New Phytologist

Research

Warming influences carbon and nitrogen assimilation between a widespread Ericaceous shrub and root-associated fungi

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Received: *7 September 2023*
Accepted: *19 October 2023*

New Phytologist (2023)
doi: 10.1111/nph.19384

Key words: boreal, climate change, *Empetrum*, ericoid mycorrhizas, mycorrhizal fungi, nitrogen mineralization, stable isotopes, warming.

Summary

- High-latitude ecosystems are warming faster than other biomes and are often dominated by a ground layer of Ericaceous shrubs, which can respond positively to warming. The carbon-for-nitrogen (C-for-N) exchange between Ericaceous shrubs and root-associated fungi may underlie shrub responses to warming, but has been understudied.
- In a glasshouse setting, we examined the effects of warming on the C-for-N exchange between the Ericaceous shrub *Empetrum nigrum* ssp. *hermaphroditum* and its root-associated fungi. We applied different ^{13}C and ^{15}N isotope labels, including a simple organic N form (glycine) and a complex organic N form (moss litter) and quantified their assimilation into soil, plant biomass, and root fungal biomass pools.
- We found that warming lowered the amount of ^{13}C partitioned to root-associated fungi per unit of glycine ^{15}N assimilated by *E. nigrum*, but only in the short term. By contrast, warming increased the amount of ^{13}C partitioned to root-associated fungi per unit of moss ^{15}N assimilated by *E. nigrum*.
- Our study suggests that climate warming affects the short-term exchange of C and N between a widespread Ericaceous shrub and root-associated fungi. Furthermore, while most isotope tracing studies use labile N sources, we demonstrate that a ubiquitous recalcitrant N source may produce contrasting results.

Introduction

One of the consequences of ongoing global climate change is that high-latitude environments, such as boreal and arctic regions, are warming faster than most other biomes on Earth (Gonzalez *et al.*, 2010; Scheffer *et al.*, 2012). There is also evidence of strong plant responses, including tree and shrub expansion, to warming in many areas (Sturm *et al.*, 2001; Elmendorf *et al.*, 2012a; Dial *et al.*, 2022). To date, observational and experimental warming studies have revealed a large heterogeneity in high-latitude plant responses to recent warming, with patterns showing many shrubs to be positively responsive to elevated temperatures in high-latitude regions where ambient temperatures are already high (Elmendorf *et al.*, 2012a,b). In addition to temperature as a driver of plant growth, nitrogen (N) is often a limiting resource at high latitudes (Högberg *et al.*, 2017) where relatively low mean annual temperatures constrain decomposition rates and consequently limit organic N mineralization (Hobbie *et al.*, 2002). Consequently, positive shrub responses to warming could also derive from an ability of shrubs to gain limiting resources, such as N, at a lower carbon (C) cost under rising temperatures. Understanding the underlying mechanisms of plant responses to rising temperatures is therefore critical for predicting ecosystem responses under projected global warming in high latitudes.

As a result of low N availability in high-latitude systems, many high-latitude plants invest in strategies to improve N mining and acquisition (Read, 1991). Along with woody plants worldwide, boreal and arctic shrubs form symbioses with mycorrhizal fungi, which forage for nutrients that can be transferred to the host plant in exchange for photosynthetically derived C (Smith & Read, 1997). The C that is partitioned to mycorrhizal symbionts and other root-associated fungi might otherwise be used for growth, defense, or reproduction (Wiley & Helliker, 2012), or may simply be surplus C exuded by the host plant (Prescott *et al.*, 2020). Temperature-driven changes in soil N availability could therefore affect how much C high latitude shrubs partition toward fungal symbionts (Olsrud *et al.*, 2004), compared with how much C that is redirected toward other functions. While many studies have investigated the effects of warming on ectomycorrhizal symbioses (Bennett & Classen, 2020), few studies have investigated the effects of warming on ericoid mycorrhizal symbioses. Nevertheless, Ericaceous dwarf-shrubs are a key ecosystem component in high-latitude environments and have been observed to respond positively to warming in the boreal-arctic transition zone, though with variation in responses among species (Wilson & Nilsson, 2009; Callaghan *et al.*, 2013). Since shifts in Ericaceous shrub cover or composition can alter litter input, rhizosphere communities, decomposition rates, and ultimately

influence C and N cycling (Hobbie, 1992, 2015; Berg & Smalla, 2009; Fanin *et al.*, 2022), better understanding of underlying mechanisms driving their response to rising temperatures can inform on potential future changes on ecosystem functions in these systems.

The relationship between host N uptake and the quantity of C partitioned to fungal symbionts may depend on the chemical form of the N source. In many boreal forest and arctic environments, feather mosses form a dense carpet and are the largest known source of biologically fixed N (DeLuca *et al.*, 2002; Lindo *et al.*, 2013; Hupperts *et al.*, 2021). Consequently, feather moss litter is one of the most common forms of complex organic N. However, following litter degradation, simple organic N forms such as amino acids can constitute up to 80% of the soil available N supply in high-latitude soils and can be readily taken up by plants and fungi (Näsholm *et al.*, 1998; Inselsbacher & Näsholm, 2012). Amino acid turnover is rapid in these soils (Jones & Kielland, 2002), and plant or fungal uptake rates may change depending on N demand or soil N availability (Näsholm *et al.*, 2013). In contrast to free amino acid uptake, N that is locked in moss litter or humus must first be liberated by saprotrophs and mycorrhizal fungi, using extracellular enzymes or nonenzymatic Fenton reactions (Lindahl & Tunlid, 2015; Hupperts *et al.*, 2017; Op De Beeck *et al.*, 2018), which presumably involve a higher C cost for mycorrhizal fungi and, by extension, the host plant (Zheng *et al.*, 2020). If warming increases photosynthetic rates of the host plant, it may partition more C toward mycorrhizal symbionts to increase N mining activity, similar to findings from CO_2 enrichment studies (Terrer *et al.*, 2018; Pellitier *et al.*, 2021). As a result, warming may increase or have no effect on the C cost of N uptake when the N is locked within moss litter, but this has not been tested, or compared with simple organic N forms such as amino acids.

Less C partitioned toward acquisition of amino acid N could underlie positive responses of Ericaceous shrubs to warming. The Ericaceous shrub *Empetrum nigrum* spp. *hermaphroditum* (hereafter, '*Empetrum*') has a circumpolar distribution in high-latitude regions of the northern hemisphere, occurring in a wide variety of habitats and ecosystem types (Popp *et al.*, 2011). *Empetrum* has been argued to be a 'niche constructor' in these high-latitude ecosystems, with positive associations between *Empetrum* and temperature being related to negative responses of herbaceous plants to temperature (Bråthen *et al.*, 2018). Furthermore, previous work has found that experimental warming may allow *Empetrum* to increase its growth rate more than some co-occurring dwarf shrub species (Parsons *et al.*, 1994; Shevtsova *et al.*, 1997). In particular, Wada *et al.* (2002) discovered that *Empetrum* grew more than co-occurring arctic and subarctic species (including other Ericaceous species) in experimentally warmed plots, suggesting that warming might confer a competitive advantage for *Empetrum*. Along with increased growth, warming can increase the leaf area index, and also the production of phenolics in *Empetrum* (Graglia *et al.*, 1997; Väisänen *et al.*, 2013), which are known to deter herbivores and competing plants (Nilsson *et al.*, 1993; Salminen & Karonen, 2011). Declining C costs for N uptake could be an underlying factor that contributes to the positive response of *Empetrum* to warming, but this important relationship has not been explored. An improved understanding of carbon and nitrogen dynamics between host plants and root-associated microbes can inform Earth system models, which often do not explicitly account for microbial processes (ASM, 2023), and therefore strengthen predictions of high-latitude ecosystem responses to climate change.

