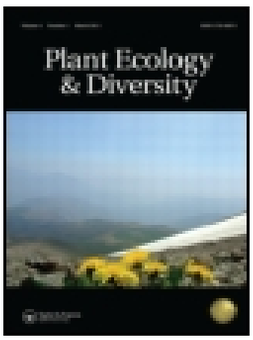

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RESEARCH ARTICLE

The presence of a foreign microbial community promotes plant growth and reduces filtering of root fungi in the arctic-alpine plant *Silene acaulis*

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ABSTRACT

Background: Climate change is expected to drive trailing-edge range redistributions of arctic-alpine plant populations, bringing together immigrant plant ecotypes and soil microbial communities associated with already resident ecotypes.

Aims: The goal of the present study was to assess growth performance and plant–microbe interactions between seedlings and native and foreign microbial communities in ecotypes of the cushion plant *Silene acaulis* from Europe and North America.

Methods: Using seed sourced from Colorado, USA, and Ireland we grew *Silene* seedlings in sterile bulk soil with live inocula added from their own local soil and each other's soil. We measured above-ground plant growth metrics, and analysed fungal and bacterial community composition using marker gene sequencing and microscopy.

Results: Seedlings growing in foreign soil inocula showed significantly greater biomass or shoot length compared to growth in home soil inocula. While seedling root microbiomes were overall convergent with each other compared to source soil inocula, significantly lower filtering of fungal taxa from the soil was observed for seedlings growing in foreign compared to home soil inocula.

Conclusions: Foreign plant ecotypes from distant habitats may experience competitively beneficial effects when growing in local soil communities; however, the nature and generality of these interactions requires further analysis.

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KEYWORDS
Bacteria; endophytes; filtering; fungi; plant-microbe interactions

Introduction

Climate significantly impacts the spatiotemporal distribution of organisms, and thus changes in climate introduce strong migratory pressures for terrestrial plants (Chen et al. 2011; Settele et al. 2014). At the local level, successful establishment by foreign climate migrants will depend not just on abiotic selective factors, but also on two-way interactions with local biotic communities (Richardson and Pyšek 2006; van der Putten et al. 2010). In this context, a key factor is the extent to which seedlings in new soil habitats adjust their soil microbial associations in the context of a novel soil microbial community (van der Putten 2012; Morriën and van der Putten 2013).

Plants and soil microbes have a reciprocal impact on each other's functioning, diversity and abundance, with complex patterns of association related to both biotic and abiotic factors (MGA et al. 2008; King et al. 2012; Philippot et al. 2013). At the ecosystem level, gross soil microbial diversity is known to vary greatly both at local and global scales, often in close alignment with abiotic soil conditions such as water availability and pH (Fierer and Jackson 2006; Tedersoo et al. 2014), and biotic conditions such as above-ground plant diversity (Prober et al. 2015; Porazinska et al. 2018).

Positive associations between plant populations and local soil microbial communities are known to stabilise with increased plant population age, conferring in some cases enhanced disease resistance and fitness on resident plants (Berendsen et al. 2012), and in others, where there is proliferation of compatible microbial pathogens and biotrophs in the soil substrate of long-established plant populations, a significant under-performance in growth of locally bred seedlings due to negative plant-soil feedbacks (Bever et al. 2012). Thus, as plant populations persist over time in a given soil habitat, commensal, mutualistic and antagonistic associations with soil microbes can develop. These kinds of established interactions can be either absent or less abundant when plant populations establish in new habitats, as occurs with invasive species. Several

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Supplemental data for this article can be accessed here.

studies have shown that there is a growth benefit for invasive species in novel soils compared to home range soils (Mitchell and Power 2003; Callaway et al. 2004; Inderjit 2010; Yang et al. 2013), consistent with the enemy-release hypothesis (Keane and Crawley 2002). On the other hand the local adaptation hypothesis posits that plant genotypes are selected for by the local environment, which includes, in part, the local soil and microbial environment (Smith et al. 2012). Therefore, specialised plant genotypes with high fitness in one soil type may have lower fitness in other soil types, which could also be partially due to a potential lack of mutualists in foreign soils.

In addition to colonisation of novel habitats by species, within-species redistribution of genotypes is also common, and climate-related migration of local genotypes within the established range of a species is widely inferred from the historical record, associated with the formation of suture and/or hybrid zones composed of distinct genetic identities with diverse biogeographic origins (Hewitt 2000). A key question is the extent to which immigrant plants (as members of a genetically diverse metapopulation) control their root microbial associations relative to the different bulk soil microbial communities that occur across their biogeographic range, and the extent to which they encourage positive associations and/or are susceptible to negative associations with local microbial communities (Weinert et al. 2011). For example, some invasive species have been shown to promote specific rhizosphere microbial communities across their ranges (Rodríguez-Caballero et al. 2020).

In this context, rapid climate warming predicted for arctic and alpine regions in the northern hemisphere (IPCC 2013) will likely drive widespread within-range displacement of endemic species ecotypes. In general, already observed levels of trailing-edge range contraction in arctic-alpine plants indicate it is mostly an incremental process (Pauli et al. 2007), and while the historical record clearly indicates that climate-mediated range changes involve genotype migration (Hewitt 2000), it is yet to be described how within-range redistribution and migration of genotypes is impacted by plant-soil microbial interactions (Ma et al. 2019). *Silene acaulis* (Linnaeus) Jacq. (moss campion) is among the circumpolar group of Arctic-alpine species likely to be affected in this way (Doak and Morris 2010). Widely distributed in tundra areas of the European and North American Arctic, the range of *S. acaulis* extends southward along mountainous terrain in the Rocky Mountains, Eastern Canada and Western and Central Europe, where it occurs above the treeline, principally in exposed rocky and gravelly areas with limited vegetation cover (Hultén 1971; Jalas and Suominen 1986; Chater et al. 1993). Existing climate change pressures are already impacting population demography in the species, especially at its southern distribution edges (Doak and Morris 2010). During earlier and less abrupt climate oscillation events in the mid- to late-Pleistocene, frequent dispersals and range expansion and contraction episodes characterised the biogeography of the species (Gussarova et al. 2015), and so novel contact between immigrant seedling genotypes and native soil microbial communities has been a necessary element of successful population establishment during these periods. In this way, the establishment of positive interactions with soil microbes is essential to the demographic stability of *S. acaulis* populations.

