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Research Article

Drivers of bacterial and fungal root endophyte communities: understanding the relative influence of host plant, environment, and space

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Abstract

Bacterial and fungal root endophytes can impact the fitness of their host plants, but the relative importance of drivers for root endophyte communities is not well known. Host plant species, the composition and density of the surrounding plants, space, and abiotic drivers could significantly affect bacterial and fungal root endophyte communities. We investigated their influence in endophyte communities of alpine plants across a harsh high mountain landscape using high-throughput sequencing. There was less compositional overlap between fungal than bacterial root endophyte communities, with four 'cosmopolitan' bacterial OTUs found in every root sampled, but no fungal OTUs found across all samples. We found that host plant species, which included nine species from three families, explained the greatest variation in root endophyte composition for both bacterial and fungal communities. We detected similar levels of variation explained by plant neighborhood, space, and abiotic drivers on both communities, but the plant neighborhood explained less variation in fungal endophytes than expected. Overall, these findings suggest a more cosmopolitan distribution of bacterial OTUs compared to fungal OTUs, a structuring role of the plant host species for both communities, and largely similar effects of the plant neighborhood, abiotic drivers, and space on both communities.

Keywords: alpine, bacteria, community assembly, endosphere, fungi, plant–microbe interactions

Introduction

Plant associations with microbes are ubiquitous and microbes residing inside the root, root endophytes (*sensu* Hardoim et al. 2015), can particularly influence plant fitness. Root endophytes include bacteria and fungi, amongst other microbes, that can enhance plant growth (Hardoim et al. 2008), increase access to nutrients (Hurek et al. 2002), and protect against pathogens (Maciá-Vicente et al. 2008). Additionally, root endophytes can include plant pathogens that may cause negative effects, such as reduced growth (Junker et al. 2012). However, studies assessing drivers of root endophyte communities typically focus on either root endophytic bacteria or fungi, hampering our ability to generalize about these different communities (but see Coleman-Derr et al. 2016, Furtado et al. 2019, Toju et al. 2019, Thiergart et al. 2020).

The distribution of bacterial and fungal root endophyte communities is shaped in part by environmental drivers, such as the soil environment and climate. Edaphic and climatic variables can act on root endophyte communities through shifts in soil microbial communities, which serve as a source community (Adair and Douglas 2017), or via shifts in plant-microbial interactions. For example, bacterial root endophyte composition shifted along a temperature and precipitation gradient (Li et al. 2012), likely due to differences in source community composition and/or altered host plant or microbe preferences. Recent research indicates soil properties to be of greater importance to bacterial root endophyte communities than to fungal root endophyte communities (Bickford et al. 2018, Thiergart et al. 2020), paralleling work demonstrating a stronger effect of abiotic drivers on soil bacteria than soil fungi (Sugiyama et al. 2008, Fanin et al. 2019).

The host plant species and the plant neighborhood (the community of plants surrounding the host plant) may also be important for bacterial and fungal root endophyte distribution. The effect of plant hosts on root endophyte composition has been somewhat variable. Studies comparing plants at the level of cultivars and strains often do not show strong host plant control (Chen et al. 2017, Leff et al. 2017). Comparisons across plant species (Kumar et al. 2017) and at coarser taxonomic levels (Glynou et al. 2018) show greater influence of the host plant, where more closely related host plants tend to have more similar root endophyte communities (Yeoh et al. 2017, Fitzpatrick et al. 2018). One possible way to resolve these differences is through the lens of functional differences across host plants: host plant control may

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be stronger in systems with more diverse and broader differences in the phenotypic traits and genetic characteristics of the host species (Francioli et al. 2020, Sweeney et al. 2021). It is possible that host plants may have differential effects on bacterial and fungal root endophyte communities. For example, different host plant genes, which determined plant morphology and physiology, shaped the diversity of bacteria and fungi in the root endosphere of *Arabidopsis thaliana* (Bergelson et al. 2019).

The composition of the plant neighborhood can also shape root endophyte communities (e.g. Ampt et al. 2022), and may do so differentially for bacteria and fungi. For example, the presence of a dominant plant species in the plant neighborhood had a negligible effect on root-associated bacterial community composition (Dean et al. 2015) but a strong effect on root-associated fungal community composition (Dean et al. 2014). A stronger effect of the plant neighborhood has been also seen for soil fungal communities compared to soil bacterial communities and may underlie parallel patterns in root endophyte communities (Sugiyama et al. 2008, Fanin et al. 2019).

Geographic distance, or space, has also been shown to shape the distribution of bacterial and fungal root endophyte communities. Effects of geographic distance can occur through several pathways including heterogeneity in the environmental variables described above (Ramette and Tiedje 2007). Alternatively, historical events such as spatial isolation (Papke and Ward 2004) or dispersal limitation (Martiny et al. 2006) may explain effects of geographic distance. Dispersal limitation is an outcome of geographic distance to which the distribution of bacterial and fungal root endophyte communities may respond differently (Bonito et al. 2014). The strength of dispersal limitation on microbes varies by size, where larger organisms, such as many fungi, are typically more dispersal limited than smaller organisms, such as bacteria (Bonito et al. 2014, Schmidt et al. 2014, Chen et al. 2020, Li et al. 2020). It is worth noting, however, that fungi, including fungal root endophytes, can have a wide distribution (Queloz et al. 2011, Cox et al. 2016, Glynou et al. 2017). Overall, space, perhaps via dispersal limitation, may differentially impact bacterial and fungal root endophyte communities.

Few studies have simultaneously examined the role of the host plant, environmental drivers, and space on both bacterial and fungal root endophyte communities. Those studies that have concurrently investigated these drivers in natural systems have primarily done so at broad scales (e.g. Coleman-Derr et al. 2016, Thiergart et al. 2020) indicating a need for parallel investigations at smaller scales. We capitalized on a spatially heterogenous gradient of plant density and richness, snowpack, and edaphic properties (i.e. soil pH, nutrient availability, soil texture) across a relatively small spatial scale (greatest distance between plots was 1900 m) to examine bacterial and fungal root endophyte communities associated with nine alpine plant species in the Colorado Front Range, using high-throughput sequencing. The alpine was an ideal location for this study due to the high environmental heterogeneity found within a small area, the strong abiotic drivers, and the moderate plant richness. Because our sampling encompassed a diverse set of plant species across a small spatial scale, we hypothesized that host plant species, reflecting morphological differences amongst host plants, would be the strongest driver of both bacterial and fungal root endophyte communities (H1). We also hypothesized that abiotic variables would have a stronger influence on bacterial root endophytes relative to fungal root endophytes, reflecting stronger associations between bacteria and abiotic drivers (H2). In contrast, we hypothesized that the plant neighborhood and space would explain more variation in fungal root endophytes than bacterial root endophytes, reflecting the stronger influence of the plant community and dispersal limitation on fungi (H3).

