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REGULAR ARTICLE

Litter-driven feedbacks influence plant colonization of a high elevation early successional ecosystem

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Abstract
Aims Plant-microbe interactions are crucial components of ecosystem development but are understudied during early succession. The goal of this study was to investigate species-specific effects of plants on unvegetated soils being colonized by plants as climate changes, and assess how plant-soil feedbacks influence plant succession.
Methods We used lab and field litter additions in an early successional unvegetated ecosystem in the Front Range of the Colorado Rocky Mountains to examine litter-driven changes in soil bacterial and fungal communities. We then used plant litter-trained soil as inocula in a greenhouse experiment to test plant-soil feedbacks.
Results We found species-specific effects of litter additions on bacterial and fungal communities in unvegetated soils, which are likely due to both differences in tissue litter chemistry and differences in the litter microbiome. We identified a negative effect of soil trained by litter from the conservative forb *Silene acaulis* on the growth of the fast-growing bunchgrass *Deschampsia cespitosa*, likely due to changes in microbial communities that resulted in lowered nitrification rates or to a litter-driven increase in N-immobilization.
Conclusions Our study demonstrates the importance of plant specificity and potential negative litter-driven feedbacks in primary succession, which could lead to patchy distribution of plant colonists as climate change allows colonization of these areas.

Keywords Plant-microbe interactions · Succession · Plant-soil feedback · Litter

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Introduction

Plant-microbe interactions are important drivers of broad vegetation patterns in ecosystems, affecting plant species composition, abundance, and diversity patterns (van der Heijden et al. 2008). Further, plant-soil feedbacks via the microbial community, whereby plants affect microbial communities and microbial communities feedback and affect plants, also have broad implications for plant community assembly (Reynolds et al. 2003; Bever et al. 2010; van der Putten et al. 2013). However, plant-soil feedbacks and plant-microbe interactions have been understudied in early successional environments, particularly those with non-leguminous plants (but see van der Putten et al. 1988, 1993), a context in which they could exert even more influence on plant community composition. Furthermore, while it is well known that plant species can have differential

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effects on soil microbial communities (Grayston et al. 1998; Kowalchuk et al. 2002; Berg and Smalla 2009; Knelman et al. 2012; Leff et al. 2018), it is possible that in extremely carbon-limited environments such as unvegetated soils, any carbon input from plants, regardless of type or quality, could overwhelm any plant species-specific effects (Tscherko et al. 2005). The goal of the present study was to determine how the litter of different colonizer plants affects soil microbial communities, and how this feeds back to affect plant growth.

Ecosystem development begins with the rapid colonization of exposed substrates by microbial communities, which catalyzes the early stages of soil development and nutrient cycling prior to plant arrival (Nemergut et al. 2007; Schmidt et al. 2008a; Sattin et al. 2010). Studies of microbial communities in early successional environments have shown that they can be limited by carbon (King et al. 2008; Bueno de Mesquita et al. 2017), though nitrogen and phosphorus may also be limiting or co-limiting (King et al. 2008; Knelman et al. 2014; Schmidt et al. 2016; Darcy et al. 2018). Furthermore, microbes in unvegetated soils are more carbon limited than microbes in nearby vegetated soils (Porazinska et al. 2018). As plants arrive, they directly interact with soil microbial communities via litter inputs and root exudates (Bardgett et al. 2005), as well as by potentially carrying new microbes with them on their seeds (Schiltz et al. 2015). This can lead to shifts in microbial community composition compared to unvegetated soils, with decreases in phototrophs and nitrogen-fixers and increases in overall biomass, plant-associated taxa, heterotrophs, r-selected bacteria, and fungal to bacteria ratios (Ohtonen et al. 1999; Edwards et al. 2006; Miniaci et al. 2007; Knelman et al. 2012, 2018). While previous studies on the effects of multiple colonizer plants have found species-specific effects on soil bacterial communities (Knelman et al. 2012, 2018), we lack a mechanistic understanding of bacterial and fungal community responses to plants with different litter traits and how that feeds back on plant growth.

Plant-soil feedbacks in primary succession have been reported as being positive (plants alter microbes in ways that benefit themselves or other plants), whereas in other contexts they are typically reported as negative (Reynolds et al. 2003; van der Putten et al. 2013). This could be due to a bias of a focus on interactions with nitrogen-fixing bacteria in primary succession (Chapin et al. 1994; Titus and Del Moral 1998; Corti et al. 2002), whereby early colonizer plants associate with nitrogen-fixers and alleviate nutrient limitation for plants arriving later. Yet plants that associate with nitrogen fixers are not always the first colonizers (Cázares et al. 2005), and the nature of plant-soil feedbacks in primary succession without nitrogen-fixers remains unclear. Work in a successional sand dune ecosystem demonstrated positive overall effects of the microbes trained by early successional plant species on later successional plant species, but not due to direct positive effects. Species-specific pathogens increased in the early colonizer plant causing a negative intraspecific feedback, while having neutral effects on later-arriving plants (van der Putten et al. 1988, 1990, 1993; van der Putten and Troelstra 1990).

Indeed, pathogen buildup can be a key mechanism for negative intraspecific plant-soil feedbacks, and if the pathogens are host-specific, this may not necessarily translate into a negative interspecific feedback (Reynolds et al. 2003). On the other hand, positive intraspecific and interspecific feedbacks can occur when there is a buildup of mutualists, including mycorrhizal fungi in addition to nitrogen-fixing bacteria, but the nature of these feedbacks is also dependent on how host-specific the mutualisms are (Bever 2002; Reynolds et al. 2003). Another important mechanism of plant-soil feedbacks through the microbial community is mediated through microbes responsible for nutrient cycling that are not necessarily symbionts with plants (van der Putten et al. 2016). For example, feedbacks can be driven by plant litter from conservative plant species that slows down nutrient cycling that would otherwise benefit faster growing plant species (Suding et al. 2008).

