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Respiratory costs of P uptake in arbuscular mycorrhizal roots supplied with NH_4^+ and NO_3^- nutrition

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Abstract

P uptake by arbuscular mycorrhizal (AM) roots can impose a C drain on host resources. However, it is not known how P uptake is influenced by the effects of inorganic N supply on AM colonisation and host root respiration. The respiratory costs of P uptake was studied in AM roots, supplied with NO_3^- and NH_4^+ nutrition. P uptake was higher in AM roots at both sources of inorganic N supply. This was correlated with the enhanced AM root respiration in host plants. In spite of AM colonisation being lower with NH_4^+ supply than in NO_3^--fed roots, the respiratory O_2 consumption and CO_2 release rates were still higher in AM roots than in non-AM roots at both forms of N. The uptake rates of NO_3^- and NH_4^+ were unaffected by AM colonisation. This was correlated with the partitioning of C from the incorporated root-zone CO_2, into amino acid, also being unaffected by AM colonisation. The lower incorporation of root-zone CO_2 by AM roots at both NO_3^- and NH_4^+ sources, concurs with the higher respiratory gas exchange quotient (Rq). These results show that the sink activity of AM roots is related to improved P uptake, irrespective of the influence of NO_3^- or NH_4^+ nutrition on AM colonisation and respiratory N metabolism.

Keywords: P uptake, arbuscular mycorrhiza, respiration, root-zone CO_2, N nutrition

1. Introduction

Although mineral acquisition (Marschner, 1994) consumes a large proportion of the photosynthate allocated to roots (Lambers et al., 1989; Lambers et al., 1991), the uptake and assimilation of nutrients impose a large carbon cost of this allocation (Bloom et al., 1992). In particular with P, arbuscular mycorrhizal (AM) plants rely on the AM symbionts to improve P uptake as well as plant growth in poor soils (Bolan, 1991; Graham, 2000; Mortimer et al., 2005). The respiratory C cost to the host is a pivotal part of AM symbiosis and although AM root respiration under low P conditions has been well published (Pang and Paul, 1980; Snellgrove et al., 1982; Harris et al., 1985; Peng et al., 1993; Jifon et al., 2002), very little is known about the effect of N nutrition on the P uptake of AM roots. AM colonisation and activity is known to be influenced by NH_4^+ and NO_3^- supply (Chambers et al., 1980; Johnson et al., 1984; Azcon et al., 1992; Valentine et al., 2001, 2002) and this may affect the respiratory C metabolism during P uptake.

For non-AM roots, the source of N has a great influence on the root respiration, in that inorganic C in the soil is utilised as an anaplerotic C source during amino acid synthesis during dark CO_2 fixation by roots (Viktor and Cramer, 2005). Most plant roots encounter inorganic C as root-zone CO_2, of which the concentrations exceed 5000 ppm (Brook, et al., 1983). The inorganic C in the soil comprises a pH-dependent combination of CO_2, HCO_3^- and CO_3^{2-} in solution and is collectively referred to as dissolved inorganic C (DIC) (Norstadt and Porter, 1984). The extent of DIC utilisation is influenced by the inorganic source of N and it was found for non-AM roots that NH_4^+ nutrition resulted in higher DIC incorporation rates than NO_3^- nutrition (Cramer and Lewis, 1993).

The respiratory C metabolism of DIC by symbiotic mycorrhizal root systems is not well known. For ecto-mycorrhizal fungi, Wingler et al. (1996) suggested that the major pathway of CO_2 incorporation appears to be through replenishment of the dicarboxylic acids of the TCA cycle, which are used for amino acid synthesis. Studies on AM fungi *in vitro* have shown that elevated CO_2 concentrations can stimulate hyphal growth from spores in a medium of root extracts (Becard et al., 1989, 1992). However, in the symbiotic state, AM fungi do not engage in dark CO_2 fixation of DIC (Pfeffer et al., 1999), although the host

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root component may still separately fix CO_2. It is therefore likely that this dark CO_2 fixation of DIC during NH_4^+ and NO_3^- nutrition may impact on the respiratory metabolism of AM root systems.

