Fungal Ecology 71 (2024) 101361

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Species-specific reliance of trees on ectomycorrhizal fungi for nitrogen supply at an alpine treeline

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ARTICLE INFO

Handling Editor: Prof. Karine Engelbrecht Clemmensen

Keywords:
^{14}C
Belowground allocation
Coniferous
Ectomycorrhizal hydrophobicity
Isotopic fractionation
Mycorrhizal fungi
Saprotrophic fungi
Soil horizons
Stable isotopes

ABSTRACT

Low temperatures near alpine treelines limit microbial release of soil nitrogen and tree growth. Ectomycorrhizal fungi can increase nitrogen supply for trees, but the importance of this exchange of carbon for nitrogen at the treeline remains unclear. Our bomb radiocarbon measurements indicated that trees transferred carbon fixed <2 years previously to fungi. The allocated carbon likely included sugars involved in starch synthesis, as $\delta^{13}C$ in fungal caps closely resembled that of fine-root starch. Mass balance of nitrogen isotopes along the plant-fungi-soil continuum revealed that *Larix decidua* trees relied less on fungal nitrogen (0–35% of N uptake), compared to *Pinus mugo* trees (up to 41%). We estimated that treeline trees allocate up to 18% of photoassimilates to fungi. Our findings suggest that at alpine treelines, trees allocate to ectomycorrhizal symbionts relatively high amount of carbon compared to the reverse nitrogen flux, but the exact exchange is tree species-specific.

1. Introduction

Ectomycorrhizal fungi are recognized as pivotal components of nitrogen (N) and carbon (C) cycling in boreal forests (Read and Perez-Moreno 2003). Less is known about ectomycorrhizal fungi in alpine treeline ecotones, but it is likely that they are similarly important in the cold climate and N-poor soils in these ecosystems (Sullivan et al., 2015; Hagedorn et al., 2019). Non-N-fixing plants acquire N in the form of small molecules such as ammonium (NH_4^+), nitrate (NO_3^-), and amino acids released by microbes from organic matter. Since microbial activity is sensitive to temperature, the concentrations of these N molecules are low in cold environments (Schimel and Bennett 2004; Hagedorn et al., 2019; Fetzer et al., 2024). Association with ectomycorrhizal fungi can enhance N uptake of plants because fungal hyphae utilize larger soil volumes than roots (Miller and Allen 1992). Ectomycorrhizal fungi are also capable of mineralizing organic N through the secretion of extracellular enzymes (Chalot and Brun 1998; Lindahl and Tunlid 2015). The enzymatic capabilities of ectomycorrhizal fungi, however, are lower than those of saprotrophic fungi, possibly because ectomycorrhizal fungi are not dependent on organic matter as a C source and instead acquire C from roots (Colpaert and vanLaere, 1996; Colpaert and vanTichelen, 1996). The C transfer from roots to ectomycorrhizal fungi is assumed to be in the form of soluble sugars and can be a significant component of the tree C balance. Under nutrient limitation, this C transfer can consume up to 20% of plant productivity (Vogt et al., 1982; Fogel and Hunt 1983; Ryan et al., 1996; Hobbie 2006).

The question of whether tree growth at alpine treeline ecotones is limited by N remains controversial. Due to low mineralization rates at cold temperatures, trees are assumed to be N-limited, which in turn suggests that large amounts of C must be allocated to ectomycorrhizal symbionts to acquire N (Read and Perez-Moreno 2003; Loomis et al., 2006; Thébault et al., 2014; Solly et al., 2017a; Möhl et al., 2019). In addition, and regardless of N status, low temperatures directly limit cell production and differentiation and thus plant growth (Körner 1998). The reduced plant growth is expected to suppress N demand, suggesting that soil N supply may have little effect on tree growth. Low-temperature growth limitation can also affect the amount of C allocated belowground, as photosynthesis proceeds at the critical low temperature for

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https://doi.org/10.1016/j.funeco.2024.101361
Received 31 May 2023; Received in revised form 17 May 2024; Accepted 3 June 2024
Available online 20 June 2024
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growth of 6.4–6.7 °C (Körner 2003b; Körner and Paulsen 2004). It has been suggested that the photoassimilates that cannot be used for growth are preferentially allocated to roots and mycorrhiza (Prescott et al., 2020). At even colder temperatures of 4–5 °C, however, trees stop allocating assimilates to their root systems (Ferrari et al., 2016). Therefore, the extent to which trees support ectomycorrhizal fungi for nutrient acquisition remains unresolved.

Studying C and N exchange between plants and fungi is notoriously difficult because roots and fungal mycelia are hidden belowground. One way to study this exchange is to measure natural ^{13}C and ^{15}N abundances and bomb ^{14}C in fungal sporocarps (Högberg et al., 1999; Kohzu et al., 1999; Hobbie et al., 2002; Taylor and Fransson 2007; Hobbie and Högberg 2012). Nitrogen isotope ratios (^{15}N:^{14}N expressed as $\delta^{15}N$) can indicate N metabolism and N sources in the plant-fungi-soil system (Högberg 1997; Hobbie et al., 2000). Saprotrophic fungi that do not transfer N to hosts usually have $\delta^{15}N$ values around 0‰, similar or slightly ^{15}N enriched compared with both its substrate and with atmospheric N_2, the ultimate N source (Mayor et al., 2009). Ectomycorrhizal fungi, in contrast, transfer N to plant hosts. Since this transferred N is ^{15}N-depleted, the remaining fungal N is enriched in ^{15}N compared to the substrate and the plant (Hobbie et al., 1999). The ^{15}N enrichment is generally smaller in hydrophilic fungi with small exploration volume and higher in hydrophobic fungi with greater exploration volume (Agerer 2006; Mayor et al., 2009; Hobbie and Agerer 2010; Hobbie et al., 2012). Isotopic fractionation during N uptake by roots is negligible in N-limited ecosystems (Högberg 1997), so this N source of plants has the $\delta^{15}N$ signature of the soil substrate, with typically higher $\delta^{15}N$ in deeper soil (Hobbie and Högberg 2012). Isotopic mass balance models considering $\delta^{15}N$ in soils, ectomycorrhizal fungi, and plants provide estimations for the fungal N partitioning between retention and transfer to plant hosts, and for the contribution of fungal N to total plant N uptake (Hobbie et al., 2000; Hobbie and Hobbie 2006). In combination with fungal C and N stoichiometry the mass-balance estimates allow to calculate the fraction from gross primary productivity (GPP) allocated to ectomycorrhizal symbionts ($fGPP_{ECM}$).

The C isotope ratios (^{13}C:^{12}C expressed as $\delta^{13}C$, ^{14}C:^{12}C expressed as $\Delta^{14}C$) can indicate the source of C compounds feeding mycorrhizal fungi and the overall C status of the plant. $\delta^{13}C$ is typically higher in saprotrophic fungi than in ectomycorrhizal fungi that in turn have higher values than host leaves (Högberg et al., 1999; Mayor et al., 2009; Hobbie et al., 2012). While these general patterns have been observed in several different sites, the source of this differentiation is unclear. Isotopic fractionation during post-photosynthetic processes might lead to ^{13}C enrichment in root nonstructural compounds that are transferred to ectomycorrhizal fungi, explaining the fungal high $\delta^{13}C$ (Hobbie and Werner 2004; Boström et al., 2008; Hobbie et al., 2012).

The time elapsed since the C in the root nonstructural compounds was photosynthetically fixed can be estimated using the bomb-^{14}C approach. The approach is based on the observed decline of atmospheric $\Delta^{14}CO_2$ since the nuclear weapon testing peak in the early 1960s (Trumbore et al., 2016). This results in a unique atmospheric $\Delta^{14}CO_2$ signature each year, which is transferred into biomass via photosynthesis. Thus, comparing ^{14}C in tissues to the atmospheric $\Delta^{14}CO_2$ record allows estimation of the mean time elapsed since C fixation, or in other words the C 'age'. Note that the mass-dependent isotopic fractionations that change $\delta^{13}C$ are corrected for when expressing $\Delta^{14}C$ (Stuiver and Polach 1977), so these can be ignored and the $\Delta^{14}C$ indicates time only. When a plant transfers newly fixed C into mycorrhizal symbiont, the $\Delta^{14}C$ of the symbiont equals the atmospheric signature, i.e. the symbiont ^{14}C age is 0.^{14}C ages of 0–2 years measured for mycorrhizal fungi in Douglas fir forest therefore indicate young transferred C from plants, in contrast with >10 years ages measured for saprotrophic fungi that feed on aged litter (Hobbie et al., 2002). But if the C transferred from host to symbiont originates from storage reserves in the plant, the $\Delta^{14}C$ of the symbiont may be higher (i.e. older) than that of the current atmosphere. To date, the ages of C in ectomycorrhizal fungi have indicated recent C sources. In cold boreal forests, however, respired CO_2 from coniferous fine roots, that likely have a similar C substrate as that transferred to mycorrhiza, can be up to 5 years old (Czimczik et al., 2006; Schuur and Trumbore 2006; Carbone et al., 2011). We speculate that ^{14}C ages in mycorrhiza can reflect the use of old storage C of a plant, whereby a greater reliance of root metabolism on storage C lead to older ^{14}C ages.

