositional nestedness and transmission across compartments. We expected the differentiation between the above-ground (i.e. leaves and bulbils) and below-ground (i.e. roots) compartments to reflect differences in the richness of fungal guilds known to interact in those compartments, such as epiphytes and endophytes in the leaves and ectomycorrhizal fungi in the roots. We specifically hypothesized that ectomycorrhizal fungi (which are known to interact with *B. vivipara*) would have stronger associations with host plants at high altitudes, supporting the stress gradient hypothesis which suggests that positive interactions among organisms become stronger as abiotic stress increases (Bertness & Callaway, 1994). In general, reflecting differential selection for interacting fungal species depending on altitude, we expected the occurrence probability of some of the selected fungal species to be higher under specific conditions such as the conditions at higher or lower altitudes, relative to the available species pool.

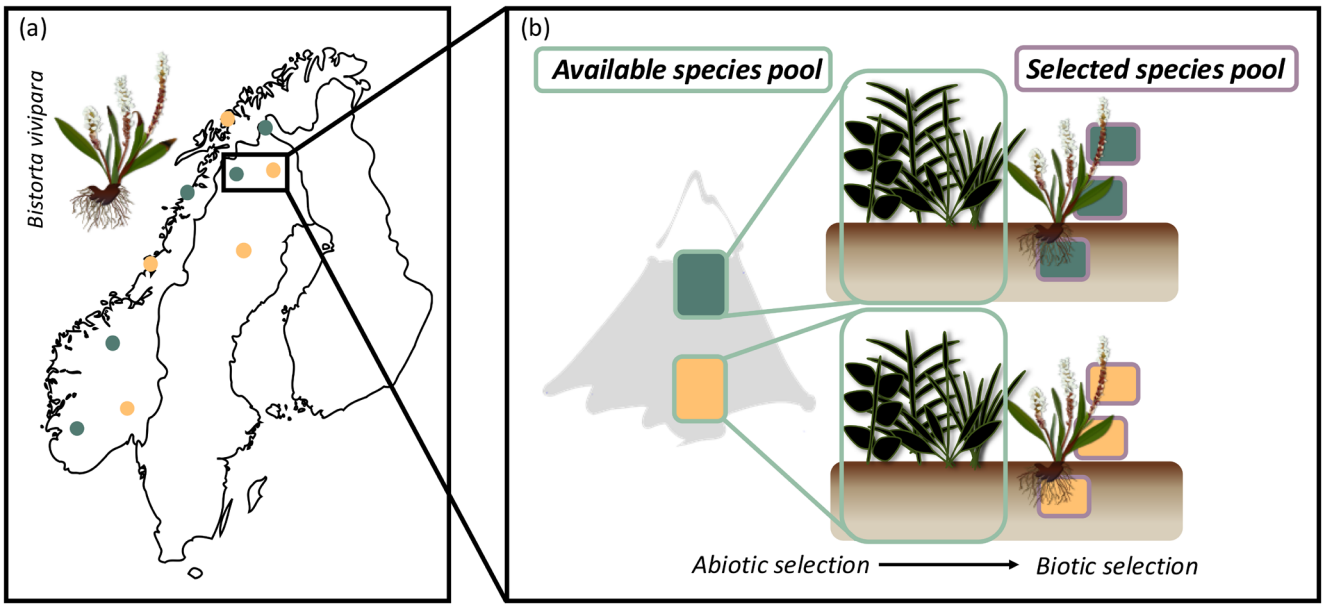


FIGURE 1 Experimental setup showing the locations of the plots around Fennoscandia (a) and the sampling design across high and low altitudes (b). Cyan represents high-altitude plots, and orange low-altitude plots. In (b), the available fungal species pool, which is selected based on the abiotic environmental conditions, is represented by the surrounding soil and plants in each plot, while the selected species pool, which we propose is selected by the plant (biotic selection), is represented by the fungal communities found in the focal plant roots, leaves and bulbils.

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2 | MATERIALS AND METHODS

2.1 | Study area and sampling design

The study area (regional species pool) covers the whole Fennoscandian (Finland, Sweden and Norway) mountainous region (Figure 1a). In July 2022, a field survey was conducted on 10 populations of *B. vivipara*, representing both high and low-altitude locations (above tree line and below tree line, respectively). All sampling sites were composed of natural tundra plants, but the high-altitude locations were mainly dominated by dwarf shrubs such as *Salix arctica* and *Dryas octopetala*, while the low-altitude sites were dominated by graminoids and forbs. To ensure that the sampled plant populations represented high- and low-altitude conditions, the populations were selected so that their surroundings (within at least a 10 km radius) represented the same altitudinal zone as the focal population. Likewise, to avoid pseudo-replication of spatially connected populations, we selected populations at least 100 km away from each other. The low- and high-altitude populations were spatially interspersed as much as possible, given the availability of low- and high-altitude populations. Additionally, we assigned population pairs between high and low sites that were closest to each other, with the average distance between population pairs being 191 km and the shortest distance between pairs being 119 km. To confirm the abiotic differences between the high- and low-altitude plots, we applied a PCA to the following variables: altitude, satellite-derived normalized difference vegetation index (NDVI), snow cover duration, snow melt date, annual precipitation, annual mean temperature and human footprint index (Venter et al., 2016; Figure S1; Table S1). Median NDVI and snow cover duration were calculated from Landsat-8 imagery for 2014–2023 (Niittynen et al., 2018). Climate variables represent averages of 1981–2010 and were extracted from Chelsa downscaled gridded climate dataset (Karger et al., 2017). Confirming the presence of abiotic differences between the high- and low-altitude plots, we found that at the high-altitude plots, environmental stressors imposed on the plants increased, such as a later snow melt date and more days of snow cover (Niittynen et al., 2020; Rissanen et al., 2023), as well as lower mean temperatures (Körner, 2003). In addition, lower NDVI values, which were found at the high-altitude locations, are associated with less healthy and abundant vegetation (Raynolds et al., 2006; Figure S1; Table S1).

Permission was not required for plant and soil collection at eight populations. For Population G in Stora Sjöfallet/Stuor Muorkke National Park (Sweden), permission was granted by the Länsstyrelsen Norrbotten County Administrative Board (ref. 521-6229-2022). For Population J in the Saanatunturi area (Finland), permission was granted by Metsähallitus (ref. MH 3296/05.04.01/2022). The Finnish Food Authority Plant Protection Service approved soil transfer and quarantine from Norway to Finland (ref. 3532/04.00.05.01.01/2022).

