Functional Ecology 2004
18, 605–611

Grass populations control nitrification in savanna soils

J.-C. LATA,* V. DEGRANGE,† X. RAYNAUD,* P.-A. MARON,‡ R. LENSI§ and L. ABBADIE¶

**Laboratoire d'Ecologie, Systématique et Evolution – UMR 8079, Université Paris Sud, 91405 Orsay Cedex, †Laboratoire d'Ecologie Microbienne, UMR-CNRS 5557, Université Claude Bernard Lyon I, 69622 Villeurbanne Cedex, ‡Laboratory of Geochemistry and Soil Microbiology, INRA, 17 rue Sully, BP 86510, 21065 Dijon Cedex, §CEFE/CNRS UMR 5175, 1919 Route de Mende 34293 Montpellier Cedex 5, and ¶Ecole Normale Supérieure, Laboratoire d'Ecologie, UMR-CNRS 7625, 46 rue d'Ulm, 75230 Paris Cedex 05, France*

Summary

1. Nitrification plays a key role in the functioning of many natural ecosystems. It is directly involved in plant nitrogen nutrition and soil N losses through leaching and denitrification. The control of this process by plants is poorly understood, although modifications of nitrification would allow plants to manipulate competition for N and induce changes in ecosystem N balance. In a wet tropical savanna ecosystem (Lamto, Côte d'Ivoire), the soil N cycle is characterized by distinct high- and low-nitrification sites. Previous publications showed that nitrification was positively or negatively correlated with root densities of the dominant grass covering these sites. These contrasting sites were chosen to investigate the extent to which vegetation controls long-term nitrification.

2. *In situ* experimental plots were created where grass individuals originating from high- or low-nitrifying soils were transplanted into both soils. Nitrifying enzyme activity (NEA) was measured up to 24 months after transplanting. Grasses from both sites significantly modified NEA up to rates similar to those at their respective control sites.

3. The level of individual plant control (inhibition and stimulation) was correlated with grass biomass. The potential mechanisms of this control is discussed, along with its consequences for ecosystem N cycling (such as N losses), as the denitrifying enzyme activity (DEA) is much higher in the high-nitrification site. Such results suggest that plant species can have important consequences for N cycling at the population level.

Key-words: *Hyparrhenia diplandra*, Lamto (Côte d'Ivoire), nitrification inhibition, nitrogen conservation, plant adaptation

Functional Ecology (2004) **18**, 605–611

Introduction

Large areas of forest and grassland ecosystems, such as the mixed tree–grass humid savannas in West Africa, have been reported to be systems with very low rates of nitrification (De Rham 1973; Bate 1981; Verstraete 1981; Haynes 1986; Abbadie & Lensi 1990; Lensi, Domenach & Abbadie 1992; Serça *et al*. 1998). It has been hypothesized that grasses, especially species belonging to the *Andropogoneae* supertribe, can inhibit nitrification through the exudation of allelopathic compounds by roots (Munro 1966; Meiklejohn 1968; Rice & Pancholy 1972). This hypothesis has been at the centre of a controversy for many years because of the absence of *in situ* evidence for plant involvement in nitrification inhibition (Stiven 1952; Munro 1966; Meiklejohn 1968; Rice & Pancholy 1972; Purchase 1974; Lodhi 1978; Jordan, Todd & Escalante 1979; Robertson 1984; Donaldson & Henderson 1990; McCarty, Bremner & Schmidt 1991; Stienstra, Klein Gunnewiek & Laanbroek 1994; Lata *et al*. 1999; Lata *et al*. 2000).

