Carbon Allocation, Belowground Transfers, and Lipid Turnover in a Plant–Microbial Association

Francisco J. Calderón*
USDA-ARS
Central Great Plains Research Station
40335 County Rd. GG
Akron, CO 80720

David J. Schultz
Dep. of Biology
Univ. of Louisville
Louisville, KY 40292

Eldor A. Paul
Natural Resource Ecology Lab.
Colorado State Univ.
Fort Collins, CO 80523

Radioactive tracers were used to study the C allocation to coarse and fine roots, aboveground plant tissues, mycorrhizal lipids, belowground respiration, and soil in a mycorrhizal association. *Sorghum bicolor* (L.) Moench was grown in soil with a nonmycorrhizal microbial inoculum with and without *Glomus clarum*, a mycorrhizal inoculant. Fifty-one-day-old mycorrhizal (M) and nonmycorrhizal (NM) plants were subjected to a 3-h exposure to $^{14}CO_2$ and sequentially harvested after 52, 54, 57, 64, and 76 d. Mycorrhizal plants assimilated 21% more ^{14}C than NM plants, even though they were slightly smaller in size. They also had a higher percentage and absolute allocation of ^{14}C to root tissue, belowground respiration, and soil. Mycorrhizal roots had a higher content of total lipids and total fatty acids. The fungal fatty acid 16:1ω5, usually associated with arbuscular mycorrhizal fungi, comprised up to 29.5% of the total fatty acid content of M roots, while NM roots had only trace levels of this molecule. Thin-layer chromatography was used to separate the fatty acids extracted from the roots. The ^{14}C of the various components was determined by radiography. The ^{14}C mean residence time (MRT) of the mycorrhizal fatty acid 16:1ω5 was calculated at 7.1 d. The monoenoic, saturated, and total fatty acids had MRTs ranging from 11.1 to 14.3 d. The lipids of NM roots incorporated less ^{14}C label. This underscores the difference in the lipid C cycle between the M and NM roots. Translocation of the ^{14}C to soil was 6.3% of the photosynthesized C in the M plants relative to only 2.4% in the NM plants, giving an indication of its movement into the mycorrhizal hyphae as well as to the soil.

Abbreviations: AMF, arbuscular mycorrhizal fungi; M, mycorrhizal; MRT, mean residence time; NM, nonmycorrhizal; PVC, polyvinyl chloride; TLC, thin-layer chromatography.

The mycorrhizal association is an important factor in the retention or loss of C in terrestrial ecosystems as well as in plant nutrition. The balance between above- and belowground plant productivity, as well as mycorrhizal fungal growth and rhizosphere and soil respiration depends on the type of symbiotic system, soil moisture, temperature, nutrient status, and atmospheric CO_2 levels (Rillig and Allen, 1999). It is difficult in calculating atmospheric–soil CO_2 transfers to know whether mycorrhizal respiration is to be considered as autotrophic or heterotrophic and how much the microbial associations affect belowground allocations. Hobbie (2006) estimated that ectomycorrhizal fungal net productivity could reach 21% of the total net primary production. This is above the 1 to 6% value usually applied to arbuscular mycorrhizal fungi (AMF) (Warembourg and Paul, 1973; Snellgrove et al., 1982). Plants infected with mycorrhizal fungi have higher C respiration due to increased flow of C to the root system (Jakobsen

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*Corresponding author (francisco.calderon@ars.usda.gov).

and Rosendahl, 1990) but also fix more C as photosynthate (Finlay and Soderstrom, 1992; Staddon et al., 1999).

Molecular techniques are providing quantitative analysis of mycorrhizal communities and the genes involved in transport mechanisms (Balestrini et al., 2011). Kramer and Gleixner (2006) utilized both ^{13}C and ^{14}C to differentiate the use of plant- and soil-derived C by soil microbes, but the use of ^{14}C provides greater sensitivity in the measurement of the different pools involved (Wiesenberg et al., 2010). Fatty acids are especially useful in such studies because they can be relatively easily isolated from either soil or plant parts, and individual compounds act as biomarkers for the different components of the plant–soil biota (Leake et al., 2006; Denef et al., 2009). Olsson and Johnson (2005) used ^{13}C tracers to study C dynamics and incorporation into signature fatty acids in a *Glomus–Plantago* symbiosis. It is challenging to directly measure the C allocated to intraradical mycorrhizal cells. The close association of the root and the fungus limits the feasibility of a physical separation (Tinker et al., 1994). Most studies have involved laboratory systems with and without the mycorrhizal fungi (Pang and Paul, 1980; Paul and Kucey, 1981).

Lipids play an important role in the C economy of the arbuscular mycorrhizal association and are used as the main C storage molecules (Cox et al., 1975; Bago et al., 1998). The higher lipid content of mycorrhizae indicates that infected roots have higher construction costs than nonmycorrhizal roots. Many species of AMF contain high amounts of the fatty acid 16:1ω5, which is absent in nonmycorrhizal roots (Beilby, 1980; Nagy et al., 1980; Nordby et al., 1981; Grandmougin-Ferjani et al., 1997; Calderón et al., 2009). Recent studies have shown that there is a rapid incorporation of photoassimilated C into soil phospholipids, with the greatest accumulation of radiolabel occurring as 16:1ω5 (De Deyn et al., 2011). Data are lacking, however, on C incorporation and turnover into lipids of mycorrhizal and nonmycorrhizal C_4 plants.

The AMF lipids offer the opportunity to determine the turnover of a large component of the AMF by the pulse incorporation of ^{14}C, followed by sequential measurements of the radioactive signal in AMF-specific and plant lipids, fine and coarse roots, soil, and respired CO_2.

In this experiment, we aimed to explore two hypotheses. The scarcity of data about the estimated C contribution to soil in mycorrhizal C_4 plants prompted us to hypothesize that 6% of photosynthate C will be transferred to the soil via growing AMF hypha and hyphal-derived C compounds. This is in line with what has been found for other plant–AMF symbioses growing in soil. Few studies have quantified the dynamics of 16:1ω5 C in roots to validate the assumption that it is a fungal pool in the mycorrhizal association. We hypothesized that 16:1ω5 is a *Glomus clarum* specific fatty acid, and its C turnover rate corresponds to AMF energy production and CO_2 release from mycorrhizal roots. Besides these two hypotheses, we had the following specific objectives: (i) to measure the patterns of C incorporation and retention in the plant tissues, root lipids, soil, and soil respiration by mycorrhizal and non mycorrhizal sorghum, (ii) measure the turnover of C in the AMF-specific and nonspecific lipids of the mycorrhizal roots, and (iii) calculate the turnover rate of the belowground constituents.