Within each focal population, a 100 m^2 plot was established. At each plot, roots (including the soil directly attached to the roots), leaves and bulbils from 20 *B. vivipara* plants were collected (Figure 1b). Focal plant individuals were selected evenly throughout the plot, with individuals at least 0.5 m apart, when possible. Large plant individuals were selected to ensure enough tissue for DNA extractions. The samples were separated into paper bags that were inside airtight plastic bags containing colour-indicating silica gel to dry them. The root samples were further hand-cleaned for soil particles after collection. On the same day as collection, and before drying samples with silica gel, individual *B. vivipara* characteristics were measured, including leaf width and length of the largest leaf per individual, and number of leaves, bulbils and inflorescences per individual. Since bulbils were prone to fall off during handling, the attachments on the stems were used to count bulbils. To compare the selected fungal communities in the focal plant to the available communities in the surrounding environment, we used the surrounding soil in each plot to represent the below-ground community, and the leaves of vascular plants in each plot to represent the above-ground community. Thus, at three locations within the plot, we randomly selected and separated three abundant and different vascular plant species leaves (three samples per site). At three locations evenly distributed within the plot, soil samples (250 g) were collected from the rooting zone (upper 10 cm), ensuring that no large debris was collected. *B. vivipara* roots, leaves and bulbils, and surrounding plant leaves and soil samples were stored separately at room temperature and in the dark with silica gel for 1 year before fungal DNA extraction. The amount of silica gel was adjusted for biomass and replaced any time it could no longer hold moisture to ensure the samples stayed dry. This storage procedure is known not to affect fungal DNA in samples of small biomass such as leaves and roots (Tedersoo et al., 2022) or of soil samples (Smenderovac et al., 2024; Wang et al., 2021). Soil carbon and nitrogen were measured 1 year after collection with a FlashEA 1112 NC Analyzer (Thermo Fisher Scientific), and soil pH was measured with a combination electrode in a 1:4 (v/v) suspension of soil in water.

2.2 | DNA extraction

Soil samples were prepared by sieving with a 2 mm sieve, followed by taking 250 mg of the sample and homogenising for 10 min at 25 Hz using a TissueLyser II (Qiagen). DNA was then extracted using the DNeasy PowerSoil Pro Kit (Qiagen), following the standard kit protocol. Elution at the end of the protocol was done with 100 µL of elution buffer.

Root, leaf and bulbil samples were processed using the DNEasy Plant Pro Kit (Qiagen). After ensuring the tissue samples did not have any visible disease symptoms, we randomly selected the leaves, bulbils and roots for DNA extraction. We took small sections from several leaves per plant individual and several bulbils, depending on weight. Tissues were first cut into small pieces and weighed to ca. 10 mg in the tissue disruption tube. We then added 450 µL of solution CD1 and 50 µL of Solution PS to the tissue disruption tube before lysis with the TissueLyser II (Qiagen) for 6 min at 24 Hz, after

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which the adapter was reoriented and samples were lysed for an additional 6 min at 24 Hz. If samples remained unbroken, they were lysed one more time under the same settings. The remainder of the DNA extraction followed the kit protocol with the following modification: DNA was washed with 250 μL of Solution CD2. DNA extracts for all sample types were diluted 1:10 before PCR.

2.3 | Next generation sequencing workflow

DNA samples were amplified with fITS7 (Ihrmark et al., 2012) as the forward primer and ITS4 and ITS4A (Urbina et al., 2016) as reverse primers, targeting the fungal rDNA ITS2 region. Primers were modified with variable length "phased" spacers to increase sequence diversity (Wu et al., 2015) and Illumina Nextera adapters. The forward primer was comprised of four phases of equal amounts and the reverse primers of eight phases (six ITS4 and two ITS4A) of equal amounts, which are listed in the Supporting Information (Appendix S1).

Plasmid DNA was extracted from *E. coli* strains bearing pUC57 plasmid with SynMock 1–12 inserts (Palmer et al., 2018) using the Thermo Scientific GeneJET Plasmid Miniprep Kit. The plasmid extracts were each quantified using ddPCR with the Biorad QX200 Automated Droplet Generator. Each extract was then standardized to a concentration of 4550 copies/μL, and all extracts except SynMock 5 were pooled to form a spike-in standard (50,000 copies/μL) which was added to PCR master mix at a 1:50 ratio for a final concentration of 1000 copies/μL to enable approximate quantification of samples, while plasmid 5 was used as a positive control sample.

The PCR mix contained 10 μL Phusion Plus Green PCR Master Mix (Thermo Fisher Scientific), 4 μL of DNA template, 3.5 μL of water, 0.5 μL of spike and 1 μL each of the pooled forward and pooled reverse 5 μM primers. The PCR consisted of an initial denaturation at 98°C for 30 s followed by 30 cycles of: denaturation at 98°C for 10 s; annealing at 54°C for 30 s; extension at 72°C for 1 min; final extension at 72°C for 5 min.

After the first PCR, the DNA concentration of samples was quantified with Quant-iT™ PicoGreen™ dsDNA Assay Kit from Invitrogen and subsequently normalized to get equal DNA concentrations for each sample. The second PCR was carried out to index the library using Index 1 and Index 2 fusion primers containing i5 and i7 indices from the Nextera XT Index Kit V2. The PCR mix contained 12.5 μL of Phusion Plus Green PCR Master Mix (Thermo Fisher Scientific), 5.5 μL of water, 2.5 μL of each 5 μM index primer and 2 μL of the amplicon template. The PCR programme consisted of an initial denaturation at 98°C for 30 s followed by 8 cycles ofdenaturation at 98°C for 10 s, annealing at 60°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 5 min.

All indexed PCR products (samples) were then pooled together and purified using a 1:1 volume ratio of Quantabio SparQ PureMag beads to purify the indexed library of free primers and primer dimers. The concentration of the pooled library was then quantified using a Qubit dsDNA HS Assay, and a quality control check was done using the Agilent 2200 TapeStation and the High Sensitivity D1000 ScreenTape kit to check the size distribution of the pooled library. The pool was then diluted to 4 μM and sequenced with Illumina MiSeq Reagent Kit v3 (600-cycle) and 40% PhiX for one 96-well plate and an output of 2 × 300 bp reads.