Such low-nitrification savanna systems are of major interest. First, savannas represent 25% of terrestrial biomes (Solbrig & Young 1993) and are second to tropical forests in their contribution to terrestrial primary production (Atjay, Ketner & Duvigneaud 1987). They are also predominant in the social and economic environment of Africa: more than half the surface of the African continent is covered by savannas and savanna–forest associations (Menaut 1983; Solbrig 1993). Moreover, in Africa, savannas are associated with the regions of highest human population growth (Scholes & Walker 1993). Savanna grasses from the *Andropogoneae* supertribe are of economic interest, in particular for pastures. They are widely represented in Africa, and were introduced to South America where they became invasive in some areas (Baruch, Ludlow & Davis 1985; San

Author to whom correspondence should be addressed. E-mail: jean-christophe.lata@ese.u-psud.fr

José & Fariñas 1991; Baruch & Fernández 1993; Klink 1996).

Second, savannas are generally nutrient-poor ecosystems, especially for nitrogen, which generally limits productivity. Nitrification therefore plays a key role in the functioning of savannas, as it is directly involved in plant N nutrition and N losses through leaching and denitrification (Huber *et al*. 1977; Jordan, Todd & Escalante 1979; Vitousek *et al*. 1979; Keeney 1986; Robertson 1989).

Third, little is known about the possible control of plants on nitrification. Such control could provide plants with potential advantages in competition for N, and induce changes in ecosystem N balance. Because nitrate can be lost easily from the ecosystem (through leaching or denitrification), the ability of plants to inhibit nitrification could therefore be considered as an adaptive trait allowing them to bypass microbial processes that limit productivity.

Finally, from a global change point of view these humid savannas of West Africa are considered as non-emitting areas for NO and N_2O as a result of their extremely low nitrification potential (Le Roux *et al*. 1995; Serça *et al*. 1998).

In the Lamto reserve (Côte d'Ivoire), the shrub savanna (covering almost 55% of the land surface and dominated by a perennial member of the *Andropogoneae*, *Hyparrhenia diplandra* (Hack.) Stapf) has been identified by several authors as a non-nitrifying ecosystem (De Rham 1973; Abbadie & Lensi 1990; Lensi *et al*. 1992). However, a recently discovered site of >15 ha, with similar vegetation, surprisingly exhibits 15- to 240-fold higher nitrification activity (Le Roux *et al*. 1995; Lata *et al*. 1999; Lata *et al*. 2000). A comparison between the nitrifying site (high-nitrification, HN site) and a non-nitrifying site (low-nitrification, LN site) gave us the opportunity to study *in situ* the mechanisms involved in the control of nitrification. Lata *et al*. (1999, 2000) found that while the two sites were similar in their soil physicochemical characteristics or the species composition of the grass cover, nitrification activity was positively or negatively correlated with root densities of *H. diplandra* populations in the HN and LN sites, respectively. The existence of distinct high- or low-nitrification sites in this ecosystem could result from an action of this particular grass at the population level.

Two possible mechanisms by which *H. diplandra* could alter the rate of nitrification can be hypothesized. The first is that the grass is directly involved in nitrification inhibition and that *H. diplandra* originating from the LN site can inhibit nitrification whereas the HN-site ecotype cannot. This inhibition could be due to an allelopathic effect of grasses on nitrifiers through root exudation of compounds that inhibit the activity of soil organisms (such as phenolic acids and tannins), the rate of exudation being generally linked to the size of plants (Prikryl & Vancura 1980). The second hypothesis postulates an indirect involvement of the grass in the inhibition of nitrification through an ability to compete with nitrifying bacteria for ammonium, and that the two *H. diplandra* populations (from LN and HN sites) exhibit different competitive abilities for ammonium uptake.

The first objective of this field study was to examine the extent to which *H. diplandra* individuals from LN and HN sites could influence nitrification. We designed *in situ* experimental plots with transplanted *H. diplandra* individuals collected from both high- and low-nitrifying soils, and planted in both high- and low-nitrifying soils. We measured the nitrifying enzyme activity (NEA), the ability of nitrifiers to oxidize ammonium to nitrate, which avoids the short-term variations induced by climate or other environmental factors.