Here we used litter additions and inocula collections to analyze the effects of plant litter on unvegetated soil microbial communities, and how this feeds back to affect plant growth. We hypothesized that 1) due to differences in tissue chemistry, the litter of different plant species would have different decomposition rates and house different microbial communities, 2) differences in decomposition rates and leaf microbial communities would cause species-specific effects on soil microbial communities and processes, with increases in species-specific pathogens, generalist mutualists, and nitrogen cycling rates, and 3) changes in soil microbial communities and processes would then feed back on plant growth, with negative intraspecific feedbacks due to pathogen buildup, and positive interspecific feedbacks due to increases in mutualists and nitrogen cycling rates.

Materials and methods

Study system

We collected plant seeds, leaf litter, and soil from a high elevation talus-field subnival ecosystem (3900 m.a.s.l.) on the southeast facing slope of Navajo Peak 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. Average precipitation from 1952 to 2012 was 1090 ± 230 mm $yr.^{-1}$, with a 60 mm $yr.^{-1}$ increase over that time period, driven mostly by increases in winter precipitation (Kittel et al. 2015). Recent mean annual temperatures (2011–2014) range from −4 °C to −7 °C, while mean daily summer temperatures range from 4 °C to 10 °C (Losleben 2017). Both annual and summer temperatures have been increasing over the last several decades (McGuire et al. 2012; Bueno de Mesquita et al. 2018) leading to increased positive degree days (Caine 2010) earlier lake ice-off dates (Preston et al. 2016), and earlier snowmelt (Bueno de Mesquita et al. 2018). Over the last several decades, 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. 2018) or dominated by moss (Bueno de Mesquita et al. 2017). We chose three focal plant species that are abundant at the highest elevation plant communities at our site and likely colonizers of unvegetated soils as climate changes – the generalist bunchgrass *Deschampsia cespitosa* ((L.) P. Beauv.), the arctic/alpine cushion plant *Silene acaulis* ((L.) Jacq.), and the talus specialist *Oxyria digyna* ((L.) Hill). Litter was collected in September 2017 from abundant populations of these three species found near Green Lake 4 on the floor of the valley (3550 m elev.), downslope of the litterbag addition experimental site. Litter (~20 g of each species) from multiple individual plants was clipped, placed in a paper bag and air-dried and stored at room temperature until it was used in the experiment. Foliar C:N ratios of these species were measured in summer 2017. Leaves of individuals of the three species were collected at peak biomass from Niwot Ridge, dried for 48 h at 60 °C, ground into a fine powder, and total C and total N were measured on an elemental analyzer. While we recognize that other leaf traits are important for decomposition (e.g. lignin and phenolic content), we use foliar C:N to exemplify broad differences in tissue chemistry among our focal species.

Litter decomposition and effects on microbes

We assessed litter decomposition rates in the lab and the field and the effects of litter additions on unvegetated soil bacterial and fungal communities. In the lab, we measured microbial decomposition and activity, while the field also incorporates mass loss by photodegradation. In the lab, 100 ml (~145 g) unvegetated soil was placed into 1 L air tight mason jars and watered to 90% water holding capacity to simulate conditions during decomposition following snowmelt. Two quarts of unvegetated soil were collected from the field site in the fall of 2017, and stored at 4 °C for 1 week until being placed in the jars. The starting soil was homogenized in a two-gallon Ziploc bag and subsampled five times for DNA sequencing to determine the initial microbial community composition. Then 1 g of each species' litter was added onto the soil surface. There were five replicate jars for each species litter and five control jars with no litter added. Jars were incubated for five weeks at 10 °C. Jars lids were fitted with rubber stoppers with two valves. Twice a week, CO_2 measurements were taken with an EGM-4 CO_2 analyzer (PP Systems, Amesbury, MA) by attaching the analyzer to the valves, opening them, and measuring for approximately 30 s. Once a week, after a second weekly CO_2 measurement was taken, the headspace in the jars was cleared to prevent CO_2 buildup and re-equilibrate with the atmosphere. At the end of five weeks, soils were sampled from each jar and stored at −20 °C until DNA extraction.

In the field, 1 g of litter of each species was placed in mesh litter bags, with five replicate bags per species and five control bags with no litter. Bags were placed on top of unvegetated soils on October 17, 2017 and staked to the ground with small plastic stakes. Bags were then collected on September 6, 2018 and biomass was dried and weighed. Soils were collected from immediately underneath each bag, placed in sterile bags, transported to the lab on ice, subsampled for DNA extraction (subsample stored at −20 °C), and then used as inoculum in a greenhouse experiment (see below).

Plant-soil feedback experiment

We added the live inoculum collected from under the litter bags to a 50:50 mixture of sterile bulk tundra and sand at a ratio of 1:30 inoculum to sterile bulk soil. Ammonium and nitrate levels in the four inocula were

not significantly different and were overwhelmed by the nutrient pools in the bulk soil. Inorganic nitrogen levels per gram of dry soil were on average 29 times higher in the sterile bulk soil than the inocula. Furthermore, because all of the inocula types were collected from a 2 × 2 m area, differences in other soil properties should be minimal. Bulk soil was sterilized one gallon at a time by autoclaving for 1 h at 121 °C, remixing the soil, and autoclaving again. We grew each of the three species in 10 replicate pots in four different treatments: control, inocula trained with their own litter, and inocula trained with the litter of the other two species (3 spp. × 4 treatments × 10 replicates = 120 pots). We planted 5 seeds per pot, but then thinned to one individual per pot once a true leaf formed on one of the individuals. Germination was recorded as any cotyledon that emerged out of the soil. Any plant that emerged but then lost pigmentation and moisture was considered dead. Pots were 8.255 cm wide × 8.255 cm long × 9.525 cm tall. Pots were filled with the sterile bulk soil until ~2 cm from the top, then a layer of inoculum was spread, and then 1 cm of sterile bulk soil was added on top of the inoculum. This minimized cross contamination and ensured that roots passed through a layer of inoculum. Pots were watered every day or every other day depending on solar radiation. Plants grew from September to December in the alpine room of the University of Colorado greenhouse, with natural light conditions and diurnal temperature cycles ranging from 15 °C during the day to 10 °C at night.

