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Henrique M.A.C. Fonseca · Ricardo L.L. Berbara
Melvin J. Daft

Shoot $\delta^{15}N$ and $\delta^{13}C$ values of non-host *Brassica rapa* change when exposed to ±*Glomus etunicatum* inoculum and three levels of phosphorus and nitrogen

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Abstract Glasshouse experiments were conducted to study the response of non-host *Brassica rapa* and host *Sorghum bicolor* to inoculation with the arbuscular mycorrhizal fungus (AMF) *Glomus etunicatum* when given different levels of N (0.9 mmol kg^{-1} sand, 2.7 mmol kg^{-1} sand, 8.1 mmol kg^{-1} sand) and P (3.6 µmol kg^{-1} sand, 10.7 µmol kg^{-1} sand, 32.0 µmol kg^{-1} sand) fertiliser. On both plant species, the presence of *G. etunicatum* inoculum (+AMF) was associated with significant changes of shoot $\delta^{15}N$ values, with +AMF plants having larger average $\delta^{15}N$ values than uninoculated plants (–AMF). These values are the largest average differences in shoot $\delta^{15}N$ yet recorded for AMF and nutrient effects. *B. rapa* shoot $\delta^{15}N$ average differences ranged from 1.67‰ to 2.70‰, while for *S. bicolor* they range between 2.07‰ and 4.40‰. For shoot $\delta^{13}C$ only the non-host *B. rapa* responded to ±AMF and added N. Although the harvested dry weight biomass (–35.2% *B. rapa*; +39.8% *S. bicolor*) of both plant species responded to AMF inoculation, no direct relationship was observed between isotopic discrimination and growth inhibition for the non-host *B. rapa*. In this paper we discuss some implications regarding AMF inocula on the basis of our findings and current literature.

Keywords Arbuscular mycorrhiza · Non-host · Nitrogen-15 natural abundance · Carbon-13 natural abundance · *Brassica rapa*

H.M.A.C. Fonseca (✉)
Centro de Biologia Celular, Departamento de Biologia,
Universidade de Aveiro, 3810-193, Aveiro, Portugal
e-mail: hfonseca@bio.ua.pt
Tel.: +351-23-4370772, Fax: +351-23-4426408

R.L.L. Berbara
Universidade Federal Rural do Rio de Janeiro,
Departamento de Solos, Seropédica, Itaguaí,
RJ, CEP 23851-970, Brazil

M.J. Daft
School of Life Sciences, University of Dundee,
Dundee, DD1 4HN, Scotland

Introduction

The plant genus, *Brassica*, belongs to one of the few major plants groups, Cruciferae (Capparales), that generally do not form mycorrhizal symbioses with Glomales fungi (Gerdemann 1968). Most work addressing the interaction between arbuscular mycorrhizal fungi (AMF) and *Brassica* species focuses on either the non-host impact on co-cultured arbuscular mycorrhizal host plants and/or on AMF development in host-fungal symbioses. Information about the direct impact of AMF on non-hosts has not been sufficiently explored to generalise about the consequences of this. Koide and Li (1989) reported an initial depression of growth when *B. hirta* was given a soil inoculum containing mycorrhizal propagules. Manjunath and Habte (1992) observed a reduction in biomass production when *B. nigra* was cultured with low amounts of added P and inoculated with AMF.

AM hosts normally show an improved nutritional state when inoculated with AMF, and this is thought to occur via an improved capacity to take up soil nutrients with a low mobility, especially P (Rhodes and Gerdemann 1980). AMF are also involved in the acquisition of the highly water-soluble N forms, such as NO_3^- and NH_4^+ (e.g. Smith and Smith 1990).

The use of stable isotope natural abundances to explain plant-AMF interactions is in the empirical phase of gathering information for the design of hypotheses (Handley et al. 1993, 1999; Azcón-G.-Aguilar et al. 1998), but has shown already that the colonisation of non-diazotrophic plants by AMF changes the $\delta^{15}N$ values of host plants, when compared with non-mycorrhizal treatments, and that there are interactions with abiotic factors such as drought and N supply. The problems inherent in designing such experiments, however, preclude easy mechanistic explanations.

Microbial contamination is one such problem. The implementation of large experiments requires the production and isolation of large quantities of homogeneous and viable single-species AMF inocula, making it technically impractical to use axenic inocula. To our

