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RESEARCH ARTICLE

Root traits and soil micro-organisms as drivers of plant–soil feedbacks within the sub-arctic tundra meadow

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Abstract

1. Plant–soil feedback (PSF) results from the influence of plants on the composition and abundance of various taxa and functional groups of soil micro-organisms, and their reciprocal effects on the plants. However, little is understood about the importance of fine root traits and root economic strategies in moderating microbial-driven PSF.
2. We examined the relationships between PSF and 11 chemical and morphological root traits from 18 sub-arctic meadow plant species, as well as the soil microbial community composition which we characterized using phospholipid fatty acids (PLFAs) and high-throughput sequencing. We also investigated the importance of the root economics spectrum in influencing PSF, because it indicates plant below-ground economic strategies via trade-offs between resource acquisition and conservation.
3. When we considered the entire root economics spectrum, we found that PSFs were more negative when root trait values were more acquisitive across the 18 species. In addition, PSF was more negative when values of root nitrogen content and root forks per root length were higher, and more positive when root dry matter content was higher. We additionally identified two fungal orders that were negatively related to PSF. However, we found no evidence that root traits influenced PSF through its relationship with these fungal orders.
4. *Synthesis*. Our results provide evidence that for some fine root traits, the root economics spectrum and some fungal orders have an important role in influencing PSF. By investigating the roles of soil micro-organisms and fine root traits in driving PSF, this study enables us to better understand root trait–microbial linkages across species and therefore offers new insights about the mechanisms that underpin PSFs and ultimately plant community assembly.

KEYWORDS

arctic, fine root traits, functional ecology, fungi, plant–soil feedback, root economics spectrum, tundra ecosystems

1 | INTRODUCTION

Plants influence the composition and abundance of antagonistic and mutualistic soil micro-organisms, and depending on the net outcome these effects may result in positive, negative or neutral plant–soil feedbacks (PSFs; Bever et al., 2012; Gundale & Kardol, 2021). Despite the increasing number of PSF studies, little is still understood about the importance of plant functional traits and their relationship with soil micro-organisms that drive PSFs. On the one hand, plant functional traits influence the composition and abundance of various groups of soil micro-organisms (Li et al., 2018), including mycorrhizal fungi (Eissenstat et al., 2015) and pathogens (Tomova et al., 2005). On the other hand, soil pathogens and mycorrhizal fungi influence the direction and strength of PSF (Semchenko et al., 2018). Leaf functional traits, such as specific leaf area and leaf dry matter content, have been identified as important drivers of PSF (Baxendale et al., 2014). For example, leaf litter may indirectly influence PSF (Veen, Fry, et al., 2019), by building up specific decomposer communities (Veen, Snoek, et al., 2019) that then influence the release of plant-available nutrients. However, fine root traits may be more important for predicting PSF, because fine roots grow in direct association with, or in close proximity to, soil micro-organisms. However, we know little about the role of fine root traits in driving PSFs, with recent studies focusing on only a few fine root traits such as specific root length and root nitrogen content (Cortois et al., 2016; Semchenko et al., 2018).

Fine root traits are increasingly being examined within the framework of the fine root economics spectrum (RES; Freschet et al., 2010). Here, acquisitive root trait values (e.g. high specific root length and specific root nitrogen content) indicate low-resource investment in defence compounds, but higher investment into fast nutrient acquisition (Cortois et al., 2016). In contrast, conservative root trait values (e.g. high root carbon to nitrogen ratios and thicker root diameters) indicate well-defended and slow-growing tissue, but with a stronger dependence on mycorrhizal fungi for nutrient uptake (Cortois et al., 2016; Eissenstat et al., 2015). However, it has recently been shown that fine root traits are multi-dimensional and that there are multiple gradients of the RES (Bergmann et al., 2020). For example, we previously found that the chemical axis but not the morphological axis of the RES was important for predicting the abundance of broad soil microbial groups, though neither axis could explain the relative abundance of fungal guilds, in sub-arctic tundra (Spitzer, Lindahl, et al., 2021). Nevertheless, the importance of these trait axes as predictors of PSF direction and strength has not been tested.

Apart from fine root traits, the outcome of PSF may be determined by the micro-organisms associated with fine roots (Bever et al., 2012). Previous research on PSF has often treated micro-organisms as a 'black box', but in recent years, high-throughput sequencing techniques have opened up new possibilities for understanding the role of soil microbial composition in predicting PSF using soil functional guilds. For example, Semchenko et al. (2018) showed that higher taxonomic richness of soil pathogens resulted in negative biotic PSF, while higher richness of arbuscular mycorrhizal (AM) fungi led to positive biotic PSF. However, the strong focus on very broad functional guilds overlooks the potential of the composition or identity of microbial taxa within those guilds in driving PSF (Bever et al., 2012). For example, individual pathogenic fungal species can determine the outcome of PSF of a single tree species (Bell et al., 2006). In addition, individual AM fungal taxa may have asymmetric resource exchange with closely related plant species (Kiers et al., 2011), with some taxa being associated with negative PSF and others with positive PSF (Bever, 2002). Therefore, to gain a better understanding of the role of soil microbial community composition on PSF, it is critical to examine responses and effects of various microbial taxa. However, PSF depends on specificity between plant species and microbial taxa (Bever et al., 2010, 2012; Mills & Bever, 1998) and it is unlikely that the same fungal species would be related to PSF across a large number of plant species (Liu et al., 2012). Therefore, systematic relationships of PSFs between plant species and fungal taxa are arguably best analysed at a higher taxonomic level than at the fungal species level, for example at the fungal order level.

Here, we focus on understanding the roles of fine root traits, soil micro-organisms and their interplay in explaining PSF in sub-arctic tundra meadow. We have recently found that there is a strong association between certain fine root traits and microbial taxa in this cold-climate ecosystem; for example, root carbon content is associated with high AM fungal abundance (Spitzer, Lindahl, et al., 2021). However, whether and to what extent these relationships influence PSF direction and strength is not known. Furthermore, there is a paucity of research on the drivers of PSF within arctic ecosystems. Arctic tundra plants allocate a large proportion of biomass belowground (Iversen et al., 2015), and are commonly nutrient limited (Sundqvist et al., 2014). In addition, it has been suggested that arctic plants have evolved to have a higher nutrient uptake capacity per root biomass relative to temperate plant species, to compensate for low nutrient availability (Chapin III, 1974). Therefore, acquisitive morphological root traits that are associated with increased fine root absorptive surface area have the potential to be important for predicting PSF strength in arctic ecosystems.