At the end of the experiment, aboveground biomass was clipped, dried for 48 h at 60 °C, and weighed, and roots were collected for staining and microscopy. To assess the quantity of fine root endophytes, dark septate endophytes, and arbuscular mycorrhizal fungi, roots were cleared in 10% KOH for 1 h at 90 °C, reacidified in 1% HCl for 20 min, and stained overnight in acidic glycerol trypan blue (modified from Koske and Gemma 1989, Schmidt et al. 2008b). In the morning, roots were destained in acidic glycerol and stored at 4 °C until microscopy. Microscopy was done according to McGonigle et al. (1990). Briefly, ~20 cm of roots were placed horizontally on slides and passes were made up and down the slide at random intervals such that 100 intersections between the root and ocular crosshair were observed. The presence or absence of a fungal structure was recorded at each intersection, and percent colonization was calculated.

Lastly, we assessed net nitrogen mineralization and nitrification rates in the soils after the greenhouse experiment. After harvesting the plants, the soils in the 40 pots with *Deschampsia* growing in them were placed in quart sized Ziploc bags and allowed to incubate in the greenhouse for 25 days. We focused on *Deschampsia* because of its high sample size and to investigate mechanisms of growth differences seen in the greenhouse harvest. 5 g of soil was sampled at the start and end of a 25-day incubation in the greenhouse, and inorganic nitrogen pools were extracted by shaking in 25 ml of 2 M KCl for 1 h at 125 rpm. Extracts were analyzed on an OI Analytical Flow Solution 3000 Flow Injection Analyzer at the Colorado State University Soil, Water, and Plant Testing Laboratory.

Microbial analyses

We sequenced the 16S and ITS regions of the genome on an Illumina MiSeq to describe bacterial and fungal communities, respectively, of plant litter, initial unvegetated soils, soils after the lab incubations, and soils after the field litterbag additions. DNA was extracted from 0.25 g of soil using the Qiagen PowerSoil Kit (QIAGEN, Hilden, Germany) and from 0.3 g of dry plant litter using the Qiagen DNeasy Kit following the manufacturer's protocols. Litter was first frozen in liquid nitrogen and ground into a powder with a sterile mortar and pestle. Extracted DNA was amplified via Polymerase Chain Reaction (PCR), using the 515F/806R primers for 16S (Fierer et al. 2012) and ITS1F/ITS2 primers for ITS (White et al. 1990) following the methods of the Earth Microbiome Project (Amaral-Zettler et al. 2009; Bellemain et al. 2010; Caporaso et al. 2012). The amplified DNA was then normalized with the SequalPrep Normalization Kit (Invitrogen Inc., Carlsbad, California, USA), and sequenced (paired-end 2 × 150 bp for 16S, 2 × 250 bp for ITS) at the BioFrontiers Next Generation Sequencing Facility (Boulder, Colorado, USA). Sequencing data were processed with the QIIME (Caporaso et al. 2010) and UPARSE (Edgar 2013) pipelines to demultiplex, merge, quality filter, remove singletons and select operational taxonomic units (OTUs) at 97% similarity, and remove chimeras. OTU tables (except litter 16S) were rarefied before analysis so all samples within each sample type had the same sequencing depth (Supplementary Table 1). Taxonomy was assigned using the Greengenes (DeSantis et al. 2006) and UNITE (Abarenkov et al. 2010) databases for bacteria and fungi,

respectively. Fungal functional guilds were assigned using the program FUNguild and included both highly probably and less certain assignments (Nguyen et al. 2016). Plant pathogens were only considered plant pathogens if that was their only guild assignment. Representative sequences for OTUs in this paper can be found in GenBank BioProject PRJNA529920. All other data will be available on the Niwot Ridge LTER database (http://niwot.colorado.edu).

Statistical analyses

All analyses were performed with the software R, version 3.4.4 (R Core Team 2018). To test the first hypothesis about litter chemistry, decomposition, and the litter microbiome, we first used analysis of variance (ANOVA) to test for a plant species effect on tissue C:N ratios, followed by the Tukey post-hoc for pairwise comparisons (functions aov and TukeyHSD, R package 'stats', R Core Team 2018). Cumulative carbon dioxide production in the lab was analyzed with a repeated measures ANOVA model (function gls, R package 'nlme', Pinheiro et al. 2014; and function Anova, R package 'car', Fox and Weisberg 2011) with plant species as a predictor variable, followed by a Tukey post-hoc test (function glht, R package 'multcomp', Hothorn et al. 2008). Decomposition in the field (litter mass loss) was also tested with ANOVA followed by Tukey post-hoc. Microbial communities were visualized with Principle Coordinates Analysis and differences between plant species were assessed with permutational multivariate analysis of variance (PerMANOVA, Anderson 2001, function adonis, R package 'vegan', Oksanen et al. 2013) on Bray-Curtis dissimilarity matrices calculated from Hellinger-transformed relative abundances. Following a significant result, we conducted pairwise PerMANOVAs (function pairwise.perm.manova, R package 'RVAideMemoire', Hervé 2019). To determine the taxa driving pairwise differences, we used similarity percentage (SIMPER) analysis, implemented in the 'vegan' package.

For the second hypothesis about the response of the soil microbiome and nitrogen cycling, we used PerMANOVA to test for shifts in soil communities among the four treatments (three plant litter addition types and control) and ANOVA to test for differences in mineralization and nitrification rates. To further examine changes in microbial communities, we used ANOVA to test for differences in relative abundances of fungal pathogens, mutualists, selected bacterial taxa (dominant groups and those involved in nitrogen cycling), and endophyte root colonization.

To test the third hypothesis about plant-soil feedbacks, we used ANOVA followed by Tukey post-hoc to test for the effect of inoculum type on plant biomass. Binary response variables (germination and survival) were analyzed with logistic regression models (R function glm with family = "binomial"), with inoculum as a predictor variable. Due to low germination of *Oxyria* and *Silene*, we were unable to calculate accurate pairwise interaction coefficients, which are commonly used to describe plant-soil feedbacks (Bever et al. 1997). In the case where the assumptions of ANOVA were not met, we used the nonparametric Kruskal-Wallis test (function kruskal.test) followed by Nemenyi post-hoc tests (function posthoc.kruskal.nemenyi.test, R package 'PMCMR', Pohlert 2014).

