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Nitrogen metabolism of two tropical forage grass species: nitrogen availability × cultivars

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Abstract

The understanding about N metabolism in roots and/or shoots of forage grasses should help determine how some cultivars use N more efficiently. This study was designed to determine whether two *Brachiaria brizantha* cultivars and two *Panicum maximum* cultivars differ in their N metabolism. Two cultivars of *Panicum maximum* and two cultivars of *Brachiaria brizantha* were investigated under the supply of low and high nitrogen rates in a greenhouse experiment. Some characteristics such as dry mass production, total nitrogen, nitrate and ammonium concentrations and contents; nitrate reductase (NR) and glutamine synthetase (GS) activities; and total free amino acid concentrations in shoots and roots were measured. The results showed that *Panicum* cultivars showed higher NR activity and GS activity in diagnostic leaves (DL) than *Brachiaria* cultivars. *Brachiaria* cultivars showed the highest ammonium concentrations in roots at low N supply and *Panicum* cultivars at high N supply. The total amino acid concentrations in DL were highest in *Panicum* and root total amino acid concentrations were highest in *Brachiaria*. *Panicum* and *Brachiaria* cultivars differed in their nitrogen metabolism with varying N supply. The results indicate that at low nitrogen supply to the pasture, *Brachiaria brizantha* cultivar Piatã may be an option to use in less intensive production environments, instead of high dry mass production. For those pastures with high nitrogen availability, the cultivars Mombaça and Aruana of *Panicum maximum* were clearly superior to Piatã, which suggests priority in the selection of these cultivars to be used in more intensive production systems that apply more nitrogen.

Keywords: Amino acids; Ammonium; Glutamine synthetase; Nitrate; Nitrate reductase.

Introduction

More than 25% or 3.3 billion hectares of the world's land area are used for livestock production in permanent meadows and pastures (FAO 2018). Forage grasses are present in almost all tropical and subtropical countries (Boddey et al., 2004; Vendramini et al., 2013). Among the grasses grown in tropical pastures, those from *Brachiaria* and *Panicum* genera play a key role in animal production systems, such that recently released grass cultivars have been studied for agronomic characteristics (Silveira et al., 2010), but not for nutrient metabolism. Given that nitrogen is the nutrient that yields the greatest increase in grass production (Ryser and Lambers, 1995; Martuscello et al., 2009; De Bona and Monteiro, 2010; De Bona et al., 2011; Garcez et al., 2011; Møller et al., 2011; Mokhele et al., 2012; Vilela et al., 2012; Chen et al., 2020), a better understanding of nitrogen metabolism in grasses of these genera could lead to improvements in pasture management by allowing plants to make efficient use of this nutrient (Iqbal et al., 2019), which is scarce and transient in soil solution. There is a clear gap in the knowledge related to the nitrogen metabolism in forage grass cultivars, which could contribute to nitrogen fertilization of grass pastures.

Plants preferentially absorb inorganic nitrogen in nitrate and ammonium forms (Marschner, 2018). These forms can be stored in the vacuole and/or quickly converted to organic substances such as amino acids. The assimilation process occurs through the reduction of nitrate to ammonium by NR and nitrite reductase enzymes, and ammonium in tissues is assimilated with glutamine synthase/glutamate oxoglutarate aminotransferase (GS/GOGAT) system (Lea et al., 2007; Martínez-Andújar et al., 2013; Marschner, 2018). The assimilation of these nitrogen forms in carbonic chains is a crucial physiological process for plant growth and production (Mokhele et al., 2012).

Several studies have shown that some species preferentially absorb nitrate and/or ammonium as inorganic forms of nitrogen (von Wirén et al., 1997; Wallander et al., 1997; Garnett and Smethurst, 1999). Scheurwater et al. (2002) argued that a plant that reduces nitrate to ammonium in roots may have different carbon requirements for assimilating nitrogen absorbed as nitrate than one that does so in the shoots. Species that reduce nitrate preferentially in shoots may have the advantage of using excess reducing power (NADH or NADPH and reduced ferredoxin) produced during photosynthesis (Raven et al., 1992; Kronzucker et al., 1997; Scheurwater et al., 2002), while species that reduce more nitrate in roots obtain their reducing power in pentose phosphate pathway and in glycolysis. Thus, the location

where nitrate reduction takes place in the plant influences its carbon balance (Oaks and Hirel, 1986; Bowsher et al., 1989; Marschner, 2018), and consequently forage grass yield.

Ammonium from nitrate reduction, direct absorption, photorespiration, gas fixation, and the deamination of nitrogenous compounds such as asparagine (Wickert et al., 2007; Mokhele et al., 2012) and nearly all ammonium is assimilated in the root system rather than in the shoots (Wang and Macko, 2011; Marschner, 2018). Ruiz et al. (2007) reported that all forms of inorganic nitrogen are first reduced to ammonium, since that is the only reduced form available for plants to use in the production of amino acids. Ammonium assimilated to glutamine and glutamate may be used as transport compounds and nitrogen donors, in the biosynthesis of nearly all amino and nucleic acids, and in others nitrogenous compounds such as chlorophyll (Lea et al., 2007; Mokhele et al., 2012). Asparagine is the primary component transported via xylem and phloem in higher plants (Pate, 1980; Lea et al., 2007; Martínez-Andújar et al., 2013).

Understanding nitrogen storage, transport, and assimilation by roots and/or shoots of forage plants should help to understand how some forage plant cultivars use nitrogen more efficiently than others and to seek an environmentally friendly management. The aim of this study was to determine whether the forage grasses *Brachiaria brizantha* cv. Xaraés, *B. brizantha* cv. Piatã, *Panicum maximum* cv. Mombaça, and *P. maximum* cv. Aruana differ in their nitrogen metabolism, by comparing dry mass production; total nitrogen, nitrate, and ammonium concentrations and contents; activities of NR and GS enzymes; and total free amino acid concentrations in the roots and shoots of these grasses, when grown in environments with low and high nitrogen availability.

