Radiocarbon – a low-impact tool to study nutrient transport by soil fungi under field conditions

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Summary

• Here, we present a new in-situ method to study the uptake of amino acids by soil fungi.
• We injected 14C-labeled glycine into a marshland soil and measured the rate and the 14C signature of CO2 respired from sporocarps of Pholiota terrestris over 53.5 h and 2 m. We also determined the incorporation of glycine-C into sporocarp tissue. The 14C signature of the CO2 and tissue was quantified by accelerator mass spectrometry.
• After the label application, the rate of CO2 flux and its 14C signature from chambers with sporocarps were significantly higher than from chambers without sporocarps, and then declined with time. Postlabel, the 14C signature of the sporocarp tissue increased by 35‰.
• We show that this approach can be used to study below-ground food webs on an hourly time-scale while minimizing the perturbation of competitive relationships among soil microorganisms and between plants and soil microorganisms.
• Additionally we show that care must be taken to avoid confounding effects of sporocarp senescence on rates and radiocarbon signatures of respired CO2.

Key words: plant–microbial interactions, radiocarbon, saprotrophic fungi, soil respiration, sporocarp.

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Introduction

In most terrestrial ecosystems, nitrogen (N) availability regulates species composition and biomass production (Vitousek & Howarth, 1991). Most research on microbe- and plant-available N has focused on the inorganic N compounds ammonium and nitrate.

In soils, c. 40% of N is present as organic N in proteinaceous material (amino acids, peptides, and proteins), 35% as heterocyclic compounds (e.g. nucleic acids), 5–6% as amino sugars (D-glucosamine and D-galactosamine), and 19% as NH3 (Schulten & Schnitzer, 1998). Only a small fraction of the organic N is dissolved in the soil solution as free amino acids. Microbes can absorb free amino acids efficiently from the soil solution, as can plants in many families and with every possible mycorrhizal status (Lipson & Nasholm, 2001). Free amino acids are produced during the hydrolysis of proteins and peptides by extracellular enzymes of microorganisms. Amino acids can also be released from bacterial cells or plant roots by excretion or during lysis of cells, for example during drying-rewetting or freeze-thaw events of soils. To better understand the dynamics of organic N in soils, we used soil respiration chambers to follow the transformation and transport of a radiocarbon (14C) labeled amino acid in one species of decomposer fungus under field conditions.

We injected 14C-labeled glycine into a marshland soil and tracked the release of that isotope in CO2 respired by sporocarps of Pholiota terrestris over a timeline spanning 53.5 h and over a spatial scale spanning 2 m. We determined the extent to which carbon (C) derived from the glycine was incorporated into sporocarp tissue. By focusing on sporocarps, which can be identified to species, we could tie function (i.e. glycine transformation)
Materials and Methods

We studied the uptake of \(^{14}\)C labeled glycine by fungi in the San Joaquin Freshwater Marsh Reserve, located on the campus of the University of California Irvine (CA, USA). The vegetation consisted of black willow (\textit{Salix gooddingii} Ball.) and alder (\textit{Alnus} spec.) with grass in the understorey. The sporocarps were a 2-m diameter cluster of the wood decay fungus \textit{Pholiota terrestris} overholts (\textit{Strophariaceae}).

We applied 0.175 mg glycine-C at 2 cm soil depth in one location amidst the cluster of sporocarps (Fig. 1). The \(^{14}\)C glycine (Moravek Biochemicals, catalog # MC 163) was labeled at all C positions and diluted to \(10,000–100,000 \text{ ml}^{-1}\) (136.5 µg glycine ml\(^{-1}\) in deionized water). At this level, the radioactivity is below levels that are regulated – the total added radioactivity is only 0.3–3 nCi. The glycine label was chosen because it is one of the more abundant amino acids in soil (Senwo & Tabatabai, 1998).

To track the \(^{14}\)C label from glycine to \(^{14}\)C resired from the sporocarps, we measured the rate of \(^{14}\)C eflux from the soil surface by placing a PVC chamber lid of 26-cm inner diameter with a tubing inlet and outlet on the chamber top on the soil surface. A sand-filled bag was placed around the perimeter of the chamber lid at its junction with the soil surface, to prevent diffusion of outside air into the chamber. We circulated the air in the chamber through a LI-800 infrared gas analyzer (Libor, Lincoln, NB, USA) for 3–8 min at 0.5 l min\(^{-1}\) and recorded the rate of change of \(^{14}\)C in the chamber headspace with a LI-1400 data logger. The rate of respired \(^{14}\)CO\(_2\) was calculated as described in Borken \textit{et al.} (2002). After measuring the \(^{14}\)CO\(_2\) flux, we closed the tubing on the chamber lid and let \(^{14}\)CO\(_2\) accumulate within the chamber for 10 min. We sampled the \(^{14}\)CO\(_2\) in the chamber by pumping the air from the chamber through Drierite to an activated molecular sieve 13X trap that quantitatively removes \(^{14}\)CO\(_2\), then back to the chamber for 10 min at 0.5 l min\(^{-1}\). We also collected ambient air c. 100 m upwind from the labeled site by flushing air through Drierite to a molecular sieve trap for 30 min at 0.5 l min\(^{-1}\). Gas samples were taken 19 h before the label application, and 1 h, 5 h, 28.5, and 53.5 h after the label application. After each sampling we removed the chamber lids and sandbags from the transect. The weather was stable during the experiment, with a mean daily temperature of 17°C and no precipitation.

