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Soil nitrogen recovery and seasonal changes of xylem sap amino acids of Amazonian tree species following pasture abandonment

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Abstract We conducted this study to understand the dynamics of revegetation by examining the levels of mineral nitrogen (NO_3^- and NH_4^+) in soil and the capacity of nitrogen use by the principal species growing in secondary forest in central Amazonia. For this, we measured the nitrate and ammonium content of soil, leaves and xylem sap, nitrate reductase activity of the leaves and free amino acid contents of the xylem sap in five tree species (*Vismia cayennensis, Vismia japurensis, Bellucia dichotoma, Laetia procera* and *Goupia glabra*) over a chronosequence during recovery after pasture abandonment at two seasons. Soil ammonium was higher in the dry season and nitrate higher in the wet season and increased these with pasture abandonment age. *V. japurensis, B. dichotoma* and *G. glabra* decreased foliar ammonium due to pasture abandonment in the dry season and foliar ammonium increased in *L. procera* in the wet season. *V. japurensis* and *V. cayennensis* showed a decrease in nitrate reductase activity, while *B. dichotoma* and *L. procera* showed an increase. Xylem nitrate decreased in *L. procera* and *B. dichotoma* in the dry and wet

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seasons, respectively, and ammonium increased only in *V. japurensis* and *V. cayennensis* in the wet. Xylem arginine increased in all plant species after a period of pasture abandonment (except *B. dichotoma*). Tree species growing in pasture abandoned areas didn't show the same pattern of use of nitrogen, and this can be important in order to understand the nitrogen metabolism of trees in the Amazon region during forest restoration.

Keywords Chronosequence · Deforestation · Nitrate reductase · Seasonality of precipitation · Secondary forest

Introduction

The increasing demand for agricultural commodities has caused extensive deforestation in the tropics in order to satisfy the world's increasing demand for such products (Schroth et al. 2016). The Amazon forest is suffering from severe degradation of its natural vegetation which is cleared mainly for soybean and beef production (Nepstad et al. 2014). In this context, large areas have been converted into cattle pasture in the last decades, even though most of the soils are poor in nutrients. In these areas, nitrogen becomes a limiting factor, since a large fraction is volatilized during clearing and burning of the mature forest in order to expand pasture areas, a common process that contributes to tropical deforestation (Davidson et al. 2004; Hamilton et al. 2016). Additionally, nitrogen (N) and other nutrients can be lost through erosion, soil emission of gases and hydrological leaching of nutrients (McGrath et al. 2001; Meyer et al. 2017). Consequently, such cleared areas are often abandoned after a short period of use since grass productivity rapidly decreases (primary succession process).

Soon after abandonment, the pastures are covered by emergent secondary vegetation, locally known as "capoeiras", which is adapted to nutrient-poor soils in the Amazon forest. Successful establishment of "capoeiras" depends on how efficiently primary resources, such as light, water and nutrients, are utilized by the colonizing plant species (Santos et al. 2006; Silva et al. 2008), especially with regard to the limiting contents of soil nitrogen (Holl 1999; Davidson et al. 2018). In these areas, the decline in pasture productivity facilitates establishment by woody species, dominated mainly by the genera *Vismia, Bellucia, Laetia* and *Goupia* (Silva et al. 2011). Understanding secondary forest formation has become important since there are many areas at some stage of revegetation in the Amazonian biome. These areas may be exhibit different dynamics due to the seasonal rainfall regime in the humid tropics, which results in the depletion of soil moisture in the subsoil by growing plants (Davidson et al. 2004; Silva et al. 2008). Additionally, this secondary vegetation is considered a significant carbon sink to mitigate rising atmospheric CO_2; however, there are still controversies that can be minimized through studies related to nitrogen content in secondary vegetation (Kirschbaum et al. 2008).

Photosynthetic gas exchange studies are currently underway for some native Amazonian species, but little is known about the use of nitrogen in such tropical emergent species. Silva et al. (2011) conducted an experiment to understand ecophysiological dynamics in Amazonian secondary forests and found that soil total nitrogen concentrations increased in the chronosequence. On the other hand, few studies have been carried out concerning the characteristics of utilization of mineral nitrogen forms by Amazonian species, especially in natural conditions, despite nitrogen being the fourth most abundant essential element in the plant biomass (Omena-Garcia et al. 2011). Nitrate and ammonium are both important forms of mineral N in the Amazonian soils but, depending on the conditions, either one may become prominent (Wick et al. 2005). According to Wick et al. (2005), net mineralization and net nitrification rates can be generally higher during the wet season than during the dry season, so nitrogen forms change during the year. Possibly, plants of the Amazon can efficiently utilize both nitrate and ammonium as nitrogen sources. An example is *Inga edulis*, a native species of the Amazon, which performs well when supplied with either ammonium or nitrate as an external source of N (Omena-Garcia et al. 2011; Justino et al. 2017). In the case of recently abandoned areas where N is scarce, the rate of biomass accumulation, species composition and biogeochemical natural processes could be limited by N availability (Davidson et al. 2004; Jaquetti et al. 2016; Jaquetti and Gonçalves 2017). On the other hand, nitrogen concentration does increase with time, during recovery of the vegetation after pasture abandonment in the Amazon (Feldpausch et al. 2004;

Davidson et al. 2007) where nitrate, ammonium and amino acids all increased in response to pasture age. Studies of Amazonian species with regard to nitrogen use efficiency are scarce.

Although most plants can assimilate nitrate in both the roots and leaves, once the uptake of nitrate exceeds the capacity of assimilation in the root the excess is transported via xylem for assimilation in the leaves (Smirnoff and Stewart 1985). However, some plants preferentially take up ammonium which is assimilated exclusively in the roots (Marschner 1995). Since products of nitrogen assimilation (amino acids) in the roots are exported in the xylem together with excess nitrate, the composition of the xylem sap will reflect the nitrogen metabolism of the roots. Xylem sap amino acid levels will indicate the intensity of nitrogen assimilation in the roots, whereas nitrate will indicate the extent of assimilation in the leaves. According to Aidar et al. (2003), species of different stages of succession vary in physiological characteristics, and early and late successional species are able to assimilate nitrate in roots or shoots, but nitrate reductase activity is more prominent in shoots of pioneer species. In addition, nitrate reductase activity in leaves of pioneer plants is linked to a high capacity of plants for leaf nitrate assimilation and shows their capacity to immediately respond to increased soil availability, which are important traits in conditions where the mineral nitrogen pool of NO_3^- and NH_4^+ changes in different seasons and increases during the period of abandonment of the area. For example, pioneer species assimilate most nitrate in the leaves, whereas climax species show preference for the uptake of ammonium which is assimilated in the roots (Stewart 1991; Stewart et al. 1998). Pioneer species of the Atlantic Forest transport nitrate and asparagine to shoots, while late succession species transported preferentially organic compounds such as asparagine and arginine (Aidar et al. 2003). The nitrogen compounds transported by xylem thereby reflect the form of nitrogen used by roots. Consequently, nitrogen traits of transport from roots to shoots can be used as an indicator of root nitrogen metabolism (Amarante et al. 2006). Certain nodulated legumes transport large quantities of ureides to shoots (Dakora 2000), but when nitrate is present as an external source of nitrogen, the same plants virtually abolish the transport of ureides, and amino acids, such as asparagine and glutamine, become prominent in the xylem sap (Amarante et al. 2006). Amazonian *I. Edulis* also changed its transport form of nitrogen from roots to shoots when the nitrogen source was altered (Omena-Garcia et al. 2011, Justino et al. 2017).