Since P uptake is a vital part of the AM symbiosis and its respiratory costs, it is important for AM functioning to understand how inorganic N sources can influence the P nutrition of AM roots. The aim of this investigation was to assess how P uptake in AM roots is affected by the respiratory C metabolism of NH_4^+ and NO_3^- nutrition. This was studied using AM fungal colonisation, N and P uptake, respiratory gas exchange, dark incorporation and assimilation of root-zone DIC.

2. Materials and Methods

Plant growth conditions

Seeds of *Lycoperisicon esculentum* L. Mill F114 (tomato) were germinated in 15 cm diameter pots containing sterilized sand (grain size 2 mm) during March and May in a north-facing glasshouse at the University of Stellenbosch, Stellenbosch, South Africa. The maximum daily irradiance was between 620 and 660 μmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 19/14°C and 68/27%, respectively.

Mycorrhizal inoculation

Seeds were inoculated either with 5 g of a live culture of *Glomus mosseae* Nicol. And Gerd. Gerdeman and Trappe (Agricultural Genetics Co. LTD, UK), or an autoclaved (3 h at 110°C under steam pressure at 200 kPa) batch of *G. mosseae* inoculum. The inoculum consisted of a mixture of chlamydospores and fragments of root in a clay support medium and was placed 1 cm below the seeds in each pot. The control seedlings were irrigated with a 0.5 g ml^{-1} H_2O extract of the live inoculum filtered through a 30 μm mesh to introduce non-mycorrhizal microbes, which may have been killed during the autoclaving process back into the autoclaved inoculum.

Hydroponic culture

Each pot received a total of 2 l of distilled H_2O per day. Upon germination, the seedlings were irrigated for the same period per day, except that Long Ashton (Hewitt, 1966) nutrient solution was supplied. The nutrient solution was modified to contain 50 μM P and 0.05 mM MES (pH 6). The N sources in the nutrient solution were 4 mM NH_4^+ or 4 mM NO_3^-. The pH was monitored twice daily and maintained at pH 6.0±0.1 using 0.1 M of either HCl or NaOH. Four weeks after germination the seedlings were transferred to 22 l hydroponic tanks. The solutions were changed every 5 to 6 days. The hypocotyls of seedlings were wrapped with foam rubber at their bases and inserted through holes in the lids of the tanks. An "air-lift" consisting of an open-ended, 1 cm diameter plastic tube, aerated at the bottom and discharging at the surface of the solution, was inserted through each lid which bubbled air containing 5,000 ppm CO_2. 5,000 ppm CO_2 was obtained by mixing industrial grade CO_2 (Afrox, RSA) with the ambient air. The air-lift served to gently circulate nutrient solution throughout the tank, without vigorously agitating the delicate mycorrhizal hyphae. The CO_2 concentrations in the air supplied to the roots were measured with an ADC-225-MK3 (ADC, Hertz, UK) infrared gas analyser calibrated to the required to CO_2 concentration using a LiCor gas syringe (LiCor Inc, Model 6000-01, Lincoln, Nebraska, USA) with a mixture of pure CO_2 and N_2 (Afrox, RSA). Plants were grown in hydroponic culture for 15 days and were subsequently used in separate experiments for root respiration, ^{14}C labelling and nutrient uptake.