In this study, we performed three parallel and complementary glasshouse experiments, in isolation of potential additive or interactive effects of other global climate change drivers (e.g. precipitation change and increased atmospheric CO_2 concentrations), to investigate the effects of warming on the resource exchange of C-for-N between the Ericaceous shrub, *Empetrum*, and its root-associated fungi at different snapshots in time. In a controlled glasshouse setting, we applied different ^{13}C and ^{15}N labels and quantified the assimilation of the labels into soil, plant biomass, and root fungal biomass. Experiment 1 tested our first hypothesis, H1, that warming will increase mineralization of simple organic N, causing the plant to acquire a greater proportion of inorganic to organic N. Experiment 2 tested our second hypothesis, H2, that warming will allow the plant to assimilate more of a simple ^{15}N-labeled amino acid, and will partition less ^{13}C to fungal symbionts, giving the plant a more favorable ^{13}C-for-^{15}N exchange. Experiment 3 tested our third hypothesis, H3, that warming will have little effect on the ^{13}C-for-^{15}N exchange when a more complex ^{15}N-labeled substrate, feather moss litter, is added to the soil. By testing these hypotheses, we intend to further the mechanistic understanding of how and why this widespread, high-latitude Ericaceous shrub responds to warming.

Materials and Methods

Site description and *Empetrum* collection

Empetrum nigrum spp. *hermaphroditum* (Hagerup) Böcher (hereafter, '*Empetrum*') plants (including roots) were collected from the Rosinedalsheden Experimental Forest (19.791133E, 64.163929N, 155 m a.s.l), near Vindeln, Sweden. In Vindeln, the average (1989–2021) daily high temperature in July is 17.7°C (Swedish Meteorological and Hydrological Institute (SMHI)), the mean annual (1991–2019) temperature at the nearby Svartberget Research Station is 1.8°C, and mean annual precipitation is 620 mm (Grau-Andrés *et al.*, 2021). The soil is a nutrient-poor sandy podzol with an organic mor layer thickness of *c.* 2–5 cm (Hasselquist *et al.*, 2012). *Pinus sylvestris* dominates the canopy layer, and understory vegetation is primarily comprised of Ericaceous shrubs including *Empetrum*, *Vaccinium myrtillus*, and *V. vitis-idaea* on moss mats of *Hylocomium splendens* and *Pleurozium schreberi* (Hasselquist *et al.*, 2012). *Empetrum* forms circular patches at our site, where we assumed each patch consisted of one clonal individual (Szmidt *et al.*, 2002).

We selected four different *Empetrum* clones that were at least 2 m in diameter and separated by at least 30 m. A portion of each clone was extracted from the soil, including the humus and underyling mineral sand, to a depth of 15–25 cm. Harvested *Empetrum* was subsequently kept in plastic containers and

transported back to Umeå on the day of collection, and then maintained in a glasshouse and watered daily.

Experimental design

We performed three parallel experiments that featured the same warming treatment but different application methods for ^{15}N and ^{13}C labels. *Empetrum* individuals were first divided into three sets of 48 pots (one set for each of three experiments, described below). Of the 48 pots per experiment, 24 pots (i.e. 4 clones × 6 replicates per clone) were allocated to either 'ambient' or 'warm' temperature rooms (one room per temperature treatment), corresponding to daily average temperatures of 17.6°C (i.e. average daily high temperature in July near collection site) and 21.6°C, respectively. Our warming treatment of 4°C above ambient falls within the range of predicted summer temperature deviations (years 2071–2100) in northern Sweden (1.5–5.5°C above current temperatures, depending on RCP model scenario; SMHI, 2023). Overall, there were 144 pots (3 experiments × 2 temperature treatments × 24 pots per temperature treatment = 144 pots), each containing one *Empetrum* plant. Lastly, we set aside four additional pots with *Empetrum* (one pot from each clone) in each temperature treatment (i.e. eight pots total) to use for natural background $\delta^{15}N$ and $\delta^{13}C$ abundance. Relative air humidity was set to 60%, with 18 light hours (120–150 PAR) and 6 dark hours per day. Pots were watered daily with deionized water.

Experiment 1: ^{13}C-^{15}N-glycine labeling

In this experiment, we applied dual-labeled glycine to test our first hypothesis (H1) that warming will increase organic N mineralization. First, 48 *Empetrum* plants were planted into 440-ml round plastic pots (diameter × height = 6.5 × 20 cm) that contained a humus layer (13 cm depth) over a sand layer (*c.* 4.5 cm depth) collected from respective clones. Care was taken to minimize variation in plant size. To quantify amino acid mineralization, uptake, and partitioning, we labeled all 48 pots (24 pots in each temperature, divided evenly among clones) with a solution containing 1.6 mg of $^{13}C_{(2)}$-^{15}N-labeled glycine (98 atom percent; Sigma-Aldrich) mixed with 10 ml of deionized water, 128 d after initial transplanting (Supporting Information Table S1). The amount of glycine added was calculated based on estimates of N demand, glycine ^{15}N enrichment, and previous studies (e.g. Rains & Bledsoe, 2007), with a goal to increase leaf ^{15}N concentration by *c.* 1.5 times above natural background levels (i.e. to *c.* 0.55 atom percent). The solution was injected at 1-cm soil depth in five evenly arranged locations around the stem (i.e. 2 ml injected at each location), using a 20-ml syringe (BD Plastipak™). The injection at different locations ensured that $^{13}C_{(2)}$-^{15}N-glycine would reach fine roots and minimize leaching.

Seven days after ^{13}C-^{15}N-glycine labeling, we destructively harvested half of the pots (12 pots in each temperature, divided evenly among clones). The stem was severed at the base and all aboveground biomass was oven-dried at 70°C for at least 48 h. After oven-drying, *Empetrum* leaves were analyzed for total N, total C, $\delta^{15}N$, and $\delta^{13}C$ using a Flash EA 2000 elemental analyzer connected to a DeltaV Isotope ratio mass spectrometer. Roots and all surrounding soil (including humus) were placed in plastic bags and stored at −18°C until further processing, when soil samples were thawed, and fine roots carefully hand-washed to remove humus and sand. Cleaned fine roots were freeze-dried for 48 h. A portion of freeze-dried fine root material was oven-dried at 70°C for 24 h, after which these samples were placed in 1.5-ml Eppendorf tubes, finely ground using a Retsch MM400 ball mill grinder (Retsch Inc., Newtown, CT, USA) and analyzed for total N, total C, $\delta^{15}N$ and $\delta^{13}C$ using the same equipment described above. The same was done for remaining fresh humus and sand samples.

To investigate temporal dynamics of amino acid mineralization, we harvested the remaining pots 7 d later (i.e. 14 d after $^{13}C_{(2)}$-^{15}N-glycine labeling), and prepared and analyzed fine roots and soil (including humus) for total N, total C, $\delta^{15}N$ and $\delta^{13}C$ using the same procedures described above.