Determining conditions for recuperation of the soil nitrogen pool and the strategies of nitrogen use by secondary vegetation is an environmentally sensitive step in order to understand how to rehabilitate these important, large abandoned areas in the Amazon. Here, we hypothesized that strategies of nitrogen use (including nitrate reductase activity of the leaves and free amino acid contents of the xylem sap) may be different among species of different successional stages, as well as those from same successional stage (including from same genus), growing in the same site conditions. We also hypothesized that forms of the inorganic nitrogen (nitrate or ammonia) might change in the plants over time after abandonment and seasonality in the Amazon forest. Therefore, the aim of this work was to determine whether the factors (soil nitrogen content, strategies of nitrogen use, successional status and seasonality) might be important in determining the utilization and transport of nitrogen from roots to shoots of tree species after 20 years of abandonment.

Materials and methods

Site description

The study was conducted in the Amazonas state, Brazil, on a chronosequence of secondary vegetation growing on abandoned areas of pasture (Site of the Biological Dynamics of Forest Fragments Project), located at Km 63 and 72 of the BR-174 highway, to the north of Manaus (02°34′S, 60°05′W and 02°42′S, 59°88′W). The regional climate is type *Afi* according to the classification of Köppen (1948), and the mean annual temperatures were 26 and 28 °C (wet and dry seasons, respectively). Total annual rainfall was 2.300 mm, with an average monthly precipitation of 300 mm from March to May (wettest months) and 100 mm from August to October (driest months). The soil of the region is classified as dystrophic and poor in nutrients (Chauvel 1982; EMBRAPA 1999).

We selected secondary forest areas based on pasture abandonment age, according to the supervised classification of satellite images performed by Moreira

Table 1 Mineral nitrogen at two soil depths from a chronosequence of secondary vegetation growing in abandoned pasture areas during the dry and wet seasons of 2010–2011, near Manaus, Amazonas, Brazil

Age of abandonment	Season	Soil mineral-N (µg g^{-1} $soil^{-1}$)						Ratio
		NO_3^-		NH_4^+		µg N g^{-1} $soil^{-1}$ (NO_3^- + NH_4^+)		NO_3^-:NH_4^+
		0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	
0–4	Dry	Aa 8.1 ± 3.5 B	Aa 6.8 ± 3.8 B	Ba 8.4 ± 3.6 A	Ba 7.7 ± 3.2 A	Aa 16.5 ± 5.3 A	Aa 14.5 ± 5.9 B	1.0
0–4	Wet	Ba 17.0 ± 8.7 A	ABa 17.7 ± 8.0 A	Ba 3.6 ± 1.0 B	Ca 3.2 ± 1.5 B	Ba 20.6 ± 8.4 A	Aa 20.9 ± 8.5 A	6.0
8–12	Dry	Aa 6.3 ± 3.7 B	Aa 5.4 ± 2.5 B	ABa 11.4 ± 4.7 A	ABa 13.0 ± 9.9 A	Aa 17.8 ± 6.0 A	Aa 16.0 ± 5.5 B	0.5
8–12	Wet	Ba 14.1 ± 5.2 A	Ba 14.7 ± 4.9 A	Aa 6.4 ± 3.0 B	Aa 7.4 ± 3.5 B	Ba 20.6 ± 5.2 A	Aa 22.1 ± 4.9 A	2.6
14–16	Dry	Aa 8.4 ± 4.1 B	Ab 5.0 ± 3.0 B	ABa 9.8 ± 3.0 A	ABa 9.6 ± 4.1 A	Aa 18.2 ± 6.6 A	Aa 14.6 ± 6.7 B	0.7
14–16	Wet	ABa 19.5 ± 10.6 A	ABa 21.0 ± 8.6 A	ABa 4.5 ± 1.7 B	BCb 3.4 ± 1.0 B	ABa 24.1 ± 11.6 A	Aa 24.4 ± 9.1 A	5.5
18–20	Dry	Aa 8.1 ± 3.4 B	Aa 8.0 ± 4.2 B	Aa 13.7 ± 6.0 A	Aa 11.8 ± 3.4 A	Aa 21.9 ± 8.1 B	Aa 19.8 ± 6.3 B	0.8
18–20	Wet	Aa 25.0 ± 5.9 A	Aa 22.6 ± 5.0 A	ABa 5.1 ± 1.8 B	Ba 4.6 ± 1.2 B	Aa 30.2 ± 5.6 A	Aa 27.1 ± 5.5 A	5.3

Mean values ± SE (n = 15). Values with different letters are statistically significant by the Tukey test ($p < 0.05$); uppercase letters (age) and lowercase letters (soil depth) on the left; and uppercase letters (season) on the right

NO_3^- nitrate, *NH_4^+* ammonium

(2003). The ages of the sites ranged between 0 and 20 years. We studied the most frequent species in these areas in (1) early successional (*Vismia japurensis* Reich. (Hypericaceae), *Vismia cayennensis* (Jacq.) Pers. (Hypericaceae), *Bellucia dichotoma* Cogn. (Melastomataceae)); (2) intermediate successional (*Laetia procera* (Poepp.) Eichler. (Salicaceae)); and (3) late successional (*Goupia glabra* aubl. (Goupiaceae) stages (Finegan 1996). The choice of the species in the field was made based on the criterion of diameter of the similar stem. Thus, we estimated that the species were approximately the same age, in each class of secondary vegetation.

Soil mineral N availability (NO_3^- and NH_4^+)

We sampled soil at two depths (0–10; 10–20 cm) during both the wet season (April–May) and dry season (September–October) of 2011. We collected three samples at different locations, and each repetition represented a composite of three soil sub-samples. NO_3^- and NH_4^+ were determined by extracting 20 g of moist soil with 40 mL of 0.5 M K_2SO_4. The samples were shaken for 1 h and then centrifuged at 10,000 ×*g*. After centrifugation, the supernatant was removed with a syringe, filtered through Whatman paper and stored in a frozen state until analysis. NH_4^+ and NO_3^- were spectrophotometrically determined by the phenol-hypochlorite and salicylic acid methods, respectively, according to (Anderson and Ingram 1993).

