NITROGEN USED STRATEGIES OF NODULATED AMAZONIAN LEGUME: INGA EDULIS

Author(s): GC Justino, RP Omena-Garcia, AMS dos Santos, LS de Camargos, L Sodek and JFC Gonçalves

Source: *Journal of Tropical Forest Science*, Vol. 29, No. 1 (January 2017), pp. 1-9

Published by: Forest Research Institute Malaysia

Stable URL: http://www.jstor.org/stable/44028269

Accessed: 25-07-2017 18:57 UTC

Journal of Tropical Forest Science 29(1): 1–9 (2017)

NITROGEN USED STRATEGIES OF NODULATED AMAZONIAN LEGUME: *INGA EDULIS*

GC Justino[1], **RP Omena-Garcia**[2], **AMS dos Santos**[3], **LS de Camargos**[4], **L Sodek**[5] & **JFC Gonçalves**[6, *]

[1]*Institute of Biological Sciences and Health - UFAL, 57010-020, Maceió, Alagoas, Brazil*
[2]*Laboratory of Plant Physiology and Biochemistry, National Institute of Amazonian Research - INPA, P.O. Box 2223, 69011-970, Manaus, Amazonas, Brazil*
[3]*Laboratory of Plant Physiology and Biochemistry, National Institute of Amazonian Research - INPA, P.O. Box 2223, 69011-970, Manaus, Amazonas, Brazil*
[4]*Department of Biology and Animal Science - UNESP, 15385-000, Ilha Solteira, São Paulo, Brazil*
[5]*Department of Plant Biology, Institute of Biology – UNICAMP, P.O. Box 6109, 13083-970 Campinas, São Paulo, Brazil*
[6]*Laboratory of Plant Physiology and Biochemistry, National Institute of Amazonian Research-INPA, P.O. Box 2223, 69011-970, Manaus, Amazonas, Brazil*

**jfc@inpa.gov.br*

Submitted July 2015; accepted March 2016

Symbiotic nitrogen fixation in legumes is strongly diminished by mineral nitrogen. Nevertheless, recent work with some tropical legumes revealed low sensitivity to mineral nitrogen or even enhancement of nodulation by ammonium. Thus, in this study, we investigated plant growth and nodulation of *Inga edulis* over a period of 128 days using different sources of nitrogen, i.e. ammonium (15 mM), nitrate (15 mM) and symbiotic nitrogen fixation. Plant growth was evaluated through dry mass, height, stem diameter and root/shoot ratio as well as nodulation by nodule number and dry mass. Nitrate, ureides and total amino acids were determined in roots, shoots and xylem sap. All three nitrogen sources were found to stimulate growth relative to the non-inoculated N-free control, however ammonium was the most effective. Nodulation, both nodule number and mass, was strongly reduced by nitrate but not ammonium. The transport of total amino acids in the xylem sap was stimulated by ammonium without change in the composition with asparagine predominating in all treatments. *Inga edulis* can benefit from fertilisation with ammonium since even at high concentrations growth was improved without impairing nodulation, a condition appropriate for restoration of soil conditions.

Keywords: Tropical tree species, biological nitrogen fixation, growth, xylem transport, asparagine

INTRODUCTION

Biological nitrogen fixation has been more widely studied in cultivated plants but little is known of its importance in tropical forests (Adams et al. 2010, Araújo et al. 2015, Aidar et al. 2003). The ability of *Inga edulis,* a legume species native to tropical forests, to both fix molecular nitrogen (N_2) and utilise different mineral nitrogen sources, provides this species an important competitive advantage in nitrogen-poor soils (Omena-Garcia et al. 2011). Despite the atmosphere containing 79% of nitrogen, paradoxically plants cannot use molecular nitrogen (Hartwig 1998, Reis 2013). However, plants of the Fabaceae family have developed a symbiotic relationship with certain microorganisms, collectively known as rizhobia, forming N_2 fixing nodules (Rogers et al. 2009, Carnicer et al. 2015). Usually, mineral nitrogen has strong inhibitory effects on nodulation in legume plants which leads to the preferential use of these sources over atmospheric nitrogen (Streeter 1988, Glyan´ko et al. 2009, Barron et al. 2011). However, in soils where nitrogen is scarce, the capacity of the Fabaceae family to use molecular nitrogen is certainly an advantage.

Although it is well established that nitrate and ammonium both lead to reduced nodule number and mass, the inhibitory effect of ammonium appears to be weaker than that of nitrate (Bollman & Vessey 2006). In the case of *I. edulis* nodule growth is actually enhanced by ammonium at 5 mM but not by nitrate which is inhibitory (Omena-Garcia et al. 2015). The positive effect of ammonium on nodulation in *I. edulis* may be an adaptation since in its natural Amazonian habitat ammonium dominates the inorganic N fraction of the soil both in the dry and wet seasons (Santiago et al. 2013). The response of *I. edulis* to mineral nitrogen is an important factor for ecological restoration of degraded lands in the Amazon since nitrogen can be used simultaneously from both atmospheric and mineral sources by this species. According to Leblanc et al. (2005), the genus *Inga* plays an important role in improving soil nutrition by providing N to degraded lands and ameliorating soil conditions in benefit of other non-legume species (Franco & DeFaria 1997, Nichols & Carpenter 2006, Lojka et al. 2012).

