available at www.sciencedirect.com

ScienceDirect

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

Fungal carbon sources in a pine forest: evidence from a ^{13}C-labeled global change experiment

Erik A. HOBBIE[a,*], *Kirsten S. HOFMOCKEL*[b], *Linda T.A. VAN DIEPEN*[c], *Erik A. LILLESKOV*[d], *Andrew P. OUIMETTE*[a], *Adrien C. FINZI*[e]

[a]*Earth Systems Research Center, University of New Hampshire, Durham, NH 03824, USA*
[b]*Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011, USA*
[c]*Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA*
[d]*US Forest Service, Northern Research Station, Houghton, MI 49931, USA*
[e]*Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA*

ARTICLE INFO

Article history:
Received 11 March 2013
Revision received 22 August 2013
Accepted 4 November 2013
Available online 16 December 2013
Corresponding editor:
Jacqueline Mohan

Keywords:
Carbon isotope
Carbon source
Duke Forest
Ectomycorrhizal fungi
Exploration type
Global change
Organic nitrogen
Saprotrophic fungi

ABSTRACT

We used natural abundance ^{13}C:^{12}C ($\delta^{13}C$) and 8 yr of labeling with ^{13}C-depleted CO_2 in a *Pinus taeda* Free Air CO_2 Enrichment (FACE) experiment to investigate carbon sources of saprotrophic fungi, ectomycorrhizal fungi, and fungi of uncertain life history. Sporocarp $\delta^{13}C$ identified *Sistotrema confluens* as ectomycorrhizal, as suspected previously from morphological characteristics. Saprotrophic $\delta^{13}C$ declined by 2 ‰–13 ‰ between ambient to elevated CO_2 treatments and corresponded to different carbon sources, including surface litter (*Rhodocollybia*, *Mycena*), pine cones (*Baeospora*), wood (*Gymnopilus*, *Pholiota*), and soil (*Ramariopsis*). Ectomycorrhizal fungi, foliage, and surficial litter declined 12 ‰ in $\delta^{13}C$ between ambient and elevated treatments, confirming that these fungi depend on recent photosynthate. The $\delta^{13}C$ of ectomycorrhizal genera correlated between treatments with a slope (4.3 ± 1.2) greater than the expected value of one. This suggested that *Inocybe* and *Cortinarius* incorporated some pre-treatment, soil-derived carbon (presumably from amino acids) whereas *Lactarius* and *Russula* only incorporated current-year photosynthate or recent, litter-derived carbon. Combining natural abundance and tracer ^{13}C measurements proved a powerful technique to examine carbon sources of different fungi.

Introduction

In most forests, fungi are key players in belowground carbon cycling. Fungi can be functionally classified based on their carbon sources, with ectomycorrhizal fungi depending primarily on recent photosynthate from plant hosts. In contrast, saprotrophic fungi access a variety of carbon sources, including leaf litter, woody debris, conifer cones, and even soil. These different resource acquisition strategies lead to a spatial separation of activity, with saprotrophic fungi dominant in the litter layer (Oi horizon), whereas ectomycorrhizal fungi are often active in organic horizons below the litter layer (Lindahl et al., 2007). Although new genetic techniques have dramatically expanded our understanding of fungal

* *Corresponding author*. Tel.: +1 603 862 3581; fax: +1 603 862 0188.
E-mail address: erik.hobbie@unh.edu (E.A. Hobbie).
1754-5048/$ – see front matter
http://dx.doi.org/10.1016/j.funeco.2013.11.001

community composition, information on many functional characteristics of fungi classified as either saprotrophic or ectomycorrhizal remains scarce.

Measuring isotope ratios in fungal sporocarps and comparing those isotope ratios against potential carbon and nitrogen sources for those fungi provides one tool to assess fungal functioning. The ^{13}C:^{12}C ratio in fungi (expressed as $\delta^{13}C$ values) generally increases in the order: ectomycorrhizal fungi < litter decay fungi < wood decay fungi (Kohzu et al., 1999; Hobbie et al., 2001). This reflects several potential mechanisms, including links between microbial efficiency and ^{13}C fractionation during respiration (Hobbie, 2005), the addition of ^{13}C-depleted CO_2 derived from fossil fuels to the atmosphere, and ^{13}C differences among substrates derived from isotopic fractionation during tissue formation. The CO_2 added over the last 150 yr has shifted the $\delta^{13}C$ of fixed carbon by about 1.5 ‰, termed the Suess effect (McCarroll and Loader, 2004). One additional factor is that the different compounds within the sporocarp are synthesized within the mycelia and then delivered to the sporocarp. Since the synthesized compounds are not isotopically uniform, the sporocarp can differ isotopically from the bulk mycelia (Hobbie and Agerer, 2010). Because sporocarps consist largely of protein (high $\delta^{13}C$), chitin (low $\delta^{13}C$), and carbohydrates (low $\delta^{13}C$) (Kalac, 2009), the relative amounts of protein, chitin, and carbohydrates can influence sporocarp $\delta^{13}C$ (Hobbie et al., 2012). As proteins have higher %N than other components, measuring sporocarp %N allows this potentially confounding factor to be assessed.

Experiments where additional CO_2 is added that differs isotopically from atmospheric CO_2 could improve assessments of fungal carbon sources by providing increased isotopic separation among the current-year photosynthate assimilated by ectomycorrhizal fungi, the relatively recent carbon assimilated by litter decay fungi and fungi colonizing pine cones, and the older carbon assimilated by saprotrophic fungi colonizing wood or soil. One such experiment began in 1996 at the Duke Free Air CO_2 Enrichment (FACE) facility. In this study, CO_2 levels within 30-m diameter rings were increased to 200 ppm above ambient. Because the added CO_2 was quite low in ^{13}C, the resulting CO_2 within FACE plots had a $\delta^{13}C$ value of ~ −20 ‰, which is 12 ‰ depleted in ^{13}C relative to normal values of −8 ‰ (Lichter et al., 2005). It could therefore assess the degree to which saprotrophic fungi access relatively new carbon (low $\delta^{13}C$) *versus* older carbon derived from litter or wood deposited before (high $\delta^{13}C$) the start of the Duke FACE experiment.

Nitrogen isotopes ($^{15}N/^{14}N$), stable carbon isotopes ($^{13}C/^{12}C$), and radiocarbon ($^{14}C/^{12}C$) have all been used at natural abundance to determine ectomycorrhizal or saprotrophic status in fungi (Hobbie et al., 2002; Mayor et al., 2009). The ^{13}C labeling introduced in global change experiments could be a new tool to trace recent photosynthate into fungal symbionts and to determine whether species are ectomycorrhizal (symbionts) or saprotrophic (free-living). Of taxa at the Duke FACE site, at least one is of uncertain life history, *Sistotrema*. Di Marino et al. (2008) suggested that *Sistotrema* was ectomycorrhizal based on genetic and morphological evidence.

The extent to which ectomycorrhizal fungi can assimilate organic nitrogen from soil or litter has been debated for many years (Chalot and Brun, 1998). Many ectomycorrhizal fungi possess proteolytic capabilities and specific transporters for amino acids and oligopeptides (Benjdia et al., 2006; Hobbie and Hobbie, 2008). The ^{13}C-depleted CO_2 introduced in the FACE plots starting in 1996 means that older soil carbon will be higher in $\delta^{13}C$ than recent photosynthate and litter carbon. This isotopic labeling can therefore potentially be used to examine whether ectomycorrhizal fungi assimilate some soil-derived or litter-derived carbon in the form of organic nitrogen (Fig 1).

