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

Permalink
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Publication Date
2008-04-01

Peer reviewed
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). 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 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.
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).

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-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 \(^{13}\)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.

Acknowledgements

We thank X. Xu and M. O. Garcia for technical assistance, Fort Greeley for access to field sites, D. S. LeBauer for comments on an earlier draft, and the UCI Keck Accelerator Mass Spectrometry Facility and UC Davis Stable Isotope Facility for sample analyses. This work was supported by grants from NSF.

**Table 1** Isofluxes and biomass of taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sporocarp isoflux (mg (^{13})C m(^{-2}) h(^{-1}))</th>
<th>Reference isoflux (mg (^{13})C m(^{-2}) h(^{-1}))</th>
<th>Biomass(^{d}) (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>3.3</td>
<td>4.7 (1)</td>
<td>3.8</td>
</tr>
<tr>
<td>Tricholomatidae</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 \(^{15}\)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, \( P > 0.05\)).

\(^{d}\) 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, \( P > 0.05\)).
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