Journal of Agricultural Science (2014), **152**, 370–378. © Cambridge University Press 2013
doi:10.1017/S002185961300004X

CROPS AND SOILS RESEARCH PAPER

The effect of arbuscular mycorrhizal fungi on total plant nitrogen uptake and nitrogen recovery from soil organic material

S. SAIA[1], E. BENÍTEZ[2], J. M. GARCÍA-GARRIDO[3], L. SETTANNI[1], G. AMATO[1]* AND D. GIAMBALVO[1]

[1] *Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy*
[2] *Department of Environmental Protection, Estación Experimental del Zaidín, CSIC, c/Prof. Albareda 1, 18008 Granada, Spain*
[3] *Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, c/Prof. Albareda 1, 18008 Granada, Spain*

(*Received 6 July 2012; revised 8 January 2013; accepted 8 January 2013; first published online 7 February 2013*)

SUMMARY

Arbuscular mycorrhizal (AM) fungi increase nitrogen (N) uptake by their host plants, but their role in plant N capture from soil organic material is still unclear. In particular, it is not clear if AM fungi compete with the host plant for the N coming from the decomposing organic matter (OM), especially when the AM extraradical mycelium (ERM) and plant roots share the same soil volume. The goal of the present research was to study the effects of AM fungi on wheat N capture after the addition of ^{15}N-labelled OM to soil. Durum wheat (*Triticum durum*) was grown under controlled conditions in a sand:soil mix and the following treatments were applied: (1) AM inoculation with *Glomus mosseae* and uninoculated control; and (2) soil amended with ^{15}N-enriched maize leaves and unamended soil. The addition of OM reduced plant growth and N uptake. The AM fungi increased both plant growth and N uptake compared with uninoculated control plants and the effect was enhanced when wheat was grown in soil amended with OM compared with the unamended control. Although AM fungi increased soil N mineralization rates and total plant N uptake, they strongly reduced wheat N recovery from OM, suggesting that AM fungi have marked effects on competition between plants and bacteria for the different N sources in soil.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are ubiquitous soil-borne micro-organisms belonging to the phylum *Glomeromycota* (Schüßler *et al*. 2001) that inhabit the roots of land plants in an obligate biotrophic relationship. These symbioses create a living connection between plant roots and soil and play a key role in promoting the uptake of nutrients, especially when such nutrients are scarce (Smith & Read 2008). Nutrient uptake is increased mainly by the highly absorbent surface of the hyphal net, its ability to explore the soil volume and its effectiveness at absorbing and utilizing nutrients (Miyasaka & Habte 2001). Although the advantages of AM symbiosis have been demonstrated with respect to the uptake of phosphorus (Barea *et al*. 2005), the effect of the symbiosis on plant nitrogen (N) status and the impact of absorbed N on plant N balance are still unclear (Hodge *et al*. 2010). Some studies have shown that AM fungi are involved in the uptake of N from inorganic sources (Ames *et al*. 1983; Johansen *et al*. 1993), namely nitrate (Tobar *et al*. 1994) and ammonium (Hawkins *et al*. 2000). In addition, AM fungi may be important in the uptake of organic N (Cliquet *et al*. 1997) and recent findings indicate that AM fungi possess at least part of the enzymatic machinery needed for the direct uptake of amino acids (Cappellazzo *et al*. 2008). Such results are consistent with work by Hodge *et al*. (2001) and Whiteside *et al*. (2009) indicating that AM fungi promote the decomposition and subsequent uptake of organic

* To whom all correspondence should be addressed. Email: gaetano.amato@unipa.it

N. The mechanisms through which AM fungi stimulate organic N mineralization are still uncertain. Recent findings have shown that colonization by AM fungi can alter rhizodeposition (Wamberg *et al*. 2003), affecting bacteria and other micro-organisms in the soil that are responsible for the decomposition of organic matter (OM). Thus AM fungi can play an indirect stimulatory role in the uptake of N from organic sources (Hodge *et al*. 2010). It has been suggested that the small size of AM hyphae (Jones *et al*. 2005) and their ability to synthesize pectinases and cellulases (García-Romera *et al*. 1991; García-Garrido *et al*. 1992) allow the mycorrhizal extraradical mycelium (ERM) to penetrate into decomposing OM; this permits the AM fungi to compete with bacteria for the decomposition products. However, although there is good evidence that AM fungi benefit from OM in the soil (Leigh *et al*. 2009; Hodge *et al*. 2010), it is still unclear whether this can be an advantage for a plant host. In some research, the presence of AM fungi did not increase plant N content (Hawkins *et al*. 2000; Hodge *et al*. 2001; Hodge 2003; Leigh *et al*. 2009), whereas other research showed that AM fungi did indeed enhance root N uptake (Faure *et al*. 1998; Leigh *et al*. 2011). Hodge *et al*. (2010) showed that AM fungi can acquire large quantities of N from OM but also that they use large proportions of it to satisfy their own nutritional needs. It is likely that even if AM fungi, plant hosts and bacteria could collaborate in accelerating the decomposition of OM, they would have to compete for the decomposition products, particularly when N is scarce.