Root respiration measurements

After 15 d in hydroponic culture the seedlings were transferred to 298 ml temperature controlled (20°C) cuvettes for the measurements of O_2 and CO_2 fluxes. The air temperature was maintained at 20°C and light was supplied at 350 μmol m^{-2} s^{-1} PAR. The cuvettes had three ports; one for the supply of air (5,000 ppm CO_2), one for the addition of inhibitors in solution and one for the sampling of gas. The cuvettes contained a similar nutrient solution in which the plants were grown, except that the MES concentration was increased to 5 mM (pH 6). The nutrient solutions were stirred in the cuvettes with magnetic stirrers at the base of each cuvette. The roots were aerated with air containing 360 ppm CO_2 and delivered through precision needle valves at a rate of *c.* 60 ml min^{-1}. After 2 to 8 h of acclimation, the roots were exposed to 5,000 ppm CO_2. The air with 5,000 ppm CO_2 was supplied from a cylinder of compressed air mixed with industrial grade CO_2 (Afrox, RSA). CO_2 flux was measured with an ADC-225-MK3 (ADC, Hertz, UK) infrared gas analyser and O_2 consumption with polarographic O_2 electrodes (YSI Co Inc., Yellow Springs, Ohio). The infrared gas analyser system was under positive pressure. Although plants were grown at 5,000 ppm CO_2, during the measurements of respiration 2,000 ppm was used as a compromise for IRGA sensitivity (Van der Westhuizen and Cramer, 1998). Upon completion of the measurements, root and shoot components were harvested separately and oven dried at 80°C for 48 h. The roots were weighed and respiration expressed on a dry weight basis.

Nitrogen uptake

After 15 d in hydroponic culture, the plants were transferred to another set of 22 l hydroponic tanks for the preparation of the NO_3^- and NH_4^+ uptake experiments. For

this preparation of uptake experiments, all the plants were fed with low N by supplying them with the standard Long Ashton nutrient solution which was modified to contain either 0.2 mM NO_3^- or 0.2 mM NH_4^+ for 5 d. After 5 d, the plants were transferred to a 12 h pre-treatment in individual 300 ml temperature regulated (20°C) cuvettes which were aerated with 5,000 ppm CO_2 and supplied with 1,400 µmol m^{-2} s^{-1} PAR. The standard Long Ashton nutrient solution was modified to contain either 0.2 mM NO_3^- or 0.2 mM NH_4^+ for the respective N uptake measurements.

After the 12 h pre-treatment, the nutrient solution in the cuvettes were replaced with a fresh solution containing 1 mM of either NO_3^- or NH_4^+ and depletion was followed over 6 h by sampling the nutrient solution hourly. The rate of N uptake was calculated from the slope of NO_3^- and NH_4^+ depletion and expressed on the basis root fresh weight. NO_3^- was determined using the copper-cadmium method (Nydahl, 1976) and NH_4^+ using a phenol-hypochloride method (Solorzano, 1969).

Phosphate uptake

After 15 d in hydroponic culture, the seedlings were transferred to another set of hydroponic tanks for the preparation of the uptake experiments. For the preparation of P uptake experiments, plants were hydroponically grown for 5 d in the standard Long Ashton nutrient solution which was modified to containing 5 µM P at both NO_3^- or NH_4^+ supply. After 5 d, the plants were transferred to a 12 h pre-treatment in a P-free Long Ashton nutrient solution. During the pre-treatment, plants were placed in individual 300 ml temperature regulated (20°C) cuvettes which were aerated with 5,000 ppm CO_2 and supplied with 1,400 µmol m^{-2} s^{-1} PAR.

After the 12 h pre-treatment, the nutrient solution in the cuvettes were replaced with a fresh solution containing 50 µM P and depletion was followed over 6 h with hourly sampling. The rate of P uptake was calculated from the slope of P depletion and expressed on the basis root fresh weight. P concentration was determined using the method of Murphy and Riley (1962).

^{14}C incorporation and fractionation

At the end of the N-uptake period (described above), the aeration was discontinued prior to the addition of 42 nmol $NaHCO_3$ containing 0.093 MBq $NaH^{14}CO_3$. The solution was aerated for 30 s every 15 minutes and the cuvettes were also swirled by hand every 5 minutes. After one hour the roots were rinsed twice in separate distilled water solutions, blotted dry and separated into root and shoot components which were immediately weighed and quenched in liquid N before storage at –20°C. Plant components were homogenised with 80% (v/v) ethanol and separated into soluble and insoluble components. The soluble component was subsequently fractionated into neutral, acidic, and basic fractions as described by Cramer et al. (1993).

