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PLANT-MICROBE-ANIMAL INTERACTIONS – ORIGINAL RESEARCH

Causes and consequences of differences in soil and seed microbiomes for two alpine plants

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Abstract

Seed and soil microbiomes strongly affect plant performance, and these effects can scale-up to influence plant community structure. However, seed and soil microbial community composition are variable across landscapes, and different microbial communities can differentially influence multiple plant metrics (biomass, germination rate), and community stabilizing mechanisms. We determined how microbiomes inside seeds and in soils varied among alpine plant species and communities that differed in plant species richness and density. Across 10 common alpine plant species, we found a total of 318 bacterial and 128 fungal operational taxonomic units (OTUs) associated with seeds, with fungal richness affected by plant species identity more than sampling location. However, seed microbes had only marginally significant effects on plant germination success and timing. In contrast, soil microbes associated with two different plant species had significant effects on plant biomass, and their effect depended both on the plant species and the location the soils were sampled from. This led to significant changes in plant-soil feedback at different locations that varied in plant density and richness, such that plant-soil feedback favored plant species coexistence in some locations and opposed coexistence at other locations. Importantly, we found that coexistence-facilitating feedback was associated with low plant species richness, suggesting that soil microbes may promote the diversity of colonizing plants during the course of climate change and glacial recession.

Keywords Soil microbes · Seed microbes · Plant-soil feedback · Alpine · Germination

Introduction

Plants are colonized by microbial consortia, on the surfaces and in the interiors of seeds (Shade et al. 2017; Nelson 2018), leaves (Stone et al. 2018), roots (Berendsen et al. 2012), flowers (Shade et al. 2013), and stems (Cregger et al. 2018). Microbial symbionts generally benefit the host plant (Vandenkoornhuyse et al. 2015); however, microbial pathogens are also common (Jackson 2009). Microbial symbionts may be especially beneficial for plants in relatively harsh abiotic environments, as symbionts can buffer against abiotic stress (Araya et al. 2020). Soil microbes associated with plant roots have received the most attention, but research on seed microbiomes and plant performance is an active and growing area of research (Nelson 2018). The seed microbiome is the only plant microbiome compartment that plants can transmit vertically from parent to offspring; therefore, seed microbiomes are expected to generally have a positive effect on plants (Rahman et al. 2018).

Both biotic and abiotic factors such as position on landscape, can structure microbial community composition. Plant microbiomes can be species-specific, with differences in microbial community composition determined by host plant species identity (Bulgarelli et al. 2013). Variation in microbiome structure, including densities of pathogens and

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mutualists, is likely to vary temporally as plant communities develop (Pugnaire et al., 2019). Seed microbiomes, because of partial maternal inheritance (Shade et al. 2017), may be structured by plant identity more than soil microbiomes. However, soil microbiomes are also influenced by plant species identity via species-specific root exudates and differences in litter quantity and litter quality (Bueno de Mesquita et al. 2019). Tedersoo et al., (2014) found a positive correlation between plant and soil fungal richness in a global study examining biogeographic patterns of fungal diversity. While biotic factors such as plant species identity are important for structuring microbial communities, Fierer (2017) and King et al., (2010) note that abiotic factors such as pH, climate, and organic carbon availability drive community structure as well. Microbes may also be limited by their own ability to disperse; fungi in particular, due to their greater size relative to bacteria, are predicted to be more dispersal-limited (Bahram et al. 2018; Chen et al. 2020), although the opposite has also been shown (Xiao et al. 2018).

The structure and composition of microbial communities can have host specific effects on aspects of plant performance such as germination (Clay and Schardl 2002) and biomass (Lugtenberg et al. 2002). Tobias et al., (2017) tested the effects of several microbe species isolated from alpine plant seeds and found their effects on *Zea mays* seedling success varied from positive to negative. Seed microbial communities from different locations can have different compositions and potentially different effects on plants (Eyre et al. 2019). Similarly, soil microbes have complex interactions with plants resulting in net positive or negative consequences for plants (van der Putten et al., 2013). Plant community composition may also impact microbial community structure with greater density of monocultures often containing a higher concentration of pathogens (Putten et al., 2013). Because soil microbiomes may be more variable than seed microbiomes, their effects on plant performance may be more variable across locations (Abdullaeva et al. 2022).

The effects of seed and soil microbiomes on different plant species can scale up to influence plant community dynamics. Plant-soil feedback (PSF) measures community level impacts of plant–microbe interactions on plant species coexistence. PSF theory posits that plant species culture species-specific soil communities that feedback to differentially influence conspecifics and heterospecifics; for a given species pair, negative pairwise PSFs occurs if the plants exhibit a lower relative performance in conspecific soil compared to heterospecific soil whereas positive pairwise PSFs occurs if the plants perform relatively better in conspecific soil (Bever et al. 1997). Theory predicts that negative PSF, acting as a density or frequency dependent mechanism, stabilizes diversity in plant communities by decreasing the relative performance of a species when it becomes more abundant while allowing rare species to recover from low abundances (Bever 2003). In contrast, positive PSFs should decrease plant diversity by promoting some species over others (Reynolds et al. 2003). If abiotic factors interact with plant species identity to influence microbial community composition, then the strength and direction of PSF may differ among sites across landscapes (Wubs and Bezemer 2016; Smith-Ramesh and Reynolds 2017).

