Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition

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Abstract

We investigated arbuscular mycorrhizal fungi (AMF) alteration of microbial mediation of litter decomposition. AMF (Glomus hoi) were either allowed access to or excluded from Plantago lanceolata L. root litter embedded in soil; litter was labeled with either ¹³C only or ¹³C and ¹⁵N. Plant N uptake was significantly increased if AMF accessed the litter, and ¹⁵N analysis of the plant material indicated that 2–3% of plant N originated from litter. Succession of the soil community mediating decomposition was assessed by phospholipid fatty acids (PLFA) combined with ¹³C-PLFA. During the first 21 days of decomposition, saprotrophic fungi and Gram-negative bacteria were the dominant consumers of litter C. As decomposition progressed however, ¹³C content of the fungal biomarkers declined substantially, and Gram-negative and Gram-positive bacteria became the primary reservoirs of labeled litter C. The putative PLFA marker for AMF (16:1ω5c) originated primarily from non-AMF sources. In AMF-invaded root litter, Gram-negative, Gram-positive, and 16:1ω5c markers became less ¹³C-enriched relative to their counterparts in non-AMF-invaded microcosms during active decomposition. These patterns of ¹³C:¹²C enrichment may result from AMF supply of ¹²C from the plant to the decomposing soil microbial community; such C inputs could alter the microbial mediation of litter decomposition.

Introduction

A key function of the arbuscular mycorrhizal (AM) symbiosis is to enhance nutrient capture for the associated host plant, while in return, the fungus obtains a supply of carbon (Smith & Read, 2008). AM fungal hyphae can explore a large volume of soil and acquire phosphorus (P) beyond the phosphate depletion zone that rapidly builds up around the root surface (Sanders & Tinker, 1973). More recently however, an arbuscular mycorrhizal fungi (AMF) role in plant N capture has been demonstrated, at least under some (Atul-Nayyar et al., 2009; Leigh et al., 2009; Barrett et al., 2011), but not all (Hodge, 2003a; Reynolds et al., 2005), conditions. In addition, a plant ammonium transporter has been identified in Lotus japonicas, which is mycorrhizal-specific and preferentially expressed in arbusculated cells (Guether et al., 2009a, b). This suggests that the arbuscule may also be involved as a site for N transfer from the fungus to the plant.

It is well established that plant roots modify the rates and characteristics of soil organic matter decomposition, in part through rhizodeposition processes (e.g. Bottner et al., 1999; Dijkstra et al., 2009; Bird et al., 2011), which in turn are influenced by a wide range of abiotic and biotic factors including AM colonization (see Hodge et al., 1997; Hodge & Millard, 1998; Paterson et al., 1999; Jones et al., 2004, 2009). There is also evidence that AMF may enhance rates of plant residue decomposition (Hodge et al., 2001; Atul-Nayyar et al., 2009). However, unlike some of the fungi involved in both the ecto- (ECM) and ericoid (ERM) mycorrhizal associations (Hodge et al., 1995; Read & Perez-Moreno, 2003), AMF have no known saprotrophic capabilities. Thus, AMF are unlikely to play a direct role in organic matter decomposition. Instead, AMF likely acquire N in inorganic form (Govindarajulu...
e.g., 2005; Leigh et al., 2011 but see Whiteside et al., 2009) and rely on members of the saprotrophic microbial community to decompose complex organic sources and release inorganic N for AM hyphal capture. As AMF are large sinks for plant photosynthate (Hodge, 1996; Johnson et al., 2002), AM hyphal exudates may stimulate microorganisms present in the mycorrhizal hyphosphere (i.e. the volume of soil influenced by AMF hyphae). However, the effect is variable: AM hyphal exudates may stimulate some microorganisms but inhibit others (see Filion et al., 1999), all of which can lead to an altered bacterial community (Toljander et al., 2007).

Results of studies specifically testing hyphosphere soil for microbial community changes are variable. While quantitative changes in bacterial numbers in the soil surrounding the AMF are seldom reported, changes in bacterial composition may occur (Olsson et al., 1996; Andrade et al., 1997). The presence of AMF may suppress microbial groups under some circumstances (Welc et al., 2010). After the addition of substrates to soil, direct interactions between AMF and other key microorganisms have been investigated (Larsen et al., 1998; Green et al., 1999; Albertsen et al., 2006; Welc et al., 2010); however, these studies have tended to concentrate on only a small number of other organisms (Larsen et al., 1998; Green et al., 1999) and often in artificial media. Consequently, little is known about the effect of AMF on the soil microbial community mediating decomposition.