In the present era, dispersal pressure will increase with ongoing climate change, resulting in immigrant plant seedling recruitment in established population areas. As accelerated biomass accumulation is key to survival of individual seedlings (Doak and Morris 2010), the way in which foreign ecotype seedlings generally interact with, and respond to, resident soil microbial communities may have a dramatic impact on their growth and survival.

Here we present results of reciprocal growth experiments for populations of *S. acaulis* from North America and Europe, grown with inoculum from their own and each other's home soil. Our objective was to determine whether two ecotypes of the same species interact differently with resident soil microbial communities and how this affects plant performance. While the distribution of these two specific genotypes are unlikely to overlap due to current climatic migration, the selected population sites represent a near maximal divergence in habitat characteristics for *S. acaulis* at the southern edge of its distribution. As such we utilise these two genotypes as an example to more generally study the extent to which genetically related seedlings filter and recruit soil microbes from their own versus distant soil inoculum and how this affects plant growth. We hypothesised that, consistent with previous work on the enemy release hypothesis, plants would grow better with microbes from distant soils than from local soils, with the prediction that there would be higher pathogen loads in the local soils. We further hypothesised that plants would filter out

more microbes from distant soil (i.e. acquire a smaller subset and have a greater degree of dissimilarity from the bulk community), as these soils contain different bulk soil microbial communities.

Materials and methods

Study organism and field sites

We analysed above-ground growth and root interior microbial communities of two populations of *S. acaulis*, one from Ireland and one from the Front Range of the Rocky Mountains, Colorado, USA, grown in soil inoculum from both sites. The two plant genotypes, distinguished by short and broad compared to long and narrow leaves, respectively (see Appendix 2), were characterised with matK and *trn*L sequencing as described in the Supplementary Material. The Irish site is a north-facing mixed alpine community at Ben Bulben Mountain, Co. Sligo (54.361498°N, 8.415649°W, 545 m. a.s.l.; Appendix 2), comprising a diverse, extensively grazed grassland habitat including *Festuca vivipara, Thalictrum alpinum, Dryas octopetala*, and *Plantago maritima*. Soil at the site is fine loamy drift with igneous and metamorphic stones, over mixed shale and slate bedrock, with pH ranging from c. 5.5 to 6.0. Mean annual air temperature at the site from 1981 to 2010 varied between 6.4 and 7.3°C, with a January average of 2.4°C and July average of 12.2°C; precipitation in the same period varied between 1690 and 1890 mm per annum, peaking in autumn (Walsh 2012).

The Colorado site is located at the Niwot Ridge Long Term Ecological Research Site (40.056177 °N, 105.589355°W, 3535 m a.s.l.; Appendix 2). Mean annual temperatures from 2011 to 2014 ranged from −4°C to −7°C (Losleben 2017). The mean growing season (June–August) temperature from 1982 to 2018 was 8.98 ± 0.29 °C, and since 1984 maximum growing season temperatures have always exceeded 15°C. Average precipitation from 1952 to 2012 was 1090 ± 230 mm year^{-1}, with an increase in average annual precipitation of 60 mm year^{-1} over that time period, driven mostly by increases in winter precipitation (Kittel et al. 2015). Soil pH is acidic (mean pH in the Green Lakes Valley where soil was collected is 5.1).

Greenhouse experiment

We collected seeds and soil in the autumn of 2015 at the Colorado site and winter of 2017 at the Irish site. Seeds were sorted to select plump seeds that would likely be viable. One litre of soil to be used as inoculum was collected from within a 2 m radius at each site. Soils were frozen at −20°C until the start of the experiment. Bulk soil used in the sterile growth medium was collected from the Colorado site (but at a different source location than the Colorado inoculum), was mixed 1:1 with sand and was sterilised by autoclaving a maximum of 4 L of the soil/sand mixture at a time, remixing the soil, and autoclaving a second time.

We sowed 5 surface-sterilised seeds into 18 replicate pots (8.255 cm wide x 8.255 cm long x 9.525 cm tall) for each treatment. Seeds were surface-sterilised by soaking in 0.6% sodium hypochlorite for at least 1 minute and then rinsing with greenhouse water. There were four treatments: Irish plant with Irish inoculum, Irish plant with Colorado inoculum, Colorado plant with Irish inoculum, Colorado plant with Colorado inoculum. Pots were sterilised by washing in 0.6% sodium hypochlorite for at least 1 minute and then rinsing with greenhouse water. To control for other soil factors, soil was added to the pots using a 30:1 ratio of sterile bulk soil to inoculum. This, for example, overwhelms any minor differences in nitrogen content in the inocula: Post-autoclaved bulk soil total inorganic nitrogen levels were 74.1 μg g dry soil^{-1}, much higher than in soils where the Colorado inoculum was collected (1.95 μg g dry soil^{-1}, Porazinska et al. 2018). Inoculum was added as a thin layer 2 cm from the top of the pot, which was then covered with 1 cm of bulk soil. Pots were watered every day to keep soils moist while seeds germinated, and then every other day following emergence. Plants were grown in the alpine room of the University of Colorado Greenhouse with natural light and temperatures ranging from 15°C during the day to 10°C at night. Seeds were sown on 16 March 2017. Due to low germination in some treatments, additional seeds were cold stratified for 2 days at 4°C and any pot with no seedlings as of 3 May 2017 received an additional five seeds. Growth variables were calculated as rates to account for differences in germination timing.

After about 2–3 months of growth in the greenhouse, on 5 July 2017 we measured leaf number, height, longest radius, and perpendicular radius to the longest radius, harvested plants to weigh above-ground biomass, and sampled roots (n = 11 from each treatment) for microscopy and sequencing. Note that the variation in length of growth period was the same across treatments and is only an additional source of within-treatment variation.

