



Growth and productive responses of tropical grass *Panicum maximum* to nitrate and ammonium supply

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ABSTRACT - This study was conducted to investigate growth response parameters and forage yield of *Panicum maximum* Jacq. plants supplied with nitrate (NO_3^-) and ammonium (NH_4^+) as N source in a nutrient solution. Six NO_3^- and NH_4^+ combinations were tested at the following proportions: 100-0, 85-15, 70-30, 55-45, 40-60 and 25-75%. Grass shoot (number of tillers and leaves and leaf surface area) and root (length and surface area) growth parameters and dry matter production exhibited highest values in *P. maximum* plants growing in the nutrient solution supplied with $\text{NO}_3^-:\text{NH}_4^+$ at the proportions ranging between approximately 70-30 and 55-45%. The use of $\text{NO}_3^-:\text{NH}_4^+$ mixture at the ratio of 55-45% instead of the NO_3^- solely as N source in the nutrient solution enhanced the production of tillers and leaves and the leaf area surface of *P. maximum* by about 30, 20 and 30%, respectively. Plants supplied with NO_3^- and NH_4^+ mixture (55-45%) showed an increased root length and root surface area by approximately two and six-fold, respectively, compared with *P. maximum* fertilized with NO_3^- exclusively in the growth medium. Supplying N in the nutrient solution as NO_3^- or NH_4^+ exceeding approximately 70 or 50%, respectively, caused a diminished *P. maximum* forage yield. Enhanced N-use efficiency associated with *P. maximum* plants supplied with a NO_3^- and NH_4^+ mixture in the growth medium supports the assumption that it is possible to increase plant production by managing N fertilization based on quality (N forms) of N fertilizers applied in pasture systems.

Key Words: forage production, nitrogen nutrition, nitrogen-use efficiency, plant development, tillering

Introduction

Forage grasses are widespread on the entire world and constitute one of the most important food sources for grazing animals. The intensity of forage production depends basically on the plant tillering and leaf development and growth processes (De Bona & Monteiro, 2010a). Such plant processes are genetically controlled and can be altered by the management of plant growth conditions (phenotype control). Among the changeable plant growth conditions, growth medium such as water and nutrient availability play a major role in determining forage dry matter yield.

Considering soil fertility and plant nutrition status, previous results have demonstrated the importance of nitrogen (N) supply on the improvement of forage grass production (Vicente-Chandler, 1973; Laurialt et al., 2002; Reich et al., 2003; De Bona & Monteiro, 2010b). In fact, many tropical forage grasses are C_4 plants, which are highly responsive to N fertilization. A classical study of Vicente-Chandler (1973), conducted in Puerto Rico soils under no water or nutrient limitations, reported that dry matter yields of tropical forage grasses such as napiergrass (*Pennisetum purpureum* Schumach.), guineagrass (*Panicum maximum* Jacq.), ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M.

Evrard) Morrone & Zuloaga] and stargrass (*Cynodon nlemfuensis* Vanderyst) increased with N rates up to 896 kg/ha. In spite of positive growth responses, very high N additions to grasslands could reduce the N-use efficiency as well as enhance the environment potential contamination by nitrate leaching (Babiker et al., 2004). Thus, to manage N fertilization aiming to enhance plant production without increasing the N rate applied is a big challenge for the scientific research and farmers practice.

Among the commercial N fertilizers, different N forms [nitrate (NO_3^-) and ammonium (NH_4^+)] or mixtures of N forms can be chosen: urea, ammonium nitrate, ammonium sulfate, mono-ammonium phosphate, di-ammonium phosphate, calcium nitrate or potassium nitrate (Havlin et al., 2005). These nitrogen sources vary not only in total N content, but also in the predominant ionic form (NO_3^- or NH_4^+) in which N is supplied to the plants. It is known that the plant physiological responses depend on the N form absorbed by the roots (Marschner, 1995). These responses certainly influence plant growth performance and dry matter yield (McCrimmon et al., 1992). Wang & Below (1992) tested two wheat (*Triticum aestivum* L.) cultivars and had higher number of tillers when they supplied NO_3^- and NH_4^+ at 75-25 or 50-50%, respectively, than solely NO_3^- or NH_4^+ .