Extraction of N compounds from leaves

Fully expanded healthy leaves were taken from the mid-region of the plants between 8:00 and 11:00 from four individuals (three replicate samples were taken for each individual). The leaves were collected in the field and taken to the laboratory under refrigeration. Leaves were then washed in distilled water, and excess moisture was removed by blotting with paper towels. The leaves were immediately ground with liquid N_2 in a pestle and mortar, and extracted for amino acid, ureide and NO_3^- determined using 10 mL of MCW (methanol/chloroform/water – 12/5/3 – v/v) per 1 g fresh weight (Bieleski and Turner 1996). The aqueous phase was recovered following phase separation on standing after addition of chloroform (1 vol.) and water (1.5 vol.) to the supernatant (4 vol.). The aqueous phase was then reduced to a known volume by evaporation at 38 °C and kept frozen until analysis.

Xylem sap collection

Xylem sap was collected in the morning using a hand vacuum pump (Pate et al. 1994) on cut shoots from four individuals with diameters between 5 to 20 mm and the sap transferred immediately to a tube kept on ice. The samples were stored at − 20 °C until analysis.

Nitrate reductase activity (NRA)

Fully expanded healthy leaves were taken from the mid-region of four plants during the morning and kept cold until analysis. (Three replicate samples were taken for each individual.) The in vivo assay for nitrate reductase (EC 1. 6. 6.1.) involved the suspension of 0.5 g (fresh weight) of leaves cut into 25 mm^2 pieces in 5 mL of 0.1 M K_2HPO_4 (pH 7,5), 0,1 M KNO_3, and 1% propanol followed by vacuum infiltration (Majerowicz et al. 2003). The assay mixture was incubated for 60 min at 30 °C in the dark. Nitrite (NO_2^-) produced by nitrate reductase action was determined by taking a 2-mL aliquot to which was added 1 mL of 0.02% sulfanilamide and 1 mL of 1% *N*-(1-nafthtyl)-ethylenediamine dihydrochloride in 3 M HCl. After 20 min, the absorbance (540 nm) was read using a spectrophotometer. Activity was calculated from a standard curve of nitrite in the range 10–100 nmol.

Quantification of xylem ammonium, nitrate and free amino acids

Nitrate was determined spectrophotometrically by the salicylic acid method (Unkovich et al. 2008). Free amino acids were separated and quantified as their OPA derivatives by C-18 reverse-phase HPLC column as described previously (Puiatti and Sodek 1999).

Statistical analysis

We tested the relationship between NH_4^+ and NO_3^- and the ages of the "capoeiras" by using polynomial regression. All other analyses were carried out by using ANOVA one-way tests (considering the differences between stations) followed by Tukey's test at a preset significance level of 0.05. All statistical analyses were calculated using SigmaPlot 13.0.0.83 free version.

Results

Soil mineral N level

The total mineral N content (NO_3^- and NH_4^+) of the soil was greater during wet than dry season at both depths analyzed (Table 1) and showed a gradual increase when related to the age of abandonment of the area (Fig. 1). Nevertheless, NO_3^- was clearly the dominant form of mineral N during the wet season while NH_4^+ was more prominent than nitrate during the dry season. During the wet season, NO_3^- was over fivefold higher than NH_4^+ at all age ranges except for the 8- to 12-year range (Table 1). On the other hand,

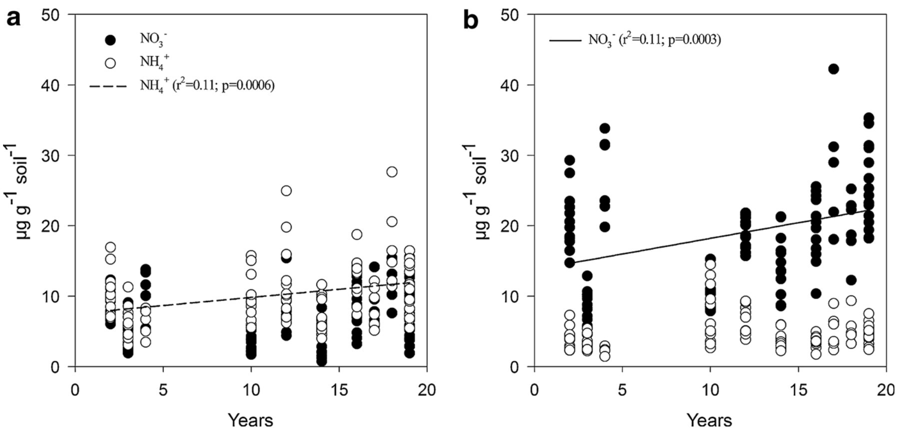

Fig. 1 Inorganic N-pools (μg NO_3^- and NH_4^+ per gram soil) from a chronosequence of secondary vegetation growing in abandoned pasture areas during the dry (**a**) and wet (**b**) seasons of 2010–2011, near Manaus, Amazonas, Brazil

