Soil nitrification as affected by Brachiarias

Adalton M. Fernandes, Emerson F.C. Souza, Gabriel J. M. Andrade, Marcelo J. Gimenes and **Ciro A. Rosolem**

College of Agricultural Sciences, São Paulo State University, Botucatu, SP, Brazil E mail rosolem@fca.unesp.br

Abstract
The nitrification process in soil may result in substantial losses of applied nitrogen through nitrate leaching and N_2O emission to the atmosphere. Nitrification control could be a key strategy in improving nitrogen recovery and agronomic N-use efficiency. This study aimed to evaluate the ability of species of *Brachiaria* in inhibiting nitrification process in the soil. The treatments consisted of growing three pasture species (*Brachiaria brizantha*, *Brachiaria ruziziensis* and *Brachiaria decumbens*) fertilized with doses of nitrogen (0, 100, 200 and 300 mg/pot) plus a control (without plants). The experiment was carried out in a greenhouse in pots containing 10/dm^3 of soil. In the absence of the brachiarias, soil NO_3^- increased with N rates. *B. ruziziensis,* when grown with 60 kg/ha of N, resulted in the lower soil nitrate concentration (an evidence of nitrification inhibition), but *B. decumbens* and *B. brizantha* had no effect on the nitrification process.

Introduction
The low use efficiency of nitrogen fertilizers and the potential pollution that comes from their losses are among the main concerns of agriculture. In soil, ammonium from fertilizers can be transformed in nitrate by nitrifying bacteria (Sousa and Lobato 2004). The nitrification of ammonium to nitrate actually is the transformation of a relatively immobile cation in a highly mobile anion that can result in losses by denitrification and/or leaching of NO_3^- (Subbarao *et al*. 2006). Thus, inhibition of nitrification may be a strategy to extend the time of residence of N in soil and improve the N use efficiency (Rodgers 1986). There is evidence of the occurrence of natural suppression of nitrification in some ecosystems, depending on the root exudation of some species (Subbarao *et al*. 2006). Tropical grasses, such as *Brachiaria humidicola,* release Braquialactona by the roots, which can reduce or even suppress nitrification in the rhizosphere soil (Subarao *et al*. 2009). In tropical environments, grasses of the genus Brachiaria are used as cover crops in no-tillage systems, so the objective of this study was to evaluate whether other species of Brachiaria, besides *B. humidicola,* used as cover crops in tropical agricultural systems, have the ability to inhibit nitrification in soil.

Material and Methods
The soil used in this experiment was collected from the surface layer (0-20 cm) of a typical Oxisol (EMBRAPA 1999), a sandy loam with pH 4.1, 18 g/dm^{3}of OM, 3.0 mg/dm^3 of P_{resin}, 0.2 , 2.0, 1.0 mmol$_c$/dm^3 of K, Ca and Mg, respectively, 6.79 and 7.49 mg/kg of NH_4^+ and NO_3^-, respectively. Lime was applied to raise pH to 5.3 and P and K were applied at 150 mg/dm^3, as superphosphate and potassium chloride. The experimental design was a randomized block with four replications in a factorial 4x4. The treatments were three grass species: *Brachiaria brizantha*, *Brachiaria decumbens* and *Brachiaria ruziziensis*, four N rates: 0, 100, 200 and 300 mg/pot, and a control without plants). At 14 days after planting, N rates of 0, 100, 200 and 300 mg/pot were applied as ammonium sulfate ($(NH_4)_2SO_4$). Forty days after nitrogen application plants shoots were cut close to the soil, washed, dried in a forced air oven for four days at 65 ^oC, ground and N (Bremner and Keeney 1966) was determined. After harvesting the plants, the soil of each pot was sampled and the rhizosphere soil was separated from bulk soil by gently shaking the root system, then the samples were dried and NH_4^+ and NO_3^- were determined by steam distillation (Bremner and Keeney 1966). The results were submitted to ANOVA and means were compared by t test (LSD, $P< 0.05$). Nitrogen effects were evaluated using regression analysis.

Results and Discussion
The increased availability of N resulted in a linear increase in dry matter yield *of B. decumbens* while in *B. brizantha* and *B. ruziziensis* dry matter yields showed quadratic responses, with maximums calculated at 248 and 297 mg/pot of N, respectively. In the absence of fertilizer, *B. decumbens* showed higher accumulation of N as compared with other brachiarias, due to its larger growth (Figure 1). However, with the supply of 200 and 300 mg/pot of N, *B. brizantha* and *B. decumbens* showed similar N uptake, and higher than that observed for *B. ruziziensis*. This shows that when N availability is a limiting factor, *B. ruziziensis* has a higher

efficiency of nutrient use. Applying N as ammonium led to small increases in the soil NH_4^+ level in the treatment without plants, and this treatment showed the lowest levels of NH_4^+ and higher concentrations of NO_3^- (Figure 2), showing that the activity of nitrifying bacteria was much higher without plants or the plants took up most of the nitrate transformed during plant growth.

