Climate and roots, not hyphal development, influence carbohydrate sharing from broad-leaved trees to ectomycorrhizal fungi under elevated CO2

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Abstract

Background

To assess the extent of belowground carbon transfer to ectomycorrhizal fungi in natural forests, we used $\delta^{13}$C and $\log_e$ C/N measurements to calculate spatial dynamics of carbon movement into ectomycorrhizal sporocarps.

Methods

Fourteen broad-leaved trees were labeled with $^{13}$C-depleted CO$_2$ from 2001–2005 in Switzerland and $^{13}$C traced into ectomycorrhizal sporocarps collected at different distances. We then used stepwise regressions on patterns of $\delta^{13}$C and $\log_e$ C/N in ectomycorrhizal sporocarps as a function of distance (zone), solar radiation, fungal genus, and association type.

Results

CO$_2$-labeled trees contributed 76 ± 5%, 36 ± 6%, and 19 ± 7% of sporocarp carbon at 0–6 m, 6–12 m, and 12–18 m from labeled trees, respectively. Literature estimates of hyphal development in different taxa did not correlate with carbon acquisition patterns. After drought in 2003, sporocarp $\log_e$ C/N was low in 2004 and $^{13}$C-depleted carbon from elevated CO$_2$ trees contributed less than in other years to sporocarps. In contrast, sporocarp $\log_e$ C/N peaked in 2005 and contributions from elevated CO$_2$ trees to the 6–12 m zone increased. Therefore, carbohydrate transport belowground decreased in 2004, reflecting plant allocation priorities, and increased in 2005. Sporocarp $\log_e$ C/N varied less among years under elevated CO$_2$ than elsewhere.

Conclusions

These patterns indicated that 1) belowground transport was influenced by climate and plant allocation, 2) root transport rather than ectomycorrhizal transport drove carbon spatial dynamics of ectomycorrhizal fungi, and 3) elevated CO$_2$ decreased the sensitivity of belowground allocation to climatic fluctuations, suggesting improved drought resistance in a high-CO$_2$ world.

Introduction

Ectomycorrhizal fungi are obligate symbionts of most temperate and boreal forest trees. They rely on sugars from their hosts and in return protect trees from root pathogens and supply nutrients and water. These sugars may move beyond the individual plant-fungal pairing and therefore allow costs of
supporting this fungal network to be shared among numerous trees while releasing fungi from reliance on individual hosts, according to work illustrating the movement of isotopically labeled carbon among multiple trees (Simard et al., 1997; Simard et al., 2012). Such studies have focused primarily on the movement of isotopic labels between donor trees and seedlings (Teste et al., 2009), between seedlings (Philip et al., 2010; Pickles et al., 2017), or between Arctic shrubs (Deslippe et al., 2016). Whether long-distance transport of carbon to ectomycorrhizal fungi is primarily through roots or through fungal pathways is not clear.

Field studies applying $^{13}$C-depleted carbon dioxide (CO$_2$) to mature forests provide unique opportunities to examine carbon movement from trees to ectomycorrhizal fungi. The mixing of $^{13}$C-depleted and ambient CO$_2$ can therefore be used to trace the movement of carbon along spatial gradients. Several studies have used this isotopic labeling in Free Air CO$_2$ Enrichment (FACE) studies to assess functioning of ectomycorrhizal fungi, including in the Duke FACE loblolly pine plantation (Hobbie et al., 2014) and in Swiss broad-leaved and coniferous trees (Keel et al., 2006; Mildner et al., 2014; Klein et al., 2016; Rog et al., 2020). Elevated CO$_2$, moisture stress, and low temperatures are conditions where surplus photosynthate may be allocated belowground (Prescott et al., 2020) but the spatial extent to which carbon moves belowground under these conditions is poorly known.

In the Swiss FACE study, both ectomycorrhizal and saprotrophic sporocarps were collected for four years in defined zones around CO$_2$-labeled broad-leaved trees (Keel et al., 2006). Here, we tested whether spatial and temporal patterns in carbon isotopes ($\delta^{13}$C), nitrogen isotopes ($\delta^{15}$N), and log$_e$ C/N of these ectomycorrhizal fungi indicated patterns of carbon fluxes from tree hosts to fungi. We also examined whether host specificity influenced these patterns, since ectomycorrhizal fungi can associate with broad-leaved trees, with conifers, or with both (Molina, 1992).

Ectomycorrhizal fungi vary widely in the hyphal development used to acquire soil resources, with contact, short-distance, medium-distance, and long-distance exploration types corresponding to increased spatial spread (Agerer, 2006). Taxa of greater hyphal spread often possess aggregated hyphae for long-distance transport of resources (rhizomorphs), hydrophobic ectomycorrhizae, and tend to have sporocarps of high $\delta^{15}$N signatures (Hobbie & Agerer, 2010). There is limited evidence from grassland/tree ecotones that such taxa are more common at greater distances from host trees (Peay et al., 2011), although whether this pattern would persist in closed canopy forests is unclear. Taxa without rhizomorphs usually have hydrophilic ectomycorrhizae, which allows direct uptake of water and soluble soil resources by these ectomycorrhizae (Lilleskov et al., 2019) but should limit long-distance transport.

The carbon to nitrogen ratio (C/N) of ectomycorrhizal sporocarps reflects site nitrogen availability (Hobbie et al., 2019; Kranabetter et al., 2019) but can also correlate with recent climate, as climate influences plant productivity and belowground carbon allocation patterns (Bader et al., 2013). In a study in northern Finland in subarctic birch forests, sporocarp C/N increased with higher temperatures and decreased with higher rainfall (Hobbie et al., 2021). Thus, higher C/N may reflect the addition of labile
carbohydrates to sporocarps, similar to rising foliar C/N due to starch accumulation during photosynthesis (Du et al., 2019). Under elevated CO₂, carbohydrate availability to ectomycorrhizal fungi should increase, potentially increasing sporocarp C/N as well.

Sporocarp composition will influence δ¹³C and C/N since fungal protein is higher in δ¹³C and lower in C/N than fungal carbohydrates or bulk sporocarps (Hobbie et al., 2012; Pollierer et al., 2020) and two-thirds of fungal nitrogen is protein (Fujihara et al., 1995). Under ambient CO₂, sporocarp C/N and δ¹³C should therefore be negatively correlated. Direct uptake of amino acids from the soil by ectomycorrhizal fungi could alter the isotopic pattern between fungal protein and carbohydrates, since the δ¹³C of such amino acids will not reflect that of recent photosynthate. The different pathways by which carbon could be supplied to sporocarps are illustrated in Fig. 1. Long-distance transport of plant-derived carbohydrates (sugars) to ectomycorrhizal fungi could be via either root or fungal pathways, whereas sporocarp amino acids could be derived from direct uptake of soil organic nitrogen or from biosynthesis from carbohydrates.