Isotope ratios were calculated per mil (‰) as $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard}) \times 1000$, where R is the molar ratio of the heavy to light isotope (i.e. $^{15}N/^{14}N$ or $^{13}C/^{12}C$) for the sample and standard. The ^{15}N standard (i.e. absolute ratio (AR)) is 0.0036764 ^{15}N abundance (atmospheric air), and the ^{13}C standard is 0.0112372 ^{13}C abundance (Vienna Pee Dee Belemnite). The ^{15}N and ^{13}C atom percent excess (i.e. the percentage of ^{15}N or ^{13}C atoms in excess of average isotopic natural abundance in the nonlabeled *Empetrum* plants) was then calculated as:

$$\text{Atom percent (AP)} = \frac{100 \times \text{AR} \times \left(\left(\delta^{15}\text{N or } \delta^{13}\text{C}\right)/1000 + 1\right)}{1 + \text{AR} \times \left(\left(\delta^{15}\text{N or } \delta^{13}\text{C}\right)/1000 + 1\right)}$$

$$\text{Atom percent excess (APE)} = \text{AP}_{\text{labeled}} - \text{AP}_{\text{background}}$$

We assumed that negative APE values indicated no enrichment beyond natural background levels, and therefore changed all negative values to zero. For ^{15}N, there were a total of three values in this study that were changed from negative to zero. For ^{13}C, the number of negative values ranged from 1 to 17 per tissue type within each experiment and temperature treatment (Dataset S1). Once negative APE values were changed to zero, we calculated the excess mass (μg) of ^{15}N and ^{13}C per gram dry mass as:

$$\text{Excess } ^{15}\text{N or} ^{13}\text{C}\left(\mu\text{g g}^{-1}\text{ dry mass}\right) = \frac{(\text{APE}/100) \times (\%\text{N or} \%\text{C}/100)}{10^6}$$

where %N and %C is the tissue nitrogen and carbon content (%), respectively. To directly compare the quantity of ^{15}N with that of ^{13}C, which has a different atomic mass, we also calculated excess moles of ^{15}N and ^{13}C per gram dry mass by dividing excess ^{15}N and ^{13}C (atoms g^{-1}) by Avogadro's constant. We used these calculations for fine roots and soil.

To investigate the effects of warming on glycine mineralization in Experiment 1, we calculated the ratio of moles of ^{13}C to moles of ^{15}N in soil and fine roots. Because $^{13}C_{(2)}$-^{15}N-enriched glycine has two ^{13}C atoms and one ^{15}N atom, a deviation from 2 is

indicative of mineralization processes. For soil, a ratio of 2.0 implies that glycine remained intact, whereas a ratio < 2.0 implies that glycine was mineralized (i.e. ^{13}C was lost via respiration; Näsholm *et al.*, 1998). For fine roots, a ratio of 2.0 implies that the plant took up glycine as an intact molecule, while a ratio < 2.0 implies that glycine was mineralized in the soil and uptake occurred as inorganic N.

Experiment 2: ^{15}N-glycine-$^{13}CO_2$ labeling

In this experiment, we applied ^{15}N-labeled glycine and ^{13}C-labeled CO_2 to test our second hypothesis (H2) that warming will influence the ^{13}C-for-^{15}N exchange between the plant and the root-associated fungi. First, 48 additional *Empetrum* plants (not used in Experiments 1 or 3) were planted into round, 560-ml plastic pots (diameter × height = 13 × 7.5 cm) that contained *c.* 5.5-cm-deep humus over *c.* 2-cm-deep sand. To quantify ^{15}N uptake from amino acid concurrently with ^{13}C assimilation from CO_2, we labeled the 48 pots (24 pots in each temperature treatment, divided evenly among clones) with $^{13}CO_2$ after 124 d of adjustment to transplanting at the two different temperatures. Pots were watered to reduce soil air porosity and promote stomatal opening, then covered with a 10-l gas sampling bag (Tedlar® PLV Gas Sampling Bag w/Thermogreen® LB-2 Septa; Sigma-Aldrich). The bag was sealed onto the outside of the pot with duct tape. The bottom of the pot remained flush with the tray. The bag was then inflated with ambient air using a bicycle pump to ensure similar headspace volume among pots. Afterwards, a gas-tight syringe (Hamilton® syringe, 1000 series GASTIGHT®, PTFE luer lock) was used to remove 50 ml of ambient air from the bag, and replaced with 50 ml $^{13}CO_2$ (99.0 atom% ^{13}C Sigma-Aldrich, MO, USA). The pot was incubated with $^{13}CO_2$ for 30 min in its respective temperature treatment (following Karst *et al.*, 2016), after which we removed the gas bag under a fume hood.

Forty-eight hours after $^{13}CO_2$ labeling (to account for speed of photosynthate delivery to roots; Högberg *et al.*, 2008), we added a solution of 1.6 mg ^{15}N-enriched glycine (98 atom%; Sigma-Aldrich) mixed with 10 ml of deionized water to each pot. The solution was added exactly as done in Experiment 1.

Pots were harvested seven and 14 d after $^{13}CO_2$ labeling, in the same way as Experiment 1. Leaves, fine roots, and soil were processed and analyzed using the same procedure as in Experiment 1.

To quantify ^{13}C partitioned to root-associated fungi, phospholipid fatty acids (PLFAs) were extracted from the freeze-dried fine roots using a modified method of Bligh & Dyer (1959) and White *et al.* (1979). We analyzed ^{13}C in phospholipids because they easily decompose via soil enzymatic processes, and we thus assume that they reflect living organisms (White *et al.*, 1979; Olsson & Johnson, 2005). Briefly, samples were injected using splitless injection and separated on a 60 m × 0.20 µm ZB-FAME column (Phenomenex, Torrance, CA, USA), then measured on a single quadruple mass spectrometer (ISQ LT; ThermoFisher Scientific, Bremen, Germany). Concentrations were calculated against an internal standard (19 : 0) and based on total ion chromatogram (TIC) signal (*m*/*z* 35–450). Extracted PLFAs were analyzed for $\delta^{13}C$ on the same equipment described above. This method has been previously used to measure $\delta^{13}C$ signatures in various microbial communities (Evershed *et al.*, 2006; Joergensen, 2022). We used PLFA 18:2 6c as a fungal marker (Willers *et al.*, 2015), which was also the most abundant PLFA in our samples. We note that some crop plants have been shown to possess this marker (Zelles, 1997), but its presence in plant root tissue in the absence of mycorrhizal or endophytic fungi has not been demonstrated. Fine root fungal PLFA ^{13}C isotope ratios and subsequent calculations were done the same as described above. Fine root fungal PLFA ^{13}C per gram of dry fine root mass was calculated using the fine root fungal PLFA concentrations.

Experiment 3: ^{15}N-moss – $^{13}CO_2$ labeling

In this experiment, we applied ^{15}N-labeled moss litter and ^{13}C-labeled CO_2 to test our third hypothesis (H3) that warming will have little effect on the ^{13}C-for-^{15}N exchange when using ^{15}N-labeled feather moss litter. First, 48 additional *Empetrum* plants (not used in Experiments 1 or 2) were planted into pots in the same way as in Experiment 2. To quantify N uptake from moss litter concurrently with C assimilation from CO_2, we labeled the 48 pots (24 pots in each temperature, divided evenly among clones) with ^{15}N-enriched feather moss (*Pleurozium schreberi*) litter and $^{13}CO_2$. The feather moss used in this study originated from a moss/Ericaceous shrub community at the nearby Svartberget Research Station, where it had been watered three times during the growing season with a ^{15}N-NH_4/^{15}N-NO_3 solution, then was harvested 2 yr later and air-dried at room temperature before grinding to a fine powder using a ball mill. The resulting $\delta^{15}N$ signature of the moss was *c.* 41 234‰. After 36 d of adjustment to transplanting, we made five *c.* 1-cm-deep incisions in the soil encircling the *Empetrum* stem, as done in Experiment 2. We then used a plastic funnel to insert 0.3 g of the dried and ground ^{15}N-enriched moss litter into each incision (1.5 g total per pot), an amount calculated by using estimates of N demand along with moss $\delta^{15}N$ enrichment. Pots were watered immediately after inserting the moss litter. Then, 82 d after ^{15}N-moss addition (to allow for organic matter decomposition), we pulse-labeled each pot with $^{13}CO_2$ using the same procedure described in Experiment 2.

