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N Sources Affect Growth, Nutrient Content, and Net Photosynthesis in Maté (*Ilex paraguariensis* St. Hil.)

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ABSTRACT

The influence of different N sources on the growth of maté (<u>Hex paragurariensis</u> St.Hil.) seedlings grown in greenhouse was studied. All seedlings received a base fertilization of 10 mg N.kg⁻¹ soil as NH₄NO₃, 60 mg P₂O₅, and 40 mg K₂O.kg⁻¹ soil as KH₂PO₄ 15 days before treatments application. Treatments were as follow: Control, with no extra N added; Urea = 100 mg N.kg⁻¹ soil as Urea; NO₃ = 100 mg N.kg⁻¹ soil as Ca(NO₃)₂; and NH₄ + 100 mg N.kg⁻¹ soil as (NH₄)₂SO₄. It was concluded that: 1) increasing N content in leaves alone was not able to promote gain in biomass production of maté seedlings; 2) seedlings receiving N-NH₄ showed a higher accumulation of P and Mg on shoot biomass; and 3) an increase in leaf area, leaf number and net photosynthesis observed at the N-NH₄ treatment was coincident with an increasing absorption of P and Mg.

Key words: Ammonium, nitrate, soil pH

INTRODUCTION

Maté (*Ilex paraguariensis*, St. Hil.) is a native tree from South Brazil, Paraguay and Argentina (Hoppe et al., 1996). It is grown mainly by small farmers which gives it a great social-economical importance. It naturally occurs on soils with low levels of nutrients and high level of Al³⁺, generally with a medium to clay texture (Oliveira and Rotta, 1985) and do not grow well in shallow soils (Dedecek, 1997). Nitrogen is consumed in high amounts during maté harvesting (Reissmann et al., 1985; Wisniewski et al., 1996), reaching up to the equivalent to 500 kg Urea.ha⁻¹.year⁻¹ (Lourenço, 1997). Therefore the replacement of N seems to be essential for maté sustainability.

Usually plants are able to take up N as nitrate (NO₃⁻) and ammonium (NH₄⁺), but some may prefer one source or another depending on plant

species (Marschner, 1995). N sources may affect plant growth via many processes within the soilplant system, and inside the plant (Wiesler, 1997). The use of different N sources may directly affect the nutrition status of plants due to changes in the rhizosphere, as a result of modification in the ionic balance in that soil fraction. The use of N-NH₄ causes an increasing H⁺ excretion, leading to a decrease in soil pH, whereas the use of N-NO₃ is associated with a decrease in H+ excretion and increasing rates of HCO₃ or OH resulting in a pH rise, especially when in combination with Ca²⁺ (Marschner and Römheld, 1996). However, when plants growing in a similar way are supplied with N-NO₃ or N-NH₄, they may differ in many aspects related to metabolic activity and ionic composition (Kandlbinder et al., 1997), as a result of different physiological responses.

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The reduction of N-NO₃ in plants consume great amount of reducing power. Considering the biochemical energy needed for NO₃ reduction, one could suppose that plant supplied with N-NH₄ would grow better than that supplied with N-NO₃ source, however in most cases the opposite occurs even altering the plant architecture of fast growing plants (Beltrano et al., 1999).

Some species show growth depression when supplied exclusively with N-NH₄, but generally this causes a decrease in soil pH which may increase the availability of some mineral nutrients such as P, Fe, Mn, Cu, and Zn (Marschner, 1995), improving the soil/plant relationship depending on soil conditions. N transfer from roots to leaves can occur in different manners (Pate, 1973) and its incorporation in different amino acids, proteins, and other N compounds are differentiated for each plant species and development stage. The limit for N-NO₃ and N-NH₄ absorption and assimilation can be carbon metabolism in the first place (depending on radiation and photosynthesis) for the carbon skeleton formation of amino acids, as well as N metabolites by itself working on the assimilation repression (Imsande and Touraine, 1994). On the other hand, using the Farquhar et al. (1980) equations, considering temperature and CO₂ concentration constant, the photosynthesis can be limited by radiation and N content (Paul and Foyer, 2001).

In the construction of a dynamic allocation model in the plant, Soussana et al. (2000) used the hypothesis of a co-limitation of growth by light and mineral N. They considered that these two resources, independently, would be used in the construction of structural compartments (shoot structural components, roots components, and leaf proteins), and among them it would be established a relationship of coordination of N absorption and photosynthesis. The aim of this research was to evaluate biomass production, nutrient content, net photosynthesis and morphogenesis in maté seedlings in response to different N sources.

