Mycorrhiza (2001) 10:287–293

ORIGINAL PAPER

Aiko Nakano · Kazushi Takahashi · Makoto Kimura

Effect of host shoot clipping on carbon and nitrogen sources for arbuscular mycorrhizal fungi

Accepted: 28 November 2000

Abstract The effect of clipping of the host-plant shoot on the sources of carbon and nitrogen for the arbuscular mycorrhizal (AM) fungus *Gigaspora margarita* was determined by measuring ^{13}C in spores and hyphae in co-cultures of C_3 and C_4 plants and by differential ^{15}N labeling. C_3 and C_4 plants, which have different $\delta^{13}C$ values, were grown in the same container separated by a series of hyphal compartments. The C_3 and C_4 plants were applied with ^{14}N- and ^{15}N-urea, respectively. After clipping of the C_3 shoots, spore $\delta^{13}C$ gradually approached that of the C_4 roots. Hyphal $\delta^{13}C$ paralleled that of spores. Spore % ^{15}N was similar to that of mineral N in the C_4 plant compartment. Thus C in *G. margarita* coming from the clipped plants decreased with time. This demonstrates that C in AM fungi comes from living plants, whilst the N in spores comes mostly from the soil.

Keywords Arbuscular mycorrhizal fungi · $\delta^{13}C$ · ^{15}N · Clipping

Introduction

Arbuscular mycorrhizal (AM) fungi develop hyphal networks which are capable of transporting various materials in the soil. Material transfer via the networks varies in direction and quantity with the fungal species, plant–fungus combination, and environmental conditions. The export of assimilate from canopy to subordinate species in plant communities may occur through hyphal networks (Grime et al. 1987) or, alternatively, the improved growth of subordinate species may be attributable to release from competition via enhanced uptake of P and N by AM (Bergelson and Crawley 1988). While much research has addressed transfer of materials between plants, there has been little discussion of the nutrition of AM fungi. Studies of C and mineral sources for AM fungi are essential for a better understanding of the AM symbiosis and material transfer.

Spore C in the AM fungus *Gigaspora margarita* comes from host plants in proportion to their proximity (Nakano et al. 1999). Spore N comes from the soil more than from host plants (Nakano et al. 2001). Our objective was to examine the effects of plant death on the origins of C and N for AM fungal spores. The C_3 plant alfalfa (Fabaceae) and the C_4 plant bahia grass (Poaceae) were grown in the same container separated only by several hyphal compartments. They were given ^{14}N- and ^{15}N-urea, respectively, and inoculated with *G. margarita*. Following clipping of the alfalfa, ^{13}C and ^{15}N was quantified in newly formed spores at various distances from the two plants.

Materials and methods

Monocultures of alfalfa or bahia grass

The experiment consisted of four treatments, two species of plants and two types of N fertilizer. There were two replicates of each treatment.

Each polystyrene cylindrical pot (113 mm diam. × 140 mm) was filled with 1.2 l of a mixture of equal volumes subsoil and sand. The subsoil (from ca. 5 m depth) was collected from the Nagoya University Farm (Red-Yellow soil, Typic Hapludult; see Soil Survey Staff 1998) and had a pH of 4.9, total C 0.67%, total N 0.05%, and <1 mg kg^{-1} of available P (Truog 1930). The sand was washed river sand. Both subsoil and sand were air-dried and sieved through a 2-mm screen before mixing. The mixture was autoclaved for 1 h at 120°C and amended with 0.4 l of soil suspension (soil:water; 1:100, sieved through a 38-µm screen) and

A. Nakano[1] · M. Kimura (✉)
Laboratory of Soil Biology and Chemistry,
School of Bioagricultural Sciences, Nagoya University,
Chikusa, Nagoya 464-8601, Japan
e-mail: kimuram@nuagr1.agr.nagoya-u.ac.jp
Fax: +81-52-7894136

K. Takahashi
Toyota Biotechnology and Afforestation Laboratory,
1099 Marune, Kurozasa, Miyoshi-cho, Nishikamo-gun,
Aichi 470-0201, Japan

Present address:
[1] Department of Natural Resource Sciences,
McGill University, Macdonald Campus, 21111 Lakeshore Road,
Ste-Anne-de-Bellevue, Québec, H9X 3V9, Canada