Some studies on plant hormones have been carried out and found that plant morphogenesis (root and shoot) may change according to the NO_3^- and NH_4^+ supply (Walch-Liu et al., 2000; Rahayu et al., 2005; Walch-Liu et al., 2006). Supported by these interesting results obtained mainly with crop plants, the growth of tropical grasses is expected to be also affected by N forms supplied in the growth medium. Thus, the objective of this study was to evaluate the general growth response parameters and forage production of the tropical grass *Panicum maximum* Jacq. supplied with NO_3^- and NH_4^+ as N in nutrient solution.

Material and Methods

The experiment was carried out during the summer season under greenhouse conditions (22-35 °C; light: dark = 16:8 hours). *Panicum maximum* Jacq. cv. Aruana (Aruana guineagrass) seeds were germinated on a sterile sand medium. After about 14 d, five grass seedlings were transplanted into plastic pots (20 cm height; 15.5 cm diameter; and 3.6 L volume) containing an inert substrate (ground quartz with diameter between 2 and 8 mm). Plants were irrigated with a complete nutrient solution based on Hoagland & Aron (1950), with six $\text{NO}_3^-:\text{NH}_4^+$ ratios (Table 1). Nitrogen, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) were homogeneously supplied in the nutrient solution at the concentrations of 15, 1, 6, 5, 2 and 2 mmol/L, respectively. The nutrient solution was replaced every 10 d and the content of N forms in the growth medium was continuously checked by using NO_3^- and NH_4^+ tests. The treatments of proportions of N in different forms were arranged in a randomized complete block design, with four replications.

Plant shoots were harvested twice: 28 d after transplanting and 22 d after the first harvest. Roots were

collected at the second shoot harvest time. The harvested material was dried in a forced air-ventilated oven at 65 °C until constant mass. At the harvesting time, the number of tillers and leaves and leaf area of plants were also determined. Leaf surface area was determined by using a leaf area machine model LI 3100 (LI-COR, NE, USA). So as to determine the root surface area and length, part of the grass root system (approximately 20%) was stained with a gentian violet solution (1000 mL distilled water + 50 mg of violet gentian) for 24 hours, put on an acetate sheet to avoid root overlapping, and scanned with a HP Scanjet 3670 (300 dpi, Hewlett-Packard Development Company, Texas, USA). Subsequently, total root area and length of scanned grass root images were calculated using the software SIARCS (Integrated Analysis System of Roots and Soil Cover, version 3.0) (Crestana et al., 1994).

The concentrations of total N, N-NO_3^- and N-NH_4^+ were determined in ground (1 mm particle) dry root and shoot material. Total N in the plant tissue was determined according to Nelson & Sommers (1973). Nitrogen content was used to estimate the amounts of N extracted by the grass from the nutrient solution. Nitrate and NH_4^+ concentrations in the plant material were determined using the micro Kjeldahl method (Tedesco et al., 1995).

Disregarding the plant root parameters, all other results (shoot parameters) presented in this study represent the mean of both harvests because the plant responses to the treatments with N forms were similar in both growth cycles. Statistical analysis was performed using the statistical software SAS (Statistical Analysis System, version 9.1.2). Data were evaluated by ANOVA for the $\text{NO}_3^-:\text{NH}_4^+$ ratios in the nutrient solution. Means were compared by Tukey's test at the 5% significance level. Regression studies for the $\text{NO}_3^-:\text{NH}_4^+$ ratios or NH_4^+ replacement for NO_3^- (%) in the nutrient solution were performed through GLM procedures.