To study the role of ectomycorrhizal fungi in C and N exchange at the alpine treeline ecotone, we sampled two coniferous trees, the deciduous *Larix decidua* L. and the evergreen *Pinus mugo* spp. *Uncinata* Ramond, and co-existing sporocarps of ectomycorrhizal and saprotrophic fungi, along an elevation gradient at Stillberg in the central Swiss Alps. We measured $\Delta^{14}C$ of fungal sporocarps and $\delta^{13}C$ and $\delta^{15}N$ in roots, branches and needles of the two tree species and fungi. In addition, we used information from previous studies at the site on soil $\delta^{15}N$ and tree biomass to estimate N and C exchange between trees and ectomycorrhizal fungi. We hypothesized the following: (1) lower temperatures (but not below 5 °C) intensify the supply of newly fixed C belowground, resulting in the youngest ^{14}C in ectomycorrhizal fungi in the coldest sites; (2) carbon in ectomycorrhizal fungi has $\delta^{13}C$ closer to the $\delta^{13}C$ values of nonstructural C in roots than to $\delta^{13}C$ values in needles; (3) at colder temperatures trees take up more fungal N and hence have lower $\delta^{15}N$ values in needles; (4) the fast-growing pioneer *Larix* relies more strongly on direct root N uptake and has higher $\delta^{15}N$ than *Pinus* with a conservative growth strategy and a stronger dependency on ectomycorrhizal symbiosis; (5) typical of nutrient-limited trees, trees in the treeline ecotone obtain most of their N from fungi in exchange for a large proportion of their GPP.

2. Materials and methods

2.1. Site and experimental design

The Stillberg ecotone (46°47'N, 9°52'E) hosts an afforestation experiment established in 1975 (Barbeito et al., 2012) and same-age trees in lower altitude. Soils are acidic (pH 3.5–4.5), NO_3^--N concentration is ~2.5 μM and uniform across elevations, and NH_4^+-N concentration is ~9 μM on average with elevational decrease (Möhl et al., 2019). In the afforestation experiment, trees of three treeline species (*Larix decidua, Pinus mugo*, and *Pinus cembra*) were planted in equal distribution in 3.5 × 3.5 m plots. However, the initial even distribution and tree size were altered during the next 30 years by intensive *Pinus* mortality with complete disappearance of *P. cembra* and by faster growth at lower elevation, resulting in *Larix* dominance and tree-height decrease with elevation (Barbeito et al., 2012). Later assessment suggests that elevation-based differences in aboveground growth weakens as growth in the lowermost afforestation may be suppressed by competition among trees for nutrients and light, and self-cooling by heavier shading (Möhl et al., 2019). For the current study we sampled trees and fungal sporocarps from three elevations: low (2000 m a.s.l., below afforestation), middle (2080 m a.s.l., within afforestation), and high (2200 m a.s.l., within afforestation). The site previously hosted various experiments including free air CO_2 fertilization (FACE), soil warming, and ^{14}C isotopic labeling in pots (Hagedorn et al., 2010; Dawes et al., 2015; Ferrari et al., 2016). The sampling sites we chose are more than 50 m away from the previous studies locations, to ensure that isotopic signatures remained unaffected.

We estimated air and soil temperatures during the growing season by air temperature measurements from a meteorological station located within the afforestation at an elevation of 2090 m a.s.l. (Bebi 2016) and soil temperature measurements from data loggers buried throughout the ecotone (Fig. 1). We defined the growing season as the period between spring and fall when the soil temperature at 10 cm depth exceeds 3.2 °C (Körner and Paulsen 2004). We calculated that the mean air temperature at 1 m height during the growing season in the years 1987–2007 was 8.6 °C. At a lapse rate of 0.5 K per 100 m (Möhl et al., 2019), the air temperature at the high altitude is 8.1 °C (Fig. 1). Soil temperature at

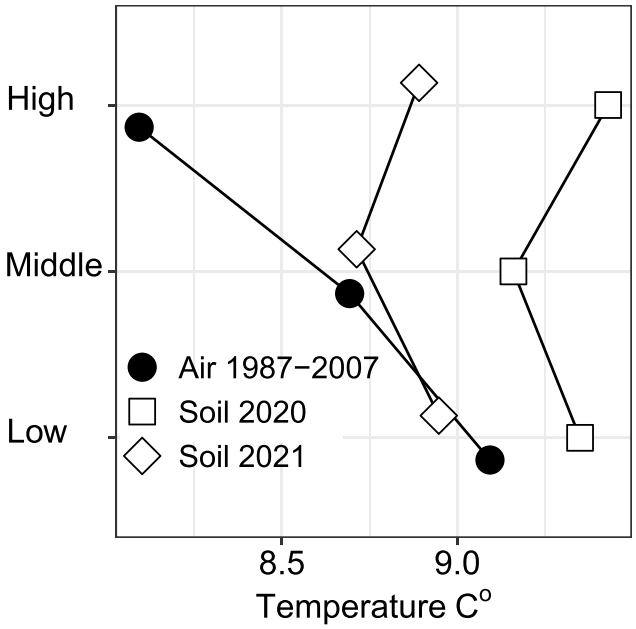

Fig. 1. Mean air and soil temperatures during the growing season across elevations. We present air temperature at 1 m above ground in a station located near the middle elevation, which is extrapolated to the other sites using lapse rate of 0.5 K per 100 m. Soil temperatures were measured by buried loggers at all elevations. Data was filtered for temperatures >3.2 °C [sensu Körner and Paulsen (2004)].

this elevation is warmer, and soil temperatures are generally more uniform across elevations (<0.2 °C variation) than air temperature, with an unexpected minimum at the middle elevation (Fig. 1). This minimum may be the result of the heavy shading from the dense *Larix* canopy. The fact that temperatures are warmer than 6.4–6.7 °C, the critical low temperature for growth, suggests that the top of the afforestation is not the 'fundamental' treeline, but rather the 'realized niche edge' (Körner and Hoch 2023). Given the presented temperatures, the maximum plant use of fungal N may not be at the highest elevation, but rather at mid-elevation where soil temperature is lowest.

During three days in the first week of September 2019 we sampled *Larix* and *Pinus mugo* (hereinafter '*Pinus*') trees. For each species × elevation combination we selected five replicate trees and excised 2-year-old branches including needles and fine roots. We traced the origin of the roots back to the tree trunks to be sure that the roots belonged to the trees studied. On the same day we sterilized the collected samples in a microwave (2 min on 900 W) to halt metabolism. On September 14th of the following year (2020) we collected over 170 ectomycorrhizal and saprotrophic fungi sporocarps. Individuals from the same species growing in proximity were regarded as replicates of the same sample. We identified 24 species and determined their guild and hydrophobicity according to published data (Table 1).

2.2. Sample handling

We analyzed tree and fungi samples for elemental and isotopic composition. Sterilized tree samples were oven-dried at 60 °C for two days. We then sorted the dry roots by diameter using a caliper to three size classes: <0.5 mm, 0.5–1 mm, and 1–2 mm. We separated the needles from the branches and scraped away the branch bark to analyze only the wood tissue. Samples were then milled and homogenized. Bulk samples (except the finest roots) were measured for δ^{15}N, %N, δ^{13}C, and %C. Fungal sporocarps were brushed clean and dried at 40 °C for two days. Cap samples were milled, homogenized, and measured for δ^{15}N, %N, δ^{13}C, %C, and Δ^{14}C. For Δ^{14}C analysis, samples were weighed into silver capsules; for the other analyses tin capsules were used.

Table 1
Fungal species collected in the study arranged by guild and hydrophobicity as defined by Agerer (2006). Classification of almost all fungi or members of the genus is according to Appendix 2 in Hobbie et al. (2012). Additional classification source is Unestam and Sun (1995).

Guild	Fungal species	Classification source
Ectomycorrhizal fungi		
Hydrophilic	*Chroogomphus rutilus*	Hobbie et al. (2012)
	Hygrophorus speciosus	Other spp. from the genus in Hobbie et al. (2012)
	Laccaria laccata	Hobbie et al. (2012)
	Lactarius porninsis	Other spp. from the genus in Hobbie et al. (2012)
	Lactarius rufus	Hobbie et al. (2012)
	Russula decolorans	Hobbie et al. (2012)
	Thelephora terrestris	Unestam and Sun (1995)
Hydrophobic	*Cortinarius cinnamomeus*	Hobbie et al. (2012)
	Cortinarius sp.	Other spp. from the genus in Hobbie et al. (2012)
	Paxillus involutus	Hobbie et al. (2012)
	Rhizopogon luteolus	Unestam and Sun (1995)
	Suillus cavipes	Other spp. from the genus in Hobbie et al. (2012)
	Suillus granulatus	Other spp. from the genus in Hobbie et al. (2012)
	Suillus grevillei	Other spp. from the genus in Hobbie et al. (2012)
	Suillus tridentinus	Other spp. from the genus in Hobbie et al. (2012)
Saprotrophic fungi		
Litter decay	*Collybia dryophila*	Other spp. from the genus in Hobbie et al. (2012)
	Clitocybe sp. *1-6*	Other spp. from the genus in Hobbie et al. (2012)
	Cystoderma carcharias	Other spp. from the genus in Hobbie et al. (2012)
	Mycena sp. *1-2*	Other spp. from the genus in Hobbie et al. (2012)
	Collybia sp.	Other spp. from the genus in Hobbie et al. (2012)
Wood decay	*Tricholomopsis rutilans*	Hobbie et al. (2012)

In addition to the bulk samples we extracted water-soluble C (a proxy for sugars) and starch from all tree tissues. For the water extraction we mixed 50-mg samples in 1.5 ml deionized water at 65 °C. The solution was then pipetted into tin capsules for analysis. We conducted two additional water washings to remove any remaining soluble C. The supernatants from the two extra washings were discarded after centrifugation, and the remaining pellet was dried for starch extraction. To convert the starch in the pellet to water soluble glucans we digested the samples with the enzyme α-amylase (Sigma cat. No. A4551) at 85 °C for 30 min (Landhäusser et al., 2018).