Results

Shoot and root dry mass production

The nitrogen × cultivar interaction was significant for shoot and root dry mass production and for the root:shoot ratio of the grasses (Figure 1). At low nitrogen rate, the highest dry mass production in shoots was found in Mombaça. Xaraés showed a similar shoot dry mass production to that of Aruana, while Piatã showed the lowest production. At high nitrogen rate, Aruana produced the highest shoot dry mass, Mombaça and Xaraés cultivars had similar productions, and Piatã showed the lowest production (Figure 1A).

At high nitrogen rate, root dry mass production was higher in Mombaça and Aruana than in Xaraés and Piatã, and lowest in Piatã cultivar. At low nitrogen rate, Mombaça and Aruana had the highest root dry mass. Root dry mass production did not differ between Xaraés and Mombaça, while Piatã showed the lowest value (Figure 1B). At the low nitrogen rate, the root:shoot ratio was higher in Aruana than in Piatã. At high nitrogen rate, Mombaça and Aruana showed the highest root:shoot ratios (Figure 1C).

Total nitrogen, nitrate, and ammonium in diagnostic leaves (DL) and roots

There was a significant nitrogen × cultivar interaction for total nitrogen concentrations and contents in DL and roots of the grasses (Figure 2). DL total nitrogen concentrations were higher in Piatã than in Mombaça. Xaraés and Aruana did not differ from the other cultivars at the low nitrogen rate. At high nitrogen rate, Xaraés had a low DL total nitrogen concentration, which differed significantly from those of Mombaça and Piatã but was similar to that of Aruana (Figure 2A). The nitrogen content in these leaves was the same in all of the cultivars at the low nitrogen rate. At high nitrogen rate, Mombaça and Aruana had higher values than Piatã, while Xaraés was not significantly different from the other cultivars (Figure 2B).

Nitrogen concentrations in roots were similar for all the cultivars at high nitrogen rate. At low nitrogen rate, Piatã had a higher nitrogen concentration than the other cultivars (Figure 2C). Root nitrogen content was higher in *Panicum* cultivars than in Piatã, but Mombaça did not differ from Xaraés at low nitrogen rate. The high nitrogen rate yielded higher root nitrogen content in *Panicum* than in *Brachiaria* cultivars (Figure 2D).

A significant nitrogen × cultivar interaction was found for nitrate and ammonium concentrations and contents in DL and roots of the grasses (Figure 3). DL nitrate concentrations did not differ among cultivars at low nitrogen rate. At high nitrogen rate, the only significant difference occurred between Aruana (which had the highest DL nitrate concentration) and Xaraés (Figure 3A). DL nitrate content was greater in Mombaça than in Xaraés and Piatã at low nitrogen rate. At high nitrogen rate, Aruana had a higher DL nitrate content than *Brachiaria* cultivars (Figure 3B).

At low nitrogen rate, Piatã had a greater root nitrate concentration than the other grasses, and *Panicum* cultivars had the lowest values. At high nitrogen rate, Piatã had a greater root nitrate concentration than Mombaça and Aruana, while Xaraés had a concentration similar to those found in the other three grasses (Figure 3C). Root nitrate content was higher in Xaraés than in *Panicum* cultivars at the low nitrogen rate. The high nitrogen rate yielded greater root nitrate content in *Panicum* than in *Brachiaria* cultivars (Figure 3D).

DL ammonium concentration was greater in Xaraés than in Mombaça at low nitrogen rate, and Aruana and Piatã did not differ from the others. At high nitrogen rate, Mombaça had the highest DL ammonium concentration and the other grasses had similar concentrations (Figure 3E). DL ammonium content did not differ among cultivars at low nitrogen rate. At high nitrogen rate, however, Mombaça showed the highest DL ammonium content, followed by Aruana, which had DL ammonium content greater than those of Xaraés and Piatã (Figure 3F).

Root ammonium concentrations were higher in Piatã cultivar than in *Panicum* cultivars at low nitrogen rate but did not differ between Xaraés and the other grasses. At high nitrogen rate, Aruana had the highest root ammonium concentration, followed by Mombaça, which had a higher concentration than those of the two *Brachiaria* cultivars (Figure 3G). Root ammonium content did not differ among these grasses at low nitrogen rate. At high nitrogen rate, Aruana had the highest root ammonium content of all the grasses (Figure 3H).

Activities of NR and GS enzymes in DL and roots

There was a significant nitrogen × cultivar interaction for nitrate reductase (NR) and glutamine synthetase (GS) activities in diagnostic leaves (DL) and roots of the grasses (Figure 4). NR activity in DL was higher in *Panicum* than in *Brachiaria* cultivars at low nitrogen rate. At high nitrogen rate, Mombaça showed the highest NR activity in DL (Figure 4A). NR activity in roots was similar in all cultivars at both nitrogen rates (Figure 4B).

GS activity in DL was greater in *Panicum* than in *Brachiaria* cultivars, at low nitrogen rate. At high nitrogen rate, Mombaça showed the highest activity of this enzyme, while Aruana cultivar showed greater activity than *Brachiaria* cultivars (Figure 4C). In the roots, GS activity was higher in Piatã than in Xaraés and *Panicum* cultivars at low nitrogen availability. At high nitrogen rate, *Brachiaria* cultivars showed higher GS activity in roots than *Panicum* cultivars (Figure 4D).

Concentrations of total free amino acids in DL and roots

The supply of nitrogen increased total concentrations of free amino acids in DL and roots of the grasses (Table 2). The high nitrogen rate yielded higher levels of total free amino acids in DL and roots of the grasses. At high N rate, Xaraés and Piatã concentrated more of asparagine and serine in DL than at low nitrogen rate. Mombaça and Aruana showed the highest DL concentrations of asparagine and glutamine, at high N supply. At high nitrogen rate, Mombaça concentrated more total free amino acids in the DL than *Brachiaria* cultivars, while Aruana did not differ from the other three grasses. At low N rate, Piatã concentrated the highest levels of free amino acids in the DL, followed by Xaraés, Mombaça, and Aruana. At low N rate, the cultivars had high concentration of arginine in DL, while Piatã had a high concentration of proline in these leaves.

