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Research

Root-associated fungi and acquisitive root traits facilitate permafrost nitrogen uptake from long-term experimentally warmed tundra

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Summary

- Root-associated fungi (RAF) and root traits regulate plant acquisition of nitrogen (N), which is limiting to growth in Arctic ecosystems. With anthropogenic warming, a new N source from thawing permafrost has the potential to change vegetation composition and increase productivity, influencing climate feedbacks. Yet, the impact of warming on tundra plant root traits, RAF, and access to permafrost N is uncertain.
- We investigated the relationships between RAF, species-specific root traits, and uptake of N from the permafrost boundary by tundra plants experimentally warmed for nearly three decades at Toolik Lake, Alaska.
- Warming increased acquisitive root traits of nonmycorrhizal and mycorrhizal plants. RAF community composition of ericoid (ERM) but not ectomycorrhizal (ECM) shrubs was impacted by warming and correlated with root traits. RAF taxa in the dark septate endophyte, ERM, and ECM guilds strongly correlated with permafrost N uptake for ECM and ERM shrubs. Overall, a greater proportion of variation in permafrost N uptake was related to root traits than RAF.
- Our findings suggest that warming Arctic ecosystems will result in interactions between roots, RAF, and newly thawed permafrost that may strongly impact feedbacks to the climate system through mechanisms of carbon and N cycling.

Introduction

Striking vegetation transformations have been observed across the circumarctic in recent decades (Sturm *et al.*, 2001; Tape *et al.*, 2006; Elmendorf *et al.*, 2012). These shifts have the potential to amplify or moderate feedbacks to the climate system (Chapin *et al.*, 2005; McGuire *et al.*, 2006; Blok *et al.*, 2010; Pearson *et al.*, 2013), yet the underlying mechanisms that facilitate vegetation change with warming are not clear. The strong nutrient limitation of tundra ecosystems (Chapin & Shaver, 1985; Mack *et al.*, 2004; DeMarco *et al.*, 2014) suggests that plant traits influencing nutrient uptake will likely affect the pace and magnitude of vegetation transitions with future warming.

Arctic tundra is underlain with permafrost soils (Brown *et al.*, 1997). With directional warming, the active layer, the seasonally thawed portion of the soil profile, deepens, releasing nitrogen (N) from previously frozen soil (Keuper *et al.*, 2012; Salmon *et al.*, 2018). As decomposition ensues, the pool of newly thawed permafrost N matches or exceeds the pool of N in active layer soils with upward of 1.8–3 g N m^{-2} released (Mack *et al.*, 2010; Keuper *et al.*, 2012), an amount that meets annual plant demand (Shaver & Chapin, 1991). To access newly thawed N, plants rely on root proliferation and mycorrhizal fungi. Recent studies document that the deep roots of nonmycorrhizal (NM) plants with low-growth potential enable them to take up permafrost N late in the growing season when the active layer thickness is greatest (Keuper *et al.*, 2017; Blume-Werry *et al.*, 2019; Hewitt *et al.*, 2019), whereas shallowly rooted but high-growth potential mycorrhizal shrubs may need to rely on mycorrhizal fungi to access newly thawed permafrost N (Hewitt *et al.*, 2020).

The traits of fine roots and identities of root-associated fungi (RAF) underlie resource acquisition strategies. For root traits, this can present as a fine-root economic spectrum, a gradient from acquisitive to conservative strategies (Freschet *et al.*, 2010). Acquisitive traits, for example, low root tissue density (RTD) and high tissue N, are suggestive of rapid acquisition of soil nutrients and intensive resource use, that is carbon (C) currency spent, to support high-growth potential. By contrast, conservative traits, such as high RTD and lower tissue N are characteristics of slow growth, low-resource use, and greater investment in well-defended slow-cycling tissues. Increasingly, studies show that variation in fine-root chemical and morphological traits is represented by multiple gradients and impacted by RAF (Weemstra *et al.*, 2016; McCormack & Iversen, 2019; Weigelt *et al.*, 2021), resulting in a multidimensional root economic space (Bergmann *et al.*, 2020). Plants can employ a resource acquisition strategy that ranges from nonmycotrophic, that is reliant on root production and high specific root length (SRL), to mycotrophic, that is nearly complete reliance on RAF and with high diameter roots that support colonization by RAF (Bergmann *et al.*, 2020). Because mycorrhizal relationships can alter root morphology and plant resource economics, they are associated with variation in root traits (Peay *et al.*, 2011; McCormack & Iversen, 2019; Spitzer *et al.*, 2021).

In tundra, ericoid (ERM) and ectomycorrhizal (ECM) fungi along with dark septate endophytes (DSE) dominate (Newsham *et al.*, 2009; Timling & Taylor, 2012), are implicated in soil nutrient acquisition (Read & Perez-Moreno, 2003; Newsham, 2011), and exhibit taxon-specific variation in nutrient transfer to host plants as well as costs of the symbiosis to plants and fungi (Smith & Read, 2008). RAF species vary in traits related to nutrient acquisition in ways akin to roots. For example, some ECM fungi produce long-distance cords to transport nutrients long distances, while others produce thinner hyphae with higher surface area for nutrient uptake (Agerer, 2001; Chen *et al.*, 2018). Furthermore, there are associations between specific plant root traits and mycorrhizal traits (Peay *et al.*, 2011), but the majority of research on root traits has not considered RAF (Schaffer-Morrison & Zak, 2023), particularly at more specific levels than broad fungal guilds.

Warming of the tundra and increasing resource availability with newly thawed permafrost may provide conditions that promote acquisitive root traits or particular fungal associations that facilitate permafrost N uptake. Plants may shift allocation to building acquisitive roots with a nonmycotrophic strategy, that is more root biomass and/or deeper exploration. Furthermore, RAF with more acquisitive strategies may colonize more acquisitive roots as plants and fungi are released from conservative resource strategies that prevail with harsh conditions. Alternatively, if plants are highly mycotrophic and reliant on RAF for resource acquisition, root traits could show little sensitivity to warming, maintaining some of the conservative strategies well-aligned with low-resource conditions. We investigated the impact of nearly three decades of experimental warming on the relationships between fine-root traits, RAF, and uptake of permafrost N. We sampled the longest-running tundra ecosystem warming experiment at Toolik Lake, Alaska, to address the following hypotheses: (H1) warming will be associated with more acquisitive and less conservative root traits because in warmed conditions, plants can allocate more resources to rapid growth; (H2) morphological root traits related to exploration will be related to N uptake from the permafrost boundary, particularly for NM plants, (H3) whereas for ERM and ECM plants, traits associated with the root tip environment where fungi occur, that is diameter, and specific RAF associations will shift to more acquisitive strategies; and lastly (H4) RAF that are proficient at permafrost N uptake will primarily associate with ECM shrubs that dominate with warming. Overall, we predict that the root traits and the specific RAF will shift to more acquisitive strategies with strong implications for C and N cycling as warming occurs.

