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To cite this version:

Clydecia M Spitzer, Björn Lindahl, David Wardle, Maja K Sundqvist, Michael Gundale, et al.. Root trait-microbial relationships across tundra plant species. New Phytologist, 2021, 229 (3), pp.1508-1520. 10.1111/nph.16982 . hal-03006806

HAL Id: hal-03006806
https://hal.inrae.fr/hal-03006806

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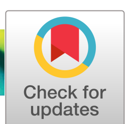

Root trait–microbial relationships across tundra plant species

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Received: *26 May 2020*
Accepted: *10 September 2020*

New Phytologist (2020)
doi: 10.1111/nph.16982

Key words: fine root traits, fungi, plant–microorganism interactions, rhizosphere, tundra ecosystems.

Summary

- Fine roots, and their functional traits, influence associated rhizosphere microorganisms via root exudation and root litter quality. However, little information is known about their relationship with rhizosphere microbial taxa and functional guilds.
- We investigated the relationships of 11 fine root traits of 20 sub-arctic tundra meadow plant species and soil microbial community composition, using phospholipid fatty acids (PLFAs) and high-throughput sequencing. We primarily focused on the root economics spectrum, as it provides a useful framework to examine plant strategies by integrating the co-ordination of belowground root traits along a resource acquisition–conservation trade-off axis.
- We found that the chemical axis of the fine root economics spectrum was positively related to fungal to bacterial ratios, but negatively to Gram-positive to Gram-negative bacterial ratios. However, this spectrum was unrelated to the relative abundance of functional guilds of soil fungi. Nevertheless, the relative abundance of arbuscular mycorrhizal fungi was positively correlated to root carbon content, but negatively to the numbers of root forks per root length.
- Our results suggest that the fine root economics spectrum is important for predicting broader groups of soil microorganisms (i.e. fungi and bacteria), while individual root traits may be more important for predicting soil microbial taxa and functional guilds.

Introduction

Plant–microorganism interactions are important drivers of plant community composition and dynamics (Klironomos, 2002; Wardle *et al.*, 2004). Plants influence soil microbial community composition by root exudation (Bais *et al.*, 2006), symbiotic interactions, and by determining the quantity and quality of litter available for decomposers (Wardle *et al.*, 2004; Veen *et al.*, 2019). These interaction pathways are known to influence the presence and abundance of mutualistic (Bonfante & Genre, 2010) and antagonistic (Philippot *et al.*, 2013) soil microorganisms. Depending on the nature of plant-induced shifts in these groups of microbes, the overall soil microbial community can exert positive, neutral or negative feedbacks on their associated plant communities (Bever *et al.*, 2012). These feedbacks can, in turn, have consequences for plant community composition, successional dynamics (Kardol *et al.*, 2007) and plant species diversity (Mangan *et al.*, 2010; Teste *et al.*, 2017). Despite the possible direct influence of root traits on the abundance of microorganisms due to plant roots and soil microorganisms being tightly connected in space within the rhizosphere, there remains a huge knowledge gap about how plant roots and their chemical and morphological traits are associated with rhizosphere soil microbial taxa and functional guilds.

Plant fine root characteristics are increasingly being examined within trait-based frameworks (Kattge *et al.*, 2011; Iversen *et al.*, 2017) in which trait values are used to indicate plant economic strategies, that is acquisitive vs conservative resource use (Freschet *et al.*, 2010). For example, acquisitive trait values indicate higher plant resource investment into the rapid acquisition of nutrients and lower investment in defence, and include high specific root lengths and small fine root diameters (Cortois *et al.*, 2016). By contrast, conservative trait values (e.g. lower specific root lengths and thicker root diameters) are associated with slower growth, well defended tissue and stronger dependence on mycorrhizal symbionts for nutrient uptake (Eissenstat *et al.*, 2015; Cortois *et al.*, 2016). Furthermore, higher concentrations of secondary defence compounds in plant tissues reduce the abundance of foliar and root pathogens (Tomova *et al.*, 2005; Maupetit *et al.*, 2018), and fine root litter polyphenol concentrations can modify saprotroph activity (Sun *et al.*, 2018). In addition, the ratios of Gram-positive to Gram-negative bacteria and of fungi to bacteria are expected to respond to those fine root traits that are linked to root quality. This is because Gram-negative bacteria utilise more labile plant-derived carbon (Fanin *et al.*, 2019a), while Gram-positive bacteria utilise more recalcitrant soil-derived carbon (Kramer & Gleixner, 2008), and increased litter recalcitrance promotes the dominance of fungi over bacteria (Strickland &

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Rousk, 2010). Hence, using a gradient of fine root trait values (i.e. across the fine root economics spectrum) will enable explicit testing of how fine root trait values affect the abundance of various rhizosphere soil microorganisms.

For the present study, we focused on the fine root trait relationships with soil microorganisms in the arctic tundra. These ecosystems are characterised by nutrient-poor soils (Sundqvist *et al.*, 2014), suggesting that their plants may be more dependent on tight root–microorganism associations for nutrient recycling than are those in other, more nutrient-rich, ecosystems. In addition, the large fine root biomass and quantity of root litter often present in tundra ecosystems (Chapin *et al.*, 2011; Iversen *et al.*, 2015) is likely to support an extensive mycorrhizal network for nutrient uptake and a high abundance of saprotrophs (McCormack & Iversen, 2019). However, to our knowledge, no study has examined the variation in morphological and chemical root traits in tundra plants, although Björk *et al.* (2007) have studied the effect of warming on some morphological fine root traits within tundra plant communities. Instead, tundra root research to date has largely focused on total root biomass (Iversen *et al.*, 2015) and phenology (Blume-Werry *et al.*, 2016). In addition, although tundra typically harbours a high abundance of cold-tolerant fungi, including yeasts (Margesin *et al.*, 2009; Treseder & Lennon, 2015), and mycorrhizal associations are common (Newsham *et al.*, 2009), we know little about moulds, saprotrophic and pathogenic fungal taxa in these ecosystems.

Sub-arctic tundra has two main vegetation types, that is meadow (dominated by graminoids and forbs) and heath (dominated by dwarf shrubs). Here, we focused on the sub-arctic meadow vegetation because it has a large species pool, while heath does not (Sundqvist *et al.*, 2011). We used a glasshouse experiment to test the effects of 11 morphological and chemical fine root traits, within and across 20 sub-arctic tundra meadow plant species, on rhizosphere microbial community composition. We characterised the microbial community both at the level of broad microbial groups (phospholipid fatty acids, PLFAs) and at the level of fungal orders and functional guilds (through high-throughput sequencing). Specifically, we tested the following hypotheses:

(**1**) Acquisitive root trait values will be associated with lower soil fungal to bacterial ratios and lower Gram-positive to Gram-negative bacterial ratios, relative to conservative root trait values.