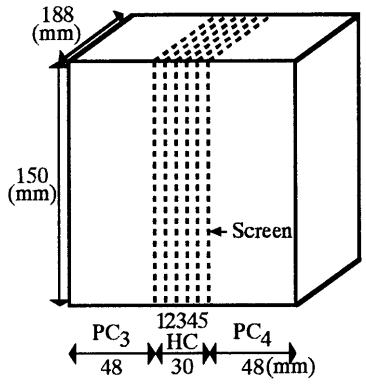

Fig. 1 PVC rectangular pot divided into 7 compartments by 26-µm screens [*HC* hyphal compartment, *PC*$_3$ C_3 plant compartment (alfalfa), *PC*$_4$ C_4 plant compartment (bahia grass)]

1.57 g of lime 10 days before pot preparation. The lime application raised the pH to 6.0–6.5. The mixture (0.369 atom % ^{15}N, AP^{15}N) was applied with 379 mg of 0:1:3 (N:P:K) fertilizer and 237 mg of coated urea (Meister-10, Chisso Corp., Japan, N 40%): ^{14}N-urea (0.366 AP^{15}N) or ^{15}N-urea (3.07 AP^{15}N). The coated urea released N to the soil slowly for 100 days.

Two hundred spores of *G. margarita* Becker & Hall (MAFF520054, National Grassland Research Institute, Japan) were inoculated into the middle of the pot. Surface-sterilized (15 min in 0.5% NaClO) seeds of the C_3 species alfalfa (*Medicago sativa* L.) and the C_4 species bahia grass (*Paspalum notatum* Fluegge) were planted. Culture was carried out in a glasshouse with natural lighting and temperature in the range 15–35°C for 116 days from May to September 1998. No nodules were found on alfalfa roots.

Cocultures of alfalfa and bahia grass

A total of eight coculture pots was established. Two replicate pots were harvested at four different times following the clipping of alfalfa shoots.

Each PVC rectangular pot (188×126×150 mm) was separated into seven compartments by screens of stainless steel mesh (26 µm): the C_3 plant compartment (PC$_3$), the C_4 plant compartment (PC$_4$), and five central AM hyphal compartments (HC1–5) (Fig. 1). The fungal hyphae could pass freely through the screens (Nakano et al. 1999), but the plant roots were restricted to their respective plant compartments. The pots were filled with 3 l of the same subsoil-sand mixture used in monocultures. The mixture was autoclaved for 1 h at 120°C and amended with 1 l of soil suspension and 3.93 g of lime as described above. The soil in the respective plant compartments was fertilized as for the monoculture pots, except that ^{14}N- and ^{15}N-urea were applied to the PC$_3$ and the PC$_4$ compartments, respectively.

Three hundred spores of *G. margarita* were inoculated into the middle of the central compartment (HC3). Surface-sterilized seeds of alfalfa and bahia grass were planted in the PC$_3$ and the PC$_4$ compartments, respectively. Culture was carried out in a glasshouse with natural lighting and temperature in the range 10–35°C from May 1998. All alfalfa shoots were clipped 116 days after planting. Culture was continued for 0 (two replicate pots, A1 and A2), 14 (pots B1 and B2), 42 (pots C1 and C2), or 70 (pots D1 and D2) days after clipping. Sprouts arising from alfalfa roots were clipped. No nodules were found on alfalfa roots.

Sample preparation and analysis

After the respective culture periods, water was withheld from the plants. Soil and plant samples were then taken from each cylindrical pot (monocultures) or from each compartment (cocultures).

Hyphae and spores of *G. margarita* were separated from each soil samples by wet sieving through a 106-µm screen. Thick-walled hyphae of >10 µm diameter (Nicolson 1959) and newly produced white spores (Nakano et al. 1999) were collected from the filtrate under a microscope. Yellow spores or those including large oil droplets were considered old (Becker and Hall 1976) and were excluded from analysis. Hyphae and spores were cleaned at least 10 times with tap water and then three times with deionized water before analysis. Hyphae or individual spores were placed into a tin capsule (5 mm diam. × 8 mm) for ^{13}C analysis and ca. 30 spores were used for ^{15}N analysis. The capsules were then closed.

Each plant sample was divided into shoot and root. A part of the root sample was kept for analysis of AM colonization (Kormanik and McGraw 1982). Shoot and root samples were cleaned separately under running water, dried for 48 h at 70°C, and pulverized in a vibrating sample mill (TI-100, Heiko Seisakusho Ltd., Japan). About 0.1 mg of the pulverized sample was put into a tin capsule for ^{13}C analysis. Approximately 0.5 and 1.5 mg aliquots of the pulverized samples of alfalfa and bahia grass, respectively, were put into tin capsules for ^{15}N analysis. The capsules were then closed.