Here, we combined information on $\delta^{13}C$ and %N patterns in sporocarps and in other ecosystem pools from both ambient and elevated CO_2 plots at the Duke FACE study to test the following hypotheses:

(1) Carbon isotopes in sporocarps from FACE experiments can be used to infer ectomycorrhizal or saprotrophic status for fungi of uncertain life history. We will specifically test the status of *Sistotrema confluens*.
(2) Comparing carbon isotopes in saprotrophic fungi suspected to utilize different carbon sources will improve the ability to determine those carbon sources. For example, litter decay fungi should shift more in $\delta^{13}C$ than wood decay fungi between ambient plots and FACE plots because much of the colonized wood will predate the start of the experiment in 1996.
(3) Taxa of ectomycorrhizal fungi that incorporate soil-derived carbon (presumably assimilated as organic nitrogen) into fungal protein will shift less in $\delta^{13}C$ between

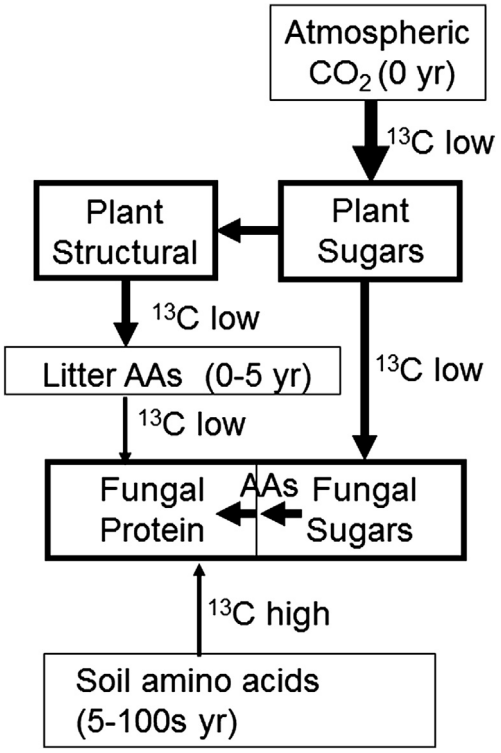

Fig 1 – Schematic of carbon fluxes with the plant-fungal-soil system, showing how uptake of organic nitrogen as amino acids can influence the $\delta^{13}C$ of ectomycorrhizal fungi when soil amino acids and fungal sugars differ in $\delta^{13}C$. AAs = amino acids.

ambient and elevated CO_2 treatments than foliage, ectomycorrhizal taxa incorporating litter-derived carbon as a component of organic nitrogen, or ectomycorrhizal taxa not incorporating organic nitrogen.

Methods

FACE experiment

The FACE experiment in the Duke Forest (35°58′41″ N, 79°05′39″ W, 163 m elevation, Orange County, North Carolina, USA) was composed of six 30 m diameter plots. Three experimental plots were fumigated with CO_2 to maintain the atmospheric CO_2 concentration at 200 ppm above ambient (i.e., 565 ppm in 1997). Three control plots were fumigated with ambient air only. The experiment began Aug. 27 1996 and was continuous (24 hr d^{-1}; 365 d yr^{-1}) until Nov. 1 2010. Additional details on FACE operation are in Hendrey et al. (1999).

The Duke Forest originated from 3 yr old loblolly pine (*Pinus taeda*) seedlings that were planted in 1983 with a 2.4 × 2.4 m spacing. In 1996, the 16 yr old pine trees were approximately 14 m tall and accounted for 98 % of the basal area of the stand. Since planting, a deciduous understory layer has recruited from nearby hardwood forests and stump sprouts. The most abundant understory tree species is sweetgum (*Liquidambar styraciflua*), with some red maple (*Acer rubrum*), redbud (*Cercis canadensis*), and dogwood (*Cornus florida*). Topographic relief is less than 1° throughout the 32 ha site. Soils are classified as being from the Enon Series (fine, mixed, active, thermic Ultic Hapludalfs). Enon soils are typically fine sandy loams derived from mafic bedrock, slightly acidic (pH = 5.75 in 0.1 M $CaCl_2$), and have well-developed soil horizons with mixed clay mineralogy. Additional site details can be found in Schlesinger and Lichter (2001) and Finzi et al. (2001).

The CO_2 used for fumigation is derived from natural gas and consequently is strongly depleted in ^{13}C relative to atmospheric CO_2, with a $\delta^{13}C$ of −43.0 ± 0.6 ‰. Because the atmospheric CO_2 concentration within the rings was raised by 200 ppm with this gas, the $\delta^{13}C$ of CO_2 within elevated CO_2 rings was −20 ‰, 12 ‰ lower than the ambient atmosphere (Lichter et al., 2005).

Sample collection and analysis

Sporocarps were collected in 2004 on Oct. 14, Oct. 25, and Oct. 27 from ambient plots (69), elevated CO_2 plots (77), and from outside the experimental plots (164). A total of 172 ectomycorrhizal collections and 141 saprotrophic collections were made. Fungal sporocarps were either air-dried or flash-frozen in the field and freeze-dried. Taxa were identified from macroscopic and microscopic morphological characteristics.

Needles were collected from the top 25 %, middle 50 % and bottom 25 % of the canopy in Sep. 2002–2005 using an upright lift (Hofmockel et al., 2011). Needles were not separated by cohort. The Oi, Oea (combined Oe and Oa), 0–15 cm and 15–30 cm horizons were sampled in Sep. 2003–2005. For the Oea, 0–15 cm and 15–30 cm horizons, soil organic matter was defined as the residual organic matter after a chloroform fumigation and a potassium sulfate extraction to assess microbial biomass (Hofmockel et al., 2011).

Samples from 2002 to 2005 were analyzed according to Hofmockel et al. (2011), with elemental (%C and %N) and isotopic analyses at the University of California Davis Stable Isotope Facility. Sporocarps collected in 2004 from both ambient FACE rings and outside the FACE plots were also measured for $\delta^{13}C$ at the University of California Davis. Values are reported in the standard notation ($\delta^{13}C$; ‰) relative to Pee Dee Belemnite, where $\delta^{13}C = [(R_{sample}/R_{standard}) - 1]$ and R is the molar ratio of $^{13}C/^{12}C$ (Lajtha and Michener, 1994). Precision of duplicate analyses was 0.1 ‰. Concurrently measured %N values on samples are also reported here.

Statistical analysis

The statistical program JMP 9.0 (SAS, Cary, North Carolina, USA) was used for analysis. Average values for needle and soil pools for each ring were used to calculate means for these pools by treatment ($n = 3$). For fungi, analyses were done on individual samples, without averaging by ring. The standard error is given unless specified as standard deviation. Statistical models in JMP explaining $\delta^{13}C$ values were run separately for ambient and elevated CO_2 treatments for saprotrophic and ectomycorrhizal fungi. Potential explanatory variables included genus and nitrogen concentration. Interactions between variables were not tested because of insufficient power and empty cells.

Results

2003–2005 sampling of ecosystem pools

Isotopic values for ecosystem pools at natural abundance are presented in Table 1. In ambient plots, $\delta^{13}C$ increased from surface litter to deeper soil horizons, with an 8 ‰ increase in $\delta^{13}C$ from surface litter to bulk soil at 15–30 cm depth. Foliage was 1.2–1.4 ‰ higher in $\delta^{13}C$ at the top of the canopy than at the bottom of the canopy, with mid-canopy foliage intermediate.