Here, we used ectomycorrhizal sporocarp samples collected from 2001 to 2005 in defined zones around CO₂-labeled broad-leaved trees as part of the Swiss Forest FACE study of responses of broad-leaved trees in a mature forest to elevated CO₂ (Keel et al., 2006). Growing season climate varied considerably over these years, including a severe drought in 2003, which affected plant productivity and allocation in both 2003 and in 2004 (Bader et al., 2013). We tested whether spatial and temporal patterns in carbon isotopes and logₑ C/N of these ectomycorrhizal fungi indicated patterns of carbon fluxes from tree hosts to fungi. We also examined whether host specificity and climate influenced these patterns.

We proposed the following three hypotheses. (1) The δ¹³C values in ectomycorrhizal sporocarps should increase with distance from elevated CO₂ trees, indicating the spatial extent of the carbon transport linking ectomycorrhizal fungi and trees. (2) Carbon transfer belowground should reflect climate and plant productivity, with greater belowground sharing with higher tree productivity. (3) The spatial extent of carbon transport belowground will vary with fungal morphology, with greater long-distance transport in taxa with hydrophobic ectomycorrhizae and with rhizomorphs than in taxa with hydrophilic ectomycorrhizae and without rhizomorphs.

**Methods**

**Field labeling, sampling, and sample analyses**

The Swiss Canopy Crane CO₂ enrichment facility is located within a mixed broad-leaved and coniferous forest at 47°28’ N, 7°30’ E; elevation 550 m. The canopy area at the site consists of 40% *Fagus sylvatica*, 15% *Carpinus betulus*, 11% *Quercus* (mostly *Quercus petraea*), 10% *Larix decidua*, 9% *Picea abies*, 5% *Tilia platyphyllos*, and 3% *Pinus sylvestris*, with five other species making up the remaining 7%. We used this facility to label new carbon assimilates on a 550 m² tree canopy area with ¹³C-depleted CO₂ (Pepin &
Körner, 2002; Körner et al., 2005). Beginning in Spring 2001, we released two tons of CO₂ per day into the crowns of 14 broad-leaved deciduous trees (maximum 32–35 m height and about 100 years old) to raise the canopy CO₂ concentration to 540 ppm (doubling pre-industrial era concentrations). The labeled trees consisted of three *Fagus sylvatica* (beech), four *Quercus petraea* (oak), four *Carpinus betulus*, one *Tilia platyphyllos*, one *Acer campestre*, and one *Prunus avium* (Fig. 2). Beech was the canopy dominant and oak the co-dominant. Pure CO₂ was mixed into the atmosphere of the tree crowns from 4 mm diameter, laser-punctured tubes which were loosely woven into the tree canopy. The concentration was computer-controlled by monitoring the CO₂ level at 24 different canopy positions. The study used the $^{13}$C signal in the added CO₂, which originated from fossil fuel (four-year mean $\delta^{13}$C was $-29.7 \pm 0.3$‰). The mixture of ambient air and fossil-derived CO₂ was depleted in $^{13}$C by $5.8 \pm 0.6$‰ relative to the ambient air $\delta^{13}$C value of $-8$‰ (Pepin & Körner, 2002; Körner et al., 2005; Keel et al., 2006). The $^{13}$C depletion in tree rings from elevated CO₂ trees to ambient trees was $4.2 \pm 0.3$‰ (Bader et al., 2013). During four growing seasons (2001, 2003, 2004, 2005), all sporocarps in and around the labelled forest (a total of 97 species of Basidiomycota, Online Resource 1) were harvested, identified morphologically, and analysed for their isotopic composition. The sporocarps were classified as either saprotrophic or ectomycorrhizal based on the taxonomic literature (Breitenbach & Kränzlin, 1981; Rinaldi et al., 2008), with only data on ectomycorrhizal taxa used in these analyses. Ectomycorrhizal sporocarps were further classified from the available literature as either associated with broad-leaved trees, coniferous trees, associated with both types, or of unknown association (Online Resource 1). We were interested in the spatial relationship between the 14 CO₂-labeled trees and sporocarps. We accordingly assigned collected sporocarps to four zones as (1) directly underneath the 550 m² labelled tree crowns (E, elevated CO₂, 0–6 m), from a 12 m wide transition zone around the labelled trees, divided into (2) an inner zone (6–12 m) and (3) an outer zone (12–18 m), each of 6 m width each, and (4) locations at > 18 m from labelled trees (ambient CO₂). For analysis of %C, %N, and isotopes (expressed as $\delta^{13}$C and $\delta^{15}$N) only caps of sporocarps were used. They were oven-dried at 80°C for 48 hours, 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). Via a variable open split interface (Conflo II, Thermo Finnigan Mat, Germany) the gas from the EA combustion was transferred to the mass spectrometer (Delta S, Thermo Finnigan Mat, Germany), which was operated in continuous flow mode. The precision for $\delta^{13}$C and $\delta^{15}$N analysis was $< \pm 0.1$‰. Some of these sporocarp $\delta^{13}$C data were previously presented in Keel et al. (2006), in which data from 2001, 2003, and 2004 were only used from the elevated CO₂ and ambient zones and data were averaged at the species level, for a presented $n$ of 51 compared to an $n$ of 250 in the current data set.

**Statistical analysis**

Temperature patterns during the growing season for the four years were assessed. We used temperature records from Binningen, Switzerland, 8 kilometers from the field site, as supplied by MeteoSwiss, the Federal Office for Meteorology and Climatology (Online Resources 2 and 3). Sporocarps were collected
between Day 225 and Day 314 in 2001, 2003, 2004, and 2005. We assumed that weather up to 40 days prior to sporocarp collection could potentially influence sporocarp carbon supply, so as factors in regressions on sporocarp $\delta^{13}$C and $\log_e$ C/N we used climate data from Day 185 to Day 314, specifically the daily temperature range (the difference between maximum and minimum daily temperature), as this climatic parameter correlated with sporocarp $\delta^{13}$C and $\log_e$ C/N in Hobbie et al. (2021).