knowledge, all published work on the effect of AMF on plant $\delta^{15}N$ and $\delta^{13}C$ was done in open, non-axenic conditions, almost certainly containing, in addition to AMF spores, free-living soil microorganisms from the parental inoculum cultures. Moreover, the production of axenic inocula in the quantity and quality required for most greenhouse or field experiments is not only difficult and labour intensive, its meaning may be distorted in an agricultural or ecological sense, since these inocula are known to lose infectivity and genetic stability with time (Vimard et al. 1999). Several authors have reported that the multiplication and maintenance of AMF in pot cultures selectively enhances particular rhizosphere, free-living microorganisms (e.g. Secilia and Bagyaraj 1987; Citernesi et al. 1996), which adhere to the surface of the AMF propagules (Fillippi et al. 1998). Under natural conditions these selective interactions can have an important role in determining plant community composition (Francis and Read 1994, 1995) and be an effective tool with which to control soil-borne plant pathogens (Christensen and Jakobsen 1993; Azcón-Aguilar and Barea 1996; Filion et al. 1999). These microbial populations, when added with AMF inocula, may play an important role in N dynamics (Ames et al. 1983), thus altering the amount, types and/or $\delta^{15}N$ of N available to the co-cultured plant (Radajewski et al. 2000). The present work extends previous empirical studies by Azcón-G.-Aguilar et al. (1998) that contrasted the effects of two sources of AMF inocula (*Glomus fasciculatum* and *Glomus mosseae*) on the $\delta^{15}N$ of a strongly symbiotic plant (*Lactuca sativa*) and a weakly symbiotic plant (*Hordeum vulgare*). Here we examine the effects of AMF inoculation with *Glomus etunicatum*, and of N and P supply, on whole-plant growth and on the $\delta^{13}C$, $\delta^{15}N$, %C, %N, %P and biomass-based water-use efficiency (WUE) of shoots in a strongly symbiotic host plant [*Sorghum bicolor* (L.) Moench var. CSH11, Gramineae] and in a non-host plant (*Brassica rapa* L. var. *rapa*, Brassicaceae).

Materials and methods

General

Two simultaneous experiments were conducted, one with *B. rapa* var. *rapa* (seeds obtained from Unwins Seeds, Histon, Cambridge, UK), which is known to be a non-mycorrhizal plant, and the other with *S. bicolor* var. CSH11, which is a mycorrhizal host plant, here used as the control species. *Sorghum* seeds were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, P.O. Andhra Pradesh 502 324, India).

Seeds of *B. rapa* and *S. bicolor* with average weights of 2.4±0.2 mg seed^{-1} and 35.4±1.0 mg seed^{-1}, respectively, were surface-sterilised [soaked for 30 s in 70% ethanol, 10 min in 10% commercial bleach (Domestos; Unilever, London)], washed with sterile water 4 times, and germinated on sterile wet sand. Germinated seedlings of uniform size were transplanted after 3 days into pots containing 1 kg sterile washed quartz sand for *B. rapa* (diameter, 12.8 cm) and 3 kg for *S. bicolor* (diameter, 17.8 cm).

Experimental design

Each set of plant species were tested in a 2×3×3 factorial design, with the mycorrhizal treatment consisting of the presence (+AMF) or absence (–AMF) of a suspension of spores which had morphological characteristics associated with *G. etunicatum* Becker and Gerdeman (Schenk and Perez 1988). The N treatment consisted of: 0.9 mmol N kg^{-1} sand (N1), 2.7 mmol N kg^{-1} sand (N2) and 8.1 mmol N kg^{-1} sand (N3), given as urea [$CO(NH_2)_2$]. The P treatment consisted of: 3.6 µmol P kg^{-1} sand (P1), 10.7 µmol P kg^{-1} sand (P2) and 32.0 µmol P kg^{-1} sand (P3), given as KH_2PO_4. There were four replicate pots per treatment, each pot containing one plant. For assessing nutrient sufficiency, we adopted the standards of Jones et al. (1991).

The spore suspension of *G. etunicatum* was obtained from a stock culture with *S. bicolor* as the host-plant. In the +AMF treatments a 1-ml freshly prepared suspension of spores (206± 21 spores ml^{-1}) was layered at 2–3 cm below the planting holes in each pot. To –AMF treatments there was added equal volumes of filtrate (45-µm mesh) from the same spore suspension. The $\delta^{15}N$ of the urea used as N was measured as –2.8‰, the same N source used by Handley et al. (1993) for their *Ricinus communis* experiment. This value would have changed during the experiment, so that no source-sink calculations could be made for $\delta^{15}N$. All plants, however, experienced the same N background. P and N amendments were supplied only during the first 2 weeks of growth. All treatments were watered weekly with 60 ml kg^{-1} sand with nutrient solution (pH 6.6) containing a total of: macronutrients, 2.4 mmol $CaCl_2.6H_2O$ kg^{-1} sand), 2.4 mmol K_2SO_4 kg^{-1} sand, 0.9 mmol $MgSO_4.7H_2O$ kg^{-1} sand; and micronutrients, 65.0 µmol $[CH_2N(CH_2COO)_2]_2FeNa$ kg^{-1} sand, 27.6 µmol H_3BO_3 kg^{-1} sand, 5.5 µmol $MnSO_4.7H_2O$ kg^{-1} sand, 0.5 µmol $ZnSO_4.7H_2O$ kg^{-1} sand, 0.3 µmol $Na_2MoO_4.2H_2O$ kg^{-1} sand, 0.2 µmol $CuSO_4.5H_2O$ kg^{-1} sand, 0.1 µmol $CoSO_4.6H_2O$ kg^{-1} sand, 0.1 µmol $NiSO_4.7H_2O$ kg^{-1} sand, and 0.1 µmol NaCl kg^{-1} sand. The experiment was planted in August 1992 and plants grown in a heated glasshouse for 16 weeks, using natural daylight, a temperature of about 25°C and ventilated with outside air (Hawkhill glasshouse, University of Dundee, Scotland).

Data collection

Plant biomass was estimated as dry weight (g) of shoots and roots after oven-drying (60°C) to a constant weight. Shoots, but not roots, were analysed for contents of C, N, P and for $\delta^{13}C$ and $\delta^{15}N$.

