Hobbie, E.A., Keel, S.G., Klein, T., Rog, I., Saurer, M., Siegwolf, R., Routhier, M.R., Körner, C. (2024). Tracing the spatial extent and lag time of carbon transfer from Picea abies to ectomycorrhizal fungi differing in host type, taxonomy, or hyphal development. *Fungal Ecology, 68*, Article 101315. https://doi.org/10.1016/j.funeco.2023.101315

October 1, 2023

Tracing the spatial extent and lag time of carbon transfer from *Picea abies* to ectomycorrhizal fungi differing in host type, taxonomy, or hyphal development

Erik A. Hobbie[1*], Sonja G. Keel[2]*, Tamir Klein[3], Ido Rog[3#], Matthias Saurer[4,5], Rolf Siegwolf[4,5], Michael R. Routhier[1], Christian Körner[2]

[1]Earth Systems Research Center, Morse Hall, University of New Hampshire, Durham, New Hampshire, 03824, USA; [2]Institute of Botany, Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, 4056 Basel; [3]Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel; [4]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, 8903, Birmensdorf, Switzerland; [5]Paul Scherrer Institute, Laboratory of Atmospheric Chemistry, CH-5232 Villigen PSI, Switzerland; *current address: Climate and Agriculture Group, Agroscope, 8046 Zurich, Switzerland; #current address: Plant-Soil Interactions Group, Research Division Agroecology and Environment, Agroscope, 8046 Zurich, Switzerland. (ORCID: EAH, 0000-0002-1629-6307; SGK, 0000-0002-2645-273X; TK, 0000-0002-3882-8845; IR, 0000-0002-9120-3617; MS, 0000-0002-3954-3534; RS, 0000-0002-0249-0651; MRR, 0000-0001-7811-6589; CK, 0000-0001-7768-7638)

*Corresponding author. E-mail: Erik.Hobbie@unh.edu; Telephone: +1-603-862-3581; fax: 1-603-862-0188.

Abstract

We used five mature *Picea abies* continuously labeled with ^{13}C-depleted CO_2 in a broadleaf-dominated Swiss forest to assess the spatial extent and lag time of carbon fluxes to ectomycorrhizal fungi differing in hyphal development and host association. We traced labeled carbon into ectomycorrhizal sporocarps collected for two seasons at different distances from labeled *Picea*. *Picea*-derived photosynthate reached conifer-specific sporocarps up to 6-12 m away and reached other sporocarps only 0-6 m away. At 0-6 m, genera of lesser hyphal development acquired more *Picea*-derived photosynthate than those of greater hyphal development, presumably from preferential fungal colonization of inner root zones by the former genera. Correlations of sporocarp $\delta^{13}C$ with daily solar radiation integrated for different periods indicated that carbon fluxes from *Picea* to sporocarps peaked 17–21 days after photosynthesis. Thus, these results provided rough estimates of the spatial extent and temporal lags of carbon transfer from *Picea* to ectomycorrhizal fungi.

Key words: saprotrophic fungi; carbon transport; host specificity; isotope tracers; exploration type, carbon dioxide, CO_2 enrichment

Introduction

Ectomycorrhizal fungi depend on recent photosynthate from their plant hosts to form sporocarps (Last et al., 1979; Lamhamedi et al., 1994). Although numerous studies have estimated the dynamics of carbon fluxes belowground and the subsequent release of CO_2 during respiration

using isotopic (^{13}C: ^{12}C ratios, commonly expressed as $\delta^{13}C$) or flux-based methods (Mencuccini and Hölttä, 2010), the lag time of carbon fluxes from photosynthesis to ectomycorrhizal sporocarps is not well-known. In a pulse-chase experiment with ^{13}C-enriched CO_2 in 3–5 m tall *Pinus sylvestris* in boreal Sweden, the label was highest in sporocarps at six and 16 days after labeling and was still at 50% of peak labeling after 23 days (Högberg et al., 2010), with an estimated decay constant of the signal of 10 days. Natural abundance levels of $\delta^{13}C$ in plants and fungi could also trace carbon fluxes. Photosynthetic $\delta^{13}C$, which is subsequently transferred to ectomycorrhizal fungi, will vary with environmental factors, including light levels (Warren et al., 2001; Gaudillère et al., 2002; Rinne et al., 2015). Carbon isotopes in ectomycorrhizal sporocarps could accordingly provide a time-sensitive and climate-sensitive indicator of the period of peak carbon movement from trees to the belowground community of ectomycorrhizal fungi. In sub-arctic Finland, climatic parameters for the three weeks prior to sporocarp collection correlated with sporocarp $\delta^{13}C$ in 5-m tall *Betula* stands (Hobbie et al., 2021). Comparable studies have not been conducted in temperate forests.

Ectomycorrhizal fungi vary widely in their host specificity (Brundrett and Tedersoo, 2018). Many ectomycorrhizal species only associate with conifers (Pinaceae), associate with broad-leaved hosts, or have a wide host (mixed) range (Breitenbach and Kränzlin, 1981; Rinaldi et al., 2008). Because tree species can differ in the $\delta^{13}C$ of sugars produced during photosynthesis and sporocarp $\delta^{13}C$ will reflect that of their host trees, sporocarp $\delta^{13}C$ at natural abundance can assess host specificity. Högberg et al. (1999) compared a 2–3‰ enrichment in ^{13}C of conifers relative to broad-leaved trees at two conifer-dominated sites in Sweden against $\delta^{13}C$ values for sporocarps of conifer-specific, broad-leaf specific, and generalist fungi to conclude that generalist fungal species primarily assimilated carbon that was derived from

conifers. Another approach to study host specificity is to supply ^{13}C-labeled CO_2 to trees and trace the resulting isotopic signal into ectomycorrhizal fungi (Epron et al., 2012). For example, such ^{13}C labeling experiments could estimate the magnitude and spatial extent of photosynthate transfer from conifers to ectomycorrhizal fungi differing in host specificity.

In addition to differing in host specificity, ectomycorrhizal fungi also differ in their hyphal exploration strategies of the soil, commonly termed exploration type (Agerer, 2001). Genera of greater hyphal development, such as *Cortinarius*, will have greater carbon demand, greater enzymatic capabilities to access soil organic nitrogen, rhizomorphs for long-distance transport, and higher ^{15}N: ^{14}N ratios (expressed as $\delta^{15}N$) in their sporocarps (Lilleskov et al., 2011). In contrast, genera of lesser hyphal development, such as *Inocybe*, will have lesser carbon demand, often lack rhizomorphs, and have low $\delta^{15}N$ values in sporocarps. Genet size also correlated with hyphal exploration strategy (Nara, 2015) and genera of greater hyphal development colonized primarily edges (low root density) rather than interiors (high root density) of tree islands (Peay et al., 2011). Thus, there are potential linkages between exploration strategies and spatial patterning of carbon fluxes to ectomycorrhizal fungi.

Although the ^{13}C-depleted CO_2 added in Free Air CO_2 Enrichment (FACE) experiments has been used several times to trace carbon fluxes from mature trees to ectomycorrhizal fungi (Keel et al., 2006; Hobbie et al., 2014), carbon movement from host plants to fungal genera differing in host preference has not been quantified in such experiments, although qualitative comparisons have begun (Rog et al., 2020). The Swiss FACE study provided opportunities to examine this in mature conifers, in which photosynthesis was traced through addition of ^{13}C-depleted CO_2 directly into the canopy of five *Picea abies* trees. In a prior study of these sites (Mildner et al., 2014), the tree needles, branchlet xylem, stemwood, fine roots, and a subset of

collected fungal sporocarps were analyzed for $\delta^{13}C$. The ^{13}C label took 12 days to reach soil CO_2. Here, we reanalyzed $\delta^{13}C$ data on ectomycorrhizal and saprotrophic sporocarps that were previously collected in defined zones around the labeled *Picea* trees. We then assessed the lag time from photosynthesis to sporocarp collection and whether carbon fluxes differed based on host specificity or on hyphal development.

To estimate the temporal and spatial dynamics of carbon movement within the plant-ectomycorrhizal system, we analyzed the sporocarps for $\delta^{13}C$ values and then tested how specific parameters influenced sporocarp $\delta^{13}C$ in multiple regressions, including (1) taxonomic groups, (2) climatic parameters integrated for different periods, specifically average daily temperature, average daily temperature range, or daily solar shortwave radiation, (3) year, (4) association with conifers or broad-leaved trees, (5) distance (zone) from labeled trees, and (6) the interactions of the last three factors, and (7) the interaction of zone and taxonomic groupings. Taxonomic groups could then be examined as to whether they differed in morphological characteristics and in carbon acquired from the CO_2-labeled *Picea*. We hypothesized that (1) $\delta^{13}C$ will correlate with time-integrated climatic parameters, indicating the timing of carbon fluxes from trees to ectomycorrhizal sporocarps, (2) conifer-specific ectomycorrhizal fungi would assimilate a higher proportion of their carbon from CO_2-labeled *Picea* photosynthesis than would other ectomycorrhizal fungi, and (3) genera of more extensive hyphal development would acquire carbon at greater distances from the CO_2-labeled *Picea* than genera with less extensive hyphal development. Concurrently collected sporocarps of saprotrophic fungi were used to test whether overall $\delta^{13}C$ of the carbon available to saprotrophs differed with distance from the labeled *Picea*.

