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# Stable coexistence of contrasted nitrification statuses in a wet tropical savanna ecosystem

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**Summary**

**1.** Wet tropical savannas are characterized by strong environmental constraints—particularly low soil nutrient availability—associated with high plant productivity. Nitrogen recycling, and especially nitrification, is supposed to be a strong determinant of the balance between conservation and loss of nutrients at the ecosystem level. Savanna facies dominated by the grass *Hyparrhenia diplandra* (Andropogoneae) are known to exhibit low levels of nitrification and thus avoid nitrate losses.

**2.** By comparing two sites in the Lamto area (Côte d'Ivoire, West Africa) with similar soil physico-chemical characteristics and equally dominated by *H. diplandra* (80% of the grass cover), it was demonstrated that, within this facies, nitrification is highly heterogeneous, with a 240-fold variation in potential nitrification within a specific site.

**3.** In order to test whether these differences can be considered as permanent in this ecosystem, nitrate reductase activities were compared on *H. diplandra* plantlets from the two sites, cultivated under identical conditions in the presence of nitrate. The leaves of plants originating from the high nitrification site were always able to reduce nitrate at a significantly higher rate than those from the low nitrification site. This observation indicates a long-term adaptation of the plants and stable nitrification behaviour.

**4.** Lamto can thus be considered as a contrasted dual ecosystem relative to its nitrogen cycle. The two sites studied therefore constitute useful models to assess the determinism of nitrification in wet savannas and the role of this process on nitrogen retention in such ecosystems.

*Key-words:* Andropogoneae, *Hyparrhenia diplandra*, Lamto, nitrate reductase activity, soil nitrogen cycle

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## Introduction

Nitrification, the bacterial oxidation of ammonium into nitrate, is known to be highly variable in time and space in various soils, including tropical soils (Lodhi 1977; Schimel & Parton 1986; Wedin & Tilman 1990; Lensi *et al*. 1991; Zak & Grigal 1991; Ross, Luizão & Luizão 1992; Davidson & Hackler 1994; De Boer, Klein Gunnewiek & Parkinson 1996). Lack of nitrification is generally linked to low nutrient availability and is supposed to improve nitrogen conservation by suppressing denitrification and limiting mineral nitrogen leaching (Huber *et al*. 1977; Jordan, Todd & Escalante 1979; Vitousek *et al*. 1979; Keeney 1986; Robertson 1989). Wet tropical savanna soils are typical examples of ecosystems characterized by low nutrient availability and low nitrification capacities. Paradoxically, some of them can support high plant productivity, which suggests efficient and conservative nutrient dynamics (Hopkins 1966; Egunjobi 1974; Menaut & César 1979; Abbadie, Mariotti & Menaut 1992).

The Lamto area, Côte d'Ivoire, West Africa, is representative of wet savanna ecosystems where nitrogen has been shown to be the major factor limiting primary production (Bate 1981). Previous studies have suggested that the potential of nitrification in this area was related to the specific composition of the plant cover (Lensi, Domenach & Abbadie 1992). In Lamto, the grass cover is generally dominated by two perennial tufted Graminaceae (Poaceae) species, either *Loudetia simplex* or *Hyparrhenia diplandra* (Menaut & César 1979). The soils from the sites dominated by *L. simplex* exhibit a substantial potential of nitrification while those dominated by *H. diplandra* generally exhibit extremely low potential of nitrification (Lensi, Domenach & Abbadie 1992). Within the *H. diplandra*-dominated sites, the potential of nitrification was even significantly lower under than between the *H. diplandra* tussocks. These results may be compared with previous data from Munro (1966), Meiklejohn (1962, 1968) and Rice & Pancholy (1973)

showing a depressive effect of many grasses on the activity of nitrifiers, especially those belonging to the Andropogoneae group. Recently, the general character of this depressive effect was questioned by Le Roux *et al*. (1995) who fortuitously discovered a site dominated by *H. diplandra* exhibiting high rates of nitrate accumulation in soil.