In elevated CO_2 plots, foliage and organic horizons were depleted in ^{13}C relative to mineral horizons, with the Oi horizon 17 ‰ depleted relative to the 15–30 cm depth. As a result, the ^{13}C depletion in elevated CO_2 plots relative to ambient plots ranged from 3 ‰ for soil organic matter at 15–30 cm depth to ~12 ‰ in the Oi horizon and foliage. These shifts across ecosystem pools and between treatments in $\delta^{13}C$ (Fig 2) provided useful data with which to evaluate isotopic patterns in fungi.

Sporocarp nitrogen concentration

Ectomycorrhizal sporocarps were lower in nitrogen concentration (3.92 ± 0.07, $n = 172$) than saprotrophic sporocarps (4.97 ± 0.15 %, $n = 141$; Wilcoxon signed-rank test, $p < 0.001$). In a model of %N as affected by genus and CO_2 treatment, CO_2 did not influence %N ($p = 0.595$). Ectomycorrhizal sporocarps varied in nitrogen concentration from 2.84 ± 0.12 % for *Sistotrema* to 5.01 ± 0.21 % for *Cortinarius*, whereas saprotrophic

Table 1 – $\delta^{13}C$ in ambient and elevated CO_2 plots at the Duke Forest FACE site for a) foliage, b) ecosystem pools, in parts per mil (‰). The ^{13}C enrichment in ambient plots relative to elevated plots is also shown as $\delta^{13}C_{amb\text{-}elev}$. Soil and litter were sampled in 2003, foliage was sampled 2003–2005. The $\delta^{13}C$ of foliage is shown separately for the top, middle, and bottom of canopy, averaged across 2003–2005. SOM = soil organic matter after a chloroform fumigation and a potassium sulfate extraction

a. Foliage 2002–2005, 1st- and 2nd-yr needles

Location	$\delta^{13}C$ (ambient)	$\delta^{13}C$ (elevated)	$\delta^{13}C$ (amb-elev)
Top	−29.2 ± 0.2	−41.2 ± 0.2	12.0 ± 0.4
Middle	−29.6 ± 0.3	−42.0 ± 0.3	12.4 ± 0.6
Bottom	−30.3 ± 0.2	−42.6 ± 0.5	12.3 ± 0.7

b. Bulk soil pools, Mar. 2003

Horizon	$\delta^{13}C$ (ambient)	$\delta^{13}C$ (elevated)	$\delta^{13}C$ (amb-elev)
Oi	−29.9 ± 0.4	−41.7 ± 0.8	11.8 ± 1.2
Oea	−29.6 ± 0.4	−38.6 ± 0.7	9.0 ± 1.1
0–15 cm	−26.5 ± 0.3	−30.4 ± 0.8	3.9 ± 1.1
15–30 cm	−21.8 ± 0.9	−24.7 ± 0.9	2.9 ± 1.8

sporocarps varied from 3.21 ± 0.09 % for *Gymnopilus* to 6.51 ± 0.12 % for *Mycena* (Fig 3). *Gymnopilus*, *Pholiota*, and *Baeospora* were more than 1 % lower in nitrogen concentration than other saprotrophic fungi, resulting in a bimodal distribution for %N of saprotrophic fungi.

$\delta^{13}C$ in fungi from ambient plots and outside treatment plots

Ectomycorrhizal taxa [−27.3 ± 0.8 ‰ (SD), $n = 127$] were lower than saprotrophic taxa [−24.6 ± 1.2 ‰ (SD), $n = 106$] in $\delta^{13}C$ (t-test, difference = 2.8 ‰, df = 232, $p < 0.001$), averaging between −26.9 ± 0.2 ‰ (*Inocybe*) and −27.5 ± 0.1 ‰ (*Russula*). Saprotrophic taxa varied from −22.6 ± 0.6 ‰ (*Baeospora*) to −26.3 ± 0.9 ‰ (*Ramariopsis*). *Sistotrema* (−27.3 ± 0.1 ‰) was similar isotopically to ectomycorrhizal fungi but not to saprotrophic fungi (Figs 4 and 5), although it overlapped

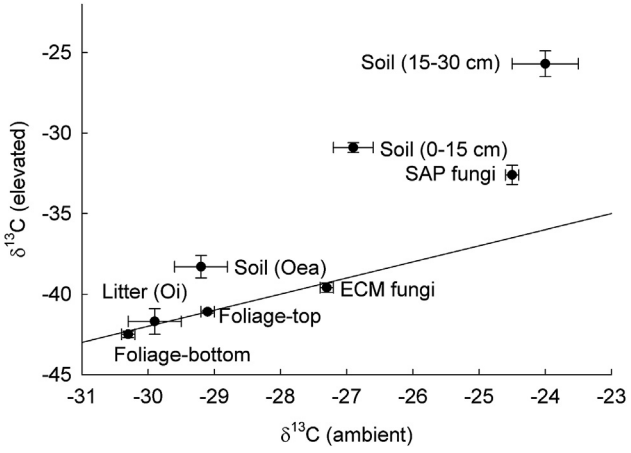

Fig 2 – Carbon isotopes of ecosystem pools (±SE) in elevated CO_2 and ambient treatments. The line fits the equation $\delta^{13}C_{elevated} = \delta^{13}C_{ambient} - 12$ ‰. ECM = ectomycorrhizal, SAP = saprotrophic.

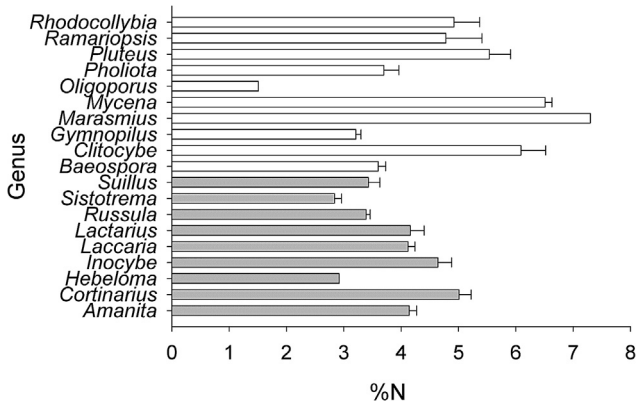

Fig 3 – %N (±SE) in saprotrophic (white bars) and ectomycorrhizal (gray bars) fungi by genus, including sporocarps from all treatments. Single values for *Oligoporus*, *Marasmius*, and *Hebeloma*.

somewhat with *Ramariopsis*. In a regression model, genus and %N significantly affected natural abundance $\delta^{13}C$ in saprotrophic fungi but not in ectomycorrhizal fungi (Table 2), with *Sistotrema* included with the ectomycorrhizal fungi. The significant influence of %N of saprotrophic fungi on $\delta^{13}C$ was driven by two taxa, *Mycena* and *Gymnopilus*, which together comprised about 65 % of the total saprotrophic samples. In those two taxa %N significantly correlated with $\delta^{13}C$ (*Mycena*: coefficient = 0.50 ± 0.14, adjusted $r^2 = 0.242$, $n = 41$, $p < 0.001$; *Gymnopilus*: coefficient = 0.47 ± 0.22, adjusted $r^2 = 0.105$, $n = 31$, $p = 0.042$).