To focus on root interior (i.e. endophyte) microbial communities, roots were surface sterilised by rinsing in water, then soaking in 70% ethanol for 1 minute, 0.6% sodium hypochlorite for 1 minute, and triple rinsing in sterile water. We subsampled ca. 20 cm of root for microscopy, which was stored in formaldehyde acetic acid alcohol (FAA) at 4°C, and 0.1 g of root for Illumina sequencing of root endophytes, which was stored at −20°C until DNA extraction.

Fungal staining and microscopy

Staining and microscopy were made following established protocols (Koske and Gemma 1989; McGonigle et al. 1990; Schmidt et al. 2008). Roots were rinsed 3 times with deionised water to remove FAA and then cleared with 10% KOH for 1 h in a 90°C water bath. Roots were rinsed with water to remove KOH and then soaked in 1% HCl at room temperature for 20 minutes. Roots were then soaked overnight in acidic glycerol with 0.05% trypan blue. The following morning, roots were destained with acidic glycerol and stored in acidic glycerol at 4°C until microscopy was performed during the subsequent 7 days. Several fine root segments and their branches, totalling 10–20 cm of root, were placed horizontally across slides, covered with a cover slip, and viewed at 160–200 x magnification under a microscope with a crosshair on the ocular. Passes were made up and down the slide at random intervals and the presence of arbuscular mycorrhizal fungi (AMF) or dark septate endophyte (DSE) structures at each of 50 intersections with the crosshair was recorded (50 intersections were used instead of 100 due to the amount of fine roots available). Per cent colonisation for each fungal group indicates the number of times this group was present across the 50 intersections, multiplied by two. We note that while the Caryophyllaceae family is often considered non-mycorrhizal, these plants are still colonised by other fungal endophytes, including DSE – which can play an important role in their ecology – as well as low levels of arbuscular mycorrhizal fungi (Bueno de Mesquita CP, Sartwell SA, Ordemann E V., et al 2018; Giesemann et al. in review).

DNA sequencing – 16S and ITS

We used sequencing of the 16S ribosomal RNA and internal transcribed spacer (ITS) marker genes to determine soil and root interior bacterial and fungal communities, respectively. For plant roots, we extracted DNA from 0.1 g wet roots using the Qiagen DNeasy plant kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We also extracted DNA from five adult individuals from Colorado and five adult individuals from Ireland. Individual adult plants from Colorado come from five individual plots as described in Bueno de Mesquita et al. (2018). Individual adult plants from Ireland were sampled across a 2 km transect, with individuals of 10–20 cm diameter cushions sampled at least 50 m apart. The age of the adult plants is unknown but is estimated in the decades based on cushion size (Morris and Doak 1998).

In addition, to characterise soil microbial communities, we took five subsamples of each inoculum, from which DNA was extracted from 0.3 g of soil using the Qiagen DNeasy power soil kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Extracted DNA was amplified via polymerase chain reaction with the 515 F and 806 R primers for 16S and ITS1f and ITS2 (White et al. 1990) primers for ITS amplicon sequencing, as done by the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/). Amplified samples were purified and normalised with the SequalPrep Normalisation Kit (Invitrogen Inc., Carlsbad, CA), combined into a single pool each of 16S and ITS amplicon libraries and sequenced on one lane each of an Illumina MiSeq2000 (2 x 300 bp paired-end) at the University of Colorado BioFrontiers Institute (Boulder, CO). 16S forward and reverse reads were trimmed to 230 bp and ITS forward and reverse reads were trimmed to 205 bp, such that all read quality scores were above 30. We used the UPARSE pipeline (Edgar 2013) to demultiplex sequences, remove singletons, and then cluster sequences (including chimera filtering) into operational taxonomic units (OTU's) of sequences with 97% similarity. Mitochondrial, chloroplast, and Archaea reads were removed from the 16S OTU table. Sequences were rarefied at 831 sequences for 16S and 16,096 sequences for ITS. Taxonomy was then assigned using default parameters in the DADA2 R package (Callahan et al. 2016) with the most recent releases of the SILVA (version 138, released 15 August 2020, McLaren 2020) and the UNITE (version 8, released 4 February 2020, Abarenkov et al. 2020) databases for 16S and ITS, respectively. OTU sequences and Raw Reads were deposited on GenBank under BioProject PRJNA661383.

Analyses

Bacterial and fungal communities were analysed at the OTU level by calculating Bray–Curtis dissimilarities on Hellinger-transformed relative abundances. Dissimilarities were visualised using Principle Coordinates Analysis and differences among sample types (soils, seedlings and adults) were tested with permutational multivariate analysis of variance (PERMANOVA, Anderson 2001). Taxa driving compositional differences among sample types were determined using similarity percentage analysis (SIMPER). Multivariate statistics were conducted in the 'vegan' package in R (Oksanen et al. 2019). Fungal guilds were assigned using FUNguild version 1.1 and included both highly probable and less certain assignments (Nguyen et al. 2016).

For analyses of seedling plant biomass, height, cushion area, longest leaf, leaf number, per cent dark septate endophyte root colonisation, per cent arbuscular mycorrhizal root colonisation, fungal pathogen relative abundance (assigned by FUNGuild as plant pathogens), and fungal mutualist (assigned by FUNGuild as dark septate endophytes or mycorrhizae), we used ANOVA with soil location, plant population and their interaction as fixed effects (function 'aov' in the R package 'stats'). To account for differences in the timing of germination among the plants, we calculated growth per day as the response variable. To satisfy assumptions of normality and homogeneity of variance, we log-transformed plant growth data. For analyses across soils, seedlings, and adult plants – bacterial richness (number of OTUs from 16S data) and fungal richness (number of OTUs from ITS data) – we used ANOVA with sample type as a fixed effect. We used Tukey's honest significant differences test to conduct pairwise comparisons among the treatments (function TukeyHSD in the R package 'stats'). Bray–Curtis dissimilarities were analysed with Wilcoxon tests. All analyses were performed using R version 3.5.3 (R Core Team 2019).

