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Short communication

Uptake of an amino acid by ectomycorrhizal fungi in a boreal forest

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ABSTRACT

We assessed the degree to which ectomycorrhizal fungi exploit organic nitrogen *in situ*. In an Alaskan boreal forest, we identified pairs of sporocarps from five taxa of ectomycorrhizal fungi. We added ^{13}C-labeled alanine to the soil surrounding one sporocarp within each pair; the second served as an unlabeled control. Peak rates of ^{13}C-respiration from alanine were higher in the labeled sporocarp plots than the controls, indicating that the ^{13}C-alanine was detectably respired from the soil. "Reference" plots adjacent to the sporocarps served as an indication of background ^{13}C-respiration rates released by the soil community as a whole. Ectomycorrhizal sporocarps displayed higher ^{13}C-respiration rates than their reference plots. Thus, the sporocarps and associated mycorrhizal mycelium appeared to contribute significantly to the release of alanine-derived $^{13}CO_2$, confirming the hypothesis that ectomycorrhizal fungi may access soil amino acid pools under natural conditions.

Ectomycorrhizal fungi have long been recognized as an important mechanism for uptake of N by plants. These fungi colonize root tips of certain plant species and extend hyphae and rhizomorphs into the soil for several meters or more (Agerer, 1992, 2001). Nitrogen and other nutrients are absorbed by the hyphal network, and a portion is then translocated to the plant in exchange for C (Smith and Read, 1997). Altogether, host plants could acquire as much as 61–86% of their total N budget from these symbionts (Hobbie and Hobbie, 2006).

Numerous culture- and greenhouse-based studies have demonstrated that ectomycorrhizal fungi can use as a sole N source a wide array of organic N compounds including amino acids, proteins, chitin, and DNA (e.g., Melin and Mikola, 1948; Abuzinadah and Read, 1986; Leake and Read, 1990). In addition, amino acid transporters have been identified in three ectomycorrhizal species (Abuzinadah and Read, 1988; Chalot and Brun, 1998; Wipf et al., 2002). These studies have established the physiological capacity of ectomycorrhizal fungi to take up organic N. Less is known about the extent to which ectomycorrhizal fungi acquire organic N under field conditions (Jones et al., 2005; Högberg and Read, 2006). In our study, we measured the respiration of ^{13}C by ectomycorrhizal sporocarps *in situ* after injections of ^{13}C-labeled alanine into the surrounding soil. We hypothesized that ectomycorrhizal sporocarps would release detectable levels of ^{13}C derived from alanine, owing to the uptake and use of this amino acid by the fungus.

To test our hypothesis, we first selected fresh sporocarps of five ectomycorrhizal taxa (Table 1) in an Alaskan black spruce forest near Delta Junction, AK (site characteristics detailed in Treseder et al., 2004, 2007) in August 2006. Each taxon was represented by two sporocarps: one to be exposed to ^{13}C-alanine, and one to remain unlabeled as a control. We established two 10 cm × 10 cm plots for each sporocarp. One plot was centered on the sporocarp. The other was located 15–40 cm away, contained no sporocarps of any species, and served as a "reference plot". Both plots received simultaneous injections of ^{13}C-alanine or deionized water, depending on the treatment. Respired CO_2 from each plot was sampled for ^{13}C analysis in order to calculate the isoflux (mg ^{13}C m^{-2} h^{-1}) of alanine-C released, following Czimczik et al. (2005). Essentially, isoflux is the product of the respiration rate and the ^{13}C:^{12}C ratio of CO_2. The isoflux from the sporocarp plot was a mixture of CO_2 respired from the sporocarp and from other microbes and plant roots present in the plot. The reference plots provided an estimation of the isoflux produced by these other sources.

^{13}C from labeled alanine was respired by the soil community within hours of injection, given that peak isofluxes from labeled plots were higher than those for non-labeled control plots (Table 1; paired *t*-test, $P = 0.009$). Specifically, peak isofluxes were higher in labeled plots than in control plots for each of the five pairs. In addition, sporocarp plots displayed greater isofluxes than did their

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Table 1
Isofluxes and biomass of taxa

Taxon[a]	Sporocarp isoflux[b] (mg ^{13}C m^{-2} h^{-1})			Reference isoflux[b] (mg ^{13}C m^{-2} h^{-1})			Biomass[c] (g dry weight)
	Average	Peak	Control	Average	Peak	Control	
Cortinarius sp. 1	2.1	2.5 (7)	1.7	2.0	2.8 (7)	1.6	1.857
Cortinarius sp. 2	2.4	5.1 (1)	2.4	3.0	5.4 (7)	3.6	1.076
Hebeloma sp.	2.0	3.5 (3)	2.0	1.7	2.3 (1)	1.9	1.109
Hydnellum peckii	3.5	4.7 (1)	3.8	2.2	3.4 (1)	1.5	2.475
Tricholomataceae	4.1	5.3 (3)	2.5	2.6	3.8 (3)	2.7	0.876

[a] Sporocarps identified to taxa based on morphology and on sequences of the ITS region of DNA. Ectomycorrhizal (versus saprotrophic) status was confirmed via measurements of $\delta^{15}N$ of sporocarp tissue.
[b] Average or peak values across sampling times (hours to peak in parentheses) for ^{13}C-labeled samples; time-averaged values are presented for controls. Isofluxes of controls did not differ significantly between sporocarp and reference plots ($P = 0.634$).
[c] For ^{13}C-labeled sporocarps collected after the final sampling. Neither average nor peak sporocarp isofluxes were significantly correlated with sporocarp biomass in labeled samples (Pearson correlations, $P > 0.05$).

reference plots (Fig. 1; $P = 0.026$ across time points), which indicates that the ectomycorrhizal sporocarps released measurable amounts of alanine-derived ^{13}C. Altogether, these results supported our hypothesis and suggest that ectomycorrhizal fungi acquired and used alanine-C from soil.