Methods

116

117 *Site*

118 The Swiss Canopy Crane CO_2 enrichment facility is within a mixed broad-leaved and coniferous
119 forest at 47.4686° N, 7.5022° E; elevation 550 m. The canopy crown area at the site consists of
120 40% *Fagus sylvatica*, 15% *Carpinus betulus*, 11% *Quercus* (mostly *Quercus petraea*), 10%
121 *Larix decidua*, 9% *Picea abies*, 5% *Tilia platyphyllos*, and 3% *Pinus sylvestris*, with five other
122 species making up the remaining 7%. The canopy (crown area) is 24% coniferous trees (all
123 ectomycorrhizal) and 76% broad-leaved trees (71% ectomycorrhizal and 5% arbuscular
124 mycorrhizal). The crown size (area) and location of each tree within the research site was
125 measured previously.

126

127 *Procedures*

128 We used this facility (Pepin and Körner, 2002; Körner et al., 2005) to label new carbon
129 assimilates of five mature *Picea abies* trees, as indicated on the crown map (Figure 1). We
130 released two tons of CO_2 per day into the crowns of these trees (30–38 m height and about 100
131 years old) to raise the canopy CO_2 concentration to 540 ppm from 2009 to 2014. From 4 mm
132 diameter, laser-punctured tubes, which were loosely woven into the tree canopy, pure CO_2 was
133 mixed into the atmosphere of the tree crowns. The concentration was computer-controlled by
134 monitoring the CO_2 level at 24 different canopy positions. The study used the ^{13}C signal in the
135 added CO_2, which originated from fossil fuel (four-year mean $\delta^{13}C$ was -29.7 ± 0.3‰ instead of -
136 8‰ of the ambient air). The mixture of ambient air and fossil-derived CO_2 was depleted in ^{13}C
137 by 5.8 ± 0.6‰ relative to the ambient air (Pepin and Körner, 2002; Körner et al., 2005; Keel et
138 al., 2006) and stem xylem of CO_2-labeled *Picea* was 4.3 ± 0.2‰ depleted in $_{13}C$ relative to stem

xylem in unlabeled *Picea*, with no evidence of assimilation of ^{13}C-depleted CO_2 by nearby trees (Klein et al., 2016). Our analysis profited from the clustering of several tall *Picea abies* trees (with their intrinsically less negative $\delta^{13}C$) combined with a clear isotopic tracer signal, forming an island among other, isotopically distinct neighbor trees. During two growing seasons (2010 and 2011), sporocarps in and around the labelled trees (97 taxa of Basidiomycota and Ascomycota, Online Resource 1) were harvested, identified morphologically, and analyzed for their isotopic composition. At collection, we classified the distance of sporocarps from CO_2-labeled *Picea* into four zones: 0–6 m from CO_2-labeled *Picea* (elevated CO_2, E), 6–12 m away (inner transition zone, IT), 12–18 m away (outer transition zone, OT), and greater than 18 m from the labelled trees (ambient CO_2, A). Sporocarps were not collected at > 18 m distance in 2010. The sporocarps were morphologically classified as either ectomycorrhizal or saprotrophic. We classified the collected ectomycorrhizal sporocarps by host association into conifer-specific, associating with broad-leaved trees, of mixed association, or of unknown association, based on the taxonomic literature (Online Resource 1) and prior results from this study (Klein et al., 2016). *Entoloma rhodopolium* was assigned to ectomycorrhizal fungi and other *Entoloma* were assigned to saprotrophic fungi. For analysis of carbon isotope (^{13}C:^{12}C) ratios (expressed as $\delta^{13}C$), only caps of the sporocarps were used if possessed of both caps and stipes. They were oven-dried at 80 °C for 48 h, ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Germany) and 0.6-0.8 mg dried powder was weighed in tin capsules. Samples were combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy). The gas from the EA combustion was transferred to the mass spectrometer (Delta S, Thermo Finnigan Mat, Germany) via a variable open split interface (Conflo II, Thermo Finnigan Mat, Germany) for isotope ratio analysis. The precision for $\delta^{13}C$ analysis was ± 0.1‰.

Statistical analysis

We used stepwise multiple regressions to evaluate the multiple potential drivers of sporocarp $\delta^{13}C$. In the regression, daily climate records lagged for different periods were used to estimate the lag time for carbon from photosynthesis to sporocarp collection. To estimate how $\delta^{13}C$ differed with distance from the labeled trees, zone was included as a factor. To test whether $\delta^{13}C$ differed by genus, plant host type, and year, these potential explanatory variables were also included. Plant host types were conifer-specific, broadleaf-specific, mixed, and unknown, whereas year was 2010 or 2011. Because fungi of different host types or genera may acquire different amounts of the ^{13}C-depleted photosynthate from the CO_2-labeled *Picea*, interactive terms, such as genera × zone, plant host × zone, or plant host × zone × year, were also tested in the regression. From these regressions, we could estimate the $\delta^{13}C$ of different classes of sporocarps in the different distance zones surrounding the CO_2-labeled *Picea*, which could then be used to estimate the relative contribution of *Picea*-derived photosynthate to the sporocarps.

To calculate the contribution from labeled *Picea* to sporocarps in a specific zone and group (fungal genera or plant host type), we must estimate the $\delta^{13}C$ of $^{13}CO_2$-labeled *Picea* photosynthate in sporocarps, termed $\delta^{13}C_{Picea\ photosynthate}$. Klein et al. (2016) estimated a 4.3‰ depletion in ^{13}C between CO_2-labeled and unlabeled *Picea* photosynthate. We accordingly estimated the $\delta^{13}C$ of $^{13}CO_2$-labeled *Picea* photosynthate in sporocarps, termed $\delta^{13}C_{Picea\ photosynthate}$, as 4.3‰ less than the $\delta^{13}C$ of conifer-specific sporocarps in the ambient zone. The equation used to calculate the contribution of labeled *Picea* is:

$$\%\ \text{contribution} = (\delta_{13}C_{ambient,\,group} - \delta_{13}C_{zone,\,group})/(\delta_{13}C_{ambient,\,group} - \delta_{13}C_{Picea}) \times 100\% \quad (1)$$

In this equation, $\delta^{13}C_{ambient,\,group}$ and $\delta^{13}C_{zone,\,group}$ are the $\delta^{13}C$ values for a given group of fungi in those zones.

We used daily temperature records for 2010–2011 from Binningen, Switzerland, 8 km from the field site, as supplied by MeteoSwiss, the Federal Office for Meteorology and Climatology and used shortwave radiation data (solar radiation, in units of kilowatt-hours m^{-2} day^{-1}) from 2010–2011 from Basel, Switzerland, supplied by Meteoblue.com (Online Resource 2). Sporocarps were collected between day of year (DoY) 215 and 308. Based on results on the time lag from *Pinus sylvestris* to sporocarps in a $^{13}CO_2$ pulse-chase experiment (Högberg et al., 2010), we assumed that weather up to 26 days prior to sporocarp collection could potentially influence sporocarp carbon supply and that carbon supply from trees to sporocarps was maximal for a given (unknown) lag time and duration. We accordingly used weather data from DoY 190 to 308, averaged for different periods, as factors in regressions on sporocarp $\delta^{13}C$ to determine this lag time and its duration.

In our study, we analyzed ectomycorrhizal and saprotrophic genera separately and included climatic information, zone (distance from labeled trees), genus, and plant host type as potential explanatory variables. Ectomycorrhizal genera comprised the following genera and sample numbers of sporocarps in this data set: *Inocybe* (42), *Lactarius* (25), *Russula* (18), *Clavulina* (9), *Hebeloma* (7), *Laccaria* (7), *Entoloma* (6), *Cortinarius* (5), *Suillus* (5), *Amanita* (4), *Hygrophorus* (4), *Peziza* (2), *Ramaria* (2), *Tricholoma* (2), *Helvella* (1), and *Xerocomus* (1). Of the 169 saprotrophic sporocarps collected across 34 genera, the most common genera were *Mycena* (37), *Collybia* (18), *Clitocybe* (16), *Entoloma* (12), *Lycoperdon* (11), *Marasmius* (10), and *Lepiota* (9). Data were analyzed using the statistical software JMP (SAS Institute, Cary,

North Carolina, USA). Sporocarp $\delta^{13}C$ values were initially analyzed by treatment using ANOVA and a Tukey post hoc test at an α-value of 0.05. The effect of hydrophobicity on $\delta^{13}C$ was analyzed by a t-test. To explore in more detail the driving factors influencing our variables of interest, sporocarp $\delta^{13}C$ was subsequently analyzed using forward stepwise regression.