This is because acquisitive root trait values are expected to be positively linked to bacterial decomposers (Wardle *et al.*, 2004) but not to mycorrhizal fungi (Ma *et al.*, 2018), and because Gram-positive bacteria are expected to associate with recalcitrant litter from plants with conservative root traits (Fanin *et al.*, 2019a).

(**2**) Conservative root traits will be associated with a higher relative abundance of mycorrhizal taxa in the fungal communities.

This is because roots of resource-conservative plants are expected to have a higher spatial capacity for accommodating mycorrhizal hyphae and are associated with greater mycorrhizal nutrient foraging (Eissenstat *et al.*, 2015; Chen *et al.*, 2016).

(**3**) Acquisitive root trait values will be associated with a higher abundance of fungal saprotrophs, pathogens, yeasts and moulds.

This is because organs of plants with acquisitive traits are expected to be less defended, more active in exudation of simple organic carbon compounds and more nutrient-rich, making them more susceptible to pathogens and more attractive to opportunists and saprotrophs (Tomova *et al.*, 2005; Botha, 2006).

Materials and Methods

Study site

The site for sourcing soils and most of the seed material for our study is located in northern Sweden, *c.* 27 km north-east of Abisko (68°21′N, 18°49′E), at the foothills of Mount Gearggečorrus at 700 m asl. The climate is sub-arctic with a growing season of *c.* 3 months. The forests at lower altitudes in this mountainous landscape are dominated by mountain birch (*Betula pubescens* ssp. *czerepanovii*), with the treeline occurring at *c.* 500–600 m asl. The vegetation above the treeline is dominated by mosaics of heath (comprised primarily of ericaceous dwarf shrubs and *Betula nana*) and meadow (comprised primarily of graminoids and forbs). The mean annual precipitation from 2005–2017 at the Abisko Scientific Research Station was 340 mm and the mean temperature for the corresponding period was +13°C in July and −9.9°C in January (the Swedish Meteorological and Hydrological Institute).

Experimental design

We collected seeds from individuals of each of 17 meadow plant species, that is graminoids and forbs at the end of the growing season in the study area in August 2016, and sourced seeds from three additional meadow species (which also occur in the study area) from a local seed company (Pratensis, Lönashult, Sweden). Shrub species are not included in our study (see Supporting Information Fig. S1 for a list of species), as they are primarily found in heath within the sub-arctic tundra (Sundqvist *et al.*, 2011). All plant species except one (*Luzula pilosa*), which is non-mycorrhizal, are potential arbuscular mycorrhizal hosts (Soudzilovskaia *et al.*, 2020). Soil was collected during late August 2016 from the rooting zone (= upper 10 cm) beneath individuals of all visible forb and graminoids species, during the expected period of peak root production (Blume-Werry *et al.*, 2016), bulked and stored in plastic bags at 4°C. The soil type is cryorthents (Darmody *et al.*, 2000) and basic soil properties are as follows: pH 5.54, moisture 25.8 %, NH_4^+ 0.009 mg g^{-1} dry soil, and PO_4^+ < 0.000 mg g^{-1} dry soil (Veen *et al.*, 2015). The bulk soil was transported to a glasshouse facility in Umeå in cool boxes, along with dried field-collected seeds (40°C for 2 d) and stored at 22°C until the setup of the experiment in February 2017. Seeds were surface sterilised by soaking for 1 min in 1% sodium hypochlorite (De Long *et al.*, 2015) and germinated on sterilised sand. To ensure that the seedlings were at a similar ontogenic stage at the time of planting, they were stored at 4°C with light (50%) as soon as they had reached a height of *c.* 3 cm, until the beginning of the experiment.

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The field-collected soil was sieved (10-mm mesh size) to remove stones and large roots, homogenised and the volume split equally into six blocks. Soil from each block was then mixed with autoclaved sand (soil : sand, 3 : 1) to facilitate better drainage. To enable acclimatisation of the soil microbial community to glasshouse conditions, we left the bulk soil at room temperature for 2 d (20°C) until the time of planting. We filled 1.4 L pots (9 × 9 × 20 cm) with 0.4 L of warm-water-washed gravel, followed by the homogenised bulk soil–sand mixture. This resulted in a soil depth of *c.* 10 cm, which is similar to the soil depth at the site where we collected the soil. We planted two seedlings of the same species in each pot (top right and bottom left), resulting in 20 species × 6 blocks = 120 pots and 12 plants per species. We randomised the location of the pots within the block every 2 wk to account for microclimatic variation in the glasshouse. Plants were grown in the glasshouse (18°C : 13°C, day : night temperature; 80% humidity, and 18 h : 6 h, light : dark regime) for 12 wk, which is similar to the length of one growing season in the Abisko region. We watered the plants every 2 d. During the first 2 wk of the experiment, we replaced any dead seedlings with live seedlings.

Harvest

We harvested the experimental units block-wise after 84 d. As soil moisture could influence the volume of soil adhering to the roots, we maintained a consistent watering and harvesting regime between blocks. Harvested blocks were stored at 4°C in the dark for a maximum of 3 d and the harvesting order of individual pots within blocks was random. After carefully removing the plants from the pots by tapping the pot, plants were gently shaken (Saj *et al.*, 2009) over trays layered with aluminium foil to obtain the rhizosphere soil surrounding the plant roots (i.e. soil stuck to plant roots) (Smalla *et al.*, 2001; Carrillo *et al.*, 2017). Soil that fell away from roots while removing plants from pots was not considered rhizosphere soil, and thus was not used in the study. We replaced the aluminium sheets in the trays between samples to avoid cross-contamination. We used this method to collect rhizosphere soil, as opposed to brushing (Clemensson-Lindell & Persson, 1992; Clemmensen *et al.*, 2008), as we wanted to minimise fine root damage (Fitz *et al.*, 2006). We bulked and transferred the rhizosphere soil from both plants within each pot into clean plastic bags and homogenised the combined sample by shaking the closed bags. Subsamples of *c.* 500 mg (dry weight equivalent) of all rhizosphere soil samples were immediately frozen at −20°C and after 1 wk freeze dried and ground using a roller grinder for subsequent PLFA and DNA analyses.