In an earlier study, we observed that 5 mM ammonium stimulated nodulation and nitrogen fixation (Omena-Garcia et al. 2015). In other studies where N_2 fixation and nodulation were tolerant to lower concentrations of mineral N, higher concentrations were found to be inhibitory (Betts & Herridge 1987, Dakora 1998, Camargos & Sodek 2010, Pal'ove-Balang et al. 2015). In order to improve the efficiency of *I. edulis* to ameliorate soil conditions in degraded areas, the beneficial use of ammonium fertiliser should preferably not be restricted to a particular low concentration in view of the difficulty of controlling its application in the field. Consequently, the present study investigated whether a higher concentration of mineral nitrogen (15 mM) could maintain a positive effect on nodulation and growth of *I. edulis.*

MATERIALS AND METHODS

Plant material and treatments

Seeds of *I. edulis* obtained from fruits harvested from trees growing in the experimental stations of the National Institute of Amazonian Research (INPA) were germinated in vermiculite in plastic trays. After 15 days, seedlings with three to four leaves, about 12 cm in height, were transferred to 3 L plastic pots (one plant per pot) containing vermiculite as substrate. The root system of the seedlings was inoculated with 5 mL of bacterial suspension taken from 11 Amazonia *Inga* species (Vincent 1970). Plants were grown between November 2011 and March 2012 in a greenhouse located at INPA under natural light and temperature conditions. Irrigation was carried out twice a week with 100 mL of full-strength modified Hoagland & Arnon's (1950) nutrient solution adjusted to pH 6.5 with three different N sources, i.e. N-free nutrient solution (N-free), nutrient solution with sodium nitrate (NO_3^-) and ammonium sulphate (NH_4^+), both added to a final concentration of 15 mM of N. The N treatments were initiated just before inoculation and the application of nutrient solution was maintained for 120 days and water supplied as necessary. Replicates were rotated every seven days to minimise effects of environmental gradients in the greenhouse.

Plant growth and quantification of nitrogen compounds

Measurements of plant height and stem diameter were taken just after transfer to plastic pots and then every fifteen days until the end of the experiment. The relative growth rate in height (RGR-h) and diameter (RGR-d) of each plant was calculated using the following formulas:

$$\text{RGR-h} = (\ln H_2 - \ln H_1)/t \qquad (1)$$

$$\text{RGR-d} = (\ln D_2 - \ln D_1)/t \qquad (2)$$

where H_1 and H_2 = initial and final height (cm) of the plant and D_1 and D_2 = initial and final stem diameter (mm) of the plant and t = experimental time (months). On day 120 after initiating treatments, immediately after harvest, leaves, roots and nodules were rinsed in distilled water and the dry mass component was determined following 72 hours in an oven at 70 °C. After initial grinding of tissues in a mortar using liquid N_2, amino acids, ureides and NO_3^- were extracted by homogenisation of 1 g of fresh weight tissue with 10 mL of MCW (methanol/chloroform/water – 12/5/3 – v/v) according to Bieleski and Turner (1966). The aqueous phase containing the soluble N compounds was recovered following phase separation on standing after addition of chloroform, water and supernatant in a ratio of 1:1.5:4 volume. The aqueous phase was then reduced to a known volume by evaporation at 38 °C and kept frozen until analysis.

Root (xylem) bleeding sap was collected for 1 h between 11:00 h and 13:00 h after cutting the stem just below the first pair of leaves (McClure & Israel 1979). The exuding sap was collected with a glass capillary and transferred to tubes kept on ice. The samples were stored at -20 °C until analysis.

Total amino acids, ureides, NO_3^- and NH_4^+ were determined colorimetrically using leucine, allantoin, NO_3^- and NH_4^+ as standards, respectively (Yemm & Cocking 1955, Vogels & Van Der Drift 1970, Cataldo et al. 1975, McCullough 1967). Free amino acids were analysed by reverse phase HPLC of their *o*-phthaldialdehyde (OPA) derivatives as described previously by Puiatti & Sodek (1999).

Statistical analysis

The experiment was carried out with 10 replicates using a randomised complete block design. The data were subjected to analyses of variance and the significance between means was determined by using the Tukey test ($p \leq 0.05$) with STATISTIC 6.0. Relationships among treatments (N-free, NO_3^- and NH_4^+) versus growth in height and diameter were investigated by regression analyses.

RESULTS

Changes in nodulation performance showed that the nitrogen source, namely ammonium, nitrate or atmospheric nitrogen produced different effects on nodulation of the legume plant *I. edulis.* Relative to the symbiotic plant grown without mineral N, nodule number and nodule dry mass were drastically reduced by nitrate at 15 mM but not by ammonium. Ammonium at 15 mM as used in this study did not stimulate nodulation in contrast to 5 mM ammonium as shown by Omena-Garcia et al. (2015) but nevertheless it was not inhibitory. It is noteworthy that dry mass accumulation by nodules (Table 1) responded to treatments in parallel with the nodule number data (Figure 1, Table 1).