Understanding the effects of microbiomes on plant species performance may be especially important for predicting the consequences of climate change in alpine ecosystems. Microbial symbionts in alpine ecosystems can benefit plants by buffering against environmental stress (Callaway et al. 2002; Bueno de Mesquita et al. 2018a; Acuña-Rodríguez et al. 2020). Some species are tracking climate change by moving uphill and newly exposed unvegetated soils from glacial retreat and earlier snowmelt are being colonized (Darcy et al. 2018; Bueno de Mesquita et al. 2018b). Consequently, plants may encounter soils with different microbial communities (Van der Putten et al. 2010; Bueno de Mesquita et al. 2020). PSF in alpine environments may be particularly important for plant community assembly as facilitation between plants and their microbial symbionts become more important in higher stress environments (Callaway et al. 2002). In addition, plants can also bring microbes with them via seeds, which could lead to priority effects in newly colonized areas, as well as increases in germination rate and success (Shade et al. 2017). Understanding which aspects of plant–microbe interactions and which microbial compartments (e.g., soil or seed) have the greatest impact on plant performance is an important step for making predictions about biodiversity.

In the present study, we first conducted a field survey of alpine plant seed microbiomes that complements previous surveys of soil and root microbiomes (Porazinska et al. 2018; Bueno de Mesquita et al. 2018a). Such surveys are foundational for first learning which microbes are present in which compartments (e.g., soil, root, seed), and which factors drive the composition of the microbial community. Our next goal was to test how those microbial communities affect plants. Thus, we used manipulative experiments to test how seed endophytes influence plant germination, and how differences in soil microbiomes affect plant growth. Our third goal was to understand how plant-soil feedbacks change across locations that vary in plant density and richness, which is a key feature of alpine landscapes. We tested three hypotheses: (1) because both plant species identity and position on landscapes have been shown to affect seed microbiome communities, these factors will also correspond to seed microbiome composition in alpine systems, (2) because seed endophytes have been shown to promote germination in other systems we hypothesize that microbiomes in seeds will increase germination proportion and rate, and 3) because soil microbial community structure varies among plant species

and as a function of plant density we hypothesize that soil from diverse and densely populated communities on the alpine landscape will produce stronger PSFs than soils from sparsely populated, species-poor communities.

Materials and methods

Field sampling

Seed and soil collection took place at the Niwot Ridge Long Term Ecological Research site and adjacent Green Lakes Valley, in the Front Range of the Rocky Mountains, Colorado, USA (40.056177°N, 105.589355°W). Exact coordinates of each seed and soil collection site are provided in Table S1. This site is characterized by mean annual temperatures of − 2.8 °C and annual total precipitation of 1205 mm [data from nearby D1 meteorological station, 1999–2018, (Kittel et al. 2019, 2021)]. Annual and summer temperatures have increased by ~ 1˚C and ~ 3˚C, respectively, over the last several decades (McGuire et al. 2012; Bueno de Mesquita et al. 2018b) and concurrent with this summer warming trend, there have been increases in cover by alpine plants in areas that were previously unvegetated (Bueno de Mesquita et al. 2018b) or dominated by moss (Bueno de Mesquita et al. 2017).

We collected seeds on August 15th and August 16th, 2018, from 10 common alpine plant species at three different meadow locations, Niwot Ridge, Green Lakes Valley, and Navajo Peak. The Niwot Ridge sampling site was in the "Saddle" near the University of Colorado tundra lab at 3535 m above sea level (m.a.s.l.), a site with expansive alpine tundra meadow communities typical of the Rocky Mountains. In the Green Lakes Valley (GLV), seeds were collected from a patch of meadow at 3505 m.a.s.l. on the valley floor to the south of a large cliff. On Navajo Peak, seeds were collected from a patch of meadow at 3935 m.a.s.l on a southeast facing slope near the continental divide at the northeast edge of the Green Lakes Valley (Fig. 1). Seeds were collected from between 20 and 50 individuals and pooled at each location for the following 10 species: *Geum rossii* (Rosaceae), *Erigeron simplex* (Asteraceae), *Silene acaulis* (Caryophyllaceae), *Oxyria digyna* (Polygonaceae), *Luzula spicata* (Juncaceae), *Kobresia myosuroides* (Cyperaceae), *Carex pyrenaica* (Cyperaceae), *Deschampsia cespitosa* (Poaceae), *Festuca brachyphylla* (Poaceae), and *Trisetum spicatum* (Poaceae). This sampling encompasses four forbs, one rush, two sedges, and three grasses (Table 1). Although the true replication of species within each site was limited, the sampling conducted allowed us to conduct a first survey of seed endophytes for many species and broadly assess the effect of species versus location on the seed microbiome. A phylogenetic tree of the 10 plant species was created with the R package V.PhyloMaker (Jin and Qian 2019) which uses published phylogenies based on DNA sequence data. Plant seed mass for 9 of the 10 species was calculated by taking the average mass of 8 replicates of 100 seeds per species.

We collected soils for a plant-soil feedback experiment on August 21, 2018, from four plots used in a previous plant-soil survey (Porazinska et al. 2018) (Table 2, Fig. S1). These four plots were selected based on plant density and plant richness combinations (high/high, high/low, low/high, low/low) and presence of two focal plant species (*T. spicatum*,