Mineral N in each soil sample from the PC$_4$ compartment was extracted by 2 M KCl, diffused by addition of Devarda alloy and MgO (Keeney and Nelson 1982), and trapped on a glass filter with $KHSO_4$ (Jensen 1991). Each filter was put into a tin capsule, which was then closed.

δ^{13}C and AP^{15}N values were determined using an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH, Bremen, Germany) coupled with an elemental analyzer (NC 2500, ThermoQuest Italia S.p.A., Milan, Italy) by an interface (ConFlo II, Finnigan MAT GmbH, Bremen, Germany).

AM colonization of root systems was determined using the gridline-intersect method after staining with trypan blue (Giovannetti and Mosse 1980) according to the following equation:

Colonization (%) = (intersections with colonized root) ÷ (total intersections with root)×100

Dry weight of colonized root was calculated by multiplying the percentage of root colonization by the dry weight of root (Rajapakse and Miller 1994).

Table 1 δ^{13}C (‰) and atom % ^{15}N (AP^{15}N) of plant shoots and roots and *Gigaspora margarita* spores and hyphae in monocultures given ^{14}N- or ^{15}N-urea to the soil

Plant species	N treatment	δ^{13}C				AP^{15}N		
		Plant		Fungus		Plant		Fungus
		Shoot	Root	Spore	Hyphae	Shoot	Root	Spore
C_3 Alfalfa	^{14}N	–30.4	–29.1	–31.1	–29.4	0.370	0.368	0.369
	^{15}N	–30.8	–29.5	–31.5	–29.9	2.45	2.51	2.25
C_4 Bahia grass	^{14}N	–13.3	–13.3	–15.5	–17.0	0.372	0.370	0.369
	^{15}N	–13.4	–13.3	–15.9	–18.0	2.42	2.47	2.31