Table 1 - Chemical composition of the nutrient solution according to the $\text{NO}_3^-:\text{NH}_4^+$ ratio supply

Salts	$\text{NO}_3^-:\text{NH}_4^+$ ratio (%)					
	100-0	85-15	70-30	55-45	40-60	25-75
KH_2PO_4 (mmol/L)	1	1	1	0	0	0
KNO_3 (mmol/L)	5	4.5	2	1	0	0
KCl (mmol/L)	0	0.5	3	5	6	6
$\text{Ca}(\text{NO}_3)_2$ (mmol/L)	5	3	2	0.75	0	0
CaCl_2 (mmol/L)	0	2	3	4.25	5	5
MgSO_4 (mmol/L)	2	2	2	2	1	0
MgCl_2 (mmol/L)	0	0	0	0	1	2
NH_4NO_3 (mmol/L)	0	2.25	4.5	5.75	6	3.75
NH_4Cl (mmol/L)	0	0	0	0	0	2.5
$\text{NH}_4\text{H}_2\text{PO}_4$ (mmol/L)	0	0	0	1	1	1
$(\text{NH}_4)_2\text{SO}_4$ (mmol/L)	0	0	0	0	1	2

All treatments received a similar content of micronutrients in the nutrient solution as follows: 46 $\mu\text{mol/L}$ of H_3BO_3 , 9 $\mu\text{mol/L}$ of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.7 $\mu\text{mol/L}$ of ZnCl_2 , 0.2 $\mu\text{mol/L}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.08 $\mu\text{mol/L}$ of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 100 $\mu\text{mol/L}$ of $\text{Fe}(\text{III})\text{-EDTA}$.

Results

The supply of N in the nutrient solution through different $\text{NO}_3^-:\text{NH}_4^+$ ratios significantly affected the *P. maximum* growth responses by promoting changes in the total tiller and leaf emission and leaf area expansion (Figures 1A, 1B and 1C, respectively). The use of the $\text{NO}_3^-:\text{NH}_4^+$ mixture at the ratio of 55-45% instead of the NO_3^- solely as N source in the nutrient solution enhanced the emission of tillers and leaves and the total leaf area of *P. maximum* by about 30, 20 and 30%, respectively.

As expected, the shoot dry matter production (Figure 1D) of the forage grass reflected the plant growth responses (Figures 1A, 1B and 1C) to the $\text{NO}_3^-:\text{NH}_4^+$ ratios in the nutrient solution. Thus, the highest forage production was found in the *P. maximum* plants grown in the nutrient solution containing $\text{NO}_3^-:\text{NH}_4^+$ at the proportions of 70-30 and 55-45%. Using N in the nutrient solution as NO_3^- or NH_4^+ exceeding about 70 or 50%, respectively, resulted in low *P. maximum* forage yield.

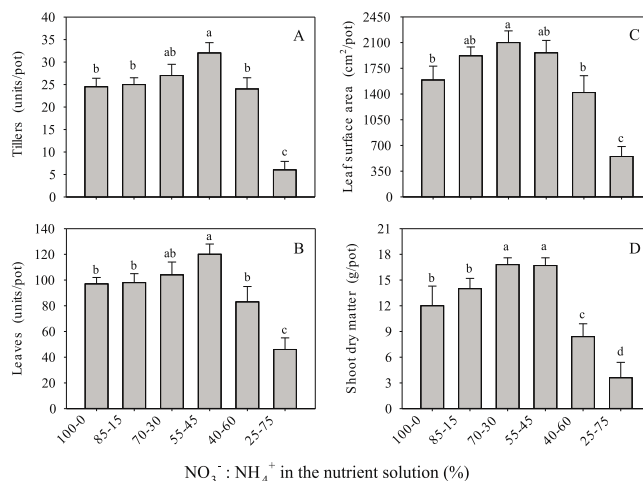
Undoubtedly, the development and growth of plant shoot depends on adequate root growth. In the present study, root length and root surface area data (Figures 2A and 2B) showed a similar response to the shoot parameters (Figures 1A, 1B and 1C) in relation to the $\text{NO}_3^-:\text{NH}_4^+$ ratio supply in the nutrient solution. Therefore, forage plants growing under a nutrient solution with N provided as a mixture of NO_3^- and NH_4^+ at the ratio of 55-45% exhibited highest root length and root surface area. Despite the response similarities, the root system (Figures 2A and 2B) of the grass plants was more intensively affected by the $\text{NO}_3^-:\text{NH}_4^+$ ratios than the shoot parameters (Figures 1A, 1B and 1C). Thus, the use of the $\text{NO}_3^-:\text{NH}_4^+$ mixture at the ratio of 55-45% instead of the NO_3^- form alone in the growth medium increased the forage grass root length and root surface area by approximately two and six-fold, respectively.