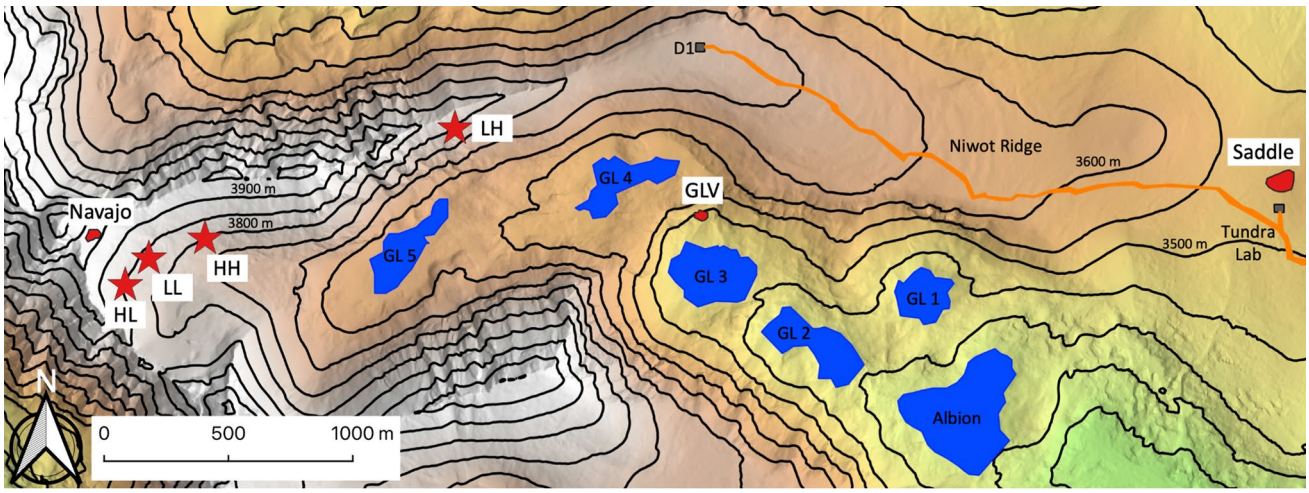

Fig. 1 Map of field sampling locations at Niwot Ridge and Green Lakes Valley, Colorado, USA. Shown are the three seed collection locations (Navajo, GLV, Saddle), the four soil collection locations (LL, LH, HL, HH for low density-low richness, low density-high richness, high density-low richness, and high density-high richness, respectively), and other landmarks. The map was made in QGIS version 3.4.13 with NAD83 / UTM zone 13 N coordinate reference system and a 2 m resolution LIDAR-based digital elevation model

Table 1 The 10 plant species sampled, along with their family, functional group, and average seed mass (g/100 seeds). NM = not measured

Species	Code	Family	Functional group	Seed Mass
Luzula spicata	LuzSpi	Juncaceae	Rush	NM
Kobresia myosuroides	KobMyo	Cyperaceae	Sedge	0.069
Carex pyrenaica	CarPyr	Cyperaceae	Sedge	0.033
Trisetum spicatum	TriSpi	Poaceae	Grass	0.03
Deschampsia cespitosa	DesCes	Poaceae	Grass	0.018
Festuca brachyphylla	FesBra	Poaceae	Grass	0.03
Geum rossii	GeuRos	Rosaceae	Forb	0.11
Oxyria digyna	OxyDig	Polygonaceae	Forb	0.047
Silene acaulis	SilAca	Caryophyllaceae	Forb	0.029
Erigeron simplex	EriSim	Asteraceae	Forb	0.012

F. brachyphylla). The high density, high richness plot represents an established and continuous tundra meadow community. The low density, low richness plot is in an area with a small patch of plants surrounded by a talus matrix and represents a sparsely vegetated area at the upper edge of alpine tundra that may be undergoing active colonization. The high/low and low/high density/richness combinations come from larger patches of vegetation in the talus matrix that are more developed than the low/low plot but are not part of the established continuous tundra meadows. In each plot, approximately 50 g of soil to a depth of 5 cm was collected from under 8 different *T. spicatum* individuals and eight different *F. brachyphylla* individuals with a sterile scoopula, placed into sterile bags, transported to the lab on ice, and then shipped on ice to the University of Houston.

Seed microbiome sequencing

Several seeds of each plant species at each site were surface sterilized in a 0.08% hypochlorite solution for 10 min, rinsed with sterilized deionized water and then frozen with liquid nitrogen and ground into a powder with a sterile mortar and pestle; DNA was extracted from 0.3 g of this powder with a DNEasy plant extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer protocols. PCR was used to amplify the 16S rRNA gene with 515F/806R primers and the ITS gene with ITS1F/ITS2 primers, according to the Earth Microbiome Project protocols (Caporaso et al. 2012). PCR products were normalized with a SequalPrep normalization kit (Invitrogen Inc., Carlsbad, California, USA), tagged with barcodes, pooled, and sequenced on a MiSeq2000 (Illumina Inc., San Diego, California, USA) at the University of Colorado BioFrontiers Institute (Boulder, Colorado, USA) with 2 × 150 base pair chemistry. Raw reads were processed with the USEARCH version 8.1.1 pipeline (Edgar 2013) to demultiplex sequences, merge paired ends, quality filter (maxee = 0.005), remove singletons, and cluster reads into operational taxonomic units (OTUs) at 97% similarity for 16S and 99% similarity for ITS. Taxonomy was assigned with the RDP Naive Bayesian Classifier algorithm (Wang et al. 2007) implemented in the dada2 R package (Callahan et al. 2016), with the SILVA (Quast et al. 2013) version 138.1 database for 16S sequences and the UNITE (Nilsson et al. 2019) version 8.3 database for ITS sequences. OTU representative sequences are publicly available on GenBank under BioProject ID PRJNA785750.

Plant germination and growth experiments

Two experiments were conducted with *T. spicatum* and *F. brachyphylla*, both of which are abundant in communities at high elevations in the Rocky Mountains and are colonizing unvegetated soils as climate changes and snowbeds melt earlier (Bueno de Mesquita et al. 2020).

In the first experiment, we tested the effects of seed endophytes on the germination of the *T. spicatum* and *F. brachyphylla* using seeds collected from the Saddle site. To isolate the effects of the seed endophytes, we sterilized half the seeds using microwave sterilization (Seaman and Wallen 1967). One live or sterilized seed was placed on water agar in an individual petri dish (30 mm) in replicates of 50 for each species and treatment and checked daily for germination. To verify sterilization efficacy, we plated seeds on malt extract agar (Difco Mfg) for 10 days; if no fungal or bacteria was detected, sterilization was considered to be effective. In a previous experiment we saw no significant difference in germination between non-sterilized seeds and seeds sterilized in the microwave and reinoculated with a seed slurry, suggesting our sterilization method did not affect germination ($F_{1,294} = 0.37$, $P = 0.69$).