All pots were harvested 7 d after $^{13}CO_2$ labeling, in the same way as Experiment 1. Leaves, fine roots, and soil were processed and analyzed using the same procedure as in Experiment 2.

Data analysis

In all analyses, we fit linear mixed effects models using the lme function in the NLME package of R v.4.2 (Pinheiro & Bates, 2023; R Core Team, 2018). Clone was included as a random effect in all analyses to account for spatial autocorrelation and natural variation among clones. Residuals were visually inspected to ensure normal distribution and homoscedasticity. R-squared values were estimated using the r.squaredGLMM function in the MuMIn package (Barton, 2020)

To test our first hypothesis (H1) that warming increases organic N mineralization, the effect of warming on leaf natural

background $\delta^{15}N$ (i.e. unlabeled control pots) was tested with background $\delta^{15}N$ as the response variable and warming treatment as a fixed effect. The effect of warming on leaf N (%) was tested in Experiment 2 using temperature, time (after ^{13}C labeling), and their interaction as categorical fixed effects. The test was repeated for Experiment 3 without a time effect (because there was only one harvest time in Experiment 3). The effect of warming on the $^{13}C:{}^{15}N$ ratio in fine roots and soil in Experiment 1 was tested using temperature and time, and their interaction, as fixed effects.

To test our second hypothesis (H2) that warming influences the ^{13}C-for-^{15}N exchange in Experiment 2, we first examined the effect of warming on excess ^{15}N and ^{13}C abundance in leaves, fine roots, and soil. Warming treatment and time (after ^{13}C labeling) were included as fixed effects in the linear mixed effects model. Second, we tested the effect of temperature and time on the ^{13}C-for-^{15}N exchange. To account for varying plant sizes, excess fine root fungal PLFA ^{13}C (nmol g^{-1} dry mass) and excess leaf ^{15}N (μmol g^{-1} dry mass) were upscaled to the whole plant level by multiplying by total fine root dry mass (g per plant) and total green leaf dry mass (g per plant), respectively. We then calculated the ratio of excess fine root fungal PLFA ^{13}C (nmol per plant) to leaf ^{15}N (μmol per plant) to obtain a ^{13}C-for-^{15}N exchange ratio. A lower ^{13}C-for-^{15}N exchange ratio implies a lower ^{13}C cost (for *Empetrum*) per unit of assimilated ^{15}N. We then fit a linear mixed effects model. Warming treatment, time (after ^{13}C labeling), and their interaction were included as fixed effects, and the ^{13}C-for-^{15}N exchange ratio was the response variable. Data were square-root-transformed to meet assumptions of normal distribution and homoscedasticity.

We then tested the effects of temperature and time on the relationship between excess fine root fungal PLFA ^{13}C (nmol per plant) and leaf ^{15}N (μmol per plant). Excess fine root fungal PLFA ^{13}C (nmol per plant) was the response variable, while leaf ^{15}N (μmol per plant), temperature, time (days after ^{13}C labeling), and their interactions were included as fixed effects. To test whether ^{13}C partitioning to fungal symbionts was independent of ^{13}C partitioning to fine roots, we repeated this analysis but replaced fine root fungal PLFA ^{13}C with fine root ^{13}C.

To test our third hypothesis (H3) that warming will have little effect on the ^{13}C-for-^{15}N exchange when using ^{15}N-labeled feather moss litter, we repeated the same analyses used for H2 but with Experiment 3 (without a time effect). For all analyses, data were square-root-, log-, or Box–Cox-transformed when necessary to meet assumptions of normal distribution and homoscedasticity, and interactions were removed if there was no effect ($\alpha = 0.10$). All figures were made with the GGPLOT2 and COWPLOT packages in R software (Wickham, 2016).

Results

N mineralization

Leaf natural background $\delta^{15}N$ (i.e. $\delta^{15}N$ of unlabeled *Empetrum* leaves) was marginally higher under warming (-0.51 ± 0.90), and near the atmospheric standard of 0‰, compared with ambient temperature (-2.42 ± 1.33, $P = 0.067$, Table S2; Fig. S1a), indicating weak evidence of less discrimination of the ^{15}N isotope during uptake. Leaf N (%) was significantly higher under warming in Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment, Table S3; Fig. S1b), but there was no warming effect on leaf N (%) in Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment).

Experiment 1, which tested our first hypothesis (H1) that warming will increase mineralization of simple organic N, revealed a clear warming effect on glycine remaining in soil. In ambient temperature, the soil $^{13}C:{}^{15}N$ ratio averaged 0.96 (±0.08), suggesting that *c.* 52% of excess ^{13}C was respired. Warming resulted in an average $^{13}C:{}^{15}N$ ratio of 0.57 (±0.06), indicating that *c.* 72% of excess ^{13}C was respired ($P = 0.0003$, Table S4a; Fig. 1a). The soil $^{13}C:{}^{15}N$ ratio was unaffected by time (Table S4a).

Experiment 1 similarly revealed that warming was linked to lower amounts of intact glycine in fine roots. The fine root $^{13}C:{}^{15}N$ ratio averaged 0.24 (±0.04) in ambient temperature, indicating that *c.* 12% of excess ^{15}N in the fine roots was taken up as intact glycine. Warming halved this amount, indicating that *c.* 6% of excess ^{15}N in the fine roots of the warming treatment was taken up as intact glycine ($P = 0.02$, Table S4b; Fig. 1b). The fine root $^{13}C:{}^{15}N$ ratio was unaffected by time (Table S4b).

C and N assimilation

We found varied effects of warming on N and C assimilation in Experiment 2, which tested our second hypothesis (H2) that warming will cause *Empetrum* to assimilate more glycine ^{15}N and partition less ^{13}C to fungal symbionts, at least in the short term. *Empetrum* leaves assimilated more ^{15}N under warming ($P = 0.005$, Table S5a; Fig. 2a), but there was no warming effect on fine root ^{15}N assimilation (Fig. 2b) or soil ^{15}N (Fig. 2c). Warming increased leaf ^{13}C assimilation, particularly the first week after the label was applied (Table S6a; Fig. 3a). Warming also tended to decrease fine root fungal PLFA ^{13}C assimilation, demonstrating weak evidence that warming caused *Empetrum* to partition less ^{13}C to fungal symbionts ($P = 0.064$, Fig. 3c), despite no change in fine root fungal PLFA concentrations ($P > 0.24$, Table S7a).