Root trait measurements

Before measuring root traits, we washed roots according to protocols outlined in Pérez-Harguindeguy *et al.,* (2013). Briefly, we carefully washed the plant roots over a sieve (4 mm) and a collection tray. Between successive rinses, we poured the flow-through from the tray over a 2 mm sieve to recover any roots that were accidentally broken during rinsing. Any remaining soil particles on the roots were washed off with light water spraying. A representative fine root (≤ 1 mm diameter) subsample was collected from the clean root system of the biggest plant of each pair from each pot (or the smaller plant whenever the bigger plant was diseased or yellowing). We used a graduated ruler to determine root diameters ≤ 1 mm. Subsamples were scanned in transparent trays (15 × 20 cm) with cold tap water using WinRhizo 2016 with a flatbed scanner (Epson Perfection V800/V850 1.9 V3.93 3.9.3.2) with the following settings: dust removal on; resolution 800 d post inoculation; root and background detection on; 128 grey detection level and 100 pixels; detection of very pale roots on; overhead lights on. We standardised the quantity of fine roots in each root subsample using the transparent tray. We selected roots until the tray was filled, while ensuring the roots did not overlap within the tray. For small plants, we scanned all fine roots ≤ 1 mm diameter. One root subsample from each pot was scanned. After each scan, we confirmed that all fine roots had the desired diameter threshold by examining the colour-coded diameter images generated by the WinRhizo software (Regent Instruments Inc., Quebec, Canada).

We selected a suite of morphological and chemical root traits from the FRED database (Iversen *et al.*, 2017) based on their potential relevance for root-associated microbial communities (see Table 1). We measured the following morphological root traits for each root subsample: number of root tips, total root length, root branching, average diameter and root surface area. After scanning, we recorded the fresh weight of each scanned sample and the dry weight following drying at 60°C for 2 d. The remaining roots were dried using the same protocol as used for the scanned root samples and the biomass was recorded. We recorded the total biomass of each scanned root subsample, and calculated specific root length (cm mg^{-1}), specific root tip abundance (tips mg^{-1}), specific root area (cm^2 mg^{-1}) and root dry matter content (dry mass per unit fresh mass; mg mg^{-1}). Root dry matter content has been shown to be a good proxy for root tissue density (Birouste *et al.*, 2014), and is a commonly used trait in belowground studies (Henneron *et al.*, 2019).

To obtain chemical root trait data we manually ground a subsample (*c.* 150 mg) of the remaining fine root material from each of the same individuals from which we obtained the scanned root sample. When necessary, we ground the roots with a Retch MM400 ball mill. We measured total root phenol concentrations from 50 ± 5 mg dry ground roots using the method of Stern *et al.* (1996). Total root carbon (C) and nitrogen (N) concentrations were analysed by dry combustion using an elemental analyser (Flash EA 2000; Thermo Fisher Scientific, Bremen, Germany).

Phospholipid fatty acid analysis

PLFAs were extracted from each freeze-dried ground rhizosphere soil sample according to Frostegård *et al.* (1991). The absolute abundance of PLFAs was expressed in nmol g^{-1} organic matter (OM). We used i14:0, 14:0, i-15:0, a-15:0, 15:0, i-16:0, 16:1ω9, 16:1ω7c, 16:1ω7t, i-17:0, a-17:0, 17:1ω8, cy-17:0, 17:0, 18:1ω7, and cy-19:0 as indicators for bacteria (Frostegård & Bååth, 1996) and 18:2ω6,9 for fungi (Kaiser *et al.*, 2010). PLFAs

Table 1 Fine root traits measured in this study with their units, as well as their ecological relevance.

	Root trait	Unit	Ecological relevance
Chemical	Carbon (C) content	%	Carbon source for microbes
	Nitrogen (N) content	%	Nutrients for microorganisms
	C : N ratio	–	Tissue quality
	Phenol : N ratio	mg g^{-1}	Investment in defence
	Total phenol	mg g^{-1}	Investment in defence
Morphological	Average diameter	mm	Space for mycorrhizal hyphae
	Dry matter content	mg mg^{-1}	Tissue density and quality
	Forks per root length	forks cm^{-1}	Soil exploration
	Specific root tip abundance	tips mg^{-1}	Soil exploration
	Specific root area	cm^2 mg^{-1}	Nutrient absorption
	Specific root length	cm mg^{-1}	Soil exploration

i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 represented Gram-positive bacteria, whereas cy-17:0, 18:1ω7, and cy-19:0 represented Gram-negative bacteria (Wardle *et al.*, 2013). Branched chained fatty acids 10me16:0, 10me17:0 and 10me18:0 were used as indicators of actinobacteria (Maaroufi *et al.*, 2019). The PLFA data were used to calculate the ratios of fungi to bacteria and Gram-positive to Gram-negative bacteria. PLFA ratios are commonly used in ecological studies to explain the response of microbial groups to plant tissue quality (Fanin *et al.*, 2019b).

Fungal community

Fungal community composition for each freeze-dried ground rhizosphere soil sample was assessed using high-throughput sequencing of amplified ITS2 markers (Clemmensen *et al.*, 2016). We extracted DNA from 300 ± 10 mg of freeze-dried rhizosphere soil using the Nucleospin® Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. We performed 50 µl polymerase chain reactions (PCRs) with 25 µl diluted DNA extracts (1 ng µl^{-1}) using a 5 µl mixture of 8-bp extended sample-specific tagged forward primer gITS7 (Ihrmark *et al.*, 2012) and reverse primer ITS4 (White *et al.*, 1990) to amplify the ITS2 region and 20 µl PCR master mixture. PCR master mixtures were prepared by mixing 8.25 µl distilled water, 0.25 µl DNA polymerase (Dream *Taq* Green; Thermo Fisher Scientific, Waltham, MA, USA), 5 µl reaction buffer, 5 µl dNTPs (2 mM each of dATP, dCTP, dGTP and dTTP) and 1.5 µl $MgCl_2$.

The PCRs were run under the following conditions: 5 min at 95°C; 26–30 cycles of 30 s at 95°C, 57 s at 57°C, 30 s at 72°C; and 7 min at 72°C. We optimised the number of PCR cycles for each sample (based on preliminary PCRs), to interrupt PCRs in the exponential phase, when amplification is quantitatively more accurate. Negative controls (both for DNA extraction and PCRs) were included and PCR products were checked by gel electrophoresis. We then purified amplicons using the Agencourt AMPure kit (Beckman Coulter, Beverly, MA, USA) and measured the concentrations fluorometrically (Qubit Fluorometer, Invitrogen, Carlsbad, CA, USA). Equimolar mixtures of amplicons from each sample were pooled into three composite samples for sequencing. Sequencing of amplicons were performed using the Pacific Biosciences Sequel Technology Platform (one single-molecule real-time (SMRT) sequencing cell per composite sample) at SciLifeLab, Uppsala, Sweden. Raw sequences were uploaded to the Sequence Read Archive of the NCBI database under accession no. PRJNA664687.