In the second experiment, we tested the effect of the soil microbiomes on plant performance by growing our focal species with a 10% subsample of their own and each other's naturally cultured field soil (described above) collected at each of four locations. A subsample of soil collected was used to isolate microbial effect and reduce potential abiotic effects between sampling locations. To reduce variability in performance that may be caused by endophyte composition

Table 2 Characteristics of the four 1-m radius plots where soils were collected for the plant-soil feedback experiment

Plot id	Plant density	Plant richness	Density richness	Elevation	WHC	May snow depth	pH	NO_3 (μg g^{-1})	NH_4 (μg g^{-1})	DOC (μg g^{-1})	MicC (μg g^{-1})	MicN (μg g^{-1})	16S Chao1	ITS Chao1
60	53	5	Low–low	3831.76	0.08	81	5.54	0.02	0.33	EQCL	83.32	7.14	5160	451
99	90	15	Low–high	3794.47	0.10	156	5047	0.49	0.44	103.92	EQCL	EQCL	4280	634
136	191	10	High–olw	3843.9	NM	180		0.76	1.68	41.85	40.60	5088	3485	501
74	265	2323	High–high	3794.17	0.21	184	5.50	0.47	1.09	75.70	578.56	62.65	5252	712

Shown are plot ID (as in previous work), plant species density and richness per 1 m radius plot, density-richness category, elevation, water holding capacity (WHC, saturated weight—dry weight/dry weight), interpolated average May snow depth from 1997 to 2015, pH from a 1:2 soil DI water slurry, nitrate, ammonium, dissolved organic carbon, microbial biomass carbon and microbial biomass nitrogen, 16S rRNA gene OTU Chao1 richness estimate and ITS gene OTU Chao1 richness estimate. Further details on these data can be found in Porazinska et al. (2018). EQCL = exceeds quality control limits. NM = not measured

or genetic variation we used seeds of *T. spicatum* and *F. brachyphylla* acquired from a single location, the Saddle at Niwot Ridge. Prior to planting, we surface sterilized the seeds in a 0.08% hypochlorite solution for 10 min and rinsed them with deionized water. Seeds were planted in sterilized play sand (Quikcrete, Atlanta, GA) and watered every 2–3 days. Play sand was sterilized by autoclaving twice at 121 °C for 1 h, with 24 h between cycles. Upon emergence of the first true leaf, seedlings were transferred into 262 mL conical pots (5 cm diameter × 17.8 cm depth; Stuewe & Sons, Tangent, OR) filled with 225 mL of sterilized (as above) background soil. The background soil consisted of metromix (SunGro Metromix 250; Agawam, MA, USA) passed through a 3 mm sieve. Pots were lined with cotton cloth to stop soil from draining out of the pots while allowing water to pass through. When transplanting the seedlings, we added 25 mL (10%) of collected field soil to the root zone from one of the two soil identities (from *T. spicatum* or *F. brachyphylla* collected from one of the four plots). Use of a 1:10 ratio of inoculum to homogenized bulk soil minimizes the confounding effects of other soil properties. Each treatment combination was replicated 8 times for a total sample size of 128 pots [4 locations with unique density/richness identities (high/high, high/low, low/high, low/low) × 2 plant-soil identities (*T. spicatum*, *F. brachyphylla*) × 2 plant species (*T. spicatum*, *F. brachyphylla*) × 8 replicates]. Plants were grown in a temperature-controlled greenhouse at the University of Houston from November 2018 to February 2019 to represent the length of the growing season. Temperatures were stable at 20 °C and relative humidity was ~ 70%. Plants were provided 50 mL of water twice a week. At the end of the experiment, we harvested aboveground and belowground plant biomass and dried the biomass at 60 °C for four days prior to weighing.

Statistical analyses

All downstream statistical analyses and graphing were performed with R version 3.4. Graphs were made with the 'ggplot2' package (Wickham 2016). OTU richness was analyzed with ANOVA (testing species, site, and their interaction) followed by Tukey's post hoc. OTU tables were not rarefied (McMurdie and Holmes 2014); instead, since OTU tables are compositional (Gloor and Reid 2016; Gloor et al. 2017), the counts were transformed with a zero-replacement function (R package 'zCompositions') followed by centered log ratio transformation (R package 'compositions'). Aitchison's distance was then calculated, and effects of species and site were analyzed with PERMANOVA (R package 'vegan'). Homogeneity of variance was tested with PERMDISP (R package 'vegan'). Ordinations of Aitchison's distances were plotted with principal components analysis (R package 'stats').

For the first experiment, we tested the effects of seed endophytes by measuring two response variables, germination success and days to germination. For germination success we used a generalized linear model with a binomial family and a logistic link applied to the germination/no germination response data. The factors used in this model were plant species and sterilization treatment. To analyze days to germination we removed the non-germinated seeds from the dataset according to Ranal and Santana (2006). We tested the factors of plant species and treatment using ANOVA with Type III sum of squares (R package 'car').

For the second experiment manipulating the soil microbiome, we tested the effect of plant species (*T. spicatum*, *F. brachyphylla)*, plant-soil identity (*T. spicatum*, *F. brachyphylla)* and collection plot on plant biomass using ANOVA with Type III sum of squares. To quantify plant-soil feedback and test whether feedback for each location was significantly different from zero, we used a priori contrasts within the significant plant species x soil identity x location interaction that isolated the strength and direction of the interaction between plant and soil identity for each species pair within each location.