We applied three measures to remove extraneous C that could influence the measured δ^{13}C and cause it to deviate from the true sample value. First, the Eppendorf tubes used through the process were extensively prewashed. Second, the enzyme was prewashed three times and ultra-filtrated with Amicon® Ultra 15 ml Centrifugal Filters (Lot R3MA06116, Merck, Darmstadt, Germany) to remove stabilizers and other hydrolysable substances found in commercial enzymes (Richter et al., 2009). Third, to remove the enzymes from the glucans, we ultra-filtered the digested solution with the Amicon® filters. The extraneous-C removal was verified by measurements of blanks and sucrose samples with known δ^{13}C (−10.8 ± 0.47‰, IAEA-C6). In the water-soluble C protocol the sucrose samples had the expected δ^{13}C values (Fig. S1). In the starch protocol, however, we found residual C in the blanks with a mean value (± standard deviation) of −29.8‰ ± 3.5, which lowered the measured δ^{13}C of sucrose samples smaller than 1 mg. However, a correction equation based on the blanks and the sucrose runs

changed the $\delta^{13}C$ value of the plant starch extracts by only 0.01‰ on average – below the measurement precision (0.1‰). We explain the negligible effect by the similarity in $\delta^{13}C$ between the extraneous C and the samples.

2.3. Mass spectrometry and elemental analysis

To measure $\delta^{15}N$ and $\delta^{13}C$, samples weighed into tin capsules were combusted using an elemental analyzer (EA; NA 1110, CE Instruments, Milan, Italy) coupled to an Isotope Ratio Mass Spectrometer (IRMS; Delta^{+}XL, Thermo Finnigan, Bremen, Germany) via a ConFlow III. $\delta^{15}N$ and $\delta^{13}C$ were calculated by equation (1):

$$\delta^{15}N, \delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad \text{Eqn. 1}$$

Where R is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in either the sample (R_{sample}) or the standard ($R_{standard}$). Reference standards are AIR-N_2 for $\delta^{15}N$ and VPDB for $\delta^{13}C$, and the internal standards are acetanilide ($\delta^{15}N$: −1.51 ± 0.1‰, $\delta^{13}C$: −30.06 ± 0.1‰) and caffeine ($\delta^{15}N$: −15.46 ± 0.1‰, $\delta^{13}C$: −40.46 ± 0.1 ‰). %C and %N were measured in separate EA analysis (vario MAX cube, Elementar Analysensysteme GmbH, Langenselbold, Germany).

To measure fungi $\Delta^{14}C$, samples were graphitized by reacting CO_2 generated on combustion with hydrogen in the presence of an iron catalyst (Steinhof et al., 2017). The radiocarbon content of the graphite was analyzed by accelerator mass spectrometry at the Max Planck Institute for Biogeochemistry in Jena, Germany (AMS; Micadas, Ionplus, Switzerland). $\Delta^{14}C$ is the deviation in permil of the $^{14}C/^{12}C$ ratio from "Modern" C (Trumbore et al., 2016):

$$\Delta^{14}C = \left[\frac{R_{-25}}{0.95 \times R_{oxalic,-19} \times e^{x - 1950/8267}} - 1\right] \times 1000 \qquad \text{Eqn 2}$$

Where R_{-25} is the sample $^{14}C/^{12}C$ ratio corrected for mass dependent fractionation by normalizing the sample's $\delta^{13}C$ to a $\delta^{13}C$ of −25‰. $R_{oxalic,-19}$ is the $^{14}C/^{12}C$ ratio in the standard, oxalic acid, normalized to $\delta^{13}C$ of −19‰, and the 0.95 term converts to the absolute radiocarbon standard (1890 wood) activity in 1950. The exponent corrects for decay of ^{14}C in the standard between 1950 and the year of measurement (x), to provide the absolute amount of ^{14}C in our samples.

2.4. Estimating carbon age

The bomb-^{14}C approach is based on a comparison between $\Delta^{14}C$ in a sample and in atmospheric CO_2. A criterion for the application of this approach is that the C in the sample was fixed from the atmosphere after the bomb peak (c. 1963). Then, higher $\Delta^{14}C$ means older C. Carbon from the trees studied, planted in 1975, meets this criterion. The criterion would be violated if saprotrophs scavenge C from pre-bomb organic matter, but saprotrophic fungi scavenge C primarily in the shallow litter layers containing tree litter (Lindahl et al., 2007; Solly et al., 2017b).

We estimated the local $\Delta^{14}CO_2$ record from annual tree rings and direct atmospheric $\Delta^{14}CO_2$ measurements (Fig. 2). For reference we also plot measured $\Delta^{14}C$ from leaves, respired CO_2 from leaves, and local atmospheric CO_2, and a global compilation of atmospheric $\Delta^{14}CO_2$ data (Hua et al., 2013). Tree rings presumably contain CO_2 fixed during the year of growth and are therefore a proxy for the local atmospheric $\Delta^{14}CO_2$. We analyzed annual rings extracted from one tree (>70 years old) growing 200 m below the low elevation site. We retrieved direct atmospheric $\Delta^{14}CO_2$ from the ICOS stations at Jungfraujoch (3450 m a.s.l), located 140 km from Stillberg in the Swiss Alps, and from Schauinsland (1205 m a.s.l), located 190 km from Stillberg at the Black Forest, Germany (Hammer and Levin 2017; Emmenegger et al., 2023). We filtered these data to use measurements from the local growing season that represent CO_2 available for photosynthesis and growth. Tree

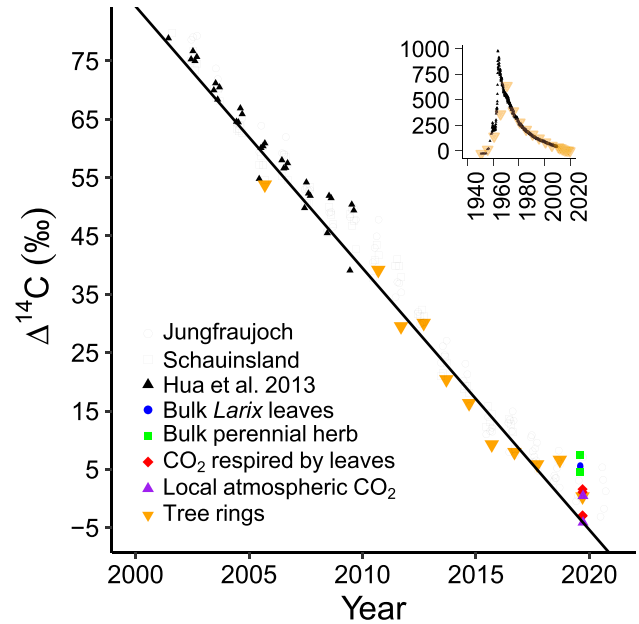

Fig. 2. Reconstruction of atmospheric $\Delta^{14}CO_2$ record in our site. Colored symbols represent new measurements, and black symbols represent two near ICOS stations were direct atmospheric $\Delta^{14}CO_2$ measurements are conducted regularly (Hammer and Levin 2017; Emmenegger et al., 2023), and global compilation of atmospheric $\Delta^{14}CO_2$ data for the period 1950–2010 (Hua et al., 2013). The black line is a linear fit for the tree rings and the ICOS stations data.

ring $\Delta^{14}C$ values were mostly within or slightly below the direct atmospheric $\Delta^{14}CO_2$ values (Fig. 2). Reasons for this could be local "Suess effect" dilution from fossil fuel emissions, which are completely depleted of ^{14}C and may be stronger lower in the atmosphere, or bias due to the low temporal resolution of the sampling (1 tree ring per year vs. 4–11 measurements per year per ICOS station). To remove possible biases from the single-tree rings, we averaged the annual atmospheric $\Delta^{14}CO_2$ for each of the ICOS stations, and then averaged an overall $\Delta^{14}CO_2$ mean including the ICOS averages and the tree-ring data. We then fitted a linear line to the overall means for the years 2000–2020, which covers most of the fungal $\Delta^{14}C$ values (the black line in Fig. 2). The resulting equation is $y = 9059–4.5x$ ($R^2 = 0.99$). According to the equation, the atmospheric $\Delta^{14}CO_2$ has been decreasing by 4.5‰ each year, and the atmospheric $\Delta^{14}CO_2$ during the 2020 growing season was −5.4‰. With this information the $\Delta^{14}C$ of a sample can be converted to age by the equation:

$$\text{Age} = \frac{\Delta^{14}C - (-5.4)‰}{4.5‰/\text{yr}} \qquad \text{Eqn 3}$$

When only tree rings are regressed the annual $\Delta^{14}CO_2$ decrease is the same and 2020 atmospheric $\Delta^{14}CO_2$ is lower by 2.7‰. We estimated the age of one sample with a $\Delta^{14}C$ value that was observed in the atmosphere before the year of 2000 by comparing it to the year with the closest atmospheric $\Delta^{14}CO_2$ value.

