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Arbuscular mycorrhiza maintains nodule function during external NH_4^+ supply in *Phaseolus vulgaris* (L.)

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Abstract The synergistic benefits of the dual inoculation of legumes with nodule bacteria and arbuscular mycorrhizae (AM) are well established, but the effect of an external NH_4^+ supply on this tripartite relationship is less clear. This effect of NH_4^+ supply was investigated with regards to the growth and function of the legume host and both symbionts. Nodulated *Phaseolus vulgaris* seedlings with and without AM, were grown in a sand medium with either 0 N, 1 mM or 3 mM NH_4^+. Plants were harvested at 30 days after emergence and measurements were taken for biomass, N_2 fixation, photosynthesis, asparagine concentration, construction costs and N nutrition. The addition of NH_4^+ led to a decline in the percentage AM colonization and nodule dry weights, although AM colonization was affected to a lesser extent. NH_4^+ supply also resulted in a decrease in the reliance on biological nitrogen fixation (BNF); however, the AM roots maintained higher levels of NH_4^+ uptake than their non-AM counterparts. Furthermore, the non-AM plants had a higher production of asparagine than the AM plants. The inhibitory effects of NH_4^+ on nodule function can be reduced by the presence of AM at moderate levels of NH_4^+ (1 mM), via improving nodule growth or relieving the asparagine-induced inhibition of BNF.

Keywords Arbuscular mycorrhiza · NH_4^+ · Nitrogen fixation · Photosynthesis · Asparagines

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Introduction

The tripartite symbiosis formed between legumes, mycorrhizae and nodule bacteria are known to benefit both the host as well as the respective symbionts (Carling et al. 1978; Mortimer et al. 2009; Vesjsadova et al. 1993). It is well established that this three-way relationship benefits the host to a greater extent than singular inoculation with either symbiont, resulting in improved nutrition and growth of the host plant (Carling et al. 1978; Goss and Varennes 2002; Kawai and Yamamoto 1986; Luis and Lim 1988; Mortimer et al. 2008; Vesjsadova et al. 1993). The benefits of dual inoculation are amplified under nutrient limiting conditions, where the P supplied by the AM and the N supplied by the nodules aid the plant in growing in these stressed conditions (Fredeen and Terry 1988; Smith and Read 1997). In exchange for the nutrients provided, the symbionts require C, which the legume host provides in the form of photosynthate (Smith and Read 1997; Vance 2002; Vessey and Layzell 1987), as has been documented for

either singular or dual inoculated legume roots (Brown and Bethlenfalvay 1987; Harris et al. 1985; Jia et al. 2004; Mortimer et al. 2008).

Conventionally, legumes rely on the N contribution of the N_2-fixing nodules for growth and development, but they also can access other sources of inorganic N in the soil. Since AM can play an important role in the uptake and supply of inorganic N to the host plants (Constable et al. 2001; Govindarajulu et al. 2005; Marschner and Dell 1994), these symbionts have also been implicated in the N nutrition of legumes (Mortimer et al. 2008, 2009). Although AM-colonized legumes have been reported to show improved N fixation rates and even increased access to inorganic soil N (Goss and de Varennes 2002; Mortimer et al. 2008, 2009), the presence of external N supply can be inhibiting to both symbionts. Inorganic N in the soil has been shown to have a negative effect on both nodulation and N fixation, although certain rhizobial strains are more sensitive to soil N than others (Awonaike et al. 1980; Müller et al. 1993; Senaratne et al. 1987). Similarly inorganic soil N, particularly NH_4^+, has been linked to a decrease in the percentage AM colonization of plant roots (Azcon et al. 1992; Chambers et al. 1980; Johnson et al. 1984; Valentine et al. 2001, 2002; Valentine and Kleinert 2006).

In the legume tripartite system, AM establishes the symbiosis with the host roots more rapidly than nodules do (Mortimer et al. 2008, 2009), and may therefore be able to influence the effects of external N supply on nodule development and functioning. In this regard, it remains uncertain at which concentration of external N supply the benefits of AM to nodules would persist. The aim of this study was therefore to investigate the range of external NH_4^+ supply at which AM confers functional benefits to legume nodules.

Materials and methods

Seed inoculation and plant growth

The seeds of the Phaseolus bean, *Phaseolus vulgaris* (var. contender), were inoculated with a rhizobial inoculum containing *Rhizobium leguminosarum* biovar *phaseoli bacteria* (StimuPlant cc, Zwavelpoort 0036) and germinated in vermiculite. The *Phaseolus* bean seeds were coated with a paste of 2 g of inoculum per 100 seeds. The seeds were spread out, away from direct sunlight, to allow the inoculum to dry until manageable. Once dry, the seeds were planted in seedling trays containing vermiculite.