We also found varied effects of warming on C and N assimilation in Experiment 3, which tested our third hypothesis (H3) that warming will have little effect on the ^{13}C-for-^{15}N exchange when using ^{15}N-labeled feather moss litter. Warming had no effect on leaf ^{15}N assimilation in Experiment 3 (Fig. 2d). These data also show that warming decreased the concentration of ^{15}N in fine root biomass (Table S5b; Fig. 2e), and had no effect on soil ^{15}N (Fig. 2f). Similar to Experiment 2, warming also increased leaf ^{13}C assimilation in Experiment 3 (Table S6a; Fig. 3e). Warming was also linked to lower soil ^{13}C (Table S6d; Fig. 3h), but had no significant effect on fine root ^{13}C (Fig. 3f) or fine root fungal PLFA ^{13}C in Experiment 3 (Fig. 3g).

^{13}C-for-^{15}N exchange

After the first sampling period after labeling, warming significantly lowered the ^{13}C-for-^{15}N exchange ratio (i.e. ratio of

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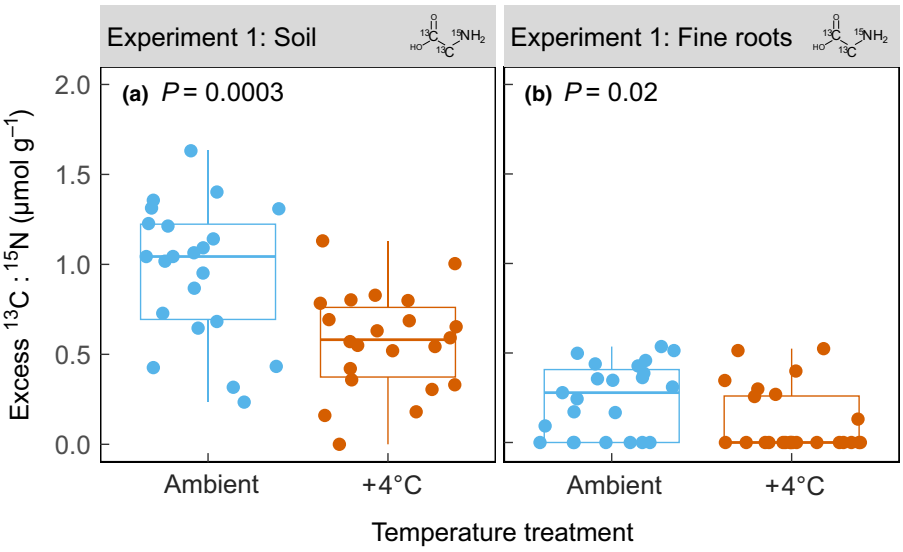


Fig. 1 Ratio of excess ^{13}C : ^{15}N (μmol g^{-1}) in (a) soil ($n = 24$ per temperature treatment; refer to Supporting Information Table S4a for statistical results) and (b) *Empetrum* fine roots ($n = 22$ per temperature treatment; refer to Table S4a for statistical results) in Experiment 1 (^{13}C-^{15}N-glycine experiment) in ambient and +4°C treatment. Time since labeling had no effect and samples from both harvest times were therefore pooled together. The middle horizontal line indicates the median value, the top and bottom lines indicate the first and third quartiles, respectively, and the whiskers extend to the largest value that is not further than 1.5 times the interquartile range. The *P* values are from linear mixed effects models.

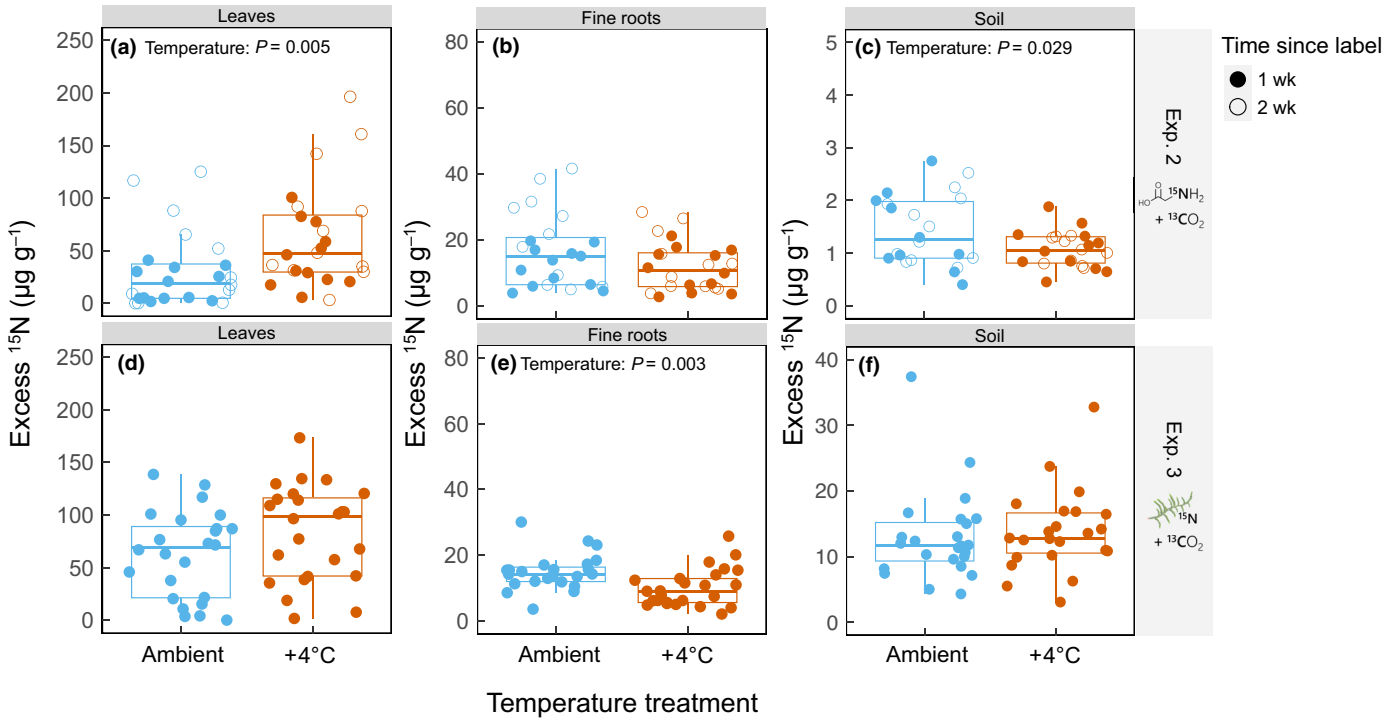


Fig. 2 Excess ^{15}N in (a–c) Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment; refer to Supporting Information Table S5 for statistical results) and (d–f) Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment; refer to Table S5 for statistical results) among different *Empetrum* tissue types (leaves, fine roots) and soil in ambient and +4°C treatment. The middle horizontal line indicates the median value, the top and bottom lines indicate the first and third quartiles, respectively, and the whiskers extend to the largest value that is not further than 1.5 times the interquartile range. The *P* values are from linear mixed effects models.

excess fine root fungal PLFA ^{13}C (nmol per plant) to excess leaf ^{15}N (μmol per plant)) in Experiment 2, indicating that less ^{13}C was partitioned to root-associated fungi per unit of assimilated glycine-derived ^{15}N (Table 1a; Fig. 4a). There was no significant effect of warming 2 wk after labeling (Fig. 4b). In contrast to Experiment 2, warming significantly increased the ^{13}C-for-^{15}N exchange ratio in Experiment 3 (Table 1b; Fig. 4c), indicating that more ^{13}C was partitioned to fungal symbionts per unit of moss-derived ^{15}N assimilated by *Empetrum*.