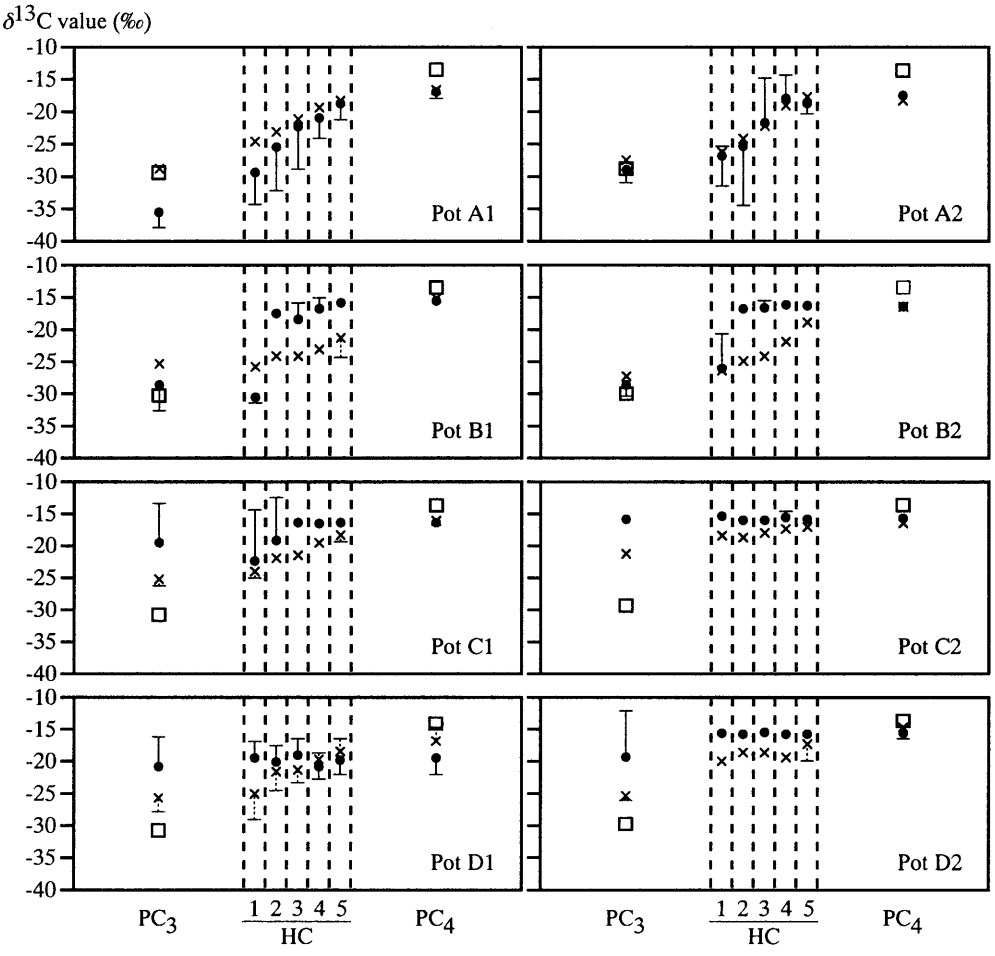

Fig. 2 Mean $\delta^{13}C$ ±SD (*bars*) of *Gigaspora margarita* spores (●) and hyphae (×) and plant roots (□) in different compartments of coculture pots. The cultures were continued for 0 (A1, A2), 14 (B1, B2), 42 (C1, C2) or 70 (D1, D2) days after clipping alfalfa

Results

Monocultures of alfalfa or bahia grass

$\delta^{13}C$ of roots of alfalfa and bahia grass were clearly different, averaging –29.3±0.3 for alfalfa and –13.3±0.1 for bahia grass (Table 1). Differences were found between $\delta^{13}C$ of roots (–30.6±0.1) and shoots (–29.3±0.1) for alfalfa ($P<0.01$) but not for bahia grass.

$\delta^{13}C$ of individual spores of *G. margarita* associated with alfalfa and bahia grass were –31.3±0.5 and –15.7±0.4, respectively, and were thus lower than those of host roots ($P<0.001$ for both plants, Table 1). Differences in $\delta^{13}C$ between spores and the roots of alfalfa and bahia grass were similar (2.0 and 2.4, respectively).

$\delta^{13}C$ of hyphae of *G. margarita* associated with alfalfa was –29.7±0.5 and was thus similar to that of host roots (Table 1). $\delta^{13}C$ for hyphae of *G. margarita* associated with bahia grass was –17.5±0.8 and was thus lower than that of host roots ($P<0.001$, Table 1). Difference in $\delta^{13}C$ between hyphae and roots was less ($P<0.001$) for alfalfa (0.4) than for bahia grass (4.3).

$AP^{15}N$ of roots in soil applied with ^{14}N- and ^{15}N-urea were clearly different ($P<0.001$), averaging 0.369±0.002 for roots with ^{14}N-urea and 2.49±0.04 for roots with ^{15}N-urea (Table 1). No differences were found in $AP^{15}N$ between roots and shoots.

$AP^{15}N$ of *G. margarita* spores in soil applied with ^{14}N- and ^{15}N-urea were clearly different ($P<0.001$), averaging 0.370±0.001 for spores with ^{14}N-urea and 2.28±0.12 for spores with ^{15}N-urea (Table 1). Differences were found in $AP^{15}N$ between spores and roots applied with ^{15}N-urea ($P<0.05$) but not with ^{14}N-urea.

Cocultures of alfalfa and bahia grass

$\delta^{13}C$ of individual spores of *G. margarita* varied between those of roots of alfalfa and bahia grass (Fig. 2). When culture was finished at the same time as alfalfa clipping, spore $\delta^{13}C$ increased linearly from the PC_3 to the PC_4 compartment (pots A1 and A2 in Fig. 2 and Table 2). When culture was continued for 14 days after clipping alfalfa, spore $\delta^{13}C$ in the PC_3 compartment and the next compartment (HC1) was –28.4±1.9 and was thus similar to that of alfalfa roots (–30.2). In contrast, spore $\delta^{13}C$ in the other compartments was –16.6±0.8 and thus similar to that of bahia grass roots (–13.2) (pots B1 and B2 in Fig. 2). When culture was continued for 42 or more days after clipping alfalfa, spore $\delta^{13}C$ in all compartments was