Table 1 Dry mass (g) components of nodules, roots, stems and leaves of nodulated *Inga edulis* as affected by N-free, NO_3^- and NH_4^+ full strength modified nutritive solution of Hoagland and Arnon (1950)

Treatments	Dry weight of plant component (g)				
	Leaf	Stem	Root	Nodule	Total dry mass
Control (-N)	2.1 ± 0.5 c	1.8 ± 0.3 c	1.7 ± 0.3 c	0.09 ± 0.02 b	5.7 ± 2.0 c
Inoculated (-N)	7.4 ± 1.4 b	4.3 ± 2.0 b	5.9 ± 1.3 b	0.52 ± 0.1 a	18.2 ± 6.2 b
NO_3^- 15 mM	6.4 ± 1.4 b	4.2 ± 1.7 b	5.9 ± 1.7 b	0.1 ± 0.1 b	16.3 ± 6.7 b
NH_4^+ 15 mM	14.1 ± 4.3 a	8.5 ± 3.0 a	10.0 ± 2.6 a	0.6 ± 0.1 a	33.3 ± 10.0 a

Means ± SE, means with different letters are significantly different ($p \leq 0.05$)

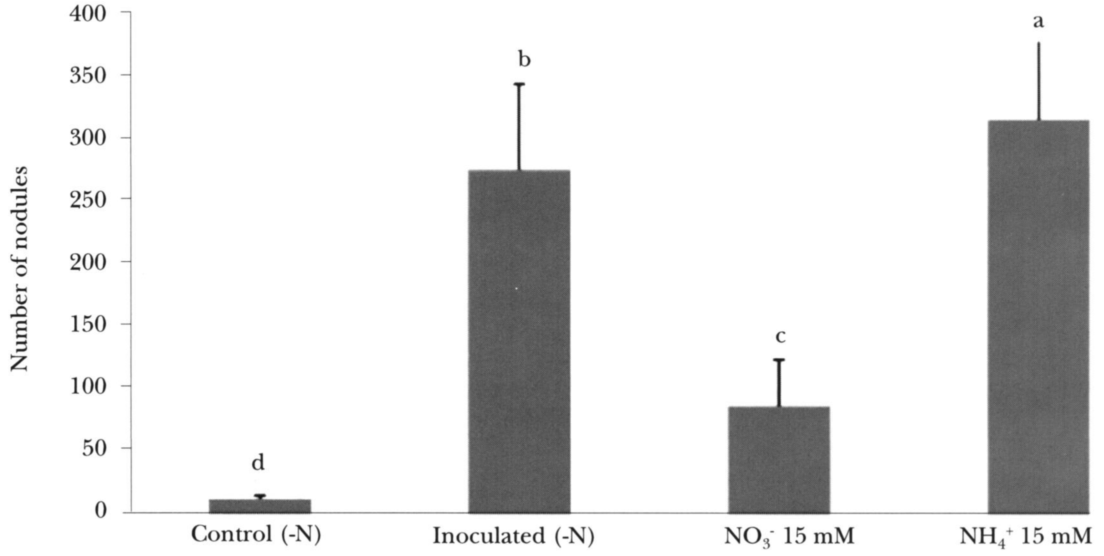

Figure 1 Nodule number of nodulated *Inga edulis* as affected by N-free, NO_3^- and NH_4^+ full strength modified nutritive solution of Hoagland and Arnon (1950); bars = SE; means with different letters are significantly different ($p \leq 0.05$)

The growth of nodulated *I. edulis* plants as measured by total plant dry mass, shoot height and stem diameter increased most in the presence of 15 mM ammonium (Figure 2, Table 1). Growth promoted by nitrate was similar to that of the symbiotic plants totally dependent on N_2 fixation (N-free). As expected, the non-inoculated N-free control grew least and clearly invested most of its resources in root growth in view of the much higher root/shoot ratio compared to all other treatments (Table 2). The same growth response was observed for all measurements, i.e dry mass accumulation (Table 1), relative growth rates for height RGR-h (cm) and stem diameter RGR-d (mm) (Table 2).

Amino acids predominated the transport of N compounds in the xylem in all treatments except non-inoculated plants fed with the N-free medium where no N compounds were detected (Table 3). There was little difference between treatments for nitrate and ureide content of the xylem sap but amino acids showed a clear increase in response to ammonium. Nitrate was conspicuously low in the xylem sap even in the treatment with nitrate. Possibly, most of the nitrate taken up was assimilated in the root with little remaining for transport to the shoot. Indeed the nitrate levels of the leaves were fairly constant between treatments, consistent with nitrate assimilation predominating in the root. The presence of nitrate in the tissues of plants not fed with nitrate may have arisen from the efficient uptake of traces of nitrate in water. Ureides were also little affected by treatment in all tissues,