We conducted glasshouse studies to test the effects of 11 fine root traits, and the RES, as well as the abundances of various groups of soil micro-organisms in predicting the direction and strength of PSF across 18 sub-arctic meadow plant species, that is, graminoids and forbs (but not dwarf shrubs, which typically do not occur in sub-arctic meadows). Soil microbial communities were characterized at the level of broad microbial groups (by phospholipid fatty acids, PLFAs) and at the level of fungal functional guilds and orders (through high-throughput sequencing). We tested the following hypotheses: (a) The fine root economics spectrum will predict PSF direction and strength, with more conservative trait values being associated with positive PSF. In addition, we aimed to identify which individual fine root traits were the best predictors of PSF strength and direction. (b) At the level of broad microbial groups, plants that elevate bacterial to fungal ratios are likely to experience positive PSF. This is because higher bacterial abundances relative to fungi are

associated with faster nutrient turnover (Wardle et al., 2004) and because the abundance of mycorrhizal fungi (which would generate positive feedbacks) is low in the sub-arctic tundra (Spitzer, Lindahl, et al., 2021). (c) At the level of finer fungal taxonomic resolution, plants that elevate the abundance of AM and opportunistic fungal orders (i.e. yeasts and moulds) will likely have positive PSF, whereas those that elevate the abundance of pathogens will likely have negative PSF. This is because the abundance of both AM and opportunistic fungi can positively influence plant growth (Botha, 2011; Smith & Smith, 2011). In addition, we determined which fungal taxa that were promoted by plants are the best predictors of PSF direction and strength. Testing these hypotheses in combination allows us to determine the importance of fine root trait–microbial relationships for predicting PSF, and will advance our understanding about the mechanisms underpinning differences in PSF among coexisting plant species.

2 | MATERIALS AND METHODS

2.1 | Study system

We sourced soils and most of the seeds for our study from a sub-arctic meadow at the foothills of Mount Vassitjåkka in northern Sweden, approximately 27 km north-east of Abisko (68°21′N 18°49′E) at 700 m above sea level. The sub-arctic meadow, which consists of graminoids and forbs, is one of the two main vegetation types in the sub-arctic tundra, the other being heath vegetation. Dwarf shrubs are very common in heath vegetation, but not in meadow vegetation, and hence were not included in this study. The soil we used is classified as cryorthents (Darmody et al., 2000). The mean annual precipitation at the sampling location was 340 mm during 2005–2017 and the mean temperature for the corresponding period was +13°C in July and −9.9°C in January, as measured by the Swedish Meteorological and Hydrological Institute.

2.2 | Soil and seed collection and seed germination

We collected meadow soil at the end of the growing season in August 2016 when root production is at its peak (Blume-Werry et al., 2016), from an area of approximately 200 m^2. Soil was collected from the rooting zone (=upper 10 cm) of several individuals of each graminoid and forb species present by excavating plants and shaking the soil directly into large plastic bags. The soil was transported to Umeå in coolers and stored at 4°C until the start of the experiment in February 2017. We bulked and homogenized all the soil prior to the beginning of the experiment. The soil properties at the beginning of the experiment were as follows: moisture = 39%/g dry soil; pH = 5.87; NH_4^+ = 1.80 mg/g dry soil; NO_3^+ = 8.02 mg/g dry soil and PO_4^+ < 0.000 mg/g dry soil. We collected seeds from 11 meadow plant species (i.e. 4 graminoids species and 7 forbs) from the Abisko area in August 2016, and sourced seeds from a local seed company (Pratensis, Lönashult, Sweden) for 7 additional plant species (i.e. 3 graminoids species and 4 forbs), resulting in 18 species in total (Table S1). The seed company sources seeds from a variety of locations throughout Sweden, cultivated or collected in the wild. All plant species except *Dryas octopetala* (which has ectomycorrhizal associations) have arbuscular mycorrhizal associations (Soudzilovskaia et al., 2020). We surface sterilized the seeds by inundation in 1% sodium hypoclorite for 1 min (De Long et al., 2015) and germinated them on sterilized sand. Germinated seedlings were stored at 4°C with light (50%) to slow their growth when they had reached an approximate height of 3 cm so that seedlings were at a similar ontogenic stage at the time of planting.

2.3 | Plant–soil feedback experiment

We conducted a two-phased PSF experiment (i.e. a conditioning phase and a feedback phase; Figure S1) using soil collected from our study system and seeds collected from our study system or sourced from a local seed company. The soil was homogenized after sieving (10 mm mesh size) to remove stones and large roots, and the volume split equally into five blocks. Autoclaved sand was then mixed with the soil from each block (soil:sand ratio of 3:1) to facilitate better drainage. Prior to planting, we allowed the soil biotic community to acclimatize to greenhouse conditions by leaving the soil at room temperature for 2 days at 20°C. Pots (1.4 L; 9 × 9 × 9 cm) were filled with 0.4 L of warm-water-washed gravel followed by the homogenized bulk soil.

2.3.1 | Conditioning phase

For the conditioning phase, two seedlings from each of the 18 plant species were planted in the same pot (top left and bottom right) in February 2017. Duplicate pots for each species were set up in each of the five blocks to ensure large enough volumes of conspecific soils (Figure S1) for the second (feedback) phase of the experiment. This resulted in 18 species × 5 blocks × 2 duplicates = 180 pots. Following planting, plants were grown for a period of 12 weeks in a glasshouse (18°C/13°C day/night temperature; 80% humidity and 18/6 hr light/dark regime). This time period roughly corresponds to the length of one growing season in the Abisko area. At the end of the experiment, plants were stored at 4°C until time of harvest, and the experiment was harvested block by block over a period of 3 weeks. The two duplicate pots for each species in each block were harvested together over an aluminium foil-lined tray. The plant shoots were clipped, and plant roots were then manually loosened from the soil and scissors were used to cut fine roots (<1 mm diameter) into smaller sections (approximately 1 cm) to enable later homogenization into the soil. Large roots (>1 mm diameter) were discarded. For each species in each block, the soil and fine roots from the two duplicate pots were homogenized in a plastic bag by shaking (2 min) and then split

in half by weighing. Soil in one bag was used as the conspecific soil for planting in the feedback phase and the other was used for creating heterospecific soil for the other plant species in the experiment. The soil was stored at 4°C for 2 weeks until it was mixed and used in the feedback phase.