Materials and methods

Study site

This study took place on a south-facing slope in the Green Lakes Valley, part of the Niwot Ridge Long Term Ecological Research site, in the Front Range of the Rocky Mountains, Colorado, USA (40° 3′ 11′ N, 105° 37′ 50′ W; Fig. 1a). We resampled a subset of spatially explicit plots established in 2007 (King et al. 2010), with the closest plots 5 m away and the farthest plots ∼2 km away from each other. The location of these circular plots (1 m in diameter) ranges in elevation from 3638 to 3870 m a.s.l. The soils are acidic with a pH that ranges from 4.52 to 5.82 (mean ± SD; 5.18 ± 0.32). Despite the small scale, the study area encompasses a large gradient in key abiotic variables such as snowpack, soil development, soil texture, nutrient concentrations, organic matter concentration, and plant community composition and density (King et al. 2010, Porazinska et al. 2018), spanning moderately vegetated patches of alpine tundra meadow (131 stems m^{-2}) to sparsely vegetated talus slopes (8 stems m^{-2}).

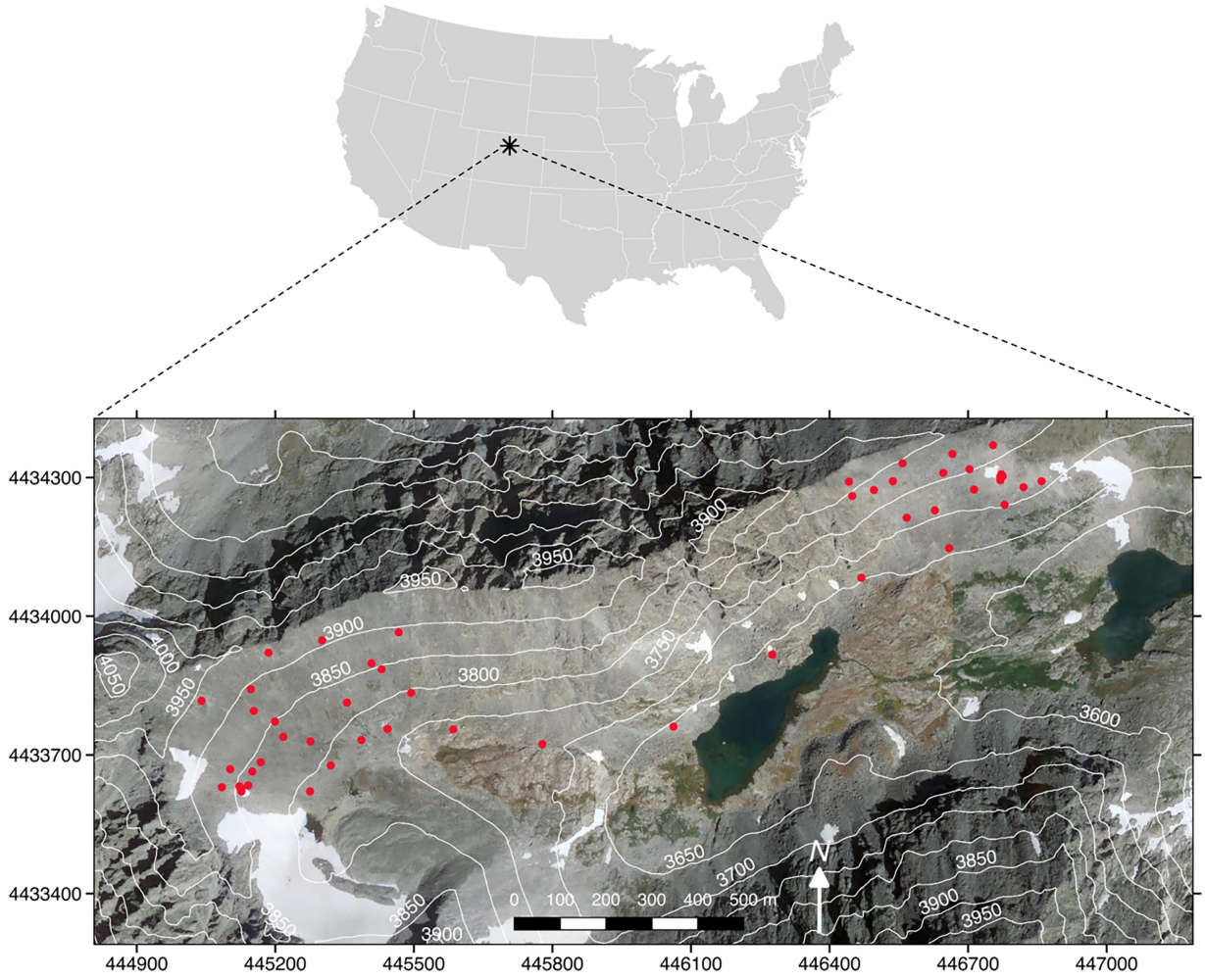
Environmental and plant characteristics

We assessed the effects of a range of environmental variables including plant neighborhood metrics and abiotic drivers (Bueno de Mesquita and King 2018, Bueno de Mesquita 2021). For our plant neighborhood metrics, we used plant density, which shapes the quantity of inputs to the soil, and plant richness, which impacts the diversity of plant inputs and is indicative of the potential number of plant species interactions. We conducted vegetation surveys between 17 August and 4 September 2015. Across our plots, we identified all plants at the species level to estimate plant richness and divided this number by the area of the plot to get the number of plant species per square meter. Plant density was calculated as the total number of stems per square meter across all species. All plants detected were perennials and due to the slow rate of vegetation turnover in the alpine, it can be assumed that vegetation composition was not changed between 2015 (vegetation surveys) and 2016 (root endophyte sampling).

Our abiotic drivers included snowpack, soil pH, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and soil texture. To assess the role that snowpack played on root endophyte communities, we estimated mean May snowpack depth at each plot by kriging interpolation of snow depth data from annual snow surveys (1997 to 2015) conducted at our study site (Bueno de Mesquita et al. 2018, Farrer et al. 2019). In these surveys, snow depth was manually measured during peak snowpack in May at an average of 483 random locations that were approximately 50 m apart.