Mycorrhizal analysis

Roots were cut into 1 cm segments and rinsed and cleared with 10% KOH for 5 minutes in an autoclave at 110°C under steam pressure of 200 kPa. The KOH was rinsed off and the segments acidified with 2 N HCl for 10 min. Thereafter the roots were stained with 0.05% (w/v) analine blue for 10 min in an autoclave at 110°C under steam pressure of 200 kPa and then destained in lactic acid overnight. Root segments were placed on slides and the infection components were determined according to Brundrett et al. (1994).

Statistical analysis

The percentage data were arcsine transformed (Zar, 1984). The influence of the factors and their interactions were tested with a three-way analysis of variance (3-way ANOVA) (Statgraphics Version 7, 1993, Statgraphics Corporation, USA). Where the ANOVA revealed significant effects by the factors, the differences between treatments were separated using a post hoc Student Newman Kuels (SNK), multiple comparison test ($P \leq 0.05$). Different letters indicate significant differences between treatments.

3. Results

Mycorrhizal infection

The NH_4^+-fed roots had approximately 30% lower AM colonisation compared to NO_3^- nutrition (Table 1). This

Table 1. Effects of NO_3^- and NH_4^+ nutrition on the arbuscular mycorrhizal infection of six-week old hydroponically-grown tomato plants infected with the arbuscular mycorrhizal fungus *Glomus mosseae*. Plants were either supplied with live inoculum (+AM), or with autoclaved inoculum (–AM) and grown with a standard Ashton nutrient solution modified to contain 50 µM P. The nutrient solution contained 4 mM NO_3^- or NH_4^+ as the nitrogen source. The plants were aerated with 5,000 ppm CO_2 in the hydroponic tanks. Different letters indicate significant differences between each treatment ($P \leq 0.05$). The values represent the means of 5 replicates.

% AM colonisation	NH_4^+		NO_3^-	
	–AM	+AM	–AM	+AM
Arbuscules	0 a	0.5 a	0 a	7.8 c
Hyphae	0 a	12.8 b	0 a	9.2 b
Vesicles	0 a	9.5 a	0 a	15.7 c
Total AMF	0 a	22.8 b	0 a	33.7 c