MATERIAL AND METHODS

Seedling preparation and treatments

Maté seedlings (four months old) were selected according to their phenotypic characteristics (size, number of leaves, and general aspect). The root system was reduced to about 50% and the seedlings were bare root transplanted to 1.3 kg

plastic bags. The soil used as substrate was an Oxisol with: pH(CaCl₂)=4,00; K⁺=0,09 cmol_c.dm⁻³; Ca²⁺=0,97 cmol_c.dm⁻³; Ca+Mg=2,31 cmol_c.dm⁻³; Al³⁺=2,38 cmol_c.dm⁻³; H+Al=8,50 cmol_c.dm⁻³; MO=24,99 g.kg⁻¹; P=1,00 mg. kg⁻¹; and Na⁺=7,00 mg.kg⁻¹. The experiment was conducted from September to December 2002, in greenhouse condition at the National Centre for Forestry Research (*Embrapa Forestry*).

Seedlings were allowed to grow with no treatment at all for 35 days from transplanting until completely established during September/October, 2002. An equilibrium nutrient solution containing 10 mg N.kg⁻¹ soil as ammonium nitrate (NH₄NO₃), 60 mg P₂O₅.kg⁻¹ soil and 40 mg K₂O.kg⁻¹ soil as potassium phosphate (KH₂PO₄) was applied in all seedlings aiming to balance the nutrient availability for all treatments. Seedlings were then allowed to grow for 15 days. After that period, four treatments were set up: Control = no extra N applied; Urea = 100 mg N. kg⁻¹ soil supplied with Urea source; N-NO₃ = 100 mg N.kg⁻¹ soil supplied with Ca(NO₃)₂; and N-NH₄= 100 mg N.kg⁻¹ soil supplied with (NH₄)₂SO₄. Seedlings were watered daily.

Net photosynthesis and morphological aspects

Eight seedlings per treatment were selected for net photosynthesis measurements and morphological characteristics determination. Selected seedlings presented 8-9 leaves and similar height, size of leaves, and general aspect. The following morphological characteristics were evaluated: seedling height (cm), internodes length (cm) leaves number, length and width and-physiological state of each leaf (mature or new). Those parameters were evaluated three times: a) one day before the treatments were applied (start); b) 10 days after treatments were applied; c) 25 days after treatments were applied. Leaf length and width were used to calculate the leaf area (LA) per plant (cm².plant-¹).

The net photosynthesis (Pn) or rate of carbon dioxide exchange (µmol CO₂.m⁻².s⁻¹) was measured using a portable analysis system (model LI-6200, LICOR, Inc. Lincoln, NE, USA). The first evaluation of photosynthesis and morphology was made before fertilization application. The leaves were marked in order to use the same ones in all evaluations. In the first evaluation, only the fully expanded leaves were marked (considered as "mature"), and after 10 days, additionally, new emitted leaves still in expansion ("young") were

also marked. The measurements of Pn were taken outside the greenhouse. The radiation microclimatic conditions were evaluated on a basis of sequential measurements obtained by LI-190AS radiation sensor, (measure photosynthetically active radiation - PPFD on the 400-700nm waveband in µmol.m⁻².s⁻¹), LI-200AS Pyranometer sensor, (measures solar irradiation in W.m⁻².s⁻¹), and by SKY 110 (660/730) sensor measures red: far-red ratio. Sensors were positioned on maté seedlings level during 9 days in greenhouse. Values were registered as means calculated for periods of 15 minutes.

Biomass and chemical composition

The first evaluation of biomass parameters was made at the same time as the 10 days observation for photosynthesis and morphological parameters. For this purpose, six plants were harvested per block and seedlings were divided in leaves, stem and roots. This material was oven dried (60°C) until constant weight and data were recorded. The shoot analysis was performed using Micro Kjeldal Sulfuric Acid Digestion for Nitrogen and Nitro-Perchloric Acid Digestion for P, K, Ca, Mg, Cu, Fe, Mn and Zn. The determination of the elements

was made using distillation and titillation for N, colorimetry for P, flame photometry for K and atomic absorption spectrometry – flame for Ca, Mg, Cu, Fe, Mn and Zn.

Experimental design and Statistical analysis

Treatments were distributed in randomized blocks in three replicates and twelve seedlings per plot in a total of 36 seedlings per treatment. Data were statistically analyzed using the Tukey test on a confidence interval of 10% of probability.

RESULTS

Average diurnal value of irradiation in plant level, for the experimentation period in glasshouse was 7.7 MJ.day⁻¹. The PPFD reaching leaves of maté reduced on maximum value of de 800μmol.m⁻².s⁻¹, in midday period (Fig. 1A) compared with values which arrive on superior canopy level in nature (to 2200μmol.m⁻².s⁻¹), on the same period of the day, year, and on the same region. The red: far-red ratio (R:FR) was not affected in the greenhouse as compared to open areas (Fig. 1B).