Across Experiment 2, we found significant evidence for a negative relationship between fine root fungal PLFA ^{13}C and leaf ^{15}N, but only 2 wk after labeling (Table S8a; Fig. S2a,b). There was no relationship between fine root fungal PLFA ^{13}C and leaf

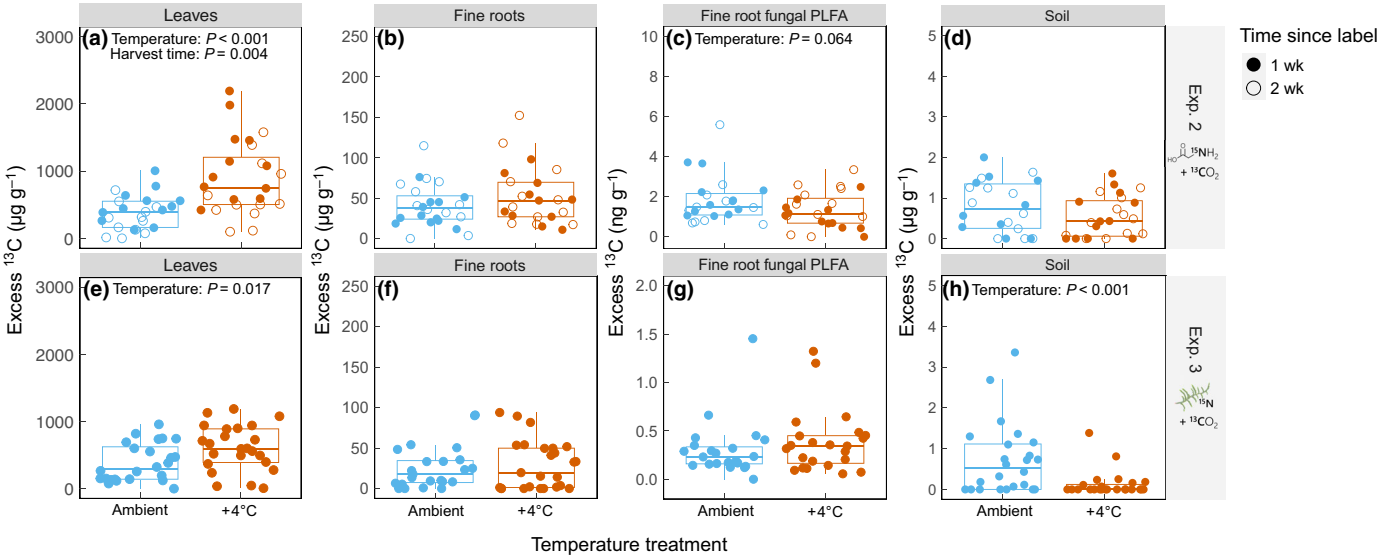
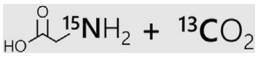


Fig. 3 Excess ^{13}C in (a–d) Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment; refer to Supporting Information Table S6 for statistical results) and (e–h) Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment, refer to Table S6 for statistical results) among different *Empetrum* tissue types (leaves, fine roots, fine root fungal PLFA) and soil in ambient and +4°C treatment. The middle horizontal line indicates the median value, the top and bottom lines indicate the first and third quartiles, respectively, and the whiskers extend to the largest value that is not further than 1.5 times the interquartile range. *P* values are from linear mixed effects models.

Table 1 Effects of temperature treatment and time (since CO_2 labeling) on the ^{13}C-for-^{15}N exchange ratio (i.e. ratio of *Empetrum* excess fine root fungal PLFA ^{13}C (nmol per plant) to *Empetrum* excess leaf ^{15}N (μmol per plant)) in (a) Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment; $n = 44$), and (b) Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment; $n = 47$), according to mixed effects model.

(a) Experiment 2

	^{13}C-for-^{15}N exchange ratio			
	$R^2_m = 0.376$; $R^2_c = 0.376$			
	DF_{num}	DF_{den}	*F* value	*P* value
Intercept	1	37	102.043	< **0.0001**
Temperature treatment	1	37	11.280	**0.002**
Time since label	1	37	9.943	**0.003**
Temperature × Time	1	37	4.643	**0.038**

(b) Experiment 3

	^{13}C-for-^{15}N exchange ratio			
	$R^2_m = 0.098$; $R^2_c = 0.098$			
	DF_{num}	DF_{den}	*F* value	*P* value
Intercept	1	39	85.543	< **0.0001**
Temperature treatment	1	39	4.675	**0.037**

Refer to Fig. 4. Bold values denote statistical significance ($P < 0.05$). R^2_m, marginal R^2 (fixed effects only); R^2_c, conditional R^2 (fixed and random effects); C, carbon; N, nitrogen; DF_{num}, degrees of freedom (numerator); DF_{den}, degrees of freedom (denominator).

^{15}N in Experiment 3 (Table S8b; Fig. S2c). Moreover, there was no relationship between fine root ^{13}C and leaf ^{15}N in Experiment 2 or Experiment 3 (Table S9).

Discussion

We performed three parallel and complementary experiments to test the effect of warming on the exchange of plant C for fungal N in a widespread Ericaceous shrub. We hypothesized (H1) that warming would increase mineralization of simple organic N; (H2) that warming would lower the ^{13}C-for-^{15}N exchange ratio (for *Empetrum*) of simple organic ^{15}N (glycine), largely due to more uptake of mineralized N which would reduce the need for mycorrhizal symbionts; and (H3) that the ^{13}C-for-^{15}N exchange ratio would remain relatively unchanged under warming when using a complex organic ^{15}N form (moss litter), due to higher C costs associated with liberating N locked within complex organic matter. Our hypotheses were partly supported; we found that warming initially increased glycine mineralization and lowered the ^{13}C-for-^{15}N exchange ratio of glycine-derived ^{15}N, but the effect disappeared over time. We also found that warming increased the ^{13}C-for-^{15}N exchange ratio when ^{15}N was derived from moss litter, indicating that the effect of warming varies depending on the form of organic N.

N mineralization

Our first hypothesis (H1) predicted that warming would increase organic N mineralization, for which we found several lines of supporting evidence. First, we found that warming halved the average $^{13}C:^{15}N$ ratio in soils of Experiment 1 (the ^{13}C-^{15}N-glycine experiment). A lower $^{13}C:^{15}N$ ratio in soil implies that more glycine was mineralized, with ^{13}C being respired (Näsholm *et al.*, 1998). Second, there was weak evidence that the natural background $\delta^{15}N$ of unlabeled *Empetrum* leaves was higher with warming ($P = 0.067$), indicating somewhat less discrimination