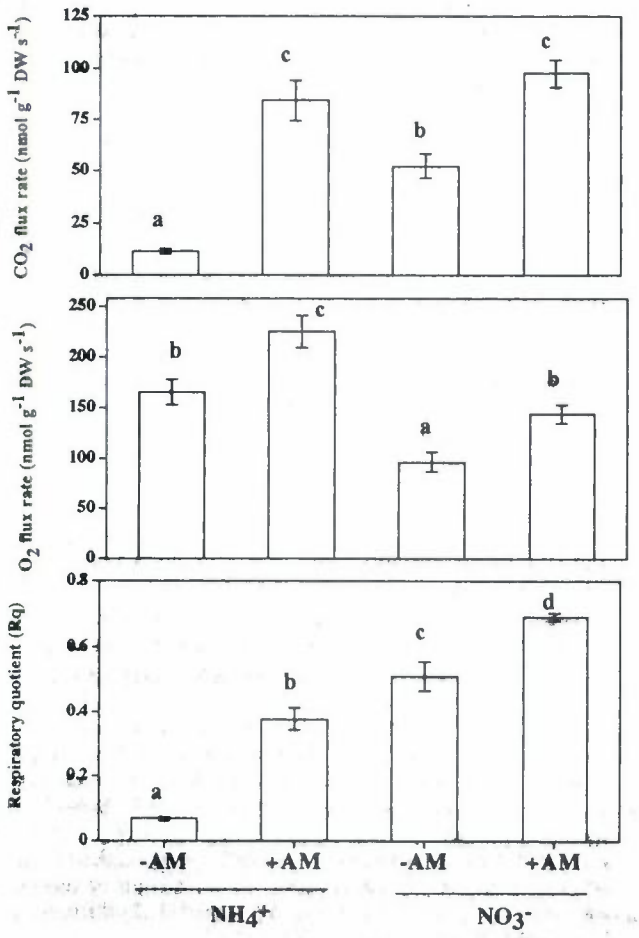

Figure 1. Effects of inorganic nitrogen on the O_2 and CO_2 respiratory fluxes and respiratory gas exchange quotient (Rq) of six-week old tomato plants grown in hydroponics. Plants were supplied with live *Glomus mosseae* inoculum (+AM), or with autoclaved inoculum (–AM) and grown with a Long Ashton nutrient solution modified to contain 50 µM P. The nutrient solution contained either 4 mM NH_4^+ or 4 mM NO_3^- as the nitrogen source. The roots were aerated with 5,000 ppm CO_2 in the hydroponic tanks. Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

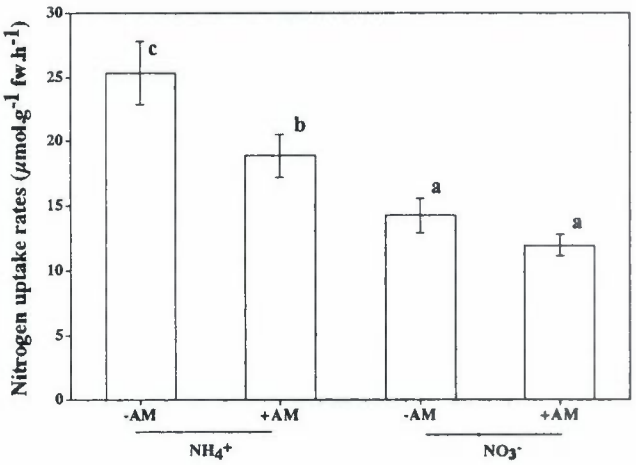

Figure 2. NH_4^+ and NO_3^- uptake rates of six-week old tomato plants grown in hydroponics. Plants were supplied with live *Glomus mosseae* inoculum (+AM), or with autoclaved inoculum (–AM) and grown with a Long Ashton nutrient solution modified to contain 50 µM P. The nutrient solution contained either 4 mM NH_4^+ or 4 mM NO_3^- as the nitrogen source. The roots were aerated with 5,000 ppm CO_2 in the hydroponic tanks. Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

decline in AM colonisation was related to the significantly lower % vesicles and % arbuscules in the NH_4^+-fed roots. Hyphal colonisation between the two nitrogen sources remained unaffected (Table 1).

Root respiration

The AM roots had a higher O_2 consumption compared to non-AM roots in both NO_3^- and NH_4^+-fed plants (Fig. 1). In general the O_2 uptake rates were higher in NH_4^+ supplied roots than NO_3^--fed roots, irrespective of AM colonisation (Fig. 1).

Table 2. Effects of NO_3^- and NH_4^+ nutrition on the incorporation of $H^{14}CO_3^-$ of six-week old hydroponically-grown tomato plants infected with the arbuscular mycorrhizal fungus *Glomus mosseae*. Plants were either supplied with live inoculum (+AM), or with autoclaved inoculum (–AM) and grown with a standard Long Ashton nutrient solution modified to contain 50 µM P. The nutrient solution contained 4 mM NO_3^- or NH_4^+ as the nitrogen source. The plants were aerated with 5,000 ppm CO_2 in the hydroponic tanks. Different letters indicate significant differences between each treatment ($P \leq 0.05$). The values represent the means of 5 replicates.