The AM treatments were inoculated with *Glomus etunicatum* (Becker & Gerdemann) (AmphiGro, Grahamstown, RSA) and the control plants received a filtered inoculum solution. The AM treatment received 50 g mycorrhizal inoculumper seedling tray (100 seeds). The inoculum was mixed with the vermiculite in the seedling tray just below the level which the seeds where planted at. For the control plants, filtered inoculum solution was prepared by filtering the inoculum through a 37-μm mesh, which removed all fungal material. The filtrate was filtered from the same inoculum and applied each time, when the plants were watered in the seedling trays.

Seedling trays were watered daily with the aid of timer controlled overhead sprayers. Seeds were germinated in an east-facing glasshouse at the University of the Western Cape, Cape Town, South Africa. The range of midday irradiances were between 570 and 650 μmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 23/16°C and 30/70%, respectively. At 10 days after emergence (dae) the seedlings were transferred to 20-cm-diameter pots containing river sand. The layout of the pots was in a randomized block design. The blocks were represented by random tables in the glasshouse and the positions of the pots on the tables, were also randomized. Once a week, the potted plants received Long Ashton nutrient solution (Hewitt 1986), modified for the respective treatments, and pH was maintained at 5.8. In addition the pots were watered with distilled water once a week. The field capacity of the river sand was calculated in the pots, to ensure the potted plants would not experience drought stress following this watering regime. The treatments were divided into AM and non-AM, both receiving three levels of N nutrition: 0 mM NH_4^+, 1 mM NH_4^+ and 3 Mm NH_4^+. NH_4^+ used was supplied as NH_4Cl^- and no NO_3 was added to the nutrient solution.

Photosynthesis

At 30 dae, the youngest fully expanded leaf for each plant was used for the photosynthetic determinations. Readings were taken using a portable infrared gas analyzer (LCA-Pro, ADC, Herts SG12 9TA, England).

Below-ground respiration

CO_2 release was measured in whole root systems, using a portable infrared gas analyzer (LCA-Pro, ADC, Herts, England). The analyzer was the same system used for photosynthesis measurements, but for total root respiration an adaptable soil hood was used.

Harvesting and nutrient analysis

Harvesting of the legumes took place at 30 dae, after the gas exchange readings had been taken. Upon harvesting the

plants were separated into roots (and nodules), stems and leaves. Sub-samples of root segments were stored in 50% ethanol in order to determine percentage AM fungal colonization at a later stage. The harvested material was then placed in a drying oven, at 80°C, for 2 days and dry weights were recorded. The dried plant material was milled using a 0.5-mm mesh (Arthur H. Thomas, California, USA).

The percentage decline in nodule dry weight, defined as the change in nodule dry weight resulting from the addition of NH_4^+, was calculated according to the following formula:

$$D = ((p_1 - p_2)/p_1) \times 100$$

where D represents the percentage decline in nodule dry weight, p_1 is the nodule dry weight with 0 mM NH_4^+ and p_2 is the nodule dry weight with supplemental NH_4^+ (either 1 or 3 mM NH_4^+, respectively).

Determination of percentage AM colonization

Roots were cut into 1 cm segments and rinsed and cleared with 20% KOH for 3 days at room temperature. KOH was rinsed off and the segments acidified with 1% HCl overnight. Thereafter the roots were stained with 0.05% (w/v) aniline blue and left overnight. The roots were then destained in a 1% HCl/glycerol mix. Root segments were placed on slides and the colonization components were determined according to the method described by Brundrett et al. (1994).

The percentage decline in mycorrhizal colonization, defined as the change in mycorrhizal colonization resulting from the addition of NH_4^+, was calculated according to the following formula:

$$D = ((p_1 - p_2)/p_1) \times 100$$

where D is the percentage decline in colonization, p_1 id the percentage mycorrhizal colonization with 0 mM NH_4^+ and p_2 is the mycorrhizal colonization with supplemental NH_4^+ (either 1 or 3 mM NH_4^+, respectively).

Determination of δN15

The $\delta^{15}N$ analyses were carried out at the Archeometry Department, University of Cape Town. The isotopic ratio of $\delta^{15}N$ was calculated as δ = 1,000‰ $[R_{sample}/R_{standard}]$, where R is the molar ratio of the heavier to the lighter isotope of the sample and standards as defined by Farquhar et al. (1989). Between 2.100 and 2.200 mg of each sample was weighed into 8×5 mm tin capsules (Elemental Microanalysis, Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyzer (Fisons Instruments, Milan, Italy). The $\delta^{15}N$ values for the nitrogen gases released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift; two in-house standards (Merck Gel and Nasturtium) and one IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO_4$.