The aim of the present work was to answer the following questions: (1) does the potential of nitrification significantly differ between the site reported by Le Roux *et al*. (1995) and a standard area where nitrification rate is known to be extremely low; (2) are these differences stable over time? To answer the first question, the average and variance of the potential of nitrification were compared between the two model sites. A potential nitrification assay was used in order to evaluate the long-term ability of microorganisms to oxidize ammonium. It was assumed that the potential activity is not affected by short-term environmental variations. To answer the second question, two sets of *H. diplandra* grown from seeds sampled in the two sites were cultivated in greenhouse under identical conditions (in particular with non-limiting nitrate supplies). The potential nitrate reductase activity (NRA) was measured in roots and leaves hypothesizing that a difference of plant ability to reduce nitrate should result from a permanent difference in nitrate availability between the soils from the two sites.

## Materials and methods

### SITE DESCRIPTION

Lamto is located in Côte d'Ivoire at latitude 6 ° 13 'N and longitude 5 ° 20 'W, at the southern limit of the 'V Baoulé', a broad grass expanse of savannas which spreads southwards far into the rain forest. The vegetation varies with the topography and five facies, from downslope to upslope, can be distinguished by tree density and the main grass species (Devineau 1976; Menaut & César 1979): a riparian forest along the Bandama river; gallery forests in the thalwegs; herbaceous savannas, without trees other than the palm *Borassus aethiopum*, dominated by the grasses *Loudetia simplex* and *Andropogon schirensis*; shrub savannas dominated by the grasses *Hyparrhenia diplandra* and *Hyparrhenia smithiana*; and dry semi-deciduous forests on the plateaux.

Temperatures are constant throughout the year (average 27 °C). Rainfall is variable and four seasons can be distinguished: the long dry season from December to February, the long wet season from March to July, the short dry season in August and the short wet season from September to November. Precipitation averages 1200 mm (statistics from Geophysical Station of Lamto). Granites and derived sands have produced tropical ferrugineous soils with a superficial gravely horizon with pH values around 6.

All the experiments were conducted during the long wet season (March–April 1995) in the shrub savannas, the dominant biotope covering almost 55% of the reserve (Gautier 1990).

### SOILS STUDIED

Soils were sampled in two savanna sites (5 km apart, more than 2·5 ha each), both dominated by *H. diplandra* (80% of the plant cover, no significant difference at $P < 0{,}05$, $n = 8$, Student's Test): the reference site A, where the potential of nitrification has been shown to be low in previous studies (Abbadie, Mariotti & Menaut 1992; Lensi, Domenach & Abbadie 1992; Le Roux *et al*. 1995) and site B, where a high potential of nitrification has been reported (Le Roux *et al*. 1995). At each site, 60 soil samples were randomly collected between the tussocks of *H. diplandra* from the uppermost layer (0–10 cm depth). The samples were immediately air-dried in the shade, homogenized, sieved (2 mm) and stored until required for the experiments.

### PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS

Soils were analysed for several physico-chemical characteristics: (1) size-class distribution of soil particles was estimated according to Feller *et al*. (1991); (2) pH was measured following the pH–KCl technique; (3) total organic C and N were determined, after crumbling up soils into a very fine powder, with an elemental analyser (NA 1500 Series 2, Fisons); results were expressed as ‰ C or N of dry soil mass; (4) bulk density of the zero to 5 cm soil layer was measured at five locations in each site using 171 $cm^3$ steel cylinders. All measurements on single samples were repeated at least three times.

### ENZYMATIC POTENTIAL OF NITRIFICATION ASSAYS

The enzymatic potential of nitrification was measured according to Lensi *et al*. (1986): the $NO_3^-$ produced during an aerobic incubation was deduced from $N_2O$ measurements after an anaerobic incubation in the presence of $C_2H_2$.

From each soil sample, 15 g subsamples ($n = 6$) were placed in 150 $cm^3$ plasma flasks. Three subsamples were used to estimate the initial $NO_3^-$ content: they were supplied with 6 $cm^3$ of a suspension of a denitrifying organism (*Pseudomonas fluorescens*, $O.D._{\cdot 580} = 2$) in a solution containing glucose and glutamic acid (final soil C content for each compound: 0·5 mg C $g^{-1}$ dry soil). This procedure ensures high denitrifying potential and electron donors in excess. The flasks were sealed with rubber stoppers and the atmosphere of each flask was replaced by an He–$C_2H_2$ mixture (90–10) to ensure anaerobic conditions and $N_2O$-reductase inhibition. The $N_2O$ accumulation was followed until a constant value (i.e. a total conversion of soil $NO_3^-$ content into $N_2O$) was reached.