We investigated the interactions of AMF hyphae with the soil fungal and bacterial communities mediating the decomposition of added labeled plant litter. We hypothesized that the presence of AMF hyphal alters the flow of litter C into and through the native soil community. Phospholipid fatty acids (PLFA) were used to broadly characterize and identify the live microbial community in the soil and litter. The root litter was labeled with $^{13}$C, thus enabling microbial C utilization to be tracked by $^{13}$C-PLFA analysis; in additional treatments, dual-labeled ($^{13}$C:$^{15}$N) root litter was added to quantify the amount of $^{15}$N acquired by the AMF and the plant host at the end of the 70-day experiment.

This approach provides a powerful tool for identifying microorganisms involved in the decomposition of litter substrates and, by following the $^{13}$C over time, mapping the movement of C through the soil community. Several taxonomic groups contain signature fatty acid biomarkers (Vestal & White, 1989), which can be used to distinguish between saprotrophic fungi, Gram-positive bacteria, Gram-negative bacteria, and actinomycetes. While there is no exclusive PLFA marker for AMF, 16:1o5c has been widely used as an indicator of AMF biomass, including for the assessment of external mycelium development (Olsson et al., 1997, 1998; Ravnskov et al., 1999; Beauregard et al., 2010). We used labeled Plantago lanceolata L. root material because most decomposing plant litter in soil is from roots and root turnover in soil can be rapid (reviewed by Eissenstat & Yanai, 1997; Fitter, 1999). Alterations by AMF of soil microbial mediation of litter decomposition could have important impacts at the ecosystem level.

Materials and methods

Litter decomposition experiment

To investigate whether the presence of AMF hyphae altered the decomposing microbial community in root litter, we used two-compartment microcosm units and a series of destructive harvests (at 10, 21, 42, and 70 days after litter addition) to track the flow of carbon (as $^{13}$C) from the $^{13}$C-labeled root litter. AMF hyphae were permitted (AMF-allowed) or denied (AMF-excluded) access to the litter by the use of nylon membrane meshes. In all cases, living roots from the AM-associated host plant in the planted compartment were denied access to the litter compartment. At each harvest, there were five replicate microcosm units from the two AM access treatments (i.e. AMF-allowed or AMF-excluded). In addition, to follow the transport of litter-derived N into the associated live host plants, a subset of 10 units (five AMF-allowed and five AMF-excluded) received $^{13}$C:$^{15}$N dual-labeled litter. These units were harvested at the end of the experiment (70 days after litter addition).

Each microcosm unit comprised two joined compartments (each 13.5 x 14.0 x 14.0 cm) separated by a double-mesh barrier (Fig. 1). The mesh was either 20 µm (John Stanier & Co., Whitefield, Manchester, UK), to allow passage of mycelia but not roots (AMF-allowed), or 0.45 µm (Anachem, Bedfordshire, UK), to exclude both mycelia and roots (AMF-excluded) but allow solute diffusion to occur (Hodge et al., 2001). In all microcosms, one compartment (planted compartment) received 120 g fresh weight (FW) inoculum of the AMF Glomus hoi (isolate number UY 110) as colonized roots of P. lanceolata L. in a sand and Terra-Green® (a calcined attapulgite clay soil conditioner; Oil-Dri, Cambridgeshire, UK) growth medium. The inoculum was mixed thoroughly with 1.85 L of a 50 : 50 mix of sand: Terra-Green® and 0.3 g L$^{-1}$ sterilized bone-meal (a complex P & N source to encourage mycorrhizal development). Plantago lanceolata seeds (Emorgase Seeds, Norfolk, UK) were planted into this compartment (one seed per microcosm). Plantago lanceolata is commonly used in AM studies because of its ability to support high levels of AM colonization; hence, this plant was selected as the model plant in this study.
A loam soil (pH 6.8 in 0.01 M CaCl₂) was collected from an experimental garden at the University of York, UK, and sieved through a 2-mm mesh. No fertilizer had been added to this experimental garden soil for at least 10 years, and we added none during the experiment except for the labeled root litter material. The sieved soil was used to fill the second compartment of the microcosms except the center, where a section of PVC pipe (internal diameter 6.5 cm) was placed at a depth of 8 cm from the soil surface. The purpose of the PVC pipe was to allow precise placement of labeled root litter while ensuring minimal disturbance to the system. The root litter (labeled with either 13C alone or 13C + 15N as described below) was added 2.5 weeks after the P. lanceolata seeds were planted, inoculated as a mixture of 2 g of labeled root material with 60 g of the loam soil, and placed at a depth of 5 cm. Patches of litter were 60 g of the loam soil, and placed at a depth of 5 cm.

The experiment was established in a randomized design in a glasshouse at the University of York, UK. The daily mean temperature over the duration of the experiment was 19.4 °C (± 0.1). Photosynthetically active radiation flux was recorded weekly at noon and averaged 190.5 (± 37.2) μmol m⁻² s⁻¹ at the plant level. All compartments were watered daily with deionized water. The plant compartment was fertilized twice weekly with 50 mL nutrient solution as described by Leigh et al. (2009). The second (soil and root litter) compartment received no additional nutrients.