2.3.2 | Feedback phase

Prior to the feedback phase, surface-sterilized seeds of the 18 species were germinated on sand as described above. The soil from one of the duplicate pots for each species within each block was used as conspecific soil in the feedback phase, and heterospecific soil for each species per block was created by homogenizing equal quantities of soil (by fresh weight) from each of the other seventeen plant species in that block (Figure S1). This resulted in a total of 1 L of conspecific and 1 L of heterospecific soil per plant species per block. After homogenization, each of the conspecific and heterospecific soils were placed in the glasshouse to acclimatize for 2 days before planting for the feedback phase. Similar to the conditioning phase, warm-water-washed gravel (0.4 L) was first placed into the plant pots (9 × 9 × 20 cm), followed by either conspecific or heterospecific soil for each plant species (1 L). This resulted in 180 experimental units [18 species × 5 blocks × 2 soil treatments (heterospecific or conspecific)], each with two seedlings. The plants were placed in the greenhouse under the same conditions as in the conditioning phase and with the same watering regime. After 10 weeks, the experiment was harvested block wise, with paired conspecific and heterospecific pots being harvested on the same day. After removal from the pots, plants roots were washed over a 4 mm sieve placed above a 1 mm sieve to capture any broken roots during the process. Any fresh roots found in the sieve after washing were weighed and split equally between the two plants from that pot. Thereafter, we dried the larger of the two plants in each pot to calculate PSF and to reduce intraspecific variability (60°C for 2 days). We used the dry weights of the plants from the conspecific and heterospecific soil treatments to calculate PSF for each species in each block, using the formula: log (plant biomass in conspecific soil) − log (plant biomass in heterospecific soil; Pernilla Brinkman et al., 2010).

2.4 | Fine root traits and soil microbial community

Fine root trait data and soil microbial community data for the same 18 plant species were obtained from a separate experiment (Spitzer, Lindahl, et al., 2021) conducted simultaneously within the same greenhouse and, therefore, under the exact same climatic conditions as the current feedback study. Hence, the microbial and root trait data were not derived from the soil used in the feedback study, but instead from other pots treated in the same way. In addition, the field-collected soil and seeds in both experiments were collected at the same time and processed in the same way. Briefly, in the study by Spitzer, Lindahl, et al. (2021), 11 fine root traits were measured from each of 21 plant species (including the 18 used in the present study) in 5 replicate blocks and both rhizosphere and bulk soil was collected from each experimental unit for PLFA analysis and high-throughput sequencing (for details, see Spitzer, Lindahl, et al., 2021). The following fine root traits were measured: carbon content (%); nitrogen content (%); carbon:nitrogen ratio; total phenol content (mg/g); phenol:nitrogen ratio (mg/g); average diameter (mm); dry matter content (dry mass per unit fresh mass; mg/mg); forks per root length (forks/cm); specific root tip abundance (tips/mg); specific root area (cm^2/mg); and specific root length (cm^2/mg). For methodological details on root trait measurements, see Spitzer, Lindahl, et al. (2021). In the present study, data from 18 (Table S1) of the 21 plant species were used, and the average trait values for each of these 18 plant species are given in Table S2. In addition, we used the PLFA and high-throughput sequencing data from both rhizosphere and bulk soil collected from the experiment reported by Spitzer, Wardle, et al. (2021), although only the rhizosphere data from that experiment were reported in Spitzer, Wardle, et al. (2021).

Detailed methods on DNA extractions, PCRs, high-throughput sequencing and sequence annotations of the soil fungal community, as well as PLFA analyses, are described in Spitzer, Lindahl, et al. (2021). Briefly, we extracted DNA from 300 ± 10 mg of freeze-dried rhizosphere or bulk soil and assessed fungal community composition by high-throughput sequencing of amplified ITS2 markers (Clemmensen et al., 2016). Rhizosphere soil in our study was defined as soil adhering to plant roots after gentle shaking, while bulk soil was soil that easily fell away when removing plants from the pots or while shaking. After conducting polymerase chain reactions (PCRs) and amplicon purification, amplicon sequencing was performed using the Pacific Biosciences Sequel Technology Platform at the SciLifeLab, Uppsala, Sweden. Sequences were processed and clustered into Species Hypotheses (SHs; Kõljalg et al., 2013) using the SCATA pipeline (Ihrmark et al., 2012). Fungal sequence reads were standardized among samples by proportional transformation. Here, we focused on the 100 most abundant SHs obtained after sequence annotation in UNITE (Kõljalg et al., 2013). Fungal SHs were assigned to the following guilds (Figure S2): ectomycorrhizal fungi, arbuscular mycorrhizal, saprotrophs–pathogens, yeasts and moulds, other root-associated (e.g. dark septate endophytes), unknown, and other (e.g. mycoparasites; see Spitzer, Lindahl, et al., 2021 for details on assignation). We extracted PLFAs from each ground freeze-dried rhizosphere and bulk soil sample as in Frostegård et al. (1991), with the absolute abundance of PLFAs being expressed in nmol/g organic matter. The total fresh weight of bulk and rhizosphere soil from each pot was recorded and gravimetric moisture content was measured from 10 ± 1 g of each bulk and rhizosphere soil sample in each pot.

2.5 | Data analysis

We performed all statistical analyses using R 3.4.0 (R Core Team, 2018).