Results

Plant growth

In the greenhouse experiment, inoculum origin had a significant effect on plant biomass (Table 1), with Irish *Silene* plants growing significantly more biomass with Colorado inoculum than with Irish inoculum (TukeyHSD, $P = 0.002$, Figure 1a). This was driven by a significantly greater amount of leaf production in Colorado soil (TukeyHSD, $P < 0.001$, Figure 1d), not by differences in height, or cushion area (TukeyHSD, $P > 0.05$, -c). There were no significant differences in the biomass of Colorado *Silene* plants between foreign and home inoculum (TukeyHSD, $P = 0.98$, Figure 1a). However, Colorado plants grew significantly taller with Irish inoculum compared to Colorado inoculum (TukeyHSD, $P = 0.003$, Figure 1c).

Table 1. ANOVA results for four different metrics of the response by *Silene acaulis*, showing the effects of plant genotype, inoculum, and their interaction.

Response variable	Predictor Variable	df	*F*	*p*
Biomass	Genotype	1	3.52	0.063
	Inoculum	1	5.43	**0.022**
	Genotype x Inoculum	1	8.42	**0.004**
	Residuals	116		
Area	Genotype	1	22.91	**< 0.001**
	Inoculum	1	0.08	0.776
	Genotype x Inoculum	1	10.72	**0.001**
	Residuals	116		
Height	Genotype	1	38.44	**< 0.001**
	Inoculum	1	1.48	0.226
	Genotype x Inoculum	1	14.57	**< 0.001**
	Residuals	116		
Leaf number	Genotype	1	5.77	**0.018**
	Inoculum	1	6.04	**0.015**
	Genotype x Inoculum	1	12.04	**< 0.001**
	Residuals	116		

Root and soil microbial communities

There were significant differences in fungal (ANOVA, $F_{7,34} = 267$, $P < 0.001$) and bacterial (ANOVA, $F_{7,32} = 52.39$, $P < 0.001$) richness among our sample types (Figure 2). These differences were primarily driven by significantly higher richness in soils compared to plant roots (TukeyHSD, $P < 0.05$). There were no differences in root fungal richness across all plant types. Adult plants collected at the Colorado site also had significantly higher bacterial richness than seedlings grown in Colorado soil inocula (TukeyHSD, $P < 0.05$). No significant differences were recorded between adult plants from the Irish site and seedlings grown in Irish soil inocula, or between groups of seedlings within each treatment (TukeyHSD, $P > 0.05$).

There were significant differences in fungal (PERMANOVA, $F_{7,34} = 3.83$, $P = 0.001$) and bacterial (PERMANOVA, $F_{7,32} = 3.91$, $P = 0.001$) community composition among our sample types (Figure 3, Figure 4). Both fungal and bacterial community composition were significantly different between the Colorado and Irish field soils (Fungal PERMANOVA, $F_{1,8} = 16.16$, $P = 0.008$; Bacterial PERMANOVA, $F_{1,8} = 10.31$, $P = 0.008$, Figure 4), and between roots of adult plants collected at the Colorado and Irish sites (Fungal PERMANOVA, $F_{1,7} = 2.22$, $P = 0.011$; Bacterial

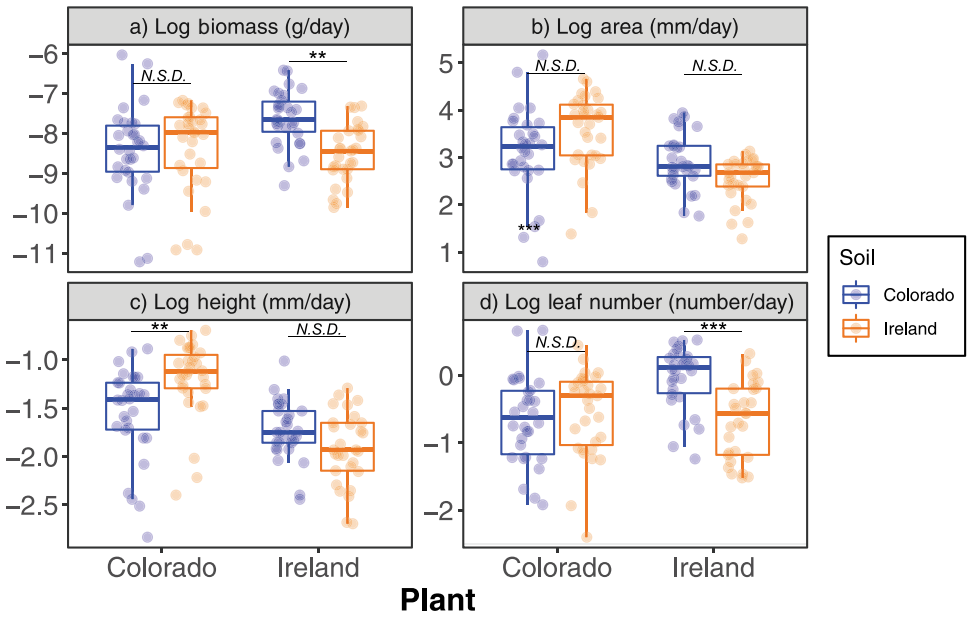

Figure 1. Growth response (log transformed biomass, cushion area, height and leaf number per day) of the two populations of *Silene acaulis* in each of the two soil inocula. Data are from 2 ~ 3 months of growth in the greenhouse (mean = 64.8 ± 1.33 SE days between germination and measurement) and are calculated as rates per day to account for differences in germination timing. Colorado plant in Colorado soil: n = 30, Colorado plant in Irish soil: n = 31, Irish plant in Colorado soil: n = 30, Irish plant in Irish soil: n = 29. **Tukey HSD $p < 0.01$.