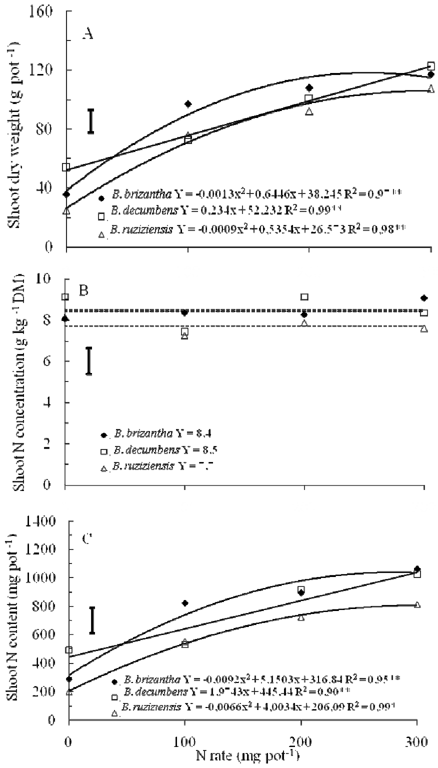

Figure 1. Shoot dry weight (A), N concentration (B) and N content (C) of pasture grasses as affected by N rates, 40 days after the application of nitrogen rate. * and ** significant at 5% and 1% probability by F test. Vertical bars indicate the value of the LSD (P < 0.05).

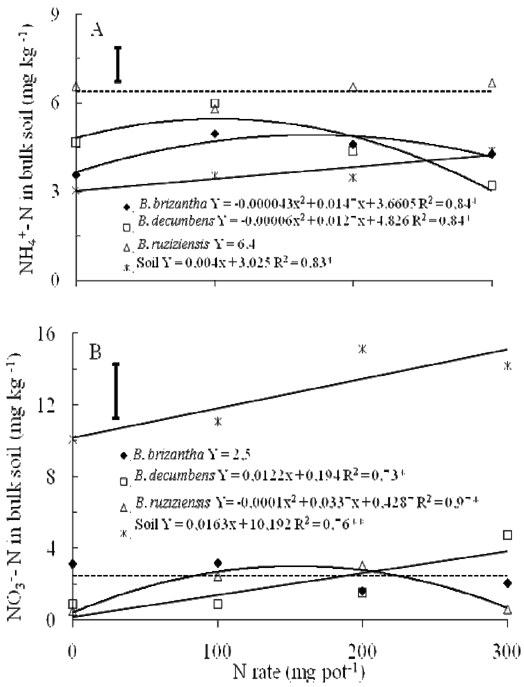

Figure 2. N - NH_4^+ (A) and N - NO_3^- (B) concentration in bulk soil of pasture grasses as affected by N rates, 40 days after the application of nitrogen rate. * and ** significant at 5% and 1% probability by F test. Vertical bars indicate the value of the LSD (P < 0.05).

Carmo *et al*. (2005), studying the N dynamics in soils with pastures and forests observed that with a mixture of *B. brizantha* and *P. maximum* soil N-NH_4^+ content was always greater than that of N-NO_3^-, 4.60 and 2.41 mg/kg, respectively, whereas in forest soil, the levels were similar for both nitrogen forms (4.83 for N-NH_4^+ and 4.21 mg/kg for N-NO_3^-). In the present experiment, the amount of NH_4^+ absorbed and/or nitrate content in the soil cropped to *B. ruziziensis* was lower than that obtained for the other forages, except for the treatment with 100 mg/pot of N (Figure 2), which is explained by the higher content of soil NH_4^+ at the end of the experiment in pots with this forage.

It was also observed that nitrification increased with N application, but there was no difference between the species as to N uptake (Figure 1c). In general, except for *B. ruziziensis*, soil NH_4^+ contents were lower than those observed in the rhizosphere (Figure 3). However, there were no differences between the NH_4^+ contents in the rhizosphere of the different species (Figure 3A). The lowest rhizosphere NO_3^- levels were observed under *B. brizantha* (Figure 3B). It can be inferred that NO_3^- uptake by this species created a gradient of concentration of N near the root surface, resulting in lower NO_3^- content in the rhizosphere. A second factor may have contributed to this low NO_3^- level, i.e., the reduction may be due an inhibitory effect of this species on biological nitrification. The results show that *B. decumbens* and *B. ruziziensis* do not inhibit the nitrification in the rhizosphere. Authors such as Sylvester-Bradley *et al*. (1988) and Ishikawa *et al*. (2003) also observed that *B. decumbens* do not inhibit nitrification as it has been reported for *B. humidicola*. Ishikawa *et al*. (2003) found that *B. humidicola* suppresses nitrification. They also observed that the suppression effect over the population of bacteria oxidizing ammonia lasted 12 days after the collection of plants, suggesting that the inhibition effect is long-lasting in the presence of plants in soil. The hypothesis of rhizosphere nitrification inhibition in this study is supported by the fact that no differences were found between species on the amount of NH_4^+ absorbed and/or nitrified, on the amount of NO_3^- absorbed and between the total amounts of inorganic N absorbed per pot. Moreover, the relationship NH_4^+/NO_3^- in the *B. brizantha* rhizosphere was greater than 15/1, while for *B. decumbens* and *B. ruziziensis* it was, respectively 3/1 and 1/1. This is evidence that there was at least some inhibitory effect of *B. brizantha* in the nitrification process, however, the effect was small and did not reflect on NH_4^+ and NO_3^- amounts in the bulk soil. In treatments that did not receive nitrogen fertilizer, 82%, 73% and 64% of total N absorbed by the species *B. decumbens*, *B. brizantha* and *B. ruziziensis* respectively, came from organic matter mineralization. When the higher N rate was applied, organic matter mineralization provided about 64% of the N absorbed by *B. brizantha* and *B. decumbents,* and 54% of the *B. ruziziensis* uptake. Subarao *et al*. (2007) observed that most of the soil inorganic N at the end of a 30-day incubation originated from the mineralization of organic N, which probably also occurred in this experiment.