$\delta^{13}C$ in fungi from elevated CO_2 plots and compared against ambient plots

The ^{13}C-depleted CO_2 used to increase CO_2 levels in the FACE plots strongly affected $\delta^{13}C$ values for both ectomycorrhizal and saprotrophic fungi, with ectomycorrhizal fungi averaging −39.6 ± 0.3 ‰ and saprotrophic fungi averaging −32.6 ± 0.6 ‰ (t-test, difference 7.0 ‰, df = 76, $p < 0.001$). The $\delta^{13}C$ of ectomycorrhizal taxa declined 11–14 ‰ from ambient to elevated CO_2 plots (Fig 4), whereas in saprotrophic taxa the decline varied from 4 ‰ for *Gymnopilus* to 13 ‰ for *Baeospora* (Fig 5), with an overall average decline of 8.1 ‰. The putative ectomycorrhizal species *S. confluens* was 12.1 ‰ lower in $\delta^{13}C$ in elevated CO_2 than in ambient plots.

Declines in $\delta^{13}C$ from ambient to elevated CO_2 plots varied by ectomycorrhizal or saprotrophic status. The $\delta^{13}C$ of ectomycorrhizal taxa declined 11–14 ‰ from ambient to elevated CO_2 plots (Fig 4). The putative ectomycorrhizal species *S. confluens* was 12.1 ‰ lower in $\delta^{13}C$ in elevated CO_2 than in ambient plots, and fell in the center of the cluster of ectomycorrhizal fungi, well separated from the saprotrophic fungi. When averaged by genus, the $\delta^{13}C$ values of ectomycorrhizal taxa in elevated CO_2 *versus* ambient plots were correlated according to the equation $\delta^{13}C_{elevated} = 4.29 \pm 1.64 \times \delta^{13}C_{ambient} + 77.4 \pm 44.7$ ‰ (adjusted $r^2 = 0.494$, $p = 0.047$, $n = 7$, Fig 4). In saprotrophic taxa the decline varied from 4 ‰ for *Gymnopilus*, 8 ‰ for *Ramariopsis*, and up to 13 ‰ for *Baeospora* (Fig 5), with an overall average decline of 8.1 ‰.

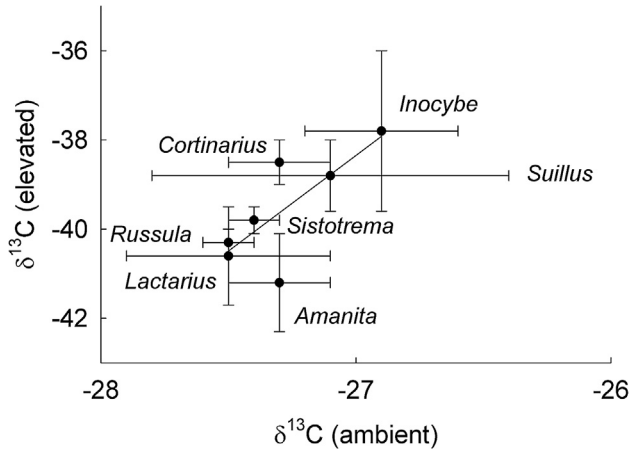

Fig 4 – Carbon isotopes of different genera of ectomycorrhizal fungi (±SE) in elevated CO_2 and ambient treatments. The regression line for mean values by genus between ambient and elevated treatments has a slope of 4.3 ± 1.2.

Discussion

Using carbon isotopes to infer ectomycorrhizal status

Carbon isotopes at natural abundance levels have been used to distinguish between ectomycorrhizal and saprotrophic fungi (Hobbie et al., 2001), with Mayor et al. (2009) reporting that ectomycorrhizal fungi averaged −25.5 ± 0.7 ‰ (SD) and saprotrophic fungi averaged −22.9 ± 0.6 ‰ (SD) across 10 different studies. Our values were somewhat lower in $\delta^{13}C$ (−27.4 ± 0.8 ‰ (SD) for ectomycorrhizal fungi and −24.7 ± 1.2 ‰ (SD) for saprotrophic fungi), but with similar differences between the groups. We made inferences about life history by comparing natural abundance $\delta^{13}C$ values with those in elevated CO_2 plots for ectomycorrhizal fungi (−39.6 ‰) and saprotrophic fungi (−32.6 ‰).

The added ^{13}C-depleted CO_2 in FACE sites provided increased resolution between the new photosynthate assimilated by ectomycorrhizal fungi and the older carbon generally assimilated by saprotrophic fungi, with ectomycorrhizal fungi averaging 2.8 ‰ lower in ^{13}C under ambient CO_2 and 7.0 ‰ lower under elevated CO_2 than saprotrophic fungi. In agreement with our first hypothesis, we conclude that having an additional axis of isotopic measurements using the elevated CO_2 plots to discriminate between ectomycorrhizal and saprotrophic fungi improved our ability to separate these two fungal strategies. The pools heavily dependent on recent photosynthate included foliage, the Oi horizon (surface litter), and ectomycorrhizal fungi (Fig 2). These pools correlate closely with the line $\delta^{13}C_{elevated} = \delta^{13}C_{ambient} - 12$ ‰, whereas other soil pools and saprotrophic fungi deviated by variable degrees from this line (Fig 2), with greater deviations reflecting less contribution of recent photosynthate to that pool and greater contribution of old (pre-1996), ^{13}C-enriched carbon.

The putatively ectomycorrhizal *S. confluens* had similar $\delta^{13}C$ to ectomycorrhizal fungi under both ambient and elevated CO_2. The only saprotrophic taxon which was somewhat similar under ambient CO_2, *Ramariopsis*, was 1 ‰ higher in $\delta^{13}C$ under ambient CO_2 and 5 ‰ higher in $\delta^{13}C$ under elevated CO_2. From the combination of morphological and genetic information previously reported for *Sistotrema* sp. (Di Marino et al., 2008) and the isotopic patterns given here (Fig 4), we conclude that *S. confluens* is ectomycorrhizal. Such isotopic measurements can strengthen inferences about ectomycorrhizal status derived from morphological and genetic studies (Wilson et al., 2007).

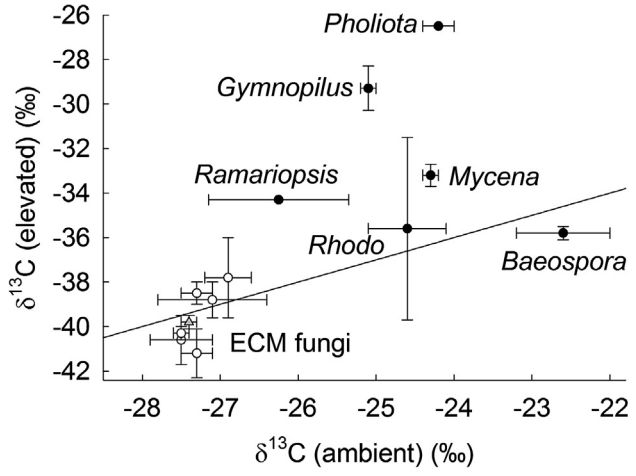

Fig 5 – Carbon isotopes of different genera of saprotrophic fungi (±SE) in elevated CO_2 (*y*-axis) and ambient (*x*-axis) treatments. Values for the putative saprobe *Ramariopis kunzei* are also included. Values for taxa of ectomycorrhizal fungi from Fig 4 are shown with clear circles and the value for *Sistotrema* is shown with a gray triangle. The line fits the equation $\delta^{13}C_{elevated} = \delta^{13}C_{ambient} - 12$ ‰. *Rhodo* = *Rhodocollybia*.