The second objective of this field study was to examine if the variations in NEA could be related to the grass biomass at the individual level. In order to easily test this hypothesis non-destructively, we first searched for correlations between above- and below-ground biomass and several size indices of individual plants. Then we looked for correlations between NEA in nitrifying and non-nitrifying sites and one of these size indices: the diameter of *H. diplandra* tussocks. These measures were done to confirm and refine, at the individual level, *in situ* measures by Lata *et al*. (2000) who found some correlations between root densities and NEA.

Finally, to discuss these results in the broader context of the N cycle, we measured potential net C and N mineralization and denitrifying enzyme activity (DEA) to test if variations in NEA between the two nitrifying sites could be related to other N-cycle processes.

Materials and methods

STUDY SITES

Lamto reserve is in Côte d'Ivoire, West Africa, at latitude 6°13′ N and longitude 5°20′ W, at the southward limit of the 'V Baoulé', a broad grass expanse of savannas which spreads southwards far into the rain forest. In the study area the vegetation is a shrub savanna dominated by the grasses *H. diplandra* and *Hyparrhenia smithiana* (Devineau 1976; Menaut & César 1979). This type of vegetation covers 55% of the surface of the Lamto reserve (Gautier 1990).

Temperatures are relatively constant throughout the year (27 °C on average). Rainfall is variable, and four seasons can be distinguished: (i) the long dry season from December to February; (ii) the long wet season from March to July; (iii) the short dry season in August; (iv) the short wet season from September to November. Annual precipitation averages 1200 mm (data from Geophysical Station of Lamto). The soils are composed of granites and derived sands, and are classified as tropical ferrugineous soils with a superficial gravelly horizon.

The LN and HN sites are 5 km apart and >15 ha each in size. Both are strongly dominated by the

perennial *H. diplandra*. In the LN site, NEA is 15–240 times lower than in the HN site (Abbadie & Lensi 1990; Lensi *et al*. 1992; Le Roux *et al*. 1995; Lata *et al*. 1999; Lata *et al*. 2000). There are no significant differences in plant species composition between the two sites (Lata *et al*. 1999).

SOIL PHYSICOCHEMICAL CHARACTERISTICS

The soil physicochemical characteristics of both sites have been described by Le Roux *et al*. (1995); Lata (1999); Lata *et al*. (1999, 2000). There are no significant differences between the two sites in soil characteristics of the 0–10 cm layer, except for DEA. The soils are sandy and near-neutral with pH-KCl values of 6·82 ± 0·26 (LN) and 6·74 ± 0·08 (HN). Bulk density is 1·64 ± 0·08 (LN) and 1·66 ± 0·02 (HN) g cm^{-3}. Total C and N contents are 9·16 ± 0·79 g C kg^{-1} and 0·55 ± 0·07 g N kg^{-1} (LN); and 10·61 ± 0·57 g C kg^{-1} and 0·63 ± 0·08 g N kg^{-1} (HN).

For this study, in addition to NEA measurements we also measured the following characteristics in undisturbed areas of both LN and HN sites (on 35 randomly sampled 10 × 10 × 10 cm cubes at 0–10 cm depth per site): (i) potential net C mineralization as described by Guillaume *et al*. (1999); (ii) potential net N mineralization as described by Nacro, Benest & Abbadie (1996); (iii) DEA as described by Abbadie & Lensi (1990). Samples were assayed in triplicate.

EXPERIMENTAL PLOTS

Fifty individual tufts randomly sampled in each site were transplanted on soil plots (15 × 15 m) where all the vegetation had been removed. We carried out four combinations of vegetation and nitrification site (HN or LN) types: two intersite transplantations (LN plant on HN site; HN plant on LN site) and two control plots where tufts were replanted in their own site (LN plant on LN site; HN plant on HN site). Distances between plots were ≈50 m. In all cases root systems were washed before replanting. After transplanting water was supplied daily for 1 month. Tufts were transplanted at a mean distance of ≈40 cm from each other to reproduce the structure of the natural grass cover.