2.5. Estimating nitrogen transfer from fungi to trees and carbon from trees to fungi

We applied two approaches of mass balance models to estimate the percentage from the N entering ectomycorrhizal fungi that is transferred to plant hosts (*T*), and the percentage from total tree N acquisition supplied by fungal N (*f*). The first approach, 'transfer only', assumes the only ^{15}N fractionation along the path soil-fungi-plant is in the fungi-plant step (Hobbie and Hobbie 2006). The process that fractionates N

isotopes is the fungal synthesis of transfer compounds that favors ^{14}N over ^{15}N with fractionation factor $\Delta = 9‰$ (Hobbie and Colpaert 2003). As a consequence of the isotopic fractionation, the $\delta^{15}N$ of the N transferred to the plant ($\delta^{15}N_{tr}$) is lower than the $\delta^{15}N$ of the remaining N in the fungi ($\delta^{15}N_{ECM}$) and lower than the $\delta^{15}N$ of the soil N available for fungal uptake ($\delta^{15}N_{soil_ECM}$). Besides fungal N, trees acquire N by direct root uptake of soil N with an isotopic signature $\delta^{15}N_{soil_roots}$. The isotopic signature of trees ($\delta^{15}N_{tree}$) is therefore a weighted average of the fungal and soil N sources. These relations are described by equations (4)–(6) (Hobbie and Hobbie 2006):

$$100 \times \delta^{15}N_{soil_ECM} = T \times \delta^{15}N_{tr} + (100 - T) \times \delta^{15}N_{ECM} \quad \text{Eqn. 4}$$

$$100 \times \delta^{15}N_{tree} = f \times \delta^{15}N_{tr} + (100 - f) \times \delta^{15}N_{soil_roots} \quad \text{Eqn. 5}$$

$$\Delta = \delta^{15}N_{soil_ECM} - \delta^{15}N_{tr} \quad \text{Eqn. 6}$$

For $\delta^{15}N_{ECM}$ and $\delta^{15}N_{tree}$, we used the values we measured at each of the three elevations for each of the hydrophilic and hydrophobic ectomycorrhizal fungal guilds ($\delta^{15}N_{ECM}$) and each of the tree species ($\delta^{15}N_{tree}$, needle values). Values for $\delta^{15}N_{soil_roots}$ and $\delta^{15}N_{soil_ECM}$ were based on previous studies near the high elevation, which we extrapolated to the lower elevations assuming the same soil $\delta^{15}N$ values (Möhl et al. 2018, 2019). For $\delta^{15}N_{soil_ECM}$ we used bulk soil measurements along the soil profile (Dawes et al., 2017). As inferred by fungal DNA abundance, the peak activity of ectomycorrhizal fungi is between the organic and mineral soil layers (Lindahl et al., 2007). The bulk soil $\delta^{15}N$ in this layer was 0.9‰ under *Larix* and −1.4‰ under *Pinus*. For $\delta^{15}N_{soil_roots}$, we used values of NH_4^+, the main plant-available N molecule in the soil: 1.4‰ under *Larix* and 1.0‰ under *Pinus* (Möhl et al., 2019). NH_4^+ was extracted from the 0–10 cm depth that hosts 94% of the fine roots under both tree species (Dawes et al., 2015). We therefore did not expect a significant N uptake at deeper depths.

In the second approach, 'saprotrophic reference', we investigated the possibility that saprotrophic fungi can serve as a reference for isotopic fractionation and N uptake in ectomycorrhizal fungi. The $\delta^{15}N$ we measured in saprotrophic fungi ($\delta^{15}N_{SAP}$) was higher than $\delta^{15}N$ in soil substrates; since the high values cannot be related to N transfer to hosts, isotopic fractionation leading to this isotopic enrichment could have contributed to the high $\delta^{15}N_{ECM}$ values. To account for the speculated isotopic fractionation, we used Eqn. (7), a modification of Eqn. (6), to adjust $\delta^{15}N_{tr}$:

$$\Delta = \delta^{15}N_{SAP} + \delta^{15}N_{soil_ECM} - \delta^{15}N_{soil_SAP} - \delta^{15}N_{tr} \quad \text{Eqn. 7}$$

For $\delta^{15}N_{SAP}$, we used the mean value we measured at each of the three elevations. $\delta^{15}N_{soil_SAP}$, the soil N taken up by saprotrophic fungi, is ^{15}N depleted compared to $\delta^{15}N_{soil_ECM}$ because saprotrophic fungi are active shallower in the soil, in the litter layer (Lindahl et al., 2007; Solly et al., 2017b). The term $\delta^{15}N_{soil_ECM}$ - $\delta^{15}N_{soil_SAP}$ compensates for the different scavenging depths. For $\delta^{15}N_{soil_SAP}$ we used the values measured for the litter layer: −0.7‰ under *Larix* and −3.8‰ under *Pinus* (Dawes et al., 2017). The resulting $\delta^{15}N_{tr}$ was then used in Eqn. (5) to estimate *f*.

It has been proposed that N transfer to hosts explains why ectomycorrhizal fungi have lower %N compared to saprotrophic fungi (Hobbie and Colpaert 2003; Trocha et al., 2016). Assuming that saprotrophic and ectomycorrhizal fungi obtain the same amount of N, a mass balance can be described:

$$N_{SAP} - N_{tr} = N_{ECM} \quad \text{Eqn. 8}$$

Where N denotes N amounts in saprotrophic fungi (SAP), ectomycorrhizal fungi to roots transfer (tr), and ectomycorrhizal fungi (ECM). An alternative estimation of *T* can be then calculated:

$$T = 100 \times \frac{N_{tr}}{N_{SAP}} = 100 \times \left(1 - \frac{N_{ECM}}{N_{SAP}}\right) \quad \text{Eqn. 9}$$

Since we lack information about fungi biomass and absolute N stocks, we rely on the assumption that N uptake and transfer are scaled with fungal biomass and hence apply the measured %N in the fungal guilds in the equation.

Accurate $\delta^{15}N$ values of soil N substrates are difficult to determine. While we assigned $\delta^{15}N$ to root and fungal substrates based on existing knowledge and previous measurements at the site, the exact soil depth of N uptake is uncertain. Rapid $\delta^{15}N$ increases with soil depth suggests that small shifts in rooting or scavenging depth can significantly affect *T* and *f*. To assess the sensitivity of *T* and *f* to substrate values we varied $\delta^{15}N_{soil_roots}$, $\delta^{15}N_{soil_ECM}$, and $\delta^{15}N_{soil_SAP}$ according to $\delta^{15}N$ values along the soil profile (from −3‰ in the litter to +3‰ in mineral soil).

To calculate $fGPP_{ECM}$, we estimated the net primary productivity (NPP) and annual N uptake (N_{uptake}) of the trees, then we estimated the annual amount of N used for fungal growth using *T* and *f*, and finally we stoichiometrically converted the fungal-growth N to C and biomass to estimate fungal C demand and growth (NPP_{ECM}). The NPP_{ECM} was also used to constrain the modeled *T* and *f* by comparison with previous assessment of fungal growth at the site. To estimate tree NPP [g C m^{-2} yr^{-1}], we used biomass per m^{-2} of the compartments needles, wood, coarse roots, and fine roots of the control trees of the FACE experiment near the high elevation (Handa et al., 2008; Dawes et al., 2015). We converted biomass to NPP by multiplying the needle and fine roots biomass by their predicted turnover rates, which are 1 yr^{-1} for *Larix* needles and fine roots of both trees (Brunner et al., 2013), and 6 yr^{-1} for *Pinus* needles (Ballian et al., 2016). In the absence of allometric equations for our site, we assumed that the annual growth of wood and coarse roots was 3% of the standing biomass, which resulted in a growth rate comparable to or slightly higher than the average NPP over the life of the trees. The %C of the plant material was assumed to be 45%. To calculate N_{uptake} [g N m^{-2} yr^{-1}] we multiplied the annual growth of each compartment by its %N (divided by 100): for needles we used %N values from litter (1.9% *Larix*, 1.3% *Pinus*, F. Hagedorn, unpublished data), for fine roots we used values reported in the same study that published the fine roots biomass [0.86% *Larix*, 0.7% *Pinus* (Handa et al., 2008)], and for wood and coarse roots we used our measurement for branch wood (0.6% for both species). We used an equation from Hobbie and Hobbie (2008) to convert N_{uptake} to fungal C demand, but decomposed the equation to calculate NPP_{ECM} and $fGPP_{ECM}$ separately:

$$NPP_{ECM} = N_{uptake} \times f \times (1/T - 1) \times C/N_{ECM} \times 1/0.45 \quad \text{Eqn. 10}$$

$$fGPP_{ECM} = NPP_{ECM} \times 0.45 \times 1/CUE_{ECM} \times CUE_{tree}/NPP \quad \text{Eqn. 11}$$