2.4 | Bioinformatic analysis

The bioinformatic analysis was performed using a pre-release development version of the OptimOTU pipeline (Furneaux et al., 2025). See Appendix S2 for more details. Briefly, we first trimmed and filtered the demultiplexed reads with Cutadapt version 4.2 (Martin, 2011) and DADA2 version 1.18 (Callahan et al., 2016) in order to remove adapters, trim low-quality ends and remove sequences that were too short, had low quality or that matched against the phiX genome. We then dereplicated, denoised, merged and chimera filtered the reads for each run independently following the standard DADA2 ITS pipeline (Callahan et al., 2020), resulting in a set of amplicon sequence variants (ASVs). Next, ASVs were taxonomically identified using Protax-fungi (Abarenkov et al., 2018) with a cutoff of 90% probability, resulting in taxonomic assignments of each ASV at ranks from phylum to species. This algorithm assesses the probability for correctly placing sequences to a particular taxonomic level and not only accounts for known reference sequences but also for species present in taxonomy but with no reference sequences, unknown species and mislabelled reference sequences. ASVs were clustered using a taxonomically informed pseudo-single-linkage clustering process at each taxonomic rank. Optimal clustering thresholds were determined at each rank using the hierarchical optimization technique developed by Dnabarcoder (Vu et al., 2022) but using the same USEARCH + BLASTCUT single-linkage clustering. We used taxonomically identified fungal sequences from the Global Spore Sampling Project (Ovaskainen et al., 2020) as references for the threshold optimization step. Reference cluster cores were formed from the sequences identified by Protax-fungi, and unidentified sequences were matched and joined to cluster cores using -usearch_global command in VSEARCH version 2.15.2 (Rognes et al., 2016), and single-linkage clustered the remaining unclustered ASVs using BLASTCUT version 2.2.26 (Dondoshansky & Wolf, 2000) and USEARCH version 11.0.667 (Edgar, 2010). The remaining clusters at the species rank were used for our analysis and referred to as operational taxonomic units (OTUs). To ensure the sampling was representative of the fungal diversity in the community, we performed rarefaction analyses, which confirmed the depth of the sequencing process (Figure S2).

2.5 | Statistical analyses

To describe general patterns in plant-associated fungal community composition, we first applied a NMDS analysis on relative species abundances (number of reads for each OTU divided by the total number of reads in each sample) using the Bray–Curtis dissimilarity measure

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with the vegan R package (Oksanen, 2011). In the NMDS, we visualized the selected communities in different *B. vivipara* plant compartments compared to the available communities according to altitude. To evaluate the representativeness of the sampling design at the plot level, we performed a sample completeness analysis (Chao et al., 2020) with the R package iNEXT.4steps. We computed incidence-based sample completeness and thus estimated the proportion of the total number of occurrences belonging to the detected species. We repeated the analyses for orders $q=1$ and $q=2$ of sample completeness, the former weighting all species equally and the latter giving more weight to the more common species (Chao et al., 2020). The sample completeness analysis demonstrated that sampling 20 focal individuals per plot was sufficient in representing the OTU diversity, especially of the more common species (Figure S3). As expected, sample completeness was lower for soil and surrounding plants for which we obtained three samples per plot, but also for these sample types, the proportion of the total number of occurrences belonging to detected species was greater than one half.

To evaluate whether the available fungal communities differed from the *B. vivipara* roots, leaves and bulbils at high and low altitudes, we analysed the data with the joint species distribution modelling (JSDM) framework Hierarchical Modelling of Species Communities (HMSC) (HMSC; Ovaskainen et al., 2017; Ovaskainen & Abrego, 2020), using Hmsc-HPC (Rahman et al., 2024), which is a high-performance computing (HPC) extension of the R package Hmsc (Tikhonov et al., 2020). HMSC is a multivariate hierarchical generalized linear mixed model fitted with Bayesian inference. The response variable contains the occurrences of the species that respond to both the measured predictors (fixed effects) as well as to latent predictors (random effects), which are jointly estimated (Ovaskainen & Abrego, 2020). This modelling framework allowed us to examine how the occurrences of the plant-associated fungal species varied among the available and selected species pools and how these depended on altitude while also accounting for the structure of the study design.

The full fungal data matrix consisted of 8670 fungal OTUs, henceforth referred to as fungal species, across the 660 sampling units (200 *B. vivipara* individuals with samples from roots, leaves and bulbils, 30 soil samples and 30 surrounding vascular plant samples). To obtain sufficient data for robust parameter estimation in the JSDM model, we removed the rarest fungal species from our analysis, defined as those that occurred in fewer than 50 out of the 660 sampling units, resulting in 253 fungal species (Table S3). We additionally excluded 19 samples from the model which contained fewer than 1000 fungal sequence reads, yielding 641 sampling units with fungal read counts ranging from 1010 to 222,629, with a median of 48,634 reads (Figure S4). We used 1000 fungal sequence reads as a cutoff to account for the negative PCR and DNA extraction controls, which on average contained 1059 fungal sequence reads (Table S4).

The main model we constructed evaluated how the fungal communities in the five sample types (available communities in the surrounding soil and plants, and selected communities in the focal roots, leaves and bulbils) varied with altitude. We considered the occurrences of the OTUs at the presence-absence level and hence assumed a Bernoulli distribution with a probit link function. The phylogenetic tree, which was constructed to visualize taxonomic patterns in the data, was approximated using the taxonomic classification of the OTUs, including pseudotaxa, with equal branch lengths for each Linnaean rank. We further evaluated the differences between fungal communities in each sample type by a variant of the main model that partitioned sample type into two categories: available or selected, and then a separate model for only the selected sample types and only the available sample types. To test whether the patterns from the main model were also generalized to the excluded rare species, we performed a sensitivity analysis by fitting Poisson regression models to the excluded and included species with the same model structure as in the main model, which showed consistent responses to the predictors (Appendix S3; Table S5). To assess whether the available communities represented the local species pool above- and below-ground (i.e. whether they varied little within the plots), we fitted separate models for soil and plant samples. The soil-only model showed that individual soil samples within each plot accounted for 11.9% of the total explained variation in fungal occurrences, and the plant-only model indicated that individual plant samples accounted for 9.9% of the total explained variation.

Additionally, we constructed a guild model, which assessed how the guild-specific fungal community richness varied between the five sample types with altitude. We constructed the guild model using a multivariate Poisson regression model to measure the responses of fungal guild assignments to the environmental variables. We extracted the trophic guild data of plant pathogens, saprotrophs, endophytes, epiphytes, ectomycorrhizas and ericoid mycorrhizas from Aguilar-Trigueros et al. (2023), which combines species-level information from the FunGuild database (Nguyen et al., 2016) with the USDA host-fungus database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm). Guilds were assigned at the genus level, and if the OTU was not assigned to a genus, it was assigned at the family level. As a result, many fungal OTUs were assigned to several trophic guilds.

As the focal environmental covariate, we considered altitude (low and high), which we included in the models by adding an interaction term between altitude and the sample type. Thus, we did not only aim to capture the effects of altitude on the selected (roots, leaves and bulbils) and available (surrounding soil and plants) communities, but also whether the selected communities varied with altitude differently than how the available communities varied. We additionally controlled for the effects of the following covariates that were measured in the field: number of leaves (at the level of the plant individual, log-transformed), mean soil carbon:nitrogen (C:N) ratio from each population and mean soil pH from each population. To assign the number of leaves to the population-level samples (surrounding soil and plants), we calculated the mean log-transformed number of leaves from each individual focal plant for each site. In addition, we controlled for variation in sequencing depth by adding a covariate consisting of the log-transformed number of sequencing reads per sample. We controlled for the study design by including as random effects the plant individual (200 levels), plot (10 levels) and pairs of high-/low-altitude populations (five levels).