**Generation of labeled of root litter**

To follow C and N during the decomposition process, labeled root litter was used in this study. Plantago lanceolata L. was labeled with 13CO₂ in an environmentally controlled chamber at the University of California, Berkeley, USA. Plantago lanceolata seeds were sown in 74 13 × 13 × 13 cm pots containing a mixture of ‘Surface Athletics’ fritted clay (Profile Products; LLC, Buffalo Grove, IL) and 30–100 mesh silica sand, 50% each by volume. At 2 weeks, seedlings were thinned to three plants per pot. Four weeks after sowing, plants were moved into a labeling chamber. Drip irrigation lines allowed delivery of either water or balanced plant nutrient solution. Plants were grown under a 14-h photoperiod, at 25–27 °C. CO₂ levels in the chamber were maintained at a minimum of 375 μmol mol⁻¹ (CO₂ evolved during dark respiration accumulated in the chamber and was reassimilated the following day) and periodically alternated the CO₂ source between natural abundance and 99 atom% 13C. At 42 days after seeding, a subset of 18 pots each received a total of 0.6 mol of 99 atom% 13CO₂. The root litter added to the experimental units contained 31% total C at 40 ± 0.3 atom% 13C. Root litter that had also received the 15N-enriched nutrient solution to create the 13C:15N dual-labeled material contained 1.16 ± 0.09% total N at 27 ± 0.6 atom% 15N. In contrast, the root litter that was only 13C-enriched and thus did not receive the 15N solution contained 0.69 ± 0.02% total N at 0.5 ± 0.008 atom% 15N. (Values are means ± standard errors.). However, despite these differences in % total N of the two root litter substrates, there were no significant differences in N capture by the shoots or roots of the experimental plants within the AMF access treatments (see Table 1).

**Mycorrhizal hyphae analysis**

To assess AMF colonization of decomposing litter, AM fungal extraradical mycelia (ERM) were extracted from the soil containing the labeled litter using a modified membrane filter technique (Hodge, 2003b). In at least 50 fields of view, hyphal length was counted at ×125 magnification using the gridline intercept method (see Hodge, 2003b). Hyphal lengths were then converted to hyphal length densities [m hyphae g⁻¹ soil dry weight (DW)].
Arbuscular mycorrhizal fungal hyphae visible at 10× under a stereoscope were hand-picked from the sand and Terra-Green® material (from planted compartment of the microcosm units) at 70 days after labeled litter application. Spores attached to the hyphae together with no, or very irregular, septae confirmed the hyphae extracted were of AM origin (see Quilliam et al., 2010). These hyphae were then dried (at 70 °C) and analyzed for 13C and 15N enrichments.

### Carbon and nitrogen in plants and hyphae

The bulk carbon (C) and nitrogen (N) content and isotopic composition of the substrate litter were determined prior to incorporation into experimental pots. In addition, the live shoot and live root material in the experimental units harvested at day 42 and day 70 were analyzed for C and N content and isotopes. Prior to analysis, material was pulverized and homogenized to a fine powder and measured into tin capsules for C and N analysis. AM hyphae collected at 70 days were transferred whole into tin capsules. Nitrogen and carbon measurements were taken on a Roboprep-CN automated N-C analyzer coupled to a model 20-20 isotope ratio mass spectrometer (Sercon, Ltd, Crewe, UK).

The C and N content and isotopic composition of the soil microbial biomass in the soil containing added root litter collected 70 days after litter application were analyzed by chloroform fumigation-extraction (CFE) of 4 g of samples (Brookes et al., 1985). Subsequently, about 18 mL of the extracts were lyophilized, and the dried crystals were analyzed for C and N content and isotope ratios as above.

We used a mixing model to estimate the amount of C and N in the plants harvested at day 70 that had originated in the dual-labeled root litter.

### Lipid extraction and analysis

PLFA were extracted from the soil material containing the labeled litter collected from all pots except those which had received 15N-labeled litter. Microbial lipids were extracted in a monophasic methanol–chloroform–phosphate buffer (Bligh & Dyer, 1959; White et al., 1998), and the neutral lipids, glycolipids, and phospholipids were separated on inert II silica columns (Burdick & Jackson, Muskegon, MI) by sequential elution with chloroform, acetone, and methanol (Gehrn & White, 1983). Phospholipids in the methanol elution were transesterified by a mild alkaline methanolysis at 37 °C (Guckert et al., 1986). Fatty acid methyl esters were extracted in hexane, dried at room temperature under N2, and stored at −20 °C. Prior to analysis, the FAMEs were resuspended in hexane with methyl decanoate (Sigma-Aldrich, St. Louis, MO) added as an internal standard. Fatty acid methyl esters were analyzed at UC Berkeley for quantity and isotope ratio as described in the study of Bird et al. (2011).
Lipid analysis was also carried out on G. hoi hyphae collected from a previous experiment (see Hodge & Fitter, 2010). The hyphae were lyophilized, then bead-beaten by adding 0.5 g each of 0.1 mm glass and 0.5 mm zirconia/silica beads (Biospec Products Inc, Bartlesville OK), and shaken in a FastPrep Instrument (Qbiogene, Inc., Irvine, CA) at 5.5 m s\(^{-1}\) for 30 s. After this preparation step, 10 mg of hyphae was analyzed by the PLFA soil extraction procedure described above to ascertain which G. hoi fatty acids would be detectable.