Increasing the nitrogen rate yielded increases in several amino acids in roots, with asparagine, arginine, glutamine, and serine showing the largest increases (Table 2). At both high and low nitrogen rates, the concentration of total free amino acids in the roots was higher in *Brachiaria* cultivars than in *Panicum* cultivars. At low nitrogen rate, higher concentrations of the amino acids arginine, asparagine, alanine, and glutamine were found in roots of *Brachiaria* cultivars.

Discussion

Previous studies have shown that increasing nitrogen supply improved shoot dry mass production of grasses grown in soil and nutrient solution (Rao et al., 1995; Martuscello et al., 2009; De Bona and Monteiro, 2010; De Bona et al., 2011; Vilela et al., 2012; Chen et al., 2020). The greatest shoot dry mass production at low nitrogen rate was observed in Mombaça, while the greatest shoot dry mass production at high nitrogen rate was observed in Aruana. Piatã cultivar showed the lowest shoot dry mass production at both nitrogen rates (Figure 1A).

Root dry mass production has also been shown to increase with increasing nitrogen rates (Ryser and Lambers, 1995; Garcez et al., 2011; Møller et al., 2011). Root dry mass production was highest in Mombaça and Aruana, while Piatã cultivar showed the lowest values at both nitrogen rates (Figure 1B). Researchers have proposed that rapid initial growth and/or the high partitioning of carbon to roots are the primary causes for the more efficient use of nutrients supplied through the soil (Rao et al., 1995; Liao et al., 2004; Noulas et al., 2010).

Root:shoot ratios were highest for Mombaça and Aruana at high nitrogen rate. At low nitrogen rate, Xaraés cultivar increased its root:shoot ratio to levels similar to those found in *Panicum* cultivars (Figure 1C). This indicates that, with high availability of nitrogen in the nutrient solution, *Panicum* cultivars (especially Mombaça) have vigorous initial growth of the root system, while Xaraés and Piatã cultivars gave greater priority to shoot growth. However, at low nitrogen rate, *Brachiaria* cultivars (especially Xaraés) showed root:shoot ratios similar to those of Mombaça and Aruana, signaling a greater plasticity and investment of nitrogen in root dry mass production, as observed by Rao et al. (1995), Santos et al. (2002), Martuscello et al. (2009), and Santos et al. (2012).

The greatest difference observed in DL nitrogen concentrations was between the nitrogen rates (Figure 2A). Increasing nitrogen rate increased nitrogen concentrations in the grass shoots, as previously reported by Batista and Monteiro (2007), Silveira and Monteiro (2010), Lavres Junior et al. (2010) and Muir et al. (2013). DL nitrogen content was similar in all cultivars at low nitrogen rate, with the increase in nitrogen favoring greater DL nitrogen content in Mombaça and Aruana (Figure 2B). Piatã showed the highest nitrogen concentration (Figure 2C) and lowest nitrogen content in roots, while the reverse was found in *Panicum* cultivars at both nitrogen rates (Figure 2D). A dilution effect of nitrogen in *Panicum* cultivars tissues may have occurred due to the continuous increase in the amount of nitrogen absorbed and increase in the proportion of structural and storage organs with low nitrogen content, which led to increased nitrogen content in shoots and roots (Greenwood et al., 1990; Primavesi et al., 2004; Corrêa et al., 2007; Yuan et al., 2007).

Nitrate assimilation occurred preferentially in shoots of the cultivars (Figures 4A and 4B). Scheurwater et al. (2002) reported that some species preferentially reduce nitrate to ammonium in roots while others do so in shoots and argued that this could affect the carbon requirements for nitrate assimilation. Species that preferentially reduce nitrate in shoots may have the advantage of using the excess reducing power (NADH or NADPH and reduced ferredoxin) produced during photosynthesis. By contrast, in the roots the reducing power is supplied via pentose phosphates, which release carbon dioxide and increase the respiratory coefficient, generating greater energy costs for the plant and consequently lower dry mass production (Oaks and Hirel, 1986; Bowsher; Hucklesby and Emes, 1989; Raven et al., 1992; Kronzucker et al., 1997; Scheurwater et al., 2002). Mombaça and Aruana had the highest nitrate contents and NR activity levels in DL, at both nitrogen rates (Figures 3A and 4A). This suggests that these grasses have a greater affinity for nitrate than for ammonium, which allows them to absorb greater amounts of that form when it is in solution, due to the greater initial growth of root system (Figure 1B), the accumulation of nitrate in roots (Figure 3D), and the rapid transport and assimilation of nitrate in shoots (Figure 4A). These grasses may also have a large number of transporters and reduced activity in the nitrate efflux channel (Burton, 1943; Mackown et al., 2009; Acuña et al., 2010).

Ammonium assimilation in DL by the GS enzyme was greater in Mombaça and Aruana than in Piatã and Xaraés (Figure 4C). High activity of this enzyme in the DL of *Panicum* cultivars may be related to the rapid root to shoot ammonium transport, to the reduction of absorbed nitrate to ammonium by the activity of NR enzyme (Figure 4A), and/or to the use of nitrogenous compounds, releasing ammonium in the DL of the grasses (Table 2). In plants like *Arabidopsis*, ammonium is generated by the reduction of nitrate, direct absorption, photorespiration, gas fixation, and the deamination of nitrogenous compounds such as asparagine (Wickert et al., 2007; Mokhele et al., 2012). According to Ruiz et al. (2007), all forms of inorganic

Table 1. Stock solution volumes employed in the preparation of nutrient solutions.

N rates		Low	High
		3 mmol L^{-1}	30 mmol L^{-1}
Stock solution		Volume (mL L^{-1})	
KH_2PO_4	1 mol L^{-1}	1	1
NH_4NO_3	1 mol L^{-1}	1.5	15
$MgSO_4$	1 mol L^{-1}	2	2
KCl	1 mol L^{-1}	7	7
$CaCl_2$	1 mol L^{-1}	5	5
Micronutrients*	-	1	1
Fe-EDTA**	-	1	1

(*) Stock solution composition of micronutrients (g L^{-1}): H_3BO_3 = 2.86; $MnCl_2.4H_2O$ = 1.81; $ZnCl_2$ = 0.10; $CuCl_2$ = 0.10 and = 0.02 $H_2MoO_4.4H_2O$. (**) We dissolved 26.1 g of disodium EDTA in 286 mL of 1 mol L^{-1} NaOH and added 24.0 g of $FeSO_4.7H_2O$. This solution was aerated for one night and the volume was completed to 1 L with deionized water.