The models were fitted assuming the default prior distributions (Ovaskainen & Abrego, 2020). We sampled the posterior distribution

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with four Markov chain Monte Carlo (MCMC) chains, each of which was run for 37,500 iterations, of which the first 12,500 were removed as burn-in. The chains were thinned by 100 to yield 250 posterior samples per chain and so 1000 posterior samples in total. We examined MCMC convergence by the potential scale reduction factors (Gelman & Rubin, 1992) of the model parameters (Appendix S4; Table S6).

The explanatory power (goodness of model fit) of the presence-absence JSDMs was assessed through AUC (Pearce & Ferrier, 2000) and the species richness with pseudo R^2 (Table S7). We applied variance partitioning to investigate the proportion of the explained variance in species occurrences, and guild-level richness attributed to each fixed and random effect included in the models. We also evaluated the β-parameters describing species-level responses to the predictors. We calculated for each plant compartment the posterior probability of a fungal species being especially present in low and high altitudes from the posterior mean of the β-parameters and the measured C:N, pH, number of leaves and sequencing depth. We examined the level of phylogenetic signal in the species responses to the environmental predictors in the model by estimating the parameter ρ, with a focus on which specific taxonomic groups showed systematic responses to the interaction between altitude and plant compartment.

We conducted all statistical analyses using R version 4.3 (R Core Team, 2023).

3 | RESULTS

3.1 | Patterns in the selected and available fungal communities at high and low altitudes

The average number of species-level fungal OTUs per sample was greater at low altitudes compared to high altitudes in all sample types except for roots (Table 1). Overall, the available fungal communities were more species rich than the fungal communities selected by *B. vivipara*. Within the focal plant's compartments, roots and leaves hosted threefold the number of species detected in bulbils (Table 1).

The most commonly occurring guild in the roots and soil was saprotrophs, followed by plant pathogens (Table 1). The most common OTUs in the roots were an unidentified *Helotiales*, *Phialocephala fortinii* (Ericoid mycorrhizal) and *Cenococcum geophilum* (ectomycorrhizal), while in the soil the most common OTUs were *Trichocladium opacum* (plant pathogen, wood saprotroph), *Pseudogymnoascus* sp. (Ericoid mycorrhizal, endophyte, saprotroph) and *Cryptococcus* sp. (epiphyte, plant pathogen, saprotroph) (Table S8). In the surrounding plants, focal leaves and focal bulbils, plant pathogens were the most commonly occurring guild, closely followed by saprotrophs and endophytes. Specifically, OTUs assigned to *Cladosporium* sp. (plant pathogen, saprotroph, endophyte, epiphyte), *Phaeosphaeria* sp. (plant pathogen, endophyte) and *Venturia* sp. (plant pathogen, endophyte) were among the most common in the surrounding plants, focal leaves and focal bulbils (Table S8). Interestingly, mycorrhizal fungi were found in all plant compartments in addition to soil, even the leaves and bulbils. Species-level OTUs assigned to mycorrhizal guilds that were found in the leaves and bulbils include Cenococcum geophilum, Pezoloma ericae, Lactarius torminosus and Tomentella lapida (Table S9). The guilds with higher species richness at low altitudes than at high altitudes were plant pathogens, saprotrophs, endophytes and epiphytes (Table 1). However, there were more saprotrophs in the roots at high altitudes than at low altitudes. Compared to the soil, the richness of every guild was smaller in the roots, bulbils and surrounding plants. Also, all guilds were less species-rich in the leaves than in soil, except for plant pathogens, which had similar richness in leaves and soil and epiphytes which were more species rich in leaves than soil.

We compared how many species were overlapping among the focal plant's compartments (i.e. how the selected fungi were allocated across plant compartments) and between each of the focal plant's compartments and the surrounding soil and plants (i.e. between the selected and available fungal communities; Figure 2a–d). Overall, there was a high degree of species overlap among the plant's compartments (Figure 2a). Within the focal plant, 32% of all species that were found in roots were also found in the leaves, and of all species found in the leaves, 34% were also found in the bulbils.

TABLE 1 Mean and standard deviation of the species richness for sample types from low and high altitudes.

	Surrounding soil		Surrounding plants		Focal roots		Focal leaves		Focal bulbils	
	Low	High	Low	High	Low	High	Low	High	Low	High
Mean number of species	265±73	255±112	134±94	82±26	124±37	142±44	131±52	102±38	41±22	31±18
Ectomycorrhizal	38±10	49±15	11±9	6±3	24±8	31±8	12±7	9±5	3±3	2±2
Ericoid Mycorrhizal	31±7	41±15	10±8	5±3	16±6	24±7	11±6	8±5	3±2	2±2
Plant pathogen	90±18	95±32	74±47	49±12	48±15	58±18	70±25	61±19	26±12	21±11
Saprotroph	148±36	145±47	69±45	43±13	71±23	80±24	66±25	55±20	23±11	18±11
Endophyte	75±16	73±23	50±33	33±8	37±12	41±15	46±17	41±13	16±8	16±8
Epiphyte	10±4	6±3	10±4	5±3	4±3	4±2	11±3	6±3	5±3	2±1

Note: The sample types represent the selected (*Bistorta vivipara's* roots, leaves and bulbils) and the available (surrounding soil and plants) communities. For species richness, the total and guild-specific number of detected fungal species per sample is given.