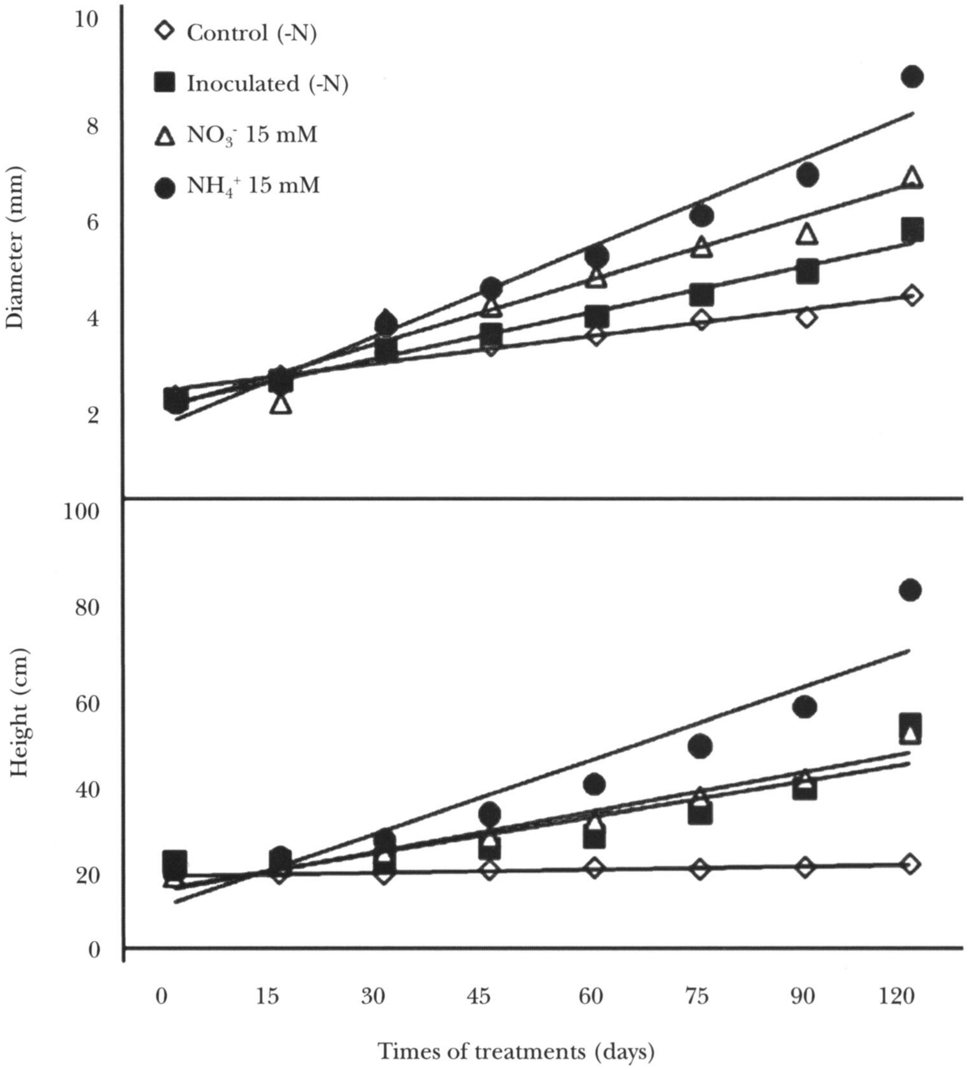

Figure 2 Treatments started at day 0 using 15-day-old seedlings; growth in height and stem diameter with time of nodulated *Inga edulis* as affected by N-free, nitrate (NO_3^-) and ammonium (NH_4^+) full strength modified nutritive solution of Hoagland and Arnon (1950)

Table 2 Relative growth rate in height (RGH – h), basal diameter (RGD – d) and root/shoot ratios of nodulated *Inga edulis* as affected by N-free, NO_3^- and NH_4^+ full strength modified nutritive solution of Hoagland and Arnon (1950)

Treatment	RGH – h (cm cm^{-1} month^{-1})	RGD - d (cm cm^{-1} month^{-1})	Root/shoot ratio
Control (-N)	0.04 ± 0.02 c	0.15 ± 0.01 c	1.15 ± 0.15 a
Inoculated (-N)	0.23 ± 0.01 b	0.23 ± 0.01 b	0.43 ± 0.08 b
NO_3^- 15 mM	0.26 ± 0.01 b	0.25 ± 0.02 b	0.46 ± 0.04 b
NH_4^+ 15 mM	0.39 ± 0.03 a	0.34 ± 0.02 a	0.35 ± 0.02 c

Means ± SE, means with different letters are significantly different ($p \leq 10.05$)

Table 3 Total amino acid, ureide and nitrate contents of nodulated *Inga edulis* as affected by N-free, NO_3^- and NH_4^+ full strength modified nutritive solution of Hoagland and Arnon (1950)