Results

Plant litter

Fundamental to our first hypothesis is the idea that the plant species had different litter tissue chemistry. Indeed, we found that plant species had significantly different foliar C:N ratios (Kruskal-Wallis, $\chi^2 = 39.54$, $df = 2$, $p < 0.001$), with significant or marginally significant pairwise differences in foliar C:N ratios among all three plant species (Nemenyi posthoc, $p < 0.06$, Fig. 1a). *Oxyria* had the lowest foliar C:N ratio followed by *Silene* and then *Deschampsia* (Fig. 1a).

As predicted in the first hypothesis, the three species' litter also had significantly different decomposition rates, as evidenced by both CO_2 production in the lab (Repeated Measures ANOVA, $\chi^2 = 256.36$, $p < 0.001$) and biomass loss in the field (ANOVA, $F = 9.94$, $p = 0.002$). Differences in CO_2 production by microbial decomposers were driven by significantly higher production in soils with *Oxyria* litter compared to the other species and unvegetated soils, and higher production in *Deschampsia* and *Silene* compared to unvegetated soils (Tukey post-hoc, $p < 0.05$ Fig. 1b). Differences in mass loss in the field were driven by significantly higher decomposition of *Oxyria* and *Silene* litter compared to *Deschampsia* litter (Tukey post-hoc, $p < 0.05$, Fig. 1c).

There were also significant differences in the bacterial (PerMANOVA, $F = 6.03$, $p = 0.001$, Fig. 2a) and

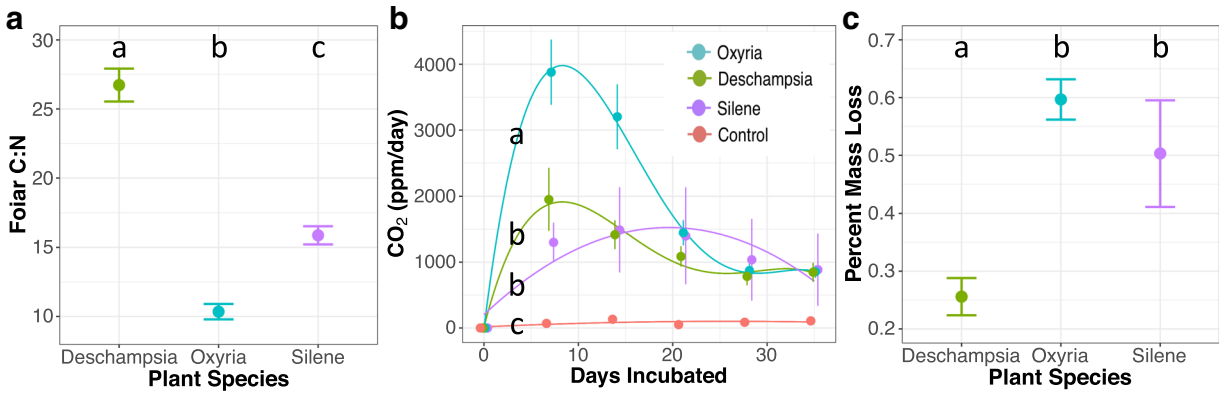

Fig. 1 Litter chemistry and decomposition. **a** Mean (± SE) foliar C:N ratios for the three species, **b** mean (± SE) decomposition rates of the three species' litter as measured by microbial respiration in a lab incubation and **c** mean (± SE) decomposition of the three species' litter as measured by mass loss after one year in the field. Lines in panel B were fit with polynomial functions. As CO_2 was measured weekly and headspace cleared each week, we calculated the rate per day for each of the five weeks of the incubation. Different letters in each panel represent significant pairwise differences (Tukey or Nemenyi post-hoc, $p < 0.05$)

fungal (PerMANOVA, F = 10.986, p = 0.001, Fig. 2b) litter microbiomes among the three plant species, with all pairwise comparisons significantly different (Pairwise PerMANOVA, p < 0.05, Supplementary Table 2).

Soil microbes and processes

As predicted by our second hypothesis, there were species-specific effects on soil microbial communities and processes. After 35 days incubation in the lab, plant litter had significant effects on soil bacterial (PerMANOVA, F = 2.42, $p = 0.001$, Fig. 3a) and fungal (PerMANOVA, F = 3.07, p = 0.001, Fig. 3b) communities, with all pairwise comparisons among the different plant litters significant (Pairwise PerMANOVA, $p < 0.05$). After 11 months in the field, there was no significant effect of the litterbag additions on unvegetated soil bacterial communities (PerMANOVA, F = 0.92, $p = 0.54$, Fig. 3c), but there was a significant effect on fungal communities (PerMANOVA, F = 1.62, p = 0.001, Fig. 3d) and all pairwise comparisons were significant (Pairwise PerMANOVA, p < 0.05) except for *Silene*-trained soils and controls (Supplementary Table 3).

In the lab, four of the nine top fungal taxa contributing to differences in litter-trained soil compared to control soil - the ectomycorrhizal/saprotroph *Sistotrema semanderi* (Di Marino et al. 2008), the endophyte *Neostagonospora elegiae* (Yang et al. 2016), and two taxa from the potentially pathogenic Ceratobasidiaceae (Sweetingham et al. 2009) - were sourced from the litter (Table 1). However, this was not the case in the field,