2.5.1 | Hypothesis 1

The fine root economics spectra were established across 17 of the plant species (i.e. excluding *D. octopetala* due to insufficient biomass for root chemical analyses) by performing a Principal Component Analysis (PCA) on the data for all the root traits, using version 2.5-6 of the VEGAN package (Oksanen et al., 2019). Prior to the analyses, data were log-transformed to fulfil the assumption of normality. Exclusion of dead plants from the experiment ($n = 2$) and three additional samples with insufficient biomass for root chemical analyses resulted in 80 experimental units. We also calculated the average PSF value for each plant species and the average root trait values for each plant species across all blocks. The first root trait axis represented an acquisitive-conservative root economics spectrum, with average diameter and root carbon content (conservative traits) being negatively related to specific root length and specific root tip abundance (acquisitive traits; Figure S3). The second root trait axis represented an additional acquisitive-conservative root economics spectrum, with root dry matter content and root carbon to nitrogen ratio (conservative traits) being negatively related to root nitrogen content (acquisitive trait). Axes scores of the first two principal components (i.e. two root economics spectra), which together accounted for 75.5% of the total variation, were then used in a multiple linear regression as predictors of PSF direction and strength (based on average PSF values across blocks) across the 17 species. Prior to using the first two principal components as explanatory variables, we conducted a parallel analysis with 9,999 iterations using the package PARAN (Alexis Dinno, 2018). Parallel analysis is used for determining the number of components to retain from a PCA. The results confirmed that the first two axes adequately explained the variation in root traits (eigenvalue = 1.06).

To find the best-fitting model with the lowest Akaike's information criterion (AIC) for individual fine root traits as predictors of PSF direction and strength, we used the function stepAIC with 'backward selection' in the package MASS (Venables & Ripley, 2002). The resulting model, which included three fine root traits (i.e. root nitrogen content, root forks per root length and root dry matter content) as predictors, was then run as a multiple regression. For both models, we used the type III ANOVA function from the CAR package (Fox & Weisberg, 2019) to obtain *p*- and *F* values for the predictors of the models. We additionally tested whether the seed source (i.e. seed company vs. field collected) influenced PSF values by including it as an additional direct effect. However, it was not significant ($p = 0.51$) and was therefore excluded as a variable from the final model. Furthermore, as closely related species may have similar trait values (Adams, 2014), we tested whether the three root traits included in the models above had a phylogenetic signal by calculating Blomberg's *K* values for each trait, and determined their significance with 1,000 permutations using the function 'phylosig' in the package PHYTOOLS (Revell, 2012).

2.5.2 | Hypothesis 2

To test the effects of fungal and bacterial PLFAs on PSF across all species, we fitted a general linear model with log-transformed absolute abundances of fungal and bacterial PLFAs as independent variables. As above, we conducted a type III ANOVA to obtain p- and *F* values for the predictors of the models.

2.5.3 | Hypothesis 3

We calculated whole pot average absolute abundances for the 100 most abundant fungal SHs at the order level (i.e. 24 orders) across all plant species using the up-scaled sub-sample sequence read abundances, and by combining the absolute abundances in the rhizosphere and bulk soils. First, we calculated the relative abundances of the 100 SH among all fungal sequences for each sample. Second, we calculated the averages of these relative abundances weighted by the relative amounts of rhizosphere and bulk soil in the pot, using the total mass of rhizosphere and bulk soil weighed at harvest. We then calculated the absolute abundances of the SHs by multiplying by the fungal PLFA concentrations for the rhizosphere or bulk soil in each pot (Fanin et al., 2019; Zhang et al., 2017). We used whole pot abundances because plant–soil feedback is typically measured based on plant growth responses to micro-organisms at the whole pot scale. Although scaling-up to whole pot abundances could potentially amplify some biases in PLFA analyses and DNA amplification and sequencing, this approach was applied equally across all samples and would therefore not have introduced biases among plant species. Furthermore, as PSF depends on the mycelial concentration of particular fungi in soil, rather than on their relative abundance in the fungal community, it is more appropriate to scale according to relative estimates of total fungal biomass (i.e. as determined from PLFAs) than to use relative abundances. In this light, recent studies have found a strong correlation between PLFA data and metabarcoding methods (Orwin et al., 2018; Smets et al., 2016), and the approach of combining high-throughput sequencing data with microbial quantification techniques (e.g. PLFA, quantitative PCR and flow cytometry) has been applied in several recent studies (Fanin et al., 2019; Lou et al., 2018; Props et al., 2017; Zhang et al., 2017).

We then performed a PCA of the absolute abundances of the fungal orders across all 85 pots after transforming the data using a Hellinger transformation. This was done to preserve the Euclidean distances among fungal orders and to meet the assumption of normality (Legendre & Gallagher, 2001). We used the first two principal component (PC) axes in a general linear model as predictors of fungal community effects on PSF across 18 plant species. We further focused on the 24 individual fungal orders in the dataset, conducting separate general linear models using the estimated biomass concentration of each of the fungal orders, as predictors of PSF. We also excluded *Antennaria alpina* from the dataset and then conducted two separate additional general linear models

with the fungal orders Mucorales and Pleosporales as predictors of PSF to assess whether this plant species was important for driving any observed relationships. Finally, we performed a multiple regression with all fungal functional guilds (except those classified as 'other' and 'unknown') as predictors of PSF strength across all 18 plant species.

2.5.4 | Plant–micro-organism–PSF relationships

To assess how root trait–microbial relationships are related to PSF, we conducted path analyses using the sem function in the LAVAAN package (Rosseel, 2012) with the root trait axis or two soil fungal orders that was significantly related to PSF in our regression models above. We expected a priori that the second root trait axis would directly contribute to PSF and indirectly via their associations with the two fungal orders (Mucorales and Pleosporales). This is because fine root traits are related to the abundance of soil fungal orders (Spitzer, Lindahl, et al., 2021). We focused on the root trait axis rather than on individual root traits, because we had insufficient statistical power to fit causal links between individual fine root traits and the fungal orders while fulfilling model fit parameter requirements [i.e. root square mean error of approximation (RMSEA) and comparative fit index (CFI)]. Hence, we stipulated two path analyses as follows: (a) the second root trait axis will directly contribute to PSF via indirect responses of the Mucorales and (b) the second root trait axis will directly contribute to PSF via indirect responses of the Pleosporales.

3 | RESULTS

3.1 | Plant–soil feedback

Three of the 18 plant species were found to have either negative or positive PSF values that were significantly different from zero, and there was no clear association between plant functional group (graminoid vs. forb) and the direction and strength of PSF (Figure 1). For example, the forbs *A. alpina* and *Geranium sylvaticum* had the strongest negative and positive PSF, respectively.