Inferring carbon sources in saprotrophic taxa

Saprotrophic taxa typically colonize a variety of substrata. Here, saprotrophic taxa colonized four main substrata, with *Baeospora myosura* commonly colonizing conifer cones (Desjardin, 1987), *Gymnopilus* and *Pholiota* commonly colonizing wood, and *Mycena* and *Rhodocollybia* commonly colonizing litter (Table 4). Whereas the other saprotrophic taxa fruit on wood or fresh litter, *Ramariopsis kunzei* typically fruits on ground, often buried in the duff (Arora, 1986), implying that it obtains carbon from soil rather than fresh litter.

Natural abundance (ambient) $\delta^{13}C$ values were only clearly separated between two saprotrophic taxa, the soil-inhabiting *Ramariopsis* and the cone-colonizing *Baeospora*, while $\delta^{13}C$ values among wood decay and litter decay fungi were similar, with the wood decomposers *Pholiota* and *Gymnopilus* and the litter decomposers *Mycena* and *Rhodocollybia* not differing in $\delta^{13}C$. However, adding a second axis of $\delta^{13}C$ values for elevated CO_2 treatments now clearly separated wood decomposers and litter decomposers (Fig 5), with wood decomposers higher than litter decomposers in $\delta^{13}C$ under elevated CO_2 because the wood decomposers assimilated more old, pre-1996 carbon that predated the addition of ^{13}C-depleted carbon to the system.

Table 2 – Regression model of $\delta^{13}C$ of saprotrophic and ectomycorrhizal fungi as a function of genus and sporocarp %N under ambient CO_2 in 2004. Data on sporocarps from outside FACE rings and from ambient treatments were used in the model. Adjusted r^2 was 0.409 ($n = 106$, $p < 0.001$) for saprotrophic fungi and −0.009 ($n = 127$, $p = 0.547$) for ectomycorrhizal fungi for the entire model

Saprotrophic			Ectomycorrhizal		
Parameter	Value ± SE	p	Parameter	Value ± SE	p
Intercept	−26.7 ± 0.5	<0.001	Intercept	−27.6 ± 0.4	<0.001
%N	0.46 ± 0.11	**<0.001**	%N	0.11 ± 0.10	0.280
Genus	–	**<0.001**	Genus	–	0.799
Genus-specific effects					
Baeospora	2.5 ± 0.4	**<0.001**	*Amanita*	−0.1 ± 0.2	0.475
Clitocybe	−1.0 ± 0.5	0.067	*Cortinarius*	−0.2 ± 0.3	0.442
Gymnopilus	0.2 ± 0.3	0.584	*Hebeloma*	0.5 ± 0.7	0.454
Marasmius	−2.1 ± 0.9	**0.022**	*Inocybe*	0.2 ± 0.2	0.442
Mycena	−0.6 ± 0.3	0.052	*Laccaria*	0.0 ± 0.3	0.915
Oligoporus	2.9 ± 0.9	**0.002**	*Lactarius*	−0.3 ± 0.3	0.248
Pholiota	0.8 ± 0.4	**0.039**	*Russula*	−0.2 ± 0.2	0.184
Pluteus	−1.1 ± 0.5	**0.018**	*Sistotrema*	0.0 ± 0.3	0.972
Ramariopsis	−1.5 ± 0.5	**0.001**			
Rhodocollybia	−0.2 ± 0.3	0.572			

Significant results are in bold.

Substratum age is clearly an important determinant of isotopic signature in fumigation experiments. Taxa assimilating carbon that predates the FACE experiment will have higher $\delta^{13}C$ signatures under elevated CO_2 than those assimilating FACE-derived carbon. Recent leaf litter and the pine cones colonized by *Baeospora* were all produced since CO_2 fumigation began, so unlike older wood, will have low $\delta^{13}C$ values reflecting the ^{13}C-depleted CO_2 added to FACE rings. This likely contributes to the lower $\delta^{13}C$ values of the litter decomposer *Rhodocollybia* and the pine cone decomposer *Baeospora* compared to wood decomposers *Pholiota* and *Gymnopilus* in the elevated CO_2 plots, as shown in the regression model of Table 3.

Shifts in $\delta^{13}C$ between ambient and elevated CO_2 plots can be used to estimate the proportion of FACE-derived carbon in fungal biomass (Fig 5). We assume that the ^{13}C shift of 12.2 ‰ between ambient and elevated CO_2 foliage represents 100 % FACE-derived photosynthate, with the shifts for *Mycena* (9.0 ‰) and *Rhodocollybia* (11.0 ‰) estimated at 73 % and 90 %, respectively, of their carbon. Thus, *Mycena* and *Rhodocollybia* are primarily colonizing litter produced since the FACE treatments began. This is consistent with the <5 yr turnover time of the organic horizon at Duke Forest (Lichter et al., 2008). The remaining two saprotrophic taxa, *Gymnopilus* and *Pholiota*, primarily assimilated wood-derived carbon that was fixed in the pre-FACE period, as their $\delta^{13}C$ only shifted 4.2 ‰ (34 %) and 2.3 ‰ (19 %) respectively between ambient and elevated FACE plots.

Causes of $\delta^{13}C$ patterns in saprotrophic taxa

Differences in $\delta^{13}C$ in saprotrophic taxa may arise from several causes, including substrate $\delta^{13}C$, sporocarp composition of different compound classes (e.g., carbohydrates, lipids, chitin, and protein), and isotopic fractionation (or enrichment) during metabolism. Substrate $\delta^{13}C$ is in turn primarily a function of three factors: the isotopic fractionations involved in the formation of specific tissues, the canopy stratum which

Table 3 – Regression model of $\delta^{13}C$ of saprotrophic and ectomycorrhizal fungi as a function of genus and sporocarp %N under elevated CO_2 in 2004. Adjusted r^2 was 0.474 ($n = 32$, $p < 0.001$) for saprotrophic fungi and 0.134 ($n = 45$, $p = 0.099$) for ectomycorrhizal fungi

Saprotrophic			Ectomycorrhizal		
Parameter	Value ± SE	p	Parameter	Value ± SE	p
Intercept	−28.0 ± 2.8	<0.001	Intercept	−40.5 ± 1.7	<0.001
%N	−0.95 ± 0.57	0.108	%N	0.24 ± 0.41	0.573
Genus	–	**0.005**	Genus	–	0.135
Baeospora	−4.2 ± 1.4	**0.007**	*Amanita*	−1.6 ± 0.8	0.056
Gymnopilus	1.6 ± 1.4	0.263	*Cortinarius*	0.9 ± 0.7	0.229
Mycena	1.0 ± 1.4	0.465	*Inocybe*	1.7 ± 1.0	0.104
Pholiota	4.9 ± 2.2	**0.032**	*Laccaria*	−0.2 ± 1.7	0.930
Ramariopsis	0.1 ± 2.4	0.953	*Lactarius*	−1.2 ± 0.8	0.144
Rhodocollybia	−3.4 ± 1.6	**0.037**	*Russula*	−0.5 ± 0.6	0.393
			Sistotrema	0.1 ± 1.4	0.938

Significant results are in bold.