Where the term $N_{uptake} \times f$ is the annual amount of N supplied to the tree by fungi and $1/T$ - 1 converts to the amount of N retained for fungal growth. For *f*, we used the values we calculated at each of the three elevations for each of the tree species. For *T*, we averaged the values for hydrophilic and hydrophobic fungi at each elevation. C/N_{ECM} is the C/N ratio of fungi that was 10.55 in average among the ecotomycorrizal sporocarps, and the term 0.45 represents fungal %C of 45%. CUE_{ECM} and CUE_{tree} are the carbon use efficiency of ectomycorrhizal fungi and trees, respectively. We applied CUE_{ECM} of 0.5 used in previous works (Hobbie and Hobbie 2006, 2008) and CUE_{tree} of 0.4 estimated for boreal forests (DeLucia et al., 2007). We also included $fGPP_{ECM}$ estimates for the lower elevations, although NPP and N_{uptake} are only available for the high elevation, because biomass partitioning between tree compartments in treeline ecotones is not expected to change with elevation (Bernoulli and Körner 1999; Körner 2003a). Accordingly, NPP and N_{uptake} in the larger trees at lower elevations increase by the same factor, which is cancelled out in the $fGPP_{ECM}$ calculation. Our $fGPP_{ECM}$ estimates for these elevations are based on the high-elevation NPP and N_{uptake} values and the local *T* and *f*.

We constrained our models by the NPP_{ECM} values, which we compared to an independent estimate of mycelial growth; Hagedorn et al. (2013) buried cylindrical bags (Ø 2 cm, 50 μm mesh) filled with

sand in the top 5 cm of the soil in the plots of the FACE experiment near the high elevation. Over the next three years, they collected the bags at the end of the growing season and estimated the in-growth of mycelial biomass per unit area using the method of Wallander et al. (2001). Here we used the data from the control plots, including 10 bags collected each year. Mycelial biomass divided by incubation years was assumed to represent 'raw' NPP_{ECM}. Among their known limitations, in-growth bags cover only a fraction of the soil profile and are blind to mycelial turnover (Ekblad et al., 2013; Wallander et al., 2013). Using fine root distribution to approximate mycelial distribution in the soil profile, we estimate that the bags buried at 0–5 cm depth represent 60% of fungal growth at 0–20 cm depth (Dawes et al., 2015). Mycelial turnover data are not available for our site, but in boreal forests mycelia turn over 1–7 times per year (Hagenbo et al., 2017). These factors were used to calculate the range of possible NPP_{ECM}.

2.6. Statistical analysis

We applied linear mixed-effect models with the R function '*lmer*' (package '*lme4*') to assess effects of explanatory variables on our measurements (Bates et al., 2015; R Development Core Team 2019). For tree samples, we tested the effects of species, elevation, and tissue while individual tree was set as a random variable. For fungal samples, we tested effects of fungal guild and elevation while fungal species was set as the random variable. Starting from a null model with the random intercept, we added sequentially the fixed factors and two-way interactions. The increasingly complex models were tested for parsimony, i.e. whether the increased complexity improves significantly the model ability to explain the data. Parsimony was tested using the 'anova' function. The χ^2 test was applied to assess the statistical significance of the stepwise model improvement. When the addition of a factor or interaction term was significant, estimated marginal means post-hoc tests were used to compare factor levels ['emmeans', package 'emmeans' (Lenth 2021)]. The response variable was log transformed when the assumption of homogeneity of variance was violated. Significant effects inferred while data was transformed were regarded as significant only when inferred also by the analysis of the untransformed variable.

3. Results and discussion

The variation in elemental and isotopic compositions among fungal species suggests diverse C and N sources and metabolic pathways (Table S1). Nonetheless, in the search for ecological generalizations we narrowed our analysis to focus on the guild level (Fig. 3). Elevation had no effect on the measured variables, and the weak elevation × guild effects found in some variables were mostly between irrelevant pairs (Table S2). In contrast, $\Delta^{14}C$, $\delta^{13}C$, %N, %C, and C/N showed a clear separation between saprotrophic fungi and the two ectomycorrhizal guilds (Tables S2 and S3). $\delta^{15}N$ was the only proxy to divide the hydrophobic and hydrophilic groups where the latter had $\delta^{15}N$ values similar to the saprotrophs (Fig. 3, Table S3). The ability of the variables to distinguish between fungal guilds in the treeline ecotone is in agreement with studies in other ecosystems, clearly demonstrating the usefulness of the variables in identifying the guild of unknown fungi (Högberg et al., 1999; Hobbie et al., 2002; Hobbie and Agerer 2010; Trocha et al., 2016).

3.1. Young C ages of ectomycorrhizal fungi indicate transfer of young carbon from plants

Mean ± SE $\Delta^{14}C$ values for ectomycorrhizal fungi (1.2 ± 0.9‰, both hydrophobicity groups) and the saprotrophs (29.6 ± 8.7‰) translate, according to the local atmospheric $\Delta^{14}CO_2$ record, to C ages of 1.5 ± 0.2 years and 7.6 ± 1.7 years, respectively. Fungal guilds also differed in C age variability. The C ages of saprotrophic fungi ranged from 1.6 to 12 years for most species sampled, while one wood decomposer (*Tricholomopsis rutilans*) reached an age of 28 years. The large variability reflects the diversity of litter sources, ranging from young needles to decades-old wood. The values mostly correspond to the 0–10 years range measured for litter decomposers in a *Pinus sylvestris* L. forest in boreal Sweden (Lindahl et al., 2007). A smaller C age variability (<3.5 years) was found in ectomycorrhizal fungi, reflecting a more uniform age of root carbohydrates.

We hypothesized younger ^{14}C ages for ectomycorrhizal fungi at colder elevations. The rationale was that the expected continuation of photosynthesis at growth-suppressing low temperatures would result in a surplus of photoassimilates that would be preferentially allocated to roots and ectomycorrhizal symbionts (Prescott et al., 2020). The uniform ^{14}C ages among elevations (Table S2), despite the observed air temperature gradients (Fig. 1), refutes this hypothesis. However, observations from the soil warming and mesocosm experiments at our site suggest that when soil is warmer than air (as at the high elevation), more C is allocated to the belowground (Hagedorn et al., 2010; Streit et al.,

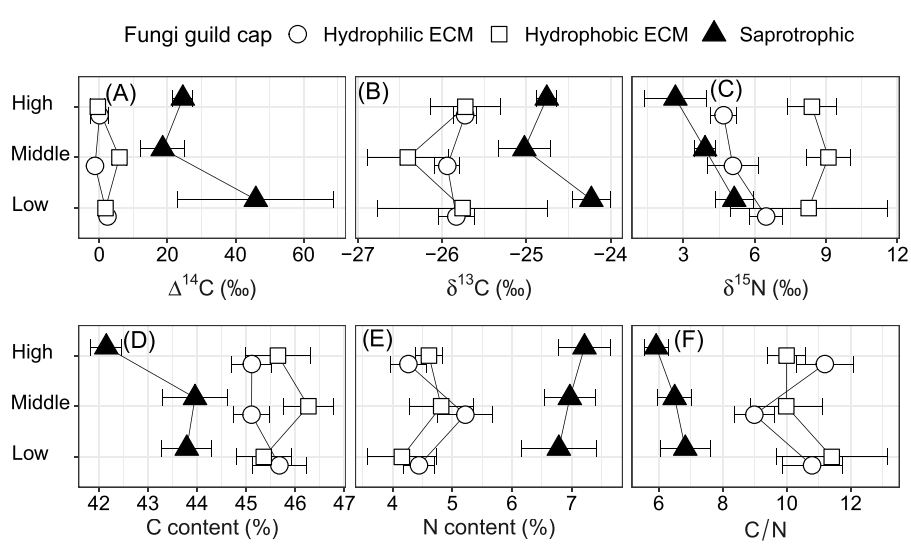

Fig. 3. Mean ± SE values for $\Delta^{14}C$, $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N ratio of fungal caps across ecotone elevations and fungal guilds (n = 3–9).

2014a; Solly et al., 2017b; Ferrari et al., 2018). A possible reason for the lack of an elevational ^{14}C trend is the low sensitivity of the ^{14}C approach to actual surplus C variability, which could be greater for a larger elevational range.

Ectomycorrhizal fungi with ages greater than 1 year suggest delay between C fixation and transfer to symbionts, but age overestimation is possible. Our predicted local atmospheric $\Delta^{14}CO_2$ during the 2020 growing season (−5.4‰) is lower than −0.5‰, the actual measurement at the Jungfraujoch station for the same year (Fig. 2). Using the Jungfraujoch value, the mean ectomycorrhizal fungi age is 0.4 years, in line with the commonly observed rapid transfer of new photoassimilates to mycorrhiza (Epron et al., 2012). Overall, the young ectomycorrhizal fungi indicate that all trees had similar surplus C in late summer, confirming the paradigm that trees near the treeline are not C-limited (Hoch and Körner 2012).