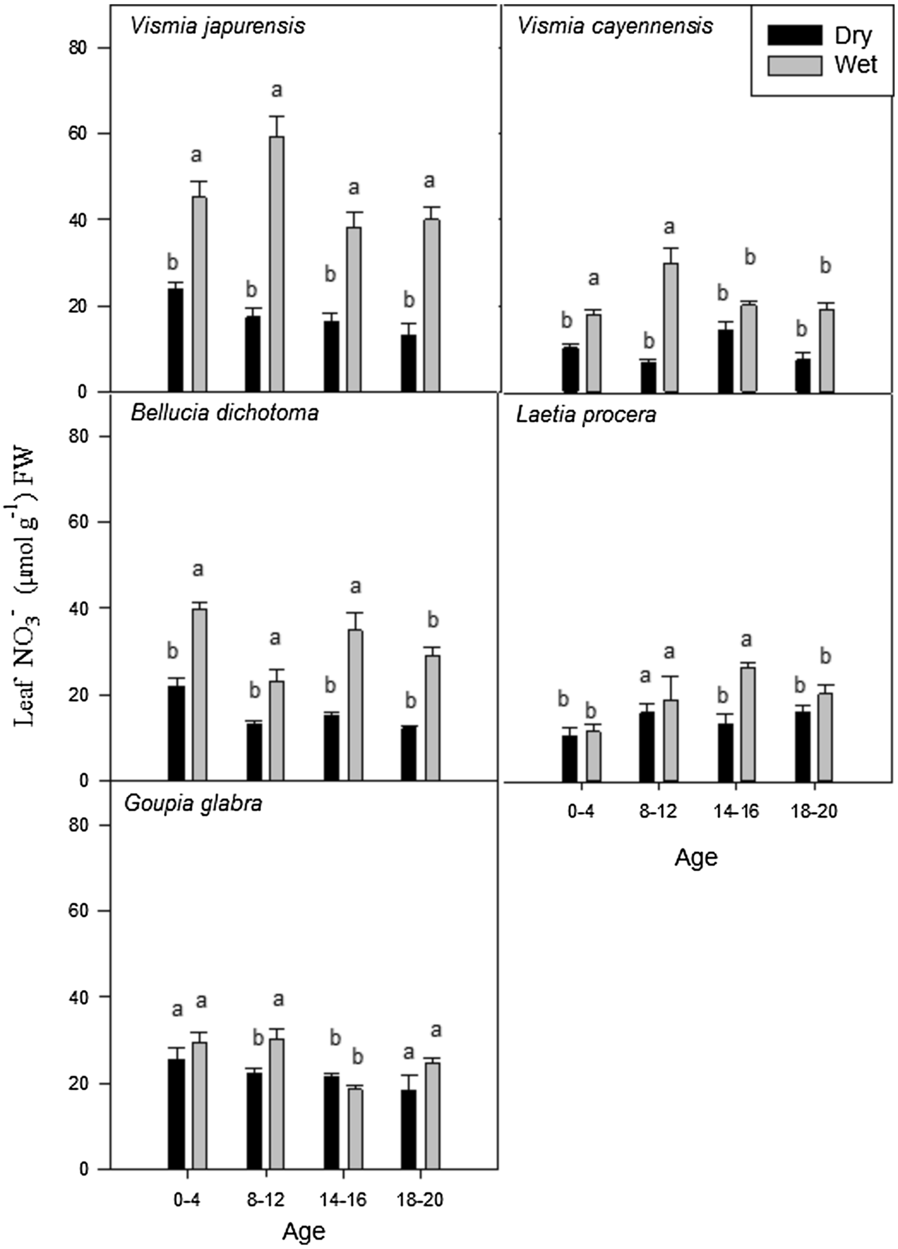

Fig. 2 Nitrate content of leaves of *Vismia japurensis, Vismia cayennensis, Bellucia dichotoma, Laetia procera and Goupia glabra* growing in abandoned pasture areas during the dry and wet seasons. Values with different letters have significant differences within species ($p < 0.05$)

NH_4^+ was slightly greater than NO_3^- during the dry season at all age ranges except for 0 to 4 year range where the amounts of NO_3^- and NH_4^+ were similar.

Leaf mineral N (NO_3^- and NH_4^+) level and Nitrate Reductase Activity (NRA)

In general, the total leaf mineral N content (NO_3^- and NH_4^+) was greater during the wet than the dry season (Figs. 2, 3). *Vismia japurensis* showed the highest values of leaf NO_3^- during dry season at all successional stages. During the dry season, leaf nitrate was prominent in *V. japurensis, B. dichotoma* and *Goupia glabra* in the 0–4 year stage, but values in general varied less between all species in the three subsequent abandonment ages (Fig. 2). Ammonium levels were higher in *V. japurensis* and *L. procera* but varied slightly with the age of abandonment during the dry season (Fig. 3). The average nitrate reductase activity was greater during the dry season in 0–4 year stage of

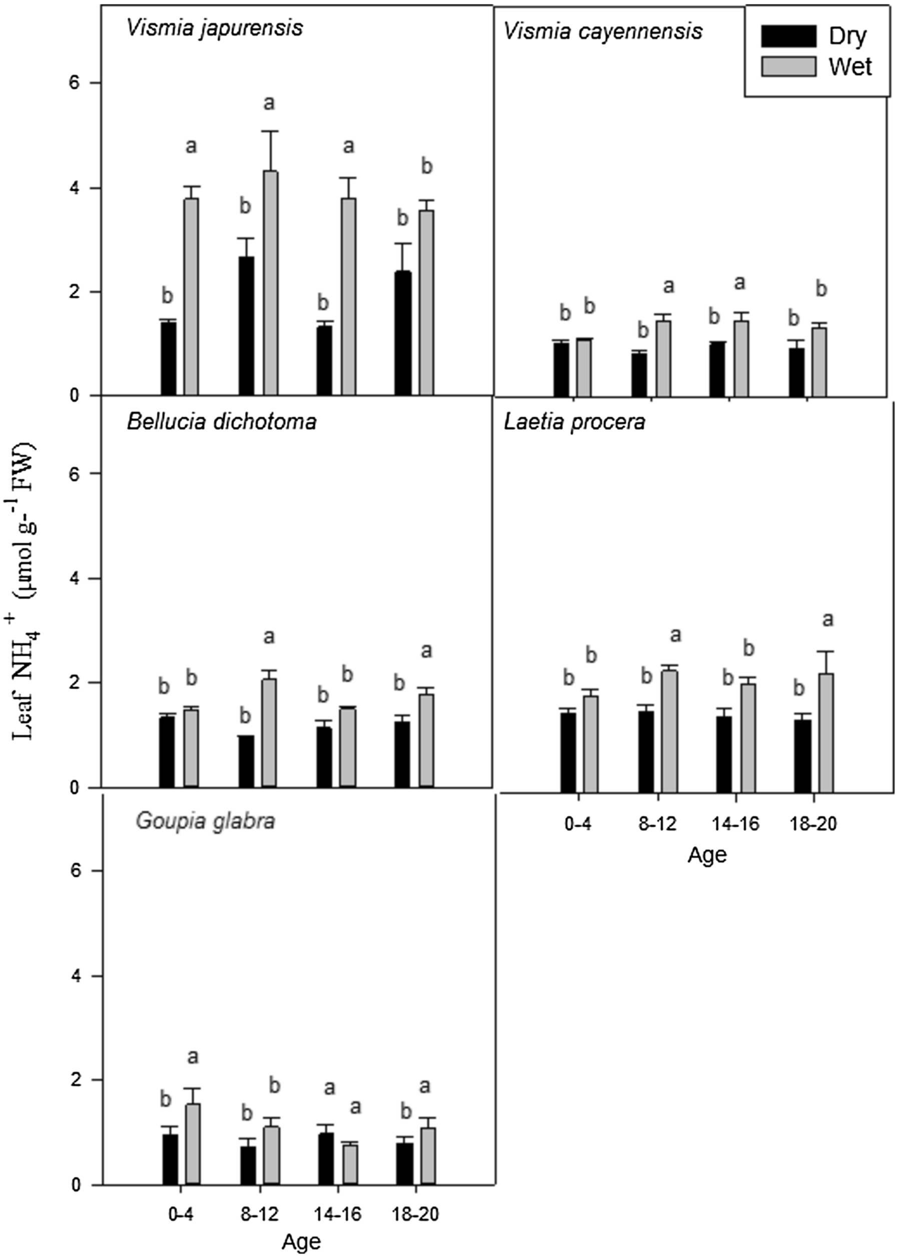

Fig. 3 Ammonium levels of leaves in *Vismia japurensis*, *Vismia cayennensis*, *Bellucia dichotoma*, *Laetia procera* and *Goupia glabra* growing in abandoned pasture areas during the dry and wet seasons. Values with different letters have significant differences within species ($p < 0.05$)

abandonment. However, in higher abandonment ages, *V. japurensis* and *V. cayennensis* showed greater activity during the wet season (Fig. 4).

When comparing N use strategies of the species along the successional gradient, we observed a decrease in NO_3^- leaves in relation to age of abandonment in three studied species (*V. japurensis*, *B. dichotomaG. glabra*) during the dry season. During the wet season, there was an increase in nitrate levels in *L. procera* and a decrease in *G. glabra.* (Table 2). Ammonium levels slightly decreased with age in *G. glabra*, but only in the wet season. Nitrate reductase activity increased for the most of the species in the dry season (except *G. glabra*), particularly in *V. japurensis* (r^2 = 0.75). During the wet season, this tendency was observed only in *V. japurensis* (Table 2).