The three other subsamples were used to determine the kinetics of $NO_3^-$ accumulation: they were enriched with 2 $cm^3$ of a $(NH_4)_2SO_4$ solution (final soil N content: 0·2 mg $g^{-1}$ dry soil) in order to ensure a moisture content equivalent to 80% W.H.C. and no limitation by ammonium (presence of $NH_4^+$ was also supposed to limit $NO_3^-$ assimilation by micro-organisms). Then, the flasks were sealed with Parafilm © (which prevents soil from drying but allows gas exchange) and incubated at 27 °C for 48 h in a horizontal position to ensure optimal and homogeneous aeration of the soil. After this aerobic incubation which allows nitrate to accumulate, the soil samples were enriched with 4 $cm^3$ of a *P. fluorescens* suspension ($O.D._{580}$ = 2) in a solution containing glucose and glutamic acid (in concentrations adequate to achieve the same final soil C content as above). Then anaerobiosis and $N_2O$ inhibition were obtained in the flasks as described above and the $N_2O$ accumulation was followed until a constant value was reached. We ascertained that neither nitrate nor nitrite remained in the *Pseudomonas* suspensions before they were added to the soil: (1) by colorimetry procedure using Morgan's and Griess Ilosway's reagents, and (2) by anaerobic incubation of suspension aliquots (with carbon and $C_2H_2$ supplies) in order to verify that no $N_2O$ was produced.

$N_2O$ was analysed on a gas chromatograph equipped with an electron capture detector. Previous studies (Abbadie & Lensi 1990; Lensi, Domenach & Abbadie 1992) have demonstrated that the nitrifying potential of different soils was not affected by drying and storage.

The enzymatic potential of nitrification was computed by subtracting the nitrate initially present in the soil from the nitrate accumulated after aerobic incubation and expressed as µg $NO_3^-$ – N produced 48 $h^{-1}$ $g^{-1}$ dry soil.

### ASSAY OF ASSIMILATORY NITRATE REDUCTASE ACTIVITY (NRA) ON FRESH TISSUES OF *HYPARRHENIA DIPLANDRA*

In each site, inflorescences of 15 randomly distributed tussocks were wrapped in bags in October 1994 and the seeds produced by selfing were collected in December 1994. After germination, two to four plantlets from each initial tussock were cultivated in a greenhouse on standard compost (solid base: loam; sand: 50 litre $m^{-3}$; pH ($H_2O$): 6·0–6·3; PG-Mix™ 12·14·24 NPK fertilizer (Hydro Agri): 1·5 kg $m^{-3}$), fertilized with $NO_3^-$ and $NH_4^+$ under the following conditions: 12 h light, 12 h dark; 28 °C; 50% air water vapour saturation deficit; water was regularly supplied in order to maintain water holding capacity.

On days 30, 45, 60, 90 and 180 after the onset of culture, two to five pieces (20–50 mg fresh material each) from five different young leaves sampled from each plantlet were shaken for 5 h in the following induction medium: $KNO_3$, 20 mM; $CaCl_2$, 1·5 mM; $MgSO_4$, 0·75 mM; $KH_2PO_4$, 0·625 mM.

*In vivo* NRA was assayed according to Hageman & Reed (1980): leaf samples were incubated in 2 $cm^3$ of the same medium (supplied with 2% isopropanol) at 30 °C under nitrogen flow in the dark. The reaction was stopped after 15 and 30 min of incubation by transferring the tube into boiling water for 5 min. The nitrite liberated in the medium was assayed by adding 500 µl of a 2% sulphanilamide solution in 3N HCl and 500 µl of N-(1-naphthyl)-ethylenediamine dihydrochloride at a 0·04% concentration, and measuring the optical density at 540 nm. The nitrate reductase activity ($NO_2^-$ produced at $t$ = 30 min minus $NO_2^-$ produced at $t$ = 15 min) was expressed as µmoles $NO_3^-$ reduced $g^{-1}$ plant matter $hour^{-1}$. As no significant differences in NRA between different parts of a single leaf and between leaves of a same plantlet were recorded, all the measurements performed on each plantlet were averaged.

The NRA was also measured in roots following the same procedure (10 20–50 mg root samples proceeding from 10 different plantlets were assayed per site at times 30 and 90 days).

For each root or leaf sample, the same measurements were repeated at least five times.