The $\delta^{15}N$ natural abundance of the legumes was corrected for the seed N, according to Boddey et al. (1995):

$$\delta^{15}\text{N enrichment (Seed corrected)} = ((\text{plant N} \times \delta^{15}\text{N}_{\text{plant}}) - (\text{seed N} \times \text{Ps} \times \delta^{15}\text{N}_{\text{seed}})/(\text{plant N} - \text{seed N}))$$

where plant N and seed N represent the respective N concentrations of the plant and seed, $\delta^{15}N$ plant and $\delta^{15}N$ seed represent the respective $\delta^{15}N$ values of the plant and seed and Ps is the proportion of the seed N that was assimilated by the legume.

The seed corrected $\delta^{15}N$ values were used to determine the percentage N derived from the atmosphere (NDFA). % NDFA was calculated according to Shearer and Kohl (1986):

$$\%\text{NDFA} = 100 \times ((\delta^{15}\text{N}_{\text{reference plant}} - \delta^{15}\text{N}_{\text{legume}})/(\delta^{15}\text{N}_{\text{reference plant}} - B))$$

where B is the $\delta^{15}N$ natural abundance of the N derived from biological N fixation (BNF) of the above-ground tissue of *Lens vulgaris*, grown in a N-free culture, according to Shearer and Kohl (1986). The B value of *L. vulgaris* was determined as −0.76 ‰.The reference plant was a non-nodulated *P. vulgaris* (var. contender), which was supplied with mineral N in a nutrient solution.

Asparagine determination

Asparagine concentrations were commercially analyzed (Central Analytical Facilities, University of Stellenbosch, RSA) using a Waters API Quattro Micro. Nodule tissue of 100 mg were hydrolyzed and subjected to EZ:Faast analysis. Asparagine levels are estimated as aspartic acid concentrations, because HCl hydrolysis causes asparagine to be deaminated to aspartic acid.

Construction costs

Construction costs, Cw (mmol $C g^{-1}$ dw), were calculated according to Mortimer et al. (2005), modified from the equation used by Peng et al. (1993):

$$Cw = [C + kN/14 \times 180/24](1/0.89)(6{,}000/180)$$

where Cw is the construction cost of the tissue (mmol C/g DW), C is the carbon concentration (mmol C/g), k is the reduction state of the N substrate (k=−3 for NH_3) and N is the organic nitrogen content of the tissue (g/g DW) (Williams et al. 1987). The constant (1/0.89) represents the fraction of the construction cost which provides reductant that is not incorporated into biomass (Williams et al. 1987; Peng et al. 1993) and (6,000/180) converts units of g glucose/g DW to mmol C/g DW.

Statistical analysis

There were three replicates for each treatment. The percentage data were arcsine transformed (Zar 1999). The effects of the factors and their interactions were tested with an analysis of variance (ANOVA) (SuperAnova). Where the ANOVA revealed significant differences between treatments the means were separated using a post hoc Student Newman–Kuehls (SNK) multiple range test ($P \leq 0.05$). Different letters indicate significant differences between treatments.

Results

Biomass

The addition of NH_4^+ resulted in a decline in the percentage AM colonization, colonization was greatest in the plants that received no supplemental NH_4^+ and lowest for those receiving 3 mM NH_4^+ (Fig. 1). The plants in the non-AM treatments remained non-mycorrhizal for the duration of the experiment. The addition of NH_4^+ also caused a drop in nodule dry weight (dw), the greatest effect being seen at 3 mM NH_4^+ (Table 1). However, the addition of NH_4^+ appeared to have a greater effect on the nodules than the AM, evidenced by the higher percentage decrease in nodule dw, than the percentage decline in percentage AM colonization (Fig. 2a, b).

It appears that AM had a positive influence on nodule growth, resulting in a larger number of nodules; larger individual nodules and a larger total nodule biomass than the non-AM plants, although once the plants were exposed to 3 mM NH_4^+ these effects were diminished (Table 1). The correlation (R^2=0.83) between AM and nodule dry weight