3.2. ^{13}C fractionation in fungal carbon uptake

$\delta^{13}C$ values increased from needles to fine roots by 0.5–0.6‰ for the water-soluble C, 0.5–1.2‰ for bulk tissue, and by 1.4–1.9‰ for starch (Fig. 4A, Tables S4 and S5). The mechanism behind this $\delta^{13}C$ increase, a widely observed phenomenon (Cernusak et al., 2009), is still in question and will not be discussed here. Elevation did not have a significant effect on $\delta^{13}C$ and hence all sites were pooled together for visualization. Compared with *Larix*, $\delta^{13}C$ increases from needles to roots were weaker in *Pinus* that also had higher $\delta^{13}C$ values overall (0.6–1.2‰). The higher *Pinus* $\delta^{13}C$ is in line with previous observations at the site and with its low stomatal conductance (Streit et al., 2014b). When less CO_2 enters the intercellular space relative to the C fixation, C fixation has a weaker isotopic fractionation and the $\delta^{13}C$ of the produced biomass is higher and closer to the atmospheric CO_2 substrate (Farquhar et al., 1982). We used water-soluble C as a proxy for soluble sugars that are used for transport and as a substrate for compound synthesis. Compared to water-soluble C, we observed ^{13}C enrichments in bulk tissues (up to 1.2‰) and starch (up to 2.8‰).

Ectomycorrhizal fungi were ^{13}C-enriched by 2.1–3.6‰ compared to bulk needles, the common reference material when comparing fungi and plants (Fig. 4B). We calculated similar enrichment of 2.5–2.8‰ when comparing the fungi with water-soluble C in the <0.5 mm roots. Smaller enrichments were calculated when ectomycorrhizal fungi were compared with bulk 0.5–1 mm roots (1.6–2.4‰) and with starch in <0.5 mm roots (1.5–1.6‰). There was no ectomycorrhizal enrichment at all when the reference was starch in larger *Pinus* roots. It should be noted that the calculated enrichment factors are approximations and only indicate general trends, since we collected the fungi one year after the tree tissues and annual differences in stomatal conductance would vary $\delta^{13}C$ values. The bulk composition of roots can be approximated by the composition of wood, where cellulose is twice as abundant as lignin (Pettersen 1984). Considering the usual higher $\delta^{13}C$ in cellulose over lignin (Bowling et al., 2008), root cellulose is even higher and closer in $\delta^{13}C$ to the ectomycorrhizal value. While it is highly unlikely that ectomycorrhizal fungi use structural cellulose as a C source, it is possible that enzymatic steps that discriminate against ^{12}C during cellulose synthesis are common with synthesis of C transfer compounds. ^{13}C fractionation during starch synthesis, which is studied more extensively than cellulose, can also suggest common metabolism with synthesis of transfer compounds. ^{13}C enrichment of starch was associated with the plastidic fructose-1,6-bisphosphate aldolase reaction that produce short-phosphorylated sugars (Gleixner et al., 1993; Lehmann et al., 2019). However, if these assumed ^{13}C-enriched sugars are indeed transferred to the fungi, they must be present in low concentrations (our water-soluble C is ^{13}C depleted) and must dephosphorylate in further steps to avoid P loss. It was already shown that ^{13}C enrichment in ectomycorrhizal caps is explained at least partially by internal fungal processes that lead to high concentrations of ^{13}C-enriched proteins in the caps (Hobbie et al., 2012). Our results point out that the ^{13}C enrichment in fungal caps can be explained at least partially by transfer of ^{13}C-enriched C compounds from tree to fungi.

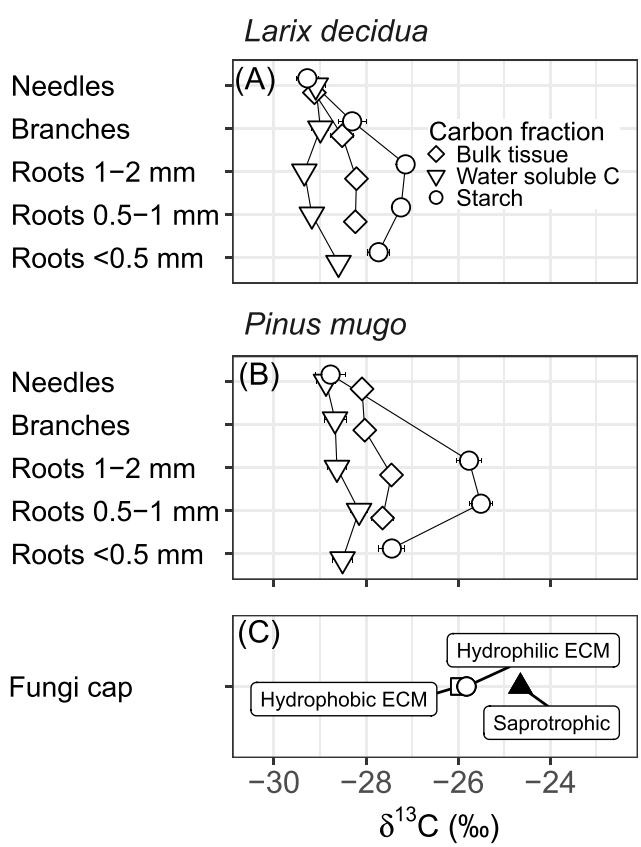

Fig. 4. Mean ± SE values for $\delta^{13}C$ in tree tissues and C fractions. (A) *Larix decidua* and (B) *Pinus mugo*, both species with n = 13–15; (C) fungal guilds pooled across elevations (n = 11–24; ECM: ectomycorrhizal).

3.3. Fungal elemental composition and $\delta^{15}N$ indicate host-ectomycorrhizal nitrogen exchange

Ectomycorrhizal fungal guilds had higher C/N ratios than the saprotrophic fungi with overall means of hydrophobic ectomycorrhizal fungi 10.4 ± 0.5, hydrophilic ectomycorrhizal fungi 10.7 ± 0.6, and saprotrophic fungi 6.5 ± 0.4 (Fig. 3F–Tables S2 and S3). The higher ratios in the ectomycorrhizal guilds can be explained by either their higher %C, or by their lower %N (Fig. 3F–E). Higher %N in saprotrophic over ectomycorrhizal fungi is explained by higher concentrations of protein and especially chitin (Trocha et al., 2016). It was further stressed, as we mentioned above, that the lower %N of the ectomycorrhizal fungi might reflect the transfer of N to hosting plants (Hobbie and Colpaert 2003; Trocha et al., 2016).

The mean $\delta^{15}N$ value for hydrophobic ectomycorrhizal fungi is 8.6 ± 0.9‰, greater than that for hydrophilic ectomycorrhizal fungi (5.5 ± 0.4‰) and saprotrophic fungi (4.1 ± 0.5‰) (Fig. 3C–Tables S2 and S3). The statistically equal values of the hydrophilic and saprotrophic fungi could be explained by similar internal processes involving similar partitioning of N isotopes, as previously suggested by Hobbie et al. (2012), who observed the same ^{15}N enrichment between stipes and caps in these fungal guilds at many sites throughout Oregon. The same authors also suggested that the high $\delta^{15}N$ in hydrophobic ectomycorrhizal fungi is due to sequestration of ^{15}N-depleted N in structural molecules in extraradical hyphae that enriches the remaining mobile N. The agreement between our results and the previous findings suggest that these

δ^{15}N trends can be generalized to other ecosystems.

3.4. Trees and soil δ^{15}N

δ^{15}N values for *Pinus* tissues were 2.6‰ lower than for the deciduous *Larix* (Fig. 5A, Table S4), in accord with Hobbie et al. (2005) who also found higher δ^{15}N of *Larix* over co-occurring evergreen conifers. It is unlikely that deeper N uptake by *Larix* explains its higher δ^{15}N values (Fig. 5C), since the fine roots of *Larix* tended to be slightly shallower than those of *Pinus* (F. Hagedorn, unpublished data). Therefore, greater N inputs through ectomycorrhizal symbionts seems a more plausible explanation for the lower δ^{15}N values for *Pinus*.

Species also differed in elevational patterns of δ^{15}N (Fig. 5A–Table S4). Values for both species decreased from low to mid-elevation as expected if there are greater fungal N inputs at lower temperatures (Fig. 1). However, species diverged from mid-to high-elevation. *Larix* δ^{15}N increased by 2.6‰ from −1.7‰ to +0.9‰, still in line with increases in soil temperature. In contrast, *Pinus* values continued to decrease (by a total of 1‰ across the ecotone). The different elevational patterns suggest that *Larix* has more flexible N acquisition. If root-available N becomes more abundant in soils, e.g. at higher temperature (Dawes et al., 2017), *Larix* trees may be able to switch from fungal to direct N uptake, an ability that *Pinus* apparently lacks. This ability might be one of the reasons why *Larix* dominates the stand. Alternatively, species might differ in their N demand. At lower elevation higher air temperature promotes tree growth which in turn increases N demand and the need of trees to supplement N through ectomycorrhizal fungi. Lower air temperature at the high elevation restricts aboveground growth and, at least for *Larix*, reduces N demand. The lower demand can be satisfied more completely by direct root uptake, requiring less N uptake via fungal symbionts.