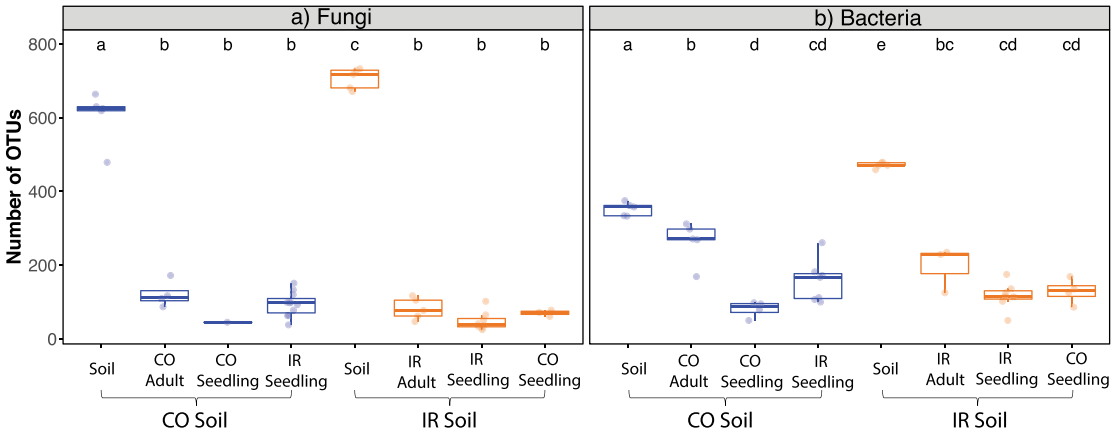

Figure 2. Richness of operational taxonomic units (OTUs) of a) Fungi and b) Bacteria among all of the soil and plant treatments. Seedlings of *Silene acaulis* were grown in soil inocula from the two sample sites; adult plants were sampled from wild stock at each sample site. Different letters represent significant pairwise differences among treatments (Tukey HSD, $p < 0.05$).

PERMANOVA, $F_{1,6} = 2.41$, $P = 0.027$, Figure 4). For the seedlings grown in inoculated soils, there was a significant effect both of soil inoculum origin and seedling population origin on fungal (PERMANOVA, inoculum origin $F_{1,20} = 3.68$, $P = 0.001$; seedling origin $F_{1,20} = 1.52$, $P = 0.036$) and bacterial (PERMANOVA, inoculum origin $F_{1,19} = 2.06$, $P = 0.001$; seedling origin $F_{1,19} = 1.71$, $P = 0.007$) community composition (Figure 4). The top taxa driving differences in the root microbiome of seedlings grown in Colorado soil were members of the bacterial genera *Buchnera, Tumebacillus, Rhodopseudomonas, Flavobacterium* and *Brevibacterium* and members of the fungal genera *Humicola, Serendipita* and *Rhodotorula* (SIMPER, Table 2). The top taxa driving differences in the root microbiome of seedlings grown in Irish soil were members of the bacterial genera *Tumebacillus, Buchnera, Flavobacterium*, and *Duganella* and members of the fungal genera *Plenodomus* and *Schizothecium* and Exidiaceae family (SIMPER, Table 2).

Root fungal communities in seedlings were more dissimilar (i.e. more filtering) to soil fungal communities in native soils compared to foreign soils

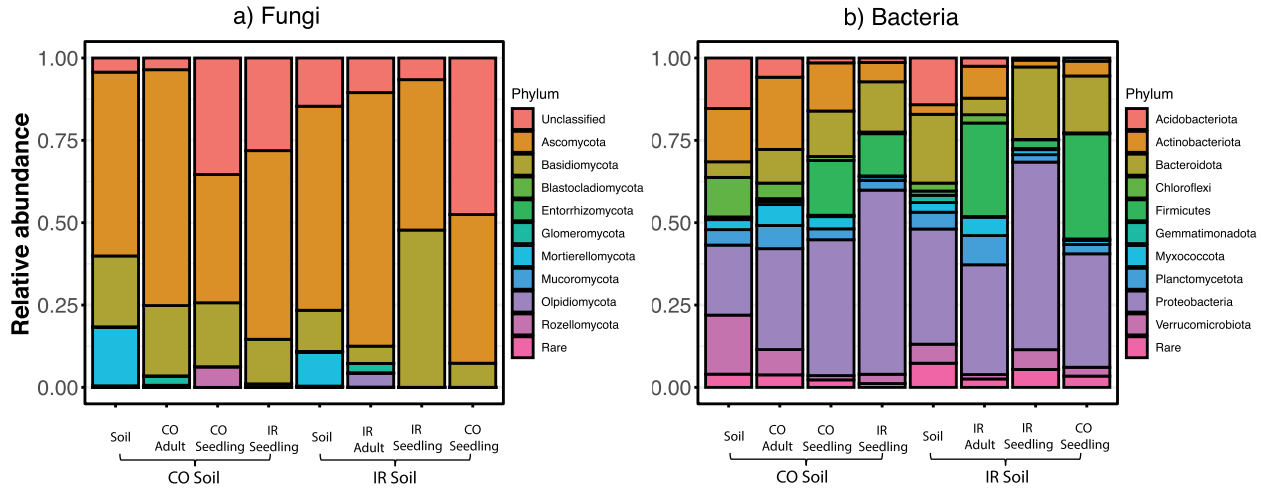

Figure 3. Relative abundances of the top 10 most abundant a) fungal phyla and b) bacterial phyla (with the 'Rare' category showing the sum of all other phyla not in the top 10) among all of the soil and plant (*Silene acaulis*) treatments.