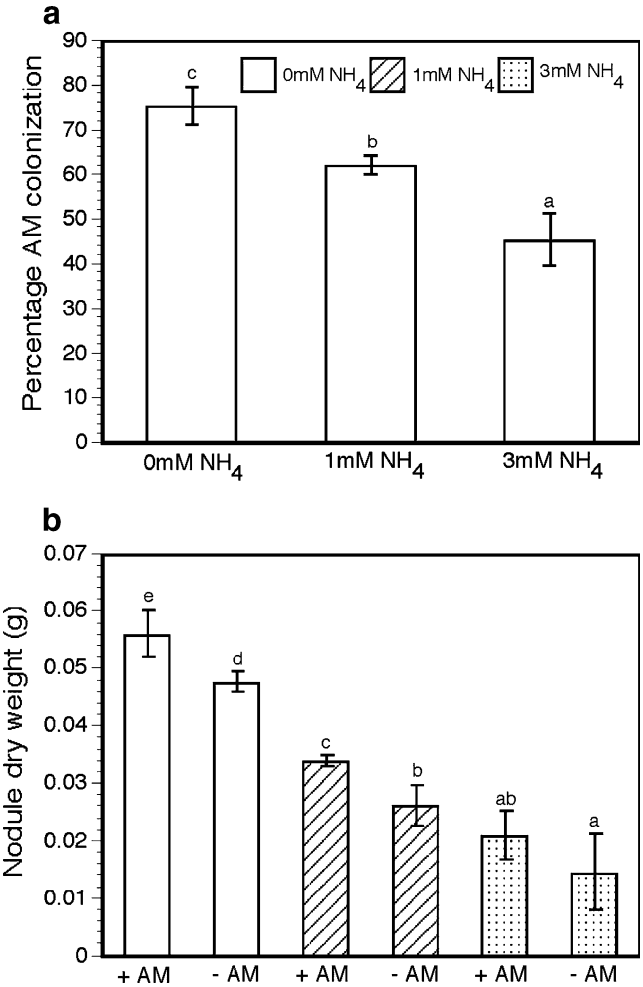

Fig. 1 Percentage mycorrhizal colonization (**a**) and the nodule dry weights (g) (**b**) of nodulated *Phaseolus vulgaris* (L.). Plants were either colonized (+*AM*) with *Glomus etunicatum* or remained uncolonized (−*AM*). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row ($P \leq 0.05$)

further attests to the positive influence of AM on nodule growth (Fig. 3a).

The addition of NH_4^+ also had an effect on host growth, resulting in a decline in root dry weight. Although the roots in the 3 mM NH_4^+ treatment were larger than those of the 1 mM treatment, the AM roots were generally smaller than the non-AM roots (Table 1). There was an opposite effect on the shoot dry weight in the plants receiving NH_4^+, non-AM, NH_4^+ fed plants had increasing shoot dry weigh with the increase in supplied N. However, the increase in shoot dw of AM plants leveled off at 1 mM NH_4^+ and differences between the AM and non-AM shoot dry weight were only apparent in the 0 N and 1 mM NH_4^+ treatments (Table 1). This relationship between the changes in the plant roots and shoots according to

Table 1 Biomass parameters (g) and nitrogen concentrations (mmol/g) of nodulated *Phaseolus vulgaris* (L.)

Plants were either colonized (+AM) with *Glomusetunicatum* or remained uncolonized (−AM). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row ($P \leq 0.05$)

	0 mM NH_4^+		1 mM NH_4^+		3 mM NH_4^+	
	+AM	−AM	+AM	−AM	+AM	−AM
Dry weights (g)						
Plant	0.894 b	0.749 a	0.977 c	0.853 b	1.073 d	0.991 cd
Roots	0.159 c	0.198 d	0.109 a	0.141 bc	0.136 b	0.154 c
Shoot	0.735 bc	0.551 a	0.868 cd	0.712 b	0.937 d	0.838 cd
N (mmol g^{-1} dw)						
Plant	1.65 b	1.349 a	1.826 c	1.712 bc	2.083d	1.886 c
Roots	1.223 b	1.017 a	1.428d	1.359 c	1.411cd	1.366 cd
Shoot	1.685 b	1.492 a	2.082 c	1.795 b	2.094 c	1.964 bc

mycorrhizal colonization and NH_4^+ treatment is also reflected in the root/shoot ratios (Table 1). The AM plants had lower root/shoot ratios than their non-AM counterparts, furthermore, the addition of NH_4^+ led to a decline in the root/shoot ratios of both AM and non-AM plants. As with the root and shoot dry weights, the addition of N in the higher concentrations (3 mM NH_4^+) no longer resulted in a significant difference between AM and non-AM root/shoot ratios (Table 1).