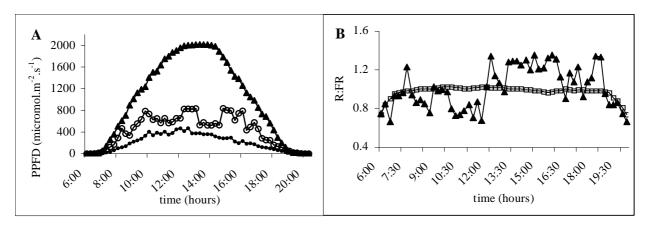


Figure 1 - (A) PPFD on plant level registered during 9-day-period (average curve), comparing the sunny (▲) and cloudy (○) days outside the glasshouse and average inside of glasshouse (●). (B) Average red: far-red ratio (R: FR) on plant level outside (▲) and inside (□) of glasshouse.

Maté seedlings showed different responses to distinct N sources (Figs. 2 to 6). Seedlings that received N-NH₄ and N-NO₃ presented a higher number of leaves after 25 days as compared to Control and Urea treatments (Fig. 2). The number of leaves was directly related to lateral branching. In the last harvest, 25 days after treatment were applied, from the eight seedlings selected to net

photosynthesis evaluation five presented lateral branching in N-NH₄, one in N-NO₃ and none in urea and control treatments.

The increase in leaf area (LA, cm².plant⁻¹) also differed depending on the N source used (Fig. 3). Seedlings exhibited a phenotypic homogeneity prior to N treatments application not showing difference among treatments at the start point of

the experiment. In the first evaluation, 10 days after treatments application, LA of plants fertilized with urea was higher than seedlings that received the sources N-NH₄ and N-NO₃ but did not differ from the control. A significant increase in LA was detected in all treatments, especially in the N-NH₄ and in the N-NO₃, between the 10th and 25th day. At that time, there was no difference among treatments, however, the inclination of the lines representing the treatments, showed steeper angles in the treatments N-NO₃ and N-NH₄ indicating a tendency of higher increase in LA in those treatments than in urea or control (Fig. 3).

The emission of ramifications, total number of leaves and LA affected the increase in shoot biomass (Fig. 4A) and the increment of both total and shoot biomass of seedlings (Fig. 4B) from the 10^{th} to the 25^{th} day after treatments application.

Distinct responses according to N source were noticed in differences on absorption capacity of nutrients by maté seedlings (Table 1). There was also an accumulation of N in the treatments that received any N source in relation to control. The N-NH₄ source showed a higher absorption of P and Mg as compared to control and N-NO₃ treatments (Table 1).

The use of different N sources was responsible for a higher biomass production per plant (Fig. 4) and higher N content in shoots (Fig. 5) as compared to control treatments It was observed yet a higher biomass increase between 10th and 25th day of N application (Fig. 4B) on urea and N-NH₄ treatments in relation to the N-NO₃ source, but no difference between N-sources in shoot N content.

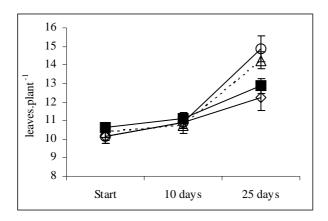


Figure 2 - Number of leaves in maté seedlings in response to N source and time after treatments (♦control; ■ urea; △nitrate; ○ ammonium).

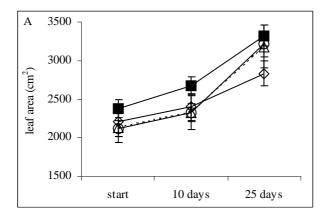


Figure 3 - Leaf area (cm².plant⁻¹) as a response to N source (♦ control, ■ urea, △ nitrate, O ammonium)

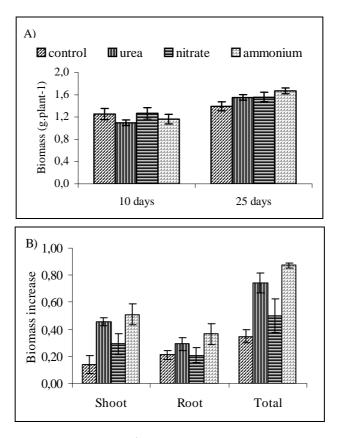


Figure 4 - (A) Shoot biomass (g. plant⁻¹) of maté seedlings at the two harvests and (B) Biomass increase (g. plant⁻¹) between the two harvests in shoot, root and total biomass according to N source.