The $\delta^{13}C$ values were analyzed using forward stepwise multiple regressions. In the stepwise regressions, nominal variables (such as genus or zone) were initially separated into two groups that maximized the explained variance and those groups could then be similarly separated. Thus, several genera could have the same model coefficients if additional separation did not further minimize values of the Bayesian Information Criterion (BIC), our metric for model selection. BIC was used instead of the Akaike Information Criterion with a correction for sample size (AICc) because the latter can overfit models (Kass and Raftery, 1995). For models with similar BIC values, other criteria for model selection may be required (Johnson and Omland, 2004). The underlying data and the statistical modeling are in Online Resources 1 and 3. Variables included zone (E, IT, OT, A), genus, three climatic parameters integrated for different periods (solar radiation, average daily temperature, and average daily temperature range), fungal associate (conifer-specific, associated with broad-leaved trees, associated with either tree type, or of unknown association), year of collection (2010 or 2011), the interactions of fungal associate, genus, or year with zone, and the three-way interaction of associate, year, and zone. To calculate the % contribution from labeled *Picea* to a fungal group and zone, we need to estimate the $\delta^{13}C$ of $^{13}CO_2$-labeled *Picea* photosynthate in sporocarps, termed $\delta^{13}C_{Picea\,\text{photosynthate}}$. This is calculated as $(\delta^{13}C_{\text{ambient}} - \delta^{13}C_{\text{zone}})/(\delta^{13}C_{\text{ambient}} - \delta^{13}C_{Picea\,\text{photosynthate}}) \times 100\%$.

Some of these sporocarp $\delta^{13}C$ data were previously presented in Mildner et al. (2014), in which only $\delta^{13}C$ data for 2010 and 2011 from 0-18 m were used (no ambient zone data were

included). In that study, data were averaged at the species level, for a presented *n* of 59 for ectomycorrhizal fungi and 98 for saprotrophic fungi, compared to an *n* of 140 for ectomycorrhizal fungi and 169 for saprotrophic fungi in the current data set. In that analysis (Mildner et al., 2014), data on ectomycorrhizal and saprotrophic fungi were combined and then tested for effects on sporocarp $\delta^{13}C$ values of zone, fungal type (ectomycorrhizal versus saprotrophic), and the interaction of zone and fungal type.

Results

Spatial analysis indicated that conifers were 72%, 54%, and 48% of the total crown area of ectomycorrhizal trees at 0-6 m (elevated CO_2 zone), 6-12 m, and 12-18 m, respectively, but only 25% across the whole site (Figure 1, Online Resource 4). From the patterns of foliar $\delta^{13}C$ in control plants (Chevillat et al., 2005) and their relative abundances in different zones, we estimated that the overall natural abundance $\delta^{13}C$ of foliar carbon should be -25.9‰ at 0-6 m from the labeled spruce but drop to -26.4‰ at 6-12 m, -26.5‰ at 12-18 m, and -26.6‰ across the whole site (Online Resource 4).

The saprotrophic fungi were classified into 34 genera (n = 169) and the ectomycorrhizal fungi into 16 genera (n = 140). Of the ectomycorrhizal sporocarps, 10% associated with broad-leaved trees, 18% with conifers, 40% were of mixed association, and 32% were of unknown association. Fungi of unknown ectomycorrhizal association included 39 samples only identified to genus as well as six *Entoloma rhodopolium* and one *Inocybe adaequata*.

Carbon isotope values averaged -24.2 ± 0.9‰ for saprotrophic fungi and -26.9 ± 1.1‰ for ectomycorrhizal fungi. Across the different zones, $\delta^{13}C$ varied from -23.8 ± 0.2‰ for

saprotrophic fungi under elevated CO_2 down to -28.1 ± 0.3‰ for conifer-specific ectomycorrhizal fungi under elevated CO_2 (Figure 2). In Tukey tests, saprotrophic fungi were similar across all zones in $\delta^{13}C$, whereas for ectomycorrhizal sporocarps, both conifer-specific and other ectomycorrhizal fungi had lowest $\delta^{13}C$ in the 0-6 m zone, closest to the CO_2-labeled trees. In *t*-tests, conifer-specific sporocarps were higher than other ectomycorrhizal sporocarps in $\delta^{13}C$ by 1.3 ± 0.3‰ in the 12-18 m zone ($p = 0.0008$) and by 1.8 ± 0.3‰ ($p < 0.0001$) in the < 18 m zone (Figure 2).

In the stepwise regression on ectomycorrhizal sporocarp $\delta^{13}C$, six terms significantly explained $\delta^{13}C$, with an adjusted r^2 of 0.525 ($n = 140$, $p < 0.0001$, Table 1). This included the average solar radiation for 17–21 days prior to sporocarp collection (Term 1, 6% of variance), two taxonomic terms in which the different genera were separated into three groups based on similarity of $\delta^{13}C$ within each group (Terms 2 and 3, 6% of variance), one term separating the elevated CO_2 zone from the other three zones (Term 4, 28% of variance), the interaction of this zonal term with the first taxonomic term (Term 5, 2% of variance), and an interaction among zone (inner transition versus ambient), year, and host tree preference (conifer-specific versus others, 10% of variance, Term 6, Table 1).

In the regression, the genera grouped into three categories that differed up to 1‰ in sporocarp $\delta^{13}C$ and also differed in their patterns of host associations (Table 1). For the genera with the lowest $\delta^{13}C$ values (Group 1; 0.7 ± 0.2‰ lower than the average; *Hebeloma*, *Helvella,* *Laccaria*, *Peziza*, and *Xerocomus*), 17 of 18 sporocarps were of mixed association, whereas for the second group, 88% were of mixed or unknown association, consisting of *Clavulina*, *Inocybe*, and *Russula* (*n* = 69, 0.1 ± 0.2‰ higher than average). The third group consisted of *Amanita*,

276 *Cortinarius*, *Entoloma*, *Hygrophorus*, *Lactarius*, *Ramaria*, *Suillus*, and *Tricholoma* (38%
277 conifer-specific, 0.3 ± 0.1‰ higher than average, n = 53).

278 Morphological characteristics of these genera are given in Table 2, together with $\delta^{15}N$
279 values for the different ectomycorrhizal genera from the broad-leaved Swiss FACE experiment
280 at the same site (Hobbie et al., 2023). With this information, we estimated average $\delta^{15}N$ for the
281 three groups. Only four ectomycorrhizal genera (6 samples total of the 140 collected) in the
282 conifer Swiss FACE were not in the broad-leaved Swiss FACE experiment, so we can assume
283 that the calculated values for the three groups are reasonable estimates. The weighted average
284 $\delta^{15}N$ values for Groups 1, 2, and 3 were 3.2‰, 1.0‰, and 6.3‰, respectively.

285 Several comparisons involving interactive terms emerged from the regression analyses in
286 Table 1. We combined Term 4, separating the elevated CO_2 (0-6 m) zone from the other three
287 zones, with Term 6, the three-way interaction of zone, host associate, and year, to calculate the
288 $\delta^{13}C$ deviation from the mean of conifer-specific sporocarps and other sporocarps in the ambient
289 (> 18 m) and inner transition (6-12 m) zone for 2011. From these data and the average ^{13}C
290 depletion of CO_2-labeled *Picea* photosynthate of 4.3‰ relative to unlabeled *Picea* photosynthate
291 (Klein et al., 2016), we calculated that *Picea* photosynthate in sporocarps under elevated CO_2
292 averaged 2.77‰ lower than the overall mean. Since Term 4 did not distinguish between conifer-
293 specific and other sporocarps, we used the estimated $\delta^{13}C$ depletion in the 0-6 m zone of 0.85 ±
294 0.10‰ for both types in our calculations. With this information, we then calculated the relative
295 contribution of the ^{13}C-labeled *Picea* photosynthate to conifer-specific fungi in the elevated CO_2
296 (55 ± 5%) and inner transition zone (34 ± 6%). The contribution to other ectomycorrhizal fungi
297 in the elevated CO_2 zone was 34 ± 7% (Table 3).

298 We then combined Term 4, separating the elevated CO_2 (0-6 m) zone from the other three
299 zones, with Term 5, the interaction of zone × group, to calculate the $\delta^{13}C$ values for each of the
300 three groups in the elevated CO_2 zone versus the other zones. The decrease to the elevated CO_2
301 zone from other zones was 2.07 ± 0.32‰ for genera of Groups 1 and 2 and 1.09 ± 0.30‰ for
302 genera of Group 3 (Table 3). With this information, we then estimated the relative contribution
303 of the ^{13}C-labeled *Picea* photosynthate to Groups 1, 2, and 3 in the 0-6 m zone at 68 ± 10%, 54 ±
304 8%, and 29 ± 8%, respectively (Table 3).