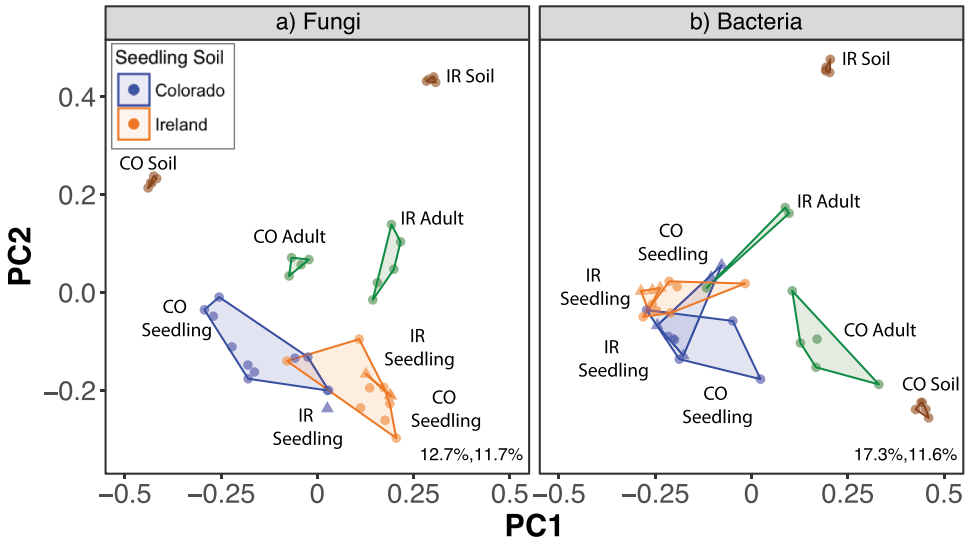

Figure 4. Principle coordinates analysis of a) Fungal community composition based on ITS DNA sequencing and b) Bacterial community composition based on 16S DNA sequencing. Numbers in the bottom right corner of each panel state the per cent variation explained by the first and second axes in each panel. Analysis was performed on Bray-Curtis dissimilarities, which were calculated on Hellinger-transformed relative sequence abundances at the OTU level. Note that the final sample sizes are less than 11 due to either not having enough root, or not passing the rarefaction cut-off. n = 5 for all soil samples. n = 5 for adult field-collected Colorado *Silene acaulis* plants and n = 3 for adult field-collected Irish plants. Irish *S. acaulis* seedling samples are shown as triangles to differentiate them from Colorado seedling samples.

(Wilcoxon test, $P < 0.001$, Figure 5a). This pattern held true for bacterial communities in Colorado plants (Wilcoxon test, $P < 0.001$, Figure 5b), however not for Ireland plants (Wilcoxon test, $P = 0.69$, Figure 5b). There were no significant effects of inoculum origin or seedling origin on the relative abundances or OTU richness of root fungal mutualists or root fungal pathogens among the treatments (ANOVA, $P > 0.05$, Figure S1), or in root colonisation by arbuscular mycorrhizal fungi or dark septate endophytes (ANOVA, $P > 0.05$, Figure S2).

Discussion

Differing responses of ecotypes to home and foreign soil inocula

The data presented indicate that the *Silene acaulis* ecotypes from Ireland (Europe) and Colorado (North America) respond differently when growing in their own versus each other's native soil inocula, and these responses are associated with significant differences in some, but not all, soil microbial associations. Both ecotypes had improved growth in at

Table 2. Similarity percentage analysis (SIMPER) of bacterial (16S) and fungal (ITS) communities in roots of *Silene acaulis* seedlings. Shown are the top 5 bacterial taxa and the top 5 fungal taxa in terms of their per cent contribution to community dissimilarity between the two plant root microbiomes in each soil (% Cont). Also shown is the cumulative percentage explained (Cum %), the OTU ID, the taxonomy from the SILVA and UNITE databases, which plant genotype the taxon was more abundant in, and the potential effect of the taxon on plant growth (Pos = Postive, Neg = Negative).

Soil	Plant with greater abundance	Potenial Effect	OTU ID	Kingdom	FunGuild	Phylum	Class	Order	Family	Genus	Species	% Cont	Cum %
Colorado	Colorado genotype	Neg	OTU_6	Bacteria		Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganellaceae	*Buchnera*		12.6	12.6
	Colorado genotype	Neg	OTU_9	Bacteria		Firmicutes	Bacilli	Alicyclobacillales	Alicyclobacillaceae	*Tumebacillus*		5.6	18.2
	Ireland genotype	Pos	OTU_10	Bacteria		Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Rhodopseudomonas*		4.6	22.8
	Colorado genotype	Neg	OTU_12	Bacteria		Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*		3.7	26.5
	Colorado genotype	Neg	OTU_179	Bacteria		Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	*Brevibacterium*		3.1	29.6
	Colorado genotype	Neg	OTU_18	Fungi	Undefined Saprotroph-Wood Saprotroph	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Humicola*	*olivacea*	8.8	8.8
	Colorado genotype	Neg	OTU_20	Fungi	Orchid Mycorrhizal	Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	*Serendipita*		5.5	14.3
	Colorado genotype	Neg	OTU_24	Fungi								4.7	19.0
	Colorado genotype	Neg	OTU_13	Fungi								4.7	23.7
	Colorado genotype	Neg	OTU_82	Fungi	Animal Endosymbiont-Animal Pathogen-Endophyte-Plant Pathogen-Undefined Saprotroph	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	*dairenensis*	4.5	28.2
Ireland	Colorado genotype	Pos	OTU_9	Bacteria		Firmicutes	Bacilli	Alicyclobacillales	Alicyclobacillaceae	*Tumebacillus*		12.8	12.8
	Ireland genotype	Neg	OTU_6	Bacteria		Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganellaceae	*Buchnera*		8.1	20.9
	Colorado genotype	Pos	OTU_12	Bacteria		Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*		4.0	24.9
	Ireland genotype	Neg	OTU_41	Bacteria		Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	*Duganella*		2.5	27.4
	Ireland genotype	Neg	OTU_38	Bacteria		Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae			2.5	29.9
	Ireland genotype	Neg	OTU_2	Fungi		Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Plenodomus*	*biglobosus*	10.6	10.6
	Colorado genotype	Pos	OTU_80	Fungi		Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium*	*curvuloides*	8.7	19.3
	Colorado genotype	Pos	OTU_13	Fungi								6.5	25.8
	Ireland genotype	Neg	OTU_3	Fungi	Endophyte-Undefined Saprotroph	Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae			6.1	31.9
	Colorado genotype	Pos	OTU_23	Fungi								11.2	37.0