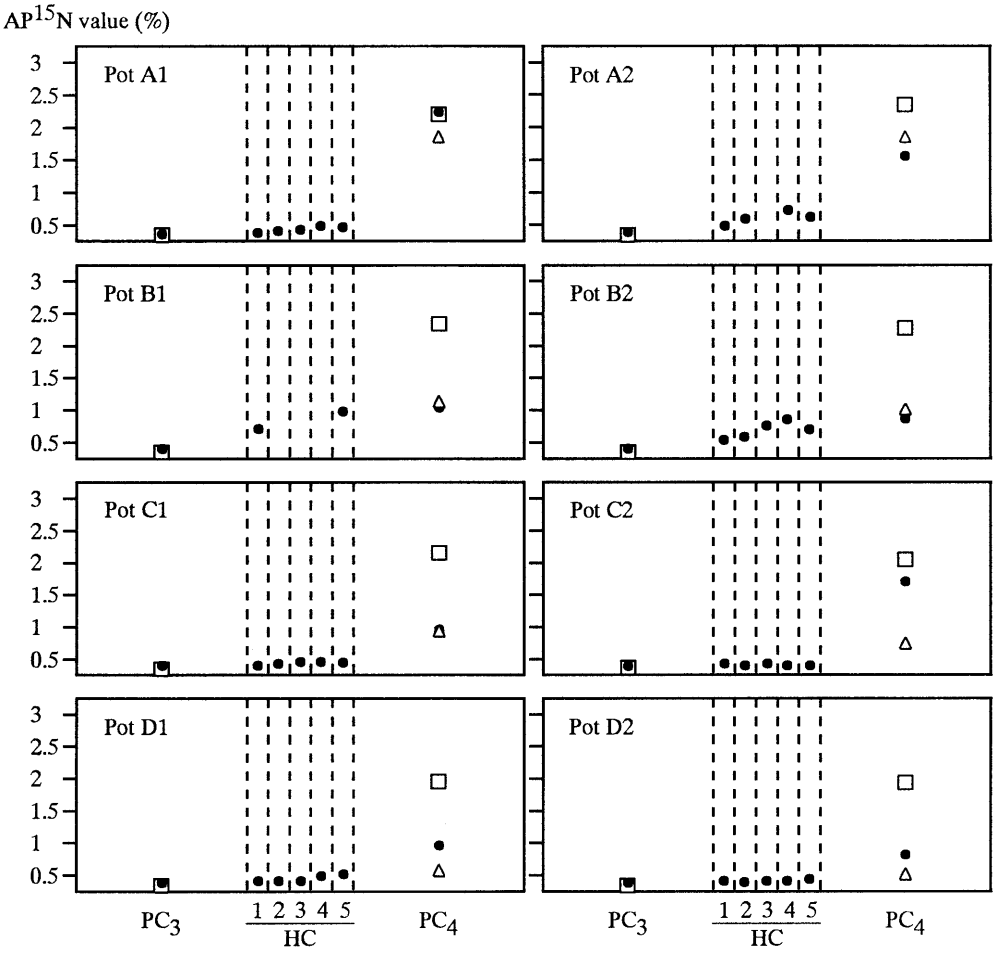

Fig. 3 Mean atom % ^{15}N ($AP^{15}N$) of *G. margarita* spores (●) and plant roots (□) in different compartments and mineral N (△) in the PC_4 compartment of coculture pots

Table 2 Mean $\delta^{13}C$ ±SD of *G. margarita* spores in cocultures of alfalfa and bahia grass

Pot	Samples	$\delta^{13}C$ (‰)	SD	Coefficient of variation	Regression slope (‰/mm)	Correlation coefficient	*P*
A1	35	–24.2	7.3	30.2	0.26	0.79	<0.001
A2	30	–22.2	6.2	27.7	0.17	0.61	<0.001
B1	30	–20.5	6.3	30.7	0.21	0.75	<0.001
B2	29	–20.2	5.8	28.7	0.20	0.76	<0.001
C1	35	–18.2	4.7	26.0	0.06	0.29	0.09
C2	28	–15.8	0.5	3.1	0.00	0.11	0.59
D1	34	–19.9	2.7	13.4	0.01	0.11	0.53
D2	34	–16.2	2.8	17.6	0.04	0.33	0.06

Table 3 Mean $\delta^{13}C$ ±SD of *G. margarita* hyphae in cocultures of alfalfa and bahia grass

Pot	Samples	$\delta^{13}C$ (‰)	SD	Coefficient of variation	Regression slope (‰/mm)	Correlation coefficient	*P*
A1	14	–21.7	4.0	18.6	0.17	0.96	<0.001
A2	14	–22.1	3.8	17.3	0.14	0.87	<0.001
B1	12	–22.3	4.0	18.0	0.14	0.84	<0.001
B2	13	–22.7	4.0	17.5	0.16	0.93	<0.001
C1	14	–21.0	3.1	14.7	0.13	0.94	<0.001
C2	14	–18.2	1.5	8.3	0.06	0.93	<0.001
D1	20	–21.4	3.7	17.2	0.13	0.76	<0.001
D2	21	–19.1	3.2	17.0	0.13	0.90	<0.001