Soils were collected between 7 and 11 September 2015 to characterize edaphic properties. We collected three soil cores of 3 cm diameter and 4 cm depth per plot, placed them in a plastic bag, gently homogenized them, and transported them on ice to the lab by the end of the day. Soils were stored at 4°C for a maximum of one week. Soil pH was measured with an Oakton benchtop pH meter (Oakton Instruments, USA) after the addition of 3 ml ultrapure water to 2 g of soil and shaking for 1 h at 175 r/m. Dissolved organic carbon and TDN were measured via soil extractions using 0.5 M K_2SO_4 and analyzed using a Shimadzu total organic C analyzer equipped with a TDN module (Shimadzu Scientific Instruments,

(A)



(B)

Angiosperm Group	Family	Host Plant Species	Samples
Dicot	Asteraceae	*Senecio fremontii*	4
Monocot	Poaceae	*Trisetum spicatum*	14
		Deschampsia cespitosa	5
		Festuca brachyphylla	22
		Elymus scribneri	5
Monocot	Cyperaceae	*Kobresia myosuroides*	4
		Carex pyrenaica	10
		Carex phaeocephala	3
		Carex albonigra	3

Figure 1. Location of the study site and aerial image highlighting the location of the 46 plots used in this study (red points) which are arrayed across the Green Lakes Valley in the Front Range of the Rocky Mountains, Colorado, USA (**A**). Values along the edge of the image indicate Universal Transverse Mercator coordinates at an interval of 300 m. Contours on the image in white indicate elevation in meters. (**B**). The host plant species and number of associated root samples (individuals collected across all plots). A cladogram on the left indicates phylogenetic relationships amongst host plant species.

Inc., USA) (Porazinska et al. 2018). In addition to those edaphic properties measured in 2015, soil texture (percentage of sand, silt, and clay) of the plots was measured by the South Dakota Soil Laboratory (Brookings, South Dakota, USA) in 2008 on soils collected in September of that year (King et al. 2010). While DOC, TDN, and pH were measured in 2015 and soil texture was measured in 2008, one year and eight years before root sampling, respectively, we do not expect that the relative differences in these soil properties across plots were significantly different from those conditions at the time of root sampling because all sampling occurred within the same seasonal time frame, despite the differences in year. See Table S1 (online supplementary material) for the range in values for plant neighborhood metrics and abiotic drivers.

Root endophyte community sampling

To assess root endophyte communities, based on plant community data established in 2015, we selected and revisited 74 plots between 15 and 25 August 2016 and harvested individual perennial plants of different species (Bueno de Mesquita et al. 2018). To minimize our impact on the populations of these plant species, only plots with > 5 individuals per species were selected, and only one random individual per species was sampled. Plants were collected during the flowering phenophase. All selected plants were at the same developmental stage, appeared healthy, and did not have visible signs of pathogenic infections or herbivory. Using a sterilized soil knife, plants were excavated at a maximum of 10 cm depth. Soil was shaken off in the field, plants were placed in plastic bags and transported to the lab on ice. Roots were selected by size such that roots < 2 mm in diameter (fine roots) were collected and surface sterilized by rinsing in deionized water, soaking in 70% ethanol for 1 min, soaking in 10% bleach for 1 min, and triple rinsing with sterile deionized water, similar to other surface sterilization protocols (Bougoure and Cairney 2005, Meade et al. 2020). Samples were then stored in a −70°C freezer. In the present study, we included only those species that had at least 3 root samples (Fig. 1b), which resulted in 70 sampled plants from 46 unique plots.

DNA extraction and analysis

Prior to DNA extraction, 0.1 g of roots were ground into a fine powder under liquid nitrogen using a sterile mortar and pestle as recommended by the DNA extraction kit manufacturer instructions (McPherson et al. 2018, Simmons et al. 2018). Roots from each individual plant were handled separately. DNA was extracted from this powder using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). We used PCR to amplify the V4 hypervariable region of the 16S rRNA gene using indexed 515f and 806r primers and the first internal transcribed spacer (ITS) region using ITS1F and ITS2 primers, following standard protocols of the Earth Microbiome Project (Amaral-Zettler et al. 2009, Caporaso et al. 2012, Smith and Peay 2014). All amplified samples were purified and normalized with the SequalPrep Normalization Kit (Invitrogen, Carlsbad, CA, USA), pooled into single 16S and ITS amplicon libraries and sequenced on a MiSeq2000 (Illumina, San Diego, CA, USA) with pair-end 2×150 bp chemistry for 16S and 2×250 bp chemistry for ITS at the University of Colorado BioFrontiers Institute (Boulder, CO, USA). For 16S, average quality scores by cycle were > 25 for forward reads and > 28 for reverse reads. For ITS, average quality scores by cycle were > 24 for forward reads and > 20 for reverse reads. Therefore, reads were not trimmed prior to analysis. Reads were demultiplexed using a custom in-house python script (prep_fastq_for_uparse_paired.py, available on GitHub https://github.com/leffj/helper-code-for-uparse). Then, the UPARSE pipeline (implemented with usearch v. 8.1) (Edgar 2013) was used to merge paired-end reads, quality filter reads for a maximum expected error rate of 0.005, dereplicate reads, remove singletons, pick operational taxonomic units (OTUs) at 97% sequence identity, remove chimeras, and create a sequence count by sample OTU table. All further processing was done in R ver. 4.1.2 (R Core Team 2021). We assigned taxonomy with the SILVA database (v. 138) (Quast et al. 2013) and UNITE database (general FASTA release, 2 April 2020) (Abarenkov et al. 2010) for bacterial and fungal reads, respectively (function 'assignTaxonomy,' package *dada2*; Callahan et al. 2016). OTUs classified as chloroplasts (2.6% of reads), mitochondria (2.3%), or archaea (0.2%) were removed from the 16S OTU table and OTUs not assigned at the domain or phylum level were removed from both 16S and ITS OTU tables (function 'filter_taxa_from_input,' package *mctoolsr;* Leff 2017). To control for differences in sequencing depth among samples, we then rarefied the 16S samples to 4094 reads per sample and the ITS samples to 5238 reads per sample (function 'single_rarefy,' package *mctoolsr*), which was enough to capture the diversity of OTUs in each sample. Relative abundances were calculated by dividing the number of each OTUs' sequence reads by the total number of reads in a sample (function 'convert_to_relative_abundances,' package *mctoolsr*). To focus analyses on abundant OTUs and thus minimize potential bias caused by stochasticity (Toju et al. 2019, Wang et al. 2021), the OTUs examined in downstream analyses were only those with a relative abundance greater than 0.05% (function 'filter_taxa_from_input,' package *mctoolsr*). The number of remaining bacterial OTUs was 302 (from 4831) and the number of remaining fungal OTUs was 183 (from 1094). Representative sequences for the OTUs are available in NCBI's GenBank via the accession numbers ON229119–ON229415 (16S) and MH238510–MH240826 (ITS). Merged demultiplexed fastq files are accessible at doi:10.6084/m9.figshare.19593805 (16S) and doi:10.6084/m9.figshare.19199969 (ITS).

Statistical analyses

To assess the relative importance of host plant species, plant neighborhood (plant density and plant richness), abiotic drivers (mean May snow depth, soil texture, TDN, DOC, and pH), and space in shaping the bacterial and fungal root endophyte communities, we conducted a distance-based redundancy analysis (dbRDA) on Bray-Curtis dissimilarity matrices using square-root transformed relative abundances for both bacterial and fungal communities (function 'dbrda,' package *vegan;* Oksanen et al., 2020). The percentage of sand was correlated with both silt and clay content ($r > 0.7$), and so only sand was retained in the model. Additionally, DOC and TDN were correlated ($r = 0.8$) and thus only TDN was retained. All continuous variables included in the dbRDA were scaled to have a mean of 0 and a standard deviation of 1. To include spatial predictors in the dbRDA, we used eigenvector mapping techniques.