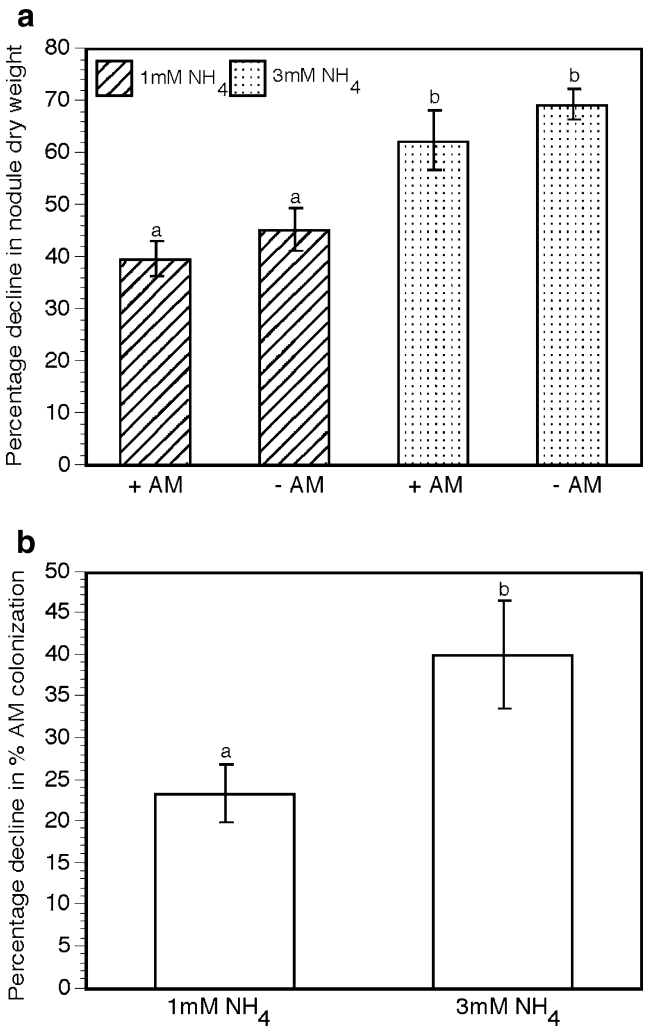

Fig. 2 Percentage decline in nodule dry weight (**a**) and percentage decline in the percentage mycorrhizal colonization (**b**) of nodulated *P. vulgaris* (L.). Plants were either colonized (+*AM*) with *G. etunicatum* or remained uncolonized (−*AM*). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row ($P \leq 0.05$)

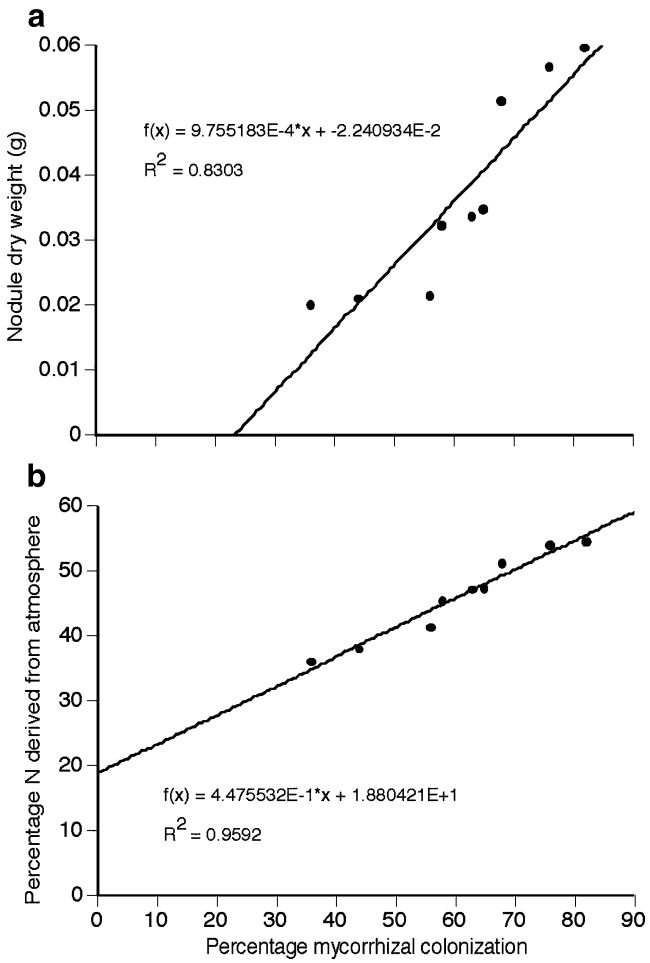

Fig. 3 Correlation between AM and nodule dry weights (**a**) and between AM and %NDFA (**b**) of nodulated *P. vulgaris* (L.). Plants were either colonized (+*AM*) *G. etunicatum* or remained uncolonized (−*AM*)

Nutrition

The percentage NDFA, which represents the amount of N gained via BNF, was greatest when no N was supplied, with the AM plants fixing the most N (Fig. 4). Further evidence for the link between AM and NDFA is shown by the positive correlation between these two parameters (R^2= 0.96) (Fig. 3b). However, the addition of NH_4^+ resulted in a decline in NDFA, although no differences between AM and non-AM plants were found at 3 mM NH_4^+.