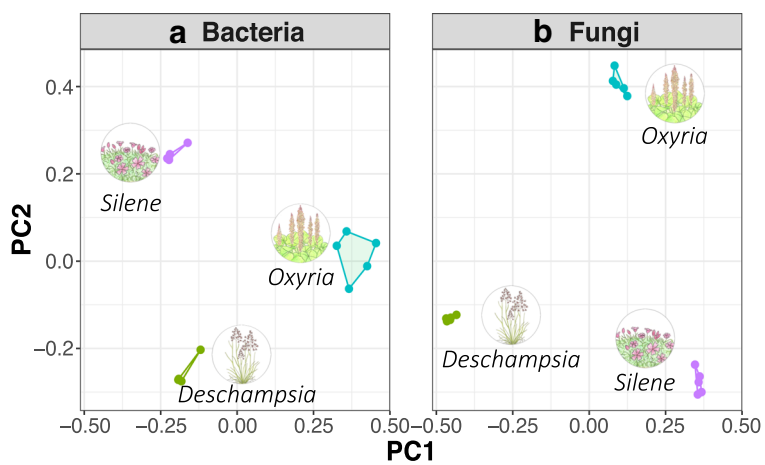

Fig. 2 Litter **a** bacterial and **b** fungal communities for the three plant species ($n = 5$ samples per species). All of the microbial communities among the species were significantly different from each other (Pairwise PerMANOVA, $p < 0.05$). Variation explained by the axes are **a**) 32.34% and 18.44%, **b**) 36.88% and 27.96%

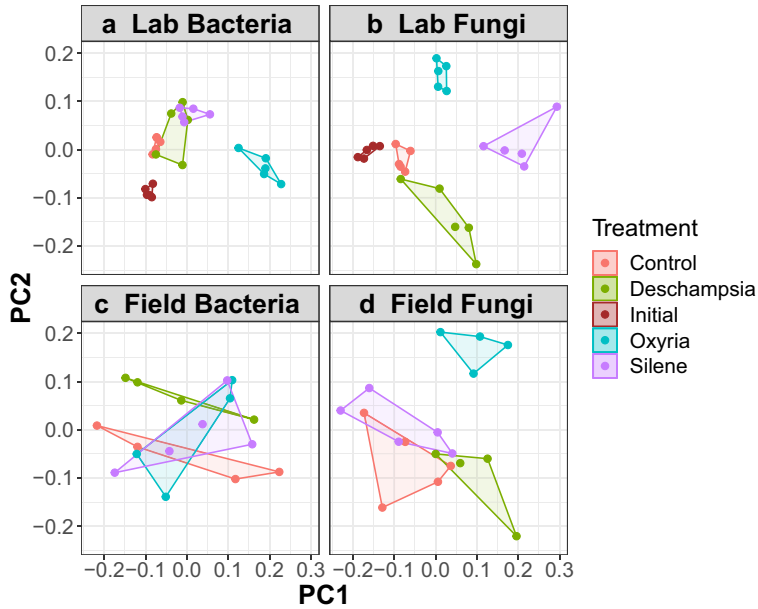

Fig. 3 Principle coordinates analysis of Bray-Curtis dissimilarities for **a** soil bacterial communities after the lab litter incubations **b** soil fungal communities after the lab litter incubations **c** soil bacterial communities from under litter bags after the field experiment, and **d** soil fungal communities from under litter bags after the field experiment. For the lab experiment, controls were soils that were incubated with no litter added. For the field experiment, controls were soils collected from under empty litterbags. For the lab experiment, we sequenced the "Initial" unvegetated soil before adding it to the jars. Analyses were done separately for each panel. Variation explained by the axes are **a**) 18.48% and 7.15%, **b**) 17.08% and 10.67%, **c**) 31.56% and 10.95%, and **d**) 11.66% and 11.19%

and there were no litter-derived bacteria among the top nine contributors to soil bacterial community dissimilarities (Table 1).

Despite changes in community composition, there were no changes in the pathogenic and mutualist portions of the fungal community, in contradiction of our predictions. There were no significant differences in fungal pathogen relative abundance or richness among the four treatments used as inocula (Kruskal-Wallis, $\chi^2 = 5.15$, $p = 0.16$, Fig. 4a) and pathogens only made up 1% of the fungal community on average. Fungal pathogen richness was not significantly different among the treatments (ANOVA, $F = 2.117$, $p = 0.114$), though litter-trained soils typically contained 2–3 more pathogenic taxa on average. There was one pathogenic fungal taxon, *Taphrina tormentillae*, which has been reported as a pathogen in other alpine plants (Petrýdesová et al. 2016), that was found in all five *Silene* trained inocula and in no other inocula.

Arbuscular mycorrhizal fungi were not detected in the sequencing data, while dark septate endophytes were detected in low abundance (0.3% on average), but did not differ by treatment (Kruskal-Wallis, $\chi^2 = 1.71$, $p > 0.05$). Root colonization of plants grown in the greenhouse by arbuscular mycorrhizal fungi, dark septate endophytes, and fine root endophytes was low and there were no significant differences in root colonization among the four treatments (ANOVA or Kruskal-Wallis, $p > 0.05$, Fig. 4b). For some of the major bacterial groups, including phototrophs, N-fixers, and plant-associated taxa, there were no significant differences in relative abundances among the treatments (ANOVA, $p > 0.05$), except for Betaproteobacteria, which were significantly lower in *Silene*-trained soil than in *Deschampsia*-trained soils, and Bacteroidetes, which were significantly lower in *Silene*-trained soil than in *Deschampsia*- and *Oxyria*-trained soils (ANOVA, $p < 0.05$, Supplementary Fig. 3). Actinobacteria and Nitrospirae abundances tended to be higher in litter-trained soils than unvegetated control soils (Supplementary Fig. 3).

With regard to nitrogen cycling, the prediction of increased cycling rates was only partially supported by the data. Nitrogen mineralization rates did not significantly differ among the four treatments (Kruskal-Wallis, $\chi^2 = 1.15$, $p = 0.76$). On the other hand, there was a

Table 1 Similarity percentage analysis (SIMPER) of bacterial and archaeal (16S) and fungal (ITS) communities in soils after litter decomposition in the lab and in the field compared to controls