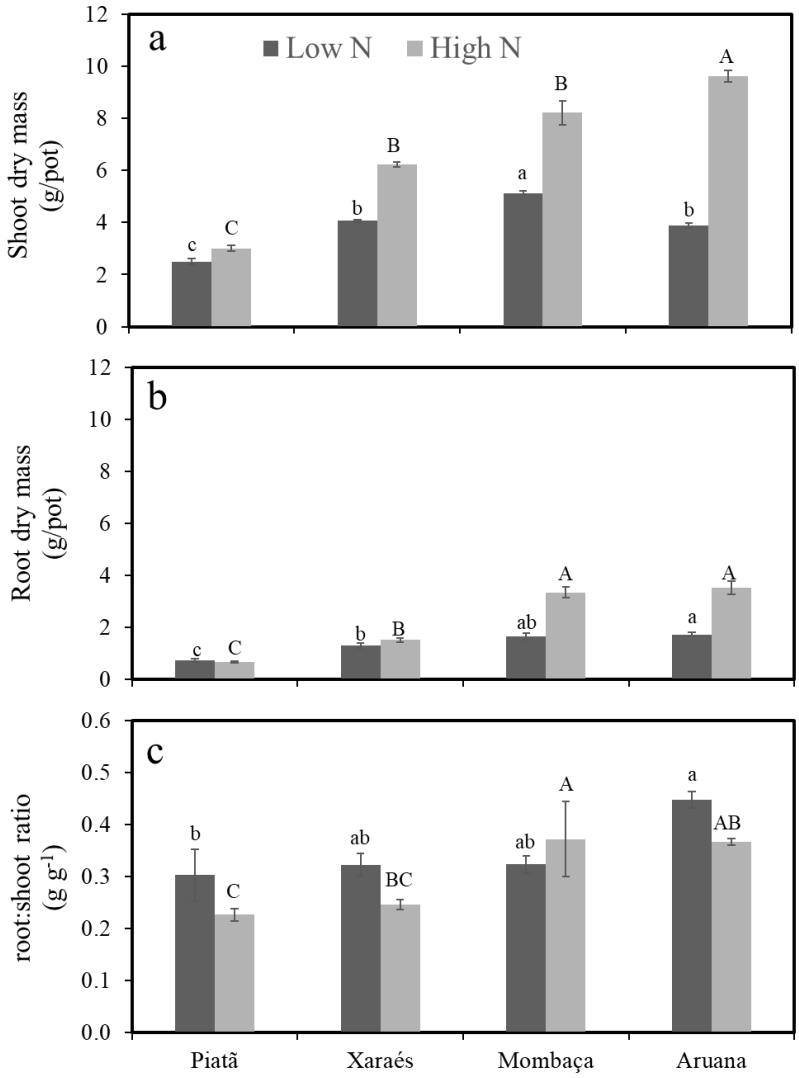

Fig 1. Shoot dry mass production (a), root dry mass production (b) and root:shoot ratio (c) of *Brachiaria spp*. and *Panicum spp*. under low and high nitrogen rates (means followed by the same lowercase letter for the low nitrogen rates and uppercase letters for the high nitrogen rates were not significant at the 5% probability level).

Table 2 Concentrations of total free amino acids in DL and roots of *Brachiaria spp*. and *Panicum spp*. cultivars grown under low and high nitrogen rates