The present research tested the hypothesis that AM fungi, in an environment of limited N, can enhance plant N uptake from OM added to soil by influencing OM decomposition through bacterial activity either directly or indirectly. To do so, wheat plants were inoculated with AM fungi, and the effects on soil enzymatic activities related to the mineralization of organic N (both in soil amended with ^{15}N-labelled-maize leaves and in untreated soil) and on further wheat N capture were examined.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the Zaidín Experimental Station (CSIC; Granada, Spain, 37° 10′ N, 3° 35′ W, 680 m a.s.l.), in a climate-controlled glasshouse with 25/19 °C day/night temperature and a photoperiod of 16 h. Additional light at a photosynthetic photon flux density of 460 µmol/m^2/s was provided, if necessary.

A complete randomized factorial design replicated four times was adopted. Treatments were (1) AM inoculation: *Glomus mosseae* (Nicol. &. Gerd.) Gerd. & Trappe isolate BEG 12 (+Myc) and uninoculated control (–Myc); and (2) addition of OM: soil amended with ^{15}N-enriched maize leaves (+OM) and unamended soil (–OM).

For mycorrhizal treatments, the inoculum was obtained from a thoroughly homogenized rhizosphere sample removed from an open-pot culture of *Sorghum bicolor* L. and consisting of soil, spores (70 spores/g soil) and mycelia. The AM inoculum was added to the appropriate pots at a rate of 1 g inoculum per pot. For the OM treatments, ^{15}N-enriched OM was prepared by cultivating maize on ^{15}N-enriched soil. Maize was harvested at anthesis and separated into roots, stems and leaves. Leaves were oven-dried at 80 °C for 1 day, chopped (*c*. 2 mm) and used as organic amendment. The N concentration of maize leaves was 19·0 g/kg (with an isotopic composition of 4·78 atom% ^{15}N) and the C:N ratio was 22·6 : 1.

Each pot (diameter 100 mm, height 110 mm) was filled with 600 g of a quartz sand:soil mixture (2 : 1). Soil properties were as follows: 37% (by volume) sand, 43% silt and 20% clay; 10·44 g/kg organic carbon (C), pH 8·1 (soil:water 1 : 2); 0·12 mS/cm saturated electrical conductivity (E.C.) (25 °C); 1·05 g/kg total N (Kjeldhal); 6·2 mg/kg phosphorus (P, as P_2O_5; Olsen); 132 mg/kg potassium (K, as K_2O); 10·06% total calcium (Ca); 99 mg/kg soluble Ca; and 16 mg/kg magnesium (Mg). Both soil and sand were sieved through a 2 mm mesh and autoclaved at 121 °C for 20 min. The ^{15}N-enriched maize biomass was added at a rate of 4·6 g dry OM/kg mixture and both OM-amended and unamended mixtures were steam-sterilized at 95 °C for 1 h on three consecutive days. Soil autoclaving and steam sterilization were performed in order to completely impair soil biological (both fungal and bacterial) activity. The bacterial microflora was extracted by suspending 500 g soil or AM inoculum in 1·5 litres distilled water. After shaking and decanting, the suspension was filtered (11 µm mesh) to discard natural AM fungi. Before starting the experiment, each pot received 30 ml of soil suspension filtrate to reintroduce the natural microbial community and 30 ml of AM inoculum suspension filtrate to normalize the starting microbial community. The amount of OM added with the AM inoculum was

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

negligible compared with the quantity added in the OM treatment.

Seeds of durum wheat (*Triticum durum* Desf.; cv Simeto) were surface-sterilized and germinated on wet filter paper in Petri dishes for 3 days. Five seedlings were transplanted into each pot; 4 days after transplantation, pots were thinned to three plants each. During the experiment, each pot received 5 ml modified Hoagland's solution (Hoagland & Arnon 1950) once every 5 days and 50 ml tap water once every 3 days. The modified Hoagland's solution used in the experiment lacked P and had only 10% N to limit the amount of inorganic N available for plant growth, as in prior studies (Reynolds *et al.* 2005).

Pots were harvested 7 and 9 weeks after transplanting (WAT). At each harvest, a bulk soil sample was taken from each pot and total plant biomass was measured. Soil samples were stored at −80 °C for further analysis.

Plant and soil analysis

Plant biomass was immediately separated into roots and shoots, and fresh weights were recorded. Roots were rinsed free of soil, cut into 10 mm fragments and mixed thoroughly. Representative root samples were taken to determine the overall colonization of roots by AM fungi and to measure metabolically active (alkaline phosphatase (ALP)) AM fungal infection. Shoots and the remaining roots were oven dried at 80 °C for 24 h and dry weights recorded. To measure total root infection by AM fungi, root samples were cleared with 100 g/l potassium hydroxide (KOH) and stained with 50 mg/l trypan blue following the method described by Phillips & Hayman (1970). To measure ALP activity, root samples were cleared (as described by Vierheilig *et al.* 2005) for 2 h in a solution containing 0·05 M Tris/citric acid (pH 9·2), 50 mg/l sorbitol, 15 units/ml cellulase and 15 units/ml pectinase (both enzymes from *Aspergillus niger*). Root samples were subsequently rinsed in distilled water and placed in the appropriate staining for ALP as described in Tisserant *et al.* (1993). Measurements of root AM infection and ALP activity were made by observing root fragments under the microscope and counting 250–300 total intersections using the grid intersect method (Giovannetti & Mosse 1980).