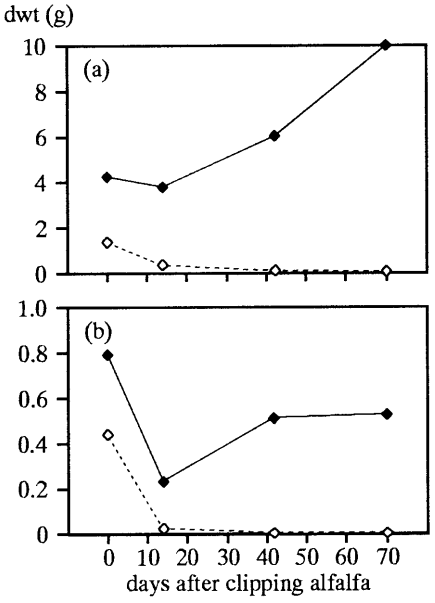

Fig. 4 Changes in dry weight (*dwt*) of (**a**) total roots and (**b**) colonized roots of alfalfa (◇) and bahia grass (◆) after clipping alfalfa in coculture pots

–22.4 to –15.4 and approached that of bahia grass roots (–13.6±0.2) (pots C1, C2, D1, and D2 in Fig. 2). Coefficients of variation in these pots were lower than those in the pots harvested earlier (Table 2). Furthermore, compared with the early-harvested pots, spore $\delta^{13}C$ in the late harvest pots did not vary with distance from the PC_3 compartment.

$\delta^{13}C$ of hyphae of *G. margarita* varied between those of the roots of alfalfa and bahia grass, similarly to that of spores (Fig. 2). Hyphal $\delta^{13}C$ increased linearly from the PC_3 to the PC_4 compartment in all eight pots (Table 3). After clipping alfalfa, hyphal $\delta^{13}C$ gradually approached that of bahia grass roots, in parallel to that of spores.

$AP^{15}N$ of *G. margarita* spores varied between those of the roots of alfalfa and bahia grass, but the pattern differed from that of $\delta^{13}C$ (Fig. 3). Spore $AP^{15}N$ in the PC_3 compartment was 0.390±0.009 and was thus similar to those of untreated soil, ^{14}N-urea, and alfalfa roots (0.373±0.004). Spore $AP^{15}N$ in the hyphal compartments (HC1–5) increased slightly from the PC_3 to the PC_4 compartment in the range 0.388–0.984. Spore $AP^{15}N$ in the PC_4 compartment initially approached those of ^{15}N-urea and bahia grass roots (2.30). After clipping alfalfa, root $AP^{15}N$ did not change in the PC_3 compartment but decreased in the PC_4 compartment ($P<0.01$). $AP^{15}N$ of mineral N decreased in the PC_4 compartment with time ($P<0.01$). Spore $AP^{15}N$ did not change in the PC_3 compartment but decreased in the PC_4 compartment with time ($P=0.16$). Spore $AP^{15}N$ decreased in the hyphal compartments (HC1–5) with time ($P<0.01$) but was higher at 14 days (0.731±0.154) than just after clipping alfalfa (0.514±0.113) ($P<0.01$).

For alfalfa, both total and colonized root dry weights decreased after clipping alfalfa in coculture pots ($P<0.05$ for total and $P=0.07$ for colonized) (Fig. 4). For bahia grass, colonized root dry weight did not change, while total root dry weight increased ($P<0.01$).

Discussion

In monocultures of alfalfa or bahia grass, $\delta^{13}C$ of individual spores of *G. margarita* was lower than those of host roots. The differences in $\delta^{13}C$ between spores and host roots did not vary significantly with plant species. These results are in accordance with the previous finding (Nakano et al. 1999), although the $\delta^{13}C$ differences between spores and host roots were somewhat smaller than those found previously (ca. 3.5). $\delta^{13}C$ of *G. margarita* hyphae also tended to be lower than those of host roots. The differences in $\delta^{13}C$ between hyphae and host roots, however, varied with plant species. This may be due to variation in content and composition of lipids in hyphae. $\delta^{13}C$ for the lipid fraction is lower than for the whole organism and other major constituents, such as proteins and carbohydrates, due to isotopic fractionation in the lipid synthetic pathway (DeNiro and Epstein 1977). The amount of phospholipids and neutral lipids in microorganisms appears to be related to biomass and storage materials, respectively (Tunlid and White 1992). Olsson et al. (1995) suggested that the ratio of fatty acid 16:1ω5 in the neutral lipid fraction to that in the phospholipid fraction may be used as an index of the physiological status of AM fungi.