Fatty acids are designated by the ratio of number of carbon atoms to the number of double bonds. For unsaturated fatty acids, \(n\) indicates the position of the first carbon of the double bond from the aliphatic end of the molecule, and \(c\) and \(t\) indicate \(cis\) and \(trans\) geometry. \(I\) and \(anteiso\) branching are indicated by the prefixes \(i\) and \(a\), mid-chain branching is indicated by \(nMe\), with \(n\) representing the branch position from the carboxyl end of the chain, and cyclopropyl fatty acids are indicated by the prefix \(cyc\).

Bacterial biomass was determined from the sum of \(15:0, 16:0, 16:1\_9c, 17:0, 17:1\_0\), \(18:1\_6c,\) and \(19:0\) (Frostegård & Bååth, 1996). \(10Me16:0\) indicates actinomycetes (Zelles, 1999); \(18:2\_6c\) (Federle et al., 1986) and \(18:6c\) (Zak et al., 2000) indicate saprotrophic fungi; \(16:1\_5c\) occurs in AMF (Olsson et al., 1997) and Gram-negative bacteria (Nichols et al., 1986); \(16:1\_7c, 17:0, 18:1\_0c,\) and \(19:0\) indicate Gram-negative bacteria (Phillips et al., 2002); and \(15:0, 16:0, 17:0,\) and \(17:0\) indicate Gram-positive bacteria (O’Leary & Wilkinson, 1988).

**Data analysis**

In plants harvested at day 42, the main effect of AMF access on atom\% \(^{13}\)C and %N of live shoots and roots was tested in ANOVAs, with plant tissue type (live shoot or root) nested within AMF access. In plants harvested at day 70, the main effect of AMF access on atom\% \(^{15}\)N of live shoots and roots was tested in an ANOVA, with plant tissue type (live shoot or root) nested within AMF access. In addition, at day 70, the main effects of AMF access and \(^{15}\)N labeling of patch litter on atom\% \(^{13}\)C and %N of live shoots and roots was tested in ANOVAs, with plant tissue type (live shoot or root) nested within the AMF access \(\times\) \(^{15}\)N labeling interaction. To determine whether litter-derived C or N occurred in plants and whether AMF affected total plant biomass or total N in plants, the main effect of AMF access on plant biomass, total plant N, litter-derived N, and litter-derived C in microcosms that had received dual-labeled litter (day 70) were tested. Plant tissue type (live shoot or root) was nested within AMF access. We tested the main effects of AMF access and time since litter patch application on the mole\% and atom\% \(^{13}\)C of PLFA biomarkers for saprotrophic fungi, Gram-negative bacteria, Gram-positive bacteria, actinomycetes, and the \(16:1\_0c\) marker; bacterial biomass; saprotrophic fungal biomass; and the sum of bacterial and saprotrophic fungal biomass. The main effects of AMF access and \(^{15}\)N labeling of patch litter on microbial C and N derived from CFE were also tested. Student’s \(t\) tests were performed on the 70-days data with respect to atom\% \(^{13}\)C, atom\% \(^{15}\)N, and %N to examine differences between AMF hyphae either allowed or denied patch access.

Linear models were performed using Statistix for Windows (Analytical Software, Tallahassee, FL). Mean comparisons in the ANOVAs were made using least significant difference. Criterion for significance for all tests is \(P < 0.05\).

**Results**

**Plant carbon and nitrogen**

CFE analysis of the day 70 \(^{13}\)C:\(^{15}\)N dual-labeled litter samples showed that total microbial biomass and C and N content from the soil containing the labeled root litter were not detectably influenced by the presence of AMF (mean ± standard error across treatments = 189 ± 16 mg C g\(^{-1}\) and 43.5 ± 3.6 mg N g\(^{-1}\)). Similarly, CFE microbial biomass atom\% \(^{13}\)C was unaffected by the AMF (mean across treatments = 18.9 ± 1.3 atom\% \(^{13}\)C). At the final harvest (70 days), 27% of soil microbial N originated from the labeled root litter.