Shoots and roots of wheat were analysed for total N and ^{15}N enrichment using an elemental analyser–isotope ratio mass spectrometer (EA–IRMS, Carlo Erba NA1500).

Total bacterial counts (TBC) were performed on soil samples (2 g) taken from each pot. Soil samples were serially diluted, and each dilution was plated onto nutrient agar (OXOID, Milan, Italy) treated with 15 mg/l nystatin to impair fungal growth (Seeley & VanDemark 1981) and aerobically incubated at 30 °C. Colony-forming units were counted after 4 days and again after 7 days to allow for the development of slower growing colonies. Analyses were carried out in duplicate.

The activity of four soil enzymes was measured: dehydrogenase, as an index of microbial activity (García *et al.* 1993); casein protease (also referred to as 'casein hydrolysing activity' or 'caseinase'), as a measure of protein hydrolysis to mono – and dipeptides (Ladd & Butler 1972); benzoylargininamide (BAA)-protease; and urease, as a measure of amino acid deamination (Kandeler & Gerber 1988; Tabatabai 1994). For the dehydrogenase assay, 1·0 g soil samples were mixed with 0·2 ml of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) at a concentration of 40 mg/l in distilled water for 20 h at 22 °C in darkness. Then 10 ml of a mixture of 1 : 1·5 ethylene chloride:acetone was added and the solution was shaken for 1 h to extract iodonitrotetrazolium formazan (INTF). The solution was filtered through Whatman no. 5 filter paper and INTF was measured spectrophotometrically at 490 nm. Dehydrogenase activity was expressed as µg INTF/g/h.

For the caseinase assay, 1·0 g soil samples were incubated in 12 ml tubes at 50 °C for 2 h in 2·5 ml of 100 mg/l casein dissolved in 0·1 M TRIS-HC1 buffer at pH 8·1. Enzyme activity was stopped by adding 1 ml of 175 ml/l trichloroacetic acid (TCA). Tubes were centrifuged, 2·0 ml supernatants were extracted, and absorbance (700 nm) was measured colorimetrically after adding 3·0 ml of 2·8 N Na_2CO_3 and 1 ml of three-fold diluted Folin–Ciocalteu reagent. After subtraction of blanks, caseinase activity was computed as µg Tyrosine/g/h.

For urease and BAA-protease assays, 1·0 g soil samples were mixed with 2 ml of 0·1 M , pH 7·0 phosphate buffer and with 0·5 ml of 6·4 M urea or 0·03 M Na-BAA for urease and BAA-protease, respectively. Shaken incubation was performed for 2 h at 37·0 °C (for urease) or 39 °C for (for BAA-protease). The ammonium released in the hydrolytic reaction was extracted by adding 8 ml of 2-M potassium chloride (KCl) (which is also meant to suppress further hydrolysis of urea). Supernatants (0·5 ml) were transferred to clean tubes and mixed

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

with 0·2 ml of a nitroprusside sodium dihydrate and sodium salicylate solution and 0·2 ml of a mixture of trisodium citrate, sodium hydroxide (NaOH) and dichloroisocyanuric acid sodium salt dehydrate, as described in García *et al.* (1993). Absorbance was measured at 690 nm and expressed as g N hydrolysed/g dry soil.

Calculations and statistical analysis

The N recovery fraction of wheat on a per-pot basis and percentage basis was calculated as follows:

$$N_{REC} = N_t \times \frac{^{15}N_a - {}^{15}N_b}{^{15}N_c - {}^{15}N_b} \; ; \; \%\, N_{REC} = \frac{N_{REC}}{f} \times 100$$

where N_{REC} is the amount of N (mg/pot) from OM detected in wheat biomass; N_t is the total *N* content (g/pot) in wheat; $^{15}N_a$, $^{15}N_b$ and $^{15}N_c$ are the ^{15}N isotopic concentrations of wheat grown with the organic amendment, without the organic amendment and of the organic amendment itself, respectively; and *f* is the total N (mg/pot) in the organic amendment. The ratio between the plant N derived from OM and total plant N capture (N_{REC}/N_t) was calculated.

Data on plant production, quality, root AM infection, ALP and soil enzymatic activities were subjected to analysis of variance (ANOVA) according to the experimental design. Variables corresponding to proportions were arcsine transformed before analysis to assure a better fit with the Gaussian law distribution. Normality was confirmed using a Kolmogorov-Smirnov test.