305 The climatic parameters of mean temperature, temperature range and shortwave solar
306 radiation were included in the stepwise regression and integrated for different periods. Solar
307 radiation correlated more strongly with sporocarp $\delta^{13}C$ than average daily temperature or daily
308 temperature range when integrated for equivalent periods (Online Resource 3). The best fits for
309 individual days of shortwave radiation were for Days 16 and 18 prior to sporocarp collection.
310 The best fit overall included the average shortwave radiation for Days 17–21 prior to sporocarp
311 collection, which correlated positively with sporocarp $\delta^{13}C$ (Figure 4). The maximum radiation
312 difference between sampling days was 3000 watt-hours m^{-2}. Based on the regression coefficient
313 for shortwave radiation of $6.49 \pm 1.63 \times 10^{-4}$, this corresponded to a shift in $\delta^{13}C$ of 1.9 ± 0.5‰.

314 In the regression of the $\delta^{13}C$ of saprotrophic sporocarps, the adjusted r^2 was 0.461 for the
315 BIC minimum model of five terms ($n = 169$, $p < 0.0001$, Table 2). The regression included four
316 taxonomic terms (45% of variance) and one zonal term (1% of variance). The zonal term
317 indicated that sporocarps closest to the CO_2-labeled trees were slightly higher in $\delta^{13}C$ (+0.34 ±
318 0.11‰) than sporocarps from the other three zones. We combined Terms 4 and 6 of Table 1 to
319 calculate the ^{13}C enrichment in the ambient zone of conifer-specific fungi and all other
320 ectomycorrhizal fungi at 1.12 ± 0.22‰ and -0.24 ± 0.05, with a difference of 1.37 ± 0.23‰.

Given the 1.4‰ enrichment of conifer-specific versus other ectomycorrhizal sporocarps in the ambient zone, we calculated that conifers contributed 0.34/1.4 (24%) more to the carbon of saprotrophic fungi in the 0-6 m zone than in the other three zones.

Discussion

Lag time of carbon fluxes in the tree-fungal system

In our first hypothesis, we proposed that climatic parameters integrated for different periods would correlate with sporocarp $\delta^{13}C$. As shown in Figure 4, the strongest correlation was of the shortwave radiation integrated for the 17–21 days prior to sporocarp collection. Shortwave radiation (~300–2000 nm) correlated closely with photosynthetically active radiation of 400–700 nm (Britton and Dodd, 1976). This time period of 17–21 days was slightly higher than the 12 days to initial detection and 16 days to maximum signal of the ^{13}C-depleted CO_2 from soil CO_2 effluxes at this site (Mildner et al., 2014). Similarly, in a multi-year study at the Swiss FACE site in which broad-leaved trees were labeled with CO_2, the $\delta^{13}C$ of soil-respired CO_2 correlated strongly with the calculated vapor pressure deficit with a lag of 10–11 days (Steinmann et al., 2004). Thus, at this location the lag from photosynthesis to belowground systems was greater than the 5-day lag from photosynthesis to soil and ecosystem respiration estimated for 30-m tall trees (Mencuccini and Hölttä, 2010). In our study, ^{13}C labeling of conifers rather than broad-leaved trees may have contributed to long lags from photosynthesis to sporocarps, as conifers transport sugars through the phloem a day or two slower than broad-leaved trees (Epron et al., 2012).

343 Few studies have linked the timing of sporocarp formation to plant photosynthesis. These
344 studies generally indicated that sporocarp production is slower than soil CO_2 efflux. In an early
345 laboratory study, after initial cap growth reached 1-2 mm in diameter in *Laccaria bicolor*
346 colonizing *Pinus strobus* seedlings, full cap expansion took 10-20 days, with slower rates at
347 lower light levels (Lamhamedi et al., 1994). In the $^{13}CO_2$ pulse labeling field study with *Pinus*
348 *sylvestris*, peak ^{13}C labeling of ectomycorrhizal sporocarps occurred 6 and 16 days after the
349 addition of $^{13}CO_2$ (Högberg et al., 2010). These studies suggest considerable variability in the
350 transit time from photosynthesis to sporocarp production, with longer times presumably
351 reflecting both tree height (Mencuccini and Hölttä, 2010) and the slow accumulation of fungal
352 carbohydrates prior to sporocarp formation.

353

354 *C fluxes to fungi differing in host association*

355 In our second hypothesis, we proposed that ^{13}C-depleted carbon from the CO_2-supplied conifers
356 would be preferentially allocated to conifer-specific ectomycorrhizal sporocarps. There was
357 some evidence for this, with CO_2-supplied *Picea* contributing more carbon to conifer-specific
358 sporocarps in the 0-6 m (54 ± 8%) and 6-12 m (34 ± 6%) zones than to other sporocarps at 0-6 m
359 (34 ± 7%) (Table 3). The pattern of conifer-specific sporocarps assimilating *Picea*-derived
360 carbon from greater distances than other fungi was also seen in stepwise regressions in which
361 conifer-specific and other sporocarps were analyzed separately, with *Picea*-derived carbon
362 detected in conifer-specific sporocarps at 0-12 m and detected in other sporocarps at 0-6 m
363 (Online Resource 5). In field studies, there are surprisingly few reports where such tracking has
364 been done into fungi of known differences in host preference, outside of the initial study using
365 natural abundance $\delta^{13}C$ in a conifer-dominated Swedish forest (Högberg et al., 1999). This may

366 partly reflect a study bias towards forests dominated by single species, as in the ^{13}C labeling
367 studies in *Pinus* sylvestris stands in boreal Sweden (Högberg et al., 2010) or the Duke FACE
368 experiment in a *Pinus taeda* plantation (Hobbie et al., 2014).

369 We note that broadleaf-associated fungi were combined with fungi of mixed association
370 and of unknown association for the interactive regression term of zone, association and year
371 (Table 1, Term 6). This combination may reflect the dominance of broad-leaved trees at this site,
372 in which ectomycorrhizal broad-leaved trees contributed 71% of crown area and ectomycorrhizal
373 conifers only 24%. In this case, fungi of mixed or unknown association would likely draw most
374 of their carbon from broad-leaved trees, and therefore have similar interactions with zone as
375 fungi restricted to broad-leaved hosts. Alternatively, we note that only 5% of sporocarps grouped
376 as 'other' at the 0-6 m distance were exclusively hosted by broadleaved trees (3 of 55), so the
377 collective response of these fungi was dominated by the contributions from fungi of mixed (29)
378 or unknown association (23).

379

380 *Functional morphology influenced spatial extent of carbon acquisition*

381 The $\delta^{13}C$ regression model separated the genera into three different groups based on similarity in
382 $\delta^{13}C$ values. Those three different groups were then included in regression analysis in an
383 interaction term with distance. The ^{13}C depletion of the third group from the 0-6 m zone
384 (elevated CO_2) to the other three zones, at 1.09‰, was much less than the calculated 2.07‰
385 depletion for Groups 1 and 2 across the same zones (Table 3). This indicated a contribution of
386 elevated CO_2 trees to Groups 3, 2, and 1 of 29 ± 8%, 54 ± 8‰, and 68 ± 10% in the 0-6 m zone,
387 respectively. We examined the characteristics of the different genera in these three groups to
388 explain this large difference in assimilation. As given in Table 2, the genera of Groups 1 and 2

were generally of contact and short-distance exploration types, with most not possessing rhizomorphs (aggregated hyphae for long-distance transport) and having hydrophilic ectomycorrhizae (Agerer, 2006). In contrast, genera of Group 3 are of various exploration types, many possess rhizomorphs and have hydrophobic ectomycorrhizae, and the percentage that were conifer-specific (38%) was higher than for the other two groups (0% and 7% for Groups 1 and 2). This suite of characteristics has been linked to higher carbon demand and greater enzymatic capabilities to access soil organic nitrogen (Weigt et al., 2012; Lilleskov et al., 2019).

Nitrogen isotope values have been linked to enzymatic capabilities, exploration types, and enzymatic capabilities to degrade organic matter (Lilleskov et al., 2002; Hobbie and Agerer, 2010). The higher estimated $\delta^{15}N$ values for Group 3 (6.3‰) than for Groups 1 and 2 (3.2‰ and 1.0‰, respectively) suggest greater spatial extent of hyphal exploration for Group 3. The lower calculated carbon contribution to Group 3 genera from CO_2-supplied trees would therefore suggest that these genera are supplied with sugars from many unlabeled trees. Essentially, Group 3 genera are drawing from a greater belowground area, and therefore the elevated CO_2 signal is diluted more by sugars supplied by other trees, with correspondingly less ^{13}C depletion of the conifer-specific sporocarps at 0-6 m relative to other zones (Table 3). In our third hypothesis, we proposed that genera of extensive hyphal development should acquire carbon from CO_2-labeled *Picea* at greater distances than genera of little hyphal development. As discussed above, there was relatively strong indirect support for this, with clear evidence of greater spatial extent of carbon acquisition by genera with presumably more extensive hyphal development.