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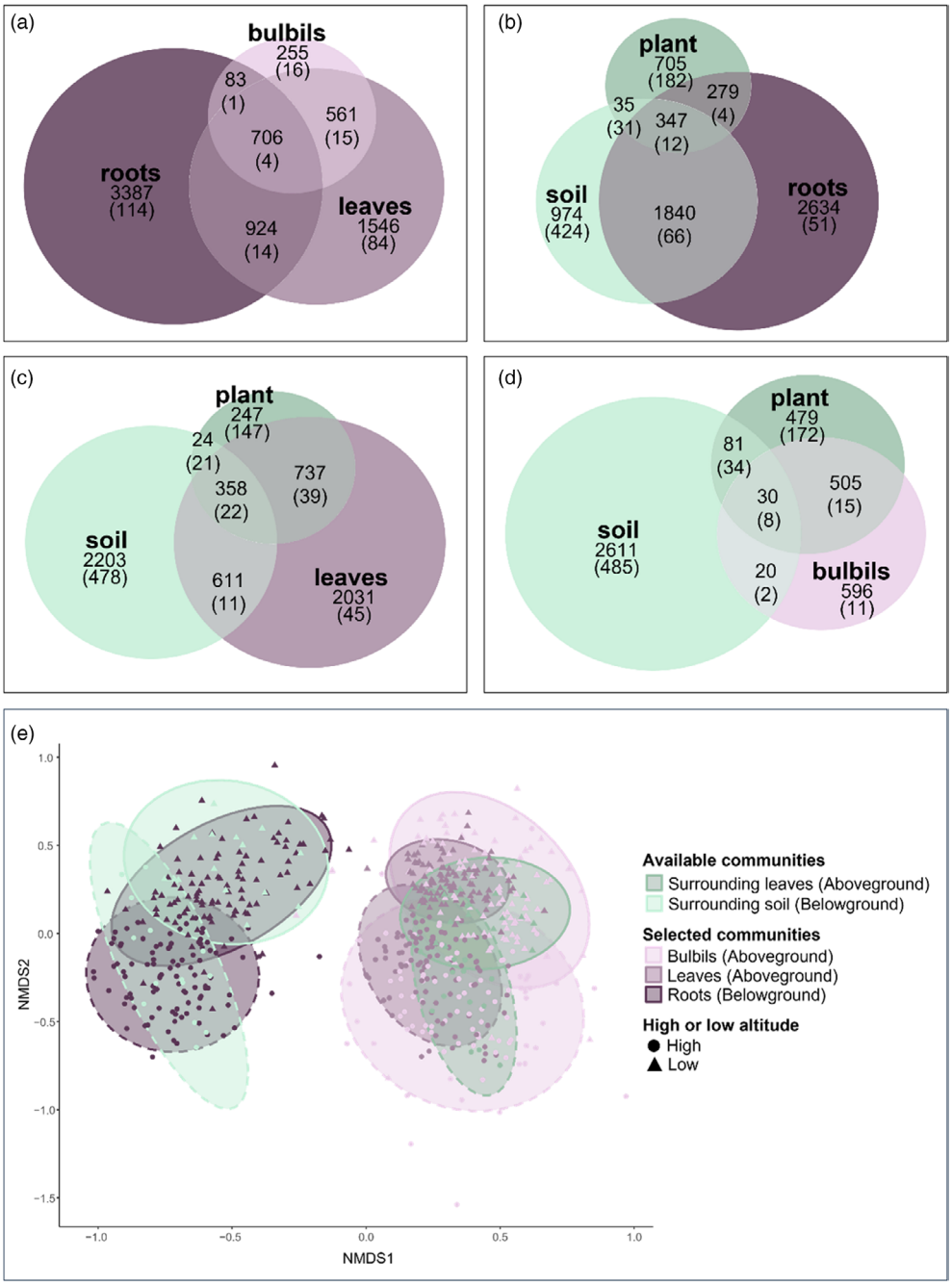


FIGURE 2 Fungal community composition between the selected (purple gradients) and available (green gradients) fungal communities. Numbers in parentheses represent the mean number of fungal species per plant individual. Panel (a) shows overlap for the selected fungal species among *B. vivipara* compartments (bulbils, leaves and roots). Panels (b–d) show, respectively, the overlap between the selected fungal communities in roots, leaves and bulbils of *B. vivipara* and the available fungal communities in the surrounding soil and plant leaves. Panel (e) shows an NMDS ordination illustrating how fungal communities within *B. vivipara* compartments (bulbils, leaves and roots, shown in purple gradients) and surrounding soil and plants (shown in green gradients) vary between high- and low-altitude sites. The ellipses cover 95% of data points (sampling individuals) within each plant compartment, with the solid ellipses covering the high-altitude samples and the dashed ellipses covering the low-altitude samples.

Furthermore, 84% of the species found in the bulbils also occurred in the leaves or roots. Likewise, there was a high degree of overlap between the selected and available fungal communities (Figure 2b–d). Between 41% and 48% of the species found in the surrounding soil and plants were also found in the roots, leaves and bulbils of the focal species. Overall, the highest overlap was between the available communities in the soil with the selected communities in the roots (68% of the species occurring in the soil were also found in the roots, and 43% of the species occurring in the roots were also found in the soil), and between the available communities in the surrounding leaves with the selected communities in the leaves (80% of the species occurring in the surrounding leaves were also found in the focal

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leaves, and 29% of the species occurring in the focal leaves were also found in the surrounding leaves) then bulbils. When examining the same patterns at the plant individual-level, the fraction of shared species among the focal plant's compartments and between each of the focal plant's compartments and the surrounding soil and plants showed consistent patterns (Figure 2a–d).

The NMDS ordination showed that despite the large overlap of the selected and available fungal species, fungal communities were still different among plant compartments and also compared to the available communities (Figure 2e). However, as expected, selected communities in roots were largely similar to the available communities in the soil, whereas the selected communities in the leaves and bulbils were largely similar to the available communities in the surrounding plant leaves. Likewise, when comparing how many species assigned to each guild overlapped between sample types, we found that most fungal guilds were consistently found across sample types, with the least overlap being between the above-ground and below-ground sample types (Figure S5; Appendix S5). Interestingly, the fungal communities clearly differed between high- and low-altitude sites both for the available and selected communities (Figure 2e).

3.2 | How much variation in the selected fungal communities does the plant compartment compared to the environment explain?

As the main analytical tool to quantify the effects of the environment on the selected versus available fungal communities, we fitted a joint species distribution model (JSDM) to data on the most prevalent 253 species-level OTUs. These analyses quantitatively confirmed the patterns observed in the NMDS, revealing that sample type captured a large amount (31.5%) of the variation in fungal species occurrences, which could be mostly explained by whether the communities were from above-ground or below-ground. Variants of the JSDM model that included sample types representing either the selected communities or the available communities captured a high amount of variation for this variable (33.3% and 38.5%, respectively) whereas a variant that indicated only whether each sample was from a selected or available community captured almost no variation for this variable (0.8%). Thus, while the fungal communities vary largely across the plant compartments and between the available above-ground and below-ground environments, they do not vary systematically between the selected and available communities. Likewise, for guild-specific species richness, most of the explained variance was captured by sample type, capturing 57.6% of the variation (Table 2; Table S10). That is, the richness of different guilds varied considerably across plant and soil compartments.

Altitude additionally explained a large proportion of the variance in the fungal communities from each sample type, explaining 19.7% of the variation in the presence-absence main model (Table 2) and more for the available (21.8%) than the selected communities (17.4%) (Table 2). The interaction between altitude and sample type, which showed whether certain fungal species were selected in each sample type depending on altitude, explained 9.6% of the variation in the presence-absence main model (Table 2). The comparison across models revealed that this pattern was mostly driven by the interaction between altitude and selected communities, not the available communities (Table 2). Altitude alone explained 9.3% of the variation in guild-specific species richness, and additionally 5.6% in interaction with sample type (Table 2, Table S10).