RESULTS

Mycorrhizal colonization and fungal activities in roots

No mycorrhizal infection was observed in uninoculated pots. In mycorrhizal plants, root AM colonization was, on average, 19·8% at 7 WAT and 34·0% at 9 WAT. Root AM colonization (Fig. 1*a*) and fungal ALP activity (Fig. 1*b*) were significantly higher ($P<0·01$) in +OM than –OM treatments in both samplings.

Plant growth and N uptake

Addition of OM markedly decreased wheat growth. At 7 WAT, the total biomass of plants in +OM treatments was 41% lower than that of –OM plants; differences between treatments diminished at 9 WAT (−15%; Table 1). The AM symbiosis had a positive effect on plant growth: mycorrhizal wheat yielded 7% more biomass than the non-mycorrhizal control at 7 WAT and the advantage increased to 20% at 9 WAT. Although the interaction between treatments (AM inoculation × OM addition) was not always significant, the benefit of AM symbiosis on shoot and root growth of wheat was greater in +OM than in –OM treatments, particularly at 9 WAT.

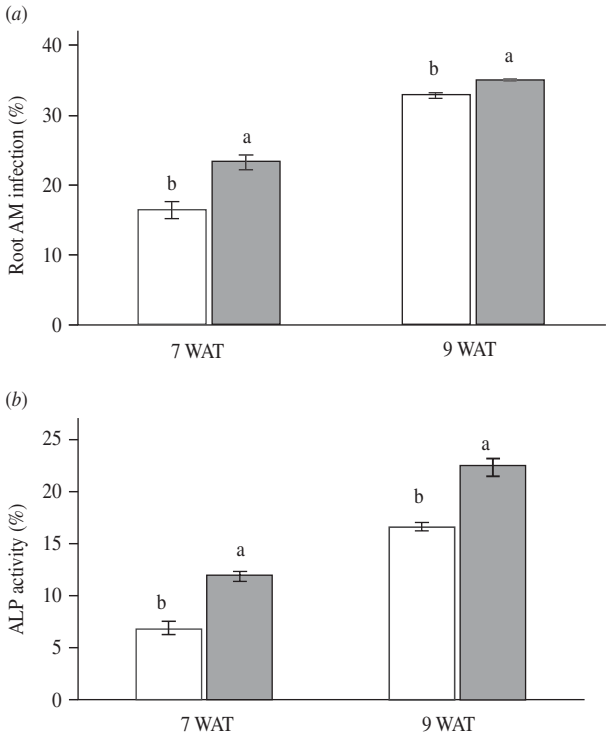

Fig. 1. Root colonization by (*a*) AM fungi and (*b*) fungal ALP activity in roots of wheat grown in soil without (open bars) or with (closed bars) added OM at 7 and 9 WAT. Different letters represent significant differences ($P<0·05$). Data are means ± S.E. ($n=4$).

The addition of OM resulted in a reduction of total N uptake by wheat at both 7 and 9 WAT. N uptake was higher in +Myc than in –Myc plants, especially in +OM treatments.

The $\%N_{REC}$ from OM detected in wheat biomass was, on average, 2·73% at 7 WAT and 5·14% at 9 WAT. In both samplings, $\%N_{REC}$ was 40% lower in +Myc than in –Myc treatments (Fig. 2*a*). Therefore, the ratio N_{REC}/N_t was significantly lower in mycorrhizal than in uninoculated plants (−55% at 7 WAT and −47% at 9 WAT; Fig. 2*b*).

Total bacterial count and enzymatic activities

The addition of OM resulted in a significant increase in TBC at both 7 and 9 WAT (Table 2). Mycorrhyzal

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

Table 1. *Shoot and root dry matter and nitrogen (N) capture in wheat inoculated with the AM fungus G. mosseae (+Myc) or not inoculated (−Myc) grown in soil amended with OM (+OM) or without OM (−OM) at different harvest time points (7 and 9 WAT)*

		Shoot DM		Root DM		Root:Shoot ratio		Shoot N		Root N		Total N	
		g/pot		g/pot		g/g		mg/pot		mg/pot		mg/pot	
		7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks
–OM	–Myc	0·31	0·69	0·37	0·31	1·22	0·45	3·82	7·84	2·15	1·94	5·97	9·78
	+Myc	0·34	0·71	0·34	0·42	1·01	0·59	4·25	9·06	1·68	1·90	5·93	10·96
+OM	–Myc	0·21	0·53	0·15	0·26	0·73	0·49	2·85	6·05	0·90	1·63	3·75	7·68
	+Myc	0·24	0·70	0·19	0·31	0·79	0·44	3·71	7·41	1·21	1·62	4·92	9·03
P-values	OM	<0·001	<0·01	<0·001	<0·01	<0·001	<0·05	<0·01	<0·05	<0·001	<0·01	<0·001	<0·01
	Myc	<0·05	<0·01	NS	<0·01	<0·05	NS	<0·05	<0·05	NS	NS	NS	<0·05
	OM×Myc	NS	<0·05	<0·01	NS	<0·001	<0·01	NS	NS	<0·01	NS	<0·05	NS