Results

Seed microbiome data

We identified a total of 318 bacterial OTUs and 128 fungal OTUs across the 10 species' seeds. We found no archaeal OTUs in the 16S rRNA gene dataset. The main bacterial phyla were Proteobacteria (55% mean relative abundance), Bacteroidota (14%), Firmicutes (8%), Actinobacteriota (7%), and Verrucomicrobiota (3%). The most abundant bacterial genera included *Pseudomonas* (13.4%), *Rhodoferax* (7.1%), and *Rugamonas* (4.6%) and were patchily distributed among the 10 plant species (i.e., abundant in some plant species and absent from others) (Figure S2). The Ascomycota phylum dominated the seed fungal community, accounting for 71% of all ITS reads. Unclassified fungi (25%), Basidiomycota (4%), Mortierellomycota (< 1%), Monoblepharomycota (< 1%), and Olpidiomycota (< 1%) were also present. The most abundant fungal genera included *Mycosphaerella* (17.9%) and *Cladosporium* (6.7%) and were even more patchily distributed among the 10 plant species than the bacterial genera (Fig. S2).

We hypothesized that position on landscape and plant species identity would affect fungal and bacterial diversity. In contrast, bacterial OTU richness in seeds was not affected by position on landscape or plant species (ANOVA, $p > 0.05$, Fig. 2a). On the other hand, fungal OTU richness was significantly affected by plant species (ANOVA, $F_{9,16} = 3.1$, $p = 0.023$) but not position on landscape (ANOVA, $p > 0.05$, Fig. 2b), with the highest richness in *Erigeron simplex* seeds. Bacterial OTU richness peaked at medium seed masses, and decreased in the lightest and heaviest seeds (polynomial regression, $p = 0.04$, $R^2 = 0.61$, Fig. S3). Fungal richness was not significantly related to seed mass (linear regression, $p > 0.05$, Fig. S3).

Bacterial community composition was significantly affected by plant species but not site (PERMANOVA, pseudo-$F_{9,17} = 1.7$, $R^2 = 0.45$, $p = 0.021$, Fig. 3a). Fungal community composition was also affected by species and not site (PERMANOVA, pseudo-$F_{9,16} = 1.6$, $R^2 = 0.45$, $p = 0.001$, Fig. 3b). Variance among sites and species was homogeneous for both bacteria and fungi (PERMDISP, $p > 0.05$).

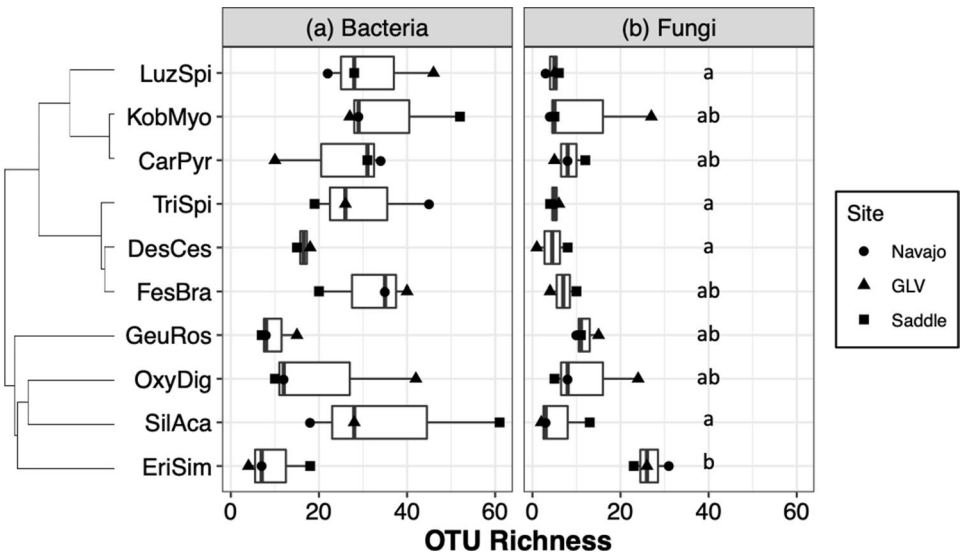

Fig. 2 OTU richness for **a** bacteria from 16S rRNA gene sequencing, and **b** fungi from ITS sequencing. Bacterial richness was not affected by species identity. Different letters in **b** represent significant differences in fungal OTU richness among species

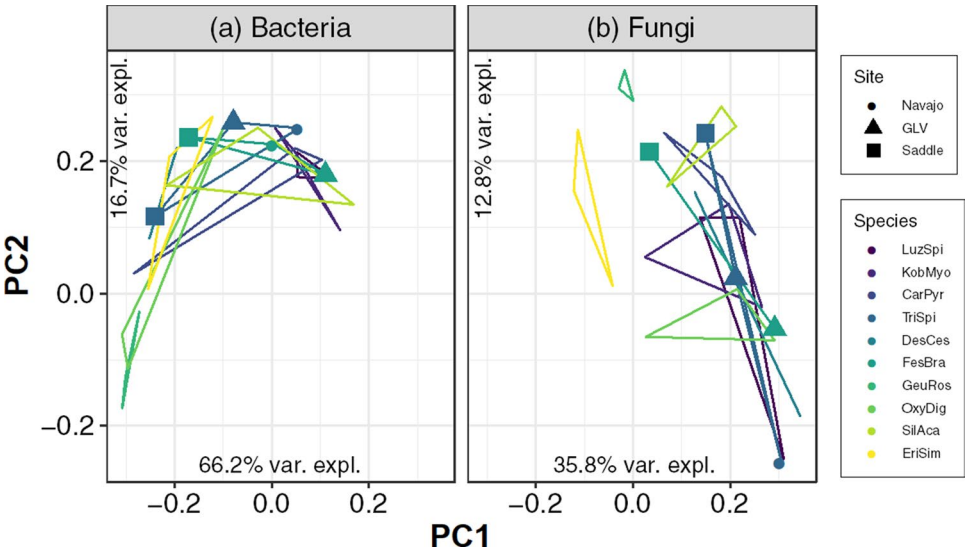

Fig. 3 Principal components analysis of seed OTU-level Aitchison's distance for **a** bacteria from 16S rRNA gene sequencing, and **b** fungi from ITS sequencing. Numbers in the bottom and left of each panel state the percent variation explained by axis 1 and axis 2, respectively. Points from the two focal species used in the germination experiment and PSF experiment, *Festuca brachyphylla* (FesBra) and *Trisetum spicatum* (TriSpi), are bolded

Seed germination

We hypothesized that seed microbiomes would increase germination proportion and rate. In contrast, sterilization of seeds did not affect the average time it took for seeds to germinate for either species ($F_{1,98} = 2.58$, $P = 0.11$, Fig. 4). Sterilization also did not affect the germination success ($\chi^2_{1,294} = 0.76$, $P = 0.68$).