3.2 | Fine root traits and PSF

We found a significant relationship between the second PC root trait axis (PC2; primarily representing root dry matter content, root forks per root length, root nitrogen content, root phenol content and root carbon to nitrogen ratio; Figure S3) and PSF strength at $p = 0.05$, and a marginally non-significant relationship between the first PC axis (PC1) and PSF (PC1: $p = 0.08$, $F_{1,14} = 3.59$; PC2: $p = 0.004$, $F_{1,14} = 11.79$; overall $r^2 = 0.46$; Figure 2a). Furthermore, as the traits became more acquisitive (Figure S3), PSF became more negative (model coefficients for predictors: PC1: −0.04; PC2: −0.13; Figure 2a). The best-fitting model for individual fine root traits as predictors of PSF ($p < 0.001$; r^2 adjusted = 0.64) contained three traits, that is, root nitrogen content, root forks per root length and root dry matter content ($p = 0.0004$; $F_{1,13} = 21.70$; $p = 0.002$; $F_{1,13} = 15.47$ and $p = 0.003$; $F_{1,13} = 12.45$, respectively). Root nitrogen content and root forks per root length were negatively related to PSF (coefficient = −0.83; coefficient = −0.37, respectively; Figure 2b), while root dry matter content was positively related to PSF (coefficient = 0.86; Figure 2b). We found no significant phylogenetic signal for root nitrogen content (Blomberg's $K = 0.34$, $p = 0.06$), root forks per root length (Blomberg's $K = 0.30$, $p = 0.19$) and root dry matter content (Blomberg's $K = 0.30$, $p = 0.16$).

3.3 | Soil microbial community and PSF

We found no significant effect of fungal to bacterial ratio ($p = 0.70$, $F_{1,16} = 0.16$; coefficient = −5.13), total fungal PLFAs ($p = 0.56$, $F_{1,15} = 0.33$ coefficient = −0.05) or total bacterial PLFAs ($p = 0.69$, $F_{1,15} = 0.16$, coefficient = −0.01) on the strength of PSF. We also

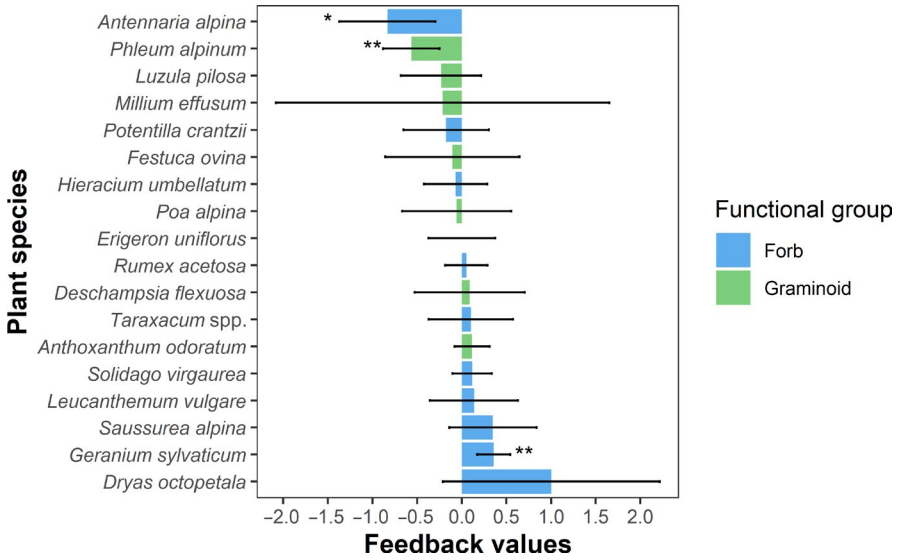


FIGURE 1 Bar plot showing plant–soil feedback for 18 tundra plant species. Bars are mean values with 95% confidence intervals ($n = 5$). Plant–soil feedback was calculated as log (plant biomass in conspecific soil) − log (plant biomass in heterospecific soil). Asterisks indicate feedback values significantly different from zero at $\alpha = 0.05$ (**$p \leq 0.01$ and *$p \leq 0.05$)