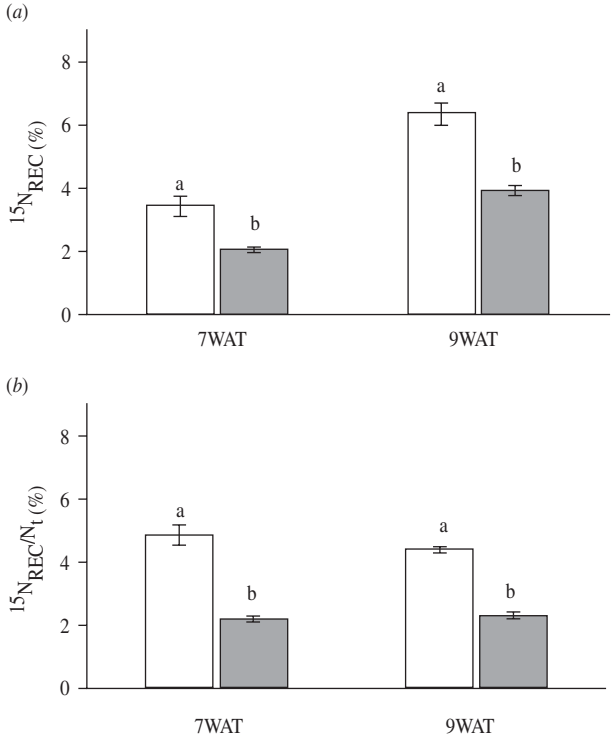

Fig. 2. (*a*) Nitrogen recovery (%$^{15}N_{REC}$) and (*b*) $^{15}N_{REC}/N_t$ of N released from OM on total wheat N in uninoculated (open bars) and mycorrhizal treatments (closed bars) 7 and 9 WAT. Different letters represent significant differences ($P<0·05$). Data are means ± S.E. ($n=4$).

inoculation did not affect TBC in –OM soil but significantly increased the concentration of bacteria in +OM soil.

On average, all enzymatic activities except for urease were significantly higher in +OM than –OM soil on both sampling dates (Table 2). Similarly, although the AM inoculum negatively affected urease, it caused an increase in all other enzymatic activities (dehydrogenase, caseinase and BAA-protease) and the effects were more evident in the +OM treatments. At 9 WAT, in particular, AM symbiosis markedly increased dehydrogenase activity in +OM treatments. At 7 WAT, caseinase was enhanced by the presence of the AM fungi, especially in the +OM treatments (+45% compared with uninoculated soil), whereas at 9 WAT, inoculation with AM fungi increased caseinase activity by 20% in both +OM and –OM soil. BAA-hydrolysing activity was markedly higher in +Myc than in –Myc treatments, especially at 9 WAT, whereas urease activity in both samplings was slightly but significantly reduced by AM fungi. The overall deamination activity (urease + BAA-protease) in soil was higher in inoculated pots than in their control counterparts.

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

Table 2. *Total bacterial counts, dehydrogenase, urease, BAA-protease and casein protease in soil amended with OM (+OM) or without OM (−OM), in which wheat was grown in symbiosis with AM fungus G. mosseae (+Myc) or grown without fungus (−Myc) at different plant harvest time points (7 and 9 WAT)*

		TBC		Dehydrogenase		Urease		BAA protease		Casein protease	
		Log colony forming units/g of soil		μg INTF g/h		μg NH_4^+ g/h		μg NH_4^+ g/h		μg Tyrosine/g/h	
		7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks	7 weeks	9 weeks
−OM	−Myc	6·98	6·83	1·26	1·11	0·91	0·89	1·93	1·89	1·14	1·03
	+Myc	6·85	6·82	1·12	0·98	0·51	0·80	1·93	2·51	1·25	1·24
+OM	−Myc	7·09	6·91	1·64	1·34	0·82	0·95	1·95	2·11	1·24	1·33
	+Myc	7·25	7·05	1·62	1·86	0·57	0·74	2·96	3·32	1·80	1·61
P-values	OM	<0·001	<0·001	<0·001	<0·001	NS	NS	<0·001	<0·001	<0·001	<0·05
	Myc	NS	NS	NS	<0·01	<0·001	<0·01	<0·001	<0·001	<0·001	<0·05
	OM × Myc	<0·01	<0·05	NS	<0·001	<0·01	NS	<0·001	<0·05	<0·001	NS

DISCUSSION

Mycorrhyzal root colonization and ALP activity were higher in wheat plants grown in soil to which OM was added than in unamended soil. This result contrasts with the findings of Hodge *et al.* (2000*a*), who found that mycorrhizal colonization of *Plantago lanceolata* roots was not affected by OM added to soil. One reason for this discrepancy could be the fact that the soil fertility, particularly the mineral N availability, was markedly lower in the present experiment. In fact, Staddon *et al.* (2004) and Gamper *et al.* (2004) found a reduction in mycorrhizal root colonization when plants were grown in nutrient-rich soils; in such soils it is easier for plants to autonomously satisfy their nutritional needs without expending photosynthates on mycorrhizae. In the present experiment, the addition of OM may have caused a temporary sequestration of nutrients (favoured by the high C : N ratio of the added organic residues, as also observed by Killham 1994 and Geisseler & Horwath 2009), thus reducing their availability and increasing plant dependence on AM symbiosis. In fact, the addition of OM increased the concentration and activity of bacteria in the soil. This means that bacteria outcompete plants for nutrients in the short term (Jackson *et al.* 1989; Kaye & Hart 1997), causing a decrease in plant growth and N uptake, as observed in other studies (Seligman *et al.* 1986; Hodge *et al.* 1998).