N supply improved the N nutrition of the plants, with the AM plants having higher concentrations of N for all treatments (Table 1). In the roots the positive effects of the N supply leveled off after 1 mM NH_4^+. Although the AM roots had greater N concentrations at 1 mM NH_4^+, no differences were found at 3 mM NH_4^+ (Table 1). In a similar fashion, shoot N concentrations were higher with the addition of NH_4^+ but leveled off after 1 mM NH_4^+ (Table 1).

Respiration, photosynthesis and construction costs

For the 0 and 1 mM NH_4^+ treatments, the dual symbiotic plants maintained higher rates of below-ground respiration, with the highest being in the dual symbiotic plants receiving no supplemental NH_4^+ (Fig. 5a). In a similar fashion, there was an increase in the rates of photosynthesis when NH_4^+ was added to the different treatments, however this increase leveled off after 1 mM NH_4^+ (Fig. 5b). The presence of AM also led to increased photosynthetic rates across all treatments.

In the absence of external N supply, the construction costs of the AM roots were higher than those of the non-AM roots; however, the opposite was true once NH_4^+ was added (Fig. 5c). The addition of NH_4^+ led to the AM roots having lower construction costs than the non-AM roots (Fig. 5c).

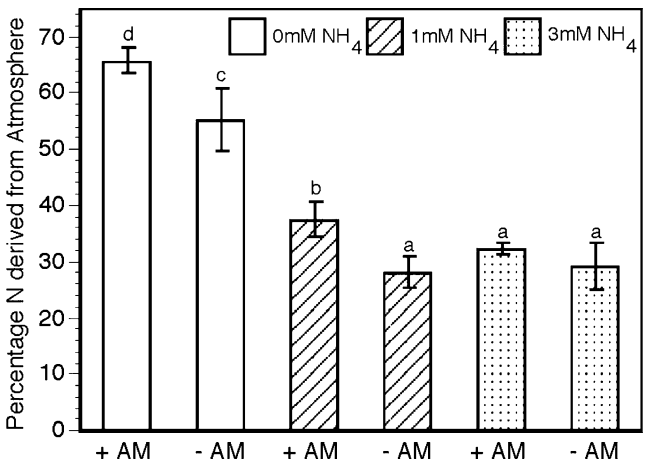

Fig. 4 Percentage N derived from the atmosphere of nodulated *P. vulgaris* (*L.*). Plants were either colonized (+*AM*) with *G. etunicatum* or remained uncolonized (−*AM*). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row (P≤0.05)