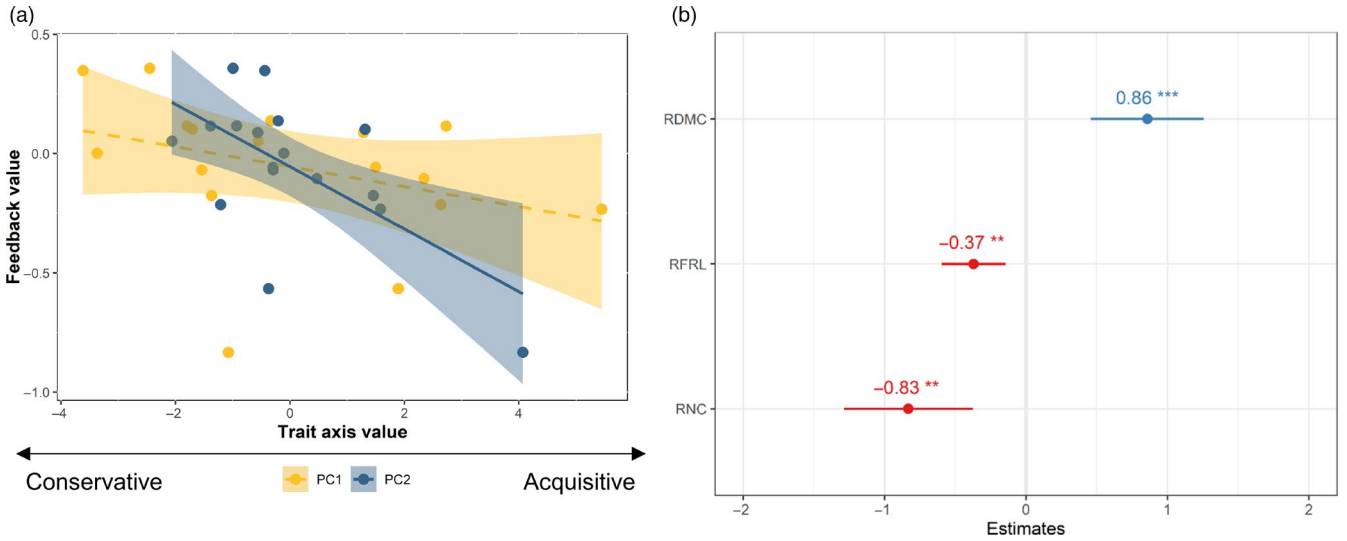
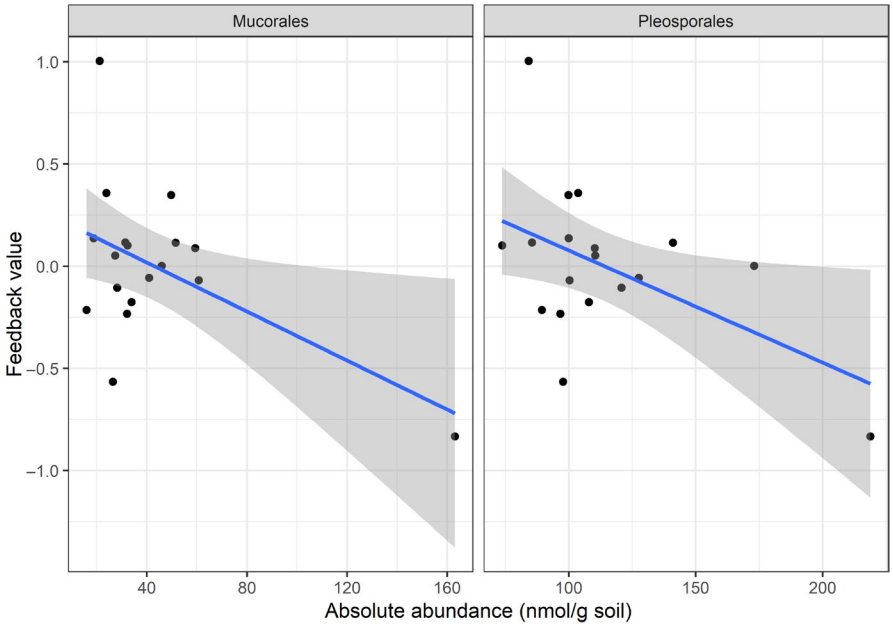


FIGURE 2 General linear model plots of plant traits as predictors of the direction and strength of plant–soil feedback (PSF). (a) First two axes (PC1 and PC2) as predictors of PSF in multiple regression; blue and orange dots represent individual plant species. The solid line indicates a significant relationship and the dotted line indicates a relationship that is not significant for the trait axes ($\alpha = 0.05$) and shaded areas indicate 95% confidence intervals. (b) Model coefficient plot of the best-fitting multiple regression model with individual root traits as predictors of plant–soil feedback across all plant species. Whiskers are the 95% confidence interval. The three fine root traits included in the multiple regression are root dry matter content (RDMC), root forks per root length (RFRL) and root nitrogen content (RNC). $^{**}p < 0.01$ and $^{***}p < 0.001$

FIGURE 3 General linear model plots of absolute abundances of Mucorales (moulds) and Pleosporales (order consisting of known parasites, saprotrophs and endophytes) as predictors of the direction and strength of plant–soil feedback (PSF). Blue lines indicate the regression lines for the trait axes and shaded areas indicate 95% confidence intervals. Black dots represent the 18 plant species used in our experiment



found no overall significant relationship between fungal functional guilds and PSF from the multiple regression ($p = 0.97$; $F_{5,12} = 0.17$).

For the PCA of the fungal orders (Figure S4), the first axis explained 44.2% of the variation and the second axis explained 14.8%. The AM fungal orders Archaeosporales and Glomerales, as well as Diaporthales (order consisting of known pathogens, saprotrophs and endophytes) explained most of the variation along the first PC axis (PC1), while Mucorales (moulds), Pleosporales (order consisting of known parasites, saprotrophs and endophytes) and Pezizales (which in our dataset consisted of saprotrophs–pathogens) explained most of the variation along the second axis (PC2; Figure S4). Strength of PSF was not significantly related to PC1 ($r^2 = 0.09$; PC1: $F_{1,15} = 3.72$; $p = 0.92$; coefficient = −0.44) and was marginally non-significantly related to PC2 ($F_{1,15} = 3.72$; $p = 0.07$; coefficient = −14.98). In post-hoc tests with individual fungal orders, PSF was significantly negatively related to Mucorales ($r^2 = 0.22$, $F_{1,16} = 5.83$; $p = 0.03$; coefficient = −0.006) and Pleosporales ($r^2 = 0.20$, $F_{1,16} = 5.34$; $p = 0.03$; coefficient = −0.005; Figure 3; Figure S5). The observed relationship between the absolute abundances of the two fungal orders and PSF was largely driven by one plant species, *A. alpina*,

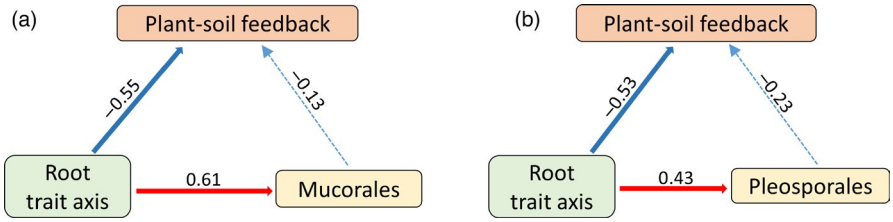


FIGURE 4 Path analysis of variables influencing plant–soil feedback. (a) Second axis of the root economics spectrum and Mucorales. (b) Second axis of the root economics spectrum and Pleosporales. Blue arrows indicate a negative relationship and the red arrows positive relationships. Solid one-directional arrows indicate a significant relationships ($\alpha = 0.05$) and dotted one-directional arrows indicate variables that are not significant

which had the strongest negative PSF (Figure 3). When *A. alpina* was excluded from the dataset, the relationships between PSF and the absolute abundances of Mucorales and Pleosporales were no longer statistically significant ($r^2 = 0.003$, $F_{1,15} = 0.04$; $p = 0.84$ and $r^2 = 0.03$, $F_{1,15} = 0.40$; $p = 0.54$, respectively).