To isolate the \(^{14}\)CO\(_2\) from the gas samples, we connected the molecular sieve traps to a vacuum line. The traps were heated to 650°C for 45 min. We froze out water in a glass trap cooled by ethanol/dry ice and \(^{14}\)CO\(_2\) in a glass trap cooled by liquid N.

Then, c. 1 mg C was frozen and sealed into an evacuated 9 mm Pyrex tube. This tube contained 25 mg Zn powder and 17 mg TiH\(_2\) powder at the bottom. Approximately 2.5 cm above the bottom, we suspended a 6 mm Pyrex tube containing 5 mg Cobalt powder. Both tubes were prebaked for 3 h at 500°C and 4 h at 550°C. The \(^{14}\)CO\(_2\) was converted to graphite, Ti, H\(_2\)O, and ZnO with Co acting as a catalyst for 3 h at 500°C and 4 h at 550°C.
Near the location of the soil chambers we sampled sporocarps and roots (2 mm diameter or less, not separated between live and dead) 20 h before the label and 93.5 h after the label. The roots were washed three times in deionized water, and then soaked in 1 mmol KCl for 20 min to remove any label absorbed onto the root surface. After a final rinse in deionized water, they were dried for 3 d at 60°C. The sporocarps were also dried for 3 d at 60°C. The solid samples were combusted to CO$_2$ in evacuated, prebaked, 6 mm quartz tubes with 0.5 mg CuO powder for 2 h at 900°C. The resulting CO$_2$ was purified and catalytically reduced to graphite as described for the gas samples above. The radiocarbon content of the graphite was measured using accelerator mass spectrometry (NEC 0.5 mV 1.5SDH-2 AMS system) at the Keck-CCAMS facility of UCI. The Instrumental error for $\Delta^{14}$C was 2.5 ± 0.5‰.

We calculated the contribution of the sporocarps in each chamber to the soil respiration for each sampling time by using a mass balance approach:

$$[\text{CO}_2]_{\text{SC}} = f [\text{CO}_2]_{\text{NSC}} + (1-f)[\text{CO}_2]_S \quad \text{Eqn 1}$$

$$1-f = 1 - \frac{[\text{CO}_2]_{\text{NSC}}}{[\text{CO}_2]_{\text{SC}} + [\text{CO}_2]_{\text{NSC}}} \quad \text{Eqn 2}$$

$$\Delta^{14}C_S = \frac{\Delta^{14}C_{\text{SC}} - f \Delta^{14}C_{\text{NSC}}}{1-f} \quad \text{Eqn 3}$$

where $[\text{CO}_2]$ is the CO$_2$ concentration and $\Delta^{14}$C is the radiocarbon signature of the CO$_2$ respired in chambers with sporocarps ($\text{SC}$), chambers without sporocarps ($\text{NSC}$), and from the sporocarps in a chamber ($S$). Variable ($f$) is the fraction of CO$_2$ respired from the soil surface in the absence of sporocarps; $(1-f)$ is the fraction of CO$_2$ respired from the sporocarps. Variable $(1-f)$ was calculated using the CO$_2$ concentrations in each chamber pair 100 s after we started the flux measurement. We assumed that the CO$_2$ in the chamber was mainly derived from soil respiration and CO$_2$ from ambient air contributed little. The CO$_2$ concentration increased during the flux measurement from c. 380 ppm to > 1000 ppm in the chambers with sporocarps and reached 700–800 ppm in the chambers without sporocarps.