We processed sequences using the bioinformatics pipeline SCATA (http://scata.mykopat.slu.se/) and carried out quality filtering and clustering with parameters as in Fanin *et al.* (2017). After discarding sequences with missing primer sequences, and missing or mismatching identification tags, 1687 418 out of a total of 2523 609 sequences (67%) passed the initial quality control. In total, 1648 502 high quality sequences were assembled into 7474 Species Hypotheses (SHs; Kõljalg *et al.,* (2013)), after removal of singletons. For this study, we focused on the relative abundances of the 100 most abundant fungal SHs. We assigned fungal SHs to functional guilds, whenever possible, by comparison with the UNITE Database and the annotated International Nucleotide Sequence Database (INSD) using the MASSBLASTer option (https://unite.ut.ee/) (Kõljalg *et al.*, 2013; Nilsson *et al.*, 2019). We considered the 10 best-matching references for annotation. In cases in which no reliable taxon name was available, we ran manual BLASTn searches against INSDC and used 98.0%, 90.0%, 85.0%, 80.0% and 75.0% similarity as criteria for assigning SHs to species, genus, family, order and class, respectively. BLASTn search results of $<e^{-50}$ were considered reliable for assigning sequences to the fungal kingdom, whereas those of $>e^{-20}$ were considered 'unknown', as proposed by Tedersoo *et al.* (2014).

We categorised fungi identified to species or genera with a well known life-form (i.e. according to published literature) into guilds: saprotrophs and pathogens, ectomycorrhizal fungi, arbuscular mycorrhizal fungi, other root-associated (e.g. dark septate endophytes), yeasts and moulds, and other (e.g. mycoparasites). Fungi in supported clades (based on phylogenetic trees produced by BLASTn searches of INSDC) that either belonged to higher taxonomic ranks with known life-form or matched with environmental database sequences derived from a specific substrate (e.g. surface-sterilised roots of ectomycorrhizal hosts) were categorised into putative functional guilds (Clemmensen *et al.*, 2013). Pathogens and saprotrophs were treated as one guild, as many pathogens and saprotrophs are capable of switching strategies from one to the other (Olson *et al.*, 2012; Zanne *et al.*, 2020). Yeasts and moulds were also merged into one composite functional guild, because they both have a limited capacity to utilise more complex C substrates (Treseder & Lennon, 2015). Clusters with insufficient information for classification into a putative functional guild were assigned as 'unknown'.

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Data analyses

All statistical analyses were carried out using R 3.4.0 (R Core Team 2018, Vienna, Austria), with the following packages: NLME v.3.1-149 (Pinheiro *et al.*, 2020), LME4 v.1.1-23 (Bates *et al.*, 2015), CAR v.3.0-8 (Fox & Weisberg, 2019), MASS v.7.3-51.6 (Venables & Ripley, 2002), TIDYVERSE v.1.3.0 (Wickham *et al.*, 2019), VEGAN v.2.5-6 (Oksanen *et al.*, 2019), APE v.5.4 (Paradis & Schliep, 2019), XLSX v.0.6.3 (Dragulescu & Cole, 2019), and PHYTOOLS v.0.7-47 (Revell, 2012). Analyses were conducted using total trait variation across all experimental units, and thus included all variation (i.e. both intraspecific and interspecific).

Fine root traits and fungal rhizosphere functional guilds The assessment of fine root trait variation and the establishment of the root economics spectrum across the 20 plant species was performed using principal component analysis (PCA). In order to fulfil the normality assumption for data analysis, data were log-transformed, and samples were removed from the analysis if they were either dead ($n = 5$ across the whole experiment) or had insufficient biomass for root chemical analyses ($n = 4$). Furthermore, we excluded one outlier (value was 132 times larger than the mean value, possibly due to a measurement error). The axes scores of the first two PCA principal components were later used as predictors in linear mixed models to represent a conservative–acquisitive root economics spectrum.

Hypothesis 1. We used general linear mixed effects models to test for the effect of the fine root economics spectrum on the ratios of fungi to bacteria and Gram-positive to Gram-negative bacteria PLFAs, using the axis scores from the first two PCA axes as fixed factors, and block as a random factor. We selected our final models based on the lowest Akaike Information Criterion (AIC) and therefore excluded the model that included species as a random factor (AIC for model with block and species as random factors = −420.44; AIC for model with block as a random factor = −422.43). Furthermore, to determine the relationships between fine root traits and the microbial groups assessed by PLFA, we first conducted a canonical correspondence analysis (CCA) with all log-transformed fine root traits as explanatory variables. As this was significant ($F = 2.16$; $P = 0.03$), we then performed post hoc Spearman's rank correlations to assess relationships between individual fine root traits and the microbial PLFA groups.

Hypotheses 2 and 3. Similar to hypothesis 1, we first conducted a CCA to determine the relationship between fine root traits and the fungal functional guilds, which was significant ($F = 1.67$; $P = 0.04$). We then performed Spearman's rank correlations to further assess the relationship between fine root traits and the various fungal guilds. In addition, we tested the effects of individual fine root traits on the relative abundances of fungal orders by performing a partial CCA (pCCA) with the 100 most abundant SHs and the fine root traits, with block included as a random variable (Ramette, 2007). For this analysis, the SHs were grouped at the order-level (24 orders in total and the functional guild 'unknown'). We tested for correlation significance using permutation tests.

Before the pCCA analysis, the relative abundances of the fungal orders were square-root-transformed, which is equivalent to a Hellinger transformation (Ramette, 2007), as it reduces the weighting of rare OTUs in ordinations (Legendre & Gallagher, 2001). We first conducted automated model selection (i.e. 'forward', 'backward' and 'ordistep' as available in the VEGAN package) and variables that were significant in any of the models were selected for inclusion into our final model. This resulted in the inclusion of root forks (ramifications) per root length, and root C and N content as explanatory variables and block as a random variable. In addition, closely related plant species possess similar trait values due to their common ancestry (Adams, 2014) and this could potentially influence root trait associations with arbuscular mycorrhizal fungi. Therefore, we further tested for phylogenetic signal of the root traits found to be associated with arbuscular mycorrhizal fungi by calculating Blomberg's K values (Adams, 2014) and determining its significance with 1000 permutations using the function 'phylosig' in the package PHYTOOLS.

Finally, we used generalised linear mixed effects models to test for the effects of the root economics spectrum on fungal functional guilds, with the axis scores from the first two PCA axes as explanatory variables and block as a random factor. We assumed a binomial distribution for our models, because our response variables were relative abundances.