On average, AM fungi resulted in an increase in the concentration and activity of bacteria in the soil only when OM was added. Several studies have shown that the presence of AM fungi can stimulate microbial growth and alter the structure of the microbial population (Secilia & Bagyaraj 1987; Marschner & Crowley 1996; Andrade 2004); such effects can lead to faster OM decomposition. Several mechanisms can explain the effects of AM fungi on soil bacteria: the bacteria can use the ERM as a vehicle for spreading into soil organic residue (Ravnskov *et al.* 1999); AM fungi can modify the amount, nature and distribution of plant-derived C compounds in the soil (Toljander *et al.* 2007); or the ERM may bring available organic C to micro-organisms in the hyphosphere, favouring the mineralization of OM in the soil (Schimel & Weintraub 2003). The different C availability could explain why AM fungi had a different effect on the concentration and activity of bacteria in the +OM and –OM treatments.

On average, plants inoculated with *G. mosseae* yielded 13% more biomass relative to non-mycorrhizal

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

plants. Several studies have shown that AM symbiosis improves plant growth and nutrient uptake, especially when plants are grown under nutrient-limiting conditions (Azcón & Ocampo 1981; Vierheilig & Ocampo 1991), as in the present study. Other studies of AM infection in wheat, however, have shown responses that vary depending on factors such as the plant and AM fungal genotypes and symbiotic efficiencies and different growth conditions (Al-Karaki & Al-Omoush 2002; Li *et al.* 2005, 2006). In accordance with other studies (Tobar *et al.* 1994; Azcón *et al.* 2001), a higher N uptake was observed in +Myc than in –Myc plants. Through AM fungi, plants can better scavenge the soil volume (Miyasaka & Habte 2001), which enhances their ability to absorb available N. This effect is particularly important when AM extraradical hyphae explore soil volumes different from those explored by the plant root system (Hodge *et al.* 2001). In pots, however, the wheat root system is often able to explore the entire soil volume. This could make AM symbiosis less advantageous for nutrient uptake. Therefore, as suggested by Hodge *et al.* (2000*b*), AM symbiosis could improve N uptake in host plants because it is more effective than roots alone in competing with soil microorganisms for inorganic N. An indirect confirmation of this mechanism can be found in the higher proteolytic activity observed in the +AM than in the –AM rhizosphere. In fact, because soil proteases are inducible enzymes released progressively (by bacteria, but not by AM fungi; Smith & Read 2008) when the organic to inorganic N ratio of soil increases (Gill & Modi 1981), the higher proteolytic activity in +AM than in –AM treatment may depend on a mycorrhizal depletion of inorganic N.

The percentage of $^{15}N_{REC}$ from OM in non-mycorrhizal treatments was 3·4% at 7 WAT and 6·4% at 9 WAT. These values are comparable with those observed by Hodge *et al.* (1998), who found, after 39 days of growth, $\%N_{REC}$ values in different grasses ranging from 3·2 to 5·0% from OM when *Lolium perenne* shoots with a 31 : 1 C : N ratio were added to soil. In the present experiment, root infection with AM fungi (+Myc treatments) strongly reduced the recovery of ^{15}N applied with OM (−41% on average compared with –Myc treatments). By contrast, Atul-Nayyar *et al.* 2009 reported that AM symbiosis increases the uptake of N from added OM; however, they found that only the ERM and not the plant roots were allowed access to the OM in the soil, whereas in the present study both mycorrhizal hyphaea and mycorrhizal roots had access to the added OM.

Therefore, the seemingly contradictory results of the present study show that although the AM fungus enhanced total plant N uptake and OM decomposition, particularly when OM was added, it still decreased plant N uptake from the added OM. AM symbiosis increased both plant uptake of inorganic N from the soil solution and the amount of bacteria in soil. Therefore, in +AM treatments compared with the uninoculated control, the higher N demand of the bacteria and the lower N availability in the soil may have induced the bacteria to rely more on the organic N; this may in turn have reduced the availability of N from OM for the plant. Another hypothesis for the contradictory results could be that, as suggested by Hodge *et al.* (2010), OM represents the major N source for the fungus's own growth, reducing the plant's uptake of N from OM. Clearly, these hypotheses should be assessed in future research and validated experimentally.

In conclusion, the present findings have practical implications for crop production in low-input systems because an effective AM symbiosis can increase both plant N uptake and soil fertility, enhancing soil biological activity.