MATERIALS AND METHODS

Each plant was grown in a polyvinyl chloride (PVC) cylinder (9.6-cm diameter, 20-cm height) containing 2 kg of sieved (5-mm) sandy loam (pH 6.2, cation exchange capacity 4.5 cmol kg^{-1}, 1.2% organic matter, 12.5 mg kg^{-1} total P, 88.0 mg kg^{-1} K, 647.5 mg kg^{-1} Ca, and 121.5 mg kg^{-1} Mg). The soil was collected from the Ap horizon of a Kalamazoo loam (a fine-loamy, mixed, mesic Typic Hapludalf) in Michigan. The soil was sterilized by irradiation with ^{60}Co (13 h, 3826 Gy h^{-1}). Seeds of *S. bicolor* were surface sterilized (70% ethanol for 30 s, then 20% bleach for 20 min). The seeds were germinated in a petri dish for 2 d over a sterile filter paper. Two germinated seeds were planted per pot and thinned to one after the first week of the experiment.

The pots in the mycorrhizal treatment (M) were inoculated with *Glomus clarum* Nicolson & Schenck (INVAM BR147B-4) by adding a 50-g mixture of infected roots and soil-borne spores. The nonmycorrhizal treatment (NM) received no roots, but a chlamydospore-free filtrate of the mycorrhizal inoculum was added to supply nonmycorrhizal soil microbes to the NM treatment.

A total of 40 plants (20 M and 20 NM) were placed inside a 5.4-m^3 Plexiglas chamber with a sealed wood frame and base and placed inside a greenhouse. The chamber was open to allow air exchange and equilibration to greenhouse conditions until the time of the pulse labeling. The plants were grown with natural light supplemented with high-pressure Na lamps placed outside the chamber. The photosynthetically active radiation ranged from 250 to 1200 μmol L^{-1} m^{-2} s^{-1} during the 16-h photoperiod. The temperature ranged from 23 to 29°C. Each pot received 300 mL of a P-free nutrient solution (1.5 mmol L^{-1} $CaCl_2$, 0.5 mmol L^{-1} K_2SO_4, 2.5 mmol L^{-1} NH_4NO_3, 0.25 mmol L^{-1} $MgSO_4$, 25 μmol L^{-1} H_3BO_3, 20 μmol L^{-1} FeDDHA, 20 μmol L^{-1} $ZnSO_4$, 0.5 μmol L^{-1} $CaSO_4$, 0.4 μmol L^{-1} H_2MoO_4, and 0.6 μmol L^{-1} $CoCl_2$, pH to 6.8 with KOH) and was watered to field capacity with distilled water every 2 d. Plants were subjected to a single exposure of $^{14}CO_2$ 51 d after planting when they were approaching the reproductive phase. A total of 92 μmol L^{-1} of labeled C in the form of $Na_2{}^{14}CO_3$, with a specific activity of 0.1542 GBq kg^{-1} C was used. The labeled $Na_2{}^{14}CO_3$ was mixed with unlabeled Na_2CO_3 to form a 0.77 mol L^{-1} solution. The concentration of the CO_2 in the chamber was maintained at ambient levels by monitoring the internal CO_2 concentration with an infrared gas analyzer and generating labeled CO_2 as needed. The $^{14}CO_2$ was produced by reacting the Na_2CO_3 solution with an excess 85% lactic acid. A total of 170.2 MBq were added to the chamber during a 3-h labeling period. The chamber was purged with fresh air at the end of the pulse labeling at a rate of 18 m^3 h^{-1}.

Five harvests were performed 1, 3, 6, 12, and 24 d after the $^{14}CO_2$ exposure. At each harvest, the shoots were separated from the roots by clipping and the soil and shoots were placed at −20°. The shoots were dried (65°C, 24 h), ground, and then stored at 5°C until the nutrient and biomass analyses. The roots were separated from the soil first by gently separating them from the bulk of the surrounding soil, then by washing any remaining soil clinging to the roots with water while retaining the roots in a sieve. After washing, the material was freeze-dried and the biomass of the fine (<1-mm-diameter) and coarse (>1-mm-diameter) roots was recorded. The root material was freeze-dried and stored at 4°C until the lipid and radioactivity analyses.

To measure shoot P concentration, samples (0.5 g) were ashed (500°C, 5 h) and digested for 1 h in 25 mL of 3 mol L^{-1} HNO_3 in 1 g kg^{-1} LiCl. The digests were filtered and mixed with a 0.3 mol L^{-1} NaOH solution (1:9 v/v). These were then analyzed colorimetrically with a Lachat Flow Injection Analyzer (Zeller Analytical). The total shoot P ranged from 10.3 to 12.2 mg $plant^{-1}$ and was statistically indistinguishable between the M and NM plants.

Ground shoot and root samples were analyzed for specific activity and C content. The samples were combusted with a biological sample converter (Europa Scientific Roboprep-CN) in series with a mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyzer). The C content of the samples was determined by comparison with sucrose standards. The CO_2 evolved by combusting the samples was trapped in Carbon 14 Cocktail (R.J. Harvey Instrument Co.) and the radioactivity was measured by liquid scintillation. The effectiveness of the traps was estimated by analyzing ^{14}C leucine of known mass and specific activity. Samples of freeze-dried fine roots (0.02 g) were analyzed by gas chromatography to measure the relative amounts and kinds of fatty acids. We used the extraction, derivatization, and procedure detailed by Calderón et al. (2009). An internal fatty acid standard (15:0, 0.001 g mL^{-1} in hexane) was used to obtain quantitative data.