Results

Fine root traits

The PCA resulted in the alignment of plant root trait values to form a conservative–acquisitive root economics trait spectrum, with the first and second PCA axes explaining 43% and 23% of the total variation in the data set, respectively (Fig. 1). Morphological root traits explained most of the variation along the first PCA axis, while the chemical root traits (except for the phenol to N ratio) and root dry matter content explained most of the variation along the second PCA axis. As such, along the first PCA axis, average diameter was negatively correlated with specific root length and specific root area. Furthermore, there was considerable variation among the 20 species for some traits (e.g. specific root length and specific root tip abundance), but not for others (e.g. average diameter and root C content; see Table S1).

PLFA markers and fine root traits

We found a significant association between the second PCA root traits axis (but not the first) and for the ratios of fungi to bacteria ($F = 4.497$; $P = 0.036$; coefficient = 0.004) and Gram-positive to Gram-negative bacteria ($F = 3.961$; $P = 0.049$; coefficient = −0.007). We further found significant, but rather weak, negative correlations between root dry matter content and the concentrations of all PLFA biomarkers except Gram-negative bacteria ($r = -0.21$ with bacteria, $r = -0.21$ with fungi,

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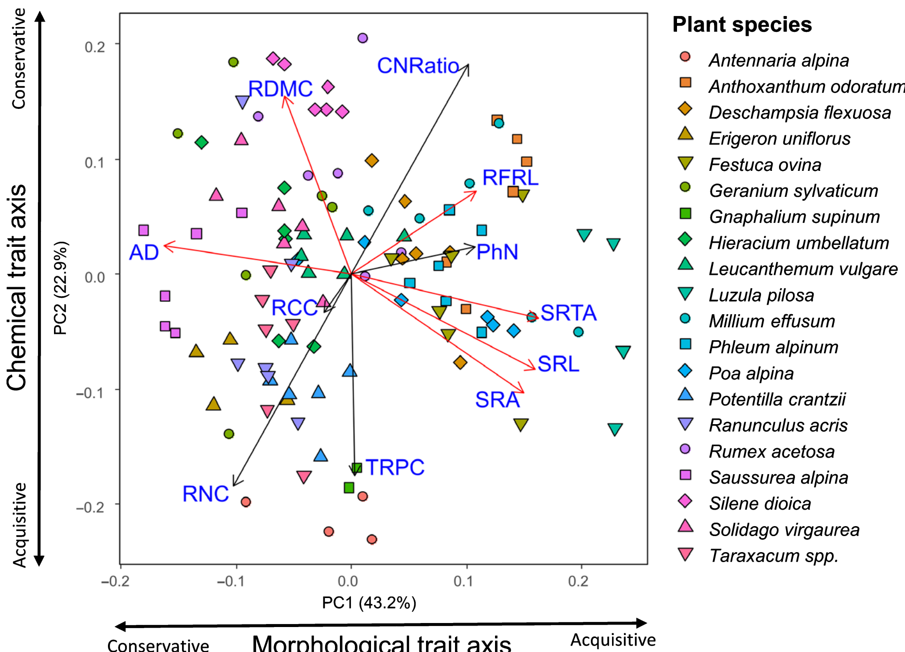

Fig. 1 Principal component analysis of fine root traits across all species. Black arrows represent chemical root traits and red arrows represent morphological root traits. Fine root traits corresponding to the bi-plot arrows are shown in blue font: average diameter (AD); root carbon content (RCC); root nitrogen content (RNC); root phenol content (TRPC); specific root area (SRA); specific root length (SRL); specific root tip abundance (SRTA); root phenol to nitrogen ratio (PhN); root forks per root length (RFRL); root carbon to nitrogen ratio (CNRatio) and root dry matter content (RDMC).

$r=-0.22$ with Gram-positive bacteria and $r=-0.24$ with actinobacteria; Fig. 2a). Furthermore, fungal to bacterial ratios were positively correlated with root forks per root length ($r=0.19$) and root C-to N-ratio ($r=0.20$) and negatively with root N content ($r=-0.23$). In addition, the ratio of Gram-positive to Gram-negative bacteria was positively and negatively correlated with root N content ($r=0.20$) and root C-to-N ratio ($r=-0.20$), respectively (Fig. 2a). However, the remaining seven fine root traits were not strongly correlated with any of the PLFA biomarkers.

Fine root traits and fungal community composition

Across all samples, saprotrophs–pathogens were the most abundant guild (mean relative abundance = 37.2% ± 5.3 SD), followed by yeasts and moulds (mean = 24.1% ± 6.6 SD), root-associated fungi (mean = 19.0% ± 6.5 SD), fungi with unknown functions (mean = 12.9% ± 2.8 SD), ectomycorrhizal fungi (mean = 3.0% ± 1.0 SD), fungi with other functions (mean = 2.8% ± 1.8 SD) and arbuscular mycorrhizal fungi (mean = 1.2% ± 1.1 SD) (see Fig. S2).

We found no evidence for a relationship between the fine root economics spectrum (represented by PCA axis scores) and the relative abundances of saprotrophs–pathogens, yeasts and moulds, and arbuscular mycorrhizal fungal functional guilds (see Table S2; Fig. 2b). However, the relative abundance of arbuscular mycorrhizal fungi was significantly and positively correlated with root C content ($r=0.44$; Fig. 2b), and negatively correlated with root forks per root length ($r=-0.39$). In addition, specific root tip abundance was negatively correlated with the relative abundance of arbuscular mycorrhizal fungi ($r=-0.25$; Fig. 2b). Furthermore, root forks per root length and the relative abundance of the fungal guild 'unknown' were positively correlated ($r=0.27$; Fig. 2b). Correlations between all individual fine root traits and the relative abundances of yeasts and moulds, ectomycorrhizal fungi and other root-associated fungi were not significant (Fig. 2b).

Overall, the fine root traits in the pCCA explained almost 10% of the variation in the relative abundances of fungal orders (Fig. 3). After accounting for block effects (accounting for 7.5% of the variation), two root traits, that is root forks per root length and root C content, significantly explained the variation in the relative abundance of fungal orders ($F=5.79$; $P=0.001$ and $F=4.45$; $P=0.001$, respectively), and the effects of a third trait (root N content) was not significant at $\alpha=0.05$ ($F=1.62$; $P=0.082$). The arbuscular mycorrhizal orders Archaeosporales and Glomerales were positively associated with root C content and negatively associated with root forks per root length. We found no phylogenetic signal for root C content (Blomberg's $K=0.16$; $P=0.87$) and root forks per root length (Blomberg's $K=0.30$; $P=0.15$). Conversely, the order Diaporthales, consisting of known pathogens, saprobes and endophytes, was associated with higher values of root forks per root length. Mucorales (moulds) was associated with higher values of root N content. Meanwhile, Sporidiobolales (yeasts) was associated with lower values of root N content, but its relative abundance did not vary with any of the morphological root traits. The orders Pezizales and Xylariales (which in our dataset consisted of saprotrophs–pathogens) were associated with higher and lower values of root C content and root N content, respectively.