Materials and Methods

Experimental design

To investigate the effects of long-term warming on belowground dynamics of moist acidic tussock tundra, we sampled the whole ecosystem warming experiment established in 1989 at the Arctic Long Term Ecological Research site (68°38′N, 149°36′W), Toolik Lake, Alaska, in August 2016. The experiment has four replicate blocks, each containing a glasshouse and control plot, for a total of four replicate warmed glasshouse ($n = 4$) and ambient control ($n = 4$) plots. Glasshouses are made of wooden frames (dimensions 5×2.5 m footprint and 1.5 m in height) with a 0.15 mm polyethylene cover mounted after snowmelt each growing season and removed for the fall, winter, and spring. In the ambient plots, the vegetation biomass is equally distributed between NM graminoids (the tussock forming sedge, *Eriophorum vaginatum* L.), ERM evergreen (*Rhododendron tomentosum* Harmaja, *Vaccinium vitis-idaea* L., *Empetrum nigrum* L., *Cassiope tetragona* (L.) D.Don) and ERM deciduous shrubs (*Vaccinium uliginosum* L.), ECM deciduous shrubs (*Betula nana* L., *Salix pulchra* Cham.), the abundant NM forb (*Rubus chamaemorus* L.), and mosses (*Sphagnum* spp., *Hylocomium splendens* (Hedw.) Schimp., and *Aulacomnium* spp., Shaver & Chapin, 1991). The NM forb *Pedicularis oederi* Vahl occurs at lower abundances. Previous studies demonstrated that experimental warming resulted in greater vascular aboveground and rhizome biomass, mostly due to increases in the deciduous shrub *B. nana*, but also by other functional groups like forbs (*R. chamaemorus*) with losses of mosses and lichens (Chapin *et al.*, 1995; Sistla *et al.*, 2013; Dunleavy & Mack, 2021). The mean growing season air temperature in warmed plots is 2°C higher, and the soil temperature at 10–40 cm depth is 1–2°C higher than in control plots (Deslippe *et al.*, 2011; Sistla *et al.*, 2013; Dunleavy & Mack, 2021). Warming increases thaw depth (*c.* 58 ± 6 cm compared with *c.* 41 ± 2 cm in control plots) and reduces precipitation but does not alter soil moisture (Clemmensen *et al.*, 2006; Deslippe *et al.*, 2011; Dunleavy & Mack, 2021).

Isotope addition

In warmed and ambient plots, we applied 98 atom % ^{15}N ammonium chloride directly above the permafrost table in August when the active layer was mostly thawed. In ambient plots, additions were made at 13 injection points within a 1 m^2 plot by inserting a frost probe, recording active layer thickness, and then inserting a sheathed stainless-steel needle (custom-built at Jon's Machine Shop, Fairbanks, Alaska) into the hole created by the frost probe. When the needle hit the permafrost table, the sheath was retracted, uncovering pin holes at the tip of the needle. A syringe was connected to the far end of the needle, tracer was injected, the needle was then retracted into the sheath, and the whole injection system was retracted from the soil. The sheath ensured that labeling occurred at maximum active layer depth by preventing the tracer from bleeding out as the needle was retracted. In ambient plots, this resulted in 250 mg of ^{15}N applied to the 1 m^2 plot. Due to space constraints in the glasshouse structures, our tracer addition was scaled to five injection points (Supporting Information Fig. S1). Twenty-four hours after application of the label, we harvested roots and mycorrhizas from active layer organic and mineral soils.

Belowground harvest

We harvested an organic soil monolith (10 × 10 cm) with a depth of the full organic horizon. Below the monolith, we cored mineral soils (7 cm diameter core) until we hit the ice at the permafrost boundary. The soil monolith and core dimensions were measured in the field and transported back to the laboratory, and the monolith and cores were separated by depth increments (0–5, 5–15, 15–25, 25–35 cm, and so on). The transition from organic to mineral soils was noted, and mineral soils were separated from organic soils.

Root processing

Live roots and rhizomes were removed from organic and mineral soils at each depth increment by hand. Roots and rhizomes were separated by size class (> or < 2 mm) and then into plant species morphotype groups based on color, texture, and branching pattern. Live roots were separated from dead roots based on turgidity, color, and tensile strength. Using microscopy (×10–40), we further assessed the consistency of appearance within species morphotype pools and removed any remaining dead roots. From this cleaned root sample, we subsampled the fine roots (< 2 mm) to analyze traits, C and N content and isotopes, and RAF taxa. First, the wet weight of a root subsample (*c.* 200 mg) was measured; these roots were scanned using WinRhizo (Regent Instruments Inc., Quebec, QC, Canada) to determine root length, projected area, and the number of tips and forks, dried at 60°C for 48 h and reweighed to obtain the dry weight. Second, from the cleaned root sample, 10 fine-root segments were preserved in RNAlater (Ambion Inc., Austin, TX, USA) for molecular verification of the plant species identity and characterization of the RAF community following methods described previously (Hewitt *et al.*, 2019, 2020) and in brief below. Third, the remaining root biomass was weighed wet, dried at 60°C, and reweighed dry. Percent C and N and isotopes were measured on a subsample, dried at 60°C for 48 h, ground, and run for C and N analysis on an Isotope Ratio Mass Spectrometer (IRMS, Delta Advantage; Thermo Fisher Scientific, Waltham, MA, USA) coupled to an Elemental Combustion Analyzer (ECS4010; Costech, Valencia, CA, USA) at Northern Arizona University.

Root identity and fungal analyses

Plant and fungal DNAs were extracted from a small section of each of the 10 root segments from each species morphotype sample using the Qiagen DNEasy Plant Mini Kit following the manufacturer's instructions with the exception of the addition of a proteinase K incubation to release DNA from protein complexes for 4 h at 55°C before step 9. We amplified the entire ITS region of the plant DNA with the primers ITS1P and ITS4 (White *et al.*, 1990). PCR products were incubated overnight at 60°C with the restriction enzyme BStuI (New England Biolabs, Ipswich, MA, USA). The digested amplicons were then run in a 3% agarose gel with control samples to assess ITS restriction fragment length polymorphism, as detailed in Hewitt *et al.* (2019). After root sample identity was confirmed, the gDNA from each species at each depth was pooled into roughly equal concentrations. The fungal ITS2 region of the pooled gDNAs was then amplified using 5.8S_FUN and ITS4_FUN (Taylor *et al.*, 2016). Fungal amplicons were sequenced using a v.3 600 cycle kit on the Illumina MiSeq (Illumina, San Diego, CA, USA) at the University of New Mexico; sequencing methods are detailed in Hewitt *et al.* (2020) and DeVan *et al.* (2023).

Bioinformatics

All bioinformatics steps were completed with the Uparse pipeline primarily using Usearch v.9.2.64_i86linux64 (Edgar, 2013). Paired ends were merged using the fastq_mergepairs command with a minimum overlap of 50 and maximum mismatch of 10%. Barcodes and adaptors were removed using the fastx_truncate command. We used the fastq_eestats2 command to find the optimum length for global trimming to maximize the number of high-quality reads. Reads were trimmed to 310 base pairs using fastx_truncate and filtered to have a maximum expected error of 1.0 using the fastq_filter command. Reads were dereplicated and counted using fastx_uniques before clustering OTUs with the cluster_otus command. PhiX was removed using the filter_phix command. Taxonomy was assigned to Usearch sin_tax using the UNITE database with an 80% confidence cut-off. For OTUs with poorly classified taxonomy, we also used the Warcup database with the RDP classifier online (Deshpande *et al.*, 2016) and blasted the most abundant taxa on NCBI Blastn excluding uncultured/environmental sample sequences. If a match to a type specimen was found with at least 97% identity to our sample, the taxonomy was assigned to genus or if there was at least 90% identity to our sample, the taxonomy was assigned to family following Timling *et al.* (2012). Query cover was always > 99%. Guilds were assigned to OTUs using FUNGuild and investigator knowledge (Nguyen *et al.*, 2016).

Statistics

Variation in tundra root traits To provide an integrated picture of the variation in all measured root traits (Table 1) and determine whether root traits in the experiment are arrayed along multiple gradients in a multidimensional root economic space, we used principal components analysis (PCA, Goodall, 1954). We used PCA because it is an ideal technique for data with approximately linear relationships among variables (McCune & Grace, 2002) and, therefore, complements the mixed effects models described below. To meet assumptions of normality, variables were log-transformed. The PCA was performed on the cross-product matrix that consisted of correlation coefficients, that is the variables were scaled. To interpret

Table 1 Root traits measured in ambient control and warmed glasshouse plots at Toolik Lake, Alaska.