Treatment	Amino acid (μmol g^{-1})	Ureide (μmol g^{-1})	Nitrate (μg g^{-1})
Leaf			
Control (-N)	10.2 ± 2.5 b	7.2 ± 1.0 a	20.5 ± 7,5 ab
Inoculated (-N)	12.8 ± 2.7 ab	5.9 ± 0.2 a	11.5 ± 5.7 b
NO_3^- 15 mM	17.6 ± 2.1 a	6.4 ± 0.7 a	19.3 ± 6.1 ab
NH_4^+ 15 mM	16.5 ± 3.4 a	5.6 ± 0.3 a	30.3 ± 4.0 a
Root			
Control (-N)	5.0 ± 2.7 a	4.4 ± 0.2 b	18.6 ± 7.4 c
Inoculated (-N)	3.9 ± 1.3 a	4.2 ± 0.4 b	18.9 ± 3.4 c
NO_3^- 15 mM	5.1 ± 2.4 a	5.1 ± 0.2 a	216.4 ± 51.9 a
NH_4^+ 15 mM	4.5 ± 1.6 a	3.3 ± 0.3 c	67.4 ± 21.9 b
Nodule			
Control (-N)	17.8 ± 10.5 b	11.1 ± 3.4 a	15.4 ± 10.0 c
Inoculated (-N)	26.5 ± 7.0 ab	4.9 ± 0.1 b	20.4 ± 4.26 c
NO_3^- 15 mM	15.0 ± 5.5 b	6.2 ± 0.4 b	99.9 ± 9.7 a
NH_4^+ 15 mM	31.6 ± 4.2 a	4.1 ± 0.3 b	65.9 ± 10.2 b
Xylem sap		μmol mL^{-1}	
Control (-N)	ND	ND	ND
Inoculated (-N)	2.6 ± 0.9 b	1.3 ± 0.1 a	0.02 ± 0.01 b
NO_3^- 15 mM	2.8 ± 2.1 b	0.9 ± 0.1 a	0.3 ± 0.1 a
NH_4^+ 15 mM	6.8 ± 1.6 a	1.3 ± 0.5 a	0.2 ± 0.1 a

ND = not detected, means ± SE, means with different letters are significantly different ($p \leq 0.05$)

even in nodules where they might be expected to respond as products of N_2 fixation. Despite the absence of N compounds in the xylem sap (not detected) of the non-inoculated N-free treatment, it is noteworthy that in the tissues of these plants the levels were similar or higher than other treatments. This may reflect the reduced mobility of N compounds in this treatment.

The amino acid composition of xylem sap was generally similar for all treatments although the asp/asn ratio was clearly higher in nodulated control plants (-N) (Figure 3). When a mineral source of N was supplied (NO_3^- or NH_4^+) asparagine represented some 70–80% of the total amino acids. In the absence of mineral N, and therefore in plants totally dependent on N_2 fixation, asparagine was lower (60%) while aspartate was more abundant (20%), compared with treatments with mineral N.

DISCUSSION

It is well established that supply of mineral nitrogen causes the inhibition of nodulation (nodule mass and number) and symbiotic nitrogen fixation (Streeter 1988, Glyan'ko et al.

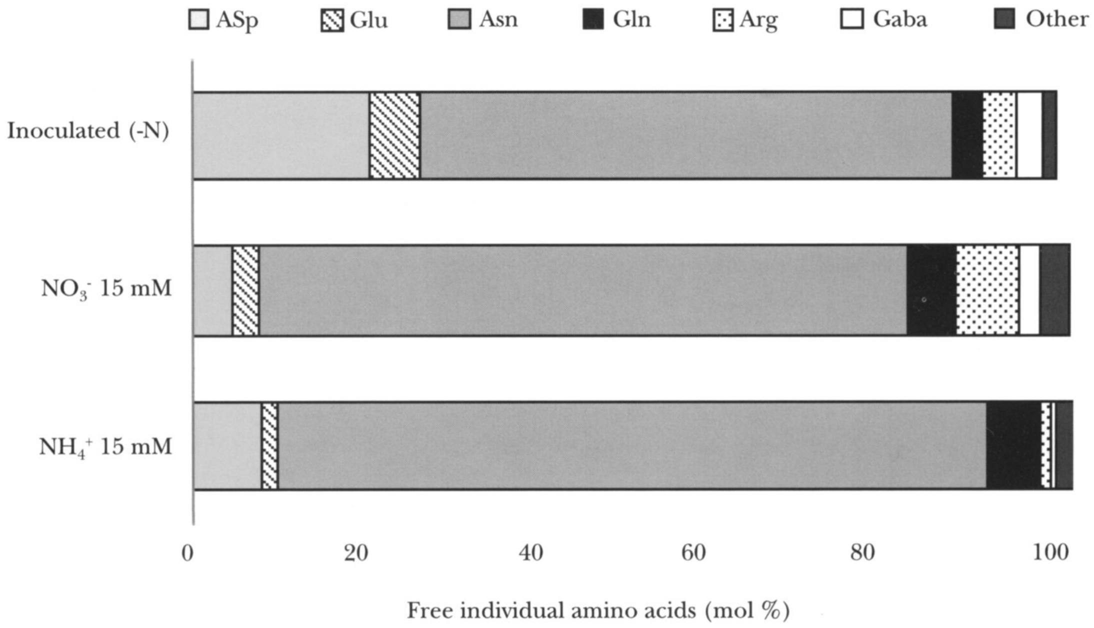

Figure 3 Amino acid composition of xylem sap of symbiotic *Inga edulis* plants grown with N-free medium (-N), with nitrate (+ NO_3^-) and with ammonium (+ NH_4^+); symbiotic plants were cultivated during 128 days and treatment with the N source initiated three days before inoculation and then twice a week following inoculation the symbiotic; Asp = aspartic acid, Glu = glutamic acid, Asn = aslaragine, Gln = glutamine, Arg = arginine, Gaba = gamma aminobutyric acid