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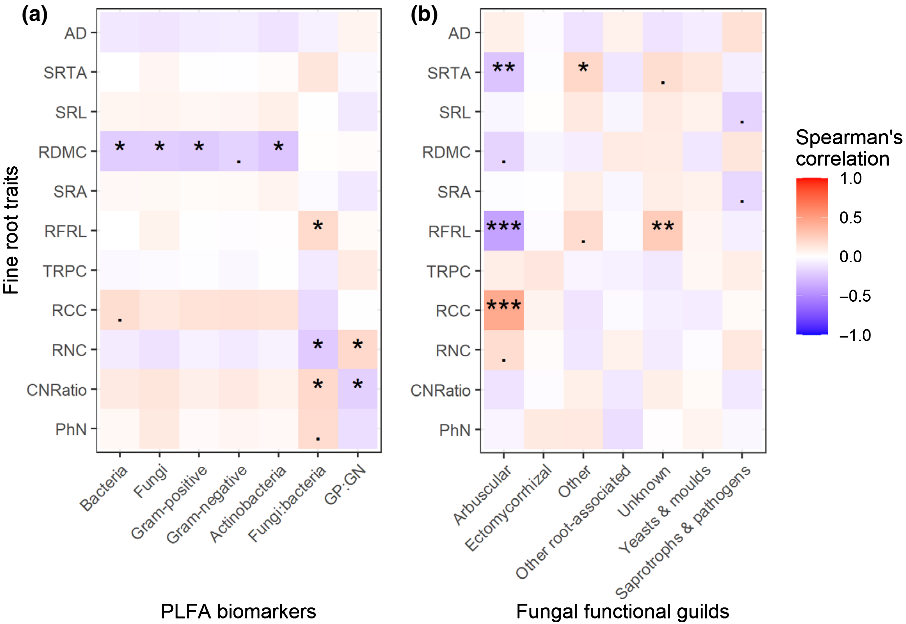

Fig. 2 Spearman's rank correlation matrices between fine root traits and absolute abundances of PLFA biomarkers, as well as for relative abundances of fungal guilds across all experimental units. (a) Fine root traits vs PLFA biomarkers. (b) Fine root traits vs fungal functional guilds. The 11 root traits tested are root phenol to nitrogen ratio (PhN), root carbon to nitrogen ratio (CNRatio), root nitrogen content (RNC), root carbon content (RCC), root phenol content (TRPC), root forks per root length (RFRL), specific root area (SRA), root dry matter content (RDMC), specific root length (SRL), specific root tip abundance (SRTA) and average diameter (AD). GP : GN means the ratio of Gram-positive to Gram-negative bacteria. Asterisks indicate statistical significance (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; '.' indicates $0.05 < P \leq 0.1$).

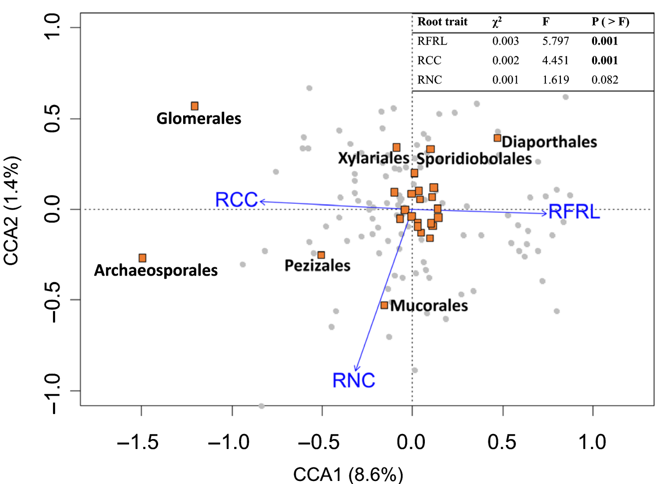

Fig. 3 Partial canonical correspondence analysis of fungal orders constrained by fine root traits across all experimental units. The blue arrows are fine root traits: root carbon content (RCC), root forks per root length (RFRL) and root nitrogen content (RNC). Grey dots represent the experimental units. Open squares are the fungal orders. Black text labels correspond to fungal orders with co-ordinates larger than ±0.25 on either axis. Plot is displayed with symmetric scaling. Fungal orders Archaeosporales and Glomerales are arbuscular mycorrhizal symbionts (Morton & Redecker, 2001; Helgason *et al*., 2003). Diaporthales consists of known pathogens, saprobes and endophytes (Rossman *et al*., 2007). Pezizales and Xylariales consist of species with various functions, but in our study the taxa for these orders are all saprotrophs–pathogens. Mucorales and Sporidiobolales are moulds and yeasts, respectively. The table in the upper right corner shows the statistical results of the model, with bold numbers indicating statistical significance at $\alpha = 0.05$.

Discussion

We showed that fine root traits of sub-arctic meadow plant species are associated with the relative abundance of various soil microbial groups and fungal guilds, but that the strength of these associations varied and were not necessarily particularly strong and/or did not apply to all measured root traits. We also found that the chemical trait axis of the fine root economics spectrum (but not the morphological trait axis) is a useful predictor of the ratios of broad microbial groups as measured by PLFA analysis. Conversely, the relative abundances of major fungal functional guilds were not associated with the root economics spectrum. However, more detailed analyses of the taxonomic composition of fungi showed that the relative abundance of several fungal orders were related to some chemical and morphological fine root traits. Below, we discuss these findings (see overview in Fig. 4) in relation to our hypotheses.

PLFA biomarkers and fine root traits

We found partial support for our first hypothesis that acquisitive fine root trait values of sub-arctic tundra meadow plant species are associated with lower ratios of fungi to bacteria and of Gram-positive to Gram-negative bacteria. The second PCA axis, which comprised primarily chemical traits (i.e. total polyphenol content and root C-to-N ratios) rather than morphological traits, was most important for explaining these ratios. Lower C : N ratios (and therefore more acquisitive trait values) may favour bacteria over fungi, because fungi have lower nutrient requirements (Fanin *et al.*, 2013) and possess a broader suite of enzymes for