An alternate explanation for these patterns is offered by a study of isolated tree islands of *Pinus* in grasslands in northern California (Peay et al., 2011). In that study, ectomycorrhizal fungi of extensive hyphal development preferentially colonized exterior roots, whereas

412 ectomycorrhizal fungi of limited hyphal development preferentially colonized interior roots. If
413 we apply this scenario to our system, the greater spatial extent of carbon sources to genera of
414 Group 3 primarily reflected that they were more likely to colonize roots further from the $^{13}CO_2$-
415 labeled trees than genera of Group 1 and 2. In support of this scenario, the spatial extent of roots
416 appears much larger than the distances across which ectomycorrhizal networks operate. For
417 example, in a German study, the dominant species here, *Fagus sylvatica*, had roots reaching 16-
418 19 m from tree stems, with root biomass roughly 50% of the maximum biomass at 5 m and 25%
419 of the maximum at 7 m (Meinen et al., 2009). In contrast, studies that have tested for movement
420 through strictly fungal networks have been limited to less than a meter (Philip et al., 2010). A
421 recent review concluded that the evidence for movement within mycorrhizal networks is weaker
422 than often cited (Karst et al., 2023), with movement of carbon within soil as one possibility.
423
424 *Insights from natural abundance $\delta^{13}C$*
425 Natural abundance $\delta^{13}C$ measurements can provide useful information about carbon fluxes from
426 different tree species to ectomycorrhizal fungi if those trees differ in $\delta^{13}C$ (Högberg et al., 1999).
427 For example, in the ambient zone here, conifer-specific fungi were 1.37 ± 0.23‰ higher in our
428 regression analysis than ectomycorrhizal fungi of our other three classifications (broad-leaved
429 trees, mixed, or unknown; as calculated from Term 6 in Table 1). This pattern agreed with
430 estimates of a 1.2‰ enrichment of *Picea* stems relative to *Fagus* stems at the site (Klein et al.,
431 2016) and also agreed with results from mixed stands of *Fagus* and *Picea* in southern Germany,
432 in which *Picea* tree rings were 1.3 ± 0.9‰ higher than *Fagus* tree rings (Schäfer et al., 2017).
433 This indicates that fungi should increase in $\delta^{13}C$ with increasing contribution of *Picea*
434 photosynthate. Broad-leaved, mixed, and unknown associated fungi were grouped together. This

435 suggested that fungi of mixed or unknown association drew most of their carbon from
436 ectomycorrhizal broad-leaved trees at the site, which accounted for 75% of ectomycorrhizal
437 crown area at the site (Online Resource 4).

438 This general trend of higher background $\delta^{13}C$ close to the CO_2-labeled trees is also
439 evident in the regression of saprotrophic $\delta^{13}C$, in which the $\delta^{13}C$ values of saprotrophic
440 sporocarps at 0-6 m from the labeled trees were 0.34 ± 0.11‰ higher than in other zones (Table
441 4). We note that multiple regression that included taxonomic groupings was necessary to show
442 this zonal difference in the $\delta^{13}C$ of saprotrophic sporocarps, as a Tukey test that only considered
443 zone did not show a statistical difference (Figure 2), nor did prior analyses at this site (Rog et al.,
444 2020). In early measurements of soil CO_2 in this experiment, CO_2 near conifers was from 0.5‰
445 to 0.1‰ enriched in ^{13}C relative to that near broad-leaved trees (Steinmann et al., 2003). This
446 agrees with the slight ^{13}C enrichment we calculated for saprotrophic fungi near the labeled *Picea*,
447 underlining the partial dependence of these fungi on old, litter-associated carbon derived from
448 *Picea abies* that was enriched in ^{13}C compared to other tree species here (Chevillat et al., 2005).
449

450 **Conclusions**

451

452 Our results indicated that photosynthate that was transferred from conifers to associated
453 ectomycorrhizal fungi spread beyond the immediate vicinity of CO_2-labeled trees (0-6 m) to
454 label sporocarps in the 6-12 m zone. Carbon fluxes from *Picea* to sporocarps peaked 17–21 days
455 after photosynthesis. Thus, these results provided rough estimates of the spatial and temporal
456 extent of carbon transport from these trees to ectomycorrhizal fungi.

457 We compared these results with prior estimates from the same location when broad-
458 leaved trees were similarly labeled (Steinmann et al., 2004). In that study, spatial patterns of ^{13}C-
459 depleted CO_2 derived from elevated CO_2-treated trees were estimated from contour plots of the
460 $\delta^{13}C$ of soil CO_2. This signal of ^{13}C-depleted CO_2 varied seasonally and spatially, and was
461 detected at perhaps 10–20 m distance from labeled trees, even farther than our signal detection in
462 sporocarps at 6–12 m. The $\delta^{13}C$ of soil CO_2 in that study correlated strongly with favorable
463 weather (as represented by the vapor pressure deficit) for 10–11 days prior to measurement,
464 somewhat shorter than our shortwave radiation signal of 16–19 days prior to collection,
465 suggesting that the mycelia accumulated recent photosynthate from trees for one to two weeks
466 prior to sporocarp emergence.
467

468 **Acknowledgements**

469 The CO_2 enrichment experiment was funded by the Swiss National Science Foundation projects
470 3100-059769.99, 3100-067775.02 and 5005-65755 (NCCR Climate), and the Swiss Canopy
471 Crane by the Swiss Agency for the Environment, Forest and Landscape. Fulbright Austria
472 supported the senior author during manuscript preparation. We thank Laura Bogar, Talia
473 Michaud, Eduardo Perez-Pazos, and several anonymous reviewers for helpful suggestions on the
474 manuscript and thank Markus Wilhelm for sporocarp identification.
475

476 **Data availability**

477 The data analyzed in the current study and the listed Online Resources are archived at Mendeley
478 Data, doi: 10.17632/gw6p48rpdb.2.
479

480 **Online Resources**

481

482 Online Resource 1. Carbon isotopes in sporocarps from the Swiss conifer web-FACE, 2010-
483 2011, DOI: 10.17632/gw6p48rpdb.2.

484 Online Resource 2. Daily climate from 2010-2011 used in regressions. Daily temperature records
485 are from Binningen, Switzerland (MeteoSwiss) and shortwave radiation records are from Basel,
486 Switzerland (Meteoblue.com).

487 Online Resource 3. Stepwise regression modeling of ectomycorrhizal and saprotrophic $\delta^{13}C$.
488 This includes stepwise regression modeling of ectomycorrhizal $\delta^{13}C$ comparing regressions with
489 daily shortwave radiation, daily temperature range, or average daily temperature, all averaged for
490 17-21 days prior to sporocarp collection.

491 Online Resource 4. A. Crown area of the different species of broad-leaved and coniferous trees
492 by zone and distance CO_2-labeled trees (E, 0-6 m; IT, 6-12 m; OT, 12-18 m; A, < 18 m). B.
493 Estimated $\delta^{13}C$ of foliage of broad-leaved trees, coniferous trees, and overall average in the four
494 zones.

495 Online Resource 5. Stepwise regression modeling of the $\delta^{13}C$ of conifer-specific ectomycorrhizal
496 sporocarps (n = 25) and of other ectomycorrhizal sporocarps (n = 115).

497

Table 1. Stepwise regression of ectomycorrhizal fungal $\delta^{13}C$ indicated that genus (separated into three groups), shortwave radiation of the prior 17-21 days, zone, the interaction of zone and group, and the interaction of year, zone, and fungal associate (conifer or others) influenced sporocarp $\delta^{13}C$. Adjusted $r^2 = 0.525$, $p < 0.0001$, $n = 140$. # = regression term number. VIF = variance inflation factor. Zones: E, IT, OT, and A refer to sporocarp distances from labeled trees of 0-6 m (elevated CO_2), 6-12 m (inner transition), 12-18 m (outer transition), and < 18 m (ambient), respectively. Fungal associate: con = conifers, others = broad-leaved, mixed, or unknown associate. Radiation units are in kilowatt-hours m^{-2} day^{-1}. Yr = Year. Regression details and genera for groups are in Online Resource 3. Number of samples for each group assigned to different associates are at end of group designations, given as broad-leaved/conifer/mixed/unknown). Group 1: -0.73 ± 0.18‰, *Hebeloma*, *Helvella*, *Laccaria*, *Peziza*, *Xerocomus* (*n* = 18, 1/0/17/0). Group 2: 0.05 ± 0.18‰, *Clavulina*, *Inocybe*, *Russula* (*n* = 69, 3/5/30/31). Group 3: 0.34 ± 0.11‰, *Amanita*, *Cortinarius*, *Entoloma*, *Hygrophorus*, *Lactarius*, *Ramaria*, *Suillus*, *Tricholoma* (*n* = 53, 9/20/10/14).