Mycorrhizal status (%) was estimated using the gridline-intersect method (Giovanneti and Mosse 1980), after clearing roots in 10% KOH (w/v) followed by staining in trypan blue (Phillips and Hayman 1970).

Pots were watered as required to maintain 60–80% of the sand's moisture-holding capacity and to avoid leaching. Evaporation was estimated by averaging the recorded water requirements of randomly placed plantless pots containing the same amount of sand as planted ones. WUE (g l^{-1}) is defined here as dry weight of harvested plant divided by the volume of water transpired. Water transpired was obtained by subtracting the volume of water lost through evaporation from the volume of water added to each plant.

Analyses of plant P, N and C contents were done on ball-milled dried shoots. P content was determined on sub-samples of shoots (0.3 g), which were ashed in a muffle furnace (stages: 200°C at 700°C h^{-1}; 300°C at 70°C h^{-1}; 600°C at 700°C h^{-1} during 6.7 h) and dissolved in 10 ml of 1 M HCl. An aliquot of supernatant was mixed with ammonium molybdate, ammonium vanadate and 1 M HCl in a 1:2:2:17 mixture (v/v/v/v) to give a final volume of 2.2 ml. After 30 min for colour development the absorbance at 440 nm was measured using a Shimadzu UV-120-02 spectrophotometer against a reagent blank. C and N content, and $\delta^{13}C$ and $\delta^{15}N$, were determined at the Scottish Crop Research Institute (Invergowrie, Dundee, Scotland) by continuous-flow iso-

tope ratio mass spectrometry using a Roboprep combustion unit coupled to a Tracer Mass [Europa Scientific, Crewe, UK; Handley et al. (1993)]. $\delta^{13}C$ and $\delta^{15}N$ were calculated as:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \quad (1)$$

where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Data were analysed by ANOVA followed by least significant difference and by multiple stepwise regression, using Statistica software (Statsoft, Norman, Okla.).

Results

Mycorrhizal colonisation and plant growth

As expected, *B. rapa* did not show colonisation by *G. etunicatum* (Table 1). There were hyphae within the cortex of *B. rapa* with an average colonisation level of about 0.01%. These occurred only in the +AMF treatment, but lacked arbuscules, vesicles or any other characteristics associated with colonisation by Glomales fungi. *S. bicolor* was successfully colonised by *G. etunicatum*, averaging 42.8% for the mycorrhizal treatment (+AMF) and 0.3% for the non-mycorrhizal treatment (–AMF). The low level infection of –AMF pots can be attributed to contamination caused by periodic random rearrangement of pots within the glasshouse space and the handling of pots for quantification of water use. The distribution of infection within contaminated plant roots in the –AMF treatment (Table 1) had the characteristics of a culture established later than the +AMF treatment cultures.

AMF inoculation (Table 2) decreased the harvested dry weight biomass (whole plants and shoots) of *B. rapa* and increased the harvested biomass (whole plant and shoots) of *S. bicolor*. Inoculation effects on plant and shoot dry weight biomasses were largest for both species when the plants were grown at the highest N supply (N3) combined with the lowest P supply (P1). At $P<0.001$, inoculation of *B. rapa* reduced shoot dry weight biomass by 33.3% and whole-plant biomass by 35.2%, relative to uninoculated plants, with a maximum reduction of 53.4% whole-plant biomass (Fig. 1; treatment P1N3). At $P<0.001$, inoculation of the AM host plant, *S. bicolor*, increased average whole-plant and shoot dry weight biomasses by 39.8% and 38.1%, respectively, relative to –AMF plants, with the largest effect observed in the P1N3 treatment where the whole-plant biomass increased to about 3 times that of uninoculated plants (Fig. 1).

Shoot %C, $\delta^{13}C$ and WUE

No significant changes were observed in measured shoot %C of both plant species due to ±AMF inoculation. Conversely, N treatments had significant effects (*B. rapa*, $P<0.007$; *S. bicolor*, $P<0.03$) on shoot %C, as shown in Table 1.

The contributions of initial seed C and N to final elemental contents and to plant isotopic values, calculated by isotopic mass balance, were found to be trivial in amount and effect on the $\delta^{13}C$ and $\delta^{15}N$ of harvested biomass.

For *B. rapa* the main effects, ±AMF and N levels, were associated with statistically significant differences ($P<0.04$) in shoot $\delta^{13}C$, of slightly less than 1‰ and probably biologically non-significant (O'Leary 1993). Larger significant effects on shoot $\delta^{13}C$ (>1.6) were seen for the interaction of ±AMF with P, in P2, and N, in N2 and N3 (Fig. 2). There were no significant differences for the $\delta^{13}C$ of *S. bicolor*.