SOIL SAMPLES

Twelve and 24 months after transplanting, soil samples were collected from under tufts of each experimental plot, and under tufts from LN and HN undisturbed areas. Soil samples were collected with a soil corer down to 10 cm (maximum microbial activity depth, De Rham 1973). In each plot 25 under-tuft samples were collected, and in each undisturbed area 30 under-tuft samples were collected. Samples were immediately air-dried in the shade, sieved (2 mm) and stored for nitrification measurements in laboratory.

NITRIFYING ENZYME ACTIVITY MEASUREMENTS

This activity is equivalent to the concentration of specific nitrifying enzyme(s) present at a given moment and in a given sample when no other factor is limiting, and without any *de novo* enzyme synthesis and cell multiplication (Tiedje 1982). Measurements were performed according to Lensi *et al*. (1986): the NO_3^- produced during an aerobic incubation was deduced from N_2O measurements after anaerobic incubation in the presence of C_2H_2. The NEA measurements are not affected by short-term environmental variations (Lensi *et al*. 1986) or by drying and storage (Abbadie & Lensi 1990; Lensi *et al*. 1992).

Subsamples of 15 g (n = 6) from each soil sample were placed in 150 ml plasma flasks. Three subsamples were used to estimate the initial NO_3^- content: they were supplied with 6 ml of a suspension of a denitrifying organism (*Pseudomonas fluorescens*, OD_{580} = 2) in a solution containing glucose and glutamic acid. The final soil C concentration for each compound was 0·5 mg C g^{-1} dry soil. This ensures a high denitrifying activity and an excess of electron donors. 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 (a total conversion of soil NO_3^- into N_2O) was reached.

The three other subsamples were used to determine the evolution of NO_3^- accumulation: they were enriched with 2 ml of an $(NH_4)_2SO_4$ solution (final soil N concentration 0·2 mg g^{-1} dry soil) in order to ensure a moisture content equivalent to 80% water-holding capacity, and no limitation by ammonium (the presence of NH_4^+ also limits NO_3^- assimilation by micro-organisms). Flasks were then 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, homogeneous aeration of the soil. After this aerobic incubation, which allows nitrate to accumulate, the soil samples were enriched with 4 ml of a *P. fluorescens* suspension (OD_{580} = 2) in a solution containing glucose and glutamic acid (in concentrations adequate for achieving the same final soil C concentration as above). Then anaerobiosis and N_2O inhibition were produced as described above, and N_2O accumulation 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 by (i) a colorimetry procedure using a solution of diphenylamine indicator for nitrate (Morgan 1930) and Griess Ilosway's reagent for nitrite (Alexander & Clark 1965); and (ii) anaerobic incubation of aliquots of the suspension (with C and C_2H_2 supplied) to verify that no N_2O was produced. The presence of N_2O was determined with a gas chromatograph equipped with an electron capture detector (HP 5890 Series II).

NEA was calculated by subtracting the nitrate initially present in the soil from that accumulated after aerobic incubation and expressed as ng NO_3^--N g^{-1} dry soil day^{-1}.

PLANT MEASUREMENTS

We measured three tuft size indices: maximum tuft height; tuft diameter; and number of leaves, on 150 tufts sampled randomly at both sites. Then we randomly sampled 30 tufts of *H. diplandra* at both sites and measured these three indices, above-ground and below-ground biomass. Above-ground biomass of individual tufts was measured by cutting the above-ground part of the tuft and weighing it after drying at 60 °C. Below-ground biomass was measured by sampling soil under tufts between 0 and 30 cm depth. Samples were immediately air-dried in the shade, homogenized and sieved (<2 mm). Roots were collected and weighed after sieving.