#	Term	%Var	Estimate ± se	P	VIF
0	Intercept	--	-29.89 ± 0.74	< 0.0001	--
1	Shortwave radiation, 17-21 days	6.1	$6.5 \pm 1.6 \times 10^{-4}$	0.0001	1.1
2	Group 1&2 – 3	3.6	-0.34 ± 0.11	0.0027	1.4
3	Group 1 – 2	2.8	-0.39 ± 0.15	0.0080	1.2
4	Zone (E – IT/OT/A)	28.2	-0.85 ± 0.10	< 0.0001	1.1
5	(Group 3 – 1&2 + 0.24) × Zone (E – IT/OT/A + 0.14)	2.2	0.250 ± 0.104	0.0174	1.0
6	(con – others + 0.64) × Zone (A – IT - 0.07) × Yr2011	9.6	0.736 ± 0.147	< 0.0001	1.1

Table 2. Morphology of genera. Genera were assigned to three groups from the regression given in Table 1, with $\delta^{13}C$ values for groups in the order 1 < 2 < 3. Fungal associates are given as b/c/m/u, with b, broad-leaved; c, conifer; m, mixed; and u, unknown. Nitrogen isotope values ($\delta^{15}N$) are averages from earlier sampling at the site in 2001-2005, as given in Mendeley Data, doi: 10.17632/yzcm5vmxf8.1. Hi/Ho = hydrophilic or hydrophobic ectomycorrhizae. Ectomycorrhizae type, presence of rhizomorphs, and exploration type follows Agerer (2006). Short, medium, and long corresponded to short-distance, medium-distance, and long-distance exploration types. Descriptive subtypes of smooth and fringe also follow Agerer (2006).[1]Based on general pattern for ascomycetes in Agerer (2006).

Genus	Group	Associate b/c/m/u	$\delta^{15}N \pm$ sd (‰)	*n*	Hi/Ho	Rhizomorph	Exploration Type
Hebeloma	1	0/0/7/0	6.0 ± 3.4	7	ho	sometimes	short, medium-fringe
Helvella	1	1/0/0/0	no data	1	hi	no	short[1]
Laccaria	1	0/0/7/0	0.4	7	hi	yes	medium-smooth
Peziza	1	0/0/2/0	no data	2	hi	no	short[1]
Xerocomus	1	0/0/1/0	no data	1	ho	yes	long
Clavulina	2	0/0/9/0	0.8 ± 1.8	9	no data	no data	no data
Inocybe	2	1/0/12/29	0.0 ± 1.4	42	hi	no	short
Russula	2	2/5/9/2	3.4 ± 5.4	18	hi	sometimes	smooth
Amanita	3	1/0/3/0	2.6 ± 0.1	4	hi (ho)	sometimes	medium-smooth
Cortinarius	3	0/1/1/3	7.9 ± 3.7	5	ho	yes	medium-fringe
Entoloma	3	0/0/0/6	7.5 ± 2.0	6	no data	yes	medium-smooth

544	*Hygrophorus*	3	1/3/0/0	5.6 ± 1.9	4	hi	no	contact, short-smooth
545	*Lactarius*	3	8/9/5/3	6.1 ± 2.2	25	hi (ho)	sometimes	contact, medium-smooth
546	*Ramaria*	3	0/0/0/2	no data	2	ho	yes	long
547	*Suillus*	3	0/5/0/0	5.8 ± 0.2	2	ho	yes	long
548	*Tricholoma*	3	0/2/0/0	11.9 ± 2.3	5	ho	yes	medium-fringe

549

Table 3. $\delta^{13}C$ shifts (± se) for different groups of ectomycorrhizal sporocarps calculated from regression terms 2-5 in Table 1 for the group × zone interaction and calculated from regression term 6 for the zone × year × associate interaction. Taxonomic groups 1, 2, and 3 are defined in Table 1; associate type designated as conifer (conifer-specific) or other. In the interaction of zone × group (Table 1, Term 5), the three outer zones were grouped together. The number of samples (*n*) is given after $\delta^{13}C$ values. The $\delta^{13}C$ shift of CO_2-labeled *Picea* photosynthate in sporocarps was calculated at -2.77‰. The % contribution from labeled *Picea* for each type and zone was calculated from Equation (1).

Type, zone (m)	Zone $\delta^{13}C$ ± se	Ambient $\delta^{13}C$ ± se	% labeled *Picea* ± se
Group 1, 0-6	-1.80 ± 0.23 (10)	0.27 ± 0.22 (8)	68 ± 10
Group 2, 0-6	-1.01 ± 0.23 (35)	1.06 ± 0.22 (24)	54 ± 8
Group 3, 0-6	-0.15 ± 0.21 (15)	0.94 ± 0.21 (38)	29 ± 8
Conifer, 0-6	-0.85 ± 0.10 (5)	1.53 ± 0.17 (15)	55 ± 5
Conifer, 6-12	0.06 ± 0.19 (3)	1.53 ± 0.17 (15)	34 ± 6
Other, 0-6	-0.85 ± 0.10 (55)	0.16 ± 0.17 (21)	34 ± 7

Table 4. Stepwise regression of saprotrophic fungi indicated that zone and genus influence sporocarp $\delta^{13}C$. Adjusted $r^2 = 0.461$, $p < 0.0001$, $n = 169$. VIF = variance inflation factor, zones are as in Table 1. Regression details and group genera are in Online Resource 3.

#	Term	%Variance	Estimate ± se	P	VIF
0	Intercept	--	-24.56 ± 0.14	< 0.0001	--
1	Zone (E – IT/OT/A)	1.2	0.17 ± 0.08	0.0277	1.0
2	Group 1, 2 – 3, 4, 5	29.0	-1.46 ± 0.14	< 0.0001	1.7
3	Group 1 – 2	4.3	-0.84 ± 0.20	< 0.0001	1.1
4	Group 3, 4 – 5	7.9	-1.01 ± 0.18	< 0.0001	1.6
5	Group 3 – 4	3.6	-0.32 ± 0.08	0.0002	1.0

583 **References**

584

585 Agerer, R., 2006. Fungal relationships and structural identity of their ectomycorrhizae Mycol. Prog.
586 5, 67-107.
587 Breitenbach, J., Kränzlin, F., 1981. Pilze der Schweiz. Lucerne: Mycologia.
588 Britton, C., Dodd, J., 1976. Relationships of photosynthetically active radiation and shortwave
589 irradiance. Agric. Meteorol., 17, 1-7.
590 Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host
591 plant diversity. New Phytol., 220, 1108-1115.
592 Chevillat, V.S., Siegwolf, R.T., Pepin, S., Körner, C., 2005. Tissue-specific variation of $\delta^{13}C$ in
593 mature canopy trees in a temperate forest in central Europe. Basic Appl. Ecol., 6, 519-534.
594 Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Högberg, P., Maillard,
595 P., Dannoura, M., Gérant, D., 2012. Pulse-labelling trees to study carbon allocation
596 dynamics: a review of methods, current knowledge and future prospects. Tree Physiol., 32,
597 776-798.
598 Gaudillère, J.P., Van Leeuwen, C., Ollat, N., 2002. Carbon isotope composition of sugars in
599 grapevine, an integrated indicator of vineyard water status. J. Exp. Bot., 53, 757-763.
600 Hobbie, E.A., Agerer, R., 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to
601 belowground exploration types. Plant Soil, 327, 71-83.
602 Hobbie, E.A., Hofmockel, K.S., van Diepen, L.T., Lilleskov, E.A., Ouimette, A.P., Finzi, A.C. 2014.
603 Fungal carbon sources in a pine forest: evidence from a ^{13}C-labeled global change
604 experiment. Fungal Ecol., 10, 91-100.