The next most relevant variable was the random effect at the level of plant population, followed by the random effect of the high–low-altitude population pairs, which explained, respectively, 11.2% and 7.2% of the variation in the presence-absence main model (Table 2). In this case as well, the pattern was mostly driven by the selected communities, not the available communities. That is, the selected communities were consistently more different across plant populations than across plant individuals, but this was not the case for the available communities (Table 2). The soil chemical variables pH and C:N explained, respectively, 3.7% and 5.3% of the variation in the presence-absence main model. These variables were more important for the available communities than for the selected communities (Table 2).

Altitude had statistically supported effects on the occurrences of most of the fungal species included in the analyses (65% of species had a statistically supported response to altitude), with the probability of occurrence of 31% of the species being greater at high altitudes, and of 34% being greater at low altitudes (Figure 3). The soil chemistry predictors (pH and C:N) had an additional effect on the fungal communities, with both having a positive effect on the occurrences of 17% of species and negative effects on 27%–32% of species (Figure 3). The number of leaves that a focal plant individual contained had small species-level effects, with between 2% and 5% of species occurrences increasing or decreasing in response to an increase in leaf number.

As previously seen in the NMDS and variance partitioning, the fungal species in the roots of the focal plant and in the surrounding soil were similar (Figure 3; responses in the roots were similar to the responses in the soil). Likewise, the fungal species in the focal plants' bulbils and leaves were similar to the fungal species in the surrounding leaves. Species occurrences especially differed when comparing the selected communities in the above-ground compartments (leaves and bulbils) with the available communities in the soil: 85% of species in the leaves and 70% of species in the bulbils had statistically supported differences in occurrence probabilities in comparison to the soil. The probability of occurrence was greater in the focal leaves compared to the soil for 51% of species, and lower for 34% of species.

3.3 | Are associations with fungi dependent on altitude?

In the main model, the interaction term between altitude and sample type revealed that in low and high altitudes, certain fungal species were more (or less) likely to be found across the sample types

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TABLE 2 Variance partitioning across the included predictors (rows) for the different models (columns).

	Main model	Guild model	Selected versus available model	Selected model	Available model
	Presence-absence	Species richness	Presence-absence	Presence-absence	Presence-absence
High-low population pair	7.2	3.7	11.5	8.2	3.7
Plant population	11.2	3.3	14.7	7.9	4.3
Plant individual	5.8	15.2	16.6	5.4	5.3
Sequencing depth	5.5	0.1	20.5	6.5	5.5
Number of leaves	0.4	0.2	0.5	0.5	NA
Soil pH	3.7	0.8	4.2	4.4	6.8
Soil C:N	5.3	4.1	5.3	6.3	9.1
Altitude	19.7	9.3	23.8	17.4	21.8
Altitude × sample type	9.6	5.6	2.1	10	5
Sample type	31.5	57.6	0.8	33.3	38.5
Sample type levels for each model	Surrounding soil, surrounding plants, focal roots, focal leaves, focal bulbils	Surrounding soil, surrounding plants, focal roots, focal leaves, focal bulbils	Available (Surrounding soil, surrounding plants), versus Selected (focal roots, focal leaves, focal bulbils)	Focal roots, focal leaves, focal bulbils	Surrounding soil and surrounding plants



FIGURE 3 Fungal species-level responses to each predictor, measured through the β-parameters from a presence-absence probit model. Panel (a) shows the statistically supported responses for each species; panel (b) shows the percentage of species showing a statistically supported response. Red indicates a positive response of fungal species with ≥95% posterior probability, and blue indicates a negative response with ≥95% posterior probability. Concerning the categorical variables, surrounding soil and low altitude are used as base levels to which the other levels are compared to. See Table S3 for a list of fungal species.

relative to their occurrences in the soil (Figure 3). In other words, the patterns seen in the interaction between altitude and sample type indicate that, even when accounting for the availability of fungal species in the soil, the plant is associating with different fungal communities at high and low altitudes. Seven per cent of the fungal species included in the model had a more positive response to altitude in the roots than in the soil. In the leaves, bulbils and surrounding plants, between 10% and 20% of species had a more positive response to altitude compared to their occurrences in the soil. Conversely, 10%–12% of species found in the leaves, bulbils and surrounding plants had a more negative response to altitude compared to soil. In the fungal guild model, the species richness in the soil decreased with altitude for all guilds besides ectomycorrhizae and ericoid mycorrhizae. The interaction term between

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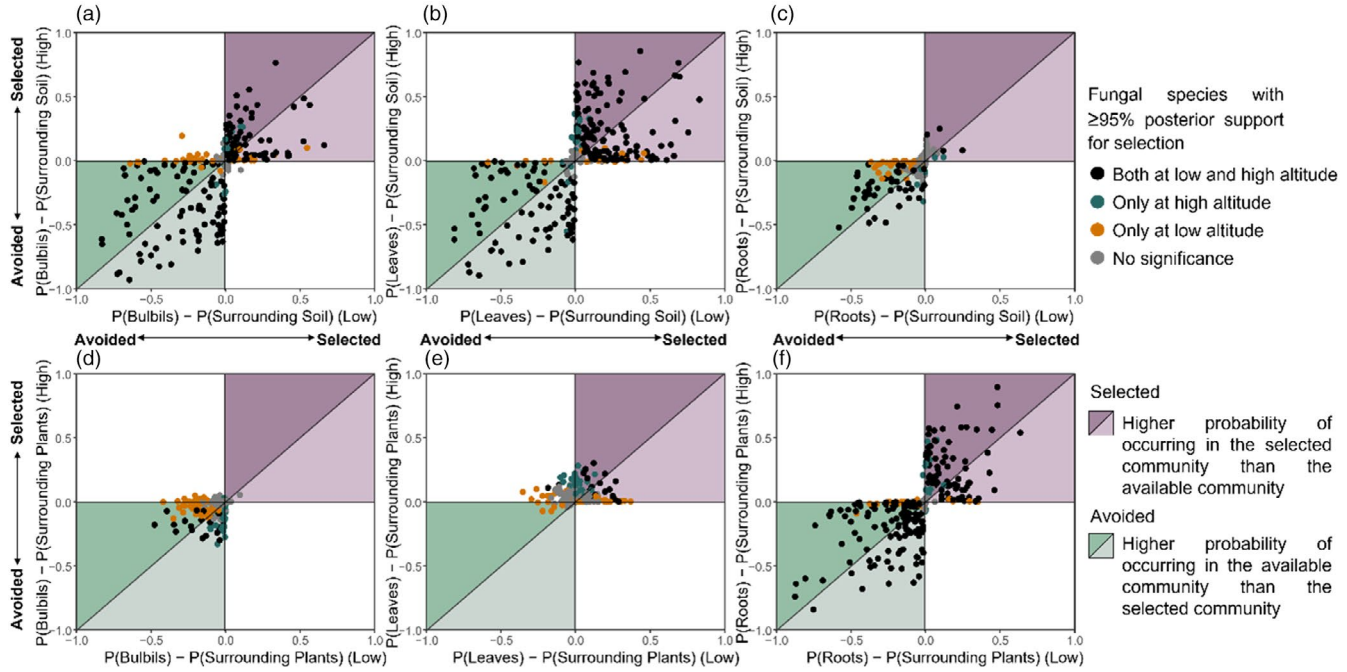