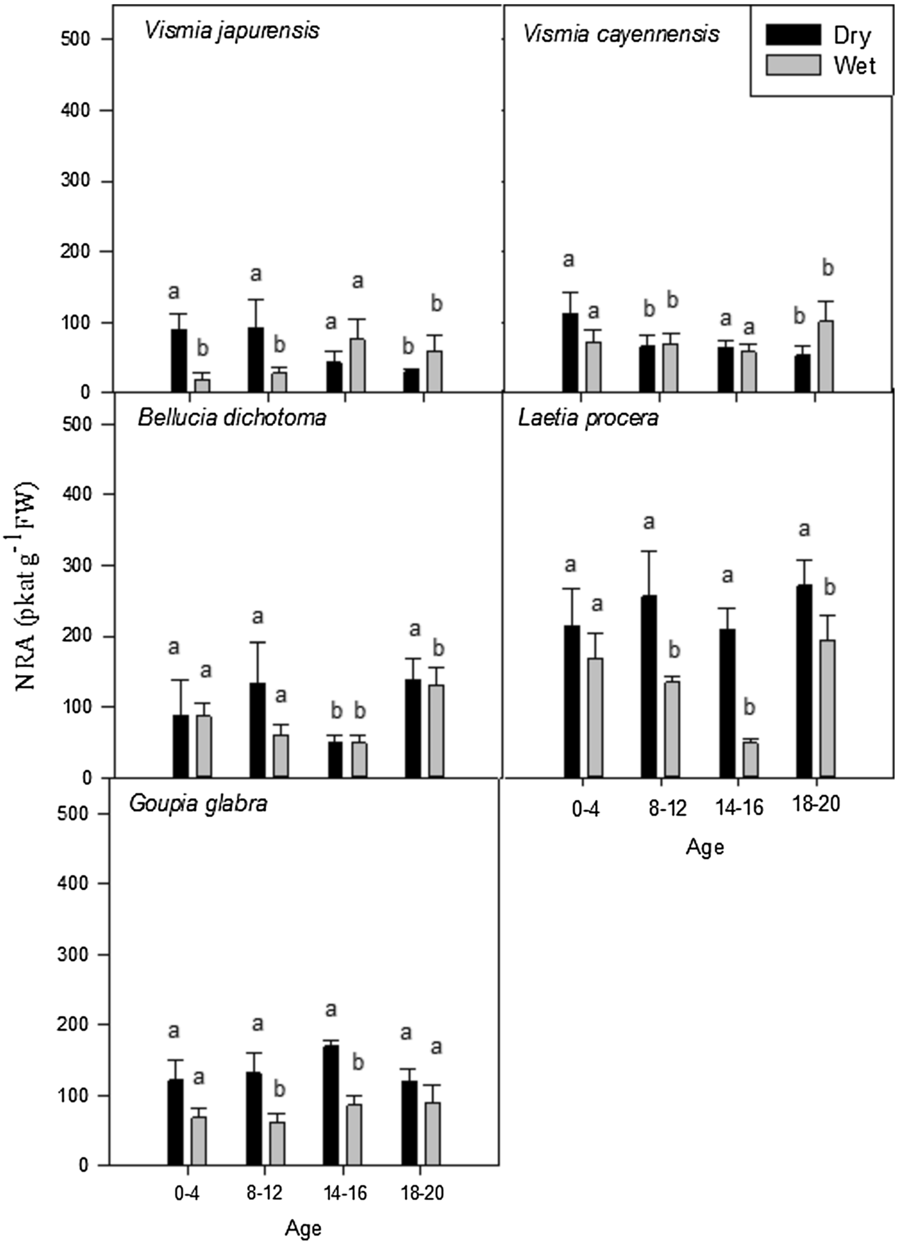

Fig. 4 NRA of leaves of *Vismia japurensis*, *Vismia cayennensis*, *Bellucia dichotoma*, *Laetia procera* and *Goupia glabra* growing in abandoned pasture areas during the dry and wet seasons. Values with different letters have significant differences within species ($p < 0.05$)

Xylem sap NO_3^-, NH_4^+ and amino acids

In general, there was little difference in xylem NH_4^+ and NO_3^- levels irrespective of seasons for all species (Figs. 5, 6). During the dry season, we observed a decrease in NO_3^- in relation to age of abandonment only in *L. procera*. There was a little increase in nitrate levels in *B.dichotoma* during the wet season (Table 3). Ammonium levels increased with age of abandonment in early successional (*V. japurensis* and *V. cayennensis)* (Table 3). With regard to xylem sap amino acids (Fig. 7), *L. procera* transported large quantities of asparagine from roots to leaves (24.35% of the total amino acid content for the wet season). For all other species, the values of asparagine in the sap varied from approximately 0.3 to 4.5%, and here the transport of glycine predominated in the dry and wet seasons. *V. japurensis, V. cayennensis* and *G. glabra* transported high levels of arginine from roots to shoots, especially in the wet season (Fig. 7). It is

Table 2 Regressions between nitrate, ammonium and NRA levels and age of pasture abandonment in *Vismia japurensis, Vismia cayennensis, Bellucia dichotoma, Laetia procera* and *Goupia glabra* during dry and wet seasons

Parameter		Species				
		V. japurensis	*V. cayennensis*	*B. dichotoma*	*L. procera*	*G. glabra*
Dry						
NO_3^-	r^2	0.44	ns	0.54	ns	0.22
	p	< 0.05		< 0.05		< 0.05
	a	− 0.64		− 0.54		− 0.42
NH_4^+	r^2	ns	ns	ns	ns	ns
	p					
	a					
NRA	r^2	0.75	0.30	0.23	0.34	ns
	p	< 0.05	< 0.05	< 0.05	< 0.05	
	a	− 0.20	− 3.85	4.27	6.20	
Wet						
NO_3^-	r^2	ns	ns	ns	0.30	0.27
	p				< 0.05	< 0.05
	a				0.57	− 0.52
NH_4^+	r^2	ns	ns	ns	ns	0.21
	p					< 0.05
	a					− 0.04
NRA	r^2	0.38	ns	ns	ns	ns
	p	< 0.05				
	a	1.95				

noteworthy that the levels of glutamine were, in general, proportionally higher in the dry season, for all species.