The spatial component, which accounts for unmeasured drivers such as dispersal and spatially structure environmental variables (Peres-Neto and Legendre 2010), consisted of eigenvectors from the calculation of distance-based Moran's Eigenvector Maps (dbMEM; Dray et al. 2006). The first several eigenvectors characterize larger distances amongst plots and subsequent eigenvectors represent smaller distances amongst plots (Bauman et al. 2018). The dbMEM consists of running a principal coordinate analysis (PCoA) on a truncated Euclidean (geographic) distance matrix constructed from spatial coordinates, with diagonal

values that are four times a threshold value (the shortest distance that maintains a connection between all plots [i.e. the longest edge of a minimum spanning tree]) (function 'dbmem,' package *adespatial*; Dray et al. 2020). The resulting eigenvectors were subjected to a global test of significance where all eigenvectors from the dbMEM were included in a dbRDA (function 'dbrda,' package *vegan*) with bacterial and fungal root endophyte communities (Bray-Curtis dissimilarity matrices using square-root transformed relative abundances) as response variables; the significance of the overall model was tested and an adjusted R^2 was obtained (Blanchet et al. 2008, Bauman et al. 2018). Next, to avoid model overfitting and to enhance predictive power (Gauch 1993, Bauman et al. 2018), forward selection with double stopping criterion was employed (function 'forward.sel', package *adespatial*, 9 999 permutations); the two criteria are a significance level of 0.05 and the global adjusted R^2 from the aforementioned dbRDA (Blanchet et al. 2008, Bauman et al. 2018).

Our dbMEM resulted in a total of 13 eigenvectors (MEM; Fig. S1, see online supplementary material), during which forward selection retained MEM 4 (the fourth eigenvector) for downstream bacterial analyses and MEM 1, 4, and 7 for downstream fungal analyses. We considered the first four eigenvectors to represent broader spatial patterns and the next four to represent more intermediate spatial patterns (Bauman et al. 2018). To understand the relationship between the selected spatial variables and environmental heterogeneity, we regressed our subset of eigenvectors against several abiotic and biotic variables (mean May snow depth, soil texture [% sand], TDN, pH, plant density, plant richness).

Variation partitioning was done to determine the amount of variation explained by host plant species, plant neighborhood, abiotic drivers, and space for both bacterial and fungal root endophyte communities (function 'varpart,' package *vegan*). We used a permutation test on partial dbRDA to determine the significance of testable components (function 'anova.cca,' package *vegan*). To test whether dispersion in community composition (i.e. variation in community composition) differed between bacteria and fungi, we conducted a permutational multivariate analysis of dispersion on Bray-Curtis dissimilarity matrices using square-root transformed relative abundances for both bacterial and fungal communities (function 'betadisper', package *vegan*; permutations: 999; metric: centroid). More specifically, we considered dispersion for each sample to be the distance between that sample and the group centroid for both bacteria and fungi in multivariate space, as calculated by 'betadisper'. We additionally tested whether the dispersion in community composition between community types was correlated (function 'cor.test', package *stats*). The correlation between host plant phylogeny and the bacterial and fungal root endophyte communities was assessed with a Mantel test (function 'mantel', package *vegan*). Host plant phylogenetic difference was calculated as the pairwise distances between terminal taxa using branch lengths ('cophenetic.phylo,' *ape*; Paradis and Schliep 2019). We did not have a molecular phylogeny of our plant species and thus subset our taxa from the molecular phylogeny provided by Zanne et al. (2014) (identified to species and sampled at least three times) using the software Phylomatic (Webb and Donoghue, 2005). Synthesis-based trees have been shown to be robust for common phylogenetic analyses (Li et al. 2019).We ran a Mantel test and a Mantel correlogram (function 'mantel.correlog,' package *vegan*) to determine the relationship between bacterial and fungal root endophyte communities and geographic distance (Borcard and Legendre 2012). We also ran an indicator species analysis to determine associations between host plant species and bacterial and fungal OTUs (function 'multipatt', package *indicspecies*; 999 permutations) (De Cáceres and Legendre 2009).

Lastly, to investigate the relative role of stochastic and deterministic processes in community assembly, we calculated nearest taxon index (NTI) (Stegen et al. 2012) for root bacterial and fungal communities (function 'NTI.p', package *iCAMP*) (Ning et al. 2020). To build a hierarchical classification tree of fungal OTUs to use in phylogenetic analyses, we used the taxonomy_to_tree.pl script to map our UNITE-assigned taxonomy table to the fungal backbone classification tree (Tedersoo et al. 2018). For a single community, an NTI value greater than 2 indicates phylogenetic clustering of the community, while an NTI value less than −2 indicates phylogenetic overdispersion of the community, either of which are suggestive of deterministic processes governing community assembly. NTI values between −2 and 2 are suggestive of stochastic processes governing community assembly (Stegen et al. 2012). All statistical analyses and visualizations were performed in R ver. 4.1.2 (R Core Team 2021).

Results

Spatial variables and the environment

All spatial variables were related to a subset of the environmental drivers (Table S2; Fig. S2). Every spatial variable was associated with plant richness and/or density ($P < 0.05$). Two of the broadest spatial variables (MEM 1 and 4) were related to snow depth (MEM 1: $P = 0.003$; MEM 4: $P < 0.001$). Additionally, MEM 1 was related to soil chemical properties (TDN: $P = 0.02$; pH: $P < 0.001$) and MEM 4 was related to soil physical properties (sand; $P = 0.04$).

Characterization of root endophyte communities

There was a mean observed richness of 157 ± 28 (mean ± standard deviation) bacterial OTUs per sample. Three of the 16 phyla comprised 75% of the bacterial reads: Proteobacteria made up the bulk of reads (on average, 42% of reads), followed by Actinobacteriota (17%), and Bacteroidota (16%) (Fig. 2A; Fig. S3a displays a rank abundance of all phyla, see online supplementary material). The most abundant bacterial family was Xanthobacteraceae (on average, 9% of reads) (Fig. 2B, Fig. S3b displays a rank abundance of all families, see online supplementary material).

There was a mean observed richness of 27 ± 8 fungal OTUs per sample. At the phylum level, Ascomycota (on average, 81% of reads) and Basidiomycota (14% of reads) were the dominant fungal phyla (Fig. 2C; Fig. S4a displays a rank abundance of all phyla, see online supplementary material). Despite a previous microscopy study demonstrating these plant roots are colonized by AMF, only 1.5% of reads belonged to Glomeromycota (AMF; Bueno de Mesquita et al. 2018a) and this low percentage was not due to the filtering of rare taxa (1.9% of reads when all OTUs were considered). The most abundant fungal family was Hyaloscyphaceae (26% of reads on average) (Fig. 2D, Fig. S4b displays a rank abundance of all families, see online supplementary material).