Data	Pair	OTU ID	Litter	Kingdom	Phylum	Class	Order	Family	Genus	Species	% Cont.	Cum. %
Lab	Con-Des	OTU_14	N	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	DA101		2.30	2.30
		OTU_17	N	Bacteria	AD3	ABS-6					1.19	3.49
		OTU_16	N	Bacteria	Gemmatimonadetes	Gemmatimonadetes	N1423WL				0.94	4.43
		OTU_3	Y	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Incertae sedis	Sistotrema	semanderi	12.50	12.50
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	hyalina	8.78	21.28
		OTU_31	Y	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Neostagonospora	elegiae	5.18	26.46
	Con-Oxy	OTU_51	N	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Mucilagnibacter	gracilis	6.10	6.10
		OTU_31	N	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium		2.83	8.93
		OTU_67	N	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylophilus*		2.47	11.40
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	hyalina	8.46	8.46
		OTU_12	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	hyalina	8.35	16.81
		OTU_38	N	Fungi	Ascomycota	Leotiomycetes	Helotiales				6.98	23.79
	Con-Sil	OTU_14	N	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	DA101		1.72	1.72
		OTU_19	N	Bacteria	Acidobacteria	iii1–8	DS-18				1.72	3.44
		OTU_17	N	Bacteria	AD3	ABS-6					1.59	5.03
		OTU_5	Y	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae			19.09	19.09
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	hyalina	11.19	30.28
		OTU_3073	Y	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae			7.72	38.00
Field	Con-Des	OTU_2	N	Bacteria	Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Phormidium		5.53	5.53
		OTU_1	N	Bacteria	Gemmatimonadetes	Gemmatimonadetes	N1423WL				2.36	7.89
		OTU_4	N	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	Nitrososphaera	SCA1145	2.09	9.98
		OTU_2	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	horticola	8.47	8.47
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	camargensis	7.68	16.15
		OTU_24	N	Fungi	Entomophthoromycota	Basidiobolomycetes	Basidiobolales	Basidiobolaceae	Basidiobolus	magnus	6.56	22.71
	Con-Oxy	OTU_2	N	Bacteria	Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Phormidium		4.87	4.87
		OTU_1	N	Bacteria	Gemmatimonadetes	Gemmatimonadetes	N1423WL				2.42	7.29
		OTU_13	N	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	DA101		2.02	9.31
		OTU_5	N	Fungi	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Tetracladium		15.79	15.79
		OTU_2	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	horticola	8.73	24.52
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	camargensis	5.96	30.48
	Con-Sil	OTU_2	N	Bacteria	Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Phormidium		5.04	5.04
		OTU_1	N	Bacteria	Gemmatimonadetes	Gemmatimonadetes	N1423WL				2.81	7.85
		OTU_13	N	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	DA101		2.01	9.86
		OTU_2	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	horticola	9.73	9.73

Table 1 (continued)

Data	Pair	OTU ID	Litter	Kingdom	Phylum	Class	Order	Family	Genus	Species	% Cont.	Cum. %
		OTU_1	N	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	camargensis	7.02	16.75
		OTU_14	N	Fungi	Ascomycota	Leotiomycetes	Helotiales	Leotiaceae			5.06	21.81

Shown are the top three taxa contributing to community dissimilarity, their percent contribution to dissimilarity, the cumulative percent dissimilarities, and whether or not the taxon was sourced from the litter (Y = yes, N = no). Taxonomy was assigned by the Greengenes and UNITE databases

marginally significant treatment effect on nitrification rates (Kruskal-Wallis, $\chi^2 = 7.44$, $p = 0.06$), driven by marginally significant differences between the *Silene* trained soil and both *Deschampsia* trained soil and controls (Nemenyi post-hoc, $p < 0.1$, Fig. 5). However, nitrification rates had no significant effect on *Deschampsia* biomass (Linear Regression, df = 38, $p = 0.59$).

Plant-soil feedbacks

Results from the plant-soil feedback contradicted our third hypothesis. Instead of negative intraspecific feedbacks and positive interspecific feedbacks, we identified one negative interspecific feedback. There was a significant negative effect of inoculum on *Deschampsia* biomass (Kruskal-Wallis, $\chi^2 = 8.81$, $p = 0.03$, Fig. 6a), with significantly lower biomass in *Silene*-trained soil compared to *Oxyria*-trained soil, and marginally significantly lower biomass in *Silene*-trained soil compared to control soils. *Deschampsia* had the highest germination rates, while both *Oxyria* and *Silene* had low germination rates. There were no significant differences in germination or survival among the inoculum treatments for any species (Logistic regression Tukey post-hoc $p > 0.05$, Supplementary Figs. 1 and 2). There were no significant differences in *Oxyria* or *Silene* biomass among the four treatments (Fig. 6b and c), though it is important to note that replication was decreased due to low germination, and individuals that did germinate experienced low growth rates.

Discussion

Our results demonstrate the species-specific effects of plant litter on unvegetated soil microbial communities, particularly fungi, and that these effects can lead to negative interspecific plant-soil feedbacks. These feedbacks are potentially mediated by microorganisms responsible for nitrification, or some as yet unknown mechanism. We do not find strong evidence for other feedback mechanisms driven by pathogens or mutualists.

Plant litter

Foliar C:N ratios were partially related to decomposition and soil microbiome response, but not litter microbiome structure, which only partially supports our first hypothesis. *Oxyria* had the lowest C:N ratio and the highest