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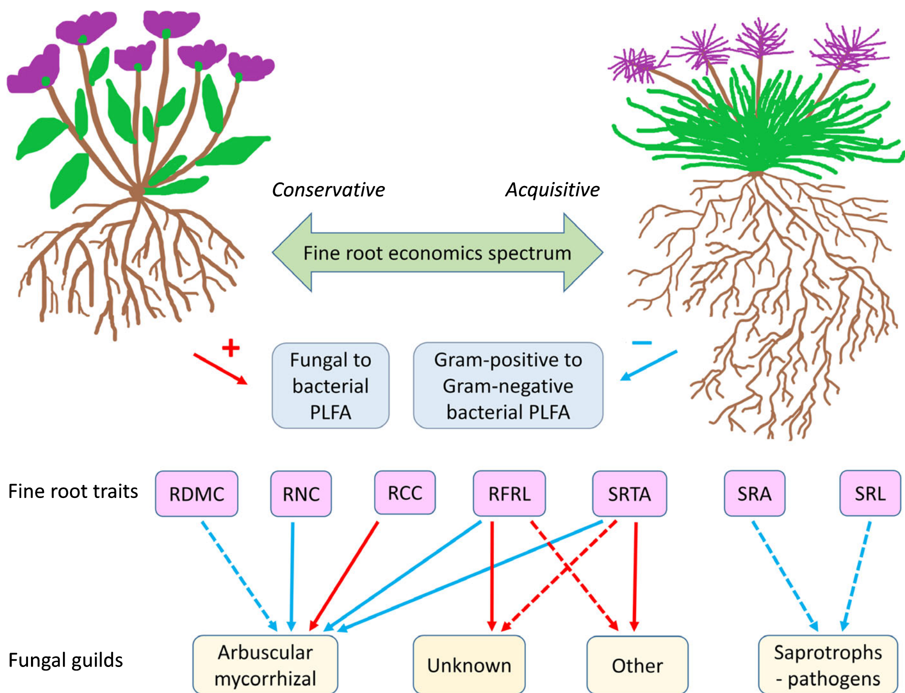

Fig. 4 Conceptual figure synthesising the new insights from this study into observed associations between fine roots and soil microorganisms. The fine root economics spectrum consists primarily of chemical root traits. Only fine root traits with significant or nearly significant associations with any fungal functional guild at $\alpha = 0.05$ are included: root nitrogen content (RNC), root carbon content (RCC), root forks per root length (RFRL), specific root area (SRA), root dry matter content (RDMC), specific root length (SRL), and specific root tip abundance (SRTA). Red arrows indicate positive relationships, while blue arrows indicate negative relationships. Solid arrows indicate a significant association and broken arrows a nearly significant association at $\alpha = 0.05$.

decomposition (Strickland & Rousk, 2010; Soares & Rousk, 2019). Conversely, the negative association that we found between the ratio of Gram-positive to Gram-negative bacteria and fine roots with higher C : N ratios may be due to a higher need by Gram-positive bacteria for nutrients to enable decomposition of more complex C forms (Orwin *et al.*, 2018; Fanin *et al.*, 2019a). Prior studies have shown differential responses of Gram-positive bacteria and fungi to N addition (Maaroufi *et al.*, 2015). Meanwhile, the morphological root trait axis was not associated with either ratios of soil fungi to bacteria or Gram-positive to Gram-negative bacteria, except for the positive correlation between root forks per root length and fungal to bacterial ratios. Nevertheless, root dry matter content could be potentially important for influencing the soil microbial biomass, because it was negatively correlated with the total abundance of all PLFA biomarkers except Gram-negative bacteria. Together, these relationships suggest that both some chemical components of the fine root economics spectrum, as well as root dry matter content, can predict the biomass of broad soil microbial groups and their ratios within the sub-arctic tundra meadow. Hence, within the cold and nutrient-poor tundra, these broad microbial groups may respond to tissue stoichiometry and nutrients rather than morphological root traits.

Fine root traits and fungal functional guilds

Contrary to our second hypothesis, neither the chemical nor the morphological trait axes of the fine root economics spectrum explained the relative abundances of any of the fungal guilds. This was unexpected because the allocation of plant resources to fine roots is expected to be linked to nitrogen acquisition (Reich, 2014), which is coupled to fungal guilds involved in nutrient cycling. In particular, the morphological trait axis, which is similar to the collaborative gradient presented in Bergmann *et al.* (2020), was expected to be important for predicting the relative abundance of arbuscular mycorrhizal fungi. However, some individual fine root traits did explain a significant proportion of the variation in fungal taxa and functional guilds, which indicates that the fine root economics spectrum may not necessarily be the 'holy grail' when examining root–microorganism interactions. For example, the relative abundances of arbuscular mycorrhizal fungal orders had strong positive associations with root C content. This could be due to the production of C-rich cellular structures by conservative plants that depend more on mycorrhiza for nutrient uptake under the nutrient-limited conditions (Li *et al.*, 2015) that characterise the sub-arctic tundra. However, caution should be taken with this interpretation, because some mycorrhizal hyphae, and the lipids that they store in their vesicles (Luginbuehl *et al.*, 2017), may be included in the measurements of total root C content, potentially elevating estimates of root C content. Nevertheless, as the biomass of plant roots is several magnitudes greater than that of the arbuscular fungi in the roots (Hart & Reader, 2002), it is unlikely that this could explain the relationship between root C content and the relative abundance of arbuscular mycorrhizal fungi.

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Furthermore, we found a significant negative correlation between the relative abundance of arbuscular mycorrhizal fungi and root forks per root length. This is likely to have been because roots colonised with arbuscular mycorrhizal fungi can have lower branching intensity (Comas *et al.*, 2014) due to their increased dependence on mycorrhizal foraging (Eissenstat *et al.*, 2015; McCormack *et al.*, 2015). However we did not find average fine root diameter to be associated with the relative abundance of arbuscular mycorrhizal fungi, despite previous studies showing a positive relationship between average root diameter and mycorrhizal root colonisation (Ma *et al.*, 2018; McCormack & Iversen, 2019). This is likely to have been due to the focus of our study on colonisation of arbuscular mycorrhizal fungi in the rhizosphere soil (i.e. the soil zone surrounding roots; Bais *et al.*, 2006), as opposed to in the fine roots themselves. Furthermore, although arbuscular mycorrhizal associations within arctic ecosystems are ubiquitous, there is a high variation in fine root colonisation across species (Gardes & Dahlberg, 1996; Newsham *et al.*, 2009). As such, the plant-mediated factors contributing to arbuscular mycorrhizal colonisation rates in arctic ecosystems require further investigation.

Fine root traits, saprotrophs-pathogens and yeasts and moulds

By contrast with our expectations, the saprotroph–pathogen guild was not significantly associated with any fine root trait, despite it being the most abundant one in our study. Other studies have found that high specific root length is associated with a shorter root life span (Weemstra *et al.*, 2020), which could in turn stimulate oligotrophic saprotrophs through a greater production of high quality root litter. However, faster root turnover could also serve as a mechanism to achieve a lower abundance of pathogens (Dietze & Matthes, 2014). In addition, the nutrient resorption rates for sub-arctic plant species are high (Freschet *et al.*, 2010), which should reduce the quality of root litter inputs and in turn negatively affect the relative abundance of copiotrophic decomposers. In addition, we found evidence that the biomass of bacteria, which are largely saprotrophic, is favoured by acquisitive root trait values. This suggests that saprotrophic bacteria may have stronger links than fungi to fine root traits (particularly those related to root N content), which could be of particular importance in sub-arctic meadows (rather than heath communities) where there may be a high abundance of bacteria relative to fungi (Sundqvist *et al.*, 2011).