There were 154 bacterial OTUs (51% of bacterial OTUs; Table S3, see online supplementary material) and 14 fungal OTUs (8% of fungal OTUs; Table S4) found in at least one individual of all nine host plant species. The 154 bacterial OTUs were from 14 different phyla but were primarily composed of Proteobacteria (31% of occurrences), Actinobacteriota (16% of occurrences), and Bacteroidota (16% of occurrences). The most prevalent bacterial families found in at least one individual of all nine host plant species included Sphingobacteriaceae and Chitinophagaceae (both 6% of all occurrences). The 14 fungal OTUs came

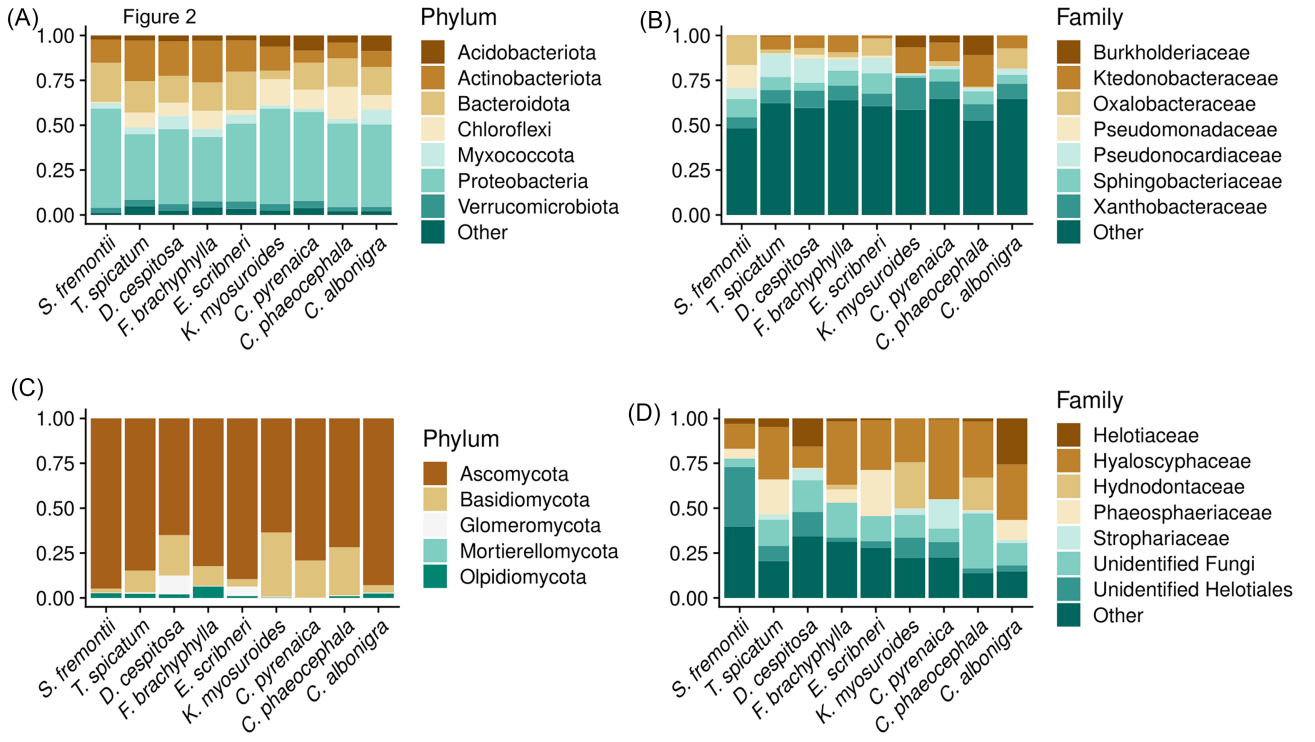


Figure 2. Comparison of the relative abundances of the top seven bacterial phyla (**A**) and bacterial families (**B**), all fungal phyla (**C**), and the top seven fungal families (**D**) by host plant species.

from two phyla: Ascomycota (93% of occurrences) and Basidiomycota (7% of occurrences), and the most prevalent fungal family was Hyaloscyphaceae (36% of occurrences). There were four bacterial OTUs found across all 70 samples (all individuals of all nine species): two OTUs from unknown genera in the Comamonadaceae and Xanthobacteraceae families, an OTU in the genus *Mucilaginibacter*, and an OTU in the genus *Sphingomonas*. There were no fungal OTUs found across all 70 samples.

The dispersion, or variation, in community composition was greater for fungal than bacterial root endophyte communities (Fig. S5a, see online supplementary material; $F_{1,138} = 388$, $P < 0.001$) and was correlated such that individuals with greater variability in bacterial root endophyte composition also had greater variability in fungal root endophyte composition (Fig. S5b, see online supplementary material; $r = 0.47$, $P < 0.001$).

Effects of host plant species, plant neighborhood, space, and abiotic drivers on root endophyte communities

All four categories of variables analyzed (i.e. host plant species, plant neighborhood, space, and abiotic effects) significantly influenced both bacterial and fungal root endophyte composition (Table 1; Fig. 3a-d). Host plant species was a significant driver of both bacterial and fungal root endophytes communities (bacterial: $F_{8,54} = 1.6$, $P = 0.001$; fungal: $F_{8,52} = 1.4$, $P = 0.001$). In terms of plant neighborhood effects, plant richness (bacteria: $F_{1,54} = 2.6$, $P = 0.003$; fungi: $F_{1,52} = 2.32$, $P = 0.001$) and plant density (bacteria: $F_{1,548} = 2.2$, $P = 0.009$; fungi: $F_{1,528} = 1.6$, $P = 0.01$) shaped both microbial communities. Mean May snow depth was the only abiotic driver that explained variation in both the bacterial ($F_{1,54} = 4.6$, $P = 0.001$) and fungal root endophyte communities ($F_{1,52} = 3.1$, $P = 0.001$). There was an additional effect of soil pH on bacterial communities ($F_{1,54} = 2.2$, $P = 0.005$) but not on fungal communities.

Table 1. Effects of the host plant species, plant neighborhood, abiotic, and spatial predictors from the dbRDA. Bolded values highlight significant effects ($P < 0.05$). MEM 1 and MEM 7 were not selected for the bacterial community and their absence is represented by a hyphen (-).