A separate set of sample fractions was used to measure the radioactivity in the total lipids and the lipid fractions and then in the fatty acids by thin layer chromatography (TLC). The total lipids of root sample fractions (30 mg) were extracted using the method of Bligh and Dyer (1959). Briefly, 1 mL of 0.15 mol L^{-1} acetic acid and 3.75 mL of 2:1 methanol/chloroform was added to each sample. The mixture was vortexed, then 1.25 mL of chloroform and 1 mL of water were added. The mixture was vortexed again, then centrifuged at low rpm to separate the phases. The bottom chloroform layer containing the total lipid was dried under N_2 and resuspended in 1 mL of chloroform. An aliquot of known volume was placed in a glass fiber filter of known mass and dried at 75°C for 1 h. The weight of the recovered lipids was measured gravimetrically and the specific activity of the lipid material in the filter was determined using the same procedure as for the plant biomass. With these data, the lipid mass and radioactivity per unit of root weight were calculated.

The fatty acid methyl esters (FAMEs) were obtained by methylating the lipid extract using the procedure of Morrison and Smith (1964). The methylation products were dried, resuspended in hexane, and used for liquid scintillation analysis and argentation TLC. The argentation TLC allowed a physical separation of the FAMEs by the number and position of double bonds as well as C chain length. Plate preparation, development, and visualization were performed following the methods detailed by Cahoon and Ohlrogge (1994). The FAME bands were identified by comparison with known standards (16:1, 18:1, 20:1, and 22:1, Sigma-Aldrich Chemical Co.). A commercial standard for fatty acid 16:1ω5 was not available. For this purpose, the FAME extract from an *Escherichia coli* clone (pDES 16) that produces 16:1ω5, 16:1ω7, and 18: ω9 as the dominant monoenoic fatty acids was included (Schultz et al., 1996). The argentation TLC achieved a full resolution of saturated, dienoic 16:1ω5, 16:1ω7, 18:1ω7, 18:1ω9, and 20:1 FAMEs. The plates were analyzed by radiography using a Packard Instant Imager (Packard Instrument Co.) at a scan time of 1.5 h to measure the radiation contributed by each fatty acid band. Liquid scintillation analysis of corresponding extracts analyzed by argentation TLC plates was used to calculate the radioactivity per unit of root mass of the different FAME bands.

First-order exponential decay curves were fitted to the fatty acid radiolabel data using the Global Curve Fit feature of Sigmaplot Version 11.0 (Systat Software Inc.). In all fatty acid classes from the M roots, the first-order fit had a higher R^2 than a linear order fit. The mean residence time was calculated as MRT = $1/k$, where k is the decomposition rate constant from the first-order decay fit.

The soil atmosphere was sampled from the plants designated for the last harvest through a port located at the bottom of the each PVC cylinder of the 4 M and 4 NM plants allocated to the last harvest and was measured starting at 8 h after the pulse label. The soil atmosphere was flushed for 10 min before the first sampling period, then each pot was sampled by continuously extracting air from the belowground airspace at a rate of 0.02 m^3 h^{-1}. The CO_2 was trapped with 350 mL of 3 mol L^{-1} NaOH, and the radioactivity was estimated using a Packard 1500 Tri-carb Liquid Scintillation Analyzer. Six sampling periods of 8 h were separated by 1-h intervals between samplings. The total belowground respiration was calculated using the recovery of respired ^{14}C between 8 and 59 h after the pulse label. The shoot respiration was not determined separately for each plant. To calculate the shoot respiration, we assumed that the radiolabel that was not accounted for by the shoot, root, soil, and belowground respiration was the shoot respiration. Another assumption was that the shoot respiration was uniform for M and NM plants. Thus, we estimated a maximum value of 0.2 MBq of shoot ^{14}C respiration.

Root-free soil samples were dried at 65°C and the specific activity of the soil C was determined in the same manner as the plant biomass. To confirm the absence of radiolabel in soil carbonates, soil samples were acidified (0.5 L of

Table 1. Biomass of inoculated (M) and uninoculated (NM) plants after the pulse label (n = 4). The plants were 51 d old at the time of the pulse exposure to $^{14}CO_2$.

	Plant biomass									
	Day 52		Day 54		Day 57		Day 64		Day 76	
Plant part†	M	NM	M	NM	M	NM	M	NM	M	NM
	g									
Shoot biomass	5.8 (0.1)‡	6.4 (0.1)	6.1 (0.4)	6.7 (0.3)	7.1 (0.3)	7.6 (0.4)	9.6 (0.1)	9.7 (0.5)	14.7 (0.5)	16.9 (1.3)
Fine root biomass	5.6 (0.3)	5.6 (0.4)	5.1 (0.5)	5.2 (0.5)	5.4 (0.7)	4.8 (0.4)	5.0 (0.4)	5.3 (0.3)	7.4 (0.1)	6.2 (0.4)
Total root biomass	7.4 (0.5)	8.1 (0.6)	6.7 (0.5)	7.4 (0.5)	7.4 (0.8)	7.2 (0.4)	7.0 (0.4)	8.2 (0.3)	10.9 (0.2)	10.0 (0.4)

† Shoot biomass had significant time and mycorrhizae main effects and no significant interaction according to ANOVA ($P < 0.05$); fine root biomass had a significant time main effect and no significant mycorrhizae effect or interaction; total root biomass had a significant time main effect and no significant mycorrhizae effect or interaction.

‡ Averages with SEM in parentheses.

1 mol L^{-1} HCl kg^{-1} soil) and their specific activity was compared with unacidified samples.

All the soils from the M treatment were sampled for the concentration and radioactivity of mycorrhizal spores. The spores were obtained from 15-g soil samples by wet sieving (38-μm mesh), followed by sucrose-gradient centrifugation modified from Daniels and Skipper (1982). The spores were then transferred into a petri dish for counting under a dissecting microscope. The spores were separated from the solution by filtration (Gelman Sciences Type NE glass fiber filter), dried (65°C, 24 h), then analyzed for the specific activity in the same way as the soil and plant material.

Statistical Analysis

The pots were placed in four blocks as a randomized, split-plot design, with sampling time as the main plots and mycorrhizal treatment as the subplots. The sampling times and mycorrhizal addition treatments were analyzed using the Split-Plot Analysis of Variance of SAS (SAS Institute, Cary, NC), with n = 4 plants per mycorrhizae and time treatment combination.