605 Hobbie, E.A., Bendiksen, K., Thorp, N.R., Ohenoja, E., Ouimette, A.P., 2021. Climate records,
606 isotopes, and C:N stoichiometry reveal carbon and nitrogen flux dynamics differ between
607 functional groups of ectomycorrhizal fungi. Ecosystems, 25, 1207-1217.
608 Hobbie, E., Steinmann, K., Wilhelm, M., Saurer, M., Siegwolf, R., Körner, C., Keel, S., 2023.
609 Nitrogen and carbon isotopes and concentrations in ectomycorrhizal and saprotrophic
610 sporocarps from the Swiss FACE experiment, 2001-2005. Mendeley Data, v2,
611 http://dx.doi.org/10.17632/yzcm5vmxf8.2.
612 Högberg, M.N., Briones, M.J., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton,
613 B., Hurry, V., Linder, S., Näsholm, T., 2010. Quantification of effects of season and nitrogen
614 supply on tree below- ground carbon transfer to ectomycorrhizal fungi and other soil
615 organisms in a boreal pine forest. New Phytol., 187, 485-493.
616 Högberg, P., Plamboeck, A.H., Taylor, A.F.S., Fransson, P.M.A., 1999. Natural ^{13}C abundance
617 reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed
618 forests. Proc. Natl. Acad. Sci. U.S.A., 96, 8534-8539.
619 Johnson, J.B., Omland, K.S., 2004. Model selection in ecology and evolution. Trends Ecol. Evol., 19,
620 101-108.
621 Karst, J., Jones, M.D., Hoeksema, J.D., 2023. Positive citation bias and overinterpreted results lead to
622 misinformation on common mycorrhizal networks in forests. Nature Ecol. Evol. 7, 501-511.
623 Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc., 90, 773-795.
624 Keel, S.G., Siegwolf, R.T.W., Körner, C., 2006. Canopy CO_2 enrichment permits tracing the fate of
625 recently assimilated carbon in a mature deciduous forest. New Phytol., 172, 319-329.
626 Klein, T., Siegwolf, R.T., Körner, C., 2016. Belowground carbon trade among tall trees in a
627 temperate forest. Science, 352, 342-344.

628 Körner, C., Asshoff, R., Bignucolo, O., Hättenschwiler, S., Keel, S.G., Peláez-Riedl, S., Pepin, S.,
629 Siegwolf, R.T., Zotz, G., 2005. Carbon flux and growth in mature deciduous forest trees
630 exposed to elevated CO_2. Science, 309, 1360-1362.
631 Lamhamedi, M.S., Godbout, C., Fortin, J.A., 1994. Dependence of *Laccaria bicolor* basidiome
632 development on current photosynthesis of *Pinus strobus* seedlings. Can. J. For. Res., 24,
633 1797-1804.
634 Last, F.T., Pelham, J., Mason, P.A., Ingleby, K., 1979. Influence of leaves on sporophore production
635 by fungi forming sheathing mycorrhizas with *Betula* spp. Nature, 280, 169-169.
636 Lilleskov, E.A., Hobbie, E.A., Fahey, T.J., 2002. Ectomycorrhizal fungal taxa differing in response
637 to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance
638 of nitrogen isotopes. New Phytol., 154, 219-231.
639 Lilleskov, E., Hobbie, E., Horton, T., 2011. Conservation of ectomycorrhizal fungi: exploring the
640 linkages between functional and taxonomic responses to anthropogenic N deposition.
641 Fungal Ecol. 4:174-183
642 Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I., Hobbie, E.A., 2019. Atmospheric nitrogen
643 deposition impacts on the structure and function of forest mycorrhizal communities: a
644 review. Environ. Pollut., 246, 148-162.
645 Meinen, C., Leuschner, C., Ryan, N.T., Hertel, D., 2009. No evidence of spatial root system
646 segregation and elevated fine root biomass in multi-species temperate broad-leaved forests.
647 Trees, 23, 941-950.
648 Mencuccini, M., Hölttä, T., 2010. The significance of phloem transport for the speed with which
649 canopy photosynthesis and belowground respiration are linked. New Phytol., 185, 189-203.

650 Mildner, M., Bader, M.K.-F., Leuzinger, S., Siegwolf, R.T., Körner, C., 2014. Long-term ^{13}C
651 labeling provides evidence for temporal and spatial carbon allocation patterns in mature
652 *Picea abies*. Oecologia, 175, 747-762.
653 Nara, K. 2015. The role of ectomycorrhizal networks in seedling establishment and primary
654 succession. Horton, T.R. (Ed.), Mycorrhizal Networks. Springer, New York, pp. 177-201.
655 Peay, K.G., Kennedy, P.G., Bruns, T.D., 2011. Rethinking ectomycorrhizal succession: are root
656 density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecol.,
657 4, 233-240.
658 Pepin, S., Körner, C., 2002. Web-FACE: a new canopy free-air CO 2 enrichment system for tall trees
659 in mature forests. Oecologia, 133, 1-9.
660 Philip, L., Simard, S., Jones, M. 2010. Pathways for below-ground carbon transfer between paper
661 birch and Douglas-fir seedlings. Plant Ecol. Divers. 3, 221-233.
662 Rinaldi, A., Comandini, O., Kuyper, T.W., 2008. Ectomycorrhizal fungal diversity: seperating the
663 wheat from the chaff. Fungal Divers., 33, 1-45.
664 Rinne, K.T., Saurer, M., Kirdyanov, A.V., Bryukhanova, M.V., Prokushkin, A.S., Churakova, O.,
665 Siegwolf, R.T., 2015. Examining the response of needle carbohydrates from Siberian larch
666 trees to climate using compound-specific $\delta^{13}C$ and concentration analyses. Plant Cell
667 Environ., 38, 2340-2352.
668 Rog, I., Rosenstock, N.P., Körner, C., Klein, T., 2020. Share the wealth: Trees with greater
669 ectomycorrhizal species overlap share more carbon. Mol. Ecol., 29, 2321-2333.
670 Schäfer, C., Grams, T.E., Rötzer, T., Feldermann, A., Pretzsch, H., 2017. Drought stress reaction of
671 growth and $\Delta^{13}C$ in tree rings of European beech and Norway spruce in monospecific versus
672 mixed stands along a precipitation gradient. Forests, 8, 177, doi:10.3390/f8060177.

673 Steinmann, K., Siegwolf, R.T., Saurer, M., Körner, C., 2004. Carbon fluxes to the soil in a mature
674 temperate forest assessed by ^{13}C isotope tracing. Oecologia, 141, 489-501.
675 Warren, C.R., McGrath, J.F. & Adams, M.A. 2001. Water availability and carbon isotope
676 discrimination in conifers. Oecologia, 127, 476-486.
677 Weigt, R.B., Raidl, S., Verma, R., Agerer, R., 2012. Exploration type-specific standard values of
678 extramatrical mycelium - a step towards quantifying ectomycorrhizal space occupation and
679 biomass in natural soil. Mycol. Prog. 11, 287-297.
680

Figure Legends

Figure 1. Map of tree crowns at the Swiss canopy crane. Conifers include *Abies alba* (dark green, Ta), *Larix decidua* (orange, Lä), *Picea abies* (red, Fi), and *Pinus sylvestris* (olive green, Fö). The CO_2-labeled *Picea abies* are Fi1, Fi2, Fi6, Fi7, and Fi8, with a blue bar under the designation. Broad-leaved taxa are *Acer campestre* (green, Ah), *Carpinus betulus* (green, Ha), *Fagus sylvatica* (blue, Bu), *Prunus avium* (purple, Ki), *Quercus sp.* (yellow, Ei), and *Tilia platyphyllos* (green, Li). Diameter of circle is 60 m.

Figure 2. $\delta^{13}C$ values for sporocarps at different zones (distances) surrounding CO_2-labeled trees (E, 0-6 m; IT, 6-12 m, OT, 12-18 m; A > 18 m) and different fungal associates (saprotrophic, clear circles; conifer-specific ectomycorrhizal (ECM), clear triangles; other ectomycorrhizal, black circles). Tukey test comparisons of zones within each category are indicated by letters name (saprotrophic, lower-case italics; conifer-specific ectomycorrhizal, upper-case letters; other ectomycorrhizal, lower-case). Zones where conifer-specific and other ectomycorrhizal sporocarps differed indicated by asterisks.

Figure 3. Shortwave radiation for individual days (black circles) or averaged for different periods (white triangles, range indicated by bars) prior to sporocarp collection (black circles) had different $\log_{10}$ probabilities (significance, y-axis) when included in stepwise regressions on the $\delta^{13}C$ of ectomycorrhizal sporocarps. Days shown on x-axis. Probabilities for all individual days are shown; for clarity, only periods (ranges) of highest probability are included. The strongest probability is for radiation from Days 17-21 prior to sporocarp collection (regression term number 1 in Table 1).