Because we were interested in the carbon dynamics between trees and ectomycorrhizal fungi and prior work had not shown that $\delta^{13}$C values of saprotrophic fungi were altered by elevated CO$_2$ in this experiment (Keel et al., 2006), we restricted our statistical analysis to ectomycorrhizal taxa, which comprised the following genera and sample numbers ($n$) in this data set: *Amanita* (3), *Clavariadelphus* (9), *Clavulina* (6), *Cortinarius* (17), *Entoloma* (7), *Hebeloma* (7), *Hygrophorus* (56), *Inocybe* (102), *Laccaria* (1), *Lactarius* (30), *Russula* (4), *Suillus* (2), and *Tricholoma* (9). Data were analyzed using the statistical software JMP (SAS Institute, Cary, North Carolina, USA). The $\delta^{13}$C and $\log_e$ C/N values were analyzed using forward stepwise multiple regressions. In the stepwise regressions, nominal variables (such as genera, associate type, 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. Nominal variables are generally presented as, for example, Group 1 – Group 2. If the estimate for the ‘Group’ parameter is 0.5‰, then the modeled coefficient for Group 1 would be + 0.5‰ and for Group 2 would be -0.5‰. Interactive terms are generally presented as (Group 1 – Group 2 + $a$), where $a$ is a constant. In this case, if the modeled coefficient is 0.5, then for Group 1 samples, the value is (1 + $a$) × 0.5; for Group 2 samples, the value is (-1 + $a$) × 0.5.

The underlying data are in Online Resource 1. Variables included genus, associate type, zone (E [elevated CO$_2$], inner transition [IT], outer transition [OT], A [ambient]), year, the interaction of zone and year, day of year, temperature range during the 40 days prior to sporocarp collection averaged for different periods, and the interaction of zone with the temperature range integrated for different periods. Because fungal protein content influences $\delta^{13}$C (Hobbie et al., 2020) and the C/N of protein is lower than other compounds, $\log_e$ C/N was included as a continuous variable in the $\delta^{13}$C regression. Since only broad-leaved trees were labeled, we included an interaction between associate and $\log_e$ C/N. To test whether sporocarp carbohydrates and protein differ in their origin, we included interaction terms between zone and $\log_e$ C/N and among zone, year, and $\log_e$ C/N as potentially affecting sporocarp $\delta^{13}$C. Rhizomorph presence and hydrophobicity of ectomycorrhizae were also included as independent variables related to ectomycorrhizal morphology. Stepwise regressions of $\log_e$ C/N were also done with the above set of variables. $\log_e$ C/N rather than C/N was used throughout because C/N is not normally distributed.

**Contributions of $^{13}$C-labeled trees to ectomycorrhizal fungi**

We calculated the initial contributions of the $^{13}$C-labeled trees to ectomycorrhizal fungi at different zones by assuming that carbohydrates supplied to fungi from trees were 4.2‰ depleted in $^{13}$C at the elevated
zone relative to the ambient zone, as observed in tree rings (Bader et al., 2013). Accordingly, the contribution of $^{13}$C-labeled trees to sporocarps at different years and zones was estimated from the $^{13}$C depletion of those sporocarp relative to ambient sporocarps (calculated from regression analyses) divided by 4.2‰.

**Results**

Over the four sampling years, 250 sporocarps were collected that were subsequently classified as ectomycorrhizal, with 40%, 14%, 19%, and 27% in the ambient, outer transition, inner transition, and elevated CO$_2$ zones, respectively. Sporocarps primarily associated with broad-leaved trees ($n$ = 104, 42%) or with both broad-leaved and coniferous trees (mixed, $n$ = 99, 40%), with 16% of unknown associate ($n$ = 41) and very few ($n$ = 6, 2%) associated strictly with coniferous trees. Sporocarps classified only to genus were designated as being of unknown associate. Of the 100 sporocarps from the ambient zone, 56% associated with broad-leaved trees and 23% were of mixed association. In contrast, of the 67 sporocarps collected under elevated CO$_2$, 27% were broad-leaved and 61% were mixed. Few sporocarps were collected in the inner transition, outer transition, and ambient zones in 2003, the year of highest daily temperature range and a severe drought in central Europe (Table 1). Numbers of sporocarps produced under elevated CO$_2$ were not obviously affected. The proportion of *Inocybe* sporocarps by zone increased with proximity to CO$_2$-labeled trees, with 21%, 45%, and 73% of total sporocarps in the ambient plus outer transition, inner transition, and elevated CO$_2$ zones, respectively (Online Resource 4).

Average values ($\pm$ standard deviation) of ectomycorrhizal sporocarps for %C, %N, C/N, $\log_e$ C/N, and $\delta^{15}$N were 41.97 $\pm$ 2.60%, 5.01 $\pm$ 1.23%, 8.95 $\pm$ 2.72, and 2.15 $\pm$ 0.27, and 3.9 $\pm$ 4.2‰, respectively. Carbon isotope values at the four zones were lowest under elevated CO$_2$ (-28.9 $\pm$ 1.7‰, $n$ = 69), intermediate in the inner transition (-27.1 $\pm$ 1.3‰, $n$ = 47), and highest in the outer transition (-26.2 $\pm$ 0.9, $n$ = 36), and ambient samples (-25.5 $\pm$ 1.3‰, $n$ = 101), for a maximum decrease of 3.4‰. In Tukey tests, these were three distinct groups (elevated < inner transition < outer transition, ambient). In addition to sporocarp $\delta^{13}$C variability at the four zones, sporocarp $\delta^{13}$C also varied across the four years of sampling, with lowest and highest $\delta^{13}$C at the ambient zone in 2004 and 2003, respectively, and lowest $\delta^{13}$C at the outer transition and inner transition zones in 2005 (Fig. 3). Sporocarp C/N varied across the different years, with sporocarp C/N highest in 2005 and lowest in 2004. In addition, sporocarp C/N varied across the different harvest dates, with sporocarps in 2005 increasing dramatically in C/N in the latter part of the harvest period (Fig. 4). Sporocarp $\delta^{15}$N varied widely across the different genera, with highest values in *Tricholoma* (11.9 $\pm$ 0.9‰), *Clavariadelphus* (11.0 $\pm$ 0.5‰), *Cortinarius* (7.9 $\pm$ 0.9‰), and *Entoloma* (7.5 $\pm$ 0.7‰). Lowest values were in *Inocybe* (0.0 $\pm$ 0.1‰), *Clavulina* (0.8 $\pm$ 0.7‰), *Amanita* (2.6 $\pm$ 0.1‰), and *Russula* (3.4 $\pm$ 2.7‰).

$\delta^{13}$C regressions
In the stepwise regression analysis of ectomycorrhizal $\delta^{13}C$, the minimum BIC was with a seven-term model (adjusted $r^2 = 0.567$, $n = 250$). The dominant factor was the zone of sporocarp collection, with the three zonal terms in the regression accounted for 45% of variance (Table 2, Terms 1–3). The other four terms included genus (5% of variance), an interaction of zone with year (4% of variance), an interaction of $\log e \ C/N$ with associate (2.1% of variance), and an interaction of $\log e \ C/N$, zone, and year (1.5% of variance). The four zonal categories reflected the declining contribution of photosynthesis from the $^{13}C$-depleted $CO_2$ to sporocarps at increasing distance from the 14 $CO_2$-labeled trees. The regression model estimates of the $^{13}C$ depletion relative to ambient sporocarps under elevated $CO_2$, inner transition, and outer transition zones were 3.26‰, 1.38‰, and 0.82‰ (Table 2).