	Bacterial Community			Fungal Community		
Predictor Variable	**df**	**F-value**	**P-value**	**df**	**F-value**	**P-value**
Host plant						
Species	8	1.6	**0.001**	8	1.4	**0.001**
Plant neighborhood						
Plant Richness	1	2.6	**0.003**	1	2.2	**0.001**
Plant Density	1	2.2	**0.009**	1	1.6	**0.01**
Abiotic						
Mean Snow	1	4.6	**0.001**	1	3.1	**0.001**
Sand (%)	1	1.4	0.11	1	1.2	0.16
TDN	1	1.1	0.30	1	1.1	0.26
pH	1	2.2	**0.005**	1	1.1	0.21
Spatial						
MEM 1	-	-	-	1	1.8	**0.01**
MEM 4	**1**	**2.1**	**0.009**	1	1.7	**0.01**
MEM 7	-	-	-	1	2.5	**0.001**
Residual	54			52		

TDN, total dissolved nitrogen; MEM, spatial eigenvectors from Moran's Eigenvector Maps

All spatial variables included in the models for both bacteria and fungi were significant (Table 1).

Among the factors examined, the unique contribution of host plant species explained the largest amount of variation for bacterial (6%) and fungal (5%) root endophyte communities (Fig. 3ef). Furthermore, host plant phylogenetic distance was positively correlated with both bacterial and fungal root endophyte community dissimilarity such that more closely related host plant species

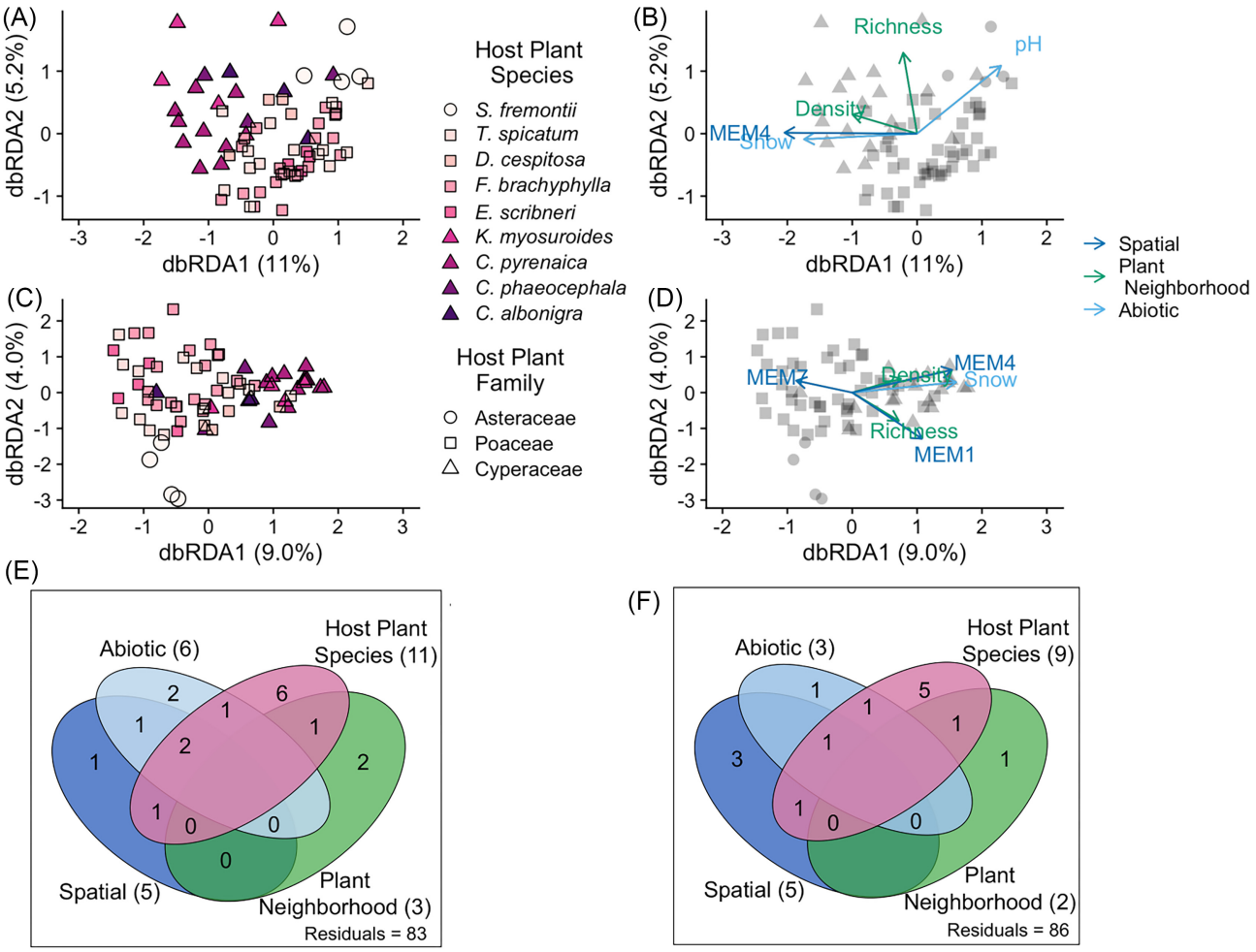


Figure 3. Host plant species shaped both bacterial (**A**) and fungal (**C**) root endophyte communities. A selection of spatial, plant neighborhood, and abiotic variables were also related to bacterial (**B**) and fungal (**D**) root endophyte communities. Venn diagram displaying the contributions of host plant species, plant neighborhood, space, and abiotic predictors in shaping (**E**) the bacterial and (**F**) fungal root endophyte communities. The numbers in parentheses indicate the total explained variation by the driver. The numbers inside the ovals indicate the unique percentage of explained variation and blank spaces indicate values less than zero. All four groups of predictors made a unique and significant contribution ($P < 0.05$).

had more similar bacterial (Mantel: r = 0.23, P = 0.004) and fungal root endophyte communities (Mantel: r = 0.18, P = 0.001). An indicator species analysis resulted in 18 bacterial OTUs (Table S5, see online supplementary material) and 10 fungal OTUs (Table S6, see online supplementary material) significantly associated with different combinations of plant species. In particular, there was one bacterial OTU in the genus *Edaphobaculum* which was predominantly associated with graminoids (P = 0.05) and one fungal OTU in the genus *Acaulospora* associated with the one dicot, *S. fremontii* (P = 0.02). *Carex albonigra* and *K. myosuroides*, both in the Cyperaceae family, shared the greatest number of associated OTUs (five bacterial OTUs), which included an OTU in the genus *Bauldia*, two OTUs in the families Ktedonobacteraceae and Micromonosporaceae, one OTU in the order Ktedonobacterales, and one OTU in the class Gammaproteobacteria. *Carex phaeocephala* was the host plant with the greatest number of associated OTUs, which included three fungal OTUs (an OTU in the genus *Cistella*, the genus *Cadophora*, and an unknown genus in the Helotiales order) and one bacterial OTU in the genus *Pedobacter*. While the number of host plant species associated with bacterial OTUs ranged from one to eight, there were typically fewer host plants associated with fungal OTUs (a maximum of three host plant species).