Tissues had statistically identical δ^{15}N values (Fig. 5a), which could result from the uptake of reduced N (e.g. NH_4^+), as opposed to NO_3^- uptake, where the site of the ion reduction can cause tissue δ^{15}N to vary (Högberg 1997). The δ^{15}N values of the tree tissues are passed on to the litter layer that maintains the species difference (Fig. 5C). In deeper soil depths the species difference weakens, in parallel to loss of N during organic matter decomposition. Losses through denitrification or leaching of compounds with low δ^{15}N (represented by dissolved N), while microbial biomass and fungal hyphae retain N with high δ^{15}N in the soil, can explain the increase in δ^{15}N with depth (Fig. 5B and C).

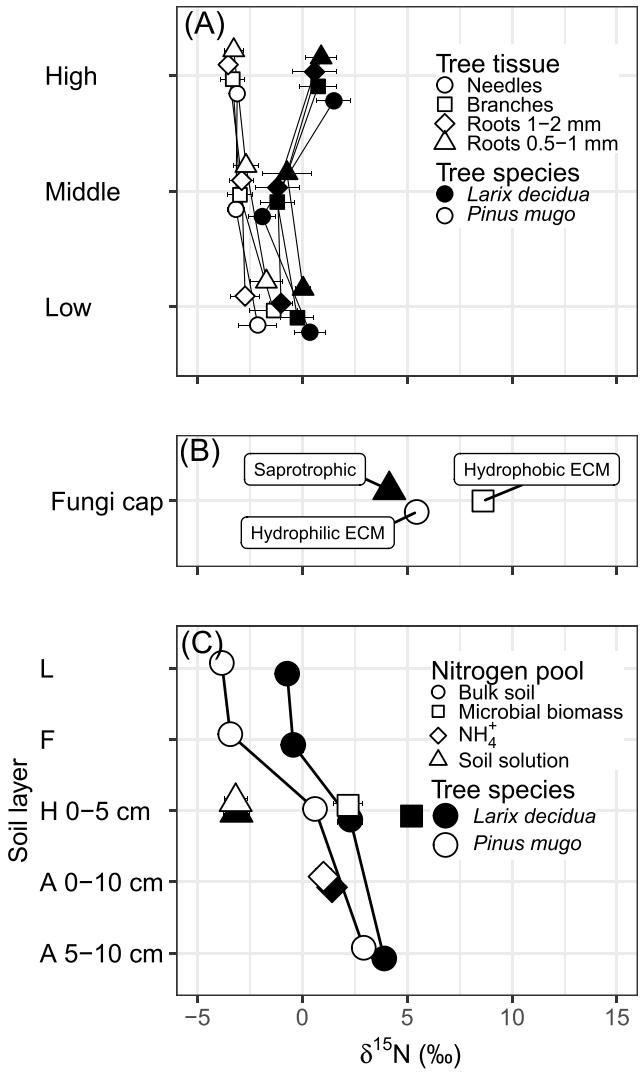

Fig. 5. Mean ± SE values for δ^{15}N in trees, fungi, and soils. (A) *Larix decidua* and *Pinus mugo* values across elevations and among tree tissues (n = 5); (B) fungal guilds pooled across elevations (n = 11–24; ECM: ectomycorrhizal); (C) soil N stocks over depth profile (L = litter layer; F = fermentation layer; H = humified layer; A = A horizon). NH_4^+ data retrieved from Möhl et al. (2019) and represents means over the afforestation. The remainder of the data were collected at the high elevation (Dawes et al., 2017).

3.5. Tree-fungi carbon and nitrogen exchange

The percentage of N taken up by ectomycorrhizal fungi that was delivered to hosts (*T*) and the fungal N contribution to tree N uptake (*f*) differed between species, fungal guilds, model approaches, and model parameters. *f* was rather uniform across elevations in the *Pinus*: 32–41% by the transfer-only approach and 96–100% by the saprotrophic-reference approach (Table 2). *Larix* trees at the high elevation were a special case of almost identical δ^{15}N in the needles and NH_4^+-source in the soil, dictating one possible model solution of $f = 0\%$ – exclusive root N uptake with no fungal N input. At lower elevations, *f* of the *Larix* remained relatively low with a peak of 35% (transfer only) or 68% (saprotrophic reference) at the middle elevation. The percentage of N transferred to hosts (*T* values) was less variable with range of 26–40% across fungal guilds and modeling approaches, supporting the

Table 2
Percentage of acquired nitrogen transferred to hosts by ectomycorrhizal fungi groups (*T*) and percentage of tree nitrogen taken up via fungi (*f*) estimated by the 'transfer only' and 'saprotrophic reference' approaches. The transfer only approach assumes that the ^{15}N enrichment of ectomycorrhizal fungi is exclusively due to transfer of ^{15}N-depleted compounds to plant hosts. The saprotrophic reference approach assumes additional ^{15}N enrichment of the ectomycorrhizal fungi by fungal-internal processes. The saprotrophs are used as a reference for those processes since they do not transfer N to hosts. The range of values was obtained by applying in the calculations δ^{15}N values corresponding to the probable depths of tree roots (between fermentation layer and mineral soil at 5–10 cm depth) and fungal N scavenging (between litter layer and mineral soil at 5–10 cm depth). For the saprotrophic reference *T* values, the uncertainty represents the propagation of standard deviation in %N values.

Elevation	Hydrophilic ECM	Hydrophobic ECM	*Larix decidua*	*Pinus mugo*
	T (%)		*f* (%)	
Transfer only				
High	36 (13–44)	49 (36–54)	0 (0–27)	41 (29–73)
Middle	41 (22–48)	49 (35–54)	35 (20–64)	37 (25–68)
Low	43 (26–49)	49 (35–54)	11 (0–39)	32 (21–62)
Saprotrophic reference				
High	40 (±7)	36 (±7)	0 (0–43)	96 (59–100)
Middle	26 (±5)	31 (±8)	68 (37–100)	100 (63–100)
Low	35 (±7)	38 (±13)	27 (0–100)	100 (69–100)

saprotrophic-reference approach assumption that ectomycorrhizal fungi have lower %N than saprotrophic fungi because they transfer N to hosts. A study using the same transfer-only approach in the Alaskan tundra estimated similar *T* values to ours, 33–43%, and *f* values of 61–86%, which is higher than what the same approach suggests for our trees (Hobbie and Hobbie 2006).

The *T* and *f* values, however, prove sensitive to the parameters related to N acquisition depth. *f* of the transfer-only approach is higher when the roots and ectomycorrhizal mycelia obtain N from deeper soil layers with higher $\delta^{15}N$ (Fig. 6A and B). In the *Pinus*, for example, *f* increases from 26% to 51% when the fungal N source is varied between the litter layer and the mineral soil at 5–10 cm depth. *T* of the transfer-only approach would decrease along this deeper scavenging (Fig. 6C). In the saprotrophic-reference approach, deeper scavenging of saprotrophic mycelia results in *f* reduction (Fig. 6D). To narrow the uncertainty of the values, we compared the isotopic models estimates for NPP_{ECM} (annual growth of ectomycorrhizal fungi) with the NPP_{ECM} estimated by the mycelial in-growth bags.

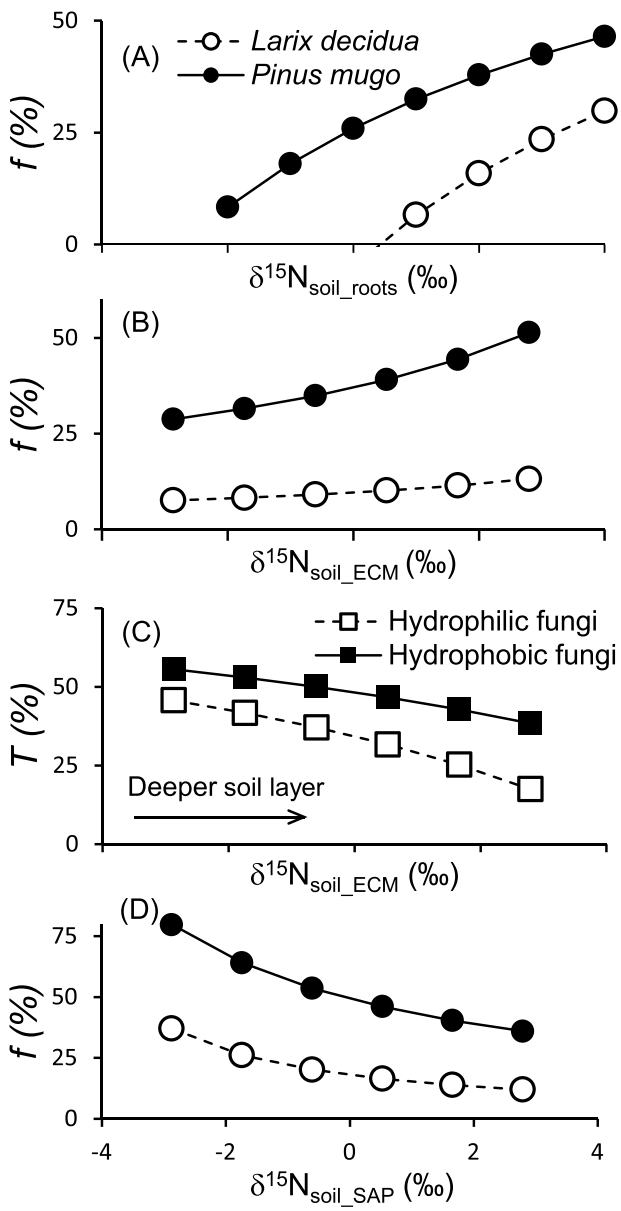

Fig. 6. The sensitivity of *T* (percentage of N acquired by ectomycorrhizal fungi and transferred to hosts) and *f* (percentage of tree N derived from fungi) to variations in $\delta^{15}N$ sources within the soil. Each panel presents the variability in *f* or *T* where all model parameters are fixed, besides the parameter in the x-axis. $\delta^{15}N_{soil_roots}$ is the $\delta^{15}N$ for N source of roots; $\delta^{15}N_{soil_ECM}$ is the N source of ectomycorrhizal fungi; $\delta^{15}N_{soil_SAP}$ is the $\delta^{15}N$ for N source of saprotrophic fungi. The $\delta^{15}N$ values on the x-axis represent the range of values in the soil profile. Note that $\delta^{15}N$ increases with soil depth. Two mass-balance approaches are used to analyze these dynamics: the 'transfer only' (panels A, B, and C) and 'saprotrophic reference' (panel D).