### STATISTICAL ANALYSIS

All statistical analyses were performed with the SAS ™ software (SAS Institute Inc 1989). We used the PROC GLM procedure, with type III sum of square, for unbalanced analyses of variance and analyses of covariance. The LSMEANS procedure was used to compare the least square means (Lsmeans) across levels of class variables. All tests were performed at the 0·01% significance level.

## Results

### PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS

The physico-chemical characteristics of soils are given for each site in Table 1. None of these characteristics was significantly different between the two sites at the $P$ = 0·05 level. Moreover, these results are consistent with previous studies (Pochon & Bacvarov 1973; Abbadie & Lensi 1990; Lensi, Domenach & Abbadie 1992; Le Roux *et al*. 1995) conducted in Lamto: total organic C and N contents are low, pH is acidic and the soil is sandy.

### POTENTIAL OF NITRIFICATION IN THE TWO SITES

The initial concentration of nitrate and the nitrate accumulated after 48 h of aerobic incubation were 15- and 90-fold higher in site B than in site A, respectively (Fig. 1). These differences are highly significant (total df = 134, $r^2$ = 0·4391, $F$ = 104·11 and $P$ = 0·0001 at

**Table 1.** Soil physico-chemical characteristics of the two studied sites. Total C and N contents, mean soil C-to-N ratio, total sands, silts and clays percentages, and mean soil pH were measured between 0 and and 10 cm depth; mean soil bulk density (BD) was measured between 0 and – 5 cm depth. Values are presented with standard errors (parenthesis). There was no difference between site A and site B for all variables at the $P = 0{\cdot}05$ level

| Site | C (‰) | N (‰) | C:N | Total sands (%) | Total silts (%) | Total clays (%) | BD (g ml$^{-1}$) | pH |
|---|---|---|---|---|---|---|---|---|
| Low nitrification zone (site A) | 9·16 (0·79) | 0·55 (0·07) | 16·89 (1·37) | 77·86 (1·68) | 13·71 (1·05) | 8·43 (1·19) | 1·64 (0·08) | 6·82 (0·26) |
| High nitrification zone (site B) | 10·61 (0·57) | 0·63 (0·08) | 17·05 (2·00) | 76·98 (1·28) | 14·5 (1·28) | 8·52 (1·04) | 1·66 (0·02) | 6·74 (0·08) |

time 0; total df = 134, $r^2 = 0{\cdot}6327$, $F = 229{\cdot}1$ and $P = 0{\cdot}0001$ after 48 h incubation). The mean value of the enzymatic potential of nitrification (obtained by subtracting the nitrate initially present in the soil from the nitrate accumulated) was 18 ng $NO_3^-$ – N produced 48 h$^{-1}$ g$^{-1}$ dry soil for site A, and 4355 ng $NO_3^-$ – N produced 48 h$^{-1}$ g$^{-1}$ dry soil for site B (i.e. in a 240-fold ratio). The intrasite variability of the potential of nitrification was very similar in the two sites (variation coefficients of 50·0 and 50·7 for site A and B, respectively).

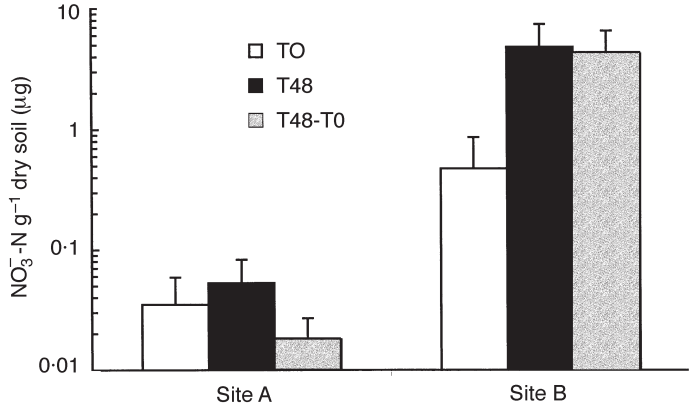


**Fig. 1.** Comparison of nitrification activities between the low nitrification site (A) and the high nitrification site (B), as µg $NO_3^-$-N g$^{-1}$ dry soil. T0, initial $NO_3^-$ content concentration; T48, $NO_3^-$ concentration after 48 h aerobic incubation with supply of ammonium; T48-T0, $NO_3^-$ produced during 48 aerobic hours incubation. The statistical analyses are shown in Results. Note the logarithmic scale for *y*-axis.