	Trait	Abbreviation	Ecological significance
Morphological	Root tissue density (g cm^{-3})	RTD	Tissue quality
	Specific root area (cm^2 g^{-1})	SRA	Nutrient absorption
	Specific root length (cm g^{-1})	SRL	Soil exploration
	Specific root tip abundance (tips g^{-1})	SRTA	Soil exploration
	Specific root fork abundance (forks g^{-1})	SRFA	Soil exploration
	Maximum depth (cm)	Depth	Proximity to permafrost N
	Diameter (mm)	Diameter	Space for mycorrhizal colonization
Chemical	%N	%N	Tissue quality
	%C	%C	Tissue quality
Ecosystem	Root density (g cm^{-3})	Density	Indicator of allocation to resource uptake
	Root biomass (g m^{-2})	Biomass	Indicator of allocation to resource uptake
	Root N pool (g N m^{-2})	N pool	Indicator of N cost for resource uptake
	Root C pool (g C m^{-2})	C pool	Indicator of C cost for resource uptake

gradients in root traits, we evaluated Pearson correlation coefficients between the first two principal components (axes 1 and 2) and the individual root traits. We tested whether variation in root traits differed by host species, mycorrhizal status (NM, ERM, and ECM), warming treatment, and the goodness of fit between variation in root traits and permafrost N uptake (g ^{15}N g^{-1} root, H1–3) with the envfit function in the VEGAN package (Oksanen *et al.*, 2022) and using the Benjamini–Hochberg procedure for *P*-value adjustment for multiple comparisons (Benjamini & Hochberg, 1995).

To test whether root traits responded to experimental warming (H1), we built generalized linear mixed effects models with the LME4 (Bates *et al.*, 2015) and GLMMTMB (Brooks *et al.*, 2017) packages. For each model, mycorrhizal status (NM, ERM, and ECM) and treatment (ambient vs warmed) were the fixed factors, the root trait was the response variable, and species nested in block was the random factor to account for spatial nonindependence and variance within mycorrhizal status attributed to species level differences. For each species in each plot, we calculated morphological and chemical root traits (RTD, SRL, specific root area (SRA), diameter, specific root fork abundance (SRFA), specific root tip abundance (SRTA), %C, and %N) as plot means of the traits observed in each soil layer and ecosystem traits (biomass, density, N pool, and C pool) were calculated as plot sums of the traits observed in each soil layer, and maximum root depth was calculated as the plot maximum (Table 1). To improve model fit and meet model assumptions of normality and homogeneity of variance, we implemented a dispersion parameter of block in five of the models and a zero-inflation parameter in three (Table S1) based on the assessment of model residuals using the DHARMA package (Hartig, 2020). To determine the significance of fixed factors, we evaluated the small sample corrected Akaike information criterion (AICc), comparing the full model to a reduced model and the null model (Zuur *et al.*, 2009). Because we tested 13 traits with the same experimental units, we also assessed the log-likelihood ratio of the final model compared with the null at a reduced $\alpha = 0.01$ and greater delta-AICc > 10 to reduce Type 1 error. We conducted *post hoc* tests of the fixed factors with the EMMEANS package (Lenth, 2022) and corrected *P*-values by controlling for the false discovery rates with the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995).

Relationships between root-associated fungi, root traits, and N uptake We tested whether variation in RAF composition among our root samples was related to root traits and N uptake (H3, H4) using nonmetric multidimensional scaling (NMDS) ordinations (Kruskal, 1964) in the VEGAN package. We used NMDS because it is well-suited to nonnormal data and the most effective ordination method for ecological community data (McCune & Grace, 2002). We subset the full OTU matrix by OTUs in the guilds ECM, ERM, and DSE, the most likely to be involved in N uptake (Read & Perez-Moreno, 2003; Newsham, 2011). After graphical analysis of an initial ordination biplot that indicated strong separation of ERM and ECM hosts in ordination space, we separately ordinated ERM and ECM host plants to analyze relationships with root traits and permafrost N uptake. Each sample in the OTU matrix was relativized by total number of OTUs per sample. We used the Bray–Curtis distance matrix and 500 iterations. Correlations between RAF composition, root traits, warming treatment, and permafrost N uptake were assessed using the envfit function in the VEGAN package separately for ERM and ECM host plants (H3, H4). We also tested for strong gradients in RAF abundance that correlated with the variation in overall RAF composition. Lastly, we evaluated Spearman correlations between the relative abundance of individual RAF taxa and permafrost N uptake. For taxa that were observed to be significantly correlated with permafrost N uptake, we assessed whether they were indicators of the ambient control or warmed treatment using the INDICSPECIES package (De Caceres & Legendre, 2009).

Relative importance of root traits and RAF composition to permafrost N access We evaluated the importance of individual root traits and for mycorrhizal plants RAF associations on the uptake of permafrost N for NM ($n = 62$), ERM ($n = 49$), and ECM ($n = 26$) plants separately (H2–4) using random forest regression trees (Breiman, 2001). First, we ranked and eliminated variables with small importance from the full suite of measured root traits, and in the case of mycorrhizal host plants, NMDS

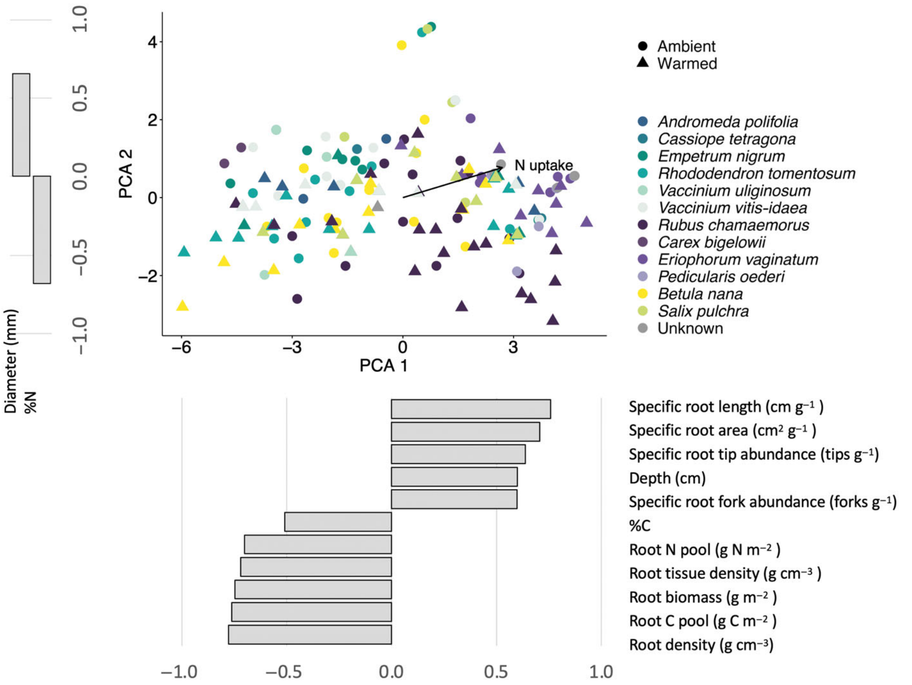

Fig. 1 Principal components analysis of root traits of tundra plant species in ambient and warmed plots at Toolik Lake, Alaska. Pearson correlation coefficients of root traits over 0.5 are depicted in relation to the correlated axis (PCA1, PCA2). Marker colors are green for ericoid mycorrhizal, purple for non-mycorrhizal, and yellow for ectomycorrhizal host plants. The vector represents the direction and magnitude of a significant correlations between root traits and permafrost N uptake ($P < 0.01$).

axes using the VSURF_thres function in the VSURF package (Genuer *et al.*, 2015). Next, we identified variables for interpretation in the random forest model by selecting variables with the smallest out-of-bag error with the VSURF_interp function. We computed variable importance scores for each selected predictor of permafrost N uptake for all plants together and individually by mycorrhizal status (NM, ERM, and ECM) using the RANDOMFOREST package (Liaw & Wiener, 2002). We then visually interpreted partial dependence plots of the selected variables.