Table 1 - Nutrient accumulation (mg.plot⁻¹) in the shoot biomass of mate seedlings between the 10th and the 25th day after treatment application

Nutrient	Control	Urea	Nitrate	Ammonium
N ^a	7.36b	56.39a	58.61a	71.51a
P	-1.08b	-0.09ab	-0.66b	0.71a
K	-1.05b	17.06a	11.95ab	21.58a
Ca	2.83b	6.54b	21.78a	7.01b
Mg	2.95b	6.89ab	3.64b	8.30a
Cu	0008b	0.0200a	0.0037ab	0.0100ab
Fe	0.1563a	0.3320a	0.1383a	0.4079a
Mn	-0.0637a	1.4060a	0.1728a	0.9682a
Zn	-0.0845a	0.1336a	0.0200a	0.1204a

^a-values followed by the same letter, in the line, do not differ at 10% of probability according to Tukey Test.

Pn was very similar among treatments at the first measurement however there were strong changes with time (Fig. 6). Seedlings from all N treatments had an increase in Pn after fertilization in both, old and new leaves which confirmed the results on the total N content. Maté seedlings fertilized with N- NH_4 source presented higher Pn in both, old and new leaves (Fig. 6). Pn was similar in new leaves and in old leaves at the N-NH $_4$ source on the 25^{th} day.

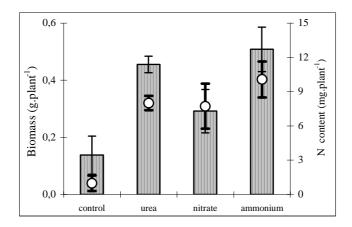


Figure 5 - Effect of N source on shoot biomass (column) and N content in shoot (circles)

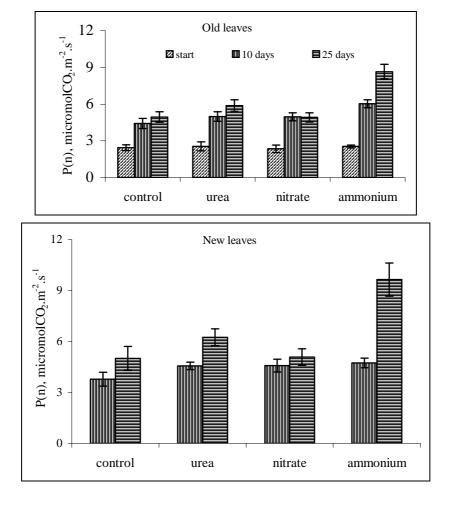


Figure 6 - Effect of N source on net photosynthesis (Pn) in old and new leaves of maté according to time after treatment application.

DISCUSSION

The radiation level inside the greenhouse was satisfactory to maté seedlings development of Pn and stomata according to results conductance from field (Rakocevic, unpublished data) where the Photosynthetic Photon Flux Density(PPFD) levels higher than 800µmol.m-².s⁻¹ were able to inhibit photosynthesis in leaves at the canopy border in pruned maté trees. Considering PPFD relatively low during the experiment the radiation would not interfere on the growth of maté seedlings. Measurements were made during the period of highest annual response to Pn of this species, which occurs from October to February (Galvão, 1986), permitting the distinction on assimilatory responses of young and mature leaves.

Low ratios R:FR may promote distinct photomorphogenetics reactions (Aphalo et al., 1999), depending on plant species (Varlet-Grancher et al., 1993). Maté tolerate shadow at any time of its cycle being able to accept higher light intensity at the adult stage (Carvalho, 1994), moreover the red : far red ratio (R:FR) was not modified in greenhouse.

The use of different N sources affected growth of maté seedlings in different ways. Plants fertilized with the N-NH₄ source presented higher number of leaves and increased branching. Plants fertilized exclusively with N-NH₄ show higher sensibility to high irradiation than plants submitted to low irradiation (Zornoza et al., 1987). Leaf area per plant (LA) receiving Urea as N source was higher than those receiving the sources N-NO₃ and N-NH₄, ten days after fertilization (Fig. 3). However, there was no difference among treatments 25 days after fertilization. Some studies showed a decrease in LA in plants nourished with N-NH4 (Lips et al., 1990; Raab and Terry, 1994), although this did not mean necessarily a decease in Pn per unit of area (Takács and Técsi, 1992).

The highest total biomass was recorded at N-NH₄ and Urea treatments (Fig. 4B). The increase in number of new leaves from the application of N sources did not interfere on biomass increase for the N-NO₃ treatment, whereas urea plants which did not present an increase in number of leaves had a significant increase in biomass despite having a similar number of leaves to the Control treatment (Fig. 3A).