Discussion

Nitrate has been reported to dominate the inorganic nitrogen content of soils of the Amazonian Forest (Matson and Vitousek 1987; Neill et al. 1995). In our chronosequence study of recovery after pasture abandonment, nitrate was found to clearly dominate the soil inorganic nitrogen fraction only in the wet season. Here, nitrate values increased with pasture abandonment age. Apparently, net nitrification was higher in the wet, in contrast to the dry season where ammonium levels were more prominent. Nevertheless, our data does show that an increment in soil inorganic nitrogen ($NO_3^- + NH_4^+$) also occurs in the dry season, but was more prominent in the wet season. By contrast, before recolonization of the area, the inorganic nitrogen fraction pool of the soil declines with pasture abandonment age (Wick et al. 2005). This decline in nitrogen is one of the reasons for the abandonment of these lands of the Amazon Basin for agriculture since the nitrogen cycle functions more slowly as pasture become older, an effect related to the microbial soil biomass (Neill et al. 1999). Pasture soil in this region becomes unsustainable after a short time of use, but nutrient levels in the soil do recover with forest restoration (Silva et al. 2011). Recovery of soil inorganic nitrogen ($NO_3^- + NH_4^+$) found in our study is consistent with the trends reported by Silva et al. (2011). The increment of soil nitrogen is

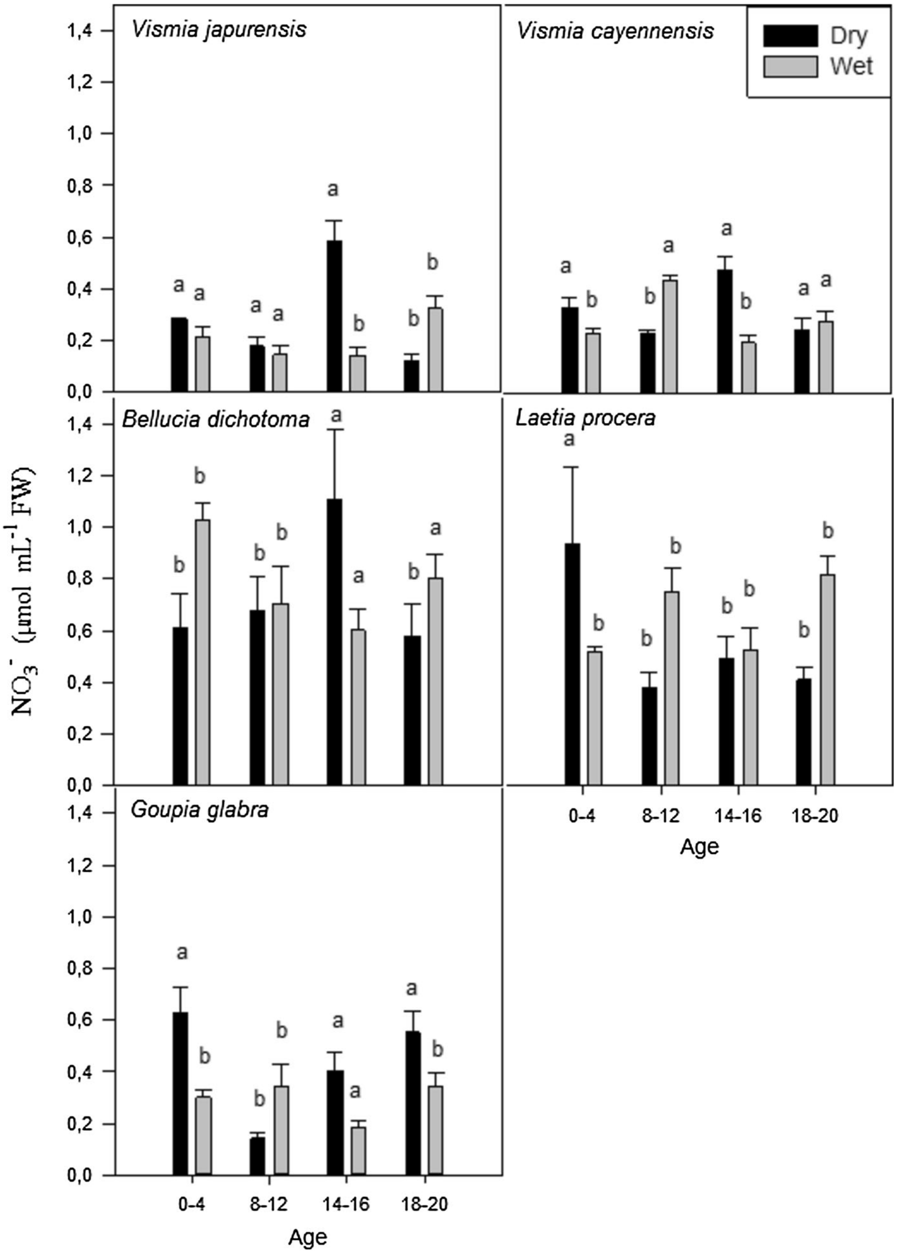

Fig. 5 Nitrate levels in the xylem sap of *Vismia japurensis*, *Vismia cayennensis*, *Bellucia dichotoma*, *Laetia procera* and *Goupia glabra* growing in abandoned pasture areas during the dry and wet seasons. Values with different letters have significant differences within species ($p < 0.05$)

apparently crucial for regenerating communities (Conrad et al. 2018). Data presented here shows that secondary forests are able to recover mineral nitrogen in the soil which is an important trait for species replacement as the forests regenerate. As we observed in our study, along pasture abandonment period, five plant species provide support to subsequent plants species by raising soil fertility and guaranteeing resources for secondary forest succession.

There was no similar trend of increased of NO_3^-, NH_4^+ or nitrate reductase activity with pasture abandonment age as expected for all species studied. Additionally, the pattern of plant responses was particularly different for species in both dry and wet season as discussed below. Diverse ecophysiological mechanisms of use associated with nitrogen mineral assimilation determine success in early establishment. *V. japurensis, V. cayennensis, B. dichotoma and L.*