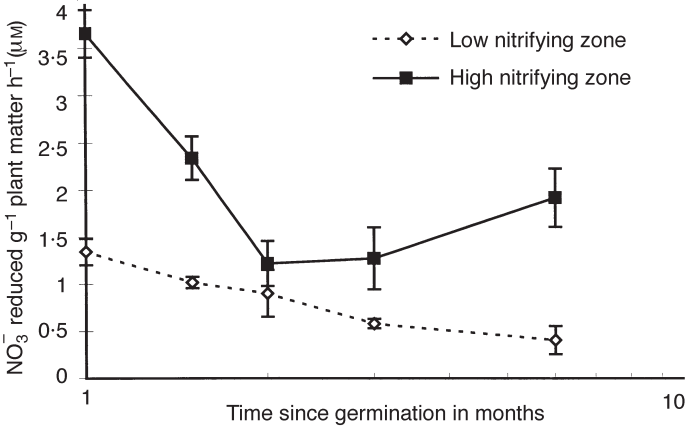


**Fig. 2.** Comparison of *in vivo* nitrate reductase activities in leaves between *Hyparrhenia diplandra* populations originating from the low nitrification site (A) and the high nitrification site (B), as µM $NO_3^-$ reduced g$^{-1}$ plant matter hour$^{-1}$ and by age class (months since germination, from 1 to 6). The statistical analyses are shown in Table 2. Note the logarithmic scale for *x*-axis.

### NITRATE REDUCTASE ACTIVITY (NRA) OF *HYPARRHENIA DIPLANDRA* PLANTLETS ORIGINATING FROM THE TWO SITES

NRA in roots was always much lower than in the leaves and often close to the detection limit of the method, and no significant difference ($n = 40$, $F = 0{\cdot}84$, $P = 0{\cdot}961$) was observed between the root NRA of the plantlets from the two sites.

The leaves of *H. diplandra* plantlets originating from site B had a significant higher NRA that those originating from site A (Fig. 2). Table 2 demonstrates that the NRA of the two subpopulations was statistically different in its entire dynamics as well as at each sampling time (i.e. at every plantlet age). After 6 months of plant growth, NRA was found to be four times higher in plants from site B than in plants from site A.

## Discussion

### POTENTIAL OF NITRIFICATION IN THE TWO SITES

The technique used to measure nitrification was well suited to this study because accurate measurements could be obtained on soil samples with low potential of nitrification after short-term incubations (reducing the interferences owing to the possible utilization of the produced nitrate by other microbial metabolic pathways). The specificity and sensitivity of this method associated with $NH_4^+$ supplies and optimal aeration offered a valuable approach to the potential of nitrification. It is therefore possible to detect very low rates of nitrification, which would be difficult by other methods based on the direct measurement of $NO_3^-$ in soil.

Despite similar soil physico-chemical characteristics and plant cover, site B displayed a much higher potential of nitrification (240-fold) than the reference site A (Fig. 1). The intrasite variability was remarkably similar between sites A and B as shown by the variation coefficient values. The virtual absence of nitrification

**Table 2.** Statistical analyses of the *Hyparrhenia diplandra* subpopulations effect [originating from the low (A) or high (B) nitrifying sites], individuals age effect and crossed effects on nitrate reductase activity in young leaves (results from unbalanced analyses of variance and covariance, type III sum of square.)

| | Nitrate reductase activity | | |
|---|---|---|---|
| Effect | df | *F* | *P* |
| Population | 2 | 110·77 | 0·0001 |
| Age of plantlet since germination | 7 | 39·41 | 0·0001 |
| Population × age | 4 | 14·28 | 0·0001 |
| Error | 137 | | |
| *r*-square | 0·8362 | | |

in the standard site A is in agreement with previous results from this site (Abbadie & Lensi 1990; Lensi, Domenach & Abbadie 1992), other *H. diplandra* sites in Lamto reserve (Pochon & Bacvarov 1973; Abbadie & Lensi 1990) or even other wet savannas dominated by Andropogoneae (Meiklejohn 1962; Bernhard-Reversat 1982). The very high values found in site B confirm the findings of Le Roux *et al*. (1995) who suggested that this site could be a high nitrification site. The site B might therefore be considered as an atypical example of a site dominated by *H. diplandra* expressing substantial nitrification activity. Consequently, the hypothesized depressive effect of *H. diplandra* on nitrification cannot be considered as a general feature of the humid savanna zone from Africa at species level. *H. diplandra*–nitrifiers interactions should only be considered at a plant infra-specific level, whatever these interactions could be. In order to test this hypothesis, further investigations must be performed to compare the two subpopulations of *H. diplandra* by the use of standard molecular tools such as RAPD, AFLP or ITS sequence variation (Welch & McLelland 1990; Baldwin 1992; Vos *et al*. 1995).