The interactive term of zone and year modified $\delta^{13}C$ values at the inner and outer transition zones in 2003 and 2004. These values were combined with those from the zonal estimates to provide yearly estimates for each zone. These estimates were then compared against yearly $\delta^{13}C$ estimates for ambient sporocarps to calculate the percent contribution from elevated $CO_2$ trees to ectomycorrhizal sporocarps by zone and year (Table 3). The contribution varied from 67% in 2004 to 94% in 2003 under elevated $CO_2$, from 22% in 2004 to 49% in 2003 in the inner transition zone, and from 0% in 2004 to 20% in 2001 and 2005 in the outer transition zone.

Given the 4.2‰ depletion of $CO_2$-labeled trees relative to ambient trees, as derived from tree ring measurements (Bader et al., 2013), and the average $^{13}C$ depletion of 3.3‰, 1.4‰, and 0.8‰ in sporocarps in the elevated $CO_2$, inner transition, and outer transition zones compared to ambient sporocarps (Table 2), this indicated that ectomycorrhizal fungi collected in the elevated $CO_2$, inner transition, and outer transition zones derived 78% (3.26/4.2), 33%, and 20% of their carbon from the $CO_2$-treated trees, respectively. At an average 6 meter separation among these three zones, this corresponded to a contribution decrease of 5% per meter from the elevated to inner transition zones and a drop of 3% per meter from the inner transition to outer transition zones.

Under ambient conditions, sporocarp protein is higher in $\delta^{13}C$ and lower in $C/N$ than bulk (Hobbie et al., 2012; Pollierer et al., 2020), which should lead to negative correlations between sporocarp $\delta^{13}C$ and $\log e \ C/N$. In the $\delta^{13}C$ regression, sporocarp $\log e \ C/N$ and $\delta^{13}C$ were negatively correlated for those associated with broad-leaved trees (coefficient, -0.96 ± 0.35, $n = 104$) and positively correlated for those of unknown association, with a coefficient of 1.61 ± 0.58, $n = 41$ (Table 2, Online Resource 5).

A three-way interaction in the $\delta^{13}C$ regression among $\log e \ C/N$, zone, and year resulted in $\log e \ C/N$ being negatively correlated with $\delta^{13}C$ in the inner transition zone in 2005 (coefficient, -1.54 ± 0.68) and in the ambient and outer transition zones in 2003 and 2004 (coefficient, -0.44 ± 0.20). This three-way interaction also led to $\log e \ C/N$ positively correlating with $\delta^{13}C$ in the inner transition zone in 2003 and 2004 (coefficient, 0.92 ± 0.15) and in the ambient and outer transition zones in 2005 (coefficient, 0.73 ± 0.17) (Table 2). We combined the two regression terms that included $\log e \ C/N$ to estimate coefficients for $\log e \ C/N$,
C/N for different sporocarp groups (Online Resource 6). Most sporocarps had negative correlations (151/250, 60%), with lesser numbers showing a positive correlation (63/250, 25%) or no correlation (36/250, 14%).

The 13 genera separated into three groups in the $\delta^{13}$C regression (Table 2, Term 5). The first group (high-$\delta^{13}$C) averaged $0.57 \pm 0.15\%$ higher than the mean and included *Hygrophorus* (56), *Lactarius* (30), *Cortinarius* (17), *Hebeloma* (7), *Entoloma* (5), and *Suillus* (2). The second group (medium-$\delta^{13}$C) included *Inocybe* (102), *Tricholoma* (9), *Clavulina* (6), *Amanita* (3), and *Laccaria* (1), and did not appear in the regression term, so were assigned a value of 0%. The third group (low-$\delta^{13}$C) included *Clavariadelphus* (9) and *Russula* (4) and averaged $0.57 \pm 0.15\%$ lower than the mean. The first group averaged higher in C/N and $\delta^{15}$N than the second group (9.52 $\pm$ 0.26 versus 8.35 $\pm$ 0.24 in C/N and 6.21 $\pm$ 0.23$\%$ versus 1.01 $\pm$ 0.32$\%$ in $\delta^{15}$N. The first and third groups primarily (70%) associated with broad-leaved trees whereas the second group was primarily classified (70%) as mixed association (both conifers and broad-leaved trees). The first and third groups were primarily from the ambient zone (58%) whereas the second group was primarily from the elevated and inner transition zones (71%).

**Log$_e$ C/N regression**

In the regression of log$_e$ C/N (Table 4), the minimum BIC required nine regression terms, with an overall adjusted $r^2$ of 0.434 (Table 4, $n = 250$). Genus accounted for 13% of variance (Terms 1–3). However, the dominant factor was the year of collection (22% of variance, Term 4), with log$_e$ C/N higher in 2005 than in other years. When combined with a positive effect of day of year on log$_e$ C/N (2% of variance, Term 5), the two interactions of year with day of year accounted for 6% of variance (Terms 6, 7). The day of year correlated highly with log$_e$ C/N in 2005 (increase of log$_e$ C/N of $1.27 \pm 0.25$% day$^{-1}$), also correlated in 2001 and 2003 (increase of $0.62 \pm 0.20$% day$^{-1}$), and was uncorrelated in 2004 (decrease of $0.08 \pm 0.23$% day$^{-1}$).

The temperature range for the 13–20 days prior to collection correlated positively with log$_e$ C/N under elevated CO$_2$ ($5.1 \pm 2.0$% increase per °C, 1% of variance) but correlated negatively with log$_e$ C/N in the other three zones ($1.9 \pm 0.7$% decrease per °C, Fig. 7, Term 8). Finally, an interaction between year and zone for 2001, 2003, and 2004 in the ambient, outer transition, and inner transition zones resulted in outer and inner transition zone sporocarps being 10% lower in log$_e$ C/N in 2004 than in 2001 and 2003, with ambient sporocarps having the opposite pattern, and elevated CO$_2$ sporocarps unaffected by these yearly differences (Term 9).