In cocultures of alfalfa and bahia grass, $\delta^{13}C$ of individual spores of *G. margarita* varied between those of the roots of alfalfa and bahia grass, and increased linearly from alfalfa to the proximity of bahia grass at the time of alfalfa clipping. This confirms the previous finding that C in *G. margarita* spores comes from host plants in inverse proportion to the distance from the plants (Nakano et al. 1999). It seems unlikely that the changes in spore $\delta^{13}C$ were caused by C uptake from plant exudates, as recent observations indicate that C uptake into AM fungi occurs through intraradical and not extraradical mycelium (Shachar-Hill et al. 1995; Pfeffer et al. 1999). After clipping alfalfa, spore $\delta^{13}C$ approached that of bahia grass roots, which suggests that, in these circumstances, C in *G. margarita* spores comes from living bahia grass plants rather than from dying alfalfa.

At the time of alfalfa clipping, $\delta^{13}C$ of hyphae of *G. margarita* varied in space and time, similarly to that of spores. This suggests that the C origins of *G. margarita* hyphe and spores were the same. After clipping alfalfa, $\delta^{13}C$ of hyphae and spores changed in parallel. Hyphal production is considered to take place throughout the culture period, while spore production occurs at the end (Smith and Read 1997). The results of a preliminary experiment suggested that more spores of *G. margarita* are produced after soil drying and plant wilting than after well-watered culture (data not shown). Hence, in this study, water was withheld from the plants after their re-

spective culture periods. It seems reasonable to suppose that hyphal $\delta^{13}C$ reflected successive contributions of respective plants to fungal C and that spore $\delta^{13}C$ reflected late contributions.

While some researchers have studied the effects of AM on C transfer between plants (Hirrel and Gerdemann 1979; Francis and Read 1984), there is no evidence that transferred C is released to plant tissues even in plants stressed by shading or clipping. Although Waters and Borowicz (1994) suggested that net C flow was away from clipped plants toward unclipped plants, they did not detect significant ^{14}C in the shoots of unclipped plants 72 h after the onset of labeling. Fitter et al. (1998) pointed out that for linked C_3 and C_4 plants the differences in $\delta^{13}C$ between roots and shoots were not significant after clipping of all plants. Our results, however, indicate that decreasing amounts of C for spore and hyphal formation in *G. margarita* came from the clipped plants.

In cocultures of alfalfa and bahia grass, the changes in $AP^{15}N$ of *G. margarita* spores differed from those in $\delta^{13}C$. In the PC_3 compartment, spore $AP^{15}N$ was slightly higher than in monocultures of alfalfa given ^{14}N-urea ($P<0.05$) and did not vary with time ($r=0.30$, $P=0.29$) or $AP^{15}N$ of alfalfa roots ($r=0.29$, $P=0.49$). In the PC_4 compartment, on the other hand, spore $AP^{15}N$ was lower than in monocultures of bahia grass given ^{15}N-urea ($P<0.05$) and decreased with time. The pattern differed from that of bahia grass roots ($r=0.21$, $P=0.61$) but was similar to that of mineral N ($r=0.69$, $P=0.06$). Thus spore $AP^{15}N$ changed with time in parallel to soil mineral N but not to roots. This confirms the previous finding that N in *G. margarita* spores comes mostly from the soil, possibly in mineral forms (Nakano et al. 2001) and suggests that little of the N in *G. margarita* spores comes from host plants, whether living or dying. It seems reasonable to suppose that the decreases in $AP^{15}N$ of roots, mineral N, and spores in the PC_4 compartment were caused by N depletion in the soil, because most of the N in the coated urea was released to the soil for 100 days, before clipping alfalfa.