Table 4 – Exploration strategies for different saprotrophic genera, including their substrata and presence of rhizomorphs (*sensu lato*)

Genus	Substrate	Growth form
Baeospora[a]	Conifer cones	Pseudorhizas on sporocarps (6 cm) (Redhead, 1974)
Clitocybe	Litter	Fairy rings, cords (Dowson et al., 1989)
Gymnopilus	Wood	Rhizomorphs (Clémençon, 2002)
Marasmius	Twigs/litter	Sometimes rhizomorphic (Townsend, 1954)
Mycena	Litter	Sometimes fine rhizomorphs (Lodge, 1988)
Oligoporus	Wood	Rhizomorphs in diffuse mycelium (Huckfeldt and Schmidt, 2006)
Pholiota	Wood	No data
Pluteus	Wood	Rhizomorphs (Takehashi and Kasuya, 2007)
Ramariopsis	Soil	Non-rhizomorphic (Garcia-Sandoval et al., 2005)
Rhodocollybia	Litter	No data

Species from these genera found at Duke Forest include the following: *Baeospora myosura*, *cf Rhodocollybia lentinoides*, *Clitocybe candicans*, *Clitocybe ramigena*, *Gymnopilus seperans*, *Marasmius fulvoferrugineus*, *Mycena cf pura*, *Mycena clavicularis*, *Mycena lilacifolia*, *Mycena pura*, *Mycena* sp., *Oligoporus caesius*, *Pholiota highlandensis*, *Pluteus cervinus*, *Ramariopsis* sp., *Rhodocollybia distorta*, *Rhodocollybia lentinoides*, *Rhodocollybia maculata*.

[a] The common *B. myosura* is restricted to conifer cones. The genus can extend to woody debris (Hutchison et al., 2012).

supplied those tissues with sugars, and whether tissues were formed before or after fumigation with ^{13}C-depleted CO_2. Source differences are likely because wood is higher in δ^{13}C than leaves or litter (Kohzu et al., 1999).

As mentioned above, canopy position could affect substrate isotopic composition. *Baeospora* fruits on pine cones and had higher δ^{13}C than any other taxa under ambient conditions. Presumably cones were enriched in ^{13}C relative to needles because pine cones at Duke Forest grow towards the top of the canopy (LaDeau and Clark, 2006), where foliar δ^{13}C values are higher by 1.2–1.4 ‰ relative to the bottom of the canopy (Table 1). Schuur et al. (2002) also found that cones were enriched in ^{13}C by 2 ‰ relative to needles. This presumably contributes to the high δ^{13}C of *Baeospora* relative to other saprotrophic fungi.

As shown in Table 2, %N is a primary factor influencing the δ^{13}C of saprotrophic fungi, with a slope of 0.46 ± 0.11 ‰ for %N. This arises because sporocarp nitrogen is predominantly protein (Hobbie et al., 2012) and therefore fungal protein content is roughly proportional to nitrogen concentration. Because protein in individual sporocarps is enriched in ^{13}C relative to carbohydrates by 4.2 ± 0.5 ‰ (Hobbie et al., 2012), linking source carbon to saprotrophic fungi using δ^{13}C will be more accurate if variations in protein content can be accounted for. We stress that the link between protein (also % N) and sporocarp δ^{13}C arises because of isotopic fractionation among compound classes within the fungal mycelia prior to delivery of those compounds to the developing primordia, as suggested previously to explain δ^{15}N patterns among different fungal taxa (Hobbie and Agerer, 2010). Thus, the sporocarp will differ in δ^{13}C depending on the proportions of ^{13}C-enriched amino acids and ^{13}C-depleted carbohydrates or chitin precursors delivered during sporocarp formation. The sporocarp will also then differ in δ^{13}C from the bulk mycelia if sporocarps are higher in ^{13}C-enriched protein than bulk mycelia, as seems likely. For example, bulk mycelia were 1.1 ‰ depleted in ^{13}C relative to ectomycorrhizal sporocarps in one field study (Boström et al., 2007), and also averaged higher in C:N (16.3, indicating less protein) than sporocarp caps (9.2), sporocarp stipes (13.6) (Hobbie et al., 2012), or bulk sporocarps (in our study, C:N of ectomycorrhizal sporocarps averaged 11.5).

Enrichment in ^{13}C during metabolism can also influence δ^{13}C. Sporocarp chitin was enriched in ^{13}C relative to source carbohydrates by 2 ‰ in saprotrophic fungi (Gleixner et al., 1993). ^{13}C enrichment during metabolism relative to source sugars ranged from −2.8 ‰ to +1.4 ‰ in hyphae of seven taxa of cultured fungi [mean of −0.3 ± 1.3 ‰ (SD)] (Hobbie et al., 2004). In another study, the ^{13}C enrichment of sporocarps and hyphae relative to spruce needles was estimated at 3.7 ‰ and 3.0 ‰, respectively (Wallander et al., 2004).

Incorporation of soil-derived organic nitrogen by ectomycorrhizal fungi

The positive correlation between δ^{13}C of ectomycorrhizal fungi in ambient and elevated CO_2 plots (Fig 4) can best be explained by uptake and incorporation of organic nitrogen into fungal protein. This scenario assumes that fungal δ^{13}C is primarily determined by the low δ^{13}C of fungal carbohydrates (including chitin), the high δ^{13}C of fungal protein (Hobbie et al., 2012), and the relative importance of protein synthesized from litter-derived organic nitrogen, deeper soil organic nitrogen, and *de novo* synthesis from plant carbohydrates. These scenarios are presented schematically in Fig 6.

In one scenario, *de novo* synthesis of amino acids using carbon from plant sugars and exogenous nitrogen is the only source for fungal protein. Fungi with more protein are higher in δ^{13}C under both ambient and elevated conditions than fungi with less protein because of the ^{13}C enrichment of protein relative to carbohydrates and chitin during *de novo* synthesis. This scenario leads to a slope of 1.0 between the δ^{13}C of fungi in ambient and elevated treatments. In a second scenario, a slope of 1.0 also results if incorporation of organic nitrogen into fungal protein is only from the Oi (litter) horizon, as carbon in organic nitrogen from the litter horizon would be of recent origin and accordingly in isotopic equilibrium with photosynthetically derived carbon. The Oi horizon shifted as much as foliage in δ^{13}C between ambient and elevated treatments, about 12 ‰.

In a third scenario, incorporation of organic nitrogen into fungal protein is possible from horizons below the Oi. Under ambient treatments, this flux would supply organic nitrogen enriched in ^{13}C by $\varepsilon_{\text{ambient}}$ (Fig 6) relative to that supplied from the Oi horizon.

$$\varepsilon_{ambient} = \delta^{13}C_{Oea\ protein} - \delta^{13}C_{Oi\ protein\ (ambient)}(ambient\ CO_2) \quad (1)$$

In contrast, under elevated CO_2 treatments, the organic nitrogen would be enriched by $\varepsilon_{\text{ambient}}$ plus $\varepsilon_{\text{elev-amb}}$, or $\varepsilon_{\text{elev}}$, relative to that supplied from the Oi horizon.