STATISTICAL METHODS

All statistical analyses were performed using SAS software (SAS Institute Inc. 1989). We used the GLM procedure, with type III sum of squares for ANOVA and covariance. The LSMEANs procedure was used to compare the least-square means across levels of class variables. All tests were performed at the 0·05 significance level. Values are given as mean ± SE.

Results

EXPERIMENTAL PLOTS

One year after transplanting, the two *H. diplandra* populations exerted some control over NEA (Fig. 1). Populations from the LN site induced a threefold decrease of NEA when planted in the HN site compared with the HN control plot. In contrast, tufts from the HN site transplanted on the LN plot completely restored NEA. The NEA values in the LN plot were not significantly different from those measured in the HN control plot. Transplanting of tufts in their native soils did not modify NEA compared with undisturbed soil (samplings at the same dates in undisturbed areas; Fig. 1), showing that any disturbance generated by transplantation was not responsible for the observed changes.

Two years after transplanting, the same pattern was observed (Fig. 1). The NEA under tufts transplanted from the LN site to the HN site was not significantly different from that in the LN control plot. The NEA under tufts transplanted from the HN site to the LN site was 19 times greater than in the LN control plot.

RELATIONSHIPS BETWEEN PLANT BIOMASS AND NITRIFYING ENZYME ACTIVITY IN UNDISTURBED AREAS

The three size indices (height, diameter and number of leaves) were all positively correlated in both LN and HN sites ($R^2 > 0{\cdot}65$). In both sites they were also positively correlated to above-ground, below-ground or total biomass of plants ($R^2 > 0{\cdot}39$, $R^2 > 0{\cdot}39$ and $R^2 > 0{\cdot}28$, respectively). We also found a positive correlation ($R^2 = 0{\cdot}57$) between above-ground and below-ground biomass, which is consistent with previous measures (Lata *et al*. 2000).

A negative correlation was observed between the diameters of *H. diplandra* tufts and the NEA in the LN site, whereas it was positive in the HN site (Fig. 2). These results were confirmed by measurements on two other dates (data not shown).

NET C AND N MINERALIZATION POTENTIALS, AND DENITRIFYING ENZYME ACTIVITY OF BOTH SITES

Potential net C mineralization at the LN and HN sites was not significantly different, and was 1·19 ± 0·14