$H^{14}CO_3^-$ incorporation	NH_4^+		NO_3^-	
	–AM	+AM	–AM	+AM
Root ^{14}C (nmol $^{14}C.g^{-1}.fw$)				
Soluble component	493.79 d	416.03 c	59.03 b	38.57 a
Insoluble component	62.04 b	57.16 b	15.07 a	14.88 a
Total incorporation	555.83 d	473.19 c	74.10 b	53.45 a
% ^{14}C in soluble fractions				
Amino acid fraction	72.94 b	75.52 b	43.82 a	39.00 a
Neutral fraction	12.55 a	7.48 a	27.33 b	29.11 b
Organic acid fraction	12.09 a	15.01 a	24.26 b	27.27 b
Lipid fraction	2.43 a	1.99 a	4.60 b	4.61 b

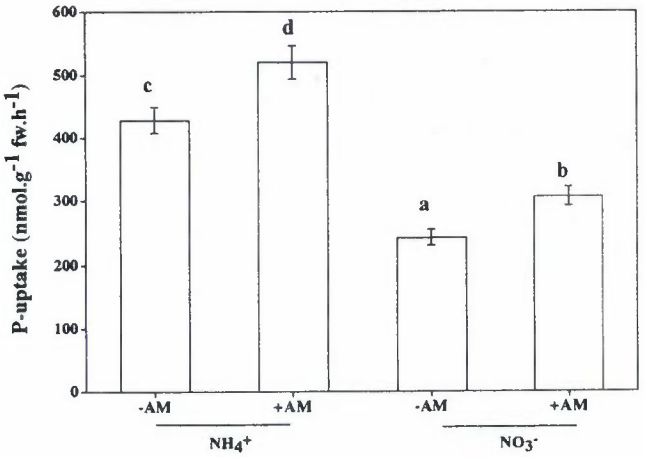

Figure 3. Effects of inorganic nitrogen on the PO_4^- uptake rates of six-week old tomato plants grown in hydroponics. Plants were supplied with live *Glomus mosseae* inoculum (+AM), or with autoclaved inoculum (–AM) and grown with a Long Ashton nutrient solution modified to contain 50 µM P. The nutrient solution contained either 4 mM NH_4^+ or 4 mM NO_3^- as the nitrogen source. The roots were aerated with 5,000 ppm CO_2 in the hydroponic tanks. Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

Furthermore, the respiratory CO_2 release rate and the respiratory gas exchange quotient, the Rq (CO_2 release rate /O_2 uptake rate = Rq) were higher in AM roots than non-AM roots supplied with either NO_3^- or NH_4^+ nutrition (Fig. 1).

^{14}C utilisation of root-zone CO_2

AM and non-AM roots supplied with NH_4^+ nutrition incorporated more root-zone DI^{14}C (dissolved inorganic carbon) than roots supplied with NO_3^- (Table 2). Compared to the non-AM control roots, the AM roots incorporated less root-zone DI^{14}C at both forms of N supply (Table 2). For the NH_4^+ and NO_3^- supplied plants, there were no differences between AM and non-AM roots in the % DI^{14}C assimilation into amino acids, organic acids neutral and lipid fractions (Table 2).

Phosphate and nitrogen uptake rates

P uptake rates in AM roots were higher than in non-AM roots at both forms of N nutrition (Fig. 3). The effect of N source was at NH_4^+ supply there were higher P uptake rates in both AM and non-AM roots, compared to NO_3^- supply (Fig. 3). AM colonisation had no effect on the NO_3^- uptake rates, but there was a decline in the NH_4^+ uptake rates in AM roots compared to non-AM roots (Fig. 2). In general, NH_4^+ uptake rates were higher than NO_3^- uptake rates for both AM and non-AM roots (Fig. 2).