RESULTS

Plant Growth

The plants were in an active growth phase during the 24 d following tracer application (Table 1). Mycorrhizal plants, the norm in most ecosystems, were comprised of 56% roots at the initiation of the labeling period. This dropped to 43% at the end of the measurement period due to the relatively higher shoot growth relative to root growth. The NM plants were larger at the time of the pulse and growing at a faster pace than the M plants. The M shoot biomass increased by 153% compared with 164% in the NM plants. This difference in shoot and root growth rate is reflected in the radioactivity measurements, which show that the shoots of the NM plants contained 56% of the label relative to 47% in the M plants (Table 2). The M plants, however, had more root growth than the NM plants. Fine root biomass in the NM treatment grew by 11%, while those of M plants grew by 32% (Table 1). The total root biomass of the M plants increased by 47% during the chase period compared with 23% in the NM roots.

Table 2. Average distribution of ^{14}C per plant for the 24-d harvest period.

Radiolabel sink	Mycorrhizal plants		Nonmycorrhizal plants	
	Total	Allocation	Total	Allocation
	MBq	%	MBq	%
Shoot	2.2 (0.1)†	47.9 (1.3)	2.1 (0.1)	56.5 (1.2)
Root	1.3 (0.1)	28.9 (1.9)	1.0 (0.1)	27.3 (1.9)
Soil	0.3 (0.0)	6.3 (0.9)	0.1 (0.0)	2.4 (0.3)
Belowground respiration	0.6 (NA‡)	11.9 (0.5)	0.4 (NA)	10.6 (1.2)
Shoot respiration	0.2 (NA)	5.0 (0.2)	0.2 (NA)	6.1 (0.7)
Total per plant	4.6 (0.2)		3.8 (0.2)	

† Averages with SEM in parentheses.

‡ NA, not available.

Radiolabel Assimilation

Inoculation with mycorrhizae affected the amount of C assimilated and the distribution of the fixed ^{14}C to the sorghum tissues and soil. The M plants allocated 47.1% of their fixed ^{14}C belowground, which includes allocation to roots, soil, and belowground respiration (Table 2). The NM plants allocated 40.3%. The M plants assimilated 21% more labeled C per plant than the NM plants (Table 2). Mycorrhizae were associated with increased belowground C allocation (root, soil, and soil respiration). Belowground respiration accounted for 11.9% of the incorporated label in the M plants and 10.6% in the NM plants (Table 2). Infection with *Glomus clarum* led to an additional 0.7 MBq of labeled C distribution to roots, soil, and soil respiration (Table 2).

The photoassimilation rate of the radiolabel by the M plants was 30% higher than that of the NM plants during the 3-h pulse, amounting to 0.26 GBq kg^{-1} shoot h^{-1} for the M treatment vs. 0.20 GBq kg^{-1} shoot h^{-1} for the NM treatment (Tables 1 and 2). The total radiolabel in the shoots and root tissues remained equal throughout the five sampling times, and this was true for both the M and the NM treatments (Table 3). Even though the M shoots had lower biomass, the total amount of radiolabel allocated to the M and NM shoot tissues was statistically indistinguishable due to higher rates of photosynthesis that produced higher specific activity in the M shoots (Table 4).

Table 3. Total radiolabel content of the tissues and soil from mycorrhizal (M) and nonmycorrhizal (NM) plants (n = 4). The plants were 51 d old at the time of the pulse exposure to $^{14}CO_2$.

Radiolabel sink†	Radiolabel content				
	Day 52	Day 54	Day 57	Day 64	Day 76
	MBq plant^{-1}				
Shoot					
M	2.2 (0.2)‡	2.6 (0.3)	2.2 (0.2)	2.0 (0.1)	2.1 (0.2)
NM	2.1 (0.4)	2.2 (0.1)	2.1 (0.1)	1.7 (0.2)	2.4 (0.3)
Whole root					
M	1.5 (0.6)	1.4 (0.1)	1.5 (0.4)	1.2 (0.3)	1.2 (0.3)
NM	1.0 (0.2)	1.0 (0.1)	1.0 (0.3)	1.1 (0.4)	1.2 (0.2)
Fine root					
M	1.1 (0.5)	0.9 (0.1)	1.1 (0.3)	0.7 (0.1)	0.9 (0.3)
NM	0.6 (0.1)	0.7 (0.1)	0.7 (0.2)	0.5 (0.1)	0.8 (0.1)
Soil					
M	0.4 (0.1)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)
NM	0.2 (0.1)	0.1 (0.0)	0.1 (0.0)	0.1 (0.2)	0.1 (0.0)

† Shoot total radiolabel had no significant main effects according to ANOVA ($P < 0.05$); whole-root radiolabel had a significant mycorrhizae main effect and no significant time effect or interaction; soil total radiolabel had a significant mycorrhizae main effect and no significant time effect or interaction.

‡ Averages with SEM in parentheses.

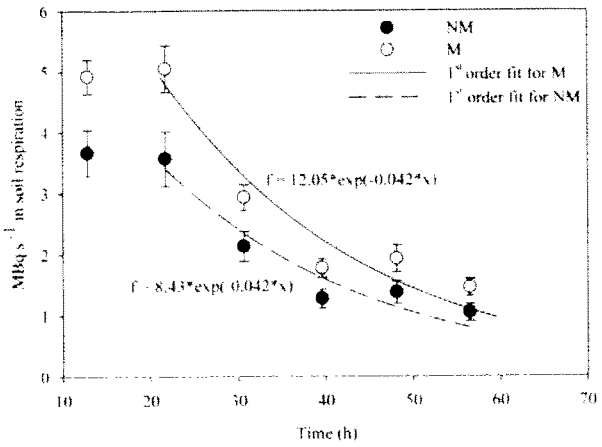

Fig. 1. Radiolabeled CO_2 respiration rates from mycorrhizal (M) and nonmycorrhizal (NM) roots.

Allocation to Belowground Respiration and Soil

The amount of $^{14}CO_2$ recovered from the belowground atmosphere decreased exponentially between 20 and 60 h following labeling and was consistently higher in the M relative to the NM treatment (Fig. 1). Each M plant evolved a total of 0.5 MBq, while NM plants respired a total of 0.40 MBq. For both treatments, 72% of the total belowground respired ^{14}C was recovered within 34 h after the pulse label. The root biomass of the M plants was equal to that of the NM plants (Tables 1 and 4), which suggests higher specific rates of respiration due to fungal association with roots.