Plants that received any N source had a higher accumulation of N in shoot biomass than control,

especially those grown on N-NH₄ source. Considering the lowest increase of shoot biomass in N-NO₃ plants comparing to other N treatments (Fig. 4), no difference in N shoot content comparing to other N treatments (Fig. 5), and the lower Pn rates in the N-NO₃ comparing to N-NH₄ plants (Fig. 6), it was reasonable to hypothesize that the N-NO₃ plants invested more on structural shoot proteins production than in protein acting on photosynthesis. The photosynthetic capacity in species were highly correlated to N concentration on leaves due to the larger fraction of leaf N photosynthetic allocated at the (Hikosaka and Hirose, 2000). N deficit necessarily promote a remobilization of N from Rubisco (Paul and Foyer, 2001). Therefore, decreasing the leaf biomass, there was a decrease in leaf protein content which lead to a decrease in net photosynthesis due to nitrogen (Soussana et al., 2000). The net photosynthesis was favored by N-NH₄ application compared to N-NO₃ source and control (Fig. 6). The young leaves emitted after the N-treatments reached high gas exchange rates even before their full expansion, and the mature leaves increased the net photosynthetic rate with time.

Claussen and Lenz (1999) highlighted that net photosynthesis may increase or decrease when plants are nourished with N-NH₄ source depending on plant species, and on the pH of the nutrient solution. *Vaccinium corymbosum*, from acidic soil, did not respond positively to liming, showing that plants from acidic soils with high levels of organic matter increased the net assimilation as much as the chlorophyll content on the application of N-NH₄. Maté grew even in soils with pH (CaCl₂) up to 3.4 (Lourenço, 1997), and also did not respond positively to liming (Reissmann et al., 1989, 1991, 1997; Pintro et al., 1998). Therefore, when determining the fertilization form one should always analyze the origin of species.

Most plant species, which react positively to N-NH₄ as compared to N-NO₃, increase Pn, diminish LA, and invest more on root biomass production, but do not affect the total biomass production (Wiesler, 1997). Maté strategy to N-NH₄ comparing with N-NO₃ was an increase in Pn (Figs. 6A and 6B) with no effect on LA (Fig. 3), investing relatively more in shoot biomass production (Fig. 4B) and with no effect on final biomass production (Fig. 4A). The lower N photosynthetic efficacy of N-NO₃ plants were probably related to their N investment focalized

more in structural shoot proteins production rather than in photosynthetic active proteins.

Growth and net photosynthesis of maté seedlings were higher in plants nourished with N-NH₄ comparing to N-NO₃, and there was no difference to urea treatment, demonstrating relation to changes in pH of the rhizosphere soil and correlated parameters (Marshner, 1995). Maté seedlings showed a higher LA as well as net photosynthesis in plants that received the N-NH₄ source differing from some other species from acidic soils that usually showed an increase in net photosynthesis per unit area but presented a diminishing LA when plants were nourished with N-NH₄. The increased absorption of P and Mg (Table 1) reflected directly on the net photosynthesis observed in this treatment. New experiments on field conditions should be crossed out considering different development stages of plants, soil types and climate conditions aiming to confirm the preference of N-NH₄ source over the others as shown in this experiment.

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RESUMO

A influência de diferentes fontes de N sobre o crescimento de mudas de erva-mate paraguariensis St.Hil.) foi estudada, em casa de vegetação. Todas as mudas receberam uma fertilização base de 10 mg N.kg⁻¹ de solo na forma de NH₄NO₃, 60 mg P₂O₅.kg⁻¹ e 40 mg K₂O.kg⁻¹ de solo na forma de KH₂PO₄ quinze dias antes da aplicação dos tratamentos. Os tratamentos foram os seguintes: Controle, sem adição extra de N; Uréia = 100 mg N.kg⁻¹ de solo como Uréia; NO₃⁻ = 100 mg N.kg⁻¹ de solo como Ca(NO₃)₂; e NH₄⁺ = 100 mg N.kg⁻¹ de solo como (NH₄)₂SO₄. Concluiuse que: 1) o aumento do conteúdo de N nas folhas, por si, não é capaz de promover ganhos na produção de biomassa em mudas de erva-mate; 2) mudas que receberam N-NH₄ apresentaram maior acumulo de P e Mg na biomassa aérea; e 3) o aumento na absorção de P e Mg coincidiu com um

aumento na área foliar, no número de folhas e na fotossíntese liquida na fonte N-NH₄.

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