	Piatã				Xaraés				Mombaça				Aruana			
N rates	Low		High		Low		High		Low		High		Low		High	
Diagnostic leaves	mg kg^{-1}															
Aspartic acid	264 ± 51		188 ± 30		305 ± 11		229 ± 66		247 ± 55		350 ± 48		230 ± 45		417 ± 40	
Glutamic Acid	83 ± 11		29 ± 6		109 ± 5		73 ± 13		72 ± 5		108 ± 39		143 ± 20		156 ± 25	
Serina	246 ± 60		404 ± 82		198 ± 17		1070 ± 58		89 ± 17		915 ± 332		70 ± 6		946 ± 217	
Glycine	36 ± 2		90 ± 19		61 ± 9		211 ± 91		16 ± 2		52 ± 19		10 ± 1		468 ± 118	
Histidine	27 ± 5		19 ± 3		18 ± 3		27 ± 10		9 ± 0		185 ± 49		5 ± 1		84 ± 26	
Arginine	764 ± 82		871 ± 26		433 ± 31		496 ± 156		413 ± 38		743 ± 24		317 ± 20		614 ± 100	
Threonine	72 ± 5		88 ± 13		52 ± 7		118 ± 45		38 ± 5		282 ± 80		37 ± 6		276 ± 75	
Alanine	105 ± 9		279 ± 87		136 ± 6		303 ± 114		101 ± 12		245 ± 25		75 ± 10		320 ± 56	
Proline	508 ± 173		747 ± 351		162 ± 21		293 ± 132		40 ± 15		368 ± 48		21 ± 3		256 ± 33	
Tyrosine	308 ± 34		571 ± 211		121 ± 31		370 ± 145		95 ± 11		239 ± 33		58 ± 5		343 ± 32	
Valine	33 ± 2		63 ± 0		23 ± 2		46 ± 13		35 ± 4		137 ± 25		26 ± 1		113 ± 16	
Methionine	17 ± 1		16 ± 2		6 ± 1		10 ± 3		6 ± 0		16 ± 3		10 ± 0		19 ± 6	
Cystine	29 ± 5		44 ± 7		23 ± 3		39 ± 11		51 ± 8		134 ± 19		41 ± 7		66 ± 10	
Isoleucine	13 ± 0		25 ± 0		9 ± 1		17 ± 4		12 ± 4		42 ± 5		14 ± 2		53 ± 5	
Leucine	18 ± 1		39 ± 6		10 ± 1		20 ± 4		20 ± 2		56 ± 5		15 ± 1		73 ± 9	
Phenylalanine	11 ± 0		19 ± 2		9 ± 0		17 ± 3		21 ± 4		66 ± 9		15 ± 2		68 ± 9	
Lysine	26 ± 2		60 ± 9		18 ± 1		60 ± 19		32 ± 4		248 ± 81		28 ± 1		171 ± 41	
Asparagine	195 ± 83		4479 ± 866		121 ± 25		5803 ± 2802		159 ± 102		10039 ± 2939		23 ± 4		6061 ± 2983	
Glutamine	161 ± 50		288 ± 102		252 ± 11		231 ± 83		207 ± 80		3424 ± 741		81 ± 12		1372 ± 385	
Total	2916 ± 500		8320 ± 1260		2066 ± 52		9092 ± 3952		1661 ± 360		17648 ± 4365		1219 ± 79		11877 ± 4029	
N rates	Low		High		Low		High		Low		High		Low		High	
Roots	mg kg^{-1}															
Aspartic acid	77 ± 9		166 ± 64		59 ± 14		105 ± 9		41 ± 5		121 ± 20		19 ± 1		75 ± 9	
Glutamic Acid	46 ± 18		54 ± 9		32 ± 6		75 ± 16		43 ± 3		116 ± 11		28 ± 3		128 ± 20	
Serina	130 ± 5		518 ± 213		161 ± 25		567 ± 70		76 ± 13		237 ± 22		51 ± 6		228 ± 22	
Glycine	33 ± 5		91 ± 30		34 ± 5		98 ± 15		13 ± 2		30 ± 3		12 ± 2		39 ± 4	
Histidine	14 ± 3		25 ± 3		10 ± 2		38 ± 5		6 ± 1		20 ± 3		7 ± 1		32 ± 3	
Arginine	628 ± 57		916 ± 72		472 ± 86		1258 ± 140		447 ± 60		820 ± 71		372 ± 45		690 ± 94	
Threonine	36 ± 3		102 ± 28		41 ± 8		114 ± 13		18 ± 3		62 ± 8		13 ± 1		80 ± 3	
Alanine	424 ± 57		508 ± 161		388 ± 69		1320 ± 125		215 ± 35		341 ± 33		172 ± 6		319 ± 56	
Proline	72 ± 19		797 ± 578		35 ± 1		102 ± 18		28 ± 6		48 ± 9		16 ± 5		38 ± 1	
Tyrosine	111 ± 11		328 ± 192		79 ± 5		114 ± 8		76 ± 5		107 ± 9		56 ± 5		102 ± 8	
Valine	70 ± 12		84 ± 7		53 ± 3		89 ± 10		48 ± 5		98 ± 16		33 ± 5		106 ± 10	
Methionine	38 ± 10		25 ± 5		18 ± 2		22 ± 4		15 ± 4		19 ± 5		11 ± 3		20 ± 4	
Cystine	78 ± 40		54 ± 24		24 ± 7		121 ± 11		29 ± 14		29 ± 6		13 ± 4		65 ± 14	
Isoleucine	25 ± 4		28 ± 2		18 ± 3		30 ± 4		16 ± 2		28 ± 4		14 ± 3		37 ± 3	
Leucine	42 ± 5		43 ± 1		26 ± 2		47 ± 8		18 ± 1		33 ± 5		13 ± 3		36 ± 5	
Phenylalanine	22 ± 2		20 ± 2		14 ± 1		19 ± 2		10 ± 1		23 ± 4		7 ± 2		22 ± 2	
Lysine	38 ± 1		69 ± 23		31 ± 2		46 ± 7		20 ± 2		47 ± 6		15 ± 3		57 ± 7	
Asparagine	540 ± 48		6437 ± 3355		293 ± 73		2785 ± 326		72 ± 14		1265 ± 198		74 ± 6		1728 ± 182	
Glutamine	249 ± 37		658 ± 137		251 ± 48		1193 ± 197		143 ± 32		844 ± 71		115 ± 5		1444 ± 71	
Total	2673 ± 6		10923 ± 4489		2037 ± 326		8143 ± 882		1335 ± 180		4287 ± 419		1040 ± 94		5244 ± 429	

Means are followed by the standard error of the mean.

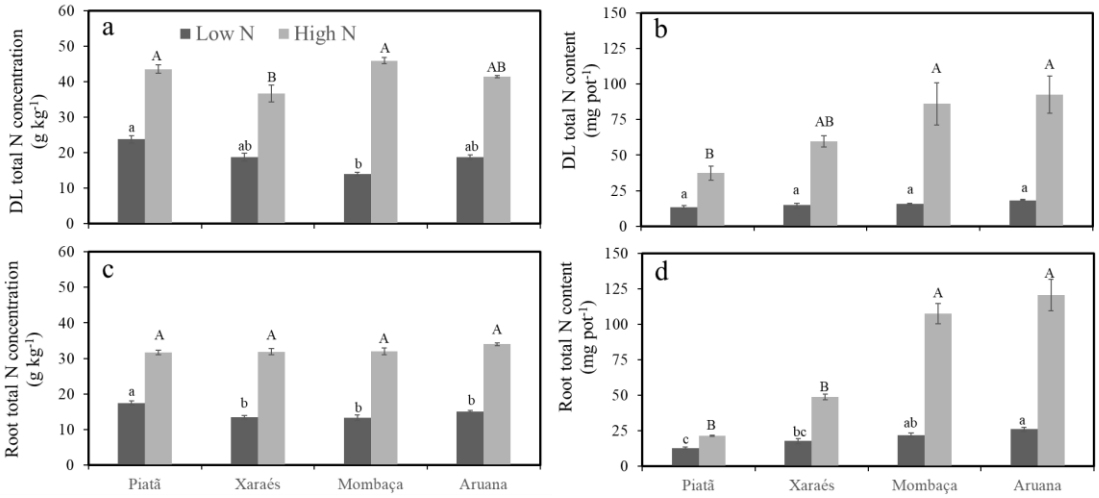

Fig 2. Total N concentration (a, c) and content (b, d) in DL and roots of *Brachiaria spp*. and *Panicum spp*. under low and high nitrogen rates (means followed by the same lowercase letter for the low nitrogen rates and uppercase letters for the high nitrogen rates were not significant at the 5% probability level).