Plant biomass/plant-soil feedback

We hypothesized that PSF would vary between sampling locations, with densely populated diverse communities generating more neutral/positive feedbacks. We found total plant biomass after four months was significantly affected by the interaction between plant species, plant-soil identity, and soil origin (Plot ID) ($F_{3,112} = 6.63$, $P < 0.001$), showing that plant-soil feedback (plant species x plant-soil identity interaction) was influenced by location (Fig. 5). The high/high

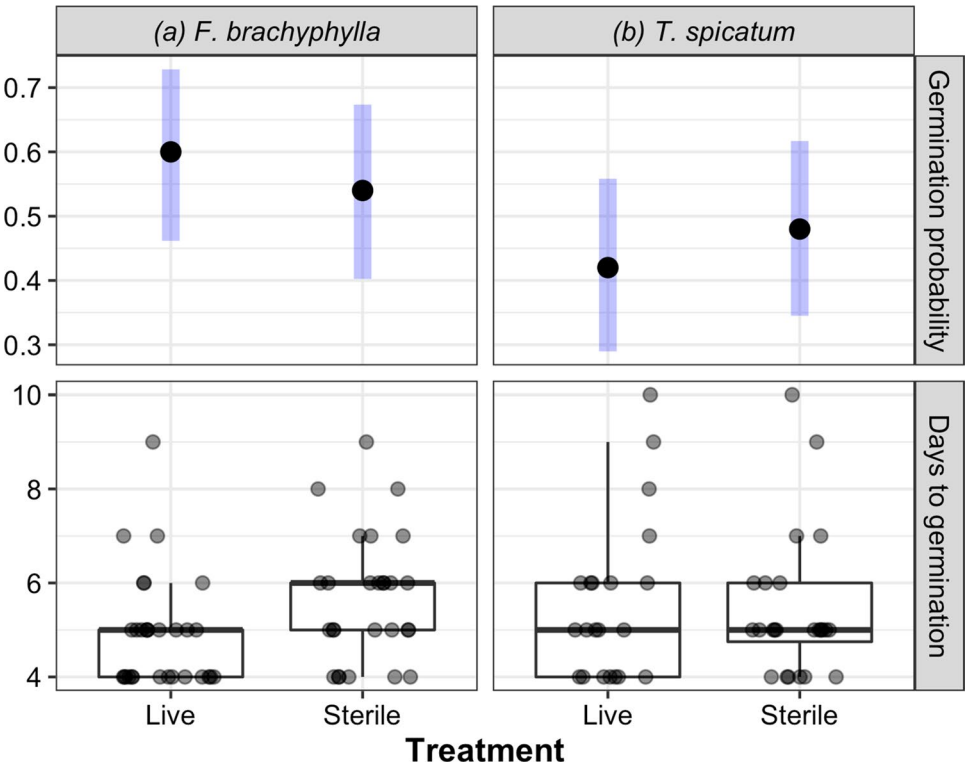

Fig. 4 Lack of effect of seed endophytes on the probability of seed germination and days to seed germination for seeds of **a** *F. brachyphylla* and **b** *T. spicatum* collected at the Saddle site. Blue segments in the top two panels are 95% confidence intervals from logistic regression models

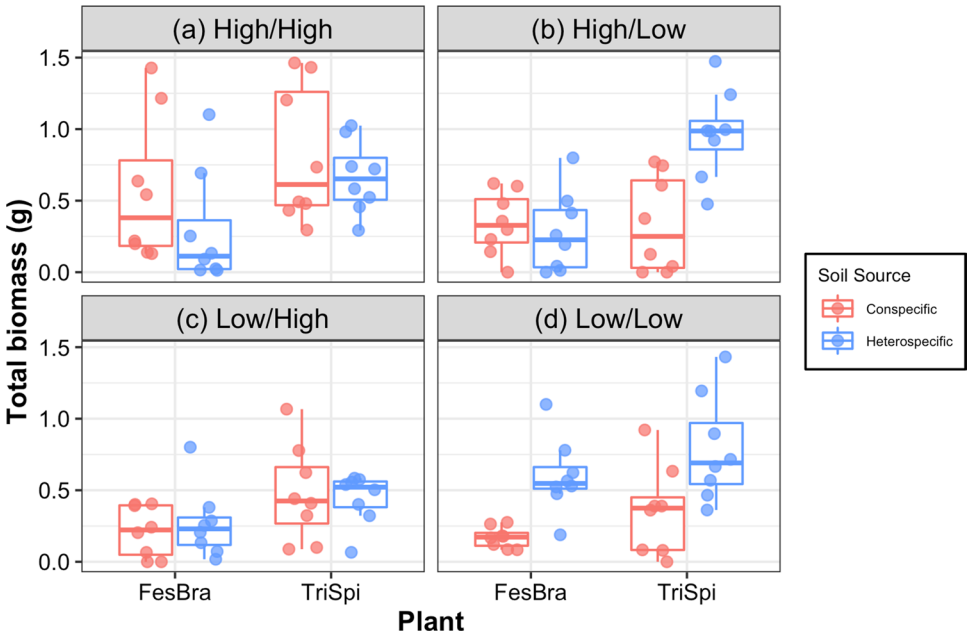

Fig. 5 Total aboveground and belowground biomass for each plant species (*T. spicatum* = TriSpi and *F. brachyphylla* = FesBra) in each soil collected from plant communities with either **a** high density, high richness (High/High), **b** high density, low richness (High/Low), **c** low density, high richness (Low/High), and **d** low density, low richness

and low/high (density/richness) plots showed positive/neutral feedback (Fig. 6). However, the high/low, low/low plots had significantly negative feedback (Fig. 6) which was in line with our hypothesis. Examining how the total biomass of each species changed depending on the soil conditioning species and plot ID helps clarify what responses drove the