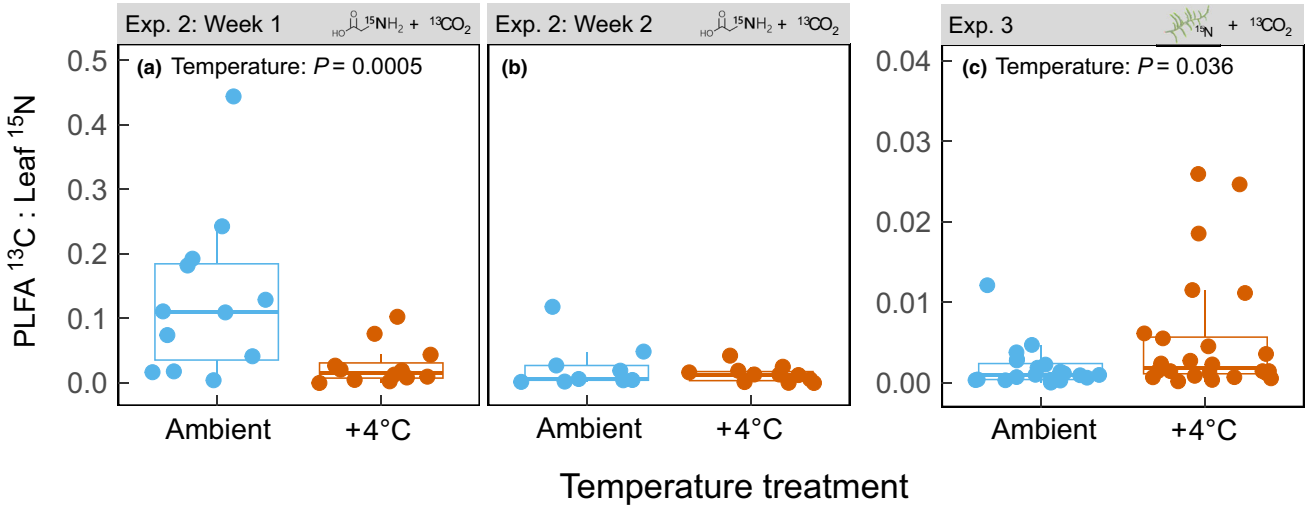


Fig. 4 The ^{13}C-for-^{15}N exchange ratio, expressed as a ratio of *Empetrum* excess fine root fungal PLFA ^{13}C (nmol per plant) to *Empetrum* excess leaf ^{15}N (μmol per plant) in (a, b) Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment), 1 and 2 wk after $^{13}CO_2$ labeling (total $n = 44$; refer to Table 1a for statistical results), and (c) Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment), 1 wk after $^{13}CO_2$ labeling (total $n = 44$; refer to Table 1b for statistical results). A smaller value indicates less ^{13}C partitioned to root-associated fungi per unit of assimilated ^{15}N. The *P* value in (a) was calculated from a *post hoc* test of multiple comparisons using least-squares means. The *P* value in (b) is from the linear mixed effects model.

against the heavier isotope during N uptake (Hobbie & Högberg, 2012). The likely explanation for this is that mycorrhizal fungi retain the heavier isotope (^{15}N) and transfer the lighter isotope (^{14}N) to the host plant due to faster reactions of the lighter isotope (Fry, 2006). Coupled with higher foliar N concentrations under warming in Experiment 2 (^{15}N-glycine-$^{13}CO_2$ experiment, Fig. S1), this suggests that *Empetrum* was overall less dependent on root-associated fungi for glycine-derived ^{15}N uptake under warming. Although temperature may not directly influence mycorrhizal colonization rates (Bennett & Classen, 2020), increasing soil N availability has been linked with declining ericoid and ectomycorrhizal fungal biomass (Hobbie & Colpaert, 2003; Nilsson *et al.*, 2005). However, we found no significant change in fine root fungal PLFA concentrations with warming in the present study, suggesting the temperature range within our experiment results in little change in mycorrhizal biomass within fine roots. Mycorrhizal biomass emanating from fine roots may have been more responsive to warming, but this could not be assessed in our study. Lastly, we found no temporal effect on soil or root ^{13}C : ^{15}N ratios. Free amino acids are known to have high turnover in soils (Jones & Kielland, 2002), suggesting that amino acid mineralization in our study may have occurred rapidly after application, with ^{13}C and ^{15}N subsequently immobilized.

^{13}C-for-^{15}N exchange when a simple organic N form is available

We found evidence to support our second hypothesis (H2) that warming will give *Empetrum* a more favorable ^{13}C-for-^{15}N exchange when a simple organic N form is available. Specifically, warming significantly lowered the ^{13}C-for-^{15}N exchange ratio (i.e. ratio of fine root fungal PLFA ^{13}C to leaf ^{15}N) in Experiment 2 at the first harvest time (i.e. 1 wk after the label was applied), indicating that less ^{13}C was partitioned to root-associated fungi per unit of assimilated ^{15}N. The lower ^{13}C-for-^{15}N exchange ratio was simultaneously driven by somewhat lower fine root fungal PLFA ^{13}C assimilation and higher leaf ^{15}N assimilation. We also found that the effect of warming on the ^{13}C-for-^{15}N exchange ratio disappeared 2 wk after the label was applied. Enhanced respiration of ^{13}C from root fungal PLFA in the warming compared with the ambient treatment could explain this difference in the ^{13}C-for-^{15}N exchange ratio between the first and second harvest. However, we did not observe a clear decline of excess ^{13}C in root fungal PLFA in the warming treatment compared with the ambient treatment over time. Furthermore, C turnover in PLFA of living biomass has been estimated as *c.* 44 d (Gunina *et al.*, 2017), and previous findings of ^{13}C retention in root fungal PLFA 16:1 5 and soil PLFA 18:2 6,9 have ranged from 8 to 16–20 d after pulse labeling (Olsson & Johnson, 2005; Birgander *et al.*, 2017). Our observed warming effect on the ^{13}C-for-^{15}N exchange ratio is thus more likely driven by N cycling than C partitioning. This is perhaps not surprising because, in addition to increased mineralization and plant uptake, warming also likely increased microbial immobilization of the ^{15}N label (Schmidt *et al.*, 2002), reducing the overall signal of our added ^{15}N-glycine pulse after 2 wk. Moreover, amino acids are relatively abundant in high-latitude soils, constituting up to 80% of available N (Kielland, 1995; Inselsbacher & Näsholm, 2012), suggesting that newly depolymerized free amino acids may have diluted the signal from the ^{15}N-glycine label. As a result, there may have been a similar warming effect after 2 wk, but it was simply undetectable because the free amino acid pool became dominated by unlabeled amino acids.

Synchronous with a diminishing warming effect on the ^{13}C-^{15}N exchange ratio, we found that a negative relationship developed between fine root fungal PLFA ^{13}C and leaf ^{15}N after 2 wk. This relationship may indicate declining ^{15}N availability and is consistent with studies of ectomycorrhizal symbioses (e.g.

Horning *et al.*, 2023). A growing body of evidence in boreal forests suggests that the plant C for fungal N exchange ratio in ectomycorrhizal symbioses can often surpass 1.0 (indicating more C partitioned to fungal symbionts per unit of assimilated N) and is potentially driven by the mycorrhizal symbiont (Näsholm *et al.*, 2013; Hasselquist *et al.*, 2016; Henriksson *et al.*, 2021). When host plants are not meeting their N demand, more C may be partitioned to ectomycorrhizal symbionts in an effort to acquire more N in return (Corrêa *et al.*, 2012). However, ectomycorrhizal fungi in boreal forests may retain N to meet their own demands, and consequently partition less N to host plants despite receiving large amounts of C (Näsholm *et al.*, 2013; Franklin *et al.*, 2014). Furthermore, in humus-rich boreal and arctic soils, ericoid and ectomycorrhizal fungi deploy a suite of extracellular enzymes to mine N from organic matter (Kohler *et al.*, 2015; Hupperts *et al.*, 2017), thus potentially increasing fungal C demands. Taken together, we speculate that the N demand of ericoid mycorrhizal fungi, coupled with potentially elevated C needs for mycorrhizal extracellular enzyme activity, may be driving the negative relationship between fungal ^{13}C partitioning and leaf ^{15}N assimilation in our study. Although we found lower fine root ^{15}N under warming, this finding could indicate that more ^{15}N is being translocated to other plant organs while simultaneously being immobilized in soil.