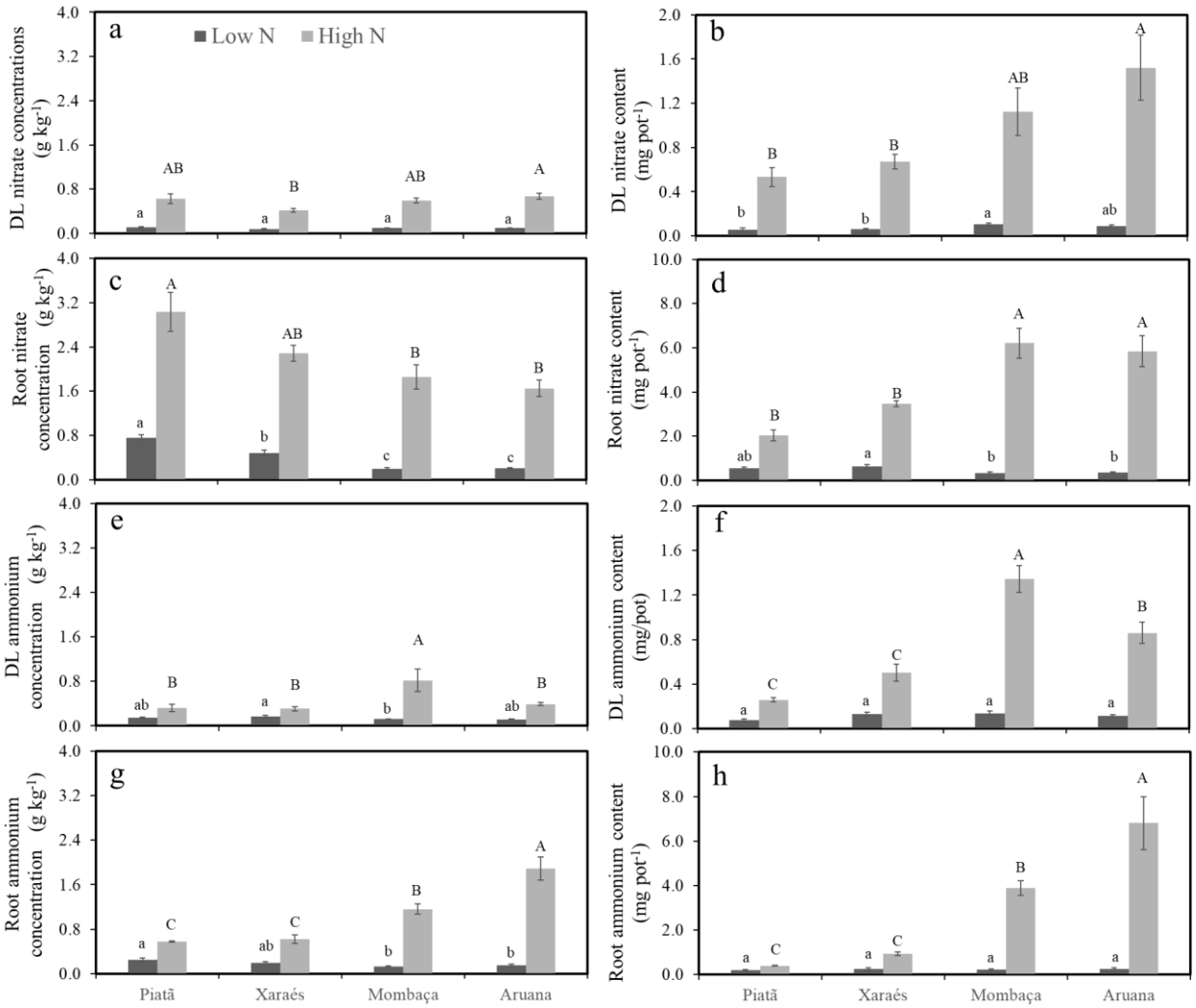

Fig 3. Nitrate and ammonium concentrations (a, c, e, g) and contents (b, d, f, h) in DL and roots of *Brachiaria spp*. and *Panicum spp*. under low and high nitrogen rates (means followed by the same lowercase letter for the low nitrogen rates and uppercase letters for the high nitrogen rates were not significant at the 5% probability level).

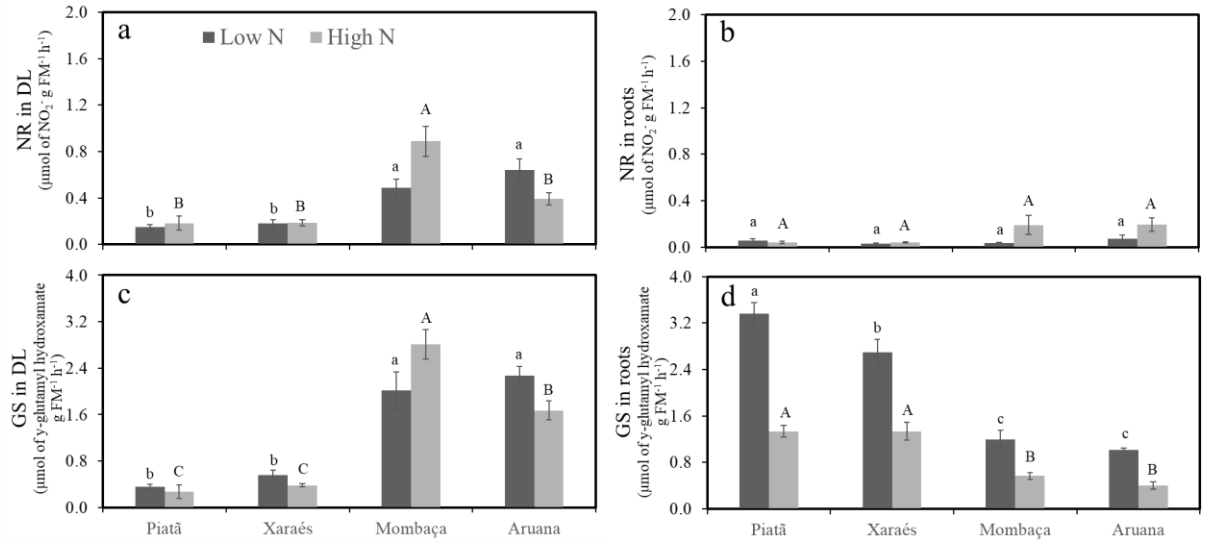

Fig 4. Nitrate reductase (NR) and Glutamine synthetase (GS) activities in DL (a, c) and roots (b, d) of *Brachiaria spp*. and *Panicum spp*. under low and high nitrogen rates (means followed by the same lowercase letter for the low nitrogen rates and uppercase letters for the high nitrogen rates were not significant at the 5% probability level).

nitrogen are first reduced to ammonium, since that is the only reduced form available for plants to use in amino acids production.