N transfer has been shown between plants colonized by AM fungi (van Kessel et al. 1985). However, net transfer of N between living plants is considered to be insignificant (Johansen and Jensen 1996). Some researchers have examined the effects of AM on N transfer from dying donor plants to living receiver plants by applying ^{15}N to the donor plants and monitoring increases in N and ^{15}N in the receiver plants. Eissenstat (1990) detected a significant increase in N but a less significant increase in ^{15}N in the receiver plants, suggesting a shift in competition between plants rather than nutrient transfer. Ikram et al. (1994) detected similar ^{15}N contents in AM and non-AM plants and suggested that the transfer occurred indirectly via root exudation and mineralization of nutrients. These studies, however, did not clarify the change in N source for AM fungi by clipping one of the host plants. Our results indicate that N in *G. margarita* spores came less from the clipped than the unclipped plants.

In conclusion, the results obtained in this study indicate that dying plants do not significantly contribute to C or N within AM fungal spores. Furthermore, they confirm that spore C in AM fungi comes from living plants and spore N mostly from the soil.

Acknowledgements The authors are grateful to Drs. S. Yoshida, T. Ezawa, and M. Taniguchi (Nagoya University), Dr. H. Ando (Yamagata University), and Dr. M. Saito (National Grassland Research Institute) for the supply of materials and advice on methods and to Dr. R. T. Koide (Pennsylvania State University) for help in revising the manuscript.

References

Becker WN, Hall IR (1976) *Gigaspora margarita*, a new species in the Endogonaceae. Mycotaxon 4:155–160

Bergelson JM, Crawley MJ (1988) Mycorrhizal infection and plant species diversity. Nature 334:202

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263

Eissenstat DM (1990) A comparison of phosphorus and nitrogen transfer between plants of different phosphorus status. Oecologia 82:342–347

Fitter AH, Graves JD, Watkins NK, Robinson D, Scrimgeour C (1998) Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. Funct Ecol 12:406–412

Francis R, Read DJ (1984) Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal myceliu. Nature 307:53–56

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Grime JP, Mackey ML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Nature 328:420–422

Hirrel MC, Gerdemann JW (1979) Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. New Phytol 83:731–738

Ikram A, Jensen ES, Jakobsen I (1994) No significant transfer of N and P from *Pueraria phaseoloides* to *Hevea brasiliensis* via hyphal links of arbuscular mycorrhiza. Soil Biol Biochem 26:1541–1547

Jensen ES (1991) Evaluation of automated analysis of ^{15}N and total N in plant material and soil. Plant Soil 133:83–92

Johansen A, Jensen ES (1996) Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. Soil Biol Biochem 28:73–81

Keeney DR, Nelson DW (1982) Nitrogen – inorganic forms. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, Wis., pp 643–698

Kormanik RP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul, Minn., pp 37–45

Nakano A, Takahashi K, Kimura M (1999) The carbon origin of arbuscular mycorrhizal fungi estimated from $\delta^{13}C$ values of individual spores. Mycorrhiza 9:41–47

Nakano A, Takahashi K, Koide RT, Kimura M (2001) Determination of the nitrogen source for arbuscular mycorrhizal fungi by ^{15}N application to soil and plants. Mycorrhiza 10:267–273

Nicolson TH (1959) Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans Br Mycol Soc 42:421–438

Olsson PA, Bååth E, Jakobsen I, Söderström B (1995) The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol Res 99:623–629

Pfeffer PE, Douds DD Jr, Bécard G, Shachar-Hill Y (1999) Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol 120:587–598

Rajapakse S, Miller JC (1994) Methods for studying vesicular-arbuscular mycorrhizal root colonization and related root physical properties. In: Norris JR, Read DJ, Varma AK (eds) Techniques for mycorrhizal research. Academic, London, pp 761–776

Shachar-Hill Y, Pfeffer PE, Douds D, Osman SF, Doner LW, Ratcliffe RG (1995) Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leek. Plant Physiol 108:7–15

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, London

Soil Survey Staff (1998) Keys to soil taxonomy. United States Department of Agriculture, Natural Resources Conservation Service, Washington, D.C.

Truog E (1930) Determination of the readily available phosphorus of soils. J Am Soc Agron 22:874–882

Tunlid A, White DC (1992) Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Stotzky G, Bollag J-M (eds) Soil biochemistry. Dekker, New York, pp 229–262

Van Kessel C, Singleton PW, Hoben HJ (1985) Enhanced N-transfer from a soybean to maize by vesicular-arbuscular mycorrhizal (VAM) fungi. Plant Physiol 79:562–563

Waters JR, Borowicz VA (1994) Effect of clipping, benomyl, and genet on ^{14}C transfer between mycorrhizal plants. Oikos 71:246–252