The \(^{13}\)C enrichment of the P. lanceolata live plant roots and shoots ranged between 1.08 and 1.10 atom\% \(^{13}\)C at the 42- and 70-days harvests (Table 1). Small differences in \(^{13}\)C enrichment of live host plant roots as a result of AMF access to the patch occurred at 42 and 70 days in the \(^{15}\)C-labeled only microcosms, where live roots in the AMF-allowed units were more enriched than live roots in the AMF-excluded units (Table 1). There was a small, but significant, increase in litter-derived C in live host plant roots of AMF-allowed plants (0.04%) compared to host roots of AMF-excluded plants (0.02%); litter-derived C in host plant roots of AMF-allowed plants was significantly higher than the shoot material from either AMF access treatments (Table 2). Plant DW was not significantly different in the AMF-allowed and AMF-excluded treatment and was not correlated with total N uptake (Table 2).

AMF hyphae harvested from the planted compartment of the dual-labeled microcosms at 70 days contained 1.09 atom\% \(^{13}\)C; there was no significant difference between the enrichment of the AMF hyphae with or without access to the \(^{13}\)C-labeled litter and no differences between hyphae and live roots by bulk IRMS analysis.
In microcosms that received $^{15}$N-enriched litter, N in the live plant shoots of the AMF-allowed treatment was 1.2 atom% $^{15}$N, significantly higher than the 0.38 atom% $^{15}$N of live shoots in AMF-excluded treatment (Table 1). This confirms that AM hyphae acquired and translocated N derived from the decomposing litter and also that simple diffusion of decomposition products from the soil + litter to the rooting compartment was not an important N transfer mechanism in this study. $^{15}$N enrichment of live roots showed a similar pattern to shoots, being significantly higher in AMF-allowed relative to AMF-excluded treatments. By 70 days, shoots in the AMF-allowed treatment had acquired significantly more total N (mg N per plant) than shoots in the AMF-excluded treatment (Table 2), although no difference in live root total N content (mg of N per plant) was observed as a result of AMF access. In the AMF-allowed treatment, there was no difference in total N acquisition between live roots and shoots.

The amount of N that had originated from the labeled root litter was negligible in AMF-excluded plants but was significantly elevated in the AMF-allowed shoots and roots (3.2% and 2.0% respectively; Table 2). Furthermore, N derived from the litter was significantly higher in the shoots than in the roots in the AMF-allowed treatment, but not in the AMF-excluded treatment. From the analysis of AM hyphae picked from the rooting compartment, we estimated the percentage of hyphal N that originated from the decomposing litter in the soil compartment; 7.3% ± 1.4 (mean ± standard error) of N in the AMF-allowed hyphae originated from the litter as compared to 0.06% ± 0.02 for the AMF-excluded treatments. This indicates that AMF hyphae acquired the majority of their N from non-litter sources.

### AM hyphae in litter

In litter from which G. hoi hyphae was excluded, AM hyphae were rarely detected throughout the course of the experiment, indicating that the mesh excluded hyphae as intended. In AMF-allowed microcosms, hyphal length densities were very low (< 0.02 m g$^{-1}$) at days 10 and 21; AMF subsequently proliferated into the decomposing root litter such that hyphal length densities averaged 1.0 ± 0.1 and 1.8 ± 0.5 m g$^{-1}$ DW (mean ± standard error) at 42 and 70 days, respectively.

### Lipids

Although there was no effect of AMF access on either total bacterial or saprotrophic fungal biomass as measured by PLFA, there was a strong effect of harvest date on both forms of microbial biomass (Fig. 2; $P < 0.0001$). Over the course of the 70-days experiment, saprotrophic fungal biomass decreased more sharply than bacterial biomass, with fungal biomass at 70 days only 28% of that at day 10, compared to 62% for bacterial biomass. This is clearly demonstrated by the ratio of saprotrophic fungal to bacterial biomass, which declined from 0.37 at 10 and 21 days to 0.17 at 42 and 70 days.

### Table 2. Total shoot and root biomass, nitrogen (N) content (mg) and N and carbon (C) from the patch as a percentage in the total plant tissue captured from $^{13}$C + $^{15}$N-labeled litter at the end of the experiment (70 days). Data are means ± standard errors ($n = 5$). Means in the same column sharing a common letter are not significantly different ($P < 0.05$); plant DW did not differ significantly between the AMF access treatments.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AMF</th>
<th>DW (g)</th>
<th>Total N (mg)</th>
<th>N derived from litter (%)</th>
<th>C derived from litter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoots</td>
<td>Allowed</td>
<td>2.17 ± 0.14</td>
<td>24.7 ± 2.4 a</td>
<td>3.18 ± 0.29 a</td>
<td>0.012 ± 0.003 b</td>
</tr>
<tr>
<td>Shoots</td>
<td>Excluded</td>
<td>2.25 ± 0.26</td>
<td>16.5 ± 1.1 b</td>
<td>0.02 ± 0.03 c</td>
<td>0.011 ± 0.002 b</td>
</tr>
<tr>
<td>Roots</td>
<td>Allowed</td>
<td>2.02 ± 0.13</td>
<td>15.2 ± 0.9 b</td>
<td>2.00 ± 0.23 b</td>
<td>0.043 ± 0.012 a</td>
</tr>
<tr>
<td>Roots</td>
<td>Excluded</td>
<td>3.28 ± 0.71</td>
<td>14.7 ± 2.0 b</td>
<td>0.10 ± 0.06 c</td>
<td>0.019 ± 0.002 b</td>
</tr>
</tbody>
</table>