The concentration of ammonium in roots was highest in Piatã and Xaraés at low nitrogen rate and in Aruana and Mombaça grasses at high nitrogen rate (Figure 3G). However, root ammonium content was highest in *Panicum* cultivars at high nitrogen rate and similar between cultivars at low nitrogen rate (Figure 3H). Marschner (2018) and Wang and Macko (2011) reported that almost all ammonium assimilation occurs in the root system rather than in shoots. In this study, ammonium assimilation by the activity of the GS enzyme was highest in the root systems of Piatã and Xaraés, at both nitrogen rates (Figure 4D). This suggests a certain preference and/or affinity of these *Brachiaria* grasses for direct absorption of the ammonium form (von Wirén et al., 1997; Wallander et al., 1997; Garnett and Smethurst, 1999), and not only the rapid assimilation of ammonium derived from the reduction of nitrate and/or the use of other nitrogenous compounds, as appears more likely for Mombaça.

Concentrations of total amino acids in DL were highest in Mombaça and Aruana at high nitrogen rate (Table 2), with asparagine and glutamine accounting for 56.8% and 19.4%, respectively, of the total amino acids in the DL of Mombaça. In roots, asparagine concentrations were highest in Piatã and Xaraés at high nitrogen rate. Ammonium, both that absorbed and that produced in the reduction of nitrate and/or other nitrogenous compounds, is assimilated to glutamine and glutamate, which can be used as transport compounds and nitrogen donors in the biosynthesis of practically any amino or nucleic acid, as well as in the production of other nitrogenous compounds, such as chlorophyll (Lea et al., 2007; Mokhele et al., 2012). Nevertheless, asparagine is the primary component in xylem and phloem in higher plants (Pate, 1980; Lea et al., 2007; Martínez-Andújar et al., 2013). Piatã and Xaraés preferentially assimilated more ammonium in the root system, keeping the construction of amino acids in the roots to use as needed, whether nitrogen is sufficient or lacking. By contrast, Mombaça and Aruana prioritize the production and rapid transport of these amino acids in the shoots. This could affect the persistence of these cultivars in production systems with low supply of nitrogen.

In general, the two cultivars of *Panicum maximum* (Mombaça and Aruana) exhibited similar or nearly the same responses in all studied parameters. On the other hand, *Brachiaria brizantha* cv. Piatã showed responses very different from those found in the two *Panicum* cultivars. Thus, it seems appropriate to detail some discussion comparing the grasses of different genera, particularly adopting Mombaça and Piatã, regarding the nitrogen metabolism and treating the roots and the shoots separately.

The root system of Piatã had a higher concentration of total nitrogen than that in Mombaça, at low nitrogen supply. As NR activity (responsible for reducing nitrogen in the form of nitrate to ammonium) was very low in these grasses, ammonium concentration was also higher in Piatã than in Mombaça. On the other hand, at high nitrogen supply, the activity of this enzyme in this part of the plant allowed the ammonium concentration to be higher in Mombaça than in Piatã. As the activity of GS (acting on the incorporation of ammonium to amino acids) was much higher in Piatã roots, under both conditions of nitrogen supply, higher concentration of free amino acids was obtained in Piatã roots than in Mombaça.

In DL (which reflect the nutritional status of the plants) nitrogen utilization by Mombaça and Piatã was different according to nitrogen supply. At low nitrogen supply, Piatã showed higher total nitrogen concentration than Mombaça, while NR activity was higher in Mombaça, so that these grasses showed similar concentrations of ammonium in leaves. Although GS activity was higher in Mombaça than in Piatã, nitrogen availability was lower and glutamine concentration was similar in both cultivars. On the other hand, under high nitrogen rate, total nitrogen concentration in leaves was similar in these two grasses, while NR activity was higher in Mombaça, so that ammonium concentration was higher in Mombaça than in Piatã. As the GS activity was higher in Mombaça, the concentration of both glutamine and free amino acids was higher in Mombaça than in Piatã.

Materials and methods

Plant materials

The experiment was carried out in a greenhouse in Piracicaba, São Paulo State, Brazil (22 42' 30'' South and 47 38' 00'' West), in a 4x2 factorial, in a randomized complete block design, with five replicates. Mean air temperature and humidity in the greenhouse during the study were 28 °C (Tmax = 45.2 ºC / Tmin = 18.2 ºC) and 63.7%, respectively. 3.6-L plastic pots were filled with ground quartz that had been rinsed first with tap water and then with deionized water. The four grasses *Brachiaria brizantha* cv. Xaraés, *B. brizantha* cv. Piatã, *Panicum maximum* cv. Mombaça, and *P. maximum* cv. Aruana were selected based on their production attributes and nitrogen accumulation in roots and shoots, in a previous experiment (Garcez and Monteiro, 2016a; Garcez and Monteiro, 2016b).

Grass seeds were allowed to germinate in plastic trays containing rinsed sand moist enough to provide favorable conditions for germination. Seedlings were transplanted 10 days after seeding, when they had two or three leaves (height > 5 cm). Fifteen seedlings were transplanted to each pot and successively thinned until 10 plants remained in each pot in order to have sufficient plant tissues for analyses at the end of the growth period (30 days after transplanted).