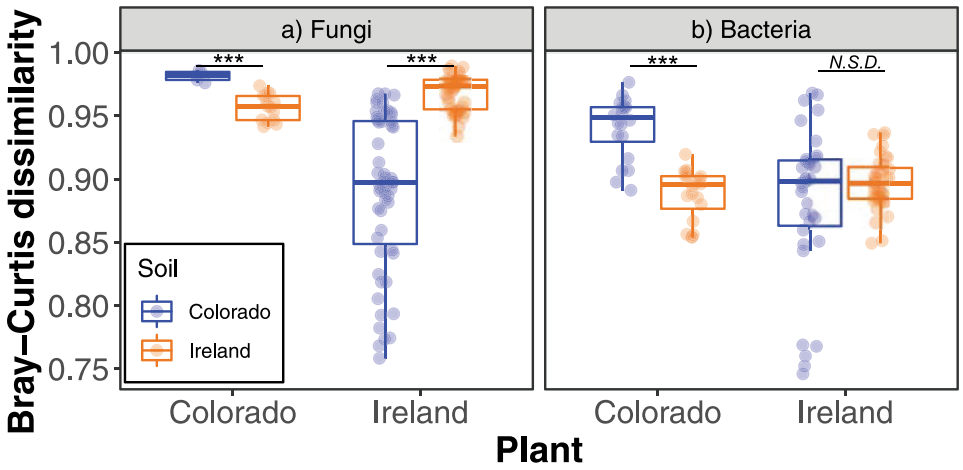

Figure 5. Bray-Curtis dissimilarities among the different *Silene acaulis* plants compared to the original soil communities they were grown in for b) Fungi and b) Bacteria. A Bray-Curtis dissimilarity of 1 means no shared taxa, and 0 means complete overlap in taxa. Thus, higher values signify a greater degree of filtering of soil microbes. ***Tukey HSD $p < 0.001$.

least one metric in foreign soil compared to home soil, in agreement with the general predictions of the enemy-release hypothesis. Overall, recruited root microbial communities differ markedly from communities in the raw soil inocula (Figure 5), and the gross microbial community on seedling roots in all four treatments are more similar to each other than to either inoculum (Figure 3). This is likely due to selective recruitment and filtering of root-associated taxa from within the bulk soil microbial pool as well as different functional groups (e.g. symbionts versus saprotrophs) predominating in roots versus bulk soils (Hirsch and Mauchline 2012; Gaiero et al. 2013; Lareen et al. 2016).

Within the seedling populations, root microbial communities are defined both by the soil inoculum and the seedling ecotype, indicating that recruitment of microbes to seedling roots is a function both of the starting microbial pool in the soil inoculum, and the genetic identity of the developing seedling, in agreement with previous studies of a wide variety of plants (Bouffaud et al. 2014; Edwards et al. 2015; Fitzpatrick et al. 2018; Sasse et al. 2018).

The data from mature adult plant roots collected at both field sites show that these plants host a greater microbial diversity and are more similar to their respective host site soils than any of the seedling cohorts grown in the greenhouse with inocula (Figure 4). This implies that total microbial recruitment is likely to be affected by plant age and duration of exposure to the host soil microbiome as others have noted (Wallander et al. 2010; Chaparro et al. 2014; Lakshmanan 2015; Edwards et al. 2018), or alternatively, by greenhouse growing conditions. The differences recorded in adult (field) root microbial populations are likely derived both from gross patterns of soil diversity at the field sites, as well as to the conditioning of the local soils by the two different ecotypes, as has been shown for two *Silene acaulis* subspecies in the Alps (Roy et al. 2018).

Filtering of root microbial associations

In terms of soil-root microbial associations across the four seedling/soil combinations, the principal difference between treatments is in the filtering of fungal and, to a lesser degree, bacterial taxa (i.e. the degree of dissimilarity between the root and bulk soil microbial communities). Plant filtering of soil microbial communities can be defined as the combination of active and passive selection pressure that results in a subset of microbes in the soils being found in plant roots.

Both Colorado and Irish plants had significantly less filtering of root fungal taxa when growing in the foreign soil inoculum compared to the home type, resulting in more similar community composition (Figure 5a). Interestingly, the difference in filtering is driven by overall community composition (Figure 5a), not richness (Figure 2a). The main fungal taxa driving differences in seedling root microbiome community composition in Colorado soils (SIMPER Analysis, Table 2) were members of the *Humicola, Serendipita*, and *Rhodotorula* genera, which could have contributed to negative growth responses in home soil. However, other species of *Humicola* have been isolated from plant roots but were not pathogenic (Menzies et al. 1998) and other

species of *Serendipita* are likely to be beneficial for plants (Osman et al. 2020). Also, although some *Rhodotorula* species can be opportunistic pathogens of animals (Wirth and Goldani 2012), all known plant-associated *Rhodotorula* species are beneficial endophytes that promote plant growth by producing auxin (indole-3-acetic acid), suppressing pathogens, and by harbouring N-fixing bacteria (Sen et al. 2019 and references cited therein). The main fungal taxa driving differences in seedling roots in Irish soils were *Plenodomus* and *Schizothecium*. *Plenodomus biglobosus* is a known pathogen known to cause disease in oilseed rape and could have contributed to diminished growth of Irish seedlings in Irish soils (Bagi et al. 2020). *Schizothecium curvuloides* is typically known as a coprophilous fungus, although the genus *Schizothecium* has also been reported from rhizosphere samples where it is associated with variable effects on plant growth (Franke-Whittle et al. 2015; Qiu et al. 2020).