The experiment was funded by the Università degli Studi di Palermo, Italy (project 'Ruolo della simbiosi micorrizica sull'efficienza di utilizzazione dell'azoto in differenti genotipi di frumento'), by MiPAF (project SICOBIOS) and by the Ministerio de Ciencia e Innovación (MICINN), Spain (project 'AGL2008-00742/AGR').

REFERENCES

Al-Karaki, G. & Al-Omoush, M. (2002). Wheat response to phosphogypsum and mycorrhizal fungi in alkaline soil. *Journal of Plant Nutrition* **25**, 873–883.

Ames, R. N., Reid, C. P. P., Porter, L. K. & Cambardella, C. (1983). Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular arbuscular mycorrhizal fungus. *New Phytologist* **95**, 381–396.

Andrade, G. (2004). Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. In *Plant Surface Microbiology* (Eds A. Varma, L. Abbott, D. Werner & R. Hampp), pp. 51–69. Berlin: Springer.

Atul-Nayyar, A., Hamel, C., Hanson, K. & Germida, J. (2009). The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. *Mycorrhiza* **19**, 239–246.

Azcón, R. & Ocampo, J. A. (1981). Factors affecting the vesicular arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytologist* **87**, 677–685.

https://doi.org/10.1017/S002185961300004X Published online by Cambridge University Press

Azcón, R., Ruiz-Lozano, J. M. & Rodríguez, R. (2001). Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil. *Canadian Journal of Botany* **79**, 1175–1180.

Barea, J. M., Werner, D., Azcón-Aguilar, C. & Azcón, R. (2005). Interactions of arbuscular mycorrhiza and nitrogen fixing simbiosis in sustainable agriculture. In *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment* (Eds D. Werner & W. E. Newton), pp. 199–222. The Netherlands: Springer.

Cappellazzo, G., Lanfranco, L., Fitz, M., Wipf, D. & Bonfante, P. (2008). Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. *Plant Physiology* **147**, 429–437.

Cliquet, J. B., Murray, P. J. & Boucaud, J. (1997). Effect of the arbuscular mycorrhizal fungus *Glomus fasciculatum* on the uptake of amino nitrogen by *Lolium perenne*. *New Phytologist* **137**, 345–349.

Faure, S., Cliquet, J.-B., Thephany, G. & Boucaud, J. (1998). Nitrogen assimilation in *Lolium perenne* colonized by the arbuscular mycorrhizal fungus *Glomus fasciculatum*. *New Phytologist*, **138**, 411–417.

Gamper, H., Peter, M., Jansa, J., Lüscher, A., Hartwig, U. A. & Leuchtmann, A. (2004). Arbuscular mycorrhizal fungi benefit from 7 years of free air CO_2 enrichment in well-fertilized grass and legume monocultures. *Global Change Biology* **10**, 189–199.

García, C., Hernandez, T., Costa, F., Ceccanti, B. & Ganni, A. (1993). Hydrolases in the organic matter fractions of sewage sludge: changes with composting. *Bioresource Technology* **45**, 47–52.

García-Garrido, J. M., García-Romera, I. & Ocampo, J. A. (1992). Cellulase production by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol and Gerd) Gerd and Trappe. *New Phytologist* **121**, 221–226.

García-Romera, I., García-Garrido, J. M., Martinez-Molina, E. & Ocampo, J. A. (1991). Production of pectolytic enzymes in lettuce root colonized by *Glomus mosseae*. *Soil Biology and Biochemistry* **23**, 597–601.

Geisseler, D. & Horwath, W. R. (2009). Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. *Pedobiologia* **53**, 87–98.

Gill, P. P. & Modi, V. V. (1981). Induction of extracellular proteases by egg-white in *Aspergillus nidulans*. *Folia Microbiologica* **26**, 78–82.

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

Hawkins, H. J., Johansen, A. & George, E. (2000). Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil* **226**, 275–285.

Hoagland, D. R. & Arnon, D. I. (1950). *Water-culture Method for Growing Plants without Soil*. California Agricultural Experiment Station Circular 347. Berkeley, CA, USA: University of California.

Hodge, A. (2003). Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. *New Phytologist* **157**, 303–314.

Hodge, A., Stewart, J., Robinson, D., Griffiths, B. S. & Fitter, A. H. (1998). Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. *New Phytologist* **139**, 479–494.

Hodge, A., Robinson, D. & Fitter, A. H. (2000*a*). An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. *New Phytologist* **145**, 575–584.

Hodge, A., Robinson, D. & Fitter, A. H. (2000*b*). Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science* **5**, 304–308.

Hodge, A., Campbell, C. D. & Fitter, A. H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**, 297–299.

Hodge, A., Helgason, T. & Fitter, A. H. (2010). Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* **3**, 267–273.

Jackson, L. E., Schimel, J. P. & Firestone, M. K. (1989). Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil Biology and Biochemistry* **21**, 409–415.

Johansen, A., Jakobsen, I. & Jensen, E. S. (1993). Hyphal transport by a vesicular–arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. *Biology and Fertility of Soils* **16**, 66–70.