Figure 2

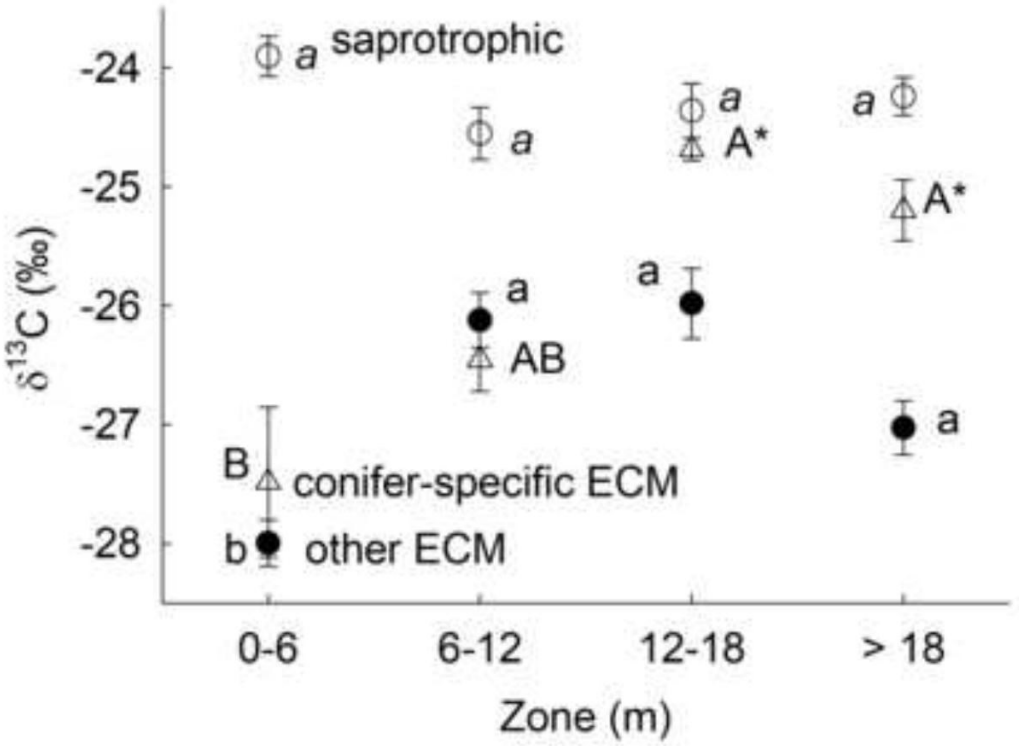

saprotrophic
conifer-specific ECM
other ECM
AB
A*
B
a
b
-24
-25
-26
-27
-28
δ13C (‰)
0-6
6-12
12-18
> 18
Zone (m)

Figure 3

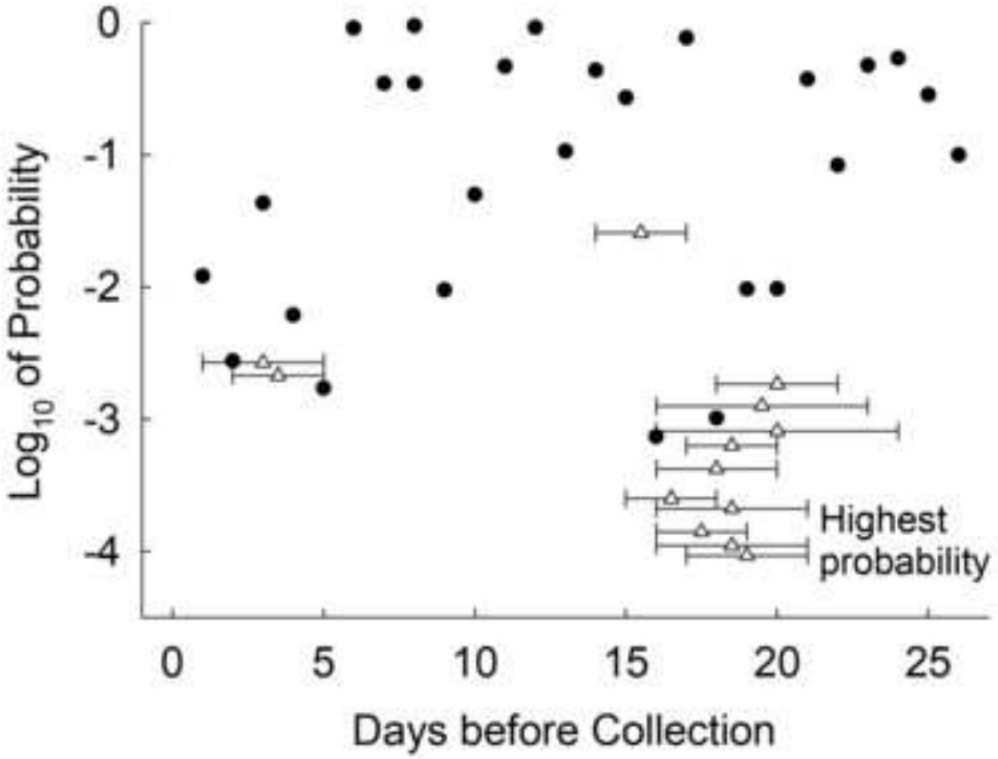
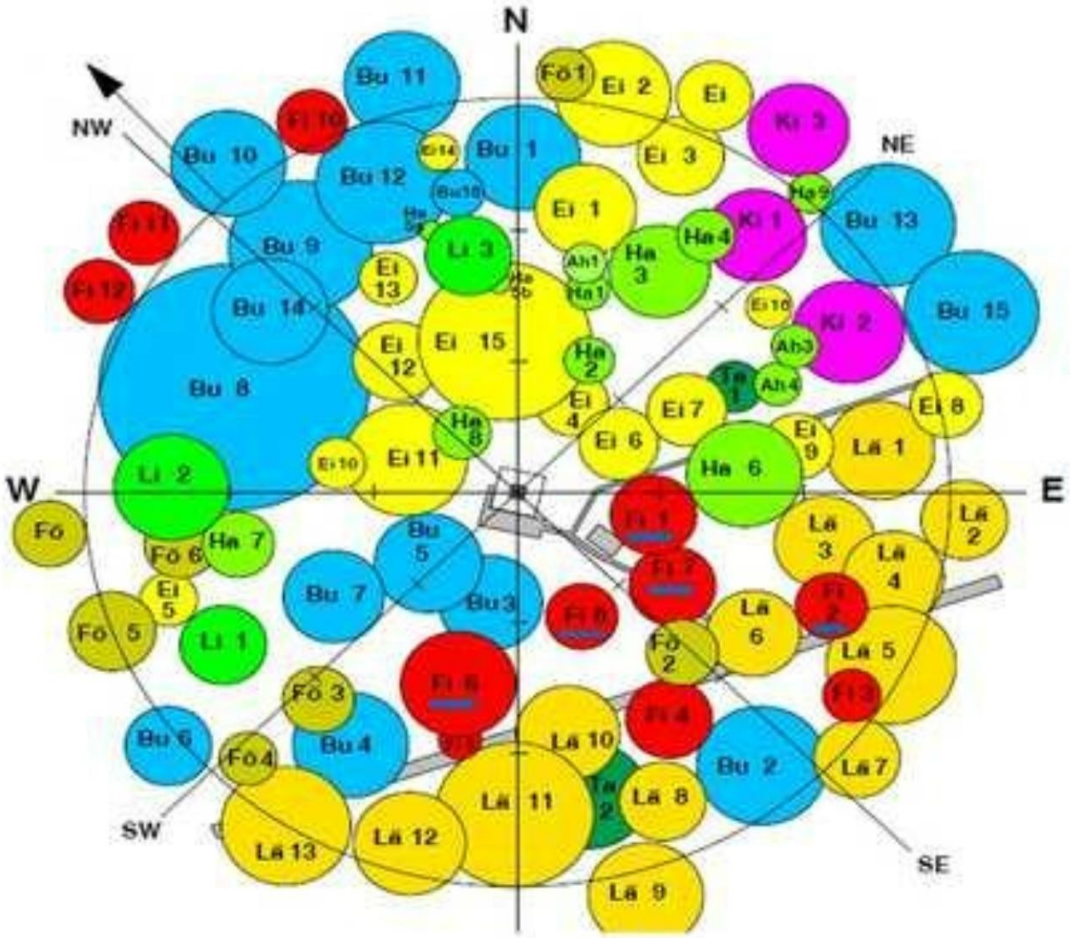

0
-1
-2
-3
-4
Log10 of Probability
0
5
10
15
20
25
Days before Collection
Highest
probability

Figure 1


N
NW
NE
W
E
SW
SE
Bu 11
Fö 1
Ei 2
Ei
Ki 3
Fi 10
Bu 10
Bu 12
Bu 1
Ei 3
Ei 1
Ki 1
Ha 4
Bu 13
Fi 11
Bu 9
Li 3
Ah1
Ha 3
Ra 1
Fi 12
Ei 13
Bu 14
Ei 15
Ki 2
Bu 15
Ei 12
Ah3
Ah4
Ha 2
Bu 8
Ei 4
Ei 7
Ei 8
Ha 8
Ei 6
Ei 9
Lä 1
Li 2
Ei 11
Ha 6
Fö
Fö 6
Ha 7
Bu 5
Lä 3
Lä 4
Lä 2
Ei 5
Bu 7
Bu 3
Fi 6
Fi 7
Fö 5
Li 1
Lä 6
Fö 2
Lä 5
Fö 3
Fi 5
Fi 4
Bu 6
Bu 4
Lä 10
Bu 2
Lä 7
Fö 4
Lä 11
Ra 2
Lä 8
Lä 13
Lä 12
Lä 9

Conflicts of interest: The authors have no relevant financial or non-financial interests to disclose.