For root bacterial communities in the seedling cohorts, the level of taxonomic overlap with source soil inocula is greater than for fungi (Figure 5b), but the decreased bacterial filtering for seedlings growing in foreign compared to home soil inoculum was only observed in the Colorado plant ecotype, not the Ireland ecotype. The consistent bacterial drivers of differences in seedling root microbiome community composition in both soils were members of the *Buchnera, Tumebacillus*, and *Flavobacterium* genera (Table 2). *Buchnera* are known symbionts of aphids (Douglas 1998), and *Flavobacterium* are commonly found in cold regions but typically in aquatic environments (Bernardet and Bowman 2006); there is no information to date, however, on the effects of these taxa on plants. *Tumebacillus* is a potentially beneficial genus as it has been associated with suppression of banana *Fusarium* wilt disease (Shen et al. 2015); however, in the dataset an OTU from this genus was more abundant in Colorado seedlings than Ireland seedlings in both soils.

Two putatively beneficial microbial OTUs, from *Duganella* and *Rhodopseudomonas*, respectively, display quite different association patterns across the treatments. The *Duganella* OTU contributed to dissimilarity between the root microbiomes in Irish soil, with higher relative abundances in the Irish plant genotype. *Duganella* are nitrogen fixers and have also been shown to produce indole-3-acetic acid which can induce a plant growth response (Fang et al. 2019); thus, our result is contrary to what might be expected, as Irish plants actually showed better growth in Colorado soil compared to Ireland soil. Alternatively, the occurrence of *Rhodopseudomonas* OTU, which have been shown to promote plant growth in other plant species (Lee et al. 2008; Wong et al. 2014) are more in line with the growth pattern results, as these were more abundant in Irish plants in Colorado soil.

Overall, we find a clear association between reduced filtering of fungal symbionts in foreign versus home soil (Figure 5a), and a corresponding growth benefit in terms of either total biomass (for Irish ecotypes in Colorado soil, Figure 1a) or growth stature (for Colorado ecotypes in Irish soil, Figure 1c). Reduced filtering of bacterial symbionts (Figure 5b) is also associated with differences in growth stature for Colorado ecotypes, but not with differences in biomass recorded for Irish ecotypes.

These functional differences between the two ecotypes can be considered in the context of their phylogeographic origins. As confirmed by DNA analysis, the sampled ecotypes belong to known *S. acaulis* genetic identities in each locality, which in turn are associated with regional population refugia that date to the mid- to late-Pleistocene in Europe and North America (Gussarova et al. 2015). In addition to prolonged reproductive separation extending back to at least 20,000 years before present, the two populations experience different selective environments. At the Ireland site, which is amongst the mildest known for *Silene acaulis* across its biogeographic range, the abiotic environment is relatively benign, with cold temperatures (4–8°C) predominating in winter, and freezing conditions (−5°C to 4°C) occurring much less frequently. Snow cover when present is intermittent and short-lived. Soil at the site is shallow but features a mature organic matter content and a more neutral pH compared to the Colorado site. Surface vegetation at the site is mostly continuous, with intermittent bare soil and gravel, and comprises a relatively species-rich upland grassland community, maintained by low-level sheep grazing. The Niwot Ridge site is more typical for *S. acaulis* populations in alpine habitats at lower latitudes, occurring at high elevation, experiencing freezing winter air conditions with prolonged snow cover, and characterised by poor soil organic development and fragmented vegetation cover.

Fitness benefit for foreign ecotypes

Native and foreign plant genotypes are known to interact differently with native soil microbial

communities (Inderjit 2010). In many cases, where local plant genotypes experience negative feedbacks when growing in local soil microbiomes, foreign genotypes experience neutral, or less often, positive feedbacks. For example, enhanced recruitment of – and receptivity to recruitment of – local AMF fungal types is indicated to improve competitive fitness in foreign invasive species versus native plant types (Zhang et al. 2017). However, these kinds of differences are also subject to an exposure continuum, and in time foreign genotypes are known to lose this soil microbial competitive advantage (Diez et al. 2010).

While our results are in line with a growth dividend predicted by the enemy-release hypothesis, our data do not directly support the hypothesis, as we find no evidence that fungal pathogen abundances or richness are greater in the home soils compared to the foreign soils. However, in terms of OTUs that drive differences in the recorded root microbiomes of home versus foreign seedlings in the two soil types (SIMPER Analysis, Table 2), it is notable 14 of the most abundant fungal and bacterial taxa have putative negative host impacts on the home ecotype. On the other hand, while the remaining six taxa in the SIMPER analysis have putatively beneficial impacts on the foreign ecotype, there is no evidence in our data of fungal mutualists being in general more common in the foreign soil. In this context, successful annotation of our sample data into broad pathogen and mutualist groups is limited; for example, FUNguild only assigned guilds to 733 or 3852 OTUs, for bacterial and fungal data, respectively, in our dataset. Thus, it remains that the observed growth response in seedlings could be driven by soil bacteria or by other fungal taxa that were not successfully assigned to any functional guild.

Conclusions

In the context of these overall patterns, the enhanced growth benefit combined with reduced filtering of fungal mutualists for foreign *S. acaulis* seedlings is broadly in keeping with observed responses for other plant species over a range of field and experimental settings. At the same time, the responses observed in the present study arise from a comparison of genotypes and soils from highly divergent habitats, and the patterns reflect interactions in the extreme case. Additional work should examine these interactions at more intermediate distances, focused on local ecotypes that are likely to come into contact due to predicted climate change impacts. Representative sampling of local soil microbiomes will be crucial in this context, both to take account of local variability, and to capture local low-elevation/southerly aspect extremes where warmer-temperature soil communities may already be present. These represent potential future soil communities at these sites.

In relation to the emerging pressure for climate-mediated redistribution of species genotypes (Jump and Peñuelas 2005; Thuiller et al. 2008; Gray and Hamann 2013), our results show that foreign seedlings can potentially experience a net neutral to positive feedback response from native soil microbial communities located elsewhere within the range of the species – especially, as is the case with *S. acaulis*, where accelerated seedling growth is associated with recruitment into the stable adult population (Doak and Morris 2010). For this reason, it seems likely that modelling of biodiversity responses to climate change would benefit from more detailed evaluation of local scale transplant analysis, as positive soil microbial feedbacks may have an important role to play in facilitating within-range migration of genotypes.

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Disclosure statement

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