Similarly to root growth parameters, the root dry matter of *P. maximum* reached the highest values when the plants grew in the nutrient solution supplied with a $\text{NO}_3^-:\text{NH}_4^+$ ratio of 55-45% (Figure 2C). The predominance of NO_3^-

(approximately 85% or more) or NH_4^+ (approximately 60% or more) as N in the growth medium was accompanied by severe grass root dry matter decreases.

Studying the variations in grass growth and productive parameters due to NH_4^+ replacement with NO_3^- as N source in the nutrient solution (Table 2), maximal plant variable responses were associated with $\text{NO}_3^-:\text{NH}_4^+$ ratios ranging from 60-40 to 72-28 (average value of 68-32).

Nitrogen concentration in shoot tissue of the forage plant was affected significantly by the $\text{NO}_3^-:\text{NH}_4^+$ ratios provided in the nutrient solution and ranged between 13.5 and 24.0 g/kg (Figure 3A). Due to the nutrient dilution phenomenon, N concentration in *P. maximum* grass shoot varied inversely to the dry matter yield changes (Figure 1D). Forage plants grown with a nutrient solution high in NH_4^+ showed decreased N absorption and accumulation. Unlike total N content, the concentration of free N- NO_3^- and N- NH_4^+ in the shoot tissue of *P. maximum* did not change in response to $\text{NO}_3^-:\text{NH}_4^+$ ratios in the nutrient solution, averaging approximately 0.50 and 0.45 g/kg, respectively.

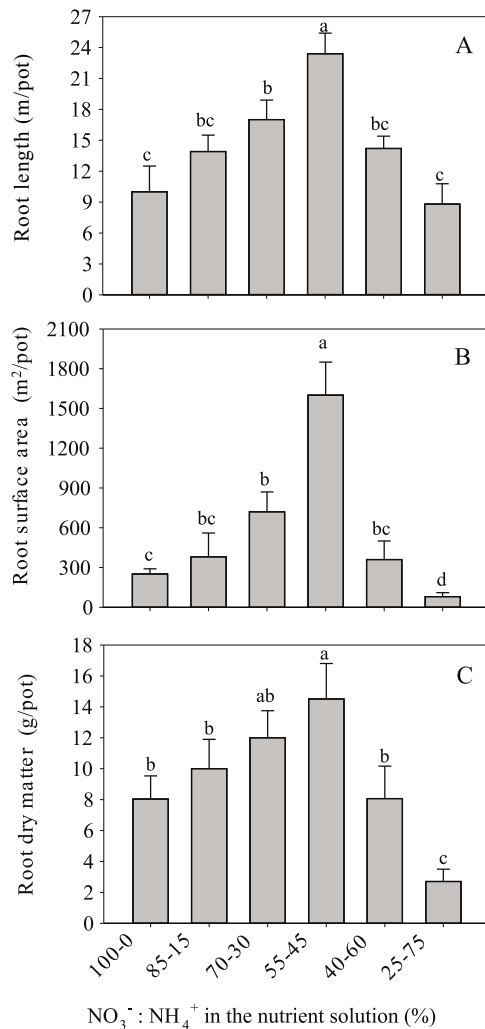


Bars with different letters differ by Tukey's test at 5% of significance (n = 4).