The average specific activity of the M soil was at least twice that of the NM soil, and this difference was measured throughout the chase period (Tables 4 and 5). This result, combined with the observed higher belowground respiration in M plants, demonstrates a higher allocation of fixed ^{14}C to the M soil relative to the NM soil. In this study, the soil specific activity at 76 d was lower than that measured at the first harvest (Table 4). The time effect was not significant (data not shown), however, suggesting that soil ^{14}C mineralization was negligible during the time frame of the chase period. Movement of the tracer to the soil from the roots or biota would tend to maintain its ^{14}C level.

Allocation to Roots and Root Lipids

The roots of the M plants had significantly higher amounts of total lipid, total fatty acids, and fatty acid 16:1ω5 than NM roots (Table 5). The average concentrations of the total lipid, total fatty acids, and the biomarker 16:1ω5 were steady throughout the 24-d chase period (Table 5). The total amount of lipids in the roots increased, however, because of the growth in root biomass (Table 1). At the time of the pulse, 16:1ω5 made up 29.5% of the total fatty acid content of the M roots (Table 5). Previous studies from our laboratory showed that 16:1ω5 percentages of 13.3% correspond to microscopic estimates of 44% for root colonization (data not shown), so the M roots had substantial fungal infection.

The radiolabel content of total lipid, total fatty acids, monoenoic fatty acids, saturated fatty acids, and 16:1ω5 was higher in M relative to NM fine roots (Fig. 2). In the M treatment, total extractable lipid, total fatty acid, and 16:1ω5 fatty acids followed a similar pattern of high radiolabel incorporation before the first harvest, followed by a decrease in the label content throughout the chase period. In the first harvest, the total lipids and fatty acids in NM roots incorporated 30% or less of

Table 4. Specific activity of the tissues and soil from mycorrhizal (M) and nonmycorrhizal (NM) plants (n = 4 plants per treatment combination). The plants were 51 d old at the time of the pulse-exposure to $^{14}CO_2$.

Radiolabel sink†	Specific activity				
	Day 52	Day 54	Day 57	Day 64	Day 76
	GBq kg^{-1} C				
Shoot					
M	0.9 (0.1)‡	0.9 (0.1)	0.7 (0.1)	0.5 (0.0)	0.3 (0.0)
NM	0.8 (0.1)	0.8 (0.1)	0.6 (0.1)	0.4 (0.1)	0.3 (0.1)
Whole root					
M	0.5 (0.2)	0.5 (0.1)	0.5 (0.1)	0.4 (0.2)	0.2 (0.1)
NM	0.3 (0.1)	0.4 (0.0)	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)
Fine root					
M	0.5 (0.2)	0.5 (0.0)	0.6 (0.1)	0.4 (0.1)	0.3 (0.1)
NM	0.3 (0.0)	0.4 (0.0)	0.4 (0.2)	0.3 (0.1)	0.3 (0.0)
Soil					
M	0.018 (0.006)	0.015 (0.004)	0.009 (0.003)	0.011 (0.004)	0.014 (0.004)
NM	0.009 (0.004)	0.004 (0.000)	0.004 (0.001)	0.002 (0.001)	0.002 (0.001)

† Shoot specific activity had significant mycorrhizae and time main effects according to ANOVA ($P < 0.05$) and no interaction; whole-root specific activity had a significant mycorrhizae main effect and no significant time effect or interaction; soil specific activity had a significant mycorrhizae main effect and no significant time effect or interaction.

‡ Averages with SEM in parentheses.

Table 5. Lipid concentrations in fine roots of mycorrhizal (M) and nonmycorrhizal (NM) plants ($n = 4$). Only the lipids with a concentration of >0.25 g kg^{-1} for any of the sampling times are shown. The plants were 51 d old at the time of the pulse exposure to $^{14}CO_2$.

	Lipid concentration									
	Day 52		Day 54		Day 57		Day 64		Day 76	
Lipid	M	NM	M	NM	M	NM	M	NM	M	NM
	g kg^{-1}									
Total lipid†	26.7(3.0)‡	19.3(1.9)	ND§	ND	ND	ND	29.5(1.9)	21.4(1.7)	24.1(2.4)	20.6(2.5)
Total fatty acid†	6.1(1.2)	4.3(0.4)	5.6(0.2)	5.1(0.9)	6.8(1.2)	4.6(0.6)	6.5(1.8)	4.9(0.9)	6.1(1.4)	5.6(0.2)
Mycorrhizal										
16:1ω5†	1.7(0.6)	t¶	1.5(0.3)	t	1.5(0.3)	0.2(0.2)	1.7(0.8)	t	1.8(0.7)	t
Monoenoic										
17:1	t	0.1(0.0)	0.3(0.1)	t	0.4(0.2)	0.3(0.2)	0.2(0.1)	0.4(0.1)	0.2(0.1)	0.2(0.2)
18:1ω9	0.3(0.0)	0.3(0.0)	0.3(0.0)	0.4(0.1)	0.4(0.1)	0.5(0.1)	0.3(0.1)	0.4(0.1)	0.3(0.1)	0.4(0.0)
18:1#	0.3(0.1)	t	0.2(0.0)	t	0.3(0.0)	t	0.2(0.1)	t	0.2(0.1)	t
19:1	0.6(0.1)	0.9(0.1)	0.4(0.1)	1.1(0.2)	0.6(0.1)	0.8(0.1)	0.5(0.1)	0.7(0.3)	0.4(0.1)	1.1(0.1)
Polyenoic										
18:2ω6	0.3(0.0)	0.5(0.1)	0.4(0.0)	0.5(0.1)	0.7(0.0)	0.6(0.1)	0.5(0.1)	0.6(0.1)	0.3(0.0)	0.6(0.1)
Saturated										
16:0	1.5(0.3)	0.8(0.1)	1.4(0.1)	0.9(0.1)	1.4(0.2)	0.8(0.3)	1.5(0.4)	1.0(0.1)	1.4(0.3)	0.9(0.1)
21:0 iso	0.3(0.1)	0.5(0.1)	0.2(0.0)	0.7(0.2)	0.4(0.2)	0.4(0.0)	0.3(0.1)	0.5(0.2)	0.2(0.1)	0.7(0.1)
21:0	0.7(0.1)	0.7(0.1)	0.5(0.1)	0.9(0.2)	0.7(0.1)	0.7(0.1)	0.7(0.2)	0.8(0.2)	0.7(0.2)	1.1(0.1)

† Significant mycorrhizae main effect according to ANOVA and no significant time effect or interaction ($P < 0.05$).