Fig. 2. Microbial biomass in amended soil. Values are means ($n = 5$). Significant differences between harvests for the bacterial biomarkers are indicated by lower-case a, b, or c, while upper-case indicates differences in the fungal biomarkers. Significant differences between harvests for the sum of bacterial and fungal biomarkers are indicated by x or y. There was no significant effect of access or exclusion of AMF.
The mole% of 13C, derived from the atom% 13C data (Fig. 4) and the quantities of the fatty acids, indicates the distribution of litter-derived 13C among the microbial biomarkers. Through the first 21 days of decomposition, saprotrophic fungi and Gram-negative bacteria were major mediators of decomposition, with their markers each in the range of 23–28 mole% 13C; this compares to <12 mole% 13C among the combined Gram-positive, actinomycete, and 16:1o5c markers during the same time. By days 42 and 70, saprotrophic fungal markers contained a substantially lower proportion of 13C (<12 mole% 13C), while Gram-negative markers remained above 26 mole% 13C. Gram-positive, actinomycete, and 16:1o5c markers initially showed lesser access to the litter carbon groups have different abundance patterns over time (Fig. 3). The main Gram-negative markers accounted for the greatest proportion throughout the experiment (25–27%) with the highest mole% occurring at 42 days, but no effect of the presence or absence of AMF was detected with this marker. The saprotrophic fungal markers 18:1o9c and 18:2o6c declined substantially over time, while the Gram-positive markers increased; neither was affected by the presence/absence of AM hyphae in the patch. The 16:1o5c marker, known to function both as a Gram-negative and as an AMF marker, increased over time and, by 70 days, was significantly higher in the root litter material to which the AMF hyphae had access (Fig. 3). The signature fatty acid for actinomycetes (10Me16:0) increased from 21 to 70 days. No significant effect of AMF access on the actinomycete marker was observed.

13C enrichment provides an indication of the substrate from which the C in a fatty acid is derived. Analysis of the atom% 13C of the lipid markers showed patterns distinct from the mole% data (Fig. 4). The highest 13C enrichments (atom% 13C) were found in the fungal markers at 10 and 21 days, which then declined substantially by 42 and 70 days. Similarly, the initially high enrichments of the Gram-negative markers declined by 70 days. The only markers to remain constant or consistently increase in 13C enrichment over time were the Gram-positive and actinomycete markers. The presence of AMF in the litter patch significantly affected the 13C enrichment of the Gram-negative, Gram-positive, and 16:1o5c markers at day 42 (the sampling date at which substantial AMF hyphal proliferation was first detected in the litter material). All three groups were more 13C-enriched in the AMF-excluded microcosms, indicating that the presence of AMF either accelerated the loss of 13C from these groups or increased the availability of 12C to the litter communities. At day 70, the 13C enrichment of all marker groups was the same in the presence and absence of AMF.

In extractions of G. hoi hyphae, the only fatty acids detected were 16:1o5c, 16:0, 18:3o6c, and 18:1o7c (0.9, 1.4, 0.1, and 0.1 nmol fatty acid g⁻¹ hyphae, respectively). That the mole% of biomarkers 18:1o9c and 18:2o6c declined at 42 and 70 days (Fig. 3) while AMF hyphal length densities significantly increased further shows that these two fatty acids indicate saprotrophic fungi, but not AMF.

Fatty acid concentrations from the soil + decomposing root litter show that the PLFA-distinguishable microbial
Arbuscular mycorrhizal hyphae effects on root litter decomposition

Fig. 4. 13C enrichment of signature fatty acids from the 13C root litter patches over time. (a) Saprotrophic fungal fatty acids (18:1ω9c, 18:2ω6c); (b) Gram-negative (16:1ω7c, cy17:0, 18:1ω7c, cy19:0); (c) Gram-positive (i15:0, a15:0, i16:0, i17:0, a17:0); (d) 16:1ω5c; (e) actinomycete 10Me16:0. Filled symbols represent treatments where AMF had access to the organic patch, while open symbols represent where AMF were denied access to the organic patch. Data are means ± SE (n = 5). Different letters within each plot indicate significant differences between data points (P < 0.05).

(10, 0.4, and 1 mole% 13C, respectively, at 10 days), but the proportion of 13C in these markers relative to the other fatty acids steadily increased, reaching 20, 2.4, and 8 mole% 13C, respectively, by 70 days.