To evaluate the variance in permafrost N uptake explained by RAF composition and root traits, we used variance partitioning analysis (Borcard *et al.*, 1992) for ERM host plants and ECM host plants separately (H3, H4) using the varpart function in the VEGAN package. We rank-transformed the response variables and z-transformed the predictor variables to meet model assumptions. The three NMDS axes represented the RAF contribution, while the 13 root traits represented the root trait contribution.

Results

Warming impacts on root traits

The first two axes of the PCA explained 72.25% of the variation in root traits with axes 1 and 2 explaining 59.52% and 12.73% of the variance, respectively. Variation in root chemical and morphological traits occurred along two main axes (Fig. 1), suggesting a multidimensional root economic space instead of a single root economic spectrum, for example %C with axis 1 and %N with axis 2. Axis 1 was positively correlated with several morphological traits like SRL, SRA, SRTA, depth, and SRFA and negatively with ecosystem variables like biomass, density, and C and N pools. Axis 2 was most strongly correlated with tissue %N and diameter. Root traits varied by host species ($r^2 = 0.30$, $P = 0.001$), mycorrhizal status ($r^2 = 0.15$, $P = 0.001$), and marginally by warming treatment ($r^2 = 0.02$, $P = 0.03$, H1).

Overall, warming promoted more acquisitive traits for plants regardless of mycorrhizal status (Figs 2, S2; Table S2), supporting H1. For NM plants, warming increased the SRTA, depth, and tissue %N, but lowered the root N pool (all contrasts $P < 0.05$). For ECM plants, warming increased RTD, SRA, SRL, SRTA, SRFA, biomass, %N, and N and C pools (all contrasts $P < 0.01$). ERM showed similar responses to ECM plants with the exception of increased rooting depth but no change in RTD or %N. We generally observed a significant interaction between plant mycorrhizal status and warming treatment for each root trait (Figs 2, S2; Table S2, all contrasts $P < 0.05$) except for fine-root diameter and %C. An additive model of treatment and mycorrhizal status was the best fit for %C with NM having lower tissue %C than ERM or ECM plants, but mycorrhizal plants not differing from each other and fine roots in the warmed plots having lower %C than those in ambient plots (Fig. S2, all contrasts $P < 0.01$). For root diameter, the model with warming treatment alone had the best fit compared with the full additive model including mycorrhizal status, and all plants responded similarly with a decline in root diameter in warmed plots (Fig. 2, all contrasts $P < 0.001$). The fine-root density model had the lowest delta AIC 5.48 between the null model and the best model and thus was excluded from our interpretation because it did not meet the criteria of AICc > 10. Within ambient and warmed plots, NM plants displayed greater fine-root SRA, SRL, SRTA, and STFA and deeper roots than ERM and ECM plants but lower RTD (Table S2, all contrasts $P < 0.001$). In ambient plots, ERM plants had higher RTD but lower SRA, SRL, and SRFA than ECM plants, but these patterns did not hold in warmed

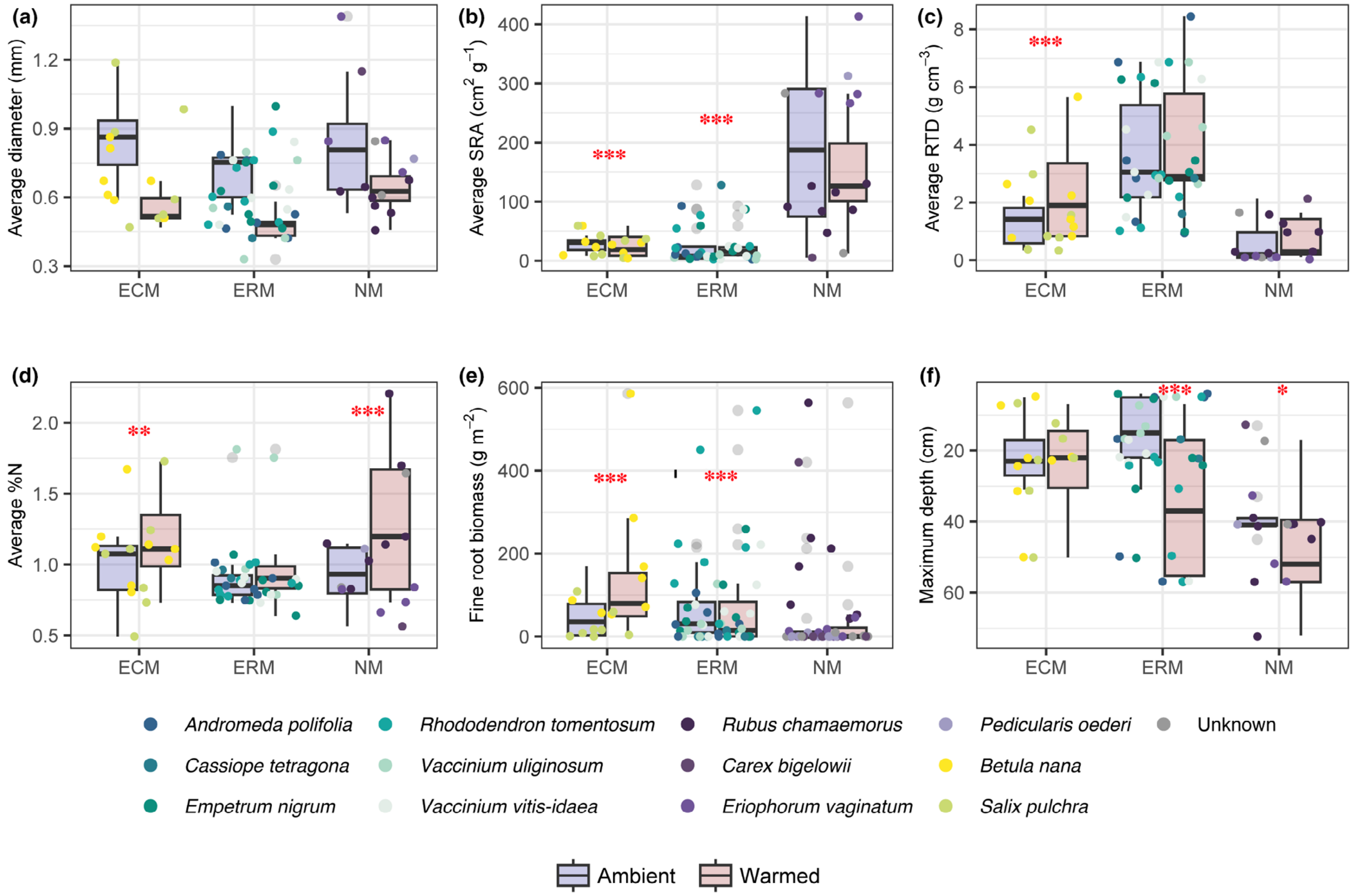

Fig. 2 Impact of experimental warming on six morphological, chemical, and ecosystem fine root traits (a–f) of non-mycorrhizal (NM), ericoid mycorrhizal (ERM) and ectomycorrhizal (ECM) host plants in ambient and warmed plots of the Arctic LTER whole ecosystem warming experiment. The lower and upper bounds of the boxplot show the first and third quartiles (the 25th and 75th percentiles), the middle line shows the median, whiskers above and below the boxplot indicate 1.5 × inter-quartile range, and points beyond the whiskers indicate outlying points. Specific root area ($cm^2 g^{-1}$, SRA); root tissue density ($g cm^{-3}$, RTD); root tissue percent nitrogen (%N). Generalized linear mixed effects models indicated a significant interaction between mycorrhizal status (NM, ERM, and ECM) and treatment (ambient vs warmed) with the exception of diameter (final model predictor treatment alone). Red asterisks indicate significant contrasts between ambient and warmed plots: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. See also Fig. S2 for seven additional traits not depicted here and Table S2 for *post hoc* contrasts.

plots. Instead, within warmed plots, ERM showed greater root depth than ECM but lower %N than NM and ECM (Table S2, all contrasts $P < 0.05$).