‡ Averages with SEM in parentheses.

§ ND, not determined.

¶ t, trace (<0.05 g kg^{-1}).

This is an unresolved mixture of several 18:1 isomers.

the label incorporated by the corresponding fractions in the M treatment (Fig. 2). The monoenoic, saturated, and 16:1ω5 fatty acids in M fine roots incorporated more label than NM and had a marked turnover of radiolabel that was not observed in the NM treatment (Fig. 2). The incorporation and turnover of radiolabel in the saturated fatty acids of M roots followed a similar first-order decay pattern to that of 16:1ω5 (Fig. 2). The 16:1ω5 MRT at 7.1 d, however, was faster than for the rest of the fatty acids, which had an MRT average of 11 to 14 d. In all cases, the concentration of the radiolabel approached the steady-state levels of the NM plants toward the end of the chase period.

The predominant polyenoic fatty acid in the M and NM roots was linoleic acid 18:2 (Table 5). The incorporation of ^{14}C in polyenoic fatty acids of M roots was small, amounting to <5% of the total fatty acids at 1 d and showing little turnover in either M or NM roots (data not shown). The mass of 18:2 fatty acid per gram of root was lower or equal in M relative to the NM roots, suggesting that the mycorrhizal fungus was not contributing to its production. The M total root lipids incorporated 97.1 kBq plant^{-1} before Day 1 of the chase period, while the NM roots incorporated 28.1 kBq (data not shown). The total fatty acids of M roots incorporated a total of 38.2 kBq (Fig. 2). Thus, the total fatty acids of M roots accounted for 39% of the radiolabel incorporated by the total lipids between 0 and 1 d of the chase period. This implies that other non-fatty-acid, lipid-soluble molecules are also responsible for the relatively high radiolabel incorporation of M root lipids. The M treatment had higher lipid concentration and also higher lipid radiolabel content in the fine roots than the NM treatment. Turnover of 16:1ω5 was significant during the chase period. In this experiment, the sporulation of the external phase of the fungus was low, with concentrations of <1 spore g^{-1} of soil recorded for all the harvest periods. Because of this, mycorrhizal spores accounted for <0.8% of the soil radiolabel content, and we were not able to detect any turnover.

DISCUSSION

Our results have given us insight into the two hypotheses tested in this experiment. The difference between the belowground C allocation in M and NM sorghum roots indicates that the AMF was responsible for a translocation of nearly 4% of the photoassimilated C. This falls within the values in the literature for a variety of plant–AMF combinations. Higher values reported for ectomycorrhizal trees may be due to the significantly different nature of ectomycorrhizae and their woody plant hosts. In this study, we showed that incorporation and turnover of 16:1ω5 is different from other fatty acids. This mycorrhizal fatty acid undergoes fast incorporation of C and turnover that is not observed in NM fatty acids. The dynamics of 16:1ω5 are in agreement with the belowground respiration of M plants and can be used as an indicator of C demand and utilization by the fungus.

Plant Growth

The lower shoot biomass of the M plants indicates that there was incomplete compensation of the C demand of the fungus in the M symbiosis. Previous experiments have shown that plants may meet the C needs of the mycorrhizal symbiont by assimilating more C (Paul and Kucey, 1981; Kucey and Paul, 1982). It has been hypothesized that the positive effect of mycorrhizal fungi on the photosynthetic rate is explained by improvement in the water balance, increased leaf tissue P, higher specific leaf area, or phytohormones associated with mycorrhizal infection (Harris et al., 1985). We observed no statistical difference in tissue P between M and NM, so

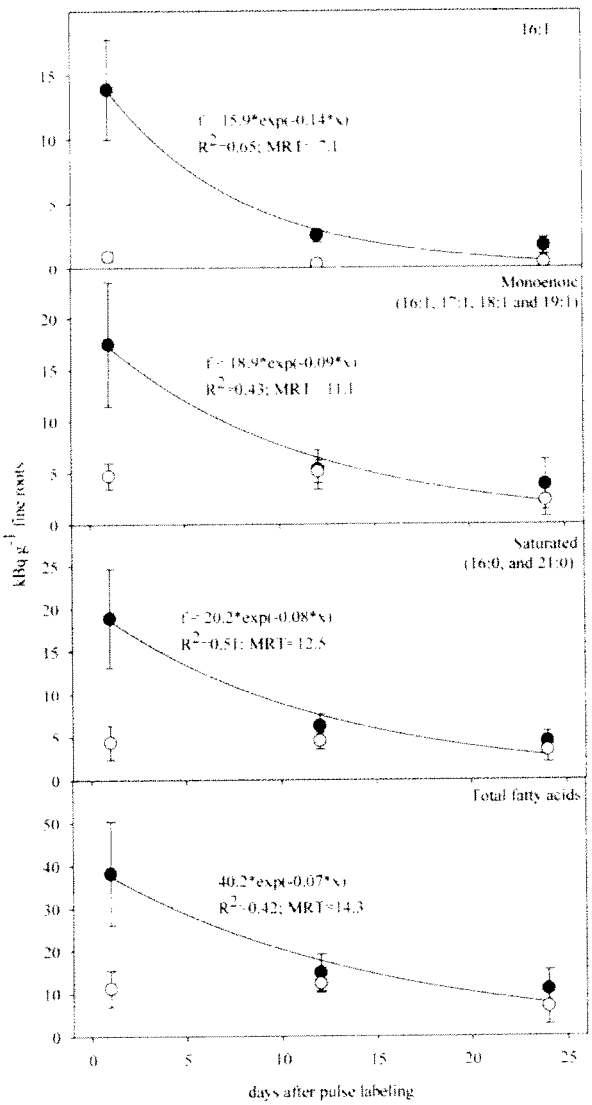

Fig. 2. Radiolabel decay in different root lipid classes from mycorrhizal (M, black circles) and nonmycorrhizal (NM, white circles) roots determined by thin-layer chromatography and radiography.

the increased photosynthetic rate in M shoots cannot be explained by P nutrition. There was no mycorrhizal effect on the root biomass, even though M roots grew more rapidly during the chase period. Other studies have shown instances where mycorrhizal plants did not significantly increase root biomass but instead increased leaf area and photosynthetic rate (Miller et al., 2002). The sink strength of mycorrhizal roots creates an additional demand that drains C from the plant and can limit vegetative growth, and this pattern increases with P deficiency (Miller et al., 2002).