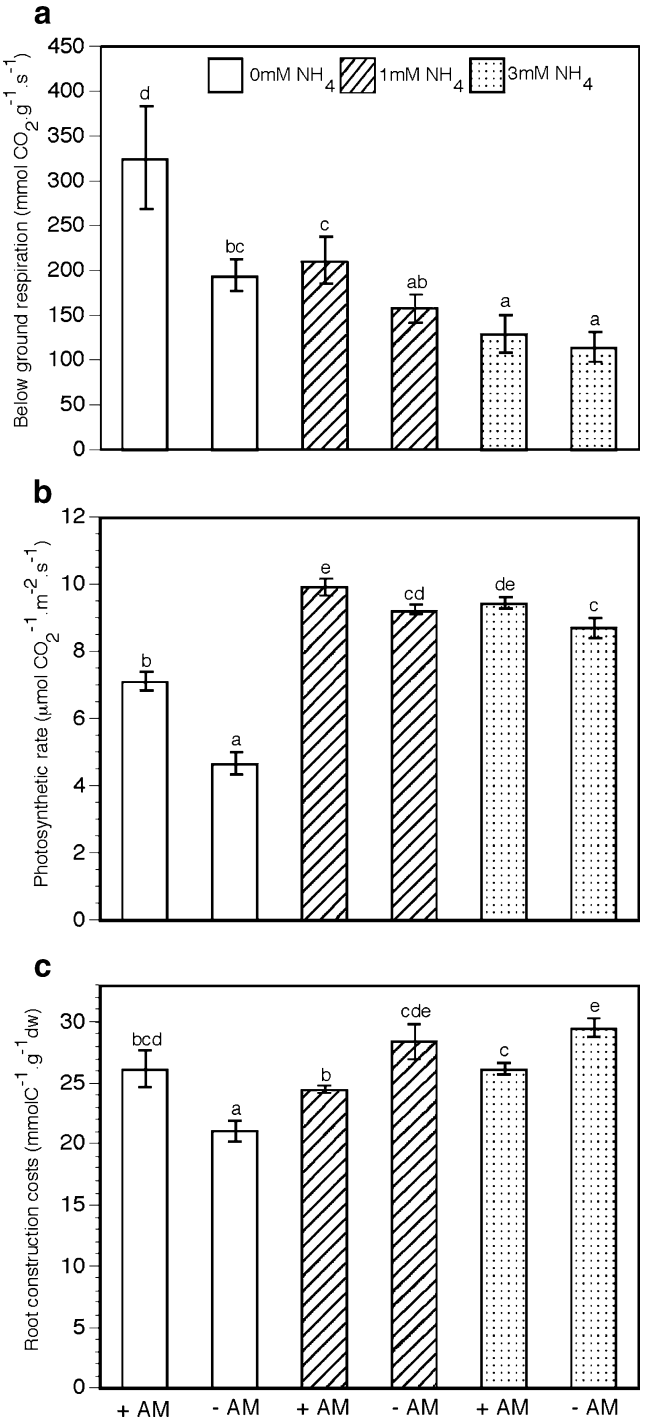

Fig. 5 Below-ground respiration (mmol CO_2 g^{-1} s^{-1}) (**a**), photosynthetic rate (mmol $CO_2^{-1}m^{-2}$ s^{-1}) (**b**) and root construction costs (mmol C^{-1} g^{-1} dw) (**b**) of nodulated *P. vulgaris* (L.). Plants were either colonized (+*AM*) *G. etunicatum* or remained uncolonized (−*AM*). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row (P≤0.05)

Asparagine content

The asparagine content (aspartic acid) between AM and non-AM plants were unaffected in the absence of external NH_4^+ supply (Fig. 6). However, with the addition of NH_4^+ the non-AM plants maintained higher levels of asparagine in both the 1 and 3 mM treatments (Fig. 6). Furthermore, the influence of aspartic acid on the %NDFA is shown in the correlation between these two factors (Fig. 7).

Discussion

In dual symbiotic legumes, NH_4^+ supply inhibited nodules to a greater extent than arbuscular mycorrhizae (AM). Furthermore, the AM appeared to benefit nodule development and maintain nodule function under moderate NH_4^+ supply.

The slower nodule development during supplemental NH_4^+ supply led to a decline in BNF, as previously reported for other legume species (Goergen et al. 2009; Malik et al. 1987; Luciñski et al. 2002). However, a novel finding of the current study is that the presence of AM can alleviate the negative effects of NH_4^+ supply, by enhancing nodule growth in comparison with the nodules of the non-AM roots. This AM effect on nodule biomass, as represented by both nodule weight and nodule number, is confirmed by the positive correlation between AM and nodule growth, irrespective of external NH_4^+ concentration. In spite of the AM-induced increase in nodule dry weights, there was still an overall decline in nodule development, represented by nodule number, specific nodule dry weight and total nodule biomass, across all treatments, as a result of the external N supply. This is in agreement with the work of Goergen et al. (2009), who found a similar pattern for nodule development when exposing *Lupinus albus* to an external source of N.

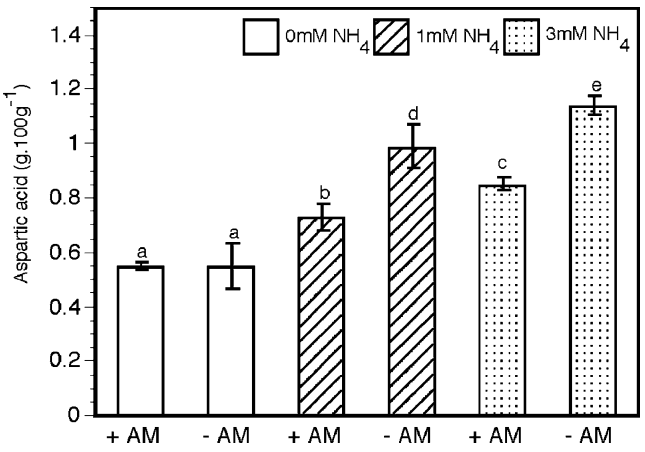

Fig. 6 Aspartic acid concentration (g/100 g) of nodulated *P. vulgaris* (L.). Plants were either colonized (+*AM*) with *G. etunicatum* or remained uncolonized (−*AM*). Values presented are the means (n=3), and the different letters indicate significant differences among the treatments for each row ($P \leq 0.05$)