For biomass-based WUE, the main effects, ±AMF and N levels, had significant and different effects on the biomass-based WUE (Tables 1, 2) of *B. rapa* and *S. bicolor*. *B. rapa* +AMF showed a substantial reduction (–32%) in WUE ($P<0.001$) relative to –AMF; conversely, *S. bicolor* showed an increased WUE ($P<0.001$) of

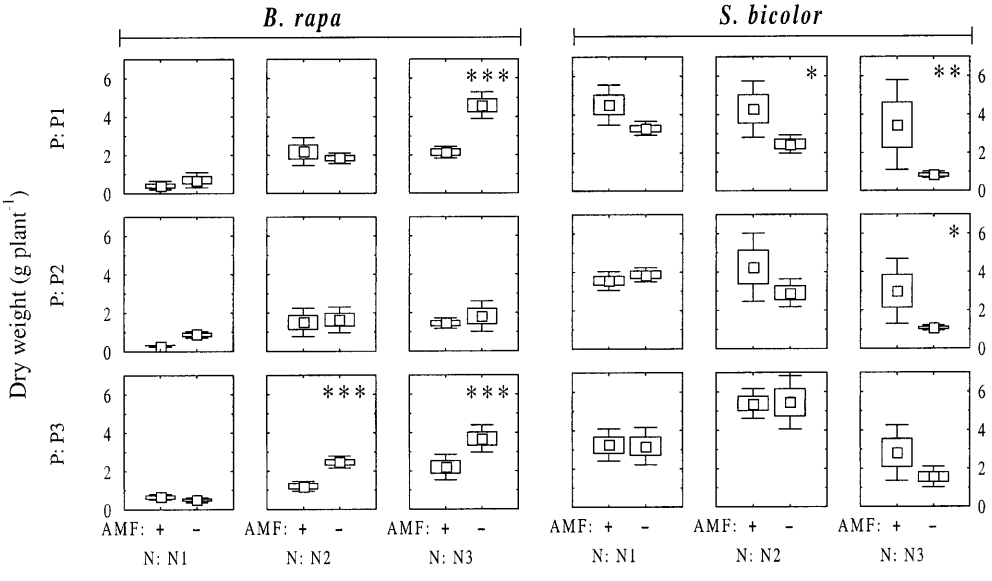

Fig. 1 *Brassica rapa* and *Sorghum bicolor*: mean (q), ±SEs (*rectangles*) and SDs (*error bars*) of whole-plant dry weight biomass measured for an arbuscular mycorrhizal fungus (*AMF*) (*Glomus etunicatum*) inoculum, N and P treatments. *Asterisks* represent statistically different pairs, within species, for AMF treatments ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). + Inoculated, – non-inoculated, *N1* 0.9 mmol N kg^{-1} sand, *N2* 2.7 mmol N kg^{-1} sand, *N3* 8.1 mmol N kg^{-1} sand [N supplied as $CO(NH_2)_2$], *P1* 3.6 µmol P kg^{-1} sand, *P2* 10.7 µmol P kg^{-1} sand, *P3* 32.0 µmol P kg^{-1} sand (P supplied as KH_2PO_4)

Table 1 *Brassica rapa* and *Sorghum bicolor* means ±SDs of mycorrhizal colonisation; shoot percentages of C, N and 10× of P; and plant water-use efficiency (*WUE*) measured for arbuscular mycorrhizal fungus (AMF) inoculum, N and P treatments. Values followed by the *same letter* within plant species and parameter do not differ significantly ($P<0.05$). *+AMF* Inoculated, *–AMF* non-inoculated, *N1* 0.9 mmol N kg^{-1} sand, *N2* 2.7 mmol N kg^{-1} sand, *N3* 8.1 mmol N kg^{-1} sand [N supplied as $CO(NH_2)_2$], *P1* 3.6 µmol P kg^{-1} sand, *P2* 10.7 µmol P kg^{-1} sand, *P3* 32.0 µmol P kg^{-1} sand (P supplied as KH_2PO_4)