Radiolabel Assimilation

Other pulse–chase experiments have shown that M plants fix more C and allocate more fixed C to M roots compared with NM plants, despite differences in growth media, pulse and chase periods, host–fungus combinations, and plant ages (Pang and Paul, 1980; Snellgrove et al., 1982; Jakobsen and Rosendahl, 1990; Tinker et al., 1994). Vandenkoornhuyse et al. (2007) showed that there is significantly higher C flux from the plant to the AMF compared with other root-inhabiting microbes. The increase in C allocation to mycorrhizae may be accounted for by several factors: (i) fungal respiration, (ii) root respiration, (iii) allocation to mycelial biomass, (iv) allocation to root biomass, (v) mycelial respiration (external), and (vi) exudation from roots or hyphae. Mycorrhizal–rhizobial plants have been found to have a higher fixation rate than uninoculated plants; however, the growth rate of the symbiotic plants was less than that that of the uninoculated plants because of the allocation of photosynthate to the symbionts (Harris et al., 1985). Snellgrove et al. (1982) found a similar pattern in *Allium* infected with *Glomus*. Other studies have found that M plants had similar or higher growth rates relative to NM plants, implying that the host compensated for the C demand from the symbionts with an increased photosynthetic rate (Pang and Paul, 1980; Paul and Kucey, 1981). Higher soil fertility may result in a parasitic-like relationship, whereas the AMF may act as a mutualist when it can help obtain limiting resources such as soil P.

Shoots of M plants received 48% of the ^{14}C, while the roots received 29% and the soil 6.3% (not taking in account respired ^{14}C). Warembourg and Paul (1973) found that 65% of the ^{14}C was retained aboveground and 35% belowground in a mixed prairie system. The lack of decay in the shoot radiolabel suggests that the majority of the C remaining in the plants 24 h after the ^{14}C pulse was incorporated into long-term storage or structural components. Root specific activity remained unchanged throughout the chase period. Warembourg and Paul (1977) found that the half-life of prairie roots in the field was 107 d, suggesting that the time frame of our experiment may have been too short to detect decay and turnover of whole roots. In our study, M roots had significantly higher specific activity and total radiolabel content than the NM roots (Tables 3 and 4). The roots in our study were actively growing during the chase period and radiolabel was incorporated into structural and storage root components.

Allocation to Belowground Respiration and Soil

Our results agree with previous studies that have shown increased root respiration of M plants relative to NM plants using pulse–chase experiments with different plant–fungus combinations (Pang and Paul, 1980; Kucey and Paul, 1982; Snellgrove et al., 1982; Harris et al., 1985; Johnson et al., 2002). The radiolabel in the M and NM belowground respiration remained stable for the initial 21 h, suggesting that this was a period of photosynthate translocation from shoots to roots (Kuzyakov and Gavrichkova, 2010). The CO_2 evolution after 60 h approached the point where movement of label from the foliage to the roots had finished and the label had been translocated to root and mycorrhizal structural and storage and soil C pools such as chitin and glomalin (Zhu and Miller, 2003).

There is great interest in C cycling studies in the separation of autotrophic from heterotrophic respiration (Hahn et al., 2006). It is hard to determine whether mycorrhizal fungal respiration is part of the autotrophic or heterotrophic cycle. The

AMF receives its C directly from the plant host and does not rely on the decomposition of plant residues. The fast cycling of the fungal fatty acids associated with the AMF in our study, together with significant movement of the tracer to the soil, leads us to suggest that although fungal in origin, this respiration is most closely associated with the autotrophic processes and should be considered as such.

Microbial decomposition of root exudates can be an important source of belowground CO_2 production (Martin and Kemp, 1986). Decreased root exudation and rhizosphere respiration has been shown in mycorrhizal plants (Graham et al., 1981; Miller et al., 2002). If the latter case is true, the increase in belowground $^{14}CO_2$ from the M soil could be accounted for by an increased specific respiration rate of the roots or the respiration of the AMF hyphae. Previous studies have shown contrasting evidence regarding increases in soil C allocation associated with AMF. Snellgrove et al. (1982) found a marginal difference, while Jakobsen and Rosendahl (1990) found that the allocation of C to the extraradical phase was twice that of NM plants and represented 3.1% of the total C fixation by the plant. Johnson et al. (2002) found that the amount of ^{14}C allocated into mycorrhizal mycelium 0 to 70 h after labeling accounted for 3.4% of the ^{14}C initially fixed by the plants. Paul and Kucey (1981) observed that extraradical mycorrhizal hyphae accounted for 1% of the fixed C in a C_3 plant. The difference between the 6.3% C allocation to the soil in the C_4 sorghum plants relative to 2.4% in the NM plants in our study could be attributed to mycorrhizal hyphae and their metabolites in the soil. If we assume that the label incorporation rates between the AMF and the plant root cells were similar and that most of the radiolabel incorporation into the soil ended up as fungal biomass, then we can estimate the extraradical AMF biomass/root ratio as 6.3/28.9 or 22%. It is important to keep in mind that this may be an overestimate because others have shown that plant-assimilated C is quickly translocated to AMF (Olsson and Johnson, 2005), followed by a slow transfer to soil microbial populations starting 4 to 5 d after labeling. Root exudation has been said to reach levels of 5 to 21% of the photosynthate (Walker et al., 2003). Hinsinger et al. (2012) have estimated C loss by deposition to approximate 11%. Our data and the results quoted for fairly mature plants in natural soils indicate much lower levels. Many of the high results have come from very young plants and NM plants grown in sand or nutrient cultures.