Relationships between root-associated fungi, root traits, and N uptake

Overall, variation in root traits was related to N uptake from the permafrost boundary ($r^2 = 0.09$, $P < 0.01$, Fig. 1). The root traits strongly correlated with axis 1 of the PCA biplot suggests deeper roots with lower tissue density and biomass but greater SRL, SRA, SRTA, and SRFA were correlated with greater N uptake.

The final ordination of RAF community composition associated with ERM hosts had three dimensions, a stress of 0.14 indicating a fair fit, a nonmetric fit $R^2 = 0.98$, and a linear fit $R^2 = 0.90$. RAF composition of ERM shrubs varied with the warming treatment and strongly correlated with root diameter and %N (Fig. 3a; Table S3) supporting H3. Higher abundances of DSE taxa, *Phialocephala fortinii* and *Cadophora finlandica*, and ERM taxa, *Meliniomyces vraolstadiae* and *Pezoloma ericae*, were associated with lower diameter and higher %N root tissue, whereas greater abundance of another ERM taxon, *Rhizoscyphus ericae*, was associated with high diameter and lower %N roots (Fig. 3a,c). The final ordination of RAF community composition associated with ECM hosts had three dimensions, a stress of 0.13 indicating a fair fit, a nonmetric fit $R^2 = 0.98$, and a linear fit $R^2 = 0.89$. RAF composition of ECM shrubs was marginally correlated with warming treatment but did not correlate with fine-root traits (Fig. 3b; Table S3). The RAF composition of ECM ($r^2 = 0.45$) but not ERM ($r^2 = 0.01$) hosts correlated strongly with the uptake of permafrost N (Fig. 3a,b; Table S3) supporting H4. The uptake of permafrost N by ECM shrubs was positively correlated with greater abundances of the RAF taxa identified as Helotiaceae sp. and *Hymenoscyphus tetracladius* (Fig. 3b,d).

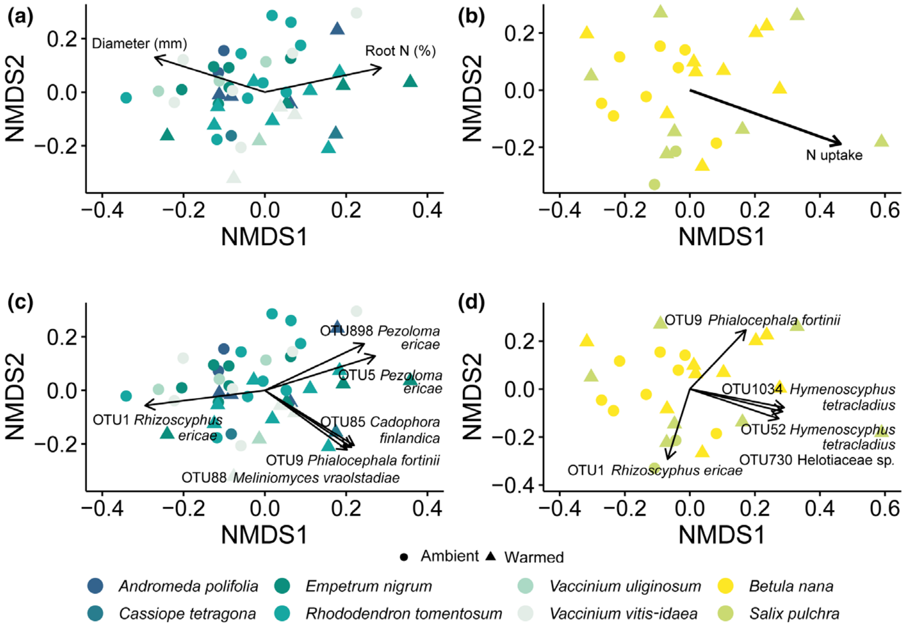

Fig. 3 Nonmetric multidimensional scaling ordination biplots representing root-associated fungi (RAF) community composition associated with ericoid (ERM) and ectomycorrhizal (ECM) host plants in ambient and warmed plots at Toolik Lake, Alaska. Vectors represent the direction and magnitude of significant correlations between RAF composition, (a, b) root traits ($P \leq 0.05$), permafrost nitrogen (N) uptake ($P < 0.01$), and gradients in the relative abundance of individual taxa (c, d, $P < 0.001$). Marker colors are green for ericoid mycorrhizal and yellow for ECM host plants.

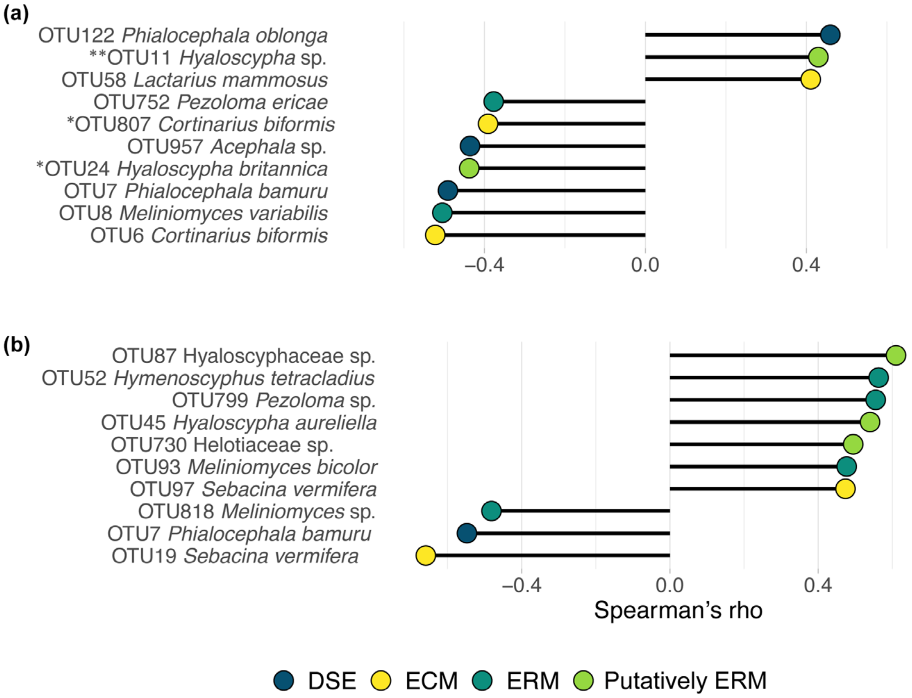

Fig. 4 Spearman correlation coefficients between the relative abundance of root-associated fungal taxa and N uptake (g ^{15}N g^{-1} root) for (a) ericoid and (b) ectomycorrhizal host plants. The highest positive and negative correlations are shown here for dark septate endophyte (DSE), ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) fungi; see also Supporting Information Table S4 for all significantly correlated taxa. **, Control plot; *, warmed plot indicator species.