Main effects	N1		N2		N3	
	+AMF	–AMF	+AMF	–AMF	+AMF	+AMF
B. rapa						
Main effects	P1					
Root levels of colonisation (%)	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
Shoot percentage						
C (%)	35.9±2.5abcd	35.4±3.7abc	38.8±1.1cde	37.5±1.4bcde	38.6±2.7cde	37.3±1.4bcde
N (%)	1.3±0.3a	1.3±0.3a	1.6±0.4a	1.3±0.3a	3.9±1.1cd	3.1±0.9cd
P (%)	4.3±1.7f	3.3±1.3bcdef	2.8±0.5abcde	2.2±0.6abc	2.1±0.2ab	2.3±0.6abcd
Plant WUE (g l^{-1})	0.5±0.2a	1.3±0.4bcd	2.2±0.9fghi	1.5±0.6cde	2.0±0.3efgh	2.7±0.7hi
Main effects	P2					
Root levels of colonisation (%)	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
Shoot percentage						
C (%)	32.8±3.2a	38.4±1.2cde	39.0±2.3cde	38.3±1.9cde	37.0±3.2bcd	36.3±1.8abcd
N (%)	1.4±0.4a	1.2±0.2a	2.8±0.4bc	1.5±0.7a	4.0±1.2d	5.2±1.9e
P (%)	3.5±1.4def	2.2±0.6abcd	2.3±0.9abcd	1.8±1.0a	2.6±0.2abcd	2.1±0.5ab
Plant WUE (g·l^{-1})	0.8±0.3ab	2.8±0.8i	1.5±0.5bcde	1.3±0.4bcd	1.8±0.3def	2.9±0.6i
Main effects	P3					
Root levels of colonisation (%)	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
Shoot percentage						
C (%)	37.4±5.3bcde	35.6±1.9abc	40.8±1.1e	36.3±2.3abcd	39.3±3.3de	34.4±1.1ab
N (%)	2.0±0.4ab	1.4±0.2a	1.6±0.1a	1.4±0.2a	3.8±1.1 cd	3.7±0.7 cd
P (%)	3.9±1.2ef	3.4±1.5cdef	2.9±0.5abcde	2.0±0.2a	2.3±0.6abcd	2.1±0.3ab
Plant WUE (g·l^{-1})	0.9±0.2abc	1.7±0.5def	1.3±0.2bcd	2.0±0.4efg	1.7±0.4def	2.5±0.4ghi
S. bicolor						
Main effects	P1					
Root levels of colonisation (%)	16.1±7.9a	1.2±1.4a	51.8±21.1bc	0.6±0.7a	55.4±17.9bc	0.0±0.0a
Shoot percentage						
C (%)	43.2±0.8c	40.4±1.6abc	41.4±0.6abc	41.4±1.3abc	40.9±1.8abc	39.0±4.1ab
N (%)	0.8±0.1a	0.8±0.0a	1.8±0.7cd	1.8±0.1 cd	2.8±0.2ef	3.4±0.7 g
P (%)	1.8±0.4bcdef	1.3±0.4abc	2.5±0.9efgh	1.2±0.5abc	2.8±0.8gh	1.4±0.4abcd
Plant WUE (g·l^{-1})	6.8±1.5defg	6.8±0.9defg	9.2±2.3gh	4.5±0.6abcd	6.1±3.8def	2.0±0.3a
Main effects	P2					
Root levels of colonisation (%)	14.2±3.7a	0.0±0.0a	49.6±8.1bc	0.0±0.0a	48.4±15.4bc	0.0±0.0a
Shoot percentage						
C (%)	43.0±1.3c	43.2±0.4c	41.8±1.8bc	41.0±0.4abc	41.1±1.3abc	41.9±1.5bc
N (%)	0.8±0.1a	0.9±0.1a	2.0±0.9cd	1.6±0.3c	3.1±0.7 fg	4.4±0.3 h
P (%)	2.1±0.3cdefg	0.9±0.1ab	2.6±0.8efgh	0.7±0.0a	3.0±0.9gh	0.9±0.1ab
Plant WUE (g·l^{-1})	6.5±0.9defg	9.0±0.7gh	7.4±2.7efg	5.0±0.9bcde	5.6±3.6def	2.4±0.4ab
Main effects	P3					
Root levels of colonisation (%)	38.9±14.6b	0.7±1.1a	58.5±6.8c	0.5±0.4a	52.2±16.0bc	0.0±0.0a
Shoot percentage						
C (%)	42.1±1.1c	41.5±2.3abc	42.2±0.7c	42.2±2.2c	42.0±1.6c	38.8±4.9a
N (%)	0.9±0.2ab	1.0±0.3ab	1.8±0.2cd	1.5±0.3bc	2.9±0.1fg	2.3±0.8de
P (%)	2.7±0.6fgh	1.3±0.6abc	2.3±0.4defgh	1.7±0.6bcde	3.0±0.4h	3.1±1.5h
Plant WUE (g·l^{-1})	5.4±1.5cdef	7.5±2.4efg	11.0±1.5h	8.2±1.8 fg	5.9±2.8def	2.7±0.7abc

Table 2 Significance levels (*P*-values) for the main effects of treatments and their interactions with *B. rapa* and *S. bicolor* variables: plant and shoot dry weight biomass; shoot $\delta^{15}N$, $\delta^{13}C$ and P:N atom ratio; and plant biomass-based WUE. For abbreviations, see Table 1

	Whole-plant biomass	Shoot biomass	Shoot $\delta^{15}N$	Shoot $\delta^{13}C$	Shoot P:N atom ratio	Plant WUE
B. rapa (Brassicaceae, dicot, C3, non-host for AMF)						
Main effects						
AMF: +/–	<0.001 *	<0.001 *	<0.001 *	0.019 *	0.356	<0.001 *
N: N1, N2, N3	<0.001 *	<0.001 *	<0.001 *	0.039 *	<0.001 *	<0.001 *
P: P1, P2, P3	<0.001 *	<0.001 *	<0.001 *	0.437	<0.002 *	0.493
Two-way interactions						
AMF-N	<0.001 *	<0.001 *	0.798	0.109	0.716	<0.001 *
AMF-P	0.167	0.090	<0.003 *	0.016 *	0.635	0.029 *
N-P	<0.001 *	<0.001 *	0.734	0.600	0.123	0.003 *
Three-way interactions						
AMF-N-P	<0.001 *	0.055	0.582	0.035 *	0.026 *	0.041 *
S. bicolor (Poaceae, monocot, C4, AMF host)						
Main effects						
AMF: +/–	<0.001 *	<0.001 *	<0.001 *	0.941	<0.001 *	<0.001 *
N: N1, N2, N3	<0.001 *	<0.001 *	<0.001 *	0.219	<0.001 *	<0.001 *
P: P1, P2, P3	0.231	0.106	0.007 *	0.787	<0.004 *	0.252
Two-way interactions						
AMF-N	0.056	<0.003 *	0.011 *	0.244	<0.002 *	<0.001 *
AMF-P	0.083	0.019 *	<0.004 *	0.911	0.009 *	0.229
N-P	0.010 *	0.102	0.173	0.533	0.947	0.024 *
Three-way interactions						
AMF-N-P	0.854	0.967	0.040 *	0.417	0.010 *	0.982