**Results**

CO$_2$ efflux from the chambers with sporocarps was highest 1 h after the label was applied and declined over time (Fig. 2). Between 1 and 28.5 h after the labeling, CO$_2$ effluxes from chambers with sporocarps (724.5 ± 76 mg C m$^{-2}$ h$^{-1}$) were significantly higher ($P < 0.01$) than from chambers without sporocarps (389.1 ± 30 mg C m$^{-2}$ h$^{-1}$). Before and 53.5 h after the labeling CO$_2$ effluxes from chambers with sporocarps and without sporocarps were similar. The mass balance calculation (Eqn 1) suggests that the proportion of CO$_2$ respired from the sporocarps at 1–28.5 h after labeling was c. 20–30% of the total CO$_2$-efflux in the chambers with sporocarps (Table 1). However, the equation could only be solved for this time interval when the CO$_2$ concentration in chambers with sporocarps was higher than in chambers without sporocarps (Fig. 2).

The $^{14}$C signature of the CO$_2$ respired from chambers with sporocarps was higher than that of chambers without sporocarps, except 53.5 h after the label application (Fig. 3). These findings were significant at 1 and 5 h after the label application (Fig. 3). In addition, the $^{14}$C signature of the CO$_2$ respired from the chambers with sporocarps changed significantly over time after the label was applied (Table 2). The $^{14}$C signature increased by c. 30‰ 1 h after the label was applied and then decreased by 5–20‰ with time. 53.5 h after the...
label, the $^{14}$C signature of the respired CO$_2$ was lower than that before the labeling. By contrast, the $^{14}$C signature of CO$_2$ from chambers without sporocarps did not change significantly with time after the label was applied (Table 2). The $^{14}$C signature of the respired CO$_2$ in all chambers was always higher than that of the ambient air (Fig. 3).

The $^{14}$C signature of the sporocarp-respired CO$_2$ (Eqn 3) was only calculated if the flux contribution of the sporocarps was larger than 10% of the total flux (1–28.5 h after labeling). The sporocarp $^{14}$C-signature increased dramatically until 5 h after the labeling (Table 1) and was much higher than that of the soil-respired CO$_2$ (Fig. 3). In addition, after the label was added the $^{14}$C signature of the sporocarp tissue increased by 35‰, while that of the root tissue did not change (Table 1).

In most cases, chambers without sporocarps had lower CO$_2$ effluxes and higher variability in CO$_2$ efflux and $^{14}$C signatures, than did chambers with sporocarps, potentially owing to a higher proportion of ambient air in the chambers without sporocarps. We note that the $\delta^{13}$C signature of the ambient air was $-9.7 \pm 0.5$‰. The low $\delta^{13}$C and $^{14}$C signature of the ambient air and its high variation can be explained by changes in the contribution of $^{14}$C-depleted CO$_2$ produced from fossil fuels by nearby traffic.

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**Table 1** The radiocarbon signature of root and sporocarp tissue and of sporocarp-respired CO$_2$

<table>
<thead>
<tr>
<th>Time since label (h)</th>
<th>Roots $\Delta^{14}$C of bulk tissue (‰)</th>
<th>n</th>
<th>Sporocarps $\Delta^{14}$C of bulk tissue (‰)</th>
<th>n</th>
<th>Estimated $^{14}$C of respired CO$_2$ from sporocarps (‰)</th>
<th>Estimated contribution of sporocarps to total CO$_2$ efflux (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-label</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-label</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500.2 (57)</td>
<td>23 (3)</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>583.6 (228)</td>
<td>25 (10)</td>
<td>2</td>
</tr>
<tr>
<td>28.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320.2 (15)</td>
<td>29 (6)</td>
<td>2</td>
</tr>
<tr>
<td>93.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>84.6 (7)</td>
<td>251.8 (17)</td>
<td>3</td>
</tr>
</tbody>
</table>

(SE in parentheses).

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**Fig. 3** $^{14}$C signature of soil respired CO$_2$ measured in the three chamber pairs and of ambient air before the label was added and with time after the label. Open symbols and dotted lines indicate chambers with sporocarps; closed symbols and solid lines, chambers without sporocarps. Pair 1, diamonds; pair 2, squares; pair 3, circles.
The CO₂ in the chambers originated from ambient air and soil respiration. Before sampling, the CO₂ concentration in the chambers reached > 1000 ppm. This implies that the contributions of CO₂ from ambient air was < 350/1000 ppm, or < 35% in all chambers, and that the δ¹³C of the soil-respired CO₂ was −24‰.