^{13}C-for-^{15}N exchange in response to a recalcitrant N form

We did not find evidence to support our third hypothesis (H3) that warming will have little effect on the ^{13}C-for-^{15}N exchange when using ^{15}N-labeled feather moss litter. In fact, warming had a positive effect on the ^{13}C-for-^{15}N exchange ratio in Experiment 3 (^{15}N-moss-$^{13}CO_2$ experiment). Furthermore, both leaf ^{15}N assimilation and fine root fungal PLFA ^{13}C assimilation were nonsignificantly higher with warming, indicating that a significantly higher ^{13}C-for-^{15}N exchange ratio was driven by only subtle changes in ^{13}C and ^{15}N partitioning. Ground-covering feather mosses and associated cyanobacteria are one of the largest known sources of biologically fixed N in high-latitude environments (DeLuca *et al.*, 2002; Lindo *et al.*, 2013; Hupperts *et al.*, 2021), and are a major source of organic matter input, thereby representing an important soil organic N source (Michelsen *et al.*, 2012; Wardle *et al.*, 2012; Jean *et al.*, 2020). In contrast to glycine, moss litter is highly recalcitrant and N incorporated therein first requires liberation (e.g. from fungal production of extracellular enzymes) before being assimilated (Strakova *et al.*, 2011). Because warming increased leaf ^{13}C assimilation (i.e. photosynthetic rates), *Empetrum* could potentially have been able to partition more ^{13}C to fungal symbionts to subsidize biomass or extracellular enzyme production for N mining. This would be consistent with previous work in N-limited boreal forests that has found positive links between host growth or photosynthetic rates and the abundance of C-demanding ectomycorrhizal fungi (Fernandez *et al.*, 2017; Hupperts & Lilleskov, 2022). It would also reflect similar findings from CO_2 enrichment studies (Terrer *et al.*, 2018; Pellitier *et al.*, 2021). Ultimately, Experiment 3 demonstrated that warming increased the ^{13}C-for-^{15}N exchange ratio with a ubiquitous litter N source, while Experiment 2 demonstrated that once amino acids are made available, warming lowered the ^{13}C-for-^{15}N exchange ratio.

There may be alternative explanations for our results. First, previous work suggests that C partitioned to root-associated fungi may not represent a cost for *Empetrum*, but instead result from the exudation of surplus C (Prescott *et al.*, 2020). Second, warming may have simply caused mycorrhizal fungi in Experiment 2 to partition more ^{13}C toward respiration or enzyme production, and less ^{13}C toward biomass production (the fungal ^{13}C pool that we measured; Forsmark *et al.*, 2021), resulting in a negligible net change in ^{13}C partitioned toward mycorrhizal fungi. However, previous work has found that temperature has little effect on ectomycorrhizal hyphal respiration rates (Heinemeyer *et al.*, 2007). Moreover, we did not find any consistent warming effect on fine root fungal PLFA concentrations, potentially suggesting that mycorrhizal biomass within fine roots was unaffected by warming. Warming may have also stimulated faster PFLA-C turnover, but one study estimated that C turnover in the PLFA of living biomass was *c.* 44 d (Gunina *et al.*, 2017), suggesting that the timeframe in our experiment was appropriate to prevent significant C turnover. Third, previous work has found that ericoid mycorrhizal fungi in pure culture can utilize amino acid residues as a sole C source (Bajwa & Read, 1986), which might cause the host plant to partition less C to the mycorrhizal symbiont, but evidence for alternative C utilization of mycorrhizas in symbiosis is still lacking. Lastly, while our methodological approach offers precise measures of ^{13}C and ^{15}N partitioning among different pools, it only captures a snapshot of the C and N that was potentially exchanged between *Empetrum* and its root-associated fungi.

Conclusions

We demonstrate that warming initially lowered the amount of ^{13}C partitioned to root-associated fungi per unit of leaf ^{15}N assimilated from a simple amino acid source, but increased the amount of ^{13}C partitioned to root-associated fungi per unit of ^{15}N assimilated from a recalcitrant moss litter source. Given that moss litter is a ubiquitous organic matter input in high-latitude environments, our findings indicate that warming may initially increase the amount of C partitioned to root-associated fungi per unit of assimilated N. However, when amino acids are made available following litter degradation, the effects of warming on the plant C for fungal N exchange ratio are reversed. Future work is need to further understand how rising temperatures interact with other global change drivers (e.g. increased atmospheric CO_2 concentrations and shifting precipitation patterns) in affecting the plant C for fungal N exchange. Ultimately, a lower C-for-N exchange ratio in response to warming could allow *Empetrum*, and perhaps other Ericaceous dwarf shrubs, to redirect C toward other functions such as growth, respiration, or defense. Our findings can be informative for Earth system models, which often do not explicitly account for microbial processes (ASM, 2023), to

improve predictions of high-latitude ecosystem responses to climate change.

Acknowledgements

MKS acknowledges funding from The Göran Gustafsson Foundations and VR (grant no. 2020-04240). SFH and MJG acknowledge funding from the Kempe Foundation (grant no. JCK-1911) and VR (grant no. 2020-03908). PK acknowledges funding from the Swedish University of Agricultural Sciences and from the Swedish Research Council Formas (project grant 2017-00366). The authors are grateful to Nils Henriksson and Marie-Charlotte Nilsson for comments on earlier versions of this manuscript. We are also deeply grateful to Niles J. Hasselquist whose expertise and passion for these research questions were critical for conceiving the ideas for this study.

Competing interests

None declared.

Author contributions

MKS conceived the idea for the study. SFH, KSI, MJG, PK and MKS contributed equally to experimental design and methodology. Fieldwork was done by KSI, SFH and PK. Isotope labeling was done by KSI and SFH; most other glasshouse and laboratory work was done by KSI. SFH conducted data analyses and wrote the manuscript with contributions from all authors.

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Data availability

The data and R script that support the findings of this study are available at https://figshare.com/projects/Empetrum_nitrogen-carbon_exchange/178239.

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Supporting Information

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

Dataset S1 Data supporting the findings of this study.

Fig. S1 Natural leaf $\delta^{15}N$ and %N in ambient and +4°C treatments.

Fig. S2 Relationship between excess fine root fungal phospholipid fatty acid ^{13}C and excess leaf ^{15}N in Experiments 2 and 3.

Table S1 Description of the three experiments in this study.

Table S2 Effect of temperature treatment on natural background Empetrum leaf $\delta^{15}N$.

Table S3 Effect of temperature treatment and harvest time on Empetrum foliar % N in Experiments 2 and 3.

Table S4 Effect of temperature treatment on soil and fine root ^{13}C : ^{15}N ratio in Experiment 1.

Table S5 Effect of temperature treatment and harvest time on ^{15}N among leaves, fine roots, and soil in Experiments 2 and 3.

Table S6 Effect of temperature treatment and harvest time on ^{13}C among leaves, fine roots, fine root fungal phospholipid fatty acid, and soil in Experiments 2 and 3.

Table S7 Effect of temperature treatment and harvest time on fine root fungal phospholipid fatty acid (18:2 6c) concentrations among the Experiments 2 and 3.

Table S8 *Empetrum* excess fine root fungal phospholipid fatty acid ^{13}C as a function of excess leaf ^{15}N, temperature, and time since label, in Experiments 2 and 3.

Table S9 *Empetrum* excess fine root ^{13}C as a function of excess leaf ^{15}N, temperature, and time since label, in Experiments 2 and 3.

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