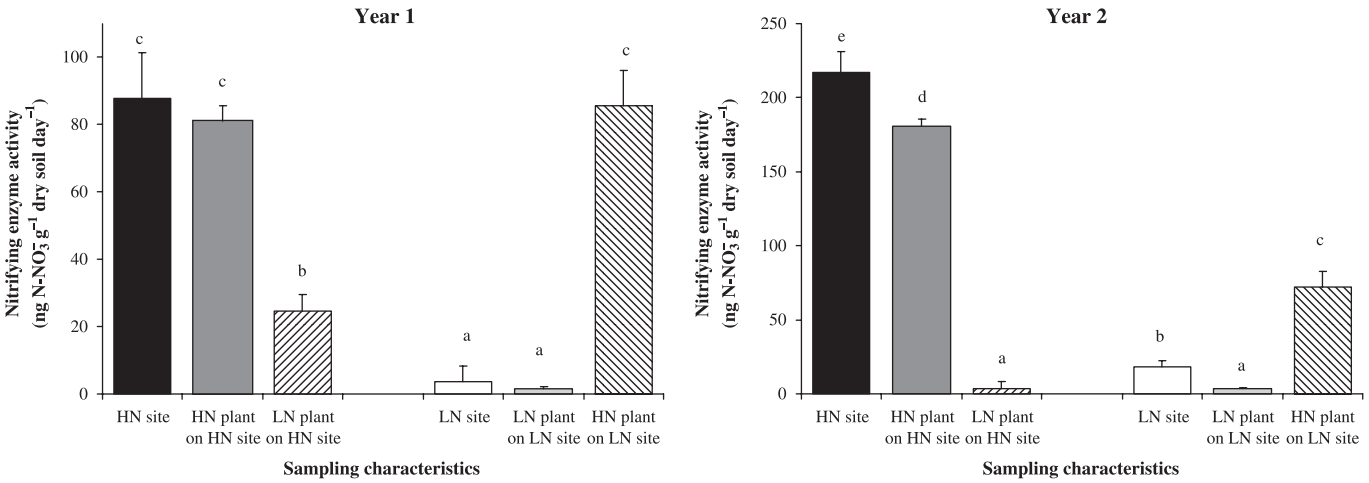

Fig. 1. Nitrifying enzyme activities in transplanted plots and undisturbed areas after 12 and 24 months of cultivation (mean ± SE). LN, low-nitrification site; HN, high-nitrification site. Sampling characteristics as follows: undisturbed area samples, LN and HN site; control plot samples, LN plant on LN site; HN plant on HN site; transplantation plot samples, HN plant on LN site; LN plant on HN site. The same letters indicate treatments that are not significantly different ($P > 0{\cdot}05$).

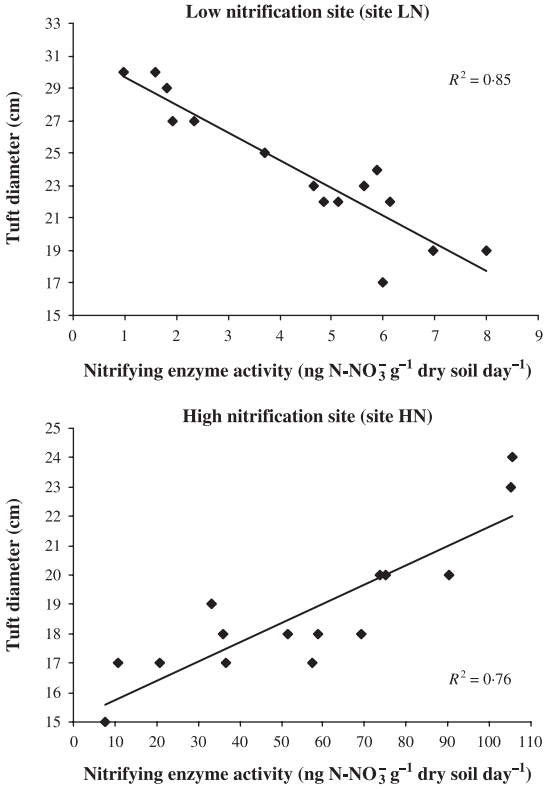

Fig. 2. Relationship between nitrifying enzyme activity under *Hyparrhenia diplandra* tufts and tuft diameter at low- and high-nitrification sites.

and 1·37 ± 0·22 μg C-CO_2 g^{-1} dry soil h^{-1}, respectively. Similarly, potential net N mineralization was not significantly different, 5·26 ± 1·09 (LN) and 6·42 ± 0·95 (HN) μg N g^{-1} dry soil day^{-1}. However, we found a significant difference in mean DEA between the two sites. The DEA was 0·09 ± 0·08 (LN) and 0·85 ± 0·37 (HN) μg N_2O-N g^{-1} dry soil day^{-1}.

Discussion

Our study is the first *in situ* demonstration of biomass-dependent control of nitrification by grasses at the individual level. In the Lamto savanna, sites occur with contrasting nitrification patterns (high and low), and individuals from these sites have a different influence on nitrification. This shows that, in this case, such control can be at the plant ecotype level (Seliskar *et al*. 2002) as well as at the species level (Wedin & Tilman 1990; Knops *et al*. 2002).

The transplantation of individuals originating from HN or LN sites into HN or LN sites showed that there was a clear plant effect on nitrification. The LN plants decreased NEA in the HN site down to values found in the LN site, whereas HN plants restored the NEA in the LN site to values found in the HN site.

Our results are consistent with previous measures showing strong correlations between nitrification and root biomass (Lata *et al*. 2000). Two hypotheses were put forward in the Introduction to explain these results.