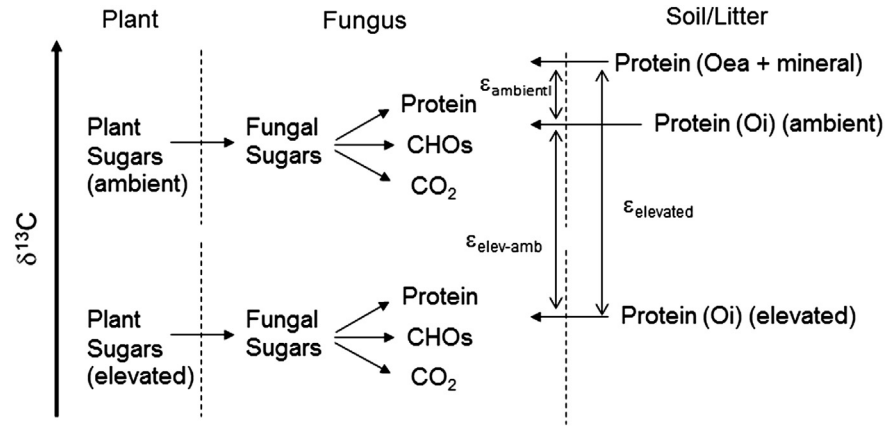

Fig 6 – Causes of carbon isotope patterns in ectomycorrhizal (ECM) fungi under ambient and elevated CO_2. Most fungal protein is synthesized *de novo* from fungal sugars plus ammonium, but a portion of fungal protein is synthesized from soil-derived organic nitrogen, such as amino acids and protein. Soil-derived organic nitrogen (protein) will differ in $\delta^{13}C$ depending on whether it is from ambient or elevated litter (Oi) or from deeper in the soil profile (Oea + mineral). $\varepsilon_{ambient}$ and $\varepsilon_{elevated}$ are the ^{13}C enrichment of protein derived from the Oea and mineral horizons relative to protein from the Oi horizon, in ambient and elevated CO_2 treatments, respectively. CHOs = carbohydrates; $\varepsilon_{elev\text{-}amb} = \varepsilon_{elevated} - \varepsilon_{ambient}$.

$$\varepsilon_{elev} = \delta^{13}C_{Oea\ protein} - \delta^{13}C_{Oi\ protein\ (elevated)}\ (elevated\ CO_2) \quad (2)$$

Subtracting Equation (1) from Equation (2) results in Equation (3).

$$\varepsilon_{elev} - \varepsilon_{ambient} = \varepsilon_{elev-amb} = \delta^{13}C_{Oi\ protein\ (ambient)} - \delta^{13}C_{Oi\ protein\ (elevated)} \quad (3)$$

The quantity $\varepsilon_{elev\text{-}amb}$ is estimated at 12.2 ‰, based on the ^{13}C enrichment of ambient *versus* elevated foliage. By rearranging Equation (3), the value of ε_{elev} can also be expressed as:

$$\varepsilon_{elev} = \varepsilon_{elev-amb} + \varepsilon_{ambient} \quad (4)$$

The slope of the regression of fungal $\delta^{13}C$ between ambient and elevated CO_2 treatments is given by the quantity $\varepsilon_{elev}/\varepsilon_{ambient}$, or $1 + \varepsilon_{elev\text{-}amb}/\varepsilon_{ambient}$, and is estimated at 4.29 ± 1.64 in Fig 4.

$$\varepsilon_{elev}/\varepsilon_{ambient} = (\varepsilon_{elev-amb} + \varepsilon_{ambient})/\varepsilon_{ambient} \quad (5)$$

$$\varepsilon_{elev}/\varepsilon_{ambient} = \varepsilon_{elev-amb}/\varepsilon_{ambient} + 1 \quad (6)$$

We can solve for $\varepsilon_{ambient}$ in Equation (6) as:

$$\varepsilon_{ambient} = \varepsilon_{elev-amb}/(\varepsilon_{elev}/\varepsilon_{ambient} - 1) \quad (7)$$

Substituting the appropriate values here leads to a value of 12.2 ‰/(4.29 – 1), or 3.7 ‰. Under elevated CO_2, the ^{13}C enrichment of the soil-derived organic nitrogen relative to that of litter-derived organic nitrogen or *de novo* synthesized protein is then 3.7 ‰ plus 12.2 ‰, or 15.9 ‰. If, based on our empirical data, we assume that ectomycorrhizal genera vary up to 2 ‰ in ^{13}C under elevated CO_2 (Fig 4) because of differences among taxa in their use of litter-derived versus soil-derived protein or in their use of organic versus inorganic nitrogen, then carbon associated with exogenous organic nitrogen derived from litter or soil contributes up to one-eighth of the fungal carbon. Based on the fungal %N in sporocarps and the probable contribution of protein to that %N (Hobbie et al., 2012), fungal carbon is roughly one-quarter protein. Up to one-half of the carbon in fungal protein would then be derived from soil organic matter. Ectomycorrhizal taxa assimilating nitrogen from below the Oi horizon, such as *Cortinarius* and *Inocybe*, should assimilate more ^{13}C-enriched soil-derived organic nitrogen than taxa assimilating nitrogen primarily from the Oi horizon, such as *Lactarius* and *Russula* (Hobbie et al., 2013a). These four taxa were 11.2 ‰, 10.9 ‰, 13.1 ‰ and 12.8 ‰ higher in ^{13}C for ambient treatments than elevated CO_2 treatments, respectively. The ^{15}N labeling patterns measured concurrently in these four taxa (Hobbie et al., 2013a) clearly indicate that *Lactarius* and *Russula* assimilate nitrogen from the Oi horizon, whereas *Cortinarius* and *Inocybe* assimilate nitrogen from deeper horizons. In addition, radiocarbon measurements on three of these taxa in a separate study also support the more recent origin of soil-derived organic nitrogen in *Lactarius* and *Russula* compared to *Cortinarius* (Hobbie et al., 2013b). Although additional data would be desirable, these results suggest that ^{13}C-enriched soil organic matter (presumably protein) may contribute to fungal protein and ultimately shift the $\delta^{13}C$ of ectomycorrhizal sporocarps. However, if we assume that $\varepsilon_{ambient}$ = 3.7 ‰ and one-eighth of sporocarp carbon is derived from organic nitrogen, then the shift would be less than 0.5 ‰ (3.7 ‰/8) at natural abundance, and unlikely to be detected given natural variations in the $\delta^{13}C$ of photosynthesis and in fungal protein content. Thus, careful measurements in global change experiments may provide new insights into the extent of organic nitrogen incorporation into ectomycorrhizal fungi.

Conclusions

Measuring carbon isotopes in fungi and in ecosystem pools at ambient and at elevated CO_2 levels can effectively test sources of carbon for different taxa. The relative age of carbon

assimilated by different saprotrophic fungi could be estimated by isotopic shifts between ambient and elevated CO_2 treatments. The high %N and $\delta^{13}C$ of fungal protein led to fungal % N correlating with $\delta^{13}C$ in saprotrophic fungi. The altered $\delta^{13}C$ signature of photosynthesis in this FACE experiment proved useful in distinguishing between ectomycorrhizal and saprotrophic strategies in fungi. Our evidence supports the hypothesis that *S. confluens* is ectomycorrhizal. Some ectomycorrhizal taxa appear to assimilate organic nitrogen from horizons below the litter layer.

Acknowledgments

This work was supported by a grant from the U.S. National Science Foundation, Division of Environmental Biology and by a Bullard Fellowship to E.H. from Harvard University. Core funding for the Duke FACE site was provided by the Office of Science (BER), U.S. Department of Energy, grant No. DE-FG02-95ER62083. Sporocarp collection and identification by Matt Henn and Rytas Vilgalys are gratefully acknowledged, as are comments on earlier versions by Sharon Billings and Rich Phillips.

REFERENCES

Arora, D., 1986. Mushrooms Demystified. Ten Speed Press, Berkeley, California.