Although the NH_4^+ fed plant maintained higher levels of N than those relying solely on BNF for N supply, this was particularly evident in the AM colonized plants, which had higher levels of both root N and shoot N. In spite of AM colonization being reduced by NH_4^+ supply, the increases in N concentration and biomass of the AM host plants, suggest that AM roots can function better with external NH_4^+ supply than nodules, especially at 1 mM NH_4^+ supply. Furthermore, this may also indicate that AM symbionts can reduce the inhibitory effects of 1 mM NH_4^+ supply on nodules, as evidenced by the higher %NDFA of the AM roots and the positive correlation between AM and %NDFA.

The AM-induced improvement in plant N nutrition has been well documented in previous studies (Constable et al. 2001; Govindarajulu et al. 2005; Marschner and Dell 1994; Mortimer et al. 2009; Valentine and Kleinert 2006) and concurs with the current findings of an AM benefit to N nutrition from NH_4^+ supply and BNF. Although the reasons for the enhanced NH_4^+ nutrition with AM are well known (Constable et al. 2001; Govindarajulu et al. 2005; Marschner and Dell 1994; Mortimer et al. 2009; Valentine and Kleinert 2006), the AM-induced increase of BNF during 1 mM NH_4^+ supply offers a fresh perspective on the dual symbiosis. This AM related increase in BNF may be related to two major reasons: the improved nodule biomass and the decrease in asparagine production. Firstly, the improved nodule growth in the presence of AM concurs with previous findings on the dual symbiosis of legume roots with AM and Rhizobial nodules (Mortimer et al. 2008, 2009). In this regard, the lower root construction costs of AM plants during NH_4^+ supply may suggest that the lower investment into AM structures (Mortimer et al. 2008, 2009) can make more C available for nodule growth. Secondly, it is known that BNF can be regulated by N-feedback inhibition (Hartwig 1998; Ruffel et al. 2008; Sulieman et al. 2010) and that asparagine is one of the major amino acids that can induce this (Almeida et al. 2000; Ruffel et al. 2008; Sulieman et al. 2010). Therefore, the lower accumulation of the asparagine derivative, aspartic acid, in AM roots, may have relieved the feedback inhibition on BNF, compared to the non-AM NH_4^+ fed roots. The study by Sulieman et al. (2010) found that an asparagine accumulation in the nodules, resulting from external N feeding, led to decreased nodular activity. This is confirmed by our work, specifically the negative correlation between aspartic acid in the phloem and the percentage nitrogen derived from the atmosphere, which is representative of nodular activity.

The plant dry weights were greatest in the AM colonized plants that received NH_4^+, although an increase in the NH_4^+ concentration above 1 mM NH_4^+ did not further benefit these plants. The improved growth of the dual symbiotic

plants occurred in spite of the increased respiratory costs of maintaining two symbionts, primarily because these costs were compensated for by greater photosynthetic rates. Thus coupled with the lower root construction costs of the dual symbiotic plants, there may have been more C available for plant growth. The higher construction costs of the non-AM roots corresponds with the increased root dry weights of these plants when exposed to an external N supply, possibly due to the higher cost of producing root tissue as opposed to fungal tissue (Harley 1989). In addition to the C saved through the lower root construction costs, the AM plants were investing proportionately less C in below-ground structures in comparison to above ground structures. These changes in the root/shoot ratios resulting from mycorrhizal colonization and/or the addition of an external N supply have been noted in other studies in the past (Snellgrove et al. 1982; Goergen et al. 2009).

The cost associated with these AM benefits to the host was an increase in the photosynthetic rate. This increase in photosynthesis is required to maintain the high C demand placed on the host by the growth and maintenance of the two symbionts and the assimilation of NH_4^+ (Harris et al. 1985; Kaschuk et al. 2009, 2010; Provorov and Tikhonovich 2003). Furthermore, the photosynthetic rates of the NH_4^+ fed plants were higher than the plants receiving no supplemental N. This increased photosynthetic rate may have resulted from the root sink stimulation of the higher C costs associated with the root metabolism of NH_4^+ (Valentine and Kleinert 2006) and the increase in shoot N concentrations (Dennis et al. 1997; Kaschuk et al. 2009; Smith et al. 1989).

In conclusion, nodules were more negatively affected than AM by the additional NH_4^+ supply, and the inhibitory effects of NH_4^+ on nodule function can be reduced by AM presence at moderate levels of NH_4^+ (1 mM).

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