4. Discussion

The source of N supply did not affect the P nutrition of AM roots. The higher P uptake rates in the AM roots are associated with the enhanced O_2 and CO_2 respiration rates of the host roots at both NO_3^- and NH_4^+ supply. Since AM fungi are able to absorb P more efficiently than their hosts and increase the absorptive area of the plant's root system (Bolan, 1991; Smith and Read, 1997), the AM symbiont may have imposed significant C costs. This was found in previous studies, where the higher root respiration was related to the costs of improved nutrient acquisition (Frey and Schüepp, 1993; Johansen et al., 1993; Johansen et al., 1994), particularly P nutrition (Bolan, 1991; Smith and Read, 1997; Mortimer et al., 2005) provided by the AM symbiont. It should be noted that the enhanced respiration of AM roots may not only be related to improved nutrient acquisition, but also to the cost of establishing and maintaining the symbiosis. In this regard, the higher respiratory O_2 consumption and CO_2 release rates in the AM roots compared to non-AM roots can also be attributed to the additional fungal respiration as previously found for O_2 (Baas and Lambers, 1988; Baas et al., 1989) and respiratory CO_2 fluxes (Peng et al., 1993). Baas et al. (1989) found that the additional AM respiration in the host roots is largely due to AM fungal growth and maintenance costs. These costs can be attributed to higher lipid concentrations associated with AM infection, as proposed by previous work (Cooper and Losel, 1978; Wright et al., 1998).

The improved P nutrition and the related enhancement of respiration in AM roots were maintained in spite of the effects of NO_3^- and NH_4^+ nutrition on AM colonisation and respiratory metabolism of root-zone CO_2.

The lower percentage AM colonisation with NH_4^+ rather than NO_3^- nutrition concurs with previous results in sand culture studies (Chambers et al, 1980; Johnson et al., 1984; Azcon et al., 1992; Valentine et al., 2001, 2002). Sand is a medium that readily accumulates CO_2 concentrations between 2,000 and 5,000 ppm (Norstadt and Porter, 1984), and the findings are therefore comparable to the 5,000 ppm CO_2 in this study. Although the sand studies have linked the decrease in AM infection to the NH_4^+-induced acidification of the sand medium, the present investigation used a hydroponic medium with a constant pH 6 for both NH_4^+ and NO_3^--fed plants. Since the NH_4^+ uptake rate was higher than the NO_3^- uptake rate in AM roots, it is therefore more likely that the root carbon costs of NH_4^+ assimilation into amino acids (Oaks and Hirel, 1985; Schweitzer and Erismann, 1985; Arnozis et al., 1988; Vourinen and Kiaser, 1997) would have diverted organic carbon away from the AM fungus. Furthermore, the decline in AM colonisation with NH_4^+ supply coincided with lower arbuscular development and may indicate lower carbohydrate availability in these roots, by virtue of the

arbuscular role in carbohydrate transfer from the host (Blee and Anderson, 1998).

The effect of N source on the AM root respiration was evident in the utilisation of root-zone CO_2. The higher Rq's in AM roots and the accompanying, lower root-zone CO_2 incorporation may indicate that the AM root system at both NH_4^+ and NO_3^- supply had lower levels of organic C skeletons available for the dark incorporation of CO_2. The further partitioning of the incorporated CO_2 may be influenced by the assimilation of inorganic N (Cramer et al., 1993). In this regard NH_4^+-fed roots have a higher anaplerotic C requirement for amino acid synthesis than NO_3^- supplied roots (Cramer et al., 1993). However, since AM roots did not improve the NH_4^+ or NO_3^- uptake rates, this was reflected by the lack of differences in the partitioning of incorporated root-zone CO_2 between AM and non-AM roots. Although the absence of an AM enhancement of N uptake does not concur with previous reports of AM hyphae utilising inorganic N (Johansen et al., 1992, 1993a,b; Frey and Schüepp, 1993), these findings do not diminish the potential role that AM symbionts have in N nutrition of host roots (Govindarajulu et al., 2005).

In conclusion, the enhanced P uptake was associated with increased respiration of AM roots at both NH_4^+ and NO_3^- sources. Although the inorganic N sources affected the colonisation of AM fungi and the respiratory metabolism of root-zone CO_2, AM roots were still able to maintain higher P uptake rates than non-AM roots. However, the increased respiratory sink activity of AM roots may not only be attributed to improved P nutrition, but also to the maintenance cost of the AM symbiosis.

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