Discussion

In this study, we found that AMF acquire N from litter decomposing in live soil and transport the N to the host plant. When AMF had access to the litter, the contribution of N from the decomposing litter to AMF hyphal N was approximately 7%. In contrast, most studies on nutrient capture by AMF have been conducted in artificial media presumably lacking ‘natural’ soil microbial communities. For example, in an experiment conducted on an artificial medium involving G. hoi accessing Lolium perenne L. leaf litter, Hodge & Fitter (2010) reported that 12–30% of hyphal N had originated in litter. In the present study, AMF were accessing litter-derived N in the presence of an active soil community as well as in soil containing other N sources. Thus, it is not surprising that in our study, AMF acquired a lower percentage of their hyphal N from decomposing litter. Some of the 15N acquired by the AMF from the decomposing litter was passed to the plant and supplied c. 2% of the live root N and 3% of shoot N when the AMF had access to the litter material. The occurrence of labeled N in the shoot material in this study is important because it demonstrates that true transfer of N from the AMF to the host plant had occurred rather than only being retained in the fungal tissue in the colonized roots. Nitrogen transfer by AMF to their associated host plant has been demonstrated previously (Hodge, 2003b; Govindarajulu et al., 2005; Leigh et al., 2009), but not from living soil.

In this experiment, we detected slight, but significant, 13C enrichment in live roots with AMF access to the labeled litter, as compared to roots without AMF access (Tables 1 and 2). Whether this resulted from the assimilation of intact organic compounds or from assimilation of H13CO3 mineralized from litter, the amount of 13C detected in the plants was small (~0.04% of biomass) and therefore is of questionable ecological relevance in this study.

We found that the presence of AMF hyphae affected C flow through the soil microbial community. 13C-PLFA analysis showed significant differences at 42 days of 13C enrichment among components of the microbial community owing to the presence of AMF. Day 42 was the first sampling date when substantial AMF hyphal proliferation into the litter was observed. Three groups of marker fatty acids (Gram-negative, Gram-positive, and 16:1ω5c) were significantly less enriched in 13C in the AMF-allowed compared to the AMF-excluded microcosms at 42 days. Because there was no difference in mole% of 16:1ω5c at day 42 between the AMF-allowed and AMF-excluded microcosms, the contribution from AMF to the atom% 13C of this fatty acid at this time point is likely small. As AMF are considerable sinks for host plant C (Hodge, 1996; Johnson et al., 2002), the lower 13C enrichment of these three groups may have resulted from the translocation of 12C plant carbon into the soil compartment through the AM hyphae. AM hyphal exudates from Glomus sp. have been documented and have been suggested
as a mechanism of AM interaction with surrounding soil microorganisms in the hyphosphere (Toljander et al., 2007).

Alternatively, the lower atom% $^{13}$C-PLFA in the AMF-allowed microcosms may have resulted from accelerated litter decomposition. While our experimental design did not allow us to directly quantify the rate of litter decomposition, accelerated litter decomposition in the presence of AMF has previously been reported (Hodge et al., 2001; Atul-Nayyar et al., 2009; Larsen et al., 2009). Acceleration of root litter decomposition resulting from plant root supply of C is a documented phenomenon (Bird et al., 2011). However, C ‘priming’ of decomposition processes can be positive, neutral, or negative (Cheng & Kuzyakov, 2005). Thus, the import of plant C by AMF to microbial decomposers may not accelerate the rate of litter decomposition.

While AMF access to the decomposing litter affected C flow through the microbial community, AMF access had no discernible effect on PLFA-detectable composition of the microbial community present in the litter-containing soil. Although PLFA has been widely used to follow gross changes in soil community composition, it does have limitations of resolution and sensitivity (see Ruess & Chamberlain, 2010; Frostegård et al., 2011). An advantage of using $^{13}$C-PLFA, however, is that it is generally more sensitive for stable isotope probing (SIP) than DNA-SIP (Radajewski et al., 2000), which still commonly requires highly enriched stable isotope labeling of target organisms (reviewed by Gutierrez-Zamora & Manefield, 2010).