### RELATIONSHIPS BETWEEN SOIL POTENTIAL OF NITRIFICATION AND NITRATE REDUCTASE ACTIVITY (NRA) OF *HYPARRHENIA DIPLANDRA*

A significant NRA has been found exclusively in the leaves of *H. diplandra*, the activity in the roots being, in several cases, below the detection limit. This result is quite different from observations made on other Graminaceae (Poaceae), such as maize and millet (Wallace 1987), where the NRA is much more equally distributed between roots and shoots. However, these different modalities of NRA occurrence inside a given plant family are not surprising. Several studies have shown that even closely related species may have different patterns of NRA occurrence: exclusively in leaves, exclusively in roots or equally distributed between leaves and roots (Haynes & Goh 1978). In our study, owing to the extremely low activity detected in roots, the analysis was restricted to the comparison of NRA in leaves.

Through the entire growth period, *H. diplandra* plantlets originating from the high nitrifying site (B) were found to display a significantly higher NRA than those originating from the low nitrifying site (A) (Fig. 2). The NRA of site B plantlets is comparable to the NRA found in cultivated millet (Wallace 1987), whereas the NRA of site A individuals is close to the activity of NR-deficient mutants of barley (Warner, Lin & Kleinhofs 1977). These differences between plantlets remained significant 6 months after the onset of culture. As NRA measurements were performed on plantlets resulting from selfing, cultivated on the same standard compost supplemented with excess $NO_3^-$, and under identical environmental conditions, it may be assumed that the results obtained reveal intrinsic differences between the two *H. diplandra* subpopulations.

The stability over time of the nitrification status observed in the two sites, which was a central point questioned by our study, is thus supported by (1) the similarity of the potential nitrification values obtained in this study with those obtained by Le Roux *et al*. (1995) in site B and by other authors many years ago in site A, and (2) the differences in NR potential activities in the leaves between the two *H. diplandra* subpopulations which strongly suggest a long-term adaptation of grasses to soil nitrogen cycles differing in their ability to produce nitrate.

In conclusion, we have shown that under the same physical environment (climate and soil) and the same plant cover, two different types of nitrogen cycling (including or excluding nitrification) coexist in savannas: the functioning of the ecosystem is patchy not only in term of intensity but even in terms of occurrence of processes such as nitrification. A strong correspondence was also demonstrated between the variation in the potential rate of nitrification in savanna and the characteristics of nitrogen nutrition of the grasses at infraspecific level. To our knowledge, it is the first time that such a relationship is shown in the field. In practice, that means that nitrification in soils cannot be analysed separately from plant physiology, the ecosystem being made of small-scale subunits, with partly independent functionings.

Given the stable character of the potential nitrification rate in soil, one can wonder if *Hyparrhenia diplandra* directly controls the level of nitrification as questioned by Munro (1966) and Meiklejohn (1962, 1968) for several species belonging to the Andropogoneae group. Their observations, today strongly debated, led them to hypothesize an allelopathic effect of these grasses on soil nitrifying communities. Only a direct approach based on plant manipulation experiments will be able to elucidate both the nature and mechanisms of Andropogoneae-nitrifiers relationships.

To explain the discrepancy between high plant productivity and nitrogen deficiency in savanna will require the understanding of the role of nitrification in

ecosystem functioning. If nitrification significantly affects ecosystem performance, a strong correlation should be observed between plant characteristics (such as productivity, structure, phenology, physiology) and potential nitrification rates. It can be hypothesized that the ability to depress nitrification should give a competitive advantage to the populations of *Hyparrhenia diplandra* or other Andropogoneae species for nitrogen acquisition. This competitive advantage could explain the invasive dynamics of some African Andropogoneae observed in South America (Baruch, Ludlow & Davis 1985; San José & Fariñas 1991; Baruch & Fernández 1993; Klink 1996).

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