There were 29 RAF taxa that were significantly positively or negatively correlated (P-value < 0.05) with the permafrost N uptake by ERM host plants (Fig. 4a; Table S4), the majority of which were in the Helotiales and including taxa identified in the genera *Meliniomyces*, *Hyaloscypha*, *Acephala*, *Phialocephala*, *Oidiodendron*, *Capronia*, and *Pezoloma* (5 DSE, 14 ERM) additionally, there were 10 ECM taxa, *Sebacina* sp. (n = 4), *Lactarius*, *Russula*, *Tricholoma*, and *Cortinarius* (n = 3), that were mostly negatively correlated with N uptake. For ERM hosts, of the top taxa positively correlated with permafrost N uptake, OTU11 *Hyaloscypha* sp. was an indicator of the control plots, and of the taxa negatively correlated with N uptake, OTUs 24 *Hyaloscypha britannica* and 807 *Cortinarius biformis* were indicators of the warmed plots. Twenty-six RAF taxa were significantly positively or negatively correlated (P-value < 0.05) with ECM host plant permafrost N uptake (Fig. 4b, Basidiomycota = 10, Ascomycota = 16). For ECM taxa, correlations with N uptake were positive and negative for taxa in the genera *Sebacina* and *Cortinarius*, and positive for *Laccaria* and *Russula*. For DSE taxa, taxa in the genera *Cadophora* had positive but *Phialocephala* had negative correlations with N uptake. Lastly, for the

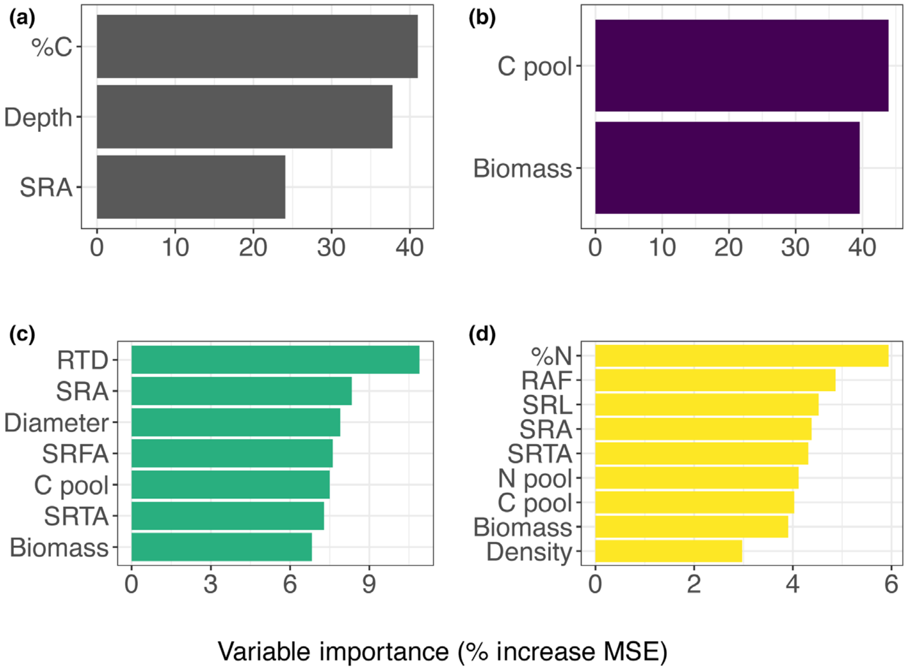

Fig. 5 Variable importance plots from random forest models explaining variation in permafrost nitrogen (N) uptake (g ^{15}N g^{-1} root) for (a) all plants, (b) nonmycorrhizal, (c) ericoid mycorrhizal, and (d) ectomycorrhizal plant species. Variable importance is reported as the percentage increase in mean squared error (MSE). Variables shown were identified for inclusion in the final model for each group of plants (a–d). Root tissue density (g cm^{-3}, RTD); specific root area (cm^2 g^{-1}, SRA); specific root length (cm g^{-1}, SRL); specific root tip abundance (tips g^{-1}, SRTA); specific root fork abundance (forks g^{-1}, SRFA); maximum depth (cm, Depth); diameter (mm); root biomass (g m^{-2}, Biomass); root N pool (g N m^{-2}, N pool); root carbon pool (g C m^{-2}, C pool); root density (g cm^{-3}, Density); root tissue percent C (%C); root tissue percent N (%N); root-associated fungi (NMDS axis 1, RAF).

14 ERM taxa, taxa identified as Helotiaceae sp., *Hyaloscypha*, *Hymenoscyphus*, and *Pezoloma* had positive correlations, *Oidiodendron* had negative, and *Meliniomyces* and taxa identified as Hyaloscyphaceae sp. had both positive and negative correlations with N uptake (Figs 3d, 4b; Table S4). The relative abundances of other NM taxa such as pathogens and saprotrophs were also correlated with permafrost N uptake (Table S4).

Relative importance of root traits and RAF composition to permafrost N uptake

We identified variables for interpretation with random forest models predicting permafrost N uptake for all plants and then separately based on mycorrhizal status. For the RF model with all plants, 36.18% of the variance in permafrost N uptake was explained with fine-root %C showing an inverse and depth and SRA showing positive relationships with permafrost N uptake (Figs 5a, S3). For NM plants, the ecosystem root traits, C pool, and biomass, were most related to permafrost N uptake with 16.19% of the variance explained (Fig. 5b) but interestingly with inverse associations (C pool positive and biomass modestly negative, Fig. S4), which contrasted with H2 that morphological traits would be instrumental to permafrost N uptake for NM plants. Acquisition of permafrost N by ERM plants was associated mostly with morphological traits with 37.01% variance explained (Fig. 5c). ERM plants had greater permafrost N uptake with higher biomass, C pool, and SRA and lower diameter, which are more acquisitive traits, but also higher RTD and lower SRFA, which are more conservative traits (Fig. S5). The variance explained for ECM permafrost N uptake was low (6.88%) and was related to both root traits and RAF composition (Fig. 5d), supporting H3 and H4. ECM shrub access to permafrost N showed contrasting relationships between root traits compared with ERM and NM plants, for example SRA and biomass (Figs S4–S6), potentially due to the important role of RAF for ECM shrubs.

For both ERM and ECM shrubs, root traits explained a greater proportion of the variation in permafrost N uptake than RAF community composition (Fig. 6a,b). There was notably low shared variance explained between root traits and RAF composition. RAF composition explains a greater amount of variance for ECM than for ERM shrubs, supporting what was observed in the RF modeling and NMDS ordination biplots and supporting H4.

Discussion

This study shows that nearly 30 yr of experimental warming in tundra resulted in changes in fine-root chemical, morphological, and ecosystem traits indicative of more acquisitive resource uptake strategies (Fig. 7), supporting H1. RAF composition of ERM shrubs changed with warming and was correlated with root diameter and %N, while RAF associated with ECM shrubs were not as responsive to warming or variation in root traits, providing partial support for H3. Deciduous ECM shrubs have increased in dominance with warming, and their plant-fungal interactions correlate with N uptake from the permafrost boundary, supporting H4. Specifically, fungi implicated in the uptake of permafrost N were DSE and ERM in addition to ECM, similar to observations from the more southerly permafrost zone where these fungi were observed beyond the root zone at the permafrost boundary (Hewitt *et al.*, 2020).