2009, Pal'ove-Balang et al. 2015). In the case of soybean (*Glycine max*), for example, nodulation is affected negatively by as little as 1.5 mM nitrate (Sodek & Silva 1996). Although nitrate (15 mM) reduced nodulation of *I. edulis* plants in this study, ammonium was totally ineffective since nodule number and mass were the same as nodulated plants grown in the absence of mineral N. In a previous study by Omena-Garcia et al. (2011), it was reported that ammonium at 5 mM actually stimulated nodulation of *I. edulis*, but in the present study, where a higher concentration of ammonium was used (15 mM), nodulation was unaffected. In general the inhibitory effects of nitrate and ammonium on nodulation and symbiotic nitrogen fixation depend on their concentration and the legume species involved (Davidson & Robson 1986). The inhibitory effect of ammonium is regarded as being less effective towards legume-rhizobium symbiosis than nitrate (Bollman & Vessey 2006), but *Sesbania sesban* is an exception (Dan & Brix 2009). In species tolerant to nitrate, such as *Calopogonium mucunoides* and *Lonchocarpus muehlbergianus*, nitrogen fixation was largely insensitive to the mineral (Camargos & Sodek 2010, Moreira et al. 2014). Nitrate and ammonium effects on nodulation may vary in a species-specific manner probably due the distinct N use strategies of plants. *I. edulis* grows naturally in the Amazon region where soils are richer in ammonium (Wick et al. 2005, Davidson et al. 2007) which may underlie the specific response to ammonium found in this study.

Despite the lack of stimulation of nodulation by higher concentration (15 mM) of ammonium used in this study, it did promote growth as found for the lower concentration (5 mM) used in previous study (Omena-Garcia et al. 2011). This was observed in growth measurements, i.e. relative growth in height (h), relative growth rate in stem diameter (d) and dry mass accumulation. Ammonium promoted growth of plants by its direct use by the plant leading to the conclusion that N_2 fixation was sub-optimal to meet the demands of the plant for N. Nitrate, on the other hand did not promote growth relative to the nodulated plants grown without mineral N. Therefore, that nitrate assimilation was less efficient than ammonium for supplying the plant with N and no more efficient than N_2 fixation. The superior growth performance of nodulated plants in the presence of 15 mM ammonium was also reported for *C. mucunoides* plants compared with plants dependent on nitrate or N_2 fixation (Camargos & Sodek 2010). The superior response of *I. edulis* to ammonium

Table 4 Plant and nodulation growth and total amino acids of the xylem bleeding sap of *Inga edulis* fed with different nitrogen sources (N-free, NC_3^- or NH_4^+)

Treatments	Plant dry weight (g plant^{-1})	Nodules dry weight (g plant^{-1})	Nodule number	Amino acid (μmol mL^{-1})
Control (-N)	9.2 ± 3.6 c	0.05 ± 0.01 b	8.0 ± 2.6 d	ND
Inoculated (-N)	21.7 ± 3.0 b	0.51 ± 0.15 a	272.0 ± 68.0 b	2.6 ± 0.9 b
NO_3^- 15 mM	19.1 ± 5.6 b	0.11 ± 0.06 b	83.0 ± 37.0 c	2.8 ± 2.1 b
NH_4^+ 15 mM	38.5 ± 3.6 a	0.59 ± 0.15 a	312.0 ± 64.0 a	6.8 ± 1.6 a

ND = not detected, means ± SE, means with different letters are significantly different ($p \leq 0.05$)

nutrition compared with nitrate is probably related to its adaptation to the ammonium-rich soils of its natural habitat.

Nitrate and ammonium treatments did not change the total free amino acid content of leaves and roots, but xylem transport of amino acids by plants treated with ammonium was significantly greater. Although ureides were also transported in the xylem, amino acids were the predominant form of N transport in *I. edulis*. Ureides predominate the xylem transport of N in nodulated legumes of the *Phaseoleae* tribe where they are known to be products of symbiotic N_2 fixation (Atkins & Smith 2007). Other symbiotic legumes produce little ureide and use asparagine as the main export form of N transported in the xylem and are often referred to as "amide-producing" legumes in contrast to "ureide-producers" (Atkins et al. 1979, Peoples et al. 1987, Tegeder 2014). The levels of ureide in the xylem sap for *I. edulis* were a little higher than 'amide-producing' legume species that transport N mainly as asparagine (Amarante et al. 2006). This, together with the predominance of asparagine in the xylem sap, suggest that *I. edulis* does not belong to the ureide-producing category but to the amide-producers. This characteristic explains the lack of response of xylem sap ureide levels between treatments found here since such levels would not reflect N_2 fixation activity as they do for the ureide-producing legume category (McClure et al. 1980, McNeil & LaRue 1984, Herridge & Peoples 1990, Herridge et al. 1990). Thus, the data only provided information as to the effects of mineral N on nodulation but not with regard to N_2 fixation.