Figure 1 - Shoot growth parameters [total number of tillers (A) and leaves (B), and leaf surface area (C)] and shoot dry matter production (D) of the forage grass *Panicum maximum* supplied with nitrate (NO_3^-) and ammonium (NH_4^+) as N source in the nutrient solution.

Table 2 - Regression analysis for estimation of *Panicum maximum* growth and productive responses as related to ammonium (0, 15, 30, 45, 60 and 75%) replacement with nitrate as N source in the nutrient solution

Plant variable	Adjusted regression	Coefficient of determination (R^2)	Estimated $\text{NO}_3^-:\text{NH}_4^+$ ratio for maximum variable response
Number of tillers	$Y = 21.66 + 0.62x - 0.010x^2$	0.83	69-31
Number of leaves	$Y = 90.07 + 1.61x - 0.028x^2$	0.85	71-29
Leaf surface area	$Y = 1550 + 39.83x - 0.70x^2$	0.99	72-28
Shoot dry matter	$Y = 11.46 + 0.35x - 0.0062x^2$	0.93	72-28
Root length	$Y = 8.76 + 0.57x - 0.0076x^2$	0.79	64-36
Root surface area	$Y = 68.93 + 49.76x - 0.66x^2$	0.55	60-40
Root dry matter	$Y = 7.16 + 0.36x - 0.056x^2$	0.88	68-32



Bars with different letters differ by Tukey's test at 5% of significance (n = 4).

Figure 2 - Root growth parameters [total root length (A) and root surface area (B)] and root dry matter production (C) of the forage grass *Panicum maximum* supplied with nitrate (NO₃⁻) and ammonium (NH₄⁺) as N source in the nutrient solution.

The supply of NO₃⁻ and NH₄⁺ in the growth medium resulted in significant changes in the N concentration of the *P. maximum* roots, which exhibited similar values to the shoot tissue (Figure 3A), ranging from 11.5 to 20.5 g/kg (Figure 3B). Forage grass growing under a nutrient solution with N provided exclusively or mostly as NO₃⁻ (100-0 and 85-15 as NO₃⁻:NH₄⁺ ratio) showed the highest concentration of total N and free N-NO₃⁻ and N-NH₄⁺ in the root tissue. Similarly to the shoot tissue (Figure 3A), plants growing under a nutrient solution where the NO₃⁻:NH₄⁺ ratio was lower than approximately 1.0 showed a strong decrease in the N extracted by these plants (Figure 3B).

Based on the plant dry matter production (Figures 1D and 2C) and the N content in plant tissue data (Figures 3A and 3B), the N-use efficiency (NUE) showed greatest

values when N was provided in the nutrient solution for *P. maximum* through the mixture of NO₃⁻ and NH₄⁺ at proportions ranging between 70-30 and 55-45% (Figure 3C).

Discussion

Since homogeneous N application was performed in the nutrient solution and no growth-limiting N concentration (Lavres Júnior & Monteiro, 2006) was noticed in the *P. maximum* tissues (Figures 3A and 3B), the grass growth responses to NO₃⁻ and NH₄⁺ treatments cannot be attributed to limited N availability or uptake. This clearly indicates that the remarkable changes in the forage production of *P. maximum* (varying from 3.6 to 16.8 g/pot) were associated with modified shoot and root development and growth parameters (Figures 1 and 2) due to the N forms supplied as N source. Involved in the increase of grass demography in field conditions and, consequently forage production potential, the number of tillers/plant was enhanced by the use of the N mixture (NO₃⁻:NH₄⁺ ratio of 55-45%), also increasing the leaf production capacity of *P. maximum* grass. This finding is very important since the number of live leaves/tiller of forage grasses remains constant throughout the life of the plant. Actually, leaves are produced continuously, but each time a new leaf appears, the oldest leaf on the tiller initiates the senescence process and dies (Hunt, 1965). Another relevant shoot modification observed in grass plants fertilized with NO₃⁻ and NH₄⁺ mixture was the increased leaf surface area. Besides directly increasing the forage yield potential, larger leaves are able to intercept more light (solar radiation), amending within certain limits the light-use efficiency and plant canopy photosynthesis (Hirose & Bazzaz, 1998).