* $P<0.05$

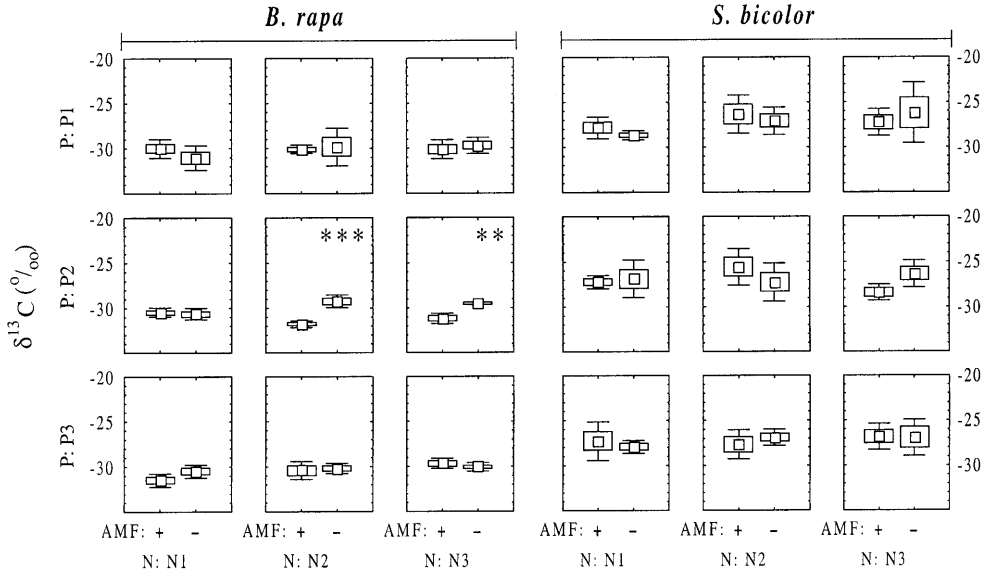

Fig. 2 Shoot $\delta^{13}C$ of *B. rapa* and shoot $\delta^{13}C\times2$ of *S. bicolor*: mean (q), ±SEs (*rectangles*) and SDs (*error bars*) measured for AMF inoculum, N and P treatments. *Asterisks* represent statistically significant differences within species for AMF treatments (**$P<0.01$, ***$P<0.001$). Treatments and abbreviations as in Fig. 1

34% for +AMF relative to –AMF. N treatments had highly significant effects on WUE, explaining more than 50% of the variation observed. Increasing N levels increased the WUE of *B. rapa* ($P<0.001$); increasing N levels decreased the WUE of *S. bicolor*, with its lowest WUE for the N3 treatment ($P<0.001$). P treatments, per se, had no effect on the WUE of either plant species.

When biomass-based WUE is known, the $\delta^{13}C$ (potential WUE) of C_3 plants can be interpreted, sensu Farquhar and Richards (1984), in terms of intrinsic WUE. Plant dry matter production and transpiration (Fig. 3) had the expected positive and linear trend (Arkley 1963). The $\delta^{13}C$ of *B. rapa* shoots was poorly correlated with intrinsic WUE in all treatments. The model explaining $\delta^{13}C$, as potential water use efficiency, does not apply to *S. bicolor*, a C4 plant.

P:N atom ratio, %P, %N and $\delta^{15}N$ of shoots

For *S. bicolor* (Table 2), +AMF was associated with an increase of about 70% ($P<0.001$) of shoot P:N atom ratio relative to –AMF, with a maximum increase of 160% in the P2N1 treatment (Fig. 4). Mycorrhizal *S. bicolor* at all P and N levels had P:N atom ratios above the critical level of 0.086 (adapted from Jones et al. 1991). For uninoculated *S. bicolor*, only at the highest P (P3) or at the lowest added N (N1) did the shoot P:N atom ratio exceed the critical value. +AMF (Table 2) had no significant effect on the shoot P:N atom ratio of the non-host, *B. rapa*. Only *S. bicolor* showed significant changes in shoot %P due to AMF inoculation, with increases of about 79% when compared with –AMF plants. Conversely, AMF inoculation did not change significantly shoot %N (Table 1).

The main effect, ±AMF, was associated with significantly different shoot $\delta^{15}N$ in the non-host, *B. rapa* and the host, *S. bicolor* (Table 2), both plants having larger shoot $\delta^{15}N$ for +AMF. Multiple stepwise regression showed that ±AMF explained 18.7% (*B. rapa*) and 39.0% (*S. bicolor*) of the total variation of shoot $\delta^{15}N$ values, and corresponding main-effect differences ($P<0.001$) of about 1.2‰ and 1.8‰ for $\delta^{15}N$ values of *B. rapa* and *S. bicolor*, respectively.