**Discussion**

**Transport among ectomycorrhizal fungi**
We used the pattern of $^{13}$C labeling to examine the yearly spatial pattern of carbon derived from CO$_2$-labeled trees. We concluded that part of the labile carbon supplied by belowground transport to ectomycorrhizal fungi was first photosynthesized at some distance from the harvest location. We combined zonal effects with the effects of the interactions of zone and year (Table 2, Terms 1–4) to estimate the yearly $\delta^{13}$C values for each zone, from which we then calculated the percent contribution of CO$_2$-labeled photosynthate to sporocarps each year for each zone (Table 3). The high calculated contribution to elevated CO$_2$ sporocarps in 2003 (94%) may reflect the drought conditions of that year, in which soil water content was quite low (Leuzinger et al., 2005). This dry soil may accordingly have limited both photosynthesis and the transport of $^{13}$C-enriched (ambient-derived) labile carbon into the elevated CO$_2$ zone. Because sporocarp fruiting depends on sufficient soil moisture and precipitation (Savoie & Largeteau, 2011; De la Varga et al., 2013), the dry soil in 2003 probably also accounted for the low numbers of sporocarps collected that year in the inner transition and outer transition zones compared to other years (three in 2003 versus 30, 25, and 25 in 2001, 2004, and 2005, respectively; Table 1). Numbers in the ambient zone were not similarly affected, although the ambient zone from which sporocarps were collected could have been larger in 2003 than in other years, since sporocarps were collected up to ~100 m from CO$_2$-labeled trees (Keel et al., 2006).

In contrast to 2003, the lower water stress in 2004 may have allowed transport of labile carbon into the elevated CO$_2$ zone. However, overall productivity appeared low in 2004. Leaf litter production in 2004 was lower than in other years, particularly for elevated CO$_2$ trees (Bader et al., 2013), and soil CO$_2$ concentrations were lower during the 2004 growing season than in 2001 or 2005 (Bader et al., 2013). Tree ring width of the canopy dominant *Fagus* was less in 2003 and 2004 than in 2001, 2002, and 2005, although this pattern was not seen in the co-dominant, deeply rooted *Quercus* (Bader et al., 2013). These patterns agreed with reduced belowground allocation in 2004 after the 2003 drought. Daily temperature range was lower in 2004 than other years and is negatively correlated with cloudiness (Rebetez & Beniston, 1998), suggesting increased cloudiness in 2004, as also indicated by fewer hours of sunlight per day in 2004 than in other years (Table 1). This reduced productivity in 2004 probably limited photosynthate transfer from the $^{13}$C-depleted elevated CO$_2$ to the outer transition (12–18 m) zone, based on the higher $\delta^{13}$C value that year for outer transition sporocarps (Table 2). This illustrated the reduced distance of carbon transport under these conditions, presumably because of reduced belowground allocation. The above results confirm our first hypothesis, that the spatial extent and amount of carbon transport belowground expand and contract as belowground allocation is enhanced or restricted by climatic conditions or plant allocation priorities.

**Functional differences in morphology are not linked to carbohydrate transport**

Ectomycorrhizal fungi differ in belowground morphology (Agerer, 2001; Agerer, 2006) including exploration type, the presence or absence of rhizomorphs (long-distance transport structures of aggregated hyphae) and whether ectomycorrhizal mantles are hydrophobic (repel water) or hydrophilic
(absorb water). These morphological characteristics are linked to different carbon demands (Weigt et al., 2012). Extensive hyphal development should increase the transport of resources among different regions.

Our third hypothesis was that fungal morphology influenced ectomycorrhizal transport. There was not strong support in our data for this, as neither the presence of rhizomorphs nor the hydrophobicity of ectomycorrhizae (Table 3 and Online Resource 7) were significant factors in our regression analyses. The most parsimonious explanation is that morphological differences in ectomycorrhizal fungi, although contributing to differences in carbon transport at small scales, are unimportant relative to the long distances by which tree sugars are transported by roots. For example, in arctic ectomycorrhizal shrubs, Deslippe et al. (2016) reported carbon transfer between *Betula nana* plants that was mediated by the rhizomorph-forming species of *Cortinarius* rather than other ectomycorrhizal species present. However, the maximum sampling distance was only about 30 cm from $^{13}$C-labeled plants. In contrast, roots of *Fagus sylvatica* and *Carpinus betulus* reached up to 16–19 m and 10 m from their stems, respectively, in mixed forests in Germany (Meinen et al., 2009). Our interpretation then indicates that in Fig. 1, pathway (2), root delivery of carbohydrates, rather than pathway (1), fungal long-distance transport of carbohydrates, is the relevant delivery system for C acquisition by fungi at distance.

**Contributions of labeled carbohydrates vary with year and association**

We explored whether sporocarp log$_e$ C/N correlated with sporocarp $\delta^{13}$C and the potential mechanisms driving any correlations. If all sporocarp amino acids are synthesized from supplied sugars, then correlations between log$_e$ C/N and $\delta^{13}$C would only arise from internal fractionation between $^{13}$C and $^{12}$C during amino acid biosynthesis. However, if ectomycorrhizal fungi assimilate soil-derived amino acids, then the source carbon for sporocarp protein and carbohydrates can differ in $\delta^{13}$C, which provides a second mechanism for altering the correlation between log$_e$ C/N and $\delta^{13}$C.

In the current experiment, the $\delta^{13}$C of photosynthate for CO$_2$-supplied trees decreased. This increased the $\delta^{13}$C of soil organic N compared to the $\delta^{13}$C of ectomycorrhizal fungal carbon derived from recent photosynthate. The simplest explanation of variability in correlations between log$_e$ C/N and $\delta^{13}$C is that it reflected increased or decreased transport of $^{13}$C-depleted carbohydrates from the elevated CO$_2$ zone coupled with some local assimilation of $^{13}$C-enriched amino acids into sporocarp protein (Fig. 1). Negative correlations reflected increased transport of $^{13}$C-depleted carbohydrates; positive correlations reflected decreased transport of $^{13}$C-depleted carbohydrates.

In our regression analyses, both associate type and an interaction between year and zone influenced how log$_e$ C/N affected sporocarp $\delta^{13}$C (Table 2, Terms 6, 7). For example, in sporocarps associated with broad-leaved trees, log$_e$ C/N correlated negatively with $\delta^{13}$C. This presumably reflected greater transport capabilities for and access to $^{13}$C-depleted sugars of these taxa than for sporocarps of unknown association (with a positive correlation) or sporocarps of mixed association (with no correlation). Carbon
flux from broad-leaved trees supplied with CO₂ to fungi associated with those trees should be greater than to fungi of other associations. Here, we see some indication that this was true.

We combined the two regression terms that incorporated interactions with logₑ C/N to examine which combinations of year, zone, and fungal associate had particularly negative coefficients with logₑ C/N in the δ¹³C regression and whether there was a consistent pattern with δ¹⁵N values for these groups (Online Resource 6). The most negative coefficient (-1.54 ± 0.68) was for sporocarps of mixed association in the inner transition zone in 2005 (adjacent to the elevated CO₂ zone). The low δ¹⁵N value of 1.3 ± 0.7‰ implies low proteolytic capabilities (Lilleskov et al., 2002). This strongly suggested that in this case, high transport was likely of ¹³C-depleted sugars derived from elevated CO₂ trees is likely.