A modified form of Hoagland and Arnon's (1950) nutrient solution was used in the experiment to provide plants with low (3 mmol L^{-1}) and high (30 mmol L^{-1}) nitrogen supply, as well as 8 mmol L^{-1} of potassium, according to Mattos et al. (2002) and Consolmagno Neto et al. (2007) (Table 1). For the first five days after they were transplanted, seedlings were kept in a diluted nutrient solution (20% of ionic strength of the complete solution). Solutions were circulated three times a day, drained into 1-L containers at night, and completed with deionized water in the following morning. The nutrient solution was renewed twice during the study: first at 14 days after transplanting and then 10 days later. The second renewal was done after a shorter period because the plants with high nitrogen rate were growing fast and the need for plant sampling to determine enzyme and free amino acid levels. Five replicates were used to guarantee that enough material was produced to carry out all laboratory analyses. Plants were harvested 30 days after transplanting. One day before harvesting, the pots were placed in a climate-controlled room and plants were acclimated to 30 °C temperature, 60% humidity, and 400 µmol m^{-2} s^{-1} PAR light intensity. Diagnostic leaves (lamina of

the two most recently expanded leaves, designated as DL) of the primary tiller (marked during the growth period) and roots were removed to determine the glutamine synthetase enzyme activities and free amino acid concentrations. These leaves and roots were first frozen in liquid nitrogen, and then placed in a freezer at -80 °C. Activity of the NR enzyme was determined immediately after collecting the plant tissues.

Measurement of traits

At the end of the growing period the shoots were harvested, sorted into diagnostic leaves (DL), other leaves, stems+sheaths and roots, and dried for 72 h in air circulating oven at 65 °C. These plant parts were ground separately in a Wiley mill and placed in plastic bags. To determine nitrogen concentrations in DL and roots, sulfuric digestion was performed, followed by distillation and titration (Sarruge and Haag, 1974). Ammonium and nitrate concentrations in DL and roots were determined according to Tedesco et al. (1985).

NR activity was determined using Mulder et al. (1959) method. Samples were collected from the middle third of DL and roots. Samples of 0.2 g of those plant tissues were incubated for two hours in a warm water bath at 35 °C, in a medium of 0.25 mol L^{-1} KNO_3 in a phosphate buffer, and shaken every 5 min. When the incubation period ended, 1 mL of the solution (without leaf fragments) was pipetted into a 50 mL volumetric flask. The flask was filled with water to approximately 25 mL, 1 mL of sulfanilic acid was added, and the mixture was left for 5 min. Subsequently, 1 mL of alpha-naphthylamine in 20% HCl and 1 mL of 2 mol L^{-1} sodium acetate buffer were added. The volume was completed and readings were taken in a spectrophotometer at 540 nm. The nitrite standard was used to calculate NO_2 concentrations in tissues.

GS activity was determined in DL and roots by using a modified version of Elliott's (1953) method. One half gram of plant tissue was collected and macerated in liquid nitrogen. Immediately afterwards it was combined with 1.5 mL of extraction buffer (50 mmol L^{-1} of tris–HCl at pH 7.5; 2 mmol L^{-1} mercaptoethanol and 1 mmol L^{-1} EDTA) and centrifuged at 10,000 rpm for 10 min at 4 ºC. Subsequently, 0.3 mL of sample supernatant was removed and combined with 0.5 mL of tris-HCl (200 mmol L^{-1} at pH 7.5), 0.2 mL of ATP (50 mmol L^{-1} at pH 7), 0.5 mL of glutamic acid (500 mmol L^{-1} at pH 7.5), 0.1 mL of magnesium sulfate (1 mol L^{-1}), 0.3 mL of hydroxylamine (100 mmol L^{-1}), and 0.1 mL of cysteine (100 mmol L^{-1}), for a total of 2 mL. For each sample, a blank was prepared by combining the sample with all the reagents except for ATP and glutamic acid, and the volume was completed with deionized water. The blank and the sample were placed in a water bath at 30 ºC for 30 min. The reaction was interrupted by adding 2 mL of the reagent containing $FeCl_3$ (0.6 mol L^{-1}), TCA (1.5 mol L^{-1}), and HCl (1 mol L^{-1}) in a 1:1:1 ratio. The mixture was immediately centrifuged at 5000 rpm and the supernatant was read at 540 nm in a spectrophotometer to detect the formation of γ-glutamyl hydroxamate, using the standard curve previously prepared.

Amino acids in DL and roots were determined using the harvested material stored in a freezer at -80 °C. The plant material was macerated using a mortar and a pestle with liquid nitrogen and a 10 mmol L^{-1} CH_2O_2 solution. Amino acids were determined using pre-column derivatization with orthophthaldehyde (OPA) and quantified by high-pressure liquid chromatography (HPLC). The Shimadzu® liquid chromatograph was fitted with a CBM 10-A communications module, a CTO 10-A temperature control module, an SIL 10-An automatic injector, an HPLC 10-AS pump, a CRB-6A oven, a C-18 150 x 4-6 mm column (Spherisorb ODS 2), and an RF 10-A fluorescence detector. To detect fluorescence, wavelengths of 340 nm and 445 nm were used for excitation and emission, respectively. The following solvents were used: 0.05 mol L^{-1} sodium acetate (pH 5-7) + 3 % v/v of tetrahydrofuran (THF) and methanol + 5 % THF. The separation time of the 19 amino acids analyzed was 30 min per sample. Concentrations of total free amino acids were determined using the Spectra Physics SP 4270 program, which integrates peak areas.

Statistical analysis

Results were analyzed using Statistical Analysis System software (SAS, 2004). Analysis of variance was performed and means were compared by Tukey's test at a significance level of 5%.

Conclusion

In practice, at low nitrogen supply to the pasture (nitrogen supply via decomposition of organic material, residue of previous application of this nutrient or other sources poor in nitrogen), Piatã showed higher concentrations of total nitrogen and free amino acids in the leaf tissues and in the roots. Thus, this cultivar of *Brachiaria brizantha* may be an option to use in less intensive production environments, instead of high dry mass production. For those pastures with high nitrogen fertilization, the cultivars of *Panicum maximum* may show similar concentrations of total nitrogen (both in the roots and in the leaves) to that of the cultivar Piatã of *Brachiaria brizantha*. Considering the amino acids concentrations and dry mass production, the grasses of the genus *Panicum* were clearly superior to Piatã, which suggests priority in the selection of the cultivars Mombaça and Aruana for systems with high nitrogen fertilization.

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