The P main effect was significant for the regression, but explained much less of the shoot $\delta^{15}N$ variation (~11%). Interaction of AMF and P main effects explained 8.7% of the variation in $\delta^{15}N$ in *B. rapa* shoots and 12.2% in *S. bicolor* shoots, contrasting with smaller interactions (0.3% and 9.8%) for AMF and N main effects that were statistically significant only for *S. bicolor* (Table 2). For individual treatments (Fig. 5; ±AMF, N and P) and *B. rapa*, shoot $\delta^{15}N$ was significantly different between +AMF and –AMF treatments at all three N levels, but only within P2. For *S. bicolor*, P treatments (P1 and P2) and N treatments (N2 and N3) produced significant differences between +AMF and –AMF for shoot $\delta^{15}N$. For all significant differences of $\delta^{15}N$ in both plant species (Fig. 5), mean differences were in the same rank order, with +AMF shoots having a larger $\delta^{15}N$ than –AMF shoots and mean differences ranging from 1.7‰ to 2.7‰ (*B. rapa*) and 2.1‰ to 4.4‰ (*S. bicolor*). These are the largest differences yet reported for AMF and nutrient effects on plant $\delta^{15}N$.

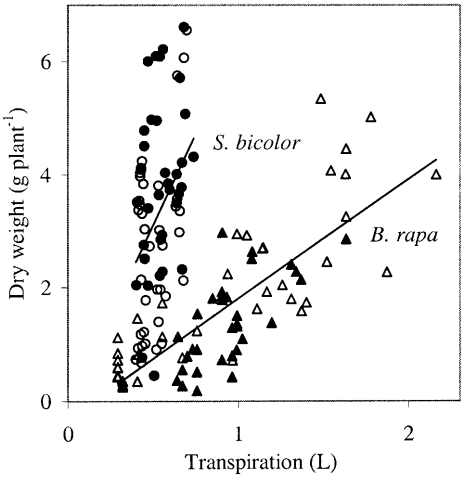

Fig. 3 Relationship between whole-plant dry weight (*DW*) and transpiration for all treatments of *B. rapa* (▲ +AMF, △ –AMF) and *S. bicolor* (● +AMF, ○ –AMF). For *B. rapa*, DW=-0.308+0.002×transpiration, r=0.78; for *S. bicolor*, DW=-0.120+0.006× transpiration, r=0.39. Treatments and other abbreviations as in Fig. 1

Discussion

Similar antagonistic effects on non-host biomass yield by +AMF inoculum were observed by Koide and Li (1989) on *B. nigra*, although well before colonisation

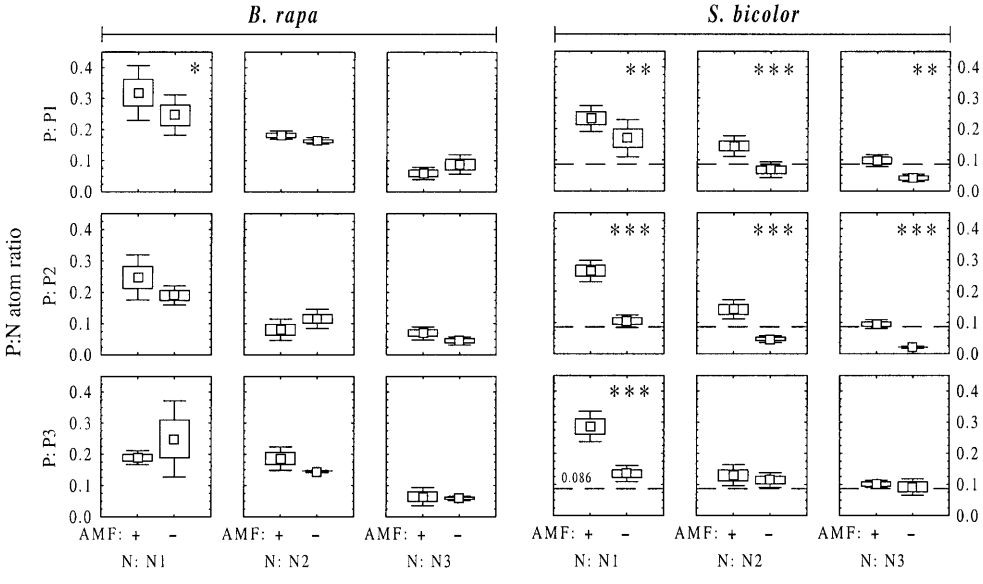

Fig. 4 *B. rapa* and *S. bicolor*: mean (q), ±SEs (*rectangles*) and SDs (*error bars*) for shoot P:N atom ratio measured in AMF inoculum, N and P treatments. *Dashed lines* mark the lower limit for optimal balance between the two elements for *S. bicolor* (adapted from Jones et al. 1991). *Asterisks* represent statistically significant differences within species for AMF treatments ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). Treatments and abbreviations as in Fig. 1