The unique contributions of abiotic (2%) and plant neighborhood variables (2%) were the second most explanatory variables for bacterial communities while spatial variables explained the second largest amount of variation in fungal communities (3%) (Fig. 3ef). Geographic distance was correlated with both bacterial (r = 0.12, $P = 0.002$) and fungal root endophyte community dissimilarity (r = 0.12, $P = 0.001$), such that communities closer than 220 m were more similar to each other than more distant communities (Fig. 4). The adjusted R^2 of shared variation amongst each fraction (host plant species, plant neighborhood, spatial, and abiotic) ranged from being negative to 1, indicating little overlap. Permutation tests revealed that the individual partitions for host plant species, plant neighborhood, abiotic drivers, and space were significant ($P < 0.05$).

The NTI values for bacterial communities ranged from −0.12 to 2.69 with a mean of 1.53 ± 0.07 SE (Fig. S6, see online supplementary material). NTI values for fungal communities ranged from −2.80 to 2.07 with a mean of 0.55 ± 0.13 SE. Thus, root bacterial and fungal communities across our data set ranged from more dominant stochastic assembly processes to more dominant deterministic assembly processes, but on average both communities were characterized by stochastic assembly processes (Stegen et al. 2012).

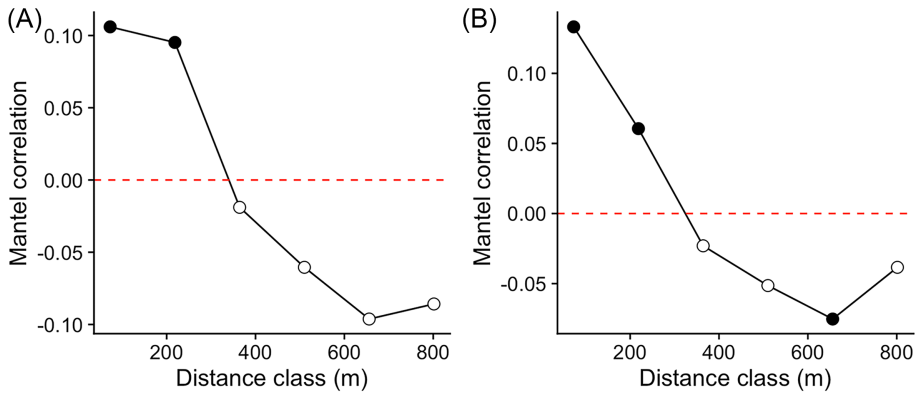


Figure 4. Mantel correlogram illustrating correlations of community similarity across geographic distance classes (m) for bacterial (**A**) and fungal root endophyte communities (**B**). The filled circles indicate significant correlations for each class ($P < 0.05$).

Discussion

Microorganisms exhibit considerable spatial heterogeneity, but it is unclear how different components of community assembly contribute to the distribution of bacterial and fungal communities (Mony et al. 2020). Our results suggest broad similarities in the factors that shape both bacterial and fungal root endophyte communities. We found that host plant species explained the greatest amount of variation in bacterial and fungal root endophyte communities across a landscape heterogeneous in its soil properties, snow depth, and plant distribution. While the drivers of bacterial and fungal root endophyte communities explained similar amounts of variation, fungal communities showed greater beta diversity which aligns with the less cosmopolitan distribution detected for fungal root endophytes. By examining drivers of root endophyte communities at a relatively small scale, we were able to show similar patterns to those occurring at much larger spatial scales (e.g. continental).

Community assembly is a balance of deterministic processes, such as environmental filtering, and stochastic processes, such as dispersal and drift (Vellend 2010, Nemergut et al. 2013). It is well known that deterministic processes influence microbial communities, including root endophyte communities (e.g. Schlaeppi et al. 2014). Less studied is the role of stochasticity, but work over the past decade has highlighted its importance for microbial community assembly (Caruso et al. 2011, Dini-Andreote et al. 2015, Debray et al. 2021). The low explained variation in both our bacterial and fungal root endophyte communities is often seen in root endophyte communities (Queloz et al. 2011, Morris et al. 2013, Glynou et al. 2016) and suggests stochasticity may be at play (Bahram et al. 2016, Maciá-Vicente and Popa 2022). This is supported by many |NTI values| < 2 for root bacterial and fungal communities in our dataset, although our samples also included some deterministically assembled communities (|NTI values| > 2). Below we discuss what variation was explained by the drivers we studied and focus on differences detected between bacteria and fungi.

Four bacterial OTUs were found across all 70 plant root samples but there were no fungal OTUs found across all samples, suggesting that bacterial root endophytes may have a more cosmopolitan distribution or that the likelihood of finding cosmopolitan bacteria is greater due to the higher number of observed OTUs. The lower number of overlapping OTUs in fungal than bacterial root endophyte communities is additionally supported by the greater dispersion, or variation, found in fungal root endophyte communities. These findings align with Thiergart et al. (2020) whose study detected greater conservation of bacterial root endophyte OTUs than fungal root endophytes. Two of the four bacterial OTUs found across all 70 samples were from the Comamonadaceae and Xanthobacteraceae families, which are commonly found in the root endosphere (Bonito et al. 2014, Kumar et al. 2017, Chen et al. 2021). Both Comamonadaceae and Xanthobacteraceae contain taxa capable of nitrogen-fixation (Oren 2014, Kumar et al. 2017), which would be particularly beneficial to plants living under the limited nitrogen conditions typical of the high alpine. The other two bacterial OTUs found across all samples were in the genera *Mucilaginibacter* and *Sphingomonas*. Both genera have been detected at a relatively high abundance in the roots of arctic plants and isolates from both genera have shown chitinolytic activity while isolates from *Sphingomonas* were also able to solubilize mineral phosphate and hydrolyze cellulose (Nissinen et al. 2012), again improving the nutritional status of their environments.

In line with H1, host plant species explained the greatest amount of variation in both bacterial and fungal root endophyte communities. The significant effect of host plant species is in line with other studies, particularly those studies examining plant species from multiple families (Mommer et al. 2018, Francioli et al. 2020). Possible drivers include exudate quality and quantity (Huang et al. 2019), immune responses (Hacquard et al. 2017), and morphological traits (Fitzpatrick et al. 2018, Bergelson et al. 2019, Sweeney et al. 2021), which can differ across plant species (Butler et al. 2011, Dietz et al. 2020). Because our host plant species were primarily monocots, more diverse sampling across plant host phyla may have resulted in an even greater effect of host plants.

Previous work examining the role of plant hosts and abiotic drivers in shaping root endophyte communities at large spatial scales demonstrates conflicting results where the effect of the host plant often varies based on the degree of phylogenetic difference between host plants, rather than the spatial scale of the study. For example, a study over 750 000 times greater in spatial extent than this study examining 23 plant species from four genera similarly found that host plant phylogeny explained the greatest amount of variation and that this level of explained variation was close to the average across bacteria and fungi in this study (5% compared to 6% in this study) (Wang et al. 2019). On the other hand, another larger scale study examining root endophytes of closely related members within a genus (*Microthlaspi*), which until recently was considered one species, found no significant effect of the host plant and instead found that abiotic drivers explained the greatest variation in root endophyte communities (Glynou et al. 2016). Together, these findings suggest that understanding the

drivers of root endophytes is complicated and may depend more strongly on plant host taxonomic divergence than spatial scale.