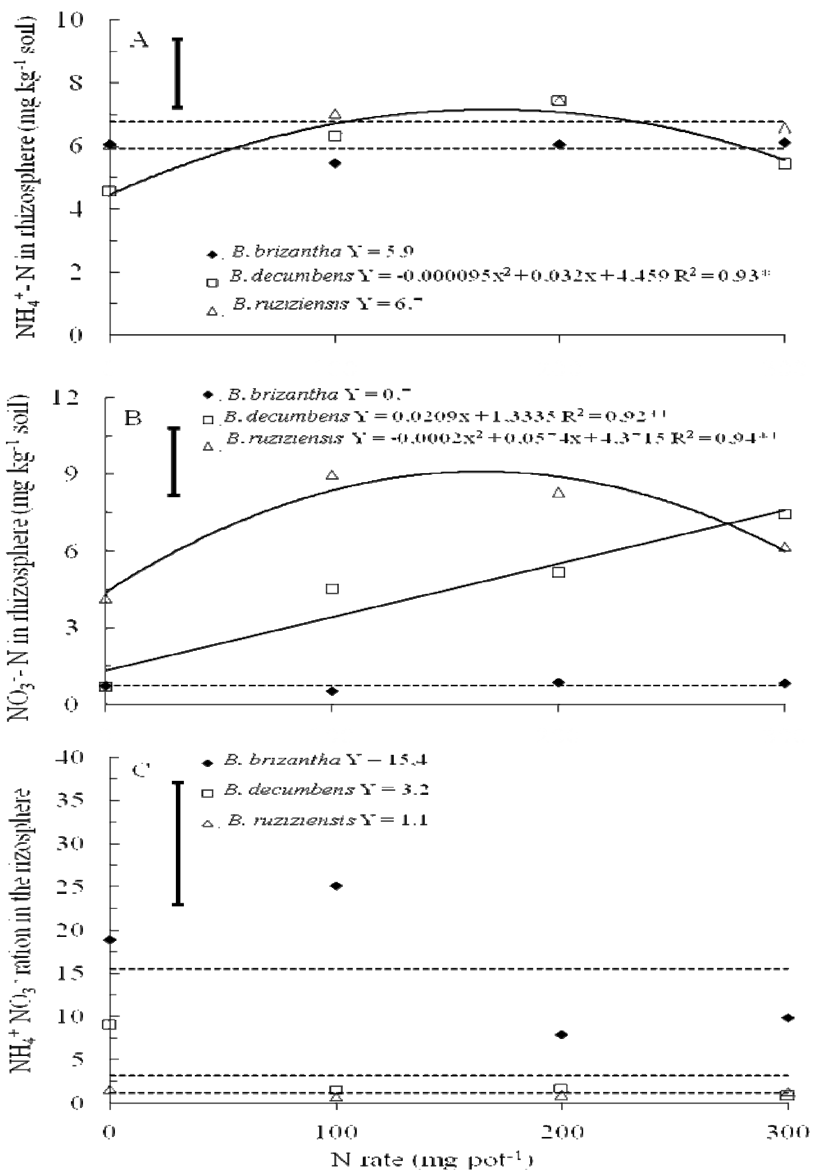

Figure 3. N-NH_4^+ (A), N-NO_3^- (B) concentration and ratio NH_4^+/NO_3^- (C) in rhizosphere of pasture grasses as affected by N rates, 40 days after fertilization. * and ** significant at 5% and 1% probability by F test. Vertical bars indicate the value of the LSD (P < 0.05).

Conclusion

In this experiment, the mineralization of organic matter supplied much of the N taken up by the forage plants. *B. decumbens* and *B. ruziziensis* do not inhibit the nitrification in the rhizosphere, and the effect of *B. brizantha* on rhizosphere nitrification was not big enough to change NH_4^+ contents in bulk soil.

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