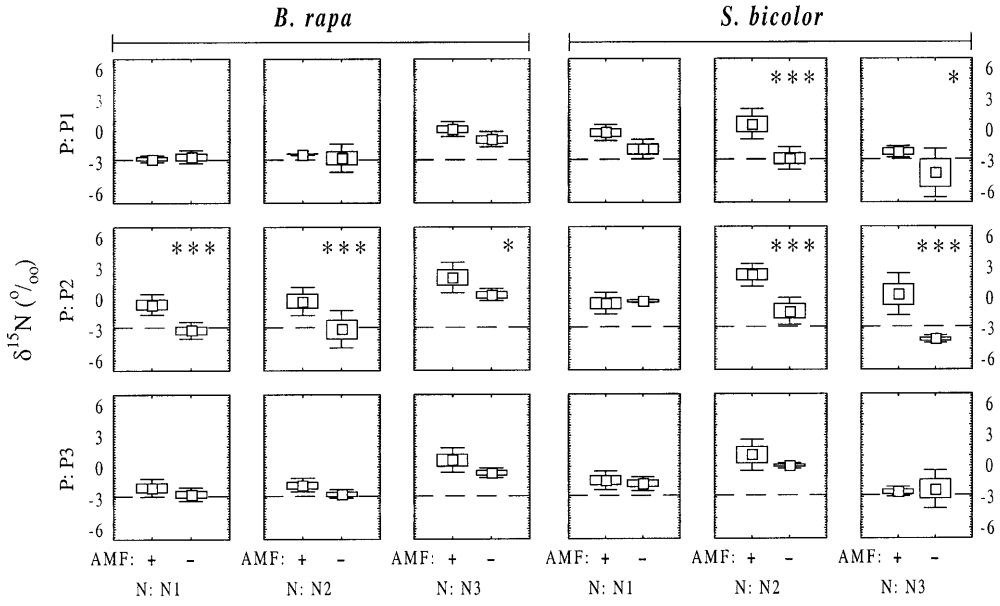

Fig. 5 *B. rapa* and *S. bicolor*: mean (q), ±SEs (*rectangles*) and SDs (*error bars*) for shoot $\delta^{15}N$ measured in AMF inoculum, N and P treatments. *Dashed line* represents the $\delta^{15}N$ value (–2.8‰) of added $CO(NH_2)_2$. *Asterisks* represent statistically significant differences within species for AMF treatments (*$P<0.05$, ***$P<0.001$). Treatments and abbreviations as in Fig. 1

had time to be established in the co-cultured host. They attributed the occurrence to other causes, rather than AMF. The well-watered regime during the present experiment not only had the effect of emphasising the dry weight component of calculated WUE; it was also the probable cause of a non-effect of AMF inoculation on shoot $\delta^{13}C$ of *S. bicolor*. These results are in accordance with those of Handley et al. (1999) who reported a loss of advantage in terms of ^{13}C discrimination conferred by fungal inoculum on well-watered plants. The improved nutritional balance between P and N, observed for mycorrhizal *S. bicolor*, is in accordance with that reported by Handley et al. (1993) for *R. communis* where mycorrhizal plants had higher average P:N atom ratios than the corresponding non-mycorrhizal plants.

The occurrence of changes in shoot $\delta^{15}N$ values of a non-mycorrhizal (non-host) species due to the effect of an AMF inoculum, even though *B. rapa* was not colonised by *G. etunicatum*, raises new questions about the nature and source of observed $\delta^{15}N$ variations. Standard methodologies for multiplication and long-term maintenance of pure cultures of Glomales species favour the growth of extra-AMF microorganisms that thrive in the rhizosphere conditions created by mycorrhizae, and in particular those that grow on hyphae and sporocarps' surfaces (Filippi et al. 1998). Most, if not all, published papers on the effect of AMF on plant $\delta^{15}N$ (e.g. Handley et al. 1993, 1999; Azcón-G.-Aguilar et al. 1998) describe procedures where plants were inoculated with different non-axenic inocula. Handley et al. (1993) using *G. clarum* as inoculum (amended with NH_4^+-N, $\delta^{15}N$=–2.8‰) reported that mycorrhizal *R. communis* was less ^{15}N enriched. Whereas, Azcón-G.-Aguilar et al. (1998) using different inocula showed patterns of variations for whole-plant and shoot $\delta^{15}N$ values that could be attributed mainly to inocula source and concentrations of soil N (see Azcón-G.-Aguilar et al. 1998: Figs. 2a and 3a). Plant species were mainly responsible for changes in the range of $\delta^{15}N$ values, rather than having a strong direct effect on the pattern-shift of measured $^{15}N/^{14}N$ discrimination. The present experiment showed a different pattern-shift for shoot $\delta^{15}N$ values, with both +AMF plants species being more ^{15}N enriched. If changes in shoot $\delta^{15}N$ due to the AMF inoculum were the result of symbiotic interaction between plant and Glomales, it would be reasonable to expect an overall non-effect from AMF-inoculated *B. rapa*, when compared with –AMF plants, or the occurrence of AMF symbiosis on *B. rapa*. The same reasoning used above for $\delta^{15}N$ may be applied to plant ^{13}C discrimination. Moreover, the recent work from Radajewski et al. (2000) pointed out that different soil bacteria populations, that are actively involved in specific metabolic processes, might induce measurable shifts in soil stable isotope availability to plants, through the cycling of C and N in the environment.

In conclusion, our data show for the first time that an AMF inoculum induces changes in shoot $\delta^{13}C$ and $\delta^{15}N$ values of a non-host plant. In addition, the ranges of shoot $\delta^{15}N$ values for both plant species are the largest yet recorded, as much as 2.7‰ for *B. rapa* and 4.4‰ for *S. bicolor*. As discussed above, our data suggest the presence of an unknown factor affecting the underlying mechanisms behind the changes in shoot $\delta^{13}C$ and $\delta^{15}N$ values due to AMF inoculation. However, the identification of the nature and source of observed isotopic discriminations is beyond the present experimental design. More detailed isotopic studies are needed to understand the partition of the effects on competition for nutrients and plant behaviour between those from extra-AMF microbes and those due alone to AMF symbiosis.

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