Warming impacts on root traits and plant–RAF associations

Plant response to warming via shifts in root traits was strong and ecologically important to permafrost N uptake. We observed a

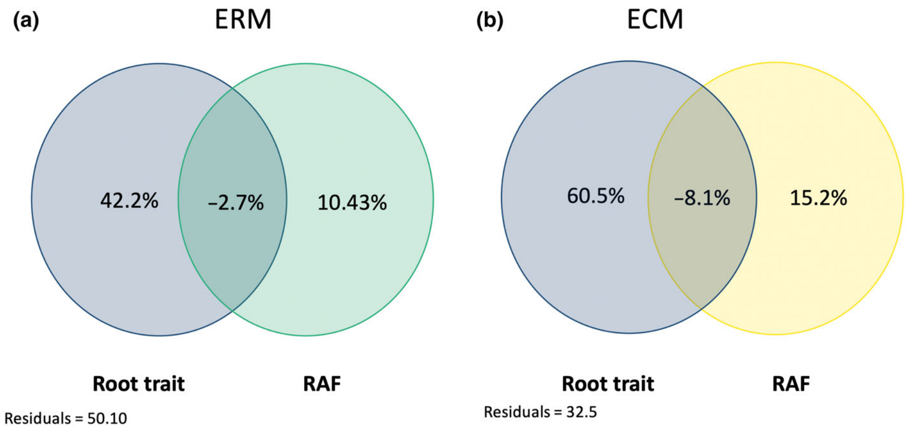

Fig. 6 Venn diagram displaying the partitioning of the variance in permafrost nitrogen (N) uptake explained by root traits and the composition of root-associated fungi (RAF) for (a) ericoid mycorrhizal (ERM) and (b) ectomycorrhizal (ECM) shrubs. Values indicate adjusted R^2 values. Negative adjusted R^2 values indicate no explanatory power of the potentially shared variance explained between RAF and root traits on permafrost N uptake.

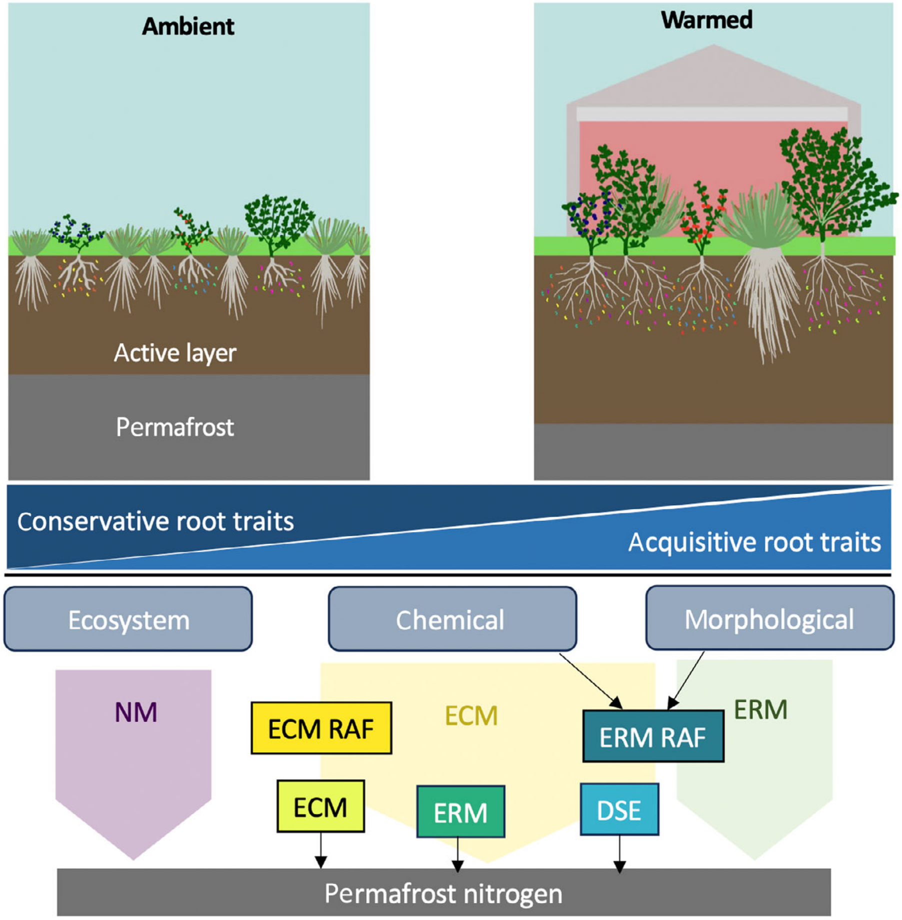

Fig. 7 Conceptual figure depicting the observations of root traits of nonmycorrhizal (NM) and mycorrhizal tundra plants and root-associated fungi (RAF) of ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) shrubs in response to long-term warming. Variance in permafrost nitrogen (N) uptake was primarily related to ecosystem fine-root traits for NM plants (light purple arrow), whereas RAF also had an important influence on N acquisition by ERM (light green arrow) and, more so, ECM (light yellow arrow) shrubs. RAF composition of ERM shrubs but not ECM shrubs was correlated with root traits (black arrows). ECM, ERM, and dark septate endophyte (DSE) fungi were associated with variation in N uptake measured in the fine-root tissue of host mycorrhizal plants (black arrows).

transition toward more acquisitive root traits with warming on the level of plant species (PCA) and grouped by mycorrhizal status (NM, ERM, and ECM). Overall, acquisitive traits were associated with greater uptake of N from the permafrost boundary. In a global meta-analysis, chemical root traits shifted to more acquisitive strategies with experimental warming with pronounced shifts in cold ecosystems (Wang *et al.*, 2021). Our study showed similar findings and additionally that morphological traits shifted toward more acquisitive strategies, and this was consistently the pattern for mycorrhizal shrubs more so than NM plants. For NM plants, warming resulted in greater depth and %N, supportive of observations of high N content in root tissue to maintain high metabolic rates (Bloom *et al.*, 1985; Reich, 2014) and rapid growth associated with soil exploration as the thaw front deepens (Blume-Werry *et al.*, 2019; Hewitt *et al.*, 2019). The uptake of N from the permafrost boundary for NM plants was related to ecosystem traits (greater C pool), whereas for ERM shrubs, morphological traits (greater SRA and smaller diameter, but also greater RTD), and for ECM shrubs, RAF, and RAF impacts on root traits (higher %N, lower SRL) were key to N uptake, which contrasted with H2 but supported H3. We observed weak relationships between RAF community composition and root traits, especially for ECM plants. This could reflect the long-term warming of these plots and the importance of perennial plant tissues, while fungi likely turnover much faster, obscuring their response to warming and contribution to nutrient uptake.

Our analysis of RAF composition and root traits supports the idea of a multidimensional space of root trait variation (Bergmann *et al.*, 2020), particularly for ERM hosts. Our findings showed root diameter and %N, the only traits that correlated with RAF, loading on PCA axis 2, while most other traits loaded on PCA axis 1; this is well-aligned with findings that show a mycotrophic gradient orthogonal to the primary acquisitive to conservative root economic spectrum. RAF of ERM plants responded to variation in traits that others have observed related to fungal colonization (diameter, %N, Freschet *et al.*, 2010; Bergmann *et al.*, 2020). For ERM hosts, lower root diameter and higher root %N were correlated with higher abundances of *P. fortinii*, *C. finlandica*, *M. vraolstadiae*, and *P. ericae*. This suggests that when plants build more narrow, high N content absorptive roots, indicative or more acquisitive and less mycotrophic strategies (Bergmann *et al.*, 2020), they are in association with these DSE and ERM taxa, whereas lower abundances of these taxa and higher abundance of *R. ericae*, another OTU of the same fungal aggregate, are associated with a more conservative and mycotrophic strategy where plants are more reliant on their fungal partners for soil resource acquisition. Of note, here, the suite of root traits that create a multidimensional root economic space do not fully mirror those observed in other studies, that is a mycotrophic gradient with tradeoffs between SRL and diameter and an acquisitive-conservative gradient with tradeoffs between RTD and root N (Bergmann *et al.*, 2020; Weigelt *et al.*, 2021). RAF of ECM shrubs showed less sensitivity to warming and root traits than RAF of ERM. Instead, as root traits of ECM plants become more acquisitive with warming, RAF composition (this study) or metrics of diversity (DeVan, 2019) did not respond. The similarity in ECM root tip communities between ambient control and warmed plots was also observed in a separate investigation of ECM enzymatic function during the same timeframe of this study (Dunleavy & Mack, 2021), but contrasts with findings of increased ECM diversity observed 10 yr earlier in the warmed plots (Deslippe *et al.*, 2011), indicating temporal variation in RAF responses to warming.