3.4 | Path analysis of fine root traits, micro-organisms and PSF

Plant–soil feedback was directly predicted by the second axis of the root economics spectrum (RES; $p = 0.02$; Figure 4a, and $p = 0.008$; Figure 4b), but not indirectly via the RES effects on the absolute abundance of Mucorales ($p = 0.58$; path analysis model fit: CFI = 1.0; RMSEA < 0.001; Figure 4a) or Pleosporales ($p = 0.25$; path analysis model fit: CFI = 1.0; RMSEA < 0.001; Figure 4b). However, the second root trait axis had a strong positive influence on the absolute abundance of the fungal orders Mucorales (moulds; $p = 0.001$; Figure 4a) and Pleosporales (order consisting of known pathogens, saprotrophs and endophytes; $p = 0.049$; Figure 4b).

4 | DISCUSSION

We showed that fine root traits can be important predictors of PSF direction and strength, but that this applied only to a few of the measured traits. We also found that two fungal orders (one that contains a mould and one that consists of known parasites, saprotrophs and endophytes) could predict part of the variation in the direction and strength of PSF, while AM fungal orders did not. However, more detailed path analyses showed that this effect may be related to their relationship with the second axis of the root economic spectrum (RES). Our findings highlight that individual plant species within the sub-arctic tundra meadow could drive relationships between soil micro-organisms and PSF within plant communities.

4.1 | Relationships between fine root traits and PSF

We found partial support for our first hypothesis that the direction and strength of PSF would be related to the RES. The second axis of the RES predicted PSF strength, with more conservative root trait values resulting in positive feedback and acquisitive root trait values resulting in negative PSF. This second axis, which is similar to the resource conservation gradient of the RES (Bergmann et al., 2020), is characterized by slow-growing and long-lived roots with higher root dry matter content at one end, and fast-growing roots with short life spans and high root nitrogen content at the other. Our results show that within our cold and nutrient-poor study system, some root traits that are related to nutrient conservation result in positive PSF. We note however that a wider spectrum of plant species, including those not found in meadow vegetation, would be needed to test the generality of the pattern across the arctic. For example, our study did not include dwarf shrubs, which are common in other types of tundra vegetation such as heath, and which typically have thicker average fine root diameters than graminoids and forbs. The inclusion of these species could potentially increase the importance of the first axis of the RES for predicting PSF, because the gradient related to average root diameter would be longer. Hence, our findings are applicable to sub-arctic tundra meadow and is therefore generalizable for only a subset of vegetation types found across the tundra biome.

Furthermore, we found that 3 of the 11 measured fine root traits along the second axis of the RES predicted PSF direction and strength, with negative PSF being associated with a high root nitrogen content, low dry matter content and a high number of branches per root length. This is probably because plant tissues with high N content and low density (which is linked to root dry matter content; Birouste et al., 2014) are more attractive for parasites (Mur et al., 2017) and root-feeding nematodes (Zhang et al., 2020) that adversely affect plant growth. These two traits have recently been found to be positively correlated with their above-ground analogues (i.e. leaf nitrogen content and leaf tissue density) and to occur at opposite ends of the resource conservation gradient, which is related to fast as opposed to slow resource return on investment (Weigelt et al., 2021). High root dry matter content, which is associated with more conservative trait strategies, resulted in positive PSF. Meanwhile, a high number of root forks per root length (associated with acquisitive trait strategies) was linked to negative PSF. This trait has not commonly been included in past ecological studies, but reduced seedling infection rates by fungal pathogens have been found in agricultural plant species (e.g. carrots) with fewer root forks (Davison & McKay, 2003). However, elucidation of the mechanism

behind the linkage between this trait and PSF requires further investigation.

Contrary to previous studies (Cortois et al., 2016; Semchenko et al., 2018), we found no relationship between average root diameter or specific root length and PSF direction and strength. This inconsistency may be due to higher AM fungal root colonization rates expected with thicker root diameters being linked to the abundance of AM fungi in the soil (Barceló et al., 2020). However, the relative abundance of AM fungi in our study is lower than what is typical for temperate grasslands (Sweeney et al., 2021) where those studies were conducted. This suggests that those two traits may not be consistent predictors of PSF across contrasting biomes.

4.2 | Relationships between PLFA biomarkers and PSF

We found no support for our second hypothesis that plants that elevate bacterial to fungal ratios are more likely to experience positive PSF. Furthermore, neither bacterial nor fungal biomass by themselves explained PSF in our study. In previous studies, PSF has been observed to be related to individual soil fungal taxa (Bell et al., 2006) or groups of fungal and bacterial taxa (Luo et al., 2019) of individual or closely related plant species. However, the effects of these individual fungal or bacterial taxa may be diluted when grouped with other organisms within these broad microbial groups. Therefore, PLFA data may have more potential for addressing broader ecological questions, such as soil C cycling (Watzinger, 2015), or changes in soil microbial communities in response to drought (Fuchslueger et al., 2014), rather than as predictors of PSF direction and strength.

4.3 | Relationships between soil fungal community and PSF

We found no support for our third hypothesis that plants which elevate the abundance of AM fungi relative to pathogens will likely have positive PSF. We found that the variation on the first fungal PC axis (Figure S4) was primarily explained by the absolute abundances of two AM fungal orders (Archaeosporales and Glomerales), a saprotrophic order (Thelebolales) and Diaporthales, which consists primarily of known saprotrophs and some pathogens (Rossman et al., 2007). However, this axis was not significantly related to PSF. Moreover, none of the individual fungal guilds was linked to PSF. This is contrary to other studies showing the relative abundance of AM fungi to be positively related to PSF and the relative abundance of fungal pathogens and saprotroph composition to be negatively related to PSF (Semchenko et al., 2018; Wilschut et al., 2019).