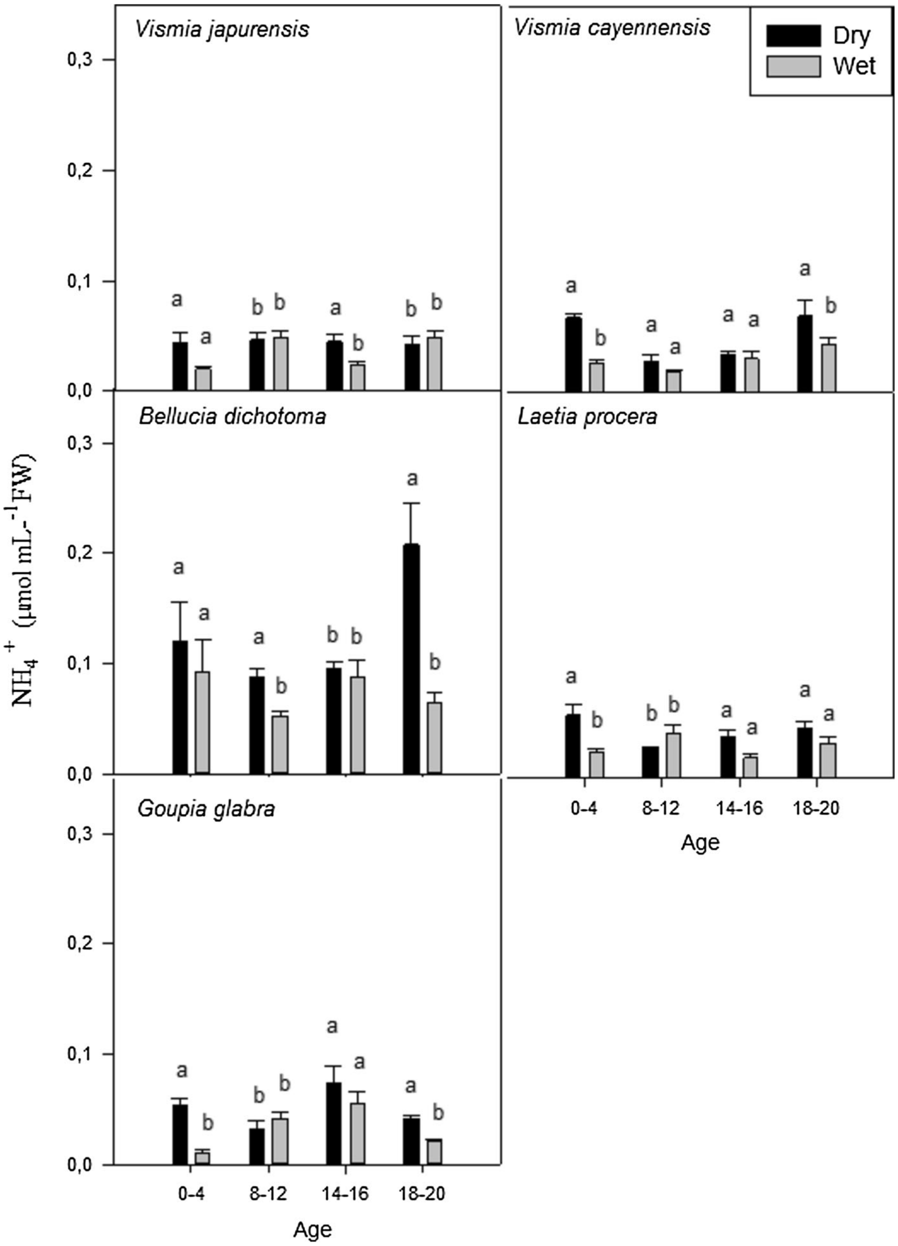

Fig. 6 Ammonium in the xylem sap of *Vismia japurensis*, *Vismia cayennensis*, *Bellucia dichotoma*, *Laetia procera* and *Goupia glabra* growing in abandoned pasture areas during the dry and wet seasons. Values with different letters have significant differences within species ($p < 0.05$)

procera, pioneer and intermediate successional plant species, respectively, showed better capacity in the use of nitrate transported to the leaves in the dry season in response to pasture abandonment age. Here, nitrogen metabolism developed an important role in the dry season as observed for nitrate reductase activity, even when amount of leaf NO_3^- was lower than values observed in the wet season. *V. japurensis* responded to the amount of soil mineral nitrogen, accumulating NO_3^- and NH_4^+ in leaves in the wet season. Nevertheless, NRA was lower, indicating that another abiotic factor could induce the activity of enzymes at natural conditions. Protein phosphorylation-dependent changes in NR activity are regulated, at least in part, by sugars and related metabolites (Krapp and Stitt 1995). As light reduces in the wet season, foliar NRA could probably be negatively affected depending on climate conditions. According to Krapp and Stitt (1995), NR activity is coordinated with the rate of photosynthesis and the availability of C skeletons by

Table 3 Regressions between nitrate, ammonium and NRA levels and age of pasture abandonment in xylem sap of *Vismia japurensis, Vismia cayennensis, Bellucia dichotoma, Laetia procera* and *Goupia glabra* during dry and wet seasons

Parameter		Species				
		V. japurensis	*V. cayennensis*	*B. dichotoma*	*L. procera*	*G. glabra*
Dry						
NO_3^-	r^2	ns	ns	ns	0.26	ns
	p				< 0.05	
	a				-0.03	
NH_4^+	r^2	ns	ns	ns	ns	ns
	p					
	a					
Wet						
NO_3^-	r^2	ns	ns	0.20	ns	ns
	p			< 0.05		
	a			-0.02		
NH_4^+	r^2	0.21	0.21	ns	ns	ns
	p	< 0.05	< 0.05			
	a	0.001	0.001			

both transcriptional and post-translational controls. The NRA of other species, such as *V. cayennensis* and *Bellucia dichotoma,* responded to a lesser extent to the increase in soil nitrogen, but in general plants transported NO_3^- from roots to shoots for reduction by the enzymes of the leaves. Nevertheless, only *L. procera* and *G. glabra* responded positively to the increase in soil nitrogen in the wet. It appears that plants did not exhibit a pattern in nitrogen use, probably driven by abiotic factors such as light, but did not show an increase in nitrogen or NRA in leaves. The NRA of *L. procera* leaves was greatest at all pasture abandonment ages in the wet season, except for the first age group. *G. glabra* also showed great values of NRA, but not significantly for 0–4 and 18–20 abandonment age groups. The performance of NRA for *L. procera* and *G. glabra* was increased in the dry season when soil NO_3^- level was lower, when compared to the wet season, indicating a different strategy for nitrogen use. Nitrate induces NRA, but it is possible that another abiotic factor, such as light, might be positively affecting enzyme activity since photosynthetically active radiation is higher in the dry season as mentioned later. Foliar nitrate decreased in the dry season, but only for *V. japurensis, B. dichotoma* and *G. glabra*. Apparently, nitrate reduction is a key factor for the establishment of these Amazonian plant species and probably leads to an accumulation of nitrate in the leaves in the wet season as appears for most species and successional stages.

Since the nitrate concentration of the xylem sap was quite similar for both wet and dry season for all species, a possible explanation for a greater accumulation of nitrate in the leaf in the wet season is that the flux of xylem metabolites was also greater in the wet. Indeed, the availability of nitrate in the soil was clearly greater in the wet. However, estimates of flux in the xylem are not possible with the present data. Another factor that may contribute to increased nitrate accumulation in the leaves is the activity of nitrate reductase. A somewhat lower mean NRA would result, as suggested above, in lower utilization of nitrate and consequently favor increased nitrate accumulation.

Despite the well-known positive influence of nitrate on NRA (Beevers and Hageman 1983), the greater availability of soil nitrate in the wet season did not result in increased leaf NRA. On the contrary, in some cases NRA was somewhat higher in the dry season. However, light is another factor that leads to higher NRA (Beevers and Hageman 1983) and the higher light intensity, which is characteristic of the dry season, could therefore explain those cases where increased nitrate reductase activity was found. Increasing light can increase nitrate reductase activity and, as observed in our study, reduction of nitrate was