It is important to note that in this study the mineralization of extramatrical hyphae might have been affected by the possible lack of soil fauna in the experimental soils. This has been conjectured before in similar experiments where soils were sterilized before inoculation with AMF (Olsson and Johnson, 2005). Reduced soil fauna could have resulted in diminished grazing of fungal structures in the soil and the observed low soil ^{14}C mineralization.

A large number of C allocation experiments in mycorrhizal systems have been performed using C_3 plants. Sorghum is a C_4 plant, and studies are showing that root exudation differs between C_3 and C_4 plants (Phillips et al., 2006). More studies are needed about possible differences between C_3 and C_4 plants regarding allocation of photosynthate to soil hyphae vs. root exudates because this may have an important influence in the priming of soil C mineralization and sequestration.

Allocation to Roots and Root Lipids

The increased accumulation of lipids in mycorrhizae has been documented by previous studies (Cooper and Losel, 1978; Nagy et al., 1980; Peng et al., 1993). A significant fraction of the lipids of several *Glomus* species may be comprised of the unusual fatty acid 16:1ω5 (Calderón et al., 2009), and this molecule has been proposed as the principal AMF storage molecule in intraradical vesicles (Pacovsky and Fuller, 1988). Because of the increased production of lipids, infection with AMF has an associated increase in the cost of root production.

It has been conjectured that the C sink strength of mycorrhizae involves the unidirectional transfer of photosynthate into fungal-specific compounds (Losel and Cooper, 1979). The concentration of 16:1ω5 fatty acid in roots increases with the formation of fungal storage structures (Graham and Hodge, 1993). Our results indicate that lipids are a dynamic C pool that could play a role in unidirectional C transfer. Wiesenberg et al. (2010) showed that in perennial ryegrass (*Lolium perenne* L.), lipid C moves from roots into the soil and that roots, rather than litterfall, are the main source of soil lipids. Pfeffer et al. (1999) showed that the AMF converts sugars inside the roots into lipids that then move to the extraradical mycelium, with no lipid synthesis in the external mycelium.

The turnover of radiolabel in NM lipids was slight or absent during the chase period. In contrast, the M fatty acids of all types showed a measurable decay in radiolabel. We hypothesize that a portion of the saturated fatty acids such as 16:0 in M roots also represents a dynamic fungal pool. The faster MRT of the 16:1ω5 may be partly due to the fast turnover of membrane-rich AMF structures such as arbuscules, which are created and senesce faster than other fungal structures (Cox and Tinker, 1976). The MRT of fatty acid 16:1ω5 at 7 d was within the turnover time frame of the fungal arbuscules reported by Cox and Tinker (1976), who calculated that fungal arbuscules have a life span of 4 to 15 d within the root cells, after which time they are reabsorbed by the plant cell. Balasooriya et al. (2008) found the mycorrhizal-specific fatty acids to have a MRT of 4 d in a grassland under field conditions. These results verify the relatively fast turnover of mycorrhizal structures. Such short turnover is probably a prerequisite to an efficient symbiotic system.

The 18:2 fatty acid, with its low amount of radiolabel incorporation and turnover, could be considered a component of plant biomass rather than of AMF biomass. This may explain the similar C cycle of 18:2 fatty acid of M and NM roots. The fact that non-fatty-acid root lipids made up a large portion of lipid label incorporation and turnover in M roots shows that other lipid forms such as alkanes and sterols are also important in the C cycling of M roots. The concentration non-fatty-acid lipids such as sterols,

waxes, and carotenoids may be affected by mycorrhizal infection (Nagy et al., 1980; Nordby et al., 1981; Schmitz et al., 1991).

Recycling of tracer may occur between lipid and non-lipid pools. In fungi, carbohydrates may be converted to fat and vice versa via the glyoxylate pathway (Weete, 1980). There is also a likelihood that some recycling occurs between the fungus and the host plant. Fatty acid 16:1ω5 may exist free in the cytoplasm or be bound to different lipid classes such as triglycerides, diglycerides, or phospholipids. Triglycerides are thought to serve as long-term storage of C, while phospholipids serve a structural function by being part of cellular membranes. We hypothesize that 16:1ω5 obtained from whole-cell extracts may consist of a mixture of different C pools, with the possibility that each pool has a C cycle of a different time span. The low sporulation found in this study indicates that the increased ^{14}C content of the M soil relative to the NM soil cannot be explained by fungal sporulation. Other factors such as fungal hyphae and C immobilization in soil saprophytes may explain the response in radiolabel content of the M soil.

CONCLUSIONS

Belowground transfers of photosynthate are especially important to the functioning of roots and their associated microbial communities. Their size needs to be known in interpreting ecosystem functioning and soil CO_2 fluxes. Previous studies have shown that photosynthate is quickly translocated to root and soil fatty acids, and 16:1ω5 receives a large portion of the C (De Deyn et al., 2011). Our study, with the benefit of a NM control, confirms that 16:1ω5 is a mycorrhizal pool. The M treatment should be viewed as the normal state of plant physiology because the NM treatment would rarely be observed in nature. We have shown that the higher root respiration and large movement of ^{14}C underground is a consequence of mycorrhizae. The allocation of 6.3% of the photosynthate to the soil is higher than other results obtained in our laboratory that tended to show values of 1 to 3%. The value of 6.3% of soil allocation represents 18% of the belowground production.

The separation of the mycorrhizal fatty acids is useful from both quantitative and qualitative standpoints because it allows, for the first time, calculation of the turnover of an important cytoplasmic component of the AMF. Our results suggest that the net effect of the AMF is to increase the C storage in root lipids, despite the high losses of C associated with the metabolism of the mycorrhizal fungus. Fatty acid 16:1ω5 is not a long-term structural molecule and does not necessarily give a total turnover of the AMF biomass. Fatty acids account for a high percentage of the AMF cytoplasm, however, so the turnover rate of the mycorrhizal fatty acids represents the C turnover of an important C pool of the fungus. In this experiment, we measured the incorporation followed by a sustained decrease in the content of ^{14}C of the 16:1ω5 fatty acid during the 24-d chase period. This pattern is absent in the nonmycorrhizal roots and represents an important difference in the C physiology and C economy of the mycorrhizal fungus and the host. This technique opens the possibility of further experiments to study environmental impacts on the turnover of mycorrhizal components.

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