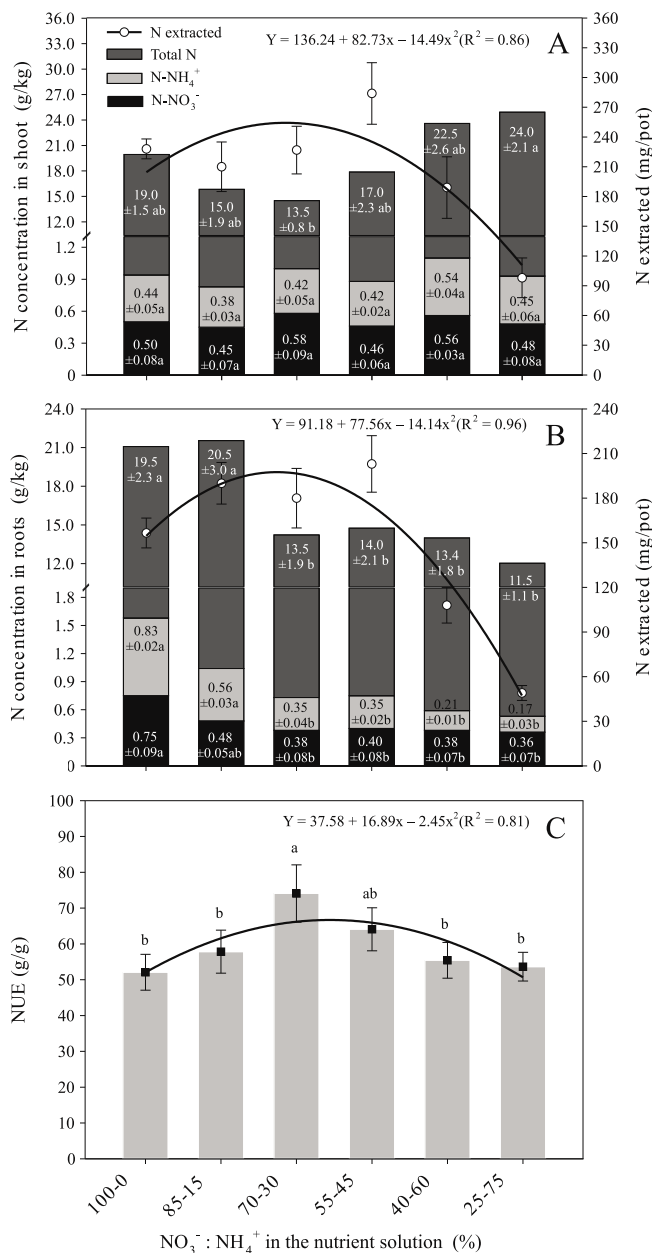
Previous studies also reported that many plant species perform better under the supply of N as a mixture of NO₃⁻ and NH₄⁺ and, comparable to the results presented in this paper, shoot yield was accompanied by increased tillering (Wang & Below, 1992; Wang & Below, 1996) and leaf surface area (Cox & Reisenauer, 1973; Walch-Liu et al., 2000; Helali et al., 2010) of NO₃⁻ and NH₄⁺ mixture-fertilized plants. Tillering of wheat plants was highly influenced by fertilization with mixed N sources (Wang & Below, 1996). Regarding leaf morphogenesis, Walch-Liu et al. (2000), investigating the effect of N forms on the growth of tobacco (*Nicotiana tabacum* L.) cultivated in a nutrient solution, found higher leaf expansion rate and leaf surface area associated with plants fertilized with a mixture of NO₃⁻ and NH₄⁺ than exclusive NH₄⁺-fertilized plants. These authors have demonstrated by microscopic analysis of the epidermis of fully expanded leaves that cell number and cell

size decrease by 50 and 30%, respectively, with application of NH_4^+ , which indicates that both cell division and cell elongation were affected. At least in part, the described results can be attributed to the action of the plant hormone cytokinin (Takei et al., 2002). Cytokinin is recognized as crucial signaling molecules that control plant growth and development by promoting cell division, cell expansion and

axillary bud outgrowth (shoot branching) (Ongaro & Leyser, 2008). Moreover, research findings have demonstrated that cytokinin synthesis and root-to-shoot translocation can be increased by applying the mixture of NO_3^- and NH_4^+ as N source in the growth medium (Smiciklas & Below, 1992; Wang & Below, 1996; Walch-Liu et al., 2000).