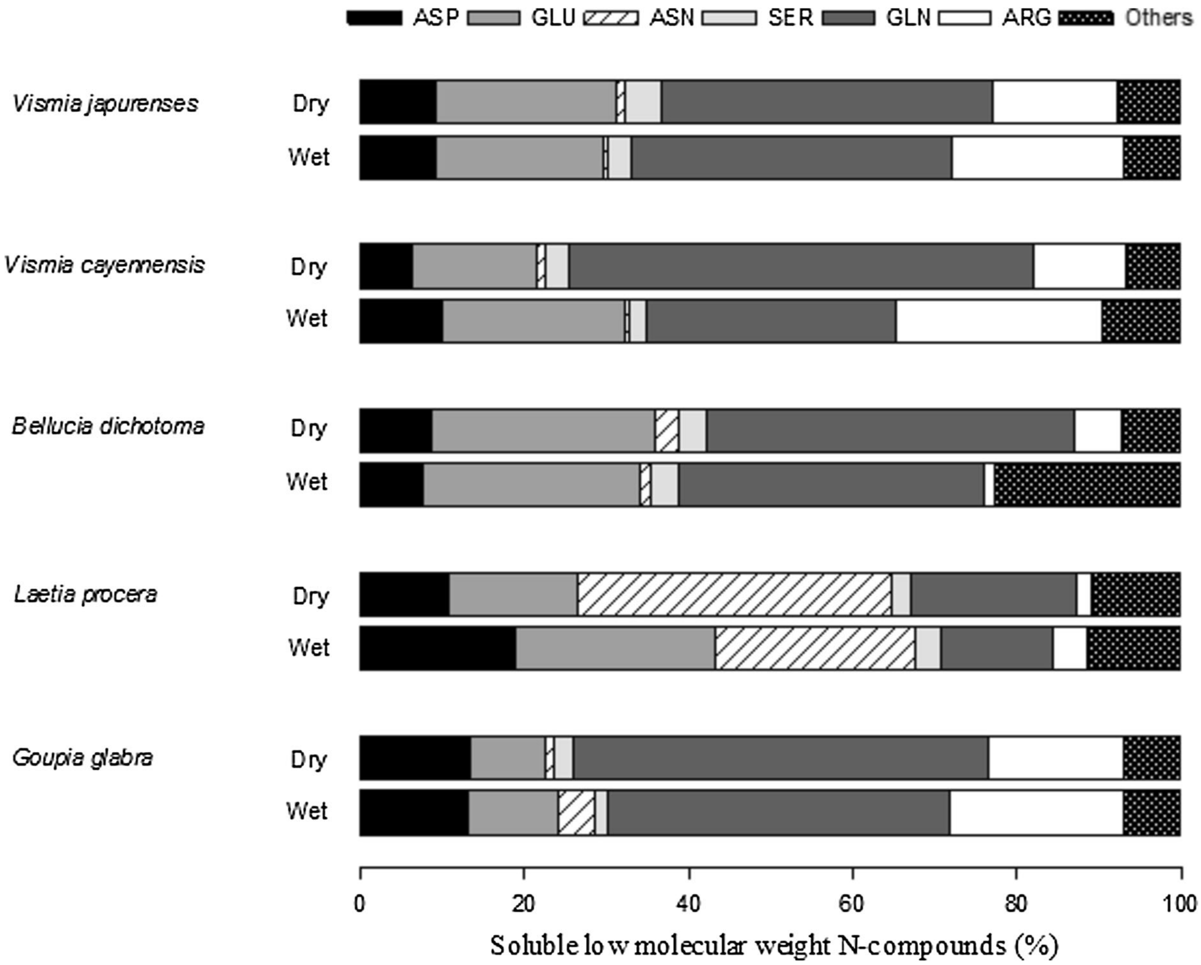

Fig. 7 Average soluble low molecular weight N compounds composition of the xylem sap (%N) for tree species growing in abandoned pasture areas during the wet and dry seasons, near Manaus, Amazonas, Brazil. *ASP* aspartate, *GLU* glutamate, *ASN* asparagine, *GLN* glutamine, *ARG* arginine, *Other* other amino acids

prominent in the dry season, when more light is available.

Overall, the late successional trees, *L. procera and G. glabra*, showed greater potential to reduce nitrate in both the dry and wet seasons than the two pioneer trees of the genera *Vismia*, the species with the lowest leaf NRA. In this respect, these Amazonian pioneer species contrast with the Atlantic Forest pioneer species (Aidar et al. 2003) which show the highest leaf NRA of all successional species. It is noteworthy that capacity to transport and reduce nitrate do not lead to a response in photosynthesis as demonstrated by Silva et al. (2011) since *Vismia*, a typical plant of early succession pasture abandonment age, exhibited better performance regarding carbon assimilation than *Goupia*. Apparently, in the successional chronosequence, plants acclimate to new conditions of irradiance with direct impact on photosynthetic rates. The same pattern was observed for nitrate reductase activity, but one must consider the successional category of the plant, which is affected by light more so than by water as demonstrated by the water use efficiency in plants such as V. *japurensis*, *V. cayennensis, B. grossularioidesL. procera* and *G. glabra* (Silva et al. 2011).

Nitrogen use and transport by the xylem are also important factors here. The presence of nitrate, together with the amino acids glutamine and asparagine in the xylem, suggests that nitrate reductase activity occurs both in roots and shoots in pioneer and secondary late successional species. All the species studied exported products of root nitrate assimilation (asparagine and glutamine) in the xylem, but *L.*

procera stood out since asparagine predominated in both the wet and dry seasons, but was very low in the other four species where glutamine predominated. This phenomenon can be attributed to activity of asparagine synthetase of the roots (Antunes et al. 2008; Beato et al. 2014; Prinsi and Espen 2015) which would appear to be active only in *L.procera*. The high N/C ratio of asparagine (2:4) makes this amino acid an ideal transport compound since nitrogen transported in this form requires the investment of less carbon, thereby economizing photosynthate. The same is true, to a lesser extent, for glutamine (N/C ratio 2:5). In the low precipitation period, export of the amides asparagine (*L. procera*) and glutamine (remaining species) was more prominent than in the higher precipitation period indicating a proportionally greater level of assimilation in the roots than in the dry season.

Silva et al. (2011) reported that photosynthesis was apparently lower in the wet season for the five species studied here, which may, in part, explain this result since C for N transport would be more limited. Abiotic conditions have been shown to cause changes in the form of N transport by xylem sap (Amarante et al. 2006; Davidson et al. 2007). The occurrence of arginine in the xylem may reflect greater internal storage and remobilization of nitrogen (Millard 1995; Aidar et al. 2003). Accordingly, our data for xylem arginine indicate that storage of nitrogen may be more efficient in the wet season at least for *V. japurensis, V. cayennensis* and *G. glabra*, and this may constitute a specific strategy for these species. Nevertheless, overall, the amino acid composition of the xylem did not show any apparent relationship to the successional category, contrasting the clear relationship observed for species of the Atlantic Forest (Aidar et al. 2003).

The data presented here indicate that nitrate reductase activities in the shoots showed that the four species (except *G. glabra*) are potentially able to reduce nitrate and accumulate nitrogen under conditions of dry weather, but, in wet seasons, only *V. japurensis* increased NRA activity, an important strategy for growth and development of trees in disturbed Amazonian areas. These results confirm our original hypothesis. Therefore, tree species growing in pasture-abandoned areas did not show the same pattern of acquisition, transport and use of nitrogen. In addition, the successional traits observed for trees growing in secondary forest process can be used to understand the influence of stress factors (for example: Seasonality of precipitation) on plantation performance in degraded tropical ecosystems during forest restoration.

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