FIGURE 4 Fungal species selection in different compartments (bulbils, leaves and roots) in high and low altitudes in relation to the available communities (surrounding soil and plants). Each datapoint represents the posterior mean difference in occurrence probability of each species between the selected and available fungal communities, shown separately for high and low altitudes and for the different available communities (Panels (a–c) shows soil; panels (d-f) shows surrounding plants) and selected communities (Panels (a) and (d) show bulbils, (b) and (e) show leaves and (c) and (f) show roots). Cyan and orange colours indicate, respectively, fungal species selected at high or low altitudes (with posterior probability ≥95%). Species close to the origin (0,0) indicate that the communities are similar between the sample types, while species further from the origin indicate that the communities are different between the sample types. The purple shadings highlight the species that are selected in the focal plant, whereas species in the green shadings highlight the species that are avoided in the focal plant. The list of the species with strong affinity to a given compartment in a given altitude are listed in Table S11.

altitude and plant compartment indicated that the species richness of all guilds had a more positive response to altitude in the roots compared to the soil (Figure S6). In the leaves, the species richness of ericoid mycorrhizae and ectomycorrhizae had a more negative response to altitude compared to the soil. In the bulbils, the species richness of ericoid mycorrhizae, ectomycorrhizae and epiphytes had a more negative response to altitude compared to the soil. In the surrounding plants, the number of ericoid mycorrhizae and ectomycorrhizae had a more negative response to altitude compared to the soil.

A comparison between the parameters measuring the affinity of the fungal species to the plant compartments with their availability in the soil or other plants confirmed that some species were especially selected or avoided depending on the altitude (Figure 4). Some species were selected in roots compared to soil (Figure 4c), and leaves compared to other plants (Figure 4e) (with ≥95% posterior probability support). Interestingly, more species were selected at high altitudes and avoided at low altitudes, as seen by the larger number of species that were plotted above the identity line in Figure 4c–e. Among the plant compartments, focal leaves were especially selecting fungal species at high altitudes and especially avoiding those same species at low altitudes (datapoints in upper left quadrant, Figure 4e).

4 | DISCUSSION

Unravelling the mechanisms by which the plant microbiome is shaped along abiotic gradients is vital for understanding plant community assembly and ecosystem functioning. In this large-scale field survey, we compared the fungal communities in the roots, leaves, bulbils and surrounding soil and plants at high and low altitudes. This design allowed us to determine whether plant-fungal associations are solely determined by the differences in the availability of fungal species at different abiotic conditions as compared to the effects of the abiotic conditions on the type and strength of the association. In agreement with our hypotheses, we found that the available and selected fungal communities consistently varied with altitude, with a fraction of the interacting species pool being selected from the available species pool. Yet, the selected fungal communities were not solely determined by the available species pool, since the occurrence probability of some fungal species increased under specific conditions relative to the available pool. Our results thus indicated that plant-fungal associations along abiotic gradients are not solely modulated by the effects of the environment on the available fungal communities, but also by the effects on the association.

There are many mechanisms by which the abiotic environment may change plant-fungal associations. Plants endure stress and

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increase fitness by associating with beneficial microbial communities which they may select and recruit through different mechanisms. One approach plants may use to endure stress is the "cry for help" strategy proposed by Ali et al. (2022), where plants modify their root exudates to alter their microbiome and activate a stress response. For example, under drought conditions, plants have been shown to adjust their microbiome to regulate the nutrient cycling of carbon and nitrogen (de Vries et al., 2019). While our study does not reveal the regulatory pathways by which plants selected different fungal species, or the mechanisms of plant-fungi communication, our results show that at different altitudes, the type and strength of the association changes, which might be critical for plant health and fitness (Burg et al., 2024). Our results show that fungal species selection was stronger at higher than lower altitudes for all plant compartments, especially leaves. We also found that all fungal guilds had a positive response to high altitudes in the roots, indicating that the strength of the association increased in stressful conditions. Furthermore, in soil and roots, there were more ectomycorrhizal occurrences at high altitudes than at low altitudes, suggesting that these types of interactions are stronger at high altitudes. However, the mycorrhizal guilds showed a negative response to altitude in the leaves, bulbils and surrounding plants, indicating that these associations mainly occur in the roots but not in the above-ground compartments. At higher altitudes, plants are exposed to strong winds, colder temperatures, shorter reproductive seasons and limited nutrients (Körner, 2003), which may activate stress responses (Freschet et al., 2018) that modify the fungal species type and strength.

Furthermore, our results demonstrated that plant-associated fungal species were compositionally nested, indicating that plants select fungi from the surrounding environment and actively select associations for different plant compartments. However, it is important to note that the fungal DNA from the soil samples was extracted from a greater biomass of material than the plant tissue samples, so the greater fungal species richness found in the soil could be due to the increased biomass. At the level of an individual plant holobiont, we also saw that there was a core microbiota, where many fungal species were found in all plant compartments. This result unifies previous opposing views in which either little or no evidence of compositional nestedness (Fang et al., 2019; Guevara-Araya et al., 2020; Wearn et al., 2012), or some stratification has been shown (Bahram et al., 2022; Bernard et al., 2021). Bulgarelli et al. (2013) suggest that community assembly of the rhizosphere and plant compartments happens in two steps: (1) The rhizosphere is the first plant-influenced habitat that soil microorganisms interact with and is thus influenced by plant metabolic pathways that can release oxygen, carbon or antimicrobial compounds into the vicinity surrounding the roots (Vandenkoornhuyse et al., 2015); (2) the host selects interacting fungal species from the available pool of microbes first recruited to the rhizosphere. Fungal communities found in the above-ground tissues were likely transmitted from both the roots and the air, considering there were some species present in the leaves and bulbils (above-ground) that were not present in the roots or soil (below-ground). Alternatively, since fungal species richness was greatest in the roots and decreased compositionally, which was similar to previous results (Guevara-Araya et al., 2020; Jin et al., 2015; Purahong et al., 2019; Wearn et al., 2012), roots may be inoculated with generalist fungi that are actively selected over time and distance from the ground. Supporting this view, we found that fungal species selection was strongest in the leaves compared to roots and bulbils. Additionally, the greater number of fungal species isolates found in below-ground tissues could be due to the longer life of roots compared to leaves (Guevara-Araya et al., 2020) and bulbils. Fungal species richness was greater in the roots compared to the above-ground compartments, and most fungal species found in the bulbils were also found in the roots or leaves, which is consistent with other studies and indicates source-sink dynamics where the soil structures much of the associated fungal communities, and the further away from the soil (e.g. the leaves and bulbils), the greater the dispersal limitation (Bahram et al., 2022; Mouquet & Loreau, 2003). While observed changes in the type and strength of fungal associations changed along altitude, we cannot determine whether plants actively select certain fungal species, and thus further experiments are needed to clarify this.