In the present study, shoot production and growth responses certainly also reflect the $\text{NO}_3^-:\text{NH}_4^+$ ratio effects on *P. maximum* root system, which was markedly more influenced by N forms in the nutrient solution than the aboveground grass tissues (Figures 1 and 2). The plant root system plays a major role in plants growing in soil because it is responsible particularly by the nutrients and water uptake. Plant N nutrition status or N availability in medium influencing the growth and development of roots has been reported by other researchers (Drew, 1975; Gerendás et al., 1997; Zhang et al., 1999; Lima et al., 2010). These findings suggested that both NO_3^- (Gerendás et al., 1997; Zhang et al., 1999) and NH_4^+ (Drew, 1975; Lima et al., 2010) are able to stimulate root branching and lateral root elongation through the expression of plant growth regulator genes (auxin and/or cytokinin) responsive to the presence of the N form in the environment. However, it is important to highlight that a very high content of available NO_3^- to the plants represses the lateral root development by a systemic inhibition possibly regulated by the accumulation of NO_3^- (Figure 3B) in the plant tissue (Zhang et al., 1999; Zhang & Forde, 2000; Walch-Liu et al., 2006). Also, a high content of NH_4^+ in the growth medium potentially impairs the root growth and development due to the NH_4^+ toxicity phenomenon (Gerendás et al., 1997; Britto & Kronzucker, 2002). Agreeing with the exposed theories, roots of *P. maximum* exhibited greatest absolute length, surface area and dry matter (Figure 2) when N was supplied in the nutrient solution at the intermediate $\text{NO}_3^-:\text{NH}_4^+$ ratio of 55-45%.

Taking into consideration the *P. maximum* shoot and root yield and growth parameters (Figures 1 and 2), it is easy to visualize that in general $\text{NO}_3^-:\text{NH}_4^+$ ratios in the nutrient solution with the predominance of NO_3^- (approximately 85% or more) or exceeding about 50% of the N as NH_4^+ resulted in low values of the response variables. Indeed, grass growth parameters were more severely impaired by high content of NH_4^+ (about 75%) in the growth medium than NO_3^- as exclusive N source. This remarkable decrease in plant growth and development when NH_4^+ was superior to NO_3^- in the nutrient solution is possibly linked to the plant NH_4^+ toxicity. The NH_4^+ toxicity depends on plant species and affects the optimal plant growth by: (i) impairing uptake of nutrients; (ii) inhibition



Bars with different letters differ by Tukey's test at 5% of significance (n = 4).

Figure 3 - Shoot and root nitrogen (N) nutritional status parameters [total N, N-nitrate (N-NO_3^-) and N-ammonium (N-NH_4^+) concentration] (A, B), N extracted by plant tissues (A, B) and N-use efficiency (NUE) (C) of the forage grass *Panicum maximum* supplied with NO_3^- and NH_4^+ as N source in the nutrient solution.