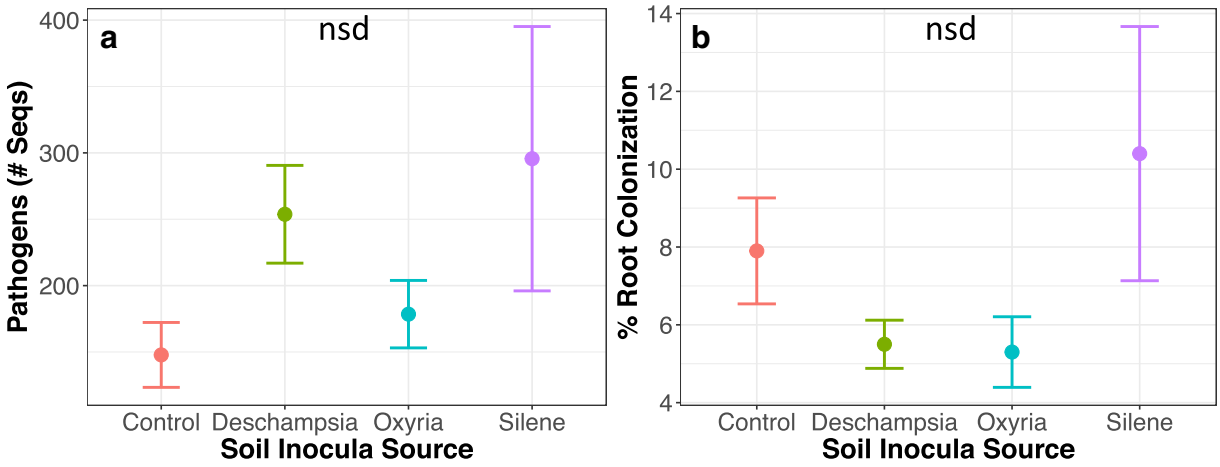

Fig. 4 **a** Number of sequences (out of 16,227) classified as plant pathogens by FUNGuild and **b** Combined percent root colonization of *Deschampsia cespitosa* roots by arbuscular mycorrhizal fungi, fine root endophytes, and dark septate endophytes after growth in the greenhouse

decomposition rates compared to *Deschampsia* and *Silene*, which is consistent with the paradigm that tissue with lower C:N ratios decomposes faster (Enríquez et al. 1993). *Silene* had higher decomposition than *Deschampsia* in the field but not the lab, which could be an artifact of a relatively greater magnitude of increase in lab decomposition compared to the field decomposition in the high C:N *Deschampsia* compared to *Silene*. Furthermore, *Silene* litterbags contained the smallest litter fragments, which could have been lost from the bags and inflated decomposition rates in the field. Another potential factor driving the difference between the lab and field results is photodegradation, which in some systems accounts for a large portion of decomposition (Austin and Vivanco 2006), but the rate of photodegradation of each species' litter is unknown. Tissue C:N was not strongly related to the litter microbiome, as all three plant species showed approximately equal pairwise separation, despite *Oxyria* and *Silene* being closer in foliar C:N. It is possible that the litter microbiome structure was driven more by differences in other plant traits such as surface structure and area, stomata traits, cuticle wax, and leaf age (Vorholt 2012).

Soil microbes and processes

Differences in litter chemistry can select for certain microbes in the original soils leading to changes in the microbial communities of the litter-trained soil (Leff et al. 2018). In the lab, the species with the highest C:N, *Deschampsia*, was consistently more similar to

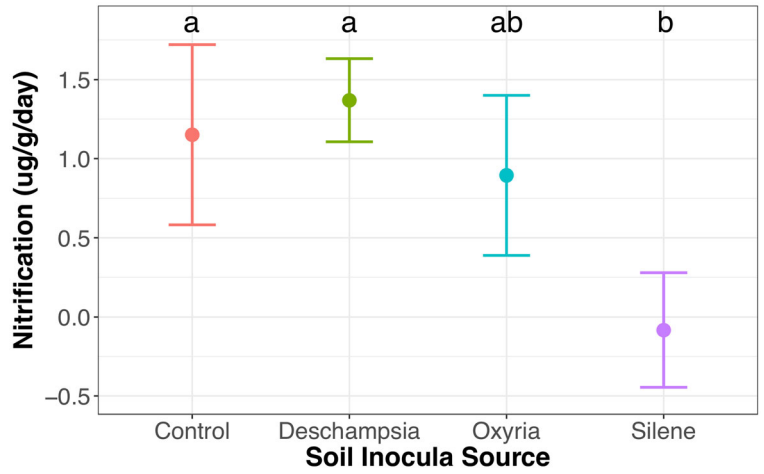

Fig. 5 Mean (±SE) net nitrification rates during a 25-day greenhouse incubation of soils from all pots with *Deschampsia* plants ($n = 40$). Different letters represent marginally significant pairwise differences (Nemenyi post-hoc, $p < 0.1$). Negative values represent net consumption of nitrate

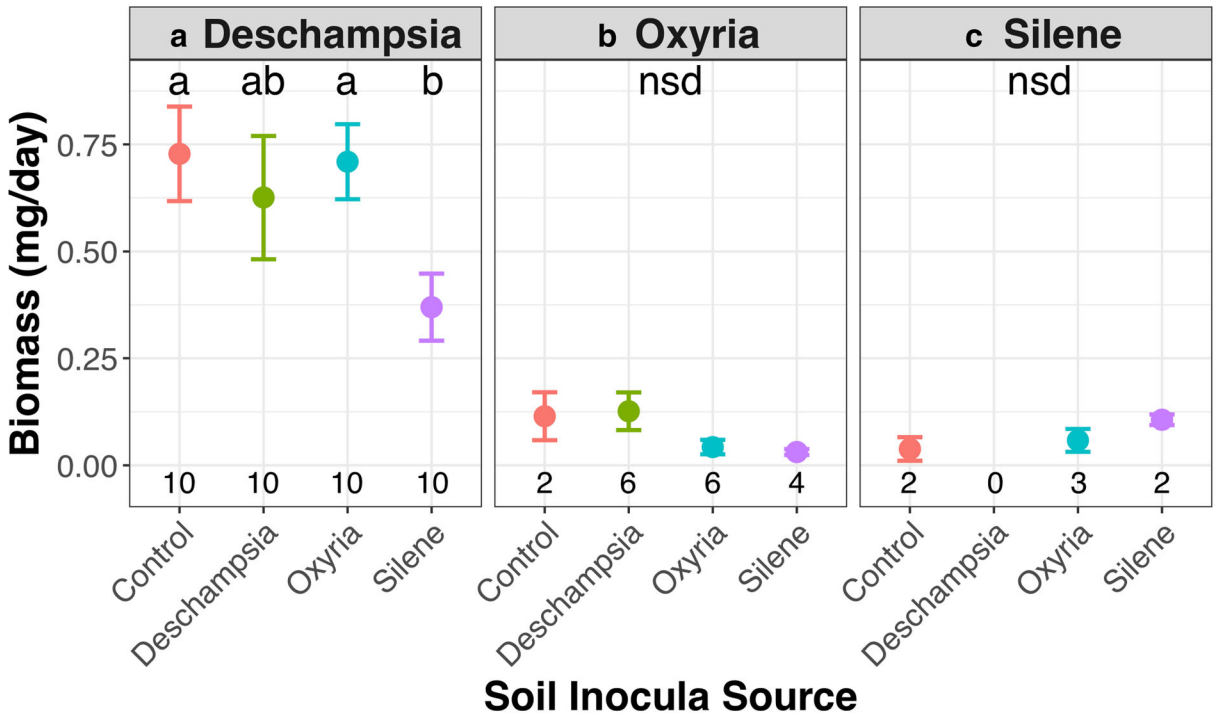

Fig. 6 Mean (±SE) biomass growth rates for **a** *Deschampsia cespitosa* **b** *Oxyria digyna* and **c** *Silene acaulis*. Small numbers at the bottom of the graphs are the final sample sizes for each treatment. Different letters show significant or marginally significant differences from the Nemenyi post-hoc test ($p < 0.06$)