The 25 sporocarps from the ambient zone in 2003 and 2004 and five sporocarps from the outer transition zone in 2004 that associated with broad-leaved trees had a similarly negative coefficient (-1.40 ± 0.40). There is no δ¹³C evidence that sporocarps from the outer transition zone in 2004 acquired C from CO₂-supplied trees (estimate, -10 ± 9%, Table 3). Enhanced transport of ¹³C-depleted carbohydrates could therefore not have caused the coefficient value. The high δ¹⁵N of these fungi (7.1 ± 0.6‰) suggests high proteolytic capabilities and therefore probable assimilation of soil-derived ¹³C-enriched amino acids. We concluded that several factors contributed to variability in the coefficient of the effect on sporocarp δ¹³C of sporocarp logₑ C/N.

**Sporocarp C/N reflected belowground system carbohydrate fluxes**

Measurements of C/N (or logₑ C/N) provided additional insight into yearly or seasonal patterns of carbon fluxes. The effect of year, in which logₑ C/N was significantly higher in 2005 than in other years, presumably reflected favorable conditions for carbon accumulation in 2005 (Table 4, Term 4). This is also suggested by increased tree ring width in 2005 in *Fagus* compared to the two prior years (Bader et al., 2013) and the positive effect of day of year on logₑ C/N in 2005 (Table 4, Term 6). This suggests that the store of labile carbohydrates in fungal mycelia increased rapidly at the end of the growing season in 2005 (Table 4 and Fig. 4). Similarly, the negative effect of day of year on logₑ C/N in 2004 (Table 4, Terms 6, 7) agreed with reduced belowground flux following the 2003 drought and a probable allocation shift towards seed production in 2004 (Hacket-Pain et al., 2017). The δ¹³C patterns in 2004 also showed reduced movement of carbon away from elevated CO₂ trees.

Two terms in the logₑ C/N regression involved interactions with zones. The inner transition, outer transition, and ambient zones interacted with years 2001, 2003, and 2004 but sporocarps in the elevated CO₂ zone were unaffected. In Table 4 (Term 8), a higher 13–20 day temperature range increased sporocarp logₑ C/N under elevated CO₂ but decreased logₑ C/N in other zones (Fig. 6), suggesting that belowground flux, and presumably overall productivity, is reduced under clear and dry conditions except under elevated CO₂. The 13–20 day temperature range was itself highly correlated with solar radiation for
the same period (adjusted $r^2 = 0.609$), with the year of measurement explaining additional variability (adjusted $r^2 = 0.900$, Online Resource 8).

The results show less climatic responsiveness of the elevated CO$_2$ zone relative to other zones. The higher soil moisture under CO$_2$-labeled trees because of reduced transpiration in this study (Bader et al., 2013) suggested that these trees experienced less water stress than other trees. As a result, the photosynthesis and allocation patterns of the elevated CO$_2$ trees should be altered less than in other trees by high temperatures and low soil moisture levels, such as during the 2003 drought. In support of this, soil CO$_2$ concentrations were generally higher under CO$_2$-labeled trees (Steinmann et al., 2004), particularly in 2005 (Bader et al., 2013), the year that elevated CO$_2$ had the greatest spatial influence on sporocarp C/N in our data.

**Conclusions**

The distance of horizontal transport was unaffected by patterns of hyphal development of different ectomycorrhizal fungi. Such an inference suggests that variable genet sizes of ectomycorrhizal fungi, such as the small network size of *Inocybe* (short-distance exploration type) and the much larger network size of *Scleroderma*, a taxon of long-distance exploration type (Nara, 2015) are not particularly informative for understanding patterns of horizontal carbon transfer.

Excess carbon fixed under elevated CO$_2$ was transported belowground as carbohydrates. After transport to other zones, presumably via roots, this $^{13}$C-depleted carbohydrate pool decreased the average $\delta^{13}$C of carbohydrates forming sporocarps under elevated CO$_2$, and therefore increased the $^{13}$C depletion of carbohydrates (no N) relative to protein (low C/N).

The spatial extent of carbon supply belowground expanded and contracted as photosynthesis and belowground transport were enhanced or restricted by climatic and soil moisture conditions. Sporocarps under elevated CO$_2$ were less sensitive to changes in climatic conditions than other sporocarps, suggesting that effects on forests of predicted increases in future drought may be offset by increased C allocation from roots to ectomycorrhizal fungi.

**Declarations**

**Acknowledgements**

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Consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data. Data for the study are archived at Mendeley Data, doi: 10.17632/yzcm5vmxf8.1.

Code availability: Not applicable.

Authors’ contributions. CK, MS, and RS planned and designed the research. SGK, and KS performed experiments and conducted fieldwork, MW identified fungal species, MW and EAH assigned fungal associations, EAH analyzed the data, all authors wrote the manuscript.

Online Resources


Online Resource 2. Daily temperature range between days 190 and 350 for 2001 (black), 2003 (red), 2004 (green), and 2005 (yellow), as a 20-day moving average.


Online Resource 4. Distribution by genus and zone (elevated CO\textsubscript{2}, inner transition, outer transition, ambient) of ectomycorrhizal sporocarps.

Online Resource 5. Stepwise regression pathways for sporocarp d\textsuperscript{13}C. Regression model of d\textsuperscript{13}C demonstrating that hydrophobicity and rhizomorphs are not significant factors is also shown.

Online Resource 6. Coefficients of log\textsubscript{e} C/N against sporocarp d\textsuperscript{13}C values, as calculated from the d\textsuperscript{13}C regression, for different groups of ectomycorrhizal fungi. d\textsuperscript{15}N values for the different groups are also given.

Online Resource 7. Stepwise regression pathways for sporocarp log\textsubscript{e} C/N.

Online Resource 8. Regression of solar radiation and year with the temperature range for the 13-20 days prior to sporocarp collection.
References


**Tables**

Table 1. Sporocarp numbers by year, zone, and associate included in regression analyses. The average daily temperature range (TR) for the 13-20 days before sporocarp sampling for each year (average of samples) is also given. Sporocarps were collected between mid-August, Day of Year (DoY) 225 and early November, DoY 314. Average sunshine in minutes (min) per day for DoY 185 to 314 for each year is also given. Assoc = association, given as conifer/broad-leaved/mixed/unknown.
Table 2. Stepwise regression of sporocarp $d^{13}$C (‰) indicates that zone, genus, the interactions of zone with year, and interactions of $\log_e C/N$ with zone, and interaction of $\log_e C/N$ with associate $\times$ year are significant factors. Adjusted $r^2 = 0.567$, $p < 0.0001$, $n = 250$. VIF = variance inflation factor. A = ambient, OT = outer transition, IT = inner transition, E = elevated CO$_2$. Values for genus, and the interaction of zone with $\log_e C/N$ are given at the bottom of the table, as calculated from the regression terms. Zone and Year $\times$ Zone effects are in Table 3. The complete regression model is in Online Resource 5. Broad = associated with broad-leaved trees, Unk = unknown association. Yr 3, 4, 5 = Year 2003, 2004, 2005. %V = %Variance.