Trait estimates for RAF, especially RAF other than arbuscular mycorrhizal (AM) and ECM fungi, are in their infancy, constraining our ability to assess whether RAF colonizing acquisitive roots are themselves acquisitive (Schaffer-Morrison & Zak, 2023 and references within). Despite a limited understanding of the C costs of ascomycetes in ERM and DSE guilds, in culture, these fungi are faster growing and more versatile in supplementing host C with saprotrophic capabilities than basidiomycetous ECM (Lukešová *et al.*, 2015; Vohník, 2020). Our observations could represent an expansion of the more mycotrophic strategy where the shrub builds more acquisitive roots to expand opportunities of colonization by RAF that are associated with N uptake. Alternatively, the transition to more acquisitive root traits with warming may indicate a shift from primarily more mycotrophic to less mycotrophic strategies; quantifying RAF colonization of root tips could inform these interpretations. Our findings suggest that a greater focus on traits of ERM and DSE fungi, in addition to the better-studied AM and ECM, is needed to elucidate the full structure of the root economic space, including a mycotrophic gradient.

RAF important to uptake of permafrost N

Ericoid mycorrhizal RAF taxa had strong correlations with the uptake of N from the permafrost boundary for both ERM and ECM host plants. Evidence is building that ERM RAF are associated with ECM hosts (Vrålstad *et al.*, 2000; Chambers *et al.*, 2008; Hewitt *et al.*, 2017). ERM RAF are important to N uptake in cold, strongly N-limited ecosystems like tundra and boreal forest biomes (Ward *et al.*, 2022 and references therein), and have been documented to facilitate uptake of permafrost N in more southerly subarctic tundra (Hewitt *et al.*, 2020). In this study, most of the RAF that significantly correlated with the permafrost N uptake were in the Helotiales, with key taxa in the genera *Meliniomyces*, *Hyaloscypha*, *Hymenoscyphus*, *Phialocephala*, and *Pezoloma*, suggesting an important role in the ecological function of non-ECM RAF (i.e. DSE and ERM) that is surprising given the dominance aboveground of ECM host plants as tundra warms. Furthermore, ECM taxa were observed on ERM shrubs as in other ecosystems (Smith *et al.*, 1995; Vrålstad, 2004) and correlated both positively and negatively with N uptake; careful further investigation documenting the anatomy of fungal interactions in the root system is needed to elucidate the functional implications. The relative abundances of pathogens and saprotrophs were also correlated with permafrost N uptake, indicating cascading effects of permafrost N acquisition throughout the fungal community, which may suggest rapid assimilation of new N sources in this N-limited system.

Implications for C and N cycling

A better understanding of the responses of fine roots and RAF to warming is key to improving the accuracy of predictions of climate change impacts in tundra ecosystems. Mycorrhizal fungi can increase N accumulation in host tissues, as seen in roots in warmed plots here, and lead to faster growth in roots and shoots (Rains & Bledsoe, 2007). Some RAF taxa were negatively correlated with the transfer of permafrost N to ERM plants and were indicators of warmed plots. This suggests that fungi responding to warming soils may play an important role in ecosystem retention of N through accumulation in plant or fungal biomass. Fungi can hold on to N and allocate a fraction of it to host plants over long periods of time, especially as resource availability increases (Näsholm *et al.*, 2013). The fate of this N as either transferred to plants or allocated to fungal growth is uncertain but likely important to the cycling of soil C and N and related to fungal identity.

Warming in tundra ecosystems results in changes in permafrost dynamics influencing fine-root traits and plant-fungal interactions; these, in turn, are critical to the cycling of C and N that influence terrestrial feedbacks to the climate system. Increasing evidence suggests that fine-root-derived C inputs as litter and exudates along with RAF are important contributors to stored C and ecosystem C balance in cold ecosystems (Clemmensen *et al.*, 2013; Iversen *et al.*, 2015; Jackson, 2017; Adamczyk *et al.*, 2019). Root litter of more acquisitive roots has higher turnover rates (Freschet *et al.*, 2010) than those with conservative traits; these faster cycling tissues along with exudates may either facilitate positive priming of soil C, especially in deeper near-permafrost soils (Pegoraro *et al.*, 2019) or alternatively be microbially processed into stabilized soil C (Cotrufo *et al.*, 2013; Sokol & Bradford, 2019). Furthermore, the ascomycetous RAF observed in warmed plots and the taxa implicated in the uptake of N from the permafrost boundary, are correlated with soil C accumulation, while some of the basidiomycetes are correlated with fast cycling of soil C (Clemmensen *et al.*, 2013, 2021). Our research highlights the importance of these belowground shifts in root traits and RAF communities to estimates of ecosystem C and N balance.

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Competing interests

None declared.

Author contributions

MCM and REH designed the field experiment; REH conducted fieldwork, laboratory analysis of root samples, and drafted the manuscript. DLT and MRD conducted molecular verification of root identities, fungal sequencing, and bioinformatics. REH, MRD and DLT analyzed the data. REH, MRD, DLT and MCM made contributions to manuscript revision.

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Data availability

Fungal sequences are archived with NCBI BioSample accessions SAMN37196287–SAMN37196363.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Schematic of field methods employed to apply nitrogen isotope tracer to the base of the active layer at the permafrost boundary in ambient control plots and warmed glasshouse plots at Toolik Lake, Alaska.

Fig. S2 Boxplots of seven morphological, chemical, and ecosystem fine-root traits of nonmycorrhizal, ericoid mycorrhizal, and ectomycorrhizal host plants in ambient control plots and warmed glasshouse plots of the Arctic LTER whole ecosystem warming experiment.

Fig. S3 Partial dependence plots from the random forest model of permafrost nitrogen uptake in relation to root traits for all plants.

Fig. S4 Partial dependence plots from the random forest model of permafrost nitrogen uptake in relation to root traits for nonmycorrhizal plants.

Fig. S5 Partial dependence plots from the random forest model of permafrost nitrogen uptake in relation to root traits and mycorrhizal associations for ericoid mycorrhizal plants.

Fig. S6 Partial dependence plots from the random forest model of permafrost nitrogen uptake in relation to fine-root traits and mycorrhizal associations for ectomycorrhizal plants.

Table S1 Generalized linear mixed effects model structure used to test variation in each root trait in relation to warming treatment and plant mycorrhizal status.

Table S2 Trait contrasts from generalized linear mixed effects model *post hoc* tests for root traits with the interaction between mycorrhizal status and warming treatment.

Table S3 Correlation coefficients between root-associated fungal composition, root traits, and permafrost nitrogen uptake for ericoid and ectomycorrhizal host plants.

Table S4 Correlations between permafrost nitrogen uptake and the relative abundance of specific root-associated fungal operational taxonomic units.