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

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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 (Hobbie and Hobbie, 2006). Together, host plants could acquire as much as 61–86% of their total N budget from these symbionts (Smith and Read, 1997). Altogether, the sporocarps and associated mycorrhizal mycelium appeared to contribute significantly to the release of alanine-derived 13CO2, confirming the hypothesis that ectomycorrhizal fungi may access soil amino acid pools under natural conditions.

To test our hypothesis, we first selected fresh sporocarps of five ectomycorrhizal taxa (Table 1) in an Alaskan boreal forest, we identified pairs of sporocarps from five taxa of ectomycorrhizal fungi. We added 13C-labeled alanine to the soil surrounding one sporocarp within each pair; the second served as an unlabeled control. Peak rates of 13C-respiration from alanine were higher in the labeled sporocarp plots than the controls, indicating that the 13C-alanine was detectably respired from the soil. “Reference” plots adjacent to the sporocarps served as an indication of background 13C-respiration rates released by the soil community as a whole. Ectomycorrhizal sporocarps displayed higher 13C-respiration rates than their reference plots. Thus, the sporocarps and associated mycorrhizal mycelium appeared to contribute significantly to the release of alanine-derived 13CO2, confirming the hypothesis that ectomycorrhizal fungi may access soil amino acid pools under natural conditions.
We collected a pre-label CO2 sample and then immediately injected the labeled alanine into each of four corners of each labeled plot. For each pair of labeled plots, we first injected alanine (CLM-2184-0.25, Cambridge Isotope Laboratories, Inc., Andover, MA) 4 cm deep from control plots in a similar design, but without the repeated measure. Microbial communities from sporocarps in our study need not have been in a growth phase to 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 (Kieland, 1994). By comparing 13CO2 release of paired sporocarp and reference plots, we could focus on uptake by the fungi (Czimczik et al., 2005).

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 each effectively uptake short-term isotope pulses of 13CO2 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-Divine 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 13C enrichment in sporocarps from labeled plots compared to controls (labeled: −25.08 ± 0.40‰; control: −25.58 ± 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 13C 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 13C to be incorporated into the sporocarp tissues. Sporocarps respire CO2 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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Table 1

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sporocarp isoflux (mg 13C m⁻² h⁻¹)</th>
<th>Reference isoflux (mg 13C m⁻² h⁻¹)</th>
<th>Biomass (g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Peak</td>
<td>Control</td>
</tr>
<tr>
<td>Cortinarius sp. 1</td>
<td>2.1</td>
<td>2.5 (7)</td>
<td>1.7</td>
</tr>
<tr>
<td>Cortinarius sp. 2</td>
<td>2.4</td>
<td>5.1 (1)</td>
<td>2.4</td>
</tr>
<tr>
<td>Hebeloma sp.</td>
<td>2.0</td>
<td>3.5 (3)</td>
<td>2.0</td>
</tr>
<tr>
<td>Hydnellum peckii</td>
<td>3.5</td>
<td>4.7 (1)</td>
<td>3.8</td>
</tr>
<tr>
<td>Tricholomataceae</td>
<td>4.1</td>
<td>5.3 (3)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*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 15N of sporocarp tissue.

*b* Average or peak values across sampling times (hours to peak in parentheses) for 13C-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 13C-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).

<table>
<thead>
<tr>
<th>isoFlux (mg 13C m⁻² h⁻¹)</th>
<th>Time after label (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
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<td>3</td>
<td>20</td>
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<td>4</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
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</table>

Fig. 1. Isofluxes of ectomycorrhizal samples in response to injections of 13C-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% 13C-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 CO2 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 CO2 samples. We obtained a second CO2 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.
References