of secondary growth by acidification of the rooting medium; (iii) alterations in intracellular pH and osmotic balance; and (iv) altered phytohormone metabolism (Gerendás et al., 1997; Kronzucker et al., 1999; Britto & Kronzucker, 2002). Unlike plant N nutrition based on NH_4^+ , even under a high content or exclusive in the growth medium, NO_3^- does not cause toxicity injuries to the plants. Yet, the results demonstrated that *P. maximum* grown in a nutrient solution where NO_3^- was the predominant (85%) or sole N source did not perform better and/or had worse performance in terms of growth response and production than forage plants grown with the NO_3^- and NH_4^+ mixture at the ratio of 70-30 or 55-45%. Actually, besides the beneficial effects associated with plant hormones, an equilibrated NO_3^- and NH_4^+ mixture is able to enhance plant growth by other ways such as increasing plant N uptake (Kronzucker et al., 1999) and saving energy costs by using reduced NH_4^+ instead of NO_3^- (Bloom et al., 1992). Based on the latter hypothesis, previous studies reported that adding NH_4^+ at a low level or up to the maximum level tolerable by a specific plant species in a growth medium containing NO_3^- promotes positive yield and growth effects (Cox & Reisenauer, 1973; Gerendás et al., 1997). It is reasonable to conclude that for *P. maximum* culture in a growth medium containing 15 mmol/L of N supplied by a NO_3^- and NH_4^+ mixture, one may use up to 6.75 mmol/L as NH_4^+ , aiming to increase the plant growth responses without or having minimal NH_4^+ toxicity risks.

Nitrogen concentration in *P. maximum* shoots and roots were distinctly affected by the $\text{NO}_3^-:\text{NH}_4^+$ ratios in the growth medium (Figures 3A and 3B). While the nutrient dilution phenomenon apparently governed the alteration in N concentration in the shoot tissue, root N concentration does not appear to follow this growth effect. In spite of the low dry matter production, roots of forage plants growing under a nutrient solution containing approximately 50% or more of the N as NH_4^+ showed no high N concentration in the root tissue. This can be attributed to a likely plant-protector mechanism (avoiding NH_4^+ uptake and/or through NH_4^+ efflux) against the NH_4^+ toxicity (Gerendás et al., 1997; Kronzucker et al., 1999; Britto & Kronzucker, 2002) caused by NH_4^+ accumulation in plant tissue.

Surprisingly, free N- NO_3^- and N- NH_4^+ concentrations in the *P. maximum* shoot tissue were unaffected by the wide range of $\text{NO}_3^-:\text{NH}_4^+$ ratios supplied in the nutrient solution (Figure 3A). In contrast, roots of grasses grown with a nutrient solution containing 85% or more N as NO_3^- exhibited higher concentration of both free N- NO_3^- and N- NH_4^+ compared with the other treatments (Figure 3B). These root and shoot data strongly suggest that the *P.*

maximum root system must play an important role in NO_3^- reduction and root-to-shoot transport of free N- NO_3^- and N- NH_4^+ . Marschner (1995) reported that NO_3^- reduction directly in the root system instead of plant leaves depends on the NO_3^- content in the growth medium and plant species.

Similarly to plant productive and growth parameters, N use efficiency (NUE) also provides interesting information regarding N fertilization for forage production. The results revealed that a balanced proportion of NO_3^- and NH_4^+ in the growth medium maximizes the NUE by the *P. maximum* plants (Figure 3C). This finding is a good evidence that knowing performance and limitations of plant species (NH_4^+ content tolerance) under NO_3^- and NH_4^+ , it is possible to manage N fertilization for yield improvement by choosing NO_3^- or NH_4^+ -releasing fertilizers with or without combination of nitrification inhibitors. In fact, many researchers have investigated the nitrification inhibitors and their effectiveness under field conditions (Weiske et al., 2001; Singh et al., 2008), but few studies have focused on intrinsic plant species response to NO_3^- and/or NH_4^+ supply.

Conclusions

The supply of a N form (NO_3^- and NH_4^+) mixture at an equilibrated proportion instead of NO_3^- or NH_4^+ predominantly or solely as N source in the growth medium enhances the forage yield of the tropical grass *P. maximum* by directly and positively affecting some shoot growth parameters (tillers and leaves) and by promoting better plant root system growth performance. The results obtained under controlled conditions provide basic knowledge of forage grass responses to NO_3^- and NH_4^+ supply. However, further field research is important, to confirm *in situ* the advantages of supplying NO_3^- and NH_4^+ together to tropical grass pastures.

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