<table>
<thead>
<tr>
<th>Year</th>
<th>Ambient</th>
<th>Transition</th>
<th>Transition</th>
<th>Elevated</th>
<th>Total</th>
<th>TR ± sd (°C) (min day$^{-1}$)</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>2/28/6/8</td>
<td>0/15/2/3</td>
<td>0/3/4/3</td>
<td>0/7/4/0</td>
<td>85</td>
<td>8.74 ± 0.30</td>
<td>340 ± 22</td>
</tr>
<tr>
<td>2003</td>
<td>1/7/5/0</td>
<td>0/0/0/0</td>
<td>0/1/2/0</td>
<td>0/5/11/1</td>
<td>33</td>
<td>8.63 ± 0.80</td>
<td>417 ± 23</td>
</tr>
<tr>
<td>2004</td>
<td>0/18/7/4</td>
<td>0/5/6/2</td>
<td>0/5/4/3</td>
<td>1/5/19/2</td>
<td>81</td>
<td>7.44 ± 0.98</td>
<td>299 ± 23</td>
</tr>
<tr>
<td>2005</td>
<td>0/3/5/6</td>
<td>0/0/2/1</td>
<td>1/0/15/6</td>
<td>1/2/7/2</td>
<td>51</td>
<td>9.04 ± 1.09</td>
<td>331 ± 22</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>36</td>
<td>47</td>
<td>67</td>
<td>250</td>
<td>8.37 ± 1.03</td>
<td></td>
</tr>
<tr>
<td>Assoc</td>
<td>3/56/23/18</td>
<td>0/20/10/6</td>
<td>1/9/25/12</td>
<td>2/19/41/5</td>
<td>6/104/99/41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# Term %V Estimate ± se P

| VIF -2.787 ± 0.11 < 0.0001 |
| 0.0001 -- |
1 Zone (A/OT/IT – E)  
37.8 1.18 ± 0.10  <  
0.0001 1.2

2 Zone (A/OT – IT)  
4.5 0.49 ± 0.12  <  
0.0001 1.3

3 Zone (A – OT)  
2.9 0.41 ± 0.13  0.0014  
1.2

4 Year (2003 – 2004 + 0.19) × (A – OT - 0.26)  
3.8 0.77 ± 0.21  0.0003  
1.0

5 Genus (Group 1 – 3)  
4.6 0.56 ± 0.15  0.0001  
1.1

6 (Broad – Unk - 0.25) × (log$_e$ C/N - 2.15)  
2.1 -1.29 ± 0.46  0.0058  
1.3

7 Yr (5 – 3&4 + 0.25) × (A/OT – IT - 0.36) × (log$_e$ C/N - 2.15)  
1.5 0.91 ± 0.39  0.0221  
1.4

**Genus:** Group 1 ($n = 119$), *Lactarius, Hygrophorus, Cortinarius, Hebeloma, Entoloma, Suillus, 0.57 ± 0.15‰;* Group 2 ($n = 118$), *Laccaria, Tricholoma, Inocybe, Amanita, Clavulina, 0‰;* Group 3 ($n = 13$), *Clavariadelphus, Russula, -0.57 ± 0.15‰.*

**Associate × (log$_e$ C/N - 2.15):** Broad-leaved, -0.96 ± 0.35; Unknown associate, 1.61 ± 0.57.

**Year × Treatment × log$_e$ C/N:** 2003/2004: IT, 0.92 ± 0.41; A/OT, -0.44 ±0.20. 2005: IT, -1.54 ± 0.68; A/OT, 0.73 ± 0.32.

Table 3. Estimates ( ± se) of d$_{13}$C deviation from the mean by year and zone. Values are derived by adding d$_{13}$C regression estimates of the zone and the zone × year interaction. Percent contribution from CO$_2$-labeled trees is given in parentheses ( ± se), as calculated from the $^{13}$C depletion relative to the ambient value divided by 4.2‰.
<table>
<thead>
<tr>
<th>Year</th>
<th>Ambient</th>
<th>Outer Transition</th>
<th>Inner Transition</th>
<th>Elevated CO&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001/5</td>
<td>2.08 ± 0.20</td>
<td>1.25 ± 0.21 (20 ± 7%)</td>
<td>0.69 ± 0.16 (33 ± 6%)</td>
<td>-1.18 ± 0.10 (78 ± 5%)</td>
</tr>
<tr>
<td>2003</td>
<td>2.76 ± 0.27</td>
<td>--</td>
<td>0.69 ± 0.16 (49 ± 8%)</td>
<td>-1.18 ± 0.10 (94 ± 7%)</td>
</tr>
<tr>
<td>2004</td>
<td>1.62 ± 0.24</td>
<td>2.04 ± 0.29 (-10 ± 9%)</td>
<td>0.69 ± 0.16 (22 ± 7%)</td>
<td>-1.18 ± 0.10 (67 ± 6%)</td>
</tr>
</tbody>
</table>

Table 4. Stepwise regression of log<sub>e</sub> C/N. Adjusted r<sup>2</sup> = 0.434, p < 0.0001, n = 250. Complete regression model is in Online Resource 7. At bottom of table, values for genus, year, the interactions of year with zone and with day of year, and the interaction of zone with the average daily temperature range for 13-20 days prior to sporocarp collection are given, as calculated from the regression terms. %Var, %Variance; VIF, variance inflation factor. TR, Temperature range; A, ambient; IT, inner transition; OT, outer transition; E, elevated CO<sub>2</sub>; DoY, Day of Year. Fungal association: Broad, broad-leaved partners; Unk, unknown partners; Year: 1, 2001; 3, 2003; 4, 2004; 5, 2005.