controls than *Oxyria* and *Silene*, but this did not hold true in the field. In the field, where decomposition is slower than in the lab, *Silene*-trained soils also clustered with *Deschampsia*-trained soils, while the lowest C:N species, *Oxyria*, showed the greatest separation in community structure, perhaps because its faster decomposition contributed more inputs to the soil. Additionally, under controlled conditions, differences in litter fungal composition can further contribute to differences in microbial community composition in litter-trained soil, but this effect is diminished in the field. This stronger influence of litter fungi may also partially explain why fungal communities shifted more than bacterial communities, and sequencing depth was much greater for ITS (mean = 110,781) than 16S (mean = 8713) in the litter samples. Interestingly, differences in nitrification were driven more by species type than foliar C:N, suggesting that other leaf traits not measured here could be important. *Silene*-trained soils had the lowest nitrification rates despite *Deschampsia* having the highest C:N ratio, but this result is in line with the conservative growth strategy of *Silene* compared to the fast-growing nature of *Deschampsia* (Suding et al. 2008).

Plant-soil feedbacks

Our hypothesis of pathogen-mediated negative intraspecific feedbacks and mutualist-mediated positive interspecific feedbacks was not supported by the data. Our results suggest that in this legume-free system, the roles of pathogens and mutualists may be relatively less important than saprotrophs and free-living taxa that are driving nutrient cycling rates. Furthermore, poorly-developed, oligotrophic, unvegetated soils are not favorable for pathogenic or mutualistic taxa that rely on plants, and are dominated by phototrophs that fix their own carbon or heterotrophs that survive off of small sources of aeolian-deposited carbon (Freeman et al. 2009a, b; Mladenov et al. 2012). Indeed, in the unvegetated soils studied here, fungal pathogens and mutualists represented a small percentage of the microbial community (~1%), as did known plant-associated bacteria. In early successional ecosystems such as ours, time can play an important role in determining microbial communities, and it could take a couple of years for pathogens to build up and affect plant growth (van der Putten et al. 1993). This idea is consistent with work

done on plant-soil feedbacks later in succession in more nutrient-rich conditions, which has shown that soil-borne pathogens play an important role (Kardol et al. 2006; van der Putten et al. 2013). Later in succession, mutualists can also play a more important role as they increase in biomass (Miller 1979; Janos 1980; van der Putten et al. 2013).

Our results also contradicted our hypothesis that litter additions would drive positive plant-soil feedbacks via nutrient cycling. Generally, in unvegetated or other carbon-limited soils, the addition of plant litter should cause the growth of saprotrophs ultimately releasing nutrients from the litter, which would then have a positive effect on plant growth. However, *Silene acaulis* is a slow-growing and long-lived plant, which would benefit from slower nutrient cycling rates if in competition with a fast-growing species (Berendse 1998). Indeed, previous work at our site on another more conservative forb (*Geum rossii*), compared to the fast-growing bunchgrass *Deschampsia*, found slower rates of nitrogen cycling in soils associated with the conservative species (Suding et al. 2008), as was found elsewhere in heathlands (Berendse 1990). Our results show that *Silene* litter can slow nitrogen cycling rates, or perhaps increase nitrogen immobilization (Fig. 5) since we measured net rates rather than gross rates (Fisk et al. 1998), and this can have negative impacts on other plants, particularly fast-growing species like *Deschampsia*. This finding agrees with the idea that the type of plant, either conservative or fast-growing, can impact the nature of plant-soil feedbacks (Kardol et al. 2006). Here, we find that the conservative species, *Silene*, to have negative soil effects that could affect faster growing species like *Deschampsia.*

Ultimately, as plants colonize unvegetated soils and ecosystems develop, these types of plant-soil feedbacks can determine the establishment of certain plants and the ultimate plant community composition (Bever et al. 2010; van der Putten et al. 2013). At our site, several different plant species are colonizing previously unvegetated soils (Bueno de Mesquita et al. 2017, 2018), and priority effects mediated by plant-soil feedbacks could be important. For example, if *Silene* establishes in a particular location, it could potentially limit *Deschampsia* colonization there. While we suggest that this was mediated through nitrogen cycling, we cannot rule out potential allelopathic effects of *Silene* litter chemical compounds on *Deschampsia*, which is an avenue for future research. Allelopathy is another mechanism of plant-soil feedback that is difficult to separate from other mechanisms (Lau et al. 2008; van der Putten et al. 2013), although there is evidence of allelopathy by both root and litter-sourced compounds (Padhy et al. 2000; Bais et al. 2003; Callaway and Ridenour 2004; Vivanco et al. 2004; Inderjit et al. 2011). At broader scales in the context of global change and species invasions, plant-microbe interactions are a particularly important type of biotic interaction that can mediate plant success as plants shift their ranges either as invaders (Keane and Crawley 2002; Mitchell and Power 2003; Van Grunsven et al. 2007; Morriën and van der Putten 2013; Yang et al. 2013) or native plants tracking suitable climate (Engelkes et al. 2008; van der Putten et al. 2010, 2016; van der Putten 2012). Future work needs to continue addressing feedbacks in these contexts at the community level, incorporate the effects of root exudates on microbial communities, and study temporal gradients in succession to capture unvegetated to recently vegetated to well developed and vegetated soils.

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