The NPP_{ECM} calculation by the isotopic models was based on our assessment of NPP and N_{uptake} at high elevation. NPP was 108–170 g C m^{-2} yr^{-1} (Table 3), slightly lower than NPP in boreal forests (181–517 g C m^{-2} yr^{-1}), a reasonable difference since the boreal sites are warmer than our site (DeLucia et al., 2007). The annual N uptake of 1.8–3.8 g N m^{-2} yr^{-1} is also reasonable – within the range of 0–5 g N m^{-2} yr^{-1} for most global cold regions (Peng et al., 2023). Combining NPP and N_{uptake} with the *T* and *f* values of the transfer-only approach for high-elevation *Pinus*, NPP_{ECM} is 23 (8–131) g m^{-2} yr^{-1} (Table 3). A three times higher value is calculated if we apply the *T* and *f* values of the saprotrophic-reference approach. Raw NPP_{ECM} from the in-growth bags data (Hagedorn et al., 2013) is significantly lower, with a mean (minimum–maximum) of 0.5 (0.1–1.7) g m^{-2} yr^{-1}. However, accounting for mycelial turnover and distribution in the soil profile yields in-growth NPP_{ECM} values of 0.2–20 g m^{-2} yr^{-1} – overlapping with the transfer-only NPP_{ECM} range. The transfer-only NPP_{ECM} would be consistent with the in-growth bags range if N uptake is shallower than our assumed depth. For example, if roots and fungi take up N from the fermentation layer (with $\delta^{15}N = -2‰$), *T* increases to 50%, *f* decreases to 19%, and NPP_{ECM} equals 10 g m^{-2} yr^{-1}. The comparison with the in-growth bags, in summary, indicates that the estimates of *T* and *f* values from the transfer-only approach appear to be more realistic than those from the saprotrophic reference approach. Furthermore, 41%, the *f* value of high-elevation *Pinus* according to our estimated rooting and scavenging depths probably marks the upper end of possible *f* values, and lower values are highly plausible. In conclusion, our results suggest that most of the N in the trees is taken up by roots and not by fungi.

Using the presumed rooting and scavenging depths corresponding to an *f* value of 41%, the fraction of GPP allocated to ectomycorrhizal fungi ($fGPP_{ECM}$) at the high elevation was 17% for *Pinus* and 0% for *Larix* (Table 3). Using instead the *T* and *f* values corresponding to N uptake from the fermentation layer, the *Pinus* $fGPP_{ECM}$ drops to 6%. The species difference is consistent with greater mycelial production in *Pinus* vs. *Larix* plots on the site (Hagedorn et al., 2013). At lower elevations, $fGPP_{ECM}$ of the *Pinus* gradually decreased, while $fGPP_{ECM}$ of the *Larix* peaked at the middle elevation. The uncertainty in $fGPP_{ECM}$, however, is large, reflecting the extreme combinations of uncertainty in NPP, *T*, and *f*. Nevertheless, the estimates are similar to $fGPP_{ECM}$ of 8–17% of Arctic tundra vegetation calculated using a similar approach (Hobbie and

Table 3
Estimates for the high elevation site of tree net primary productivity (NPP), gross primary productivity (GPP), annual N uptake, annual growth of ectomycorrhizal fungi fed by carbon from the respective tree (NPP_{ECM}) at the high elevation, and the fraction of GPP allocated to ectomycorrhizal fungi at each elevation ($fGPP_{ECM}$). The bracketed range is the uncertainty calculated for NPP, GPP, and N uptake by the range of tree compartment biomass used for the estimations, and for NPP_{ECM} and $fGPP_{ECM}$ by the extreme combinations of tree compartment range and *T* and *f* range.

	Larix decidua	Pinus mugo
NPP (g C m^{-2} yr^{-1})	170 (128–216)	108 (91–187)
GPP (g C m^{-2} yr^{-1})	425 (321–540)	271 (228–467)
N uptake (g C m^{-2} yr^{-1})	3.8 (2.8–5)	1.8 (1.7–3.5)
NPP_{ECM} (g m^{-2} yr^{-1})	0 (0–98)	23 (8–131)
$fGPP_{ECM}$ (%)		
High	0 (0–61)	17 (4–151)
Middle	18 (5–118)	14 (3–115)
Low	5 (0–65)	11 (3–95)

Hobbie 2006). Even in studies using different definitions of plant productivity and fungal C use, the allocation to ectomycorrhizal fungi was similar, 0–22% of plant productivity in forests and 9.4% on average for conifers growing in the field and in pots (Hobbie 2006; Hawkins et al., 2023). Given that the transfer-only approach suggests low tree dependence on fungal N, the Stillberg trees allocate a relatively large amount of C to the ectomycorrhizal symbionts. Our results therefore suggest that trees in the treeline ecotone are not N-limited, but still provide relatively large amounts of C to their ectomycorrhizal symbionts.

4. Conclusions

It is difficult to predict how trees near alpine treelines allocate C to and receive N from ectomycorrhizal fungi. The low temperatures may control both C allocation to roots and fungi and, through growth limitation, tree N demand. Here, we studied C and N exchange between two tree species and ectomycorrhizal fungi using elemental analysis and isotopes along a treeline ecotone. ^{14}C ages of ectomycorrhizal fungi were mostly 0–2 years without an elevational trend, suggesting that in late summer all trees are in a state of C surplus and able to allocate newly fixed C to symbionts. Fungi had rather high δ^{13}C values, which could be explained if the allocated carbon consists of short and ^{13}C-enriched sugars produced during starch synthesis. The mass balance of δ^{15}N and %N along the tree-fungi-soil continuum indicated that N acquisition varied between tree species; *Larix* was generally less dependent on fungal N and acquired all of its N through direct root uptake at the high elevation. However, at mid-elevation, where soil temperature was low and tree competition was high, *Larix* acquired 35% of its N from ectomycorrhizal fungi. *Pinus* acquired up to 41% of its N from ectomycorrhizal fungi with no significant elevation difference. The species difference is consistent with our hypothesis that *Larix*, as a pioneer species, is less dependent on ectomycorrhizal symbionts than *Pinus*. We estimated that the GPP fraction allocated to ectomycorrhizal fungi was generally higher in *Pinus*, with an overall range of 0–18%, similar to other forest ecosystems at lower elevations. In the alpine treeline ecotone, the isotopic proxies suggest that the N contribution of ectomycorrhizal fungi to trees is relatively small compared to the reverse C flux, but the exchange is tree species specific.

CRediT authorship contribution statement

Boaz Hilman: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emily F. Solly:** Writing – review & editing, Conceptualization. **Iris Kuhlmann:** Writing – review & editing, Formal analysis. **Ivano Brunner:** Writing – review & editing, Investigation. **Frank Hagedorn:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used DeepL/write in order to improve language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Boaz Hilman reports financial support was provided by Max-Planck Institute for Biogeochemistry, Biogeochemical Processes. Boaz Hilman reports financial support was provided by European Research Council. Emily F. Solly reports financial support was provided by Swiss National Science Foundation, Switzerland.

Acknowledgments

We thank Christin Leschik for preparing samples for analysis and extraction of nonstructural C, Axel Steinhof and Heike Machts for processing and measuring the ^{14}C samples, Heiko Moossen and Heike Geilmann for measuring the ^{15}N and ^{13}C, and Ines Hilke for C and N elemental analysis. B.H acknowledges funding from the Max Planck Institute for Biogeochemistry and the European Research Council Horizon 2020 Research and Innovation Programme, grant agreement 695101 (14Constraint). E. F. Solly acknowledges funding from the Swiss National Science Foundation (Ambizione grant PZ00P2_180030). The authors are grateful to Susan Trumbore and two reviewers for their constructive comments and the "Stillberg" team (in particular, Peter Bebi, Melissa Dawes, Christian Rixen) for maintaining the research site and sampling efforts of biomass and soils. The data are available in https://doi.org/10.6084/m9.figshare.24609192.v1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2024.101361.

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