<table>
<thead>
<tr>
<th>#</th>
<th>Term</th>
<th>%Var</th>
<th>Estimate ± se</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VIF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Intercept</td>
<td>--</td>
<td>0.893 ± 0.415</td>
<td>0.0322</td>
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<td></td>
<td></td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Genus (Group 1&amp;2&amp;3&amp;4 – 5)</td>
<td>9.2</td>
<td>-0.134 ± 0.019</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Genus (Group 1 – 2&amp;3&amp;4)</td>
<td>2.4</td>
<td>-0.113 ± 0.032</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Genus (Group 2 – 3)</td>
<td>1.6</td>
<td>0.089 ± 0.030</td>
<td>0.0036</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expression</td>
<td>F</td>
<td>Value</td>
<td>P-value</td>
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<tr>
<td>---</td>
<td>---------------------------------------------------------------------------</td>
<td>------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>4</td>
<td>Year (5 - 1&amp;3&amp;4)</td>
<td>21.6</td>
<td>0.284 ± 0.027</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DoY</td>
<td>2.4</td>
<td>0.0051 ± 0.0014</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Year (5 - 1&amp;3&amp;4 + 0.59) × (DoY - 286.5)</td>
<td>2.7</td>
<td>0.0047 ± 0.0013</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Year (4 - 1&amp;3 + 0.148) × (DoY - 286.5)</td>
<td>1.0</td>
<td>-0.0035 ± 0.0014</td>
<td>0.0239</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(13-20 day TR – 8.37) × (E – IT/OT/A + 0.464)</td>
<td>1.3</td>
<td>0.035 ± 0.013</td>
<td>0.0106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Year (4 - 1&amp;3 + 0.148) × (A – IT/OT – 0.068)</td>
<td>1.3</td>
<td>0.046 ± 0.018</td>
<td>0.0094</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Genus:** Group 1 (12), -0.247 ± 0.037, *Amanita, Tricholoma*; Group 2 (7), 0.067 ± 0.048, *Hebeloma*; Group 3 (102), -0.110 ± 0.048, *Inocybe*; Group 4 (41), -0.021 ± 0.037, *Clavariadelphus, Lactarius, Suillus*; Group 5 (88), 0.134 ± 0.019, *Cortinarius, Clavulina, Entoloma, Hygrophorus, Laccaria, Russula*.

**Year:** 2001, 2003, -0.180 ± 0.030; 2004, -0.346 ± 0.030; 2005, 0.263 ± 0.24.

**Year × (DoY - 286.7) [× 10^-3]:** 2001/03, 1.04 ± 1.40; 2004, -5.93 ± 1.83; 2005, 7.53 ± 2.01.

**13-20 day TR – 8.37) × Zone:** E, 0.0508 ± 0.0197; IT/OT/A, -0.0186 ± 0.0072.

**Year × Zone:** 2001/2003: A; -0.0367 ± 0.0140; OT/IT, 0.0420 ± 0.0160. 2004: A; 0.0494 ± 0.0189; OT/IT, -0.0566 ± 0.0216.

Table 5. Description of genera. Regr = regression given in Table 2 for genera. Letters after d\(^{15}\)N values are results of a Tukey test by genus, with genera not sharing any letters significantly different. Hi/Ho = hydrophilic or hydrophobic ectomycorrhizae. Ectomycorrhizae type, presence of rhizomorphs, and exploration type follows Agerer (2006), with short, medium, and long, corresponding to short-distance, medium-distance,
and long-distance exploration types. Descriptive subtypes of smooth, fringe, and mat also follow Agerer (2006).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Regr</th>
<th>d$^{13}$C</th>
<th>d$^{15}$N ± sd (%)</th>
<th>n</th>
<th>Hi/Ho</th>
<th>Rhizomorph</th>
<th>Exploration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cortinarius</strong></td>
<td>high</td>
<td>7.9 ± 3.7C</td>
<td>17 ho yes</td>
<td>medium-fringe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Entoloma</strong></td>
<td>high</td>
<td>7.5 ± 2.0CD</td>
<td>7 nd yes</td>
<td>medium-smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hebeloma</strong></td>
<td>high</td>
<td>6.0 ± 3.4CDE</td>
<td>7 ho sometimes</td>
<td>short, medium-fringe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hygrophorus</strong></td>
<td>high</td>
<td>5.6 ± 1.9DE</td>
<td>56 hi no</td>
<td>contact, short-smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lactarius</strong></td>
<td>high</td>
<td>6.1 ± 2.2CDE</td>
<td>30 hi (ho) sometimes</td>
<td>contact, medium-smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Suillus</strong></td>
<td>high</td>
<td>5.8 ± 0.2BCDEF</td>
<td>2 ho yes</td>
<td>long</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amanita</strong></td>
<td>medium</td>
<td>2.6 ± 0.1EFG</td>
<td>3 hi (ho) sometimes</td>
<td>medium-smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clavulina</strong></td>
<td>medium</td>
<td>0.8 ± 1.8FG</td>
<td>6 no data no data</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inocybe</strong></td>
<td>medium</td>
<td>0.0 ± 1.4G</td>
<td>99 hi no</td>
<td>short</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laccaria</strong></td>
<td>medium</td>
<td>0.4DEFG</td>
<td>1 hi yes</td>
<td>medium-smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tricholoma</strong></td>
<td>medium</td>
<td>11.9 ± 2.3A</td>
<td>9 ho yes</td>
<td>medium-fringe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clavariadelphus</strong></td>
<td>low</td>
<td>11.0 ± 1.6AB</td>
<td>9 ho yes</td>
<td>medium-mat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Russula</strong></td>
<td>low</td>
<td>3.4 ± 5.4DEFG</td>
<td>4 hi sometimes</td>
<td>smooth</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

Carbon moves via fungal pathways (1) or via roots (2) as carbohydrates (CHO) to supply sporocarps. Fungal amino acids are synthesized from supplied sugars (3) or taken up directly from the soil organic nitrogen (SON) (4). The $\delta^{13}C$ of photosynthetic carbon is lower under elevated CO$_2$ than under ambient CO$_2$. The $\delta^{13}C$ of amino acids are higher than carbohydrates under ambient conditions. Transfer of $^{13}C$-enriched carbohydrates from ambient to elevated will increase the $\delta^{13}C$ of carbohydrates relative to that of amino acids synthesized primarily in situ. The C/N values of protein and of carbohydrates are independent of CO$_2$ level.
Figure 2

Stem map of trees at the Swiss FACE site surrounding the canopy crane (center). 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). The 14 CO$_2$-labeled trees have a blue bar under the designation. Conifers include *Abies alba* (dark green, Ta), *Larix decidua* (orange, Lä), *Picea abies* (red, Fi), and *Pinus sylvestris* (olive green, Fö). Ring diameter is 60 m. Size corresponds to crown size.
Sporocarp $\delta^{13}C$ (‰) ± standard error at the four zones (ambient, outer transition (OT), inner transition (IT), and elevated) for collections during 2001, 2003, 2004, and 2005.
Figure 4. Sporocarp C/N ± standard error for each day of year of collections during 2001 (■), 2003 (○), 2004 (▲), and 2005. On two dates in 2004, only a single sporocarp was collected, as indicated on figure.

Figure 4

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• OnlineResource5d13Cregressionstepwise.xlsx
• OnlineResource6logCNvsd13Ccoefficientfromreg.xlsx
• OnlineResource7logCregressionstepwise.xlsx
• OnlineResource8TempRangevsSolarradiationyear.xlsx
• OnlineResourcesListOR2FigOR3OR4Table.docx