Benjdia, M., Rikirsch, E., Muller, T., Morel, M., Corratgé, C., Zimmermann, S., Chalot, M., Frommer, W.B., Wipf, D., 2006. Peptide uptake in the ectomycorrhizal fungus *Hebeloma cylindrosporum*: characterization of two di- and tripeptide transporters (HcPTR2A and B). *New Phytologist* 170, 401–410.

Boström, B., Comstedt, D., Ekblad, A., 2007. Isotope fractionation and ^{13}C enrichment in soil profiles during the decomposition of soil organic matter. *Oecologia* 153, 89–98.

Chalot, M., Brun, A., 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiology Reviews* 22, 21–44.

Clémençon, H., 2002. Mycelial morphology, rhizomorph anatomy and primordium formation of *Gymnopilus penetrans* (Cortinariaceae, Basidiomycetes). *Feddes Repertorium* 113, 63–79.

Desjardin, D.E., 1987. *The Agaricales (Gilled Fungi) of California. 7. Tricholomataceae I. Marasmioid fungi: the genera Baeospora, Crinipellis, Marasmiellus, Marasmius, Micromphale, and Strobilurus*. Mad River Press, Eureka.

Di Marino, E., Scattolin, L., Bodensteiner, P., Agerer, R., 2008. *Sistotrema* is a genus with ectomycorrhizal species – confirmation of what sequence studies already suggested. *Mycological Progress* 7, 169–176.

Dowson, C.G., Rayner, A.D.M., Boddy, L., 1989. Spatial dynamics and interactions of the woodland fairy ring fungus. *Clitocybe nebularis*. *New Phytologist* 111, 699–705.

Finzi, A.C., Allen, A.S., DeLucia, E.H., Ellsworth, D.S., Schlesinger, W.H., 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. *Ecology* 82, 470–484.

Garcia-Sandoval, R., Cifuentes, J., De Luna, E., Estrada-Torres, A., Villegas, M., 2005. A phylogeny of *Ramariopsis* and allied taxa. *Mycotaxon* 94, 265–292.

Gleixner, G., Danier, H.J., Werner, R.A., Schmidt, H.L., 1993. Correlations between the ^{13}C content of primary and secondary plant-products in different cell compartments and that in decomposing basidiomycetes. *Plant Physiology* 102, 1287–1290.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F., Nagy, J., 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* 5, 293–309.

Hobbie, E.A., 2005. Using isotopic tracers to follow carbon and nitrogen cycling of fungi. In: Dighton, J., Oudemans, P., White, J. (Eds.), *The Fungal Community: Its Organization and Role in the Ecosystem*. Marcel Dekker, New York, pp. 361–381.

Hobbie, E.A., Agerer, R., 2010. Nitrogen isotopes in ectomycorrhizal mushrooms correspond to belowground exploration types. *Plant and Soil* 327, 71–83.

Hobbie, E.A., Hobbie, J.E., 2008. Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. *Ecosystems* 11, 815–830.

Hobbie, E.A., Hofmockel, K., van Diepen, L., Lilleskov, E.A., Ouimette, A.P., Finzi, A., 2013a. Fungal functioning in a pine forest: evidence from a ^{15}N-labeled global change experiment. *New Phytologist*. http://dx.doi.org/10.1111/nph.12578.

Hobbie, E.A., Ouimette, A.P., Schuur, E.A., Kierstead, D., Trappe, J.M., Bendiksen, K., Ohenoja, E., 2013b. Radiocarbon evidence for the mining of organic nitrogen from soil by mycorrhizal fungi. *Radiocarbon*. http://dx.doi.org/10.1007/s10533-012-9779-z.

Hobbie, E.A., Sánchez, F.S., Rygiewicz, P.T., 2004. Carbon use, nitrogen use, and isotopic fractionation of ectomycorrhizal and saprotrophic fungi in natural abundance and ^{13}C-labelled cultures. *Mycological Research* 108, 725–736.

Hobbie, E.A., Sánchez, F.S., Rygiewicz, P.T., 2012. Controls of isotopic patterns in saprotrophic and ectomycorrhizal fungi. *Soil Biology & Biochemistry* 48, 60–68.

Hobbie, E.A., Weber, N.S., Trappe, J.M., 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist* 150, 601–610.

Hobbie, E.A., Weber, N.S., Trappe, J.M., van Klinken, G.J., 2002. Using radiocarbon to determine the mycorrhizal status of fungi. *New Phytologist* 156, 129–136.

Hofmockel, K.S., Gallet-Budynek, A.G., McCarthy, H.R., Currie, W.S., Jackson, R.B., Finzi, A., 2011. Sources of increased N uptake in forest trees growing under elevated CO_2: results of a large-scale ^{15}N study. *Global Change Biology* 17, 3338–3350.

Huckfeldt, T., Schmidt, O., 2006. Identification key for European strand-forming house-rot fungi. *Mycologist* 20, 42–56.

Hutchison, L., Kropp, B., Hausner, G., 2012. *Baeospora occidentalis*, a new snowbank agaric from western North America. *Mycoscience* 53, 139–143.

Kalac, P., 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: a review. *Food Chemistry* 113, 9–16.

Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K., Wada, E., 1999. Natural ^{13}C and ^{15}N abundance of field-collected fungi and their ecological implications. *New Phytologist* 144, 323–330.

LaDeau, S.L., Clark, J.S., 2006. Pollen production by *Pinus taeda* growing in elevated atmospheric CO_2. *Functional Ecology* 20, 541–547.

Lajtha, K., Michener, R. (Eds.), 1994. *Stable Isotopes in Ecology*. Blackwell Scientific Publications, Oxford.

Lichter, J., Barron, S.H., Bevacqua, C.E., Finzi, A.C., Irving, K.F., Stemmler, E.A., Schlesinger, W.H., 2005. Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment. *Ecology* 86, 1835–1847.

Lichter, J., Billings, S.A., Ziegler, S.E., Gaindh, D., Ryals, R., Finzi, A.C., Jackson, R.B., Stemmler, E.A., Schlesinger, W.H.,

2008. Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Global Change Biology* 14, 1–13.
Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* 173, 611–620.
Lodge, D.J., 1988. Three new *Mycena* species (Basidiomycota: Tricholomataceae) from Puerto Rico. *Transactions of the British Mycological Society* 91, 109–116.
Mayor, J.R., Schuur, E.A.G., Henkel, T.W., 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecology Letters* 12, 171–183.
McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* 23, 771–801.
Redhead, S., 1974. Cone-dwelling Fleshy Basidiomycetes from British Columbia. Masters Thesis. University of British Columbia.
Schlesinger, W.H., Lichter, J., 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. *Nature* 411, 466–469.
Schuur, E.A., Trumbore, S.E., Mack, M.C., Harden, J.W., 2002. Isotopic composition of carbon dioxide from a boreal forest fire: inferring carbon loss from measurements and modeling. *Global Biogeochemical Cycles* 16. http://dx.doi.org/10.1029/2001GB001840.
Takehashi, S., Kasuya, T., 2007. First record of *Pluteus chrysophaeus* and reexamination of *Pluteus leoninus* from Japan. *Mycoscience* 48, 321–325.
Townsend, B.B., 1954. Morphology and development of fungal rhizomorphs. *Transactions of the British Mycological Society* 37, 222–233.
Wallander, H., Göransson, H., Rosengren, U., 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* 139, 89–97.
Wilson, A.W., Hobbie, E.A., Hibbett, D.S., 2007. The ectomycorrhizal status of *Calostoma cinnabarinum* determined using isotopic, molecular, and morphological methods. *Botany* 85, 385–393.