The overall trajectories of the microbial communities present in the soil containing decomposing litter were indicated by the gross PLFA analysis. The decline in microbial biomass after 21 days suggests that the more labile litter constituents were becoming depleted by this time point. The increase in the ratio of cyclopropyl fatty acids to their monoenoic precursors (data not shown; $P < 0.001$) supports the possibility of bacterial starvation (Guckert et al., 1986). As has been previously shown (Denef et al., 2007; Bird et al., 2011), saprotrophic fungi rapidly process fresh carbon, with utilization shifting to certain bacterial groups during later stages of decomposition. In the present experiment, this is indicated in the fungal/bacterial ratio at 42 days, which was half of the value at 21 days. As confirmed by our findings, AMF do not contain the common saprotrophic fungal fatty acids (18:1ω9c and 18:2ω6c). So although AMF hyphae had proliferated into the decomposing litter by the 42-days harvest, their presence would not be detected among the saprotrophic fungal markers. The biomarker 16:1ω5c is known to be present in AMF (Nordby et al., 1981; Olsson et al., 1998) and was the second most abundant fatty acid detected in AMF hyphae in this study. Differences in AMF access treatments with respect to mole% of 16:1ω5c were detected at day 70; however, the abundance of that fatty acid was elevated by < 15% in the AMF-allowed treatment as compared to the AMF-excluded treatment. Despite the proliferation of AMF hyphae at day 42, the failure to detect a difference in mole% of 16:1ω5c at day 42 is not surprising. Other studies (e.g. Nichols et al., 1986; Frostegård et al., 1993; Hedlund, 2002) have found 16:1ω5c to be abundant in other members of the soil community, thereby interfering with the potential AMF signal. Thus, although this fatty acid has been used widely as a marker for AMF (Olsson et al., 1998; Gormsen et al., 2004; Labidi et al., 2007), our results suggest that in complex communities, this marker needs to be used with caution.

Hodge et al. (2001) did not detect any changes in microbial community structure using PLFA analysis of decomposing grass (L. perenne L.) shoot material in the presence of AMF. Similarly, PLFA biomarker analysis only rarely detected AM impacts on gross microbial community composition. Concurrent DNA-based analyses did, however, reveal changes in the taxonomic composition of the soil bacterial community in response to AMF hyphae in the decomposing litter (Nuccio EE, Hodge A, Herman DJ, Pett-Ridge, J & Firestone MK in preparation). Welc et al. (2010) recently reported AM suppression of soil microorganisms based on PLFA analysis. PLFA analysis characterizes microbial communities in terms of broad groupings, thus allowing direct coupling of $^{13}$C analysis with a broad community characterization and revealing groups actively utilizing litter C over time. Fungal $^{13}$C enrichment closely tracked the mole%, indicating that the size and activity of the saprotrophic fungal population was closely coupled to the early utilization of fresh litter. The high $^{13}$C enrichment of the fungal biomarkers at 10 and 21 days after labeled litter introduction indicated that the fungal population rapidly developed new biomass using the enriched litter as substrate. The decline in enrichment and mole% of fungal markers at 42 and 70 days suggests a turnover of saprotrophic fungal biomass and a consumption of other soil carbon.

While the saprotrophic fungi declined at 42 and 70 days, AMF proliferation became more apparent in the litter patch over the same timescale. Olsson et al. (1998) reported that AMF decreased soil colonization by saprophytic fungi. However, a negative interaction between these two fungal groups was unlikely in our study as the decline in saprotrophic fungi also occurred in the decomposing root litter patch to which AMF were excluded. Actinomycetes behaved similar to the saprotrophic fungi, in that the $^{13}$C enrichment changed in step with mole% of 10Me16:0. However, unlike the saprotrophic fungi,
actinomycetes became more important over time in both abundance and \(^{13}\)C content.

The importance of microbial groups in the process of litter decomposition can be seen in the total mole% of \(^{13}\)C (derived from the atom% \(^{13}\)C data and the mole% abundances of the marker fatty acids). During the first 21 days of root litter decomposition, saprotrophic fungi and Gram-negative bacteria were the dominant consumers of litter C. As decomposition of the litter progressed, however, the \(^{13}\)C content of the saprotrophic fungal markers declined substantially and Gram-negative and Gram-positive bacteria became the primary reservoirs of carbon from labeled litter. The relative abundance of Gram-negative markers remained fairly constant throughout the experiment.

The progression of microbial communities present on the decomposing *P. lanceolata* root litter provides an interesting contrast to a similar PLFA and \(^{13}\)C-PLFA analysis of microbial communities on *Avena barbata* (annual grass) roots decomposing in an annual grassland soil (Bird *et al.*, 2011). The primary microbial group present and active in the decomposition of grass root litter was Gram-positive bacteria, with Gram-negative bacteria and saprotrophic fungi substantially less important both in biomass and in \(^{13}\)C retention. The actinomycete markers accounted for roughly the same percentage of biomass and label uptake in both systems. This demonstrates that in different soils (English garden soil and California grassland soil) and with different root litter (*P. lanceolata* vs. *A. barbata*), substantially different groups of soil microorganisms play central roles in the belowground decomposition of litter.

In conclusion, amounts and isotopic composition of the nitrogen in the host plants and AMF hyphae indicated that in live soil, AM fungal hyphae can acquire N released during litter decomposition and translocate it to the host plant. While PLFA showed no difference in the overall trajectories of the microbial communities over time between the two AMF access treatments, \(^{13}\)C-PLFA (or PLFA-SIP) detected a significant decrease in the \(^{13}\)C enrichment of three marker fatty acids during the period of active decomposition. The presence of AMF appears to be supplying natural abundance plant carbon to the litter-decomposing microbial community.

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