Organic N uptake by ectomycorrhizal fungi has rarely been directly measured *in situ*. Dual isotope labeling can be used in the field to track uptake of specific organic N compounds into plants; the ratio of labeled C–N in plant material indicates the degree to which intact compounds are absorbed and transferred to the host plants (e.g., Näsholm et al., 1998; Weigelt et al., 2003). By using this technique, Näsholm et al. (1998) demonstrated that ecto-, ericoid, and arbuscular mycorrhizal host plants could each effectively acquire intact glycine from boreal forest soils. However, with this approach it is difficult to determine the degree to which ectomycorrhizal fungi contribute to uptake, since the possibility that plant roots may absorb amino acids directly cannot be excluded (Kielland, 1994). By comparing $^{13}CO_2$ release of paired sporocarp and reference plots, we could focus on uptake by the fungi (Czimczik et al., 2005).

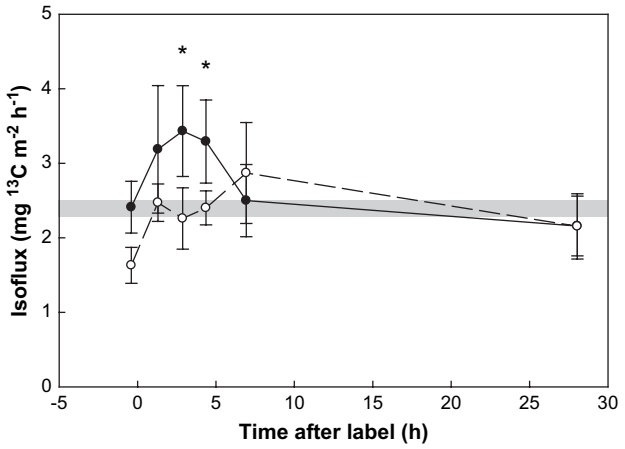

Fig. 1. Isofluxes of ectomycorrhizal samples in response to injections of ^{13}C-labeled alanine in nearby soil. Closed symbols are sporocarp plots; open symbols, reference plots. Symbols denote means ± 1 SE of 3–5 samples per time point. Grey bar represents mean ± 1 SE of control samples, including sporocarp and reference plots. Asterisks denote specific times at which differences between sporocarp and reference plots were significant ($P < 0.016$). Isofluxes varied significantly across sampling times ($P = 0.005$).

At the onset of labeling, we injected 28 ml of 0.74 mM universally labeled 99% ^{13}C-alanine (CLM-2184-0.25, Cambridge Isotope Laboratories, Inc., Andover, MA) 4 cm deep into each of four corners of each labeled plot. For each pair of labeled plots, we first collected a pre-label CO_2 sample and then immediately injected the labeled alanine. We returned to each sporocarp and reference plot approximately 1, 3, 4, 7, and 28 h after labeling. For each taxon, the sporocarp and reference plots were sampled simultaneously. For controls, we did not collect a pre-label timepoint. We injected the water and then returned 1 h later to collect CO_2 samples. We obtained a second CO_2 sample at intervals that ranged between 1 and 5 days after injection, depending on the sporocarp.

To test our hypothesis, we conducted a repeated measures analysis of variance (ANOVA) in a complete randomized block design with isoflux as the dependent variable and sporocarp status (sporocarp versus reference plot) as the independent variable. Data were blocked by sporocarp genus. We compared time-averaged isofluxes from control plots in a similar design, but without the repeated measure.

Ectomycorrhizal fungi appeared responsible for a significant portion of the $^{13}CO_2$ respired from the sporocarp-centered plots. However, the mycelium of individuals producing the sporocarps could have extended into the reference plots as well, since hyphal networks of basidiomycete individuals can span areas 2–12 m in diameter (Kretzer et al., 2004). In this sense, our approach was conservative because we focused on differences between the sporocarp and reference plots. On the other hand, microbial communities can vary over scales of centimeters (Horner-Devine et al., 2004), which could have accentuated our observed differences. We tried to minimize this effect by placing labeled and reference plots as close together as possible.

We found no evidence of ^{13}C enrichment in sporocarps from labeled plots compared to controls (labeled: $-25.08 \pm 0.40‰$; control: $-25.58 \pm 0.22‰$; $P = 0.444$). It is possible that alanine-derived C was incorporated into fast-cycling pools within the sporocarps. In this case, the ^{13}C label could have been respired quickly. In fact, isofluxes from sporocarp plots had returned to near pre-label values by the end of the sampling period. Sporocarps were collected following this measurement. In addition, the sporocarps were fully expanded and may not have been constructing new biomass. As a result, there may not have been an opportunity for ^{13}C to be incorporated into the sporocarp tissues. Sporocarps respire CO_2 strongly even after they are harvested (e.g., Hammond and Nichols, 1975; Cliffe-Byrnes and O' Beirne, 2007), so the sporocarps in our study need not have been in a growth phase to respire the alanine-C.

Amino acids are labile compounds, with half-lives in the soil of 0.3–30 h (e.g., Jones, 1999; Berthrong and Finzi, 2006). It remains to be seen whether ectomycorrhizal fungi are able to take up more recalcitrant N compounds under field conditions. An examination of this issue is an important next step in understanding the influence of ectomycorrhizal fungi on C and N dynamics, and it could be conducted by measuring isofluxes from sporocarps in a similar manner as used here.

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