It is surprising that the abundance of AM fungal orders did not promote positive PSF, as we previously showed that AM abundance is positively associated with root carbon content among plant species in the sub-arctic tundra (Spitzer, Lindahl, et al., 2021). However, although AM fungi are generally beneficial for plant growth, trees with AM associations have also been previously shown to be negatively related to PSF (Bennett et al., 2017). Hence, further studies on nutrient transfer to plants by AM fungi are required to clarify the importance of AM fungi for plant nutrition and PSF in the sub-arctic tundra. In addition, in our study, the abundance of AM fungi is low relative to other fungal functional guilds (Figure S2), and it is therefore possible that their effect on PSF was overruled by other more abundant fungal orders. Stronger positive PSF is typically expected for ectomycorrhizal species (Teste et al., 2017) and ecto- and ericoid mycorrhizal fungal guilds might be more important drivers of PSF for tundra dwarf shrubs. However, our study did not include dwarf shrubs, which are typically not present in meadow vegetation but which commonly have these mycorrhizal associations.

Two fungal orders were found to contribute to negative PSF, namely Mucorales and Pleosporales, although this relationship was largely driven by one plant species, *A. alpina*. Within our dataset, Mucorales consists of one taxon, *Mucor piriformis*, which is a known pathogen in agricultural systems (Mari et al., 2000). Pleosporales consists of five taxa, two of which are known pathogens, namely *Acicuseptoria rumicis*, *Didymella exigua*, while the others are either saprotrophic or could not be identified at a lower taxonomic level. It is therefore plausible that these orders are contributing to negative PSF via pathogenicity, particularly since *A. alpina* has the highest root nitrogen content within our study which is likely to make it more attractive for pathogens (Mur et al., 2017). However, we could not test for specific effects of pathogens within our study, because putative saprotrophs and pathogens were combined into one functional guild on the basis that many saprotrophs can switch strategy from one to the other (Olson et al., 2012). Furthermore, some fungal pathogens may have been excluded, since we focused on the hundred most abundant taxa (Species hypotheses; see Section 2), while some pathogens are commonly host specific (Raaijmakers et al., 2009) and less abundant (Barrett et al., 2009).

It was unexpected that a single forb species in our study (*A. alpina*) could drive the relationships between PSF and the abundances of Mucorales and Pleosporales. This is because while this plant species is both abundant in our study system and has a strongly negative PSF, rare plant species are generally known to have stronger negative PSF than more abundant species (Kempel et al., 2018; Klironomos, 2002). In addition, stronger negative PSF usually occurs with graminoids rather than with forbs (Cortois et al., 2016). It may therefore be difficult to generalize our findings across the tundra biome and to plant communities in which this species is not present. However, observed relationships between PSF and soil microorganisms or plant traits are still ecologically relevant provided that a large number of species that are typically present and that are abundant in a plant community are included in the study. The identification of those plant species that drive PSF within plant communities could be important, as they may be particularly sensitive to changes in the soil microbial community composition, for example as driven by climate warming, with consequences for plant community composition and diversity.

4.4 | Fine root trait–soil micro-organism relationships and PSF

Using path analysis, we found that the second axis of the RES was a direct predictor of PSF. However, we found no evidence of this root trait axis indirectly contributing to PSF through its relationship with Mucorales and Pleosporales, despite the absolute abundance of both orders being positively influenced by this trait axis. This means that although these fungal orders contributed to negative PSF, they were not mediators between the fine root traits and PSF. Here, we focused on fungal orders to assess across-species relationships between PSF and soil fungal communities because effects of individual fungal species on PSF are likely to be plant species specific (Liu et al., 2012). However, within-species linkages among fine root traits, soil fungal species and PSF could potentially be assessed by focusing on fungal indicator species, for example by using known pathogens or mycorrhizal associates of plant species (Bever, 2002; Liu et al., 2012). Other soil micro-organisms or fauna may be stronger mediators between root traits in the conditioning phase of the experiment and feedbacks in the feedback phase of the experiment. For example, higher relative abundances of root-feeding nematodes have been found to associate with plants with acquisitive fine root traits (Zhang et al., 2020) and to be involved in negative PSF (Wilschut et al., 2019). Currently, there is a limited understanding of causal linkages between fine root traits, soil organisms and PSF, and further studies that are focused on understanding these linkages would help advance our understanding of mechanisms that influence plant community productivity and community assembly.

5 | CONCLUSIONS

Our study provides new insights into fine root traits and the RES as predictors of the direction and strength of PSF within sub-arctic tundra meadow. We have investigated a large number of fine root traits and have identified three fine root traits (i.e. root dry matter content, root forks per root length and root nitrogen content) that are predictors of PSF, two of which have not previously been studied in the context of PSF. Previous research had tested the effects of root nitrogen content, but not root dry matter content and root forks per root length on PSF. Furthermore, while previous studies on PSF have focused on individual root traits, we have shown that the fine root economics spectrum is directly linked to PSF direction and strength, with acquisitive strategies being linked to negative PSF. This negative relationship could over time result in a reduced abundance of acquisitive plant species, thereby promoting increased plant species diversity. However, the fine root economics spectrum did not indirectly influence PSF through its relationship with two fungal orders that were themselves predictors of PSF. This points to a knowledge gap and the need for future research into the mechanistic effects of root trait and soil biota relationships on PSF within arctic ecosystems. Future research could investigate, for example, the root traits driving the abundances of other groups of soil organisms, that is, bacterial and nematodes that may affect PSF. Furthermore, although AM fungal abundance was not related to PSF in our experiment, expected increases in AM fungal colonization rates with warming (Rillig et al., 2002) could potentially become an important driver of arctic PSF in the future. Taken together, these findings suggest an important role for the RES and soil microbial taxa in PSF research and points to their potential role in plant community assembly within the sub-arctic tundra.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare. Paul Kardol and David Wardle are both Associate Editors of *Journal of Ecology*, but took no part in the peer review and decision-making processes for this paper.

AUTHORS' CONTRIBUTIONS

P.K., C.M.S. and D.A.W. designed the study; C.M.S. and P.K. collected the seeds and soil; C.M.S. conducted the greenhouse study and laboratory work; B.D.L. supervised the molecular laboratory work; C.M.S. conducted the fungal annotations under the supervision of B.D.L. and N.F.; C.M.S. conducted the statistical computations; C.M.S. lead the writing of the manuscript; C.M.S., D.A.W., P.K., M.K.S., B.D.L., N.F. and M.J.G. contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data for this paper are archived in Dryad Digital Repository and freely accessible via https://doi.org/10.5061/dryad.r7sqv9sbn (Spitzer, Wardle, et al., 2021).

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