**Discussion**

We added only a small amount of labeled glycine to prevent any possible ‘priming’ effects. However, after the label was applied, we observed that the flux of respired CO₂ increased by c. 400 mg C m⁻² h⁻¹ from chambers with sporocarps and by c. 200 mg C m⁻² h⁻¹ from chambers without sporocarps. To sustain this increase in respiration over a 24-h period within an area of 1 m² would require the respiration of an additional 9.6 g C for the chambers with sporocarps or 4.8 g C for chambers without sporocarps. We added only 0.000175 g of labeled C to the soil. Although the addition of glycine could have stimulated fungal respiration the increases in CO₂ efflux cannot be fully explained by the added C. The higher respiration most likely resulted from changes in the phenology of the sporocarps (growth, maturation, and decay throughout the experiment) and the activity of the mycelia. The largest part of the fungal biomass contributing to the CO₂-efflux from the soil surface is located below-ground. Changes in the activity of the mycelia could also have contributed to the increase of CO₂-efflux in the chambers without sporocarps since fungal mycelia can extend below-ground for several meters from the nearest sporocarp (Kretzer et al., 2004). Also, higher overall soil respiration could be caused by changes in root respiration of grass and trees or disturbance of the soil during the measurements.

Despite the high variability in the fluxes, the changes in the δ¹⁴C signature of the sporocarp-respired CO₂ together with the increase of the δ¹⁴C signature of the sporocarp tissue indicate that the labeled glycine was taken up and metabolized by the fungi and released from their sporocarps within 5 h. The label was even detected within the first hour in the CO₂ respired from sporocarps growing 60 cm from the injection point (‘Location 2’). This indicates that the labeled C could be transported quickly over a relatively large area, potentially through fungal rhizomorphs. The rapid decrease in the δ¹⁴C value indicates that most of the label was respired shortly after it became available. This short response time is consistent with previous findings that smaller amino acids such as glycine are taken up and processed by organisms relatively quickly (Lipson et al., 1999).

Laboratory-based studies have often used isotopic labels to examine the uptake, assimilation, or transformation of specific compounds by fungi (e.g. Kirk et al., 1975; Finlay, 1992; Steffen et al., 2002; Frey et al., 2003; Hobbie et al., 2003). Field-based applications are rare but critical; they incorporate complex biotic and abiotic interactions such as competition among microbes or between microbes and plants. In situ measurements have included incorporation of isotopically labeled C into ergosterol, a compound primarily produced by fungi (Suberkropp & Weyers, 1996). Carbon has also been tracked between ‘donor’ and ‘recipient’ plants via mycorrhizal fungi (Simard et al., 1997). The isolation of fungal tissues or signal compounds from soils has posed a logistical challenge in field studies. By contrast, sporocarps can serve as a conduit of the tracer from below-ground mycelia to more easily manageable and identifiable surface structures. However, one drawback is that sporocarps are ephemeral and only represent a portion of fungal biomass.

In many tracer studies the use of the stable δ¹³C isotope as a tracer should be chosen over δ¹⁴C due to easier handling and much lower costs. However, δ¹⁴C can be very useful when operating with very small amounts of quickly metabolized compounds. Even if we had added pure δ¹³C glycine the change in the δ¹³C ratio of the respired CO₂ would have been within the range of fractionation during photosynthetic uptake which is reflected in the δ¹³C ratio of soil respiration (Fig. 4). By contrast, the signal from the δ¹⁴C label is outside natural variation and is visible over a longer time span.

**Table 2** Statistical results of variation in Δ¹⁴C

<table>
<thead>
<tr>
<th>Time since label (h)</th>
<th>t</th>
<th>Degrees of freedom</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>−19</td>
<td>−4.489</td>
<td>1</td>
<td>0.140</td>
</tr>
<tr>
<td>1</td>
<td>−18.033</td>
<td>2</td>
<td>0.003</td>
</tr>
<tr>
<td>5</td>
<td>−7.562</td>
<td>2</td>
<td>0.017</td>
</tr>
<tr>
<td>28.5</td>
<td>−3.344</td>
<td>1</td>
<td>0.185</td>
</tr>
<tr>
<td>53.5</td>
<td>−1.889</td>
<td>2</td>
<td>0.199</td>
</tr>
</tbody>
</table>

**Repeate measures analysis of variance**

<table>
<thead>
<tr>
<th>Factor</th>
<th>F-ratio</th>
<th>Degrees of freedom</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence/absence sporocarps</td>
<td>8.163</td>
<td>1,2</td>
<td>0.104</td>
</tr>
<tr>
<td>Time</td>
<td>5.806</td>
<td>4,4</td>
<td>0.017</td>
</tr>
<tr>
<td>Time × sporocarps</td>
<td>14.868</td>
<td>4,8</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Our results suggest that the presence of saprotrophic sporocarps can significantly alter the rates of CO₂ respired from soils. In addition, growth, maturation, and decay of the sporocarps and mycelia might be coupled to changes in the composition of the ¹⁴C signature of soil respired CO₂. This should be considered in soil respiration studies. We show that small amounts of ¹⁴C combined with chamber measurements can be used as a tracer to study the fate and dynamics of individual components of soil organic matter in below ground food webs under field conditions on an hourly resolution.

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