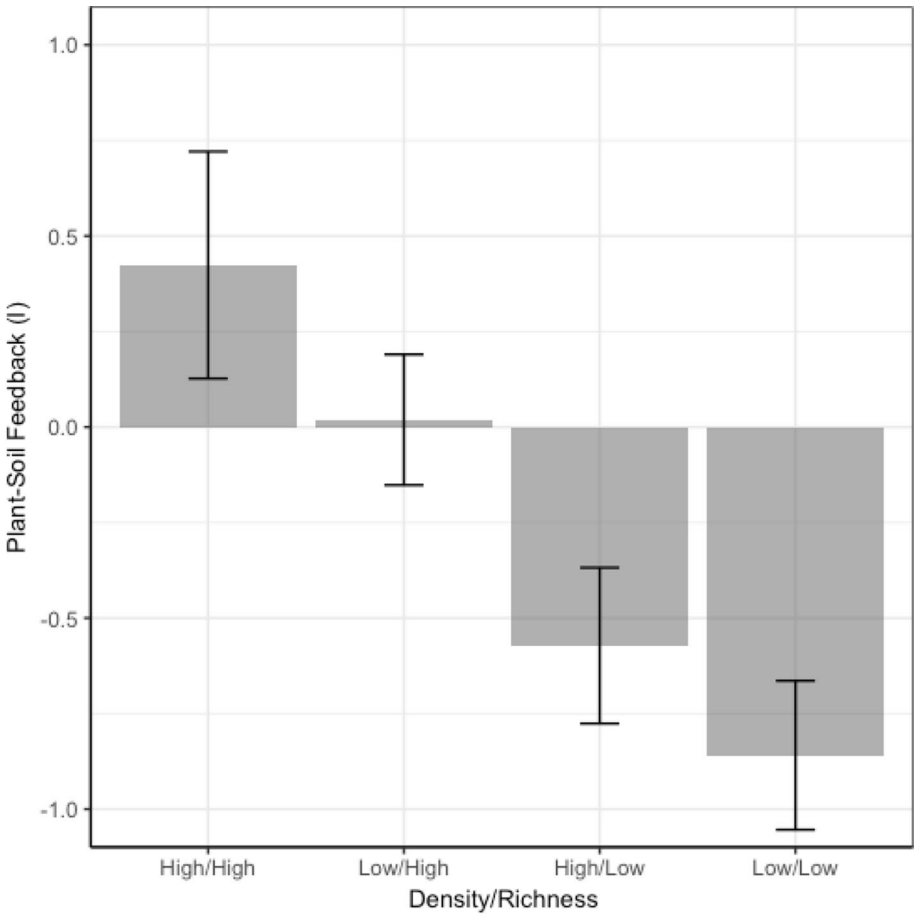

Fig. 6 Plant-soil feedbacks between *Trisetum spicatum* and *Festuca brachyphylla* in the four different types of plant density-plant richness combinations. The High/Low and Low/Low PSFs are considered significantly based on the plant-soil interaction term

differences in plant-soil feedback (Fig. 5). In soils collected from plots with low species richness *T. spicatum* performed much better in *F. brachyphylla* soil and in the plot with low diversity and low richness *F. brachyphylla* performed better in *T. spicatum* soil. This heterospecific advantage contributed to the negative plant-soil feedback. There was no significant difference between conspecific and heterospecific performance for *T. spicatum* and *F. brachyphylla* in both the high/high and low/high soils, contributing to the neutral plant-soil feedback in these soils.

Discussion

Our results show that seed microbiomes are structured more by plant species identity than landscape position, and that these microbiomes do not appear to be important for plant germination, at least under controlled conditions. On the other hand, plant density and species richness altered how PSFs affected plant biomass. This strong PSF effect on plant biomass might scale-up to influence plant community dynamics over time. Specifically, in soil sourced from a low density, low richness area typical of the ecotone between alpine tundra and unvegetated talus, late melting snowbeds, or newly exposed soils from receding glaciers, both plant species grew better in each other's soil, and worse in their own soil. This may partially explain the high degree of species richness relative to density seen in the majority of plots at the upper edge of alpine tundra or in other more environmentally harsh areas in alpine tundra such as dry meadows, fellfields, and snowbeds (Suding et al. 2015). Together, our results suggest that plant species identity strongly structures microbial communities, that soil microbial communities play a stronger role than seed microbial communities in alpine plant performance, and that feedbacks between plants and soil microbes can be highly variable across a landscape.

The effect of plant species identity on seed microbiomes is consistent with previous work (Wassermann et al. 2019) where species identity influenced seed microbiome structure. This effect is likely driven by variation in seed traits among the species, similar to what has been shown for other plant parts and the rhizosphere (Fitzpatrick et al. 2018; Ulbrich et al. 2021). Furthermore, seed microbes often co-disperse with seeds via vertical transmission from the rest of the plant, which would reinforce species-specific differences in seed microbiomes (Shade et al. 2017). This may also explain why seed microbiomes were more similar across our sites than soil microbiomes. Even so, the overall lack of effect of site is surprising because seed microbiomes are also partly derived from the soil (Shade et al. 2017; Escobar Rodríguez et al. 2020) and the soil biota in the three locations were expected to be quite different based on previous work (King et al. 2010; Porazinska et al. 2018). Indeed, 41% of the seed associated bacterial and fungal genera identified in our seed microbiome survey were also identified in the soil microbiome survey across the same landscape (Porazinska et al. 2018) (Table S2). To effectively examine landscape scale effects on plant microbiomes, more replication would be needed; future research could focus on fewer species and replicated plots at different elevations across the landscape.