The predominance of asparagine in the xylem sap of *I. edulis* was similar to another *Inga* species, *I. marginatta,* by Aidar et al. (2003). Information on the transport forms of N in the xylem can be useful since alterations in composition can result from abiotic stress like flooding or nitrogen source as verified by Justino & Sodek (2013) and Amarante et al. (2006). Here, we found that mineral nitrogen lowered the ratio asp/asn in the xylem sap which may indicate a more efficient transformation of aspartate to asparagine during mineral N assimilation compared with N_2 fixation. Indeed, an increase in the asp/asn ratio in the xylem sap of soybean plants has been associated with N deficiency, a phenomenon related to asparagine synthetase activity of the root system (Lima & Sodek 2003, Antunes et al. 2008). This is consistent with sub-optimal growth when *I. edulis* depends solely on N_2 fixation as a source of N.

CONCLUSIONS

Inga edulis showed higher performance in terms of growth when treated with ammonium, a phenomenon apparently linked to the natural growth conditions of the species. Nitrate was similar to N_2 fixation in supplying the plants with N. Nodulation was reduced by nitrate but not by ammonium. Nodulated *I. edulis* may benefit, therefore, from ammonium fertilisation without impairment of nodulation and thereby improve the potential of this species for restoration of soil conditions.

ACKNOWLEDGMENTS

The authors are grateful to the National Institute of Amazonian Research (MCTI-INPA) and National Council for Scientific and Technological Development (CNPq, Brazil) for their financial support of this study. Thanks to LAG de Souza for his assistance during project execution and JFC Gonçalves, researcher of the Brazilian Council for Research and Development.

REFERENCES

Adams MA, Simon J & Pfautsch S. 2010. Woody legumes: a (re)view from the South. *Tree Physiology* 30: 1072–1082.

Aidar MPM, Schmidt S, Moss G, Stewart GR & Joly CA. 2003. Nitrogen use strategies of neotropical rainforest trees in threatened Atlantic forest. *Plant, Cell and Environment* 26: 389–399.

Amarante L, Lima JD & Sodek L. 2006. Growth and stress conditions cause similar changes in xylem amino acids for different legume species. *Environmental and Experimental Botany* 58: 123–129.

Antunes F, Aguilar M, Pineda M & Sodek L. 2008. Nitrogen stress and the expression of asparagine synthetase in roots and nodules of soybean (*Glycine max*). *Physiologia Plantarum* 133: 736–743.

Araújo EO, Martins MR, Vitorino ACT, Mercante FM & Urquiaga SS. 2015. Effect of nitrogen fertilization associated whit diazotrophic bacteria inoculated on nitrogen use efficiency and its biological fixation by corn determined using ^{15}N. *African Journal of Microbiology Research* 9: 643–650.

Atkins CA & Smith PM. 2007. Translocation in legumes: assimilates, nutrients, and signaling molecules. *Plant Physiology* 144: 550–561.

Atkins CA, Pate JS & Layzell DB. 1979. Assimilation and transport of nitrogen in nonnodulated (NO_3^- grown) *Lupinus albus* L. *Plant Physiology* 64: 1078–1082.

Barron AR, Purves DW & Hedin LO. 2011. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. *Oecologia* 165: 511–520.

Betts JH & Herridge DF. 1987. Isolation of soybean lines capable of nodulation and nitrogen fixation under high levels of nitrate supply. *Crop Science* 27: 1156–1161.

Bieleski LR & Turner NA. 1966. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Analytical Biochemistry* 17: 278–293.

Bollman MI & Vessey JK. 2006. Differential effects of nitrate and ammonium supply on nodule initiation, development and distribution on roots of pea (*Pisum sativum*). *Canadian Journal of Botany* 84: 893–903.

Camargos LS & Sodek L. 2010. Nodule growth and nitrogen fixation of *Calopogonium mucunoides* L. show low sensitivity to nitrate. *Symbiosis* 51: 167–174.

Carnicer J, Sardans J, Stefanescu C, Ubach A, Bartons M, Asensio L & Peñuelas J. 2015. Global bio-diversity, stoichiometry and ecosystem function responses to human-induced C–N–P imbalances. *Journal of Plant Physiology* 172: 82–91.

Cataldo DA, Haroon M, Schrader LE & Youngs VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* 6: 71–80.

Dakora FD. 1998. Nodule function in symbiotic Bambara Groundnut (*Vigna subterranea* L.) and Kersting's Bean (*Macrotyloma geocarpum* L.) is tolerant of nitrate in the root medium. *Annals of Botany* 82: 687–690.

Dan TH & Brix H. 2009. Growth responses of the perennial legume *Sesbania sesban* to NH_3 and NO_3^- nutrition and effects on root nodulation. *Aquatic Botany* 91: 238–244.

Davidson EA, Carvalho CJR, Figueira AM et al. 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* 447: 995–999.

Davidson IA & Robson MJ. 1986. Effect of contrasting patterns of nitrate application on the nitrate uptake, N2-fixation, nodulation and growth of white clover. *Annals of Botany* 57: 331–338.

Franco AA & Defaria SM. 1997. The contribution of N-2-fixing tree legumes to land reclamation and sustainability in the tropics. *Soil Biology and Biochemistry* 29: 897–903.

Glyan'ko AK, Vasil'eva GG, Mitanova NB & Ishchenko AA. 2009. The influence of mineral nitrogen on legume-rhizobium symbiosis. *Plant Physiology* 36: 250–258.

Hartwig UA. 1998. The regulation of symbiotic N_2 fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 92–120.

Herridge DF & Peoples MB. 1990. Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by 15N methods. *Plant Physiology* 93: 495–503.