There were certain OTUs associated with particular plant species, which may have driven some of the bacterial and fungal root endophyte community-level differences between host plants. An OTU in the genus *Acaulospora* was associated with our one dicot, *S. fremontii*. Microbial species within the genus *Acaulospora* are AMF (phylum Glomeromycota) (Schüβler et al. 2001) shown to enhance foliar phosphorus concentrations (Klironomos 2000). There was also an OTU in the genus *Edaphobaculum* which was associated with all studied monocots (host plant species in the Cyperaceae and Poaceae families). There is only one known species in the genus *Edaphobaculum, Edaphobaculum flavum*, which was first isolated from grassland soils in China (Cao et al. 2017) and has since been detected in the roots of *Populus* (Fracchia et al. 2021). The plant species with the highest number of associated OTUs was *C. phaeocephala*, which included three OTUs in the order Helotiales. The order Heliotales includes many dark septate endophytes (Newsham et al. 2009), which are associated with plant nutrient uptake (Newsham 2011). Culture-dependent methods or metagenomic/metatranscriptomic methods may be helpful in future studies to elucidate the function of more prevalent root endophyte taxa across plants in natural systems.

In support of H2, abiotic variables explained a greater amount of variation in bacterial compared to fungal root endophyte communities. Previous studies have found edaphic properties, including resource availability and pH, to be drivers of bacterial root endophyte communities (Schlaeppi et al. 2014, Chen et al. 2017). Partially in line with that work, soil pH shaped bacterial root endophyte communities, but there was no effect of soil texture or TDN. Soil pH has been shown to be a strong driver of soil bacterial communities (Lauber et al. 2009, Delgado-Baquerizo et al. 2018), including those at this site (King et al. 2010), suggesting that the effect of soil pH on root endophyte communities may be through its effects on soil microbial communities which can serve as a source community for the root endosphere. Alternatively, soil pH can influence root architecture and therefore shape endosphere membership through a morphological pathway (Haling et al. 2011). Together, our findings demonstrate an effect of pH in shaping bacterial root endophyte communities (but not fungal root endophyte communities), either through the pool of available colonizers or via morphological effects on the host plant.

In partial agreement with H3, we found that more spatial variables were selected for the fungal communities and thus space explained greater variation in fungal than bacterial communities but that each community showed similar levels of spatial autocorrelation. While our spatial predictors could be partially explained by environmental heterogeneity, variation partitioning revealed independent spatial effects in both the bacterial and fungal communities after accounting for the other included variables. We found similar relationships to geographic distance for both communities where bacterial and fungal communities closer than 220 m were more similar to each other than more distant communities. This distance is similar to that found for the spatial autocorrelation of soil bacterial communities at this site (240 m) (King et al. 2010). Space may act as a proxy variable for multiple types of drivers including unmeasured, spatially structured biotic and abiotic factors as well as dispersal limitation (Peres-Neto and Legendre 2010, Dray et al. 2012, Hanson et al. 2012), and has previously been found to explain variation in both bacterial and fungal root endophyte communities (Glynou et al. 2016, Wang et al. 2019). The spatial extent of our study was small (~3 km^2) compared to studies which encompass large swaths of a country (2.3 million km^2; Wang et al. 2019), which may clarify why space explained less variation than has been previously seen for root endophytes.

In contrast to H3, the plant neighborhood explained more variation in bacterial than fungal root endophyte communities. This finding was unexpected, as previous research has demonstrated a stronger influence of plant variables (e.g. plant biomass, composition, richness) on fungal rather than bacterial root endophyte communities (Dean et al. 2014, 2015) and on soil fungal compared to soil bacterial microbial communities (Sugiyama et al. 2008, Fanin et al. 2019). Soil microbial communities are a likely intermediary of plant community effects on root endophyte communities; shifts in soil microbial communities occur with alterations in plant density and composition (Knelman et al. 2012, Porazinska et al. 2018, Bueno de Mesquita et al. 2019), with the effects of plants occurring via litter inputs and root exudates (Bardgett and Walker 2004, Bueno de Mesquita et al. 2019). These shifts in soil microbial communities could feedback to alter root exudate patterns (Badri and Vivanco 2009), and hence root endophyte communities. It is also possible that plant density and richness could influence root endophyte communities through plant competitive dynamics, which could then shape root endophyte communities via plant-soil feedbacks (Fitzpatrick et al. 2018). Finally, plant presence shapes the quality of the soil, including the texture and nutrient availability (Bueno de Mesquita et al. 2017), which could alter the relationship between plants and their root endophytes (Yeoh et al. 2017, Xu et al. 2020). However, there was no effect of soil texture or nutrient availability on bacterial or fungal root endophyte communities, suggesting this was not the pathway through which plant density and richness acted in our study.

Conclusion

It is paramount to study bacterial and fungal root endophyte communities concurrently in order to obtain a more complete understanding of root endophyte distribution. Our study demonstrates that bacterial and fungal root endophytes were shaped by a similar suite of drivers, but the plant neighborhood was less important for fungal endophytes than expected. For both communities, host plant species was of greater importance than space, plant neighborhood, and abiotic drivers in shaping the assembly of root endophyte communities in a diverse set of plants in a natural setting though we cannot rule out the potential for muted abiotic effects due to differences in sampling year between soils and the root endophyte communities. Overall, a low amount of variation was explained by our drivers. Together, these findings suggest broad similarities in drivers shaping bacterial and fungal root endophyte communities of alpine plants, and a likely role for stochasticity.

Author contributions

Katharine Suding, Steve Schmidt, Dorota Porazinska, and Clifton Bueno de Mesquita conceived the study. Jane Smith, Marko Spasojevic, Clifton Bueno de Mesquita, Emily Farrer, Dorota Porazinska, and Steve Schmidt collected the data. Dorota Porazinska and Clifton Bueno de Mesquita performed laboratory work, Clifton Bueno de Mesquita and Laurel Brigham processed the microbial data, and Laurel Brigham analyzed the data. Laurel Brigham led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary data

Supplementary data are available at FEMSEC online.

Conflicts of interest. None declared.

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Data availability

Merged demultiplexed fastq files are accessible at doi:10.6084/m9.figshare.19593805 (16S) and doi:10.6084/m9.figshare.19199969 (ITS)

Sequencing data is accessible via Genbank accession numbers ON229119–ON229415 (16S) and MH238510–MH240826 (ITS)

Environmental data and metadata are archived with the Environmental Data Initiative doi:10.6073/pasta/3ec2bf8bef96871ea56f9093362bcee3 and doi:10.6073/pasta/4c1017f934dce2081b6f1aecfefc55f2

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