In alpine ecosystems, germination and seedling recruitment have large effects on the population demography of some species (Forbis 2003) and are highly affected by abiotic variables which could outweigh the effects of microbiomes. While previous experiments found that fungal endophytes sourced from seeds at our sites affected *Zea mays* germination (Tobias et al. 2017), these endophytes may not necessarily have the same effect on the seeds of species that they were sourced from. Perhaps the seed microbiomes did not affect germination in our study because the wild alpine plants could simply rely on their endosperm in the controlled experimental environment which did not mimic the field conditions. It is also possible that seed microbiomes are not important for the germination of alpine plants, unlike in other systems such as tallgrass prairies (Clay and Schardl 2002) and agricultural systems (Newton et al. 2010), but this hypothesis should continue to be tested in future work. While we did not study the effects of seed microbiomes on growth after germination, seed endophytes can be transferred to the rhizosphere and can then affect growth even if they do not affect germination (Tobias et al. 2017). Aboveground endophytes can also affect belowground microbial communities, as has been demonstrated with the foliar endophyte *Epichloë* (Bell-Dereske et al. 2017). Thus, seed microbiomes could be important for alpine plants in other ways even if they did not affect germination.

The effects of the soil microbiome varied between conspecific or heterospecific sources, as well as the richness and density of plants at the locations where soil was collected. The strongest effect and only consistent result across both species was the increase in growth in heterospecific soils from sites with low plant density and low richness. This combination resulted in a strong negative PSFs that could affect plant community composition through early colonization of unvegetated to sparsely vegetated areas. Interestingly, this effect disappeared at high species richness, even when plant density was still low.

As climate warms, snowbeds melt out earlier and glaciers recede, exposing new substrates for ecosystem development. Newly exposed glacial till and periglacial soils are first colonized by microbes (Schmidt et al. 2008) and then plants, which can be affected by both the microbes, growing season length, moisture, and nutrient availability (Darcy et al. 2018; Bueno de Mesquita et al. 2020). Plant colonization can occur in either unvegetated areas, areas dominated by conspecifics, areas dominated by heterospecifics, or areas with relatively

even mixtures of conspecifics and heterospecifics. In the latter three instances, PSF can be particularly important. While plant successional position can be important for PSFs (Klironomos 2002; Kardol et al. 2006), the two plant species we studied here cannot be classified as either early successional or late-successional species as they are present in both newly colonized and well established tundra meadows. However, the high density-high richness soil could be considered late-successional, and the low density-low richness soil could be considered early successional.

Based on this perspective on the sites, the highest growth for both species occurred either in heterospecific early successional soil or in conspecific late-successional soil (Fig. S4). The first of these two results is consistent with previous findings that soil conditioned by an early successional species had positive or neutral effects on heterospecifics (Van der Putten et al. 1993; van de Voorde et al. 2011), and could be due to a lack of effect of species-specific pathogens on heterospecifics. However, this positive effect of heterospecific soil is contrary to previous results from a litter addition PSF experiment, where there was a negative effect of unvegetated soil conditioned with *Silene acaulis* litter on the growth of *Deschampsia cespitosa* (Bueno de Mesquita et al. 2019). This discrepancy could be due to differences in litter-only conditioning versus whole-plant conditioning, or by *Silene acaulis* litter characteristics that are not relevant in *T. spicatum* and *F. brachyphylla* litter.

Greater growth in soils trained by conspecifics in late-successional communities is contrary to what might be predicted from the literature. Late-successional soil that is more developed and contains more-developed plant communities has greater microbial alpha diversity (Porazinska et al. 2018) (Table 2). This greater diversity has been predicted to increase the likelihood of encountering antagonists and the likelihood of synergistic co-infections, both of which should lead to negative effects on plant growth (Wubs and Bezemer 2016). Positive PSF would also be expected to destabilize the community (Reynolds et al. 2003), yet in the 1 m radius circle surveyed, there was a rich community containing 23 plant species, suggesting that other community coexistence mechanisms are at play (Chesson 2000; Leibold et al. 2004). One such mechanism possibly preventing a pathogen-driven negative PSF is that pathogens could be diluted due to the high microbial richness. Future work could be designed to systematically address the effects of plant density and richness on PSF with experimental combinations of richness and density.

This survey of the causes and consequences of differences in seed and soil microbiomes for two alpine plants shows the importance of understanding which plant-associated microbes are relevant and how plant–microbe interactions could potentially influence plant species migration. We quantified the importance of soil microbes in both facilitation of biomass growth and community stabilizing mechanisms such as plant-soil feedback. By comparing the effect of different plant compartment microbiomes, we suggest that soil microbes in previously established areas have stronger effects on plant performance than the seed microbes which are carried with the plant. Our study provides a new strategy to assess the impact and relative importance of different plant compartments for plant–microbe interactions in a natural environment.

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Author contribution statement NCL and CPBM conceived and designed the experiments. NCL and ML performed the greenhouse and laboratory experiments. CPBM performed sequencing and bioinformatics. NCL and CPBM analyzed the data. NCL and CPBM wrote the initial draft of the manuscript, with significant edits from KMC, SKS and KNS.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability The code using during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interests to declare.

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Consent to participate All patients included in this study gave written informed consent to participate in this research.

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