We further showed that the relative abundance of yeasts and moulds in arctic soil is high (24%), compared with 0.4–14.3% found in temperate forests (Mašínová *et al.*, 2017), probably due to their tolerance for cold and stressful environments (Treseder & Lennon, 2015). Yeasts and moulds primarily derive their C from simple C compounds exuded by plant roots (Botha, 2006; Pawłowska *et al.*, 2019). While little studied, there is evidence that yeasts may have positive effects on nutrient recycling, plant growth and soil structure (Botha, 2011). Although we found no significant associations between the fine root economics spectrum and the relative abundances of yeasts and moulds, there was a strong positive relationship between root nitrogen content and the mould order Mucorales, and a negative association between root nitrogen content and the yeast order Sporidiobolales. The positive association between root nitrogen content (RNC) and Mucorales suggests a high N requirement for maintaining their rapid growth rates (Mehrotra, 1967). Conversely, the negative association between RNC and the yeast order Sporidiobolales suggests that they may be less reliant on freshly available plant-derived N.

Conclusions and future outlook

Overall, our study showed that the chemical trait axis of the fine root economics spectrum is a useful predictor of the relative abundance of major microbial groups, such as the ratios of soil fungi to bacteria and Gram-positive to Gram-negative bacteria. Conversely, individual fine root traits may be more important for understanding relationships with fungal community composition, that is, soil fungal guilds and at the taxonomic order level, although this might be influenced by root or fungal carbon storage. This suggests that it may be more useful to focus on individual fine root traits when research is aimed at understanding the effects on specific fungal guilds. Together, these findings (summarised in Fig. 4) provide new insights into the relationships between fine root traits and rhizosphere microbial communities. However, our study was focused on sub-arctic tundra meadow and not heath vegetation, which typically has higher fungal to bacterial ratios (Sundqvist *et al.*, 2011). Hence, the effects of fine root traits on fungal guilds may potentially be stronger in sub-arctic heath than in sub-arctic meadows. Based on these new insights, below we present some lessons learnt and suggest considerations for future research related to plant–microorganism interactions within the sub-arctic tundra.

First, the fine root economics spectrum represents a simplistic one-dimensional spectrum (i.e. acquisitive vs conservative), whereas there may be several dimensions of resource use (Kramer-Walter *et al.*, 2016; Kong *et al.*, 2019) that could each be linked to different subsets of the fungal community. For example, Bergmann *et al.* (2020) identified two main axes of root trait variation related to plant strategies for nutrient acquisition with implications for plant–microorganism interactions ('do it yourself vs outsourcing' and 'slow vs fast turnover'). In our study, the chemical trait axis predicted the ratios of broad microbial groups, while the morphological trait axis did not predict the abundance of microorganisms. Therefore, in order to understand fine root–microorganism interactions, the use of individual fine root traits may be more informative. However, although there are large numbers of potential fine root traits (Iversen *et al.*, 2017), the selection of fine root traits in studies related to plant–microorganism associations should be based on *a priori* knowledge of their ecological relevance. For example, in our study, root traits were selected *a priori* based on ecological relevance (Table 1) and we found associations between six out of 11 selected root traits and various soil microbial guilds. In addition, at larger scales, other factors not addressed in our study (e.g. soil OM

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quality) may also be important drivers of soil microbial groups in the tundra (Eskelinen *et al*., 2009).

Second, the resolution at which microbial communities are examined may influence the observed fine root–microbe relationships. For example, bulking of fungal taxa into functional guilds may obscure existing and ecologically relevant patterns, because some fungal taxa within a particular guild may have stronger associations with fine root traits than might others, and because different taxa could have opposing relationships with the same traits. In addition, our understanding of fine root–microorganism interactions may be obscured by a lack of knowledge about the function of several soil microbial taxa in general, but particularly in arctic ecosystems. For example, in our study there was a high relative abundance of dark septate endophytes, yeasts and other fungi with unknown functions that were associated with fine roots. Their functions and benefits to plants are currently still not well understood. Thus, a more detailed investigation of the ecological functions of these understudied organisms will be important for advancing the field of plant–microorganism–soil interactions within arctic and other 'cold' ecosystems.

Finally, understanding root trait–microbe relationships is an essential foundation for predicting responses of C cycling to global change. For example, the positive effects on arbuscular mycorrhizal fungi colonisation in fine roots that is expected with elevated temperature (Bennett & Classen, 2020) could in turn affect C sequestration (Drigo *et al*., 2010). In addition, responses of root traits to global change (Parts *et al*., 2019) could influence the relative abundance of various fungal guilds that contribute in varying degrees to soil C cycling. Taken together, it is important to understand root–microorganism interactions as a means towards understanding implications for future ecosystem C balance in arctic ecosystems.

Acknowledgements

We thank Rémy Beugnon and Tania Maxwell for assistance in the glasshouse and the laboratory. We also thank the Department of Forest Mycology and Plant Pathology, SLU, for providing workspace for DNA extractions. The authors would like to acknowledge the support of the National Genomics Infrastructure (NGI)/Uppsala Genome Centre at UPPMAX for providing assistance in massive parallel sequencing and its computational infrastructure. Work performed at NGI/Uppsala Genome Centre was funded by RFI/VR and Science for Life Laboratory, Sweden. This project was funded by a grant (grant no. 2015-04214) from the Swedish Research Council (Vetenskapsrådet) to PK.

Author contributions

CMS, PK and DAW designed the study. CMS and PK collected soils and seeds. CMS conducted the glasshouse study and laboratory work. BL supervised the molecular laboratory work. CMS conducted the fungal annotations, with supervision from BL and NF. CMS performed the statistical computations. NF verified the analytical methods. CMS took the lead in writing the manuscript. CMS, BL, DAW, MKS, MJG, NF and PK discussed the results and contributed to each draft of the manuscript.

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Fig. S1 Phylogenetic tree with the 20 tundra plant species included in the experiment and their functional group assignment.

Fig. S2 Boxplot showing relative read abundances of fungal functional guild across all experimental units.

Table S1 Mean fine root trait values for all plant species with standard deviations.

Table S2 Summary statistics of generalised linear mixed effects models with relative abundances of fungal functional guilds as explained by the root economics spectrum.

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