Herridge DF, Bergensen FJ & Peoples MB. 1990. Measurement of nitrogen fixation by soybean in the field using the ureide and natural 15N abundance methods. *Plant Physiology* 93: 708–716.

Hoagland DR & Arnon DI. 1950. *The Water Culture Method for Growing Plants Without Soil.* University of California – Agricultural Experiment Station, Bekerley.

Justino GC & Sodek L. 2013. Recovery of nitrogen fixation after short-term flooding of the nodulated root system of soybean. *Journal of Plant Physiology* 170: 235–241.

Leblanc HA, Mcgraw RL, Nygren P & Le Roux C. 2005. Neotropical legume tree *Inga edulis* forms N2-fixing symbiosis with fast-growing *Bradyrhizobium* strains. *Plant and Soil* 275: 123–133.

Lima JD & Sodek L. 2003. N-stress alters aspartate and asparagine levels of xylem sap in soybean. *Plant Science* 165: 649–656.

Lojka B, Preininger D, Van Damme P, Rollo A & Banout J. 2012. Use of the Amazonian tree species *Inga edulis* for soil regeneration and weed control. *Journal of Tropical Forest Science* 24: 89–101.

Mcclure PR & Israel DW. 1979. Transport of nitrogen in the xylem of soybean plants. *Plant Physiology* 64: 411–416.

Mcclure PR, Israel DW & Volk RJ. 1980. Evaluation of the relative ureide content of xylem sap as an indicator of N2 fixation in soybeans. *Plant Physiology* 66: 720–725.

Mccullough H. 1967. Determination of ammonia in whole blood by a direct colorimetric method. *Clinica Chimica Acta* 17: 297–304.

Mcneil DL & Larue TA. 1984. Effect of nitrogen source on ureides in soybean. *Plant Physiology* 74: 227–232.

Moreira V, Justino GC, Camargos LS & Aguiar LF. 2014. Características adaptativas da associação simbiótica e da fixação biológica do nitrogênio molecular em plantas jovens de *Lonchocarpus muehlbergianus* Hassl., uma leguminosa arbórea nativa do Cerrado. *Rodriguésia* 65: 517–525.

Nichols JD & Carpenter FN. 2006. Interplanting *Inga edulis* yields nitrogen benefits to Terminalia Amazonia. *Forest Ecology and Management* 233: 344–351.

Omena-Garcia RP, Justino GC, Araújo VBF, Souza LAG, Camargos LS & Gonçalves JFC. 2015. Mineral nitrogen associated changes in growth and xylem-N compounds in Amazonian legume tree. *Journal of Plant Nutrition* 4: 584–595.

Omena-Garcia RP, Justino GC, Sodek L & Goncalves JFC. 2011. Mineral nitrogen affects nodulation and amino acids xylem transport in the amazonian legume *Inga edulis* Mart. *International Journal of Plant Physiology and Biochemistry* 3: 215–218.

Pal'ove-Balang P, Garcíia-Calderón M, Pérez-Delgado CM, Pavlovkin J, Betti M & Márquez AJ. 2015. A *Lotus japonicus* mutant defective in nitrate uptake is also affected in the nitrate response to nodulation. *Plant Biology* 17: 16–25.

Peoples MB, Sudin MN & Herridge DF. 1987. Translocation of nitrogenous compounds in symbiotic and nitrate-fed amide-exporting legumes. *Journal of Experimental Botany* 38: 567–579.

Puiatti M & Sodek L. 1999. Waterlogging affects nitrogen transport in the xylem of soybean. *Plant Physiology and Biochemistry* 37: 767–773.

Reis VM. 2013. Como fazer uma agricultura verde usando o mais antigo processo de obtenção de nitrogênio em plantas. *Acta Scientiae and Technicae* 1: 15–23.

Rogers A, Ainsworth EA & Leakey ADB. 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiology* 151: 1009–1016.

Santiago WR, Vasconcelos SS, Kato OR, Bispo CJC, Rangel-Vasconcelos GT & Castellani DC. 2013. Nitrogênio mineral e microbiano do solo em sistemas agroflorestais com palma de óleo na Amazônia oriental. *Acta Amazonica* 43: 395–406.

Sodek L & Silva DM. 1996. Nitrate inhibitis soybean nodulation and nodule activity when applied to roots regions distant from the nodulation sites. *Revista Brasileira de Fisiologia Vegetal* 8: 187–191.

Streeter JG. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. *Critical Reviews in Plant Sciences* 7: 1–23.

Tegeder M. 2014. Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement. *Journal of Experimental Botany* 65: 1865–1878.

Vincent J. 1970. A manual for the practical study of root-nodule bacteria. Blackwell, Oxford.

Vogels GD & Van Der Drift C. 1970. Differential analyses of glyoxylate derivates. *Analytical Biochemistry* 33: 143–157.

Wick B, Veldkamp E, Mello WZ, Keller M & Crill P. 2005. Nitrous oxide fluxes and nitrogen cycling along a pasture chronosequence in Central Amazonia, Brazil. *Biogeosciences* 2: 175–187.

Yemm EM & Cocking EC. 1955. Estimation of amino acids by ninhydrin. *Analyst* 80: 209–213.