Consistent with many studies (Bernard et al., 2021; Fang et al., 2019; Guevara-Araya et al., 2020; Jin et al., 2015; Küngas et al., 2020; Peršoh, 2013; Purahong et al., 2019; Trivedi et al., 2020; Wearn et al., 2012), our results showed that the plant compartment is a major selective force shaping plant-fungal associations. Plant compartments can be distinguished by different physiochemical properties (Purahong et al., 2019), especially in above-ground compared to below-ground tissues, clarifying the results presented in this study. Horizontal transmission, through factors such as spore source (air or soil), host tissue chemistry, host genotype, temperature or precipitation, could explain the specificity of fungal communities in certain plant compartments (Bálint et al., 2013; Campisano et al., 2017; David et al., 2016; Giauque & Hawkes, 2013; Guevara-Araya et al., 2020; Hardoim et al., 2015; Purahong et al., 2019). Soil chemistry and altitude (surrounding abiotic environment) were important determinants of fungal community structure, but still not as strong a determinant as plant compartment. There were also many fungal species that were more likely to be found at high or low altitudes in all plant compartments. Thus, while abiotic factors structure the available fungal species pool, biotic selection of fungal species mediated by abiotic factors explains the interacting fungal species pool. This selection may involve plants allocating more C to beneficial fungi (Bogar et al., 2019) or the up-regulation of defence mechanisms to exclude fungi it does not want to associate with (Hortal et al., 2017). The large amount of variation explained by plant compartments suggests that biotic drivers are strong determinants of realized plant-fungal associations.

One aspect we did not study was whether host genetics regulate the interacting fungal community. However, previous studies report that plant microbiomes were more affected by abiotic and biotic factors than by plant genotype (Cregger et al., 2018; Hamonts et al., 2018; Lundberg et al., 2012), except for fungal communities

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within leaves, which are greatly affected by host genotype (Cregger et al., 2018; Qian et al., 2018; Wagner et al., 2016). Confirming these expectations, the plant populations in our study, which were likely genetically similar, explained less variation in the associated fungal communities than the plant compartments and other abiotic variables. In other words, the variation in associated fungal communities was better explained by the plant compartment than the spatial proximity of the plants.

Interestingly, root-associated fungi, including ectomycorrhizae and ericoid mycorrhizae, were found in above-ground tissues, including leaves and bulbils. One explanation is that the plant transfers helpful root-associated fungi onto its offspring, as fungal species transmission via seeds has been observed (Abdelfattah et al., 2021; Hodgson et al., 2014). Confirming a maternal effect on fungal species pools would require experimental approaches across multiple generations. Another explanation is that the guild assignments may not fully reflect actual interactions due to limitations in metabarcoding data based on database assignment. Fungi can perform diverse functions depending on the environment, life cycle phase and other factors, making guild classifications challenging (Zanne et al., 2020). Additionally, because we did not sterilize plant tissue before extraction, spores may have landed on the surface of the bulbils without interacting with the bulbils. Sterilizing bulbils before extraction or using metatranscriptomics techniques could help uncover if mycorrhizal fungi are interacting with the bulbils.

5 | CONCLUSION

The understanding of plant-microbe associations is generally underdeveloped. Previous studies have provided evidence that plant-fungal associations change depending on the environmental context (Lynn et al., 2019; Rodriguez et al., 2008; Soares et al., 2016), but our study is the first large-scale observational study to directly link abiotic environmental changes with the differential selection of fungal species. These results suggest that the environmental changes related to the ongoing climate change will have profound effects on the structure of plant-fungal associations. Our observational study provides an exciting baseline for future experimental studies focusing on how transmission of microbes from dispersal propagules influences biotic interactions, and how abiotic changes will impact plant-microbe associations.

AUTHOR CONTRIBUTIONS

Skylar Burg and Nerea Abrego conceived and designed the study, Skylar Burg and Lluís Serra-Domínguez implemented the field work, Skylar Burg, Kim Kreuze and Sari M. Viinikainen carried out the laboratory work, Brendan Furneaux applied the bioinformatics and Otso Ovaskainen led the statistical analysis with contributions from Skylar Burg, Nerea Abrego, Pekka Niittynen and Niklas Moser. Skylar Burg and Nerea Abrego wrote the first draft of the manuscript with specific contributions from Otso Ovaskainen, Brendan Furneaux, Pekka Niittynen, Kim Kreuze, Niklas Moser, Lluís Serra-Domínguez and Sari M. Viinikainen. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

We declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70075.

DATA AVAILABILITY STATEMENT

Raw sequence reads have been submitted to the European Nucleotide Archive under project PRJEB76595. The data and scripts for reproducing the results of this study are deposited in Zenodo: https://doi.org/10.5281/zenodo.13868808 (Burg et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. PCA comparing the abiotic differences between high and low altitude sites.

Figure S2. Rarefaction analyses for each plant/soil compartment.

Figure S3. Sampling completeness analysis.

Figure S4. Histogram of sequence reads per sample.

Figure S5. Fungal guild overlap.

Figure S6. Guild-level responses to each predictor.

Table S1. Abiotic differences between plots.

Table S3. List of common fungal species included in the JSDM analysis.

Table S4. Fungal sequence read counts of the negative DNA extraction kit and PCR controls.

Table S5. Parameter estimates for the species richness model.

Table S6. Potential scale reduction factor for the parameters estimated from the main model.

Table S7. Explanatory power (goodness of fit) for each model.

Table S8. Most common species occurring in each sample type.

Table S9. Species-level OTUs occurring in the focal leaves and bulbils assigned to mycorrhizal guilds.

Table S10. Variance partitioning for the guild model.

Table S11. Values associated with each species in Figure 4.

Appendix S1. Fungal ITS primers.

Appendix S2. Bioinformatic analysis.

Appendix S3. Sensitivity of main results to excluding rare species.

Appendix S4. MCMC Convergence.

Appendix S5. Results from the fungal guild overlap analyses.

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