Jones, D. L., Healey, J. R., Willett, V. B., Farrar, J. F. & Hodge, A. (2005). Dissolved organic nitrogen uptake by plants: an important N uptake pathway? *Soil Biology and Biochemistry* **37**, 413–423.

Kandeler, E. & Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* **6**, 68–72.

Kaye, J. P. & Hart, S. C. (1997). Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology and Evolution* **12**, 139–143.

Killham, K. (1994). *Soil Ecology*. Cambridge, UK: Cambridge University Press.

Ladd, J. N. & Butler, J. H. A. (1972). Short-term assays of soil proteolytic enzyme activities using proteins and dipeptides derivatives as substrates. *Soil Biology and Biochemistry* **4**, 19–30.

Leigh, J., Hodge, A. & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist* **181**, 199–207.

Leigh, J., Fitter, A. H. & Hodge, A. (2011). Growth and symbiotic effectiveness of an arbuscular mycorrhizal fungus in organic matter in competition with soil bacteria. *FEMS Microbiology Ecology* **76**, 428–438.

Li, H. Y., Zhu, Y., Marschner, P., Smith, F. A. & Smith, S. E. (2005). Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. *Plant and Soil* **277**, 221–232.

Li, H., Smith, S. E., Holloway, R. E., Zhu, Y. & Smith, F. A. (2006). Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. *New Phytologist* **172**, 536–543.

Marschner, P. & Crowley, D. E. (1996). Root colonization of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum*) by *Pseudomonas fluorescens* 2–79RL. *New Phytologist* **134**, 115–122.

Miyasaka, S. C. & Habte, M. (2001). Plant mechanisms and mycorrhizal symbioses to increase phosphorus uptake efficiency. *Communications in Soil Science and Plant Analysis* **32**, 1101–1147.

Phillips, J. M. & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

Ravnskov, S., Nybroe, O. & Jakobsen, I. (1999). Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. *New Phytologist* **142**, 113–122.

Reynolds, H. L., Hartley, A. E., Vogelsang, K. M., Bever, J. D. & Schultz, P. A. (2005). Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phytologist* **167**, 869–880.

Schimel, J. P. & Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* **35**, 549–563.

Schüßler, A., Schwarzott, D. & Walker, C. (2001). A new fungal phylum, the *Glomeromycota*, phylogeny and evolution. *Mycological Research* **105**, 1413–1421.

Secilia, J. & Bagyaraj, D. J. (1987). Bacteria and actinomycetes associated with pot cultures of vesicular–arbuscular mycorrhizas. *Canadian Journal of Microbiology* **33**, 1069–1073.

Seeley, H. W. & VanDemark, P. J. (1981). *Microbes in Action: a Laboratory Manual of Microbiology*. 3rd edn. San Francisco, USA: W. H. Freeman and Company.

Seligman, N. G., Feigenbaum, S., Feinerman, D. & Benjamin, R. W. (1986). Uptake of nitrogen from high C-to-N ratio, ^{15}N-labelled organic residues by spring wheat grown under semi-arid conditions. *Soil Biology and Biochemistry* **18**, 303–307.

Smith, S. E. & Read, D. J. (2008). *Mycorrhizal Symbiosis*. San Diego, CA, USA: Academic Press.

Staddon, P. L., Jakobsen, I. & Blum, H. (2004). Nitrogen input mediates the effect of free-air CO_2 enrichment on mycorrhizal fungal abundance. *Global Change Biology* **10**, 1678–1688.

Tabatabai, M. A. (1994). Soil enzymes. In *Methods of Soil Analysis. Part 2. Microbial and Biochemical Properties* (Eds R. W. Weaver, J. S. Angel & P. S. Bottomley), pp. 775–833. Madison, WI, USA: Soil Science Society of America.

Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S. & Gollote, A. (1993). *In planta* histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. *Mycological Research* **97**, 245–250.

Tobar, R., Azcón, R. & Barea, J. M. (1994). Improved nitrogen uptake and transport from ^{15}N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytologist* **126**, 119–122.

Toljander, J. F., Lindahl, B. D., Paul, L. R., Elfstrand, M. & Finlay, R. D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiology Ecology* **61**, 295–304.

Vierheilig, H. & Ocampo, J. A. (1991). Receptivity of various wheat cultivars to infection by VA-mycorrhizal fungi as influenced by inoculum potential and the relation of VAM-effectiveness to succinic dehydrogenase activity of the mycelium in the roots. *Plant and Soil* **133**, 291–296.

Vierheilig, H., Schweiger, P. & Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum* **125**, 393–404.

Wamberg, C., Christensen, S., Jakobsen, I., Müller, A. K. & Sørensen, S. J. (2003). The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). *Soil Biology and Biochemistry* **35**, 1349–1357.

Whiteside, M. D., Treseder, K. K. & Atsatt, P. R. (2009). The brighter side of soils: quantum dots track organic nitrogen through fungi and plants. *Ecology* **90**, 100–108.