We found a negative correlation between the biomass of LN plants and NEA, which is consistent with both hypotheses. On the other hand, we found a positive relationship between plant biomass originating from the HN site and NEA. This may seem surprising due to the mostly autotrophic character of nitrification, so this process is generally considered not to be positively affected by root exudates (Bock, Koops & Harms 1989). However, the substrate for nitrification is ammonium produced by the heterotrophic mineralization of organic matter. Lamto savanna is highly structured, and micro-organisms and the production of organic and inorganic (such as ammonium) compounds are concentrated close to roots (Abbadie, Mariotti & Menaut 1992). Thus increases in plant biomass could potentially increase ammonium availability in the soil through an increase of root-derived carbon. Depending on the competitiveness for ammonium uptake between *H. diplandra* and soil micro-organisms, this could increase NEA with increasing plant biomass. Moreover, because nitrifiers can grow in mixotrophic or heterotrophic media (Bock, Sundermeyer-Klinger & Stackebrandt 1983), and survive and multiply in soils under heterotrophic conditions (Degrange, Lensi & Bardin 1997), the increase of nitrification with plant biomass could be caused by heterotrophic nitrification (Nemergut & Schmidt 2002). Our data cannot distinguish between the competition and the inhibition hypothesis. However, preliminary laboratory measurements on NEA in mixed HN + LN soils appear to favour the inhibition hypothesis, because the presence of a very low quantity of LN soil induces a drastic fall of NEA in HN soil (data not shown).

In conclusion, the mechanisms(s) involved in the inhibition of nitrification by *H. diplandra* remain unknown, but the hypothesis of an allelopathic inhibition through exudation of product(s) by the grass roots, as previously described (see Introduction), is still possible. However, only the identification of the (hypothetical) chemical mediator(s) responsible for inhibition can answer this question. Experiments based on *in vitro* evaluation of the influence of soil extracts from the two sites on pure culture of nitrifiers are currently under way.

From an evolutionary viewpoint, a major question remains. Is the HN site a remnant of a system where high nitrification was the rule, or is it a first indication of a change towards a nitrifying system? Both systems can coexist for a long time, as suggested by physiological adaptations (genetic differences in the inducibility of the assimilatory enzyme nitrate reductase between HN and LN *H. diplandra* populations; Lata *et al*. 1999). However, plants from the HN site are smaller, and grow more slowly in both glasshouse and field conditions, than those originating from the LN site (Lata 1999). The LN plants could therefore outcompete HN plants unless particular environmental conditions or perturbations maintain HN plants in pure communities in some areas. Thus, even if we cannot state which nitrification system appeared first, it appears very unlikely that Lamto savanna changes towards a high-nitrification system.

The ability to inhibit nitrification, either through an inhibiting factor or a superior competitiveness of plants compared with micro-organisms, could give a strong competitive advantage to LN plants. In acid soils it could create better local availability of N by decreasing its losses through nitrate leaching and denitrification. Carbon- and N-mineralization potentials were not significantly different between LN and HN sites; this suggests that the initial decomposibility of the organic matter is the same in the two systems, and that nitrification plays a key role. The DEA was ≈10-fold less in the LN site than the HN site, decreasing potential atmospheric losses of N.

Acknowledgements

We thank D. Aïdara (President of the Abobo-Adjamé University), N. Salin, R. Vuattoux, J. Jantzen, E. Kouassi Kouassi, F. Kouamé N'Guessan, P. Savadogo Sadare and S. Barot for assistance. We thank P. Leadley, C. Raynaud and two anonymous referees for helpful and constructive comments on the manuscript. This study was supported by grants from the CNRS (SALT-IGBP/GCTE Core Research Programme) and Programme National Sol et Erosion (PNSE). This work is dedicated to the memory of Dr François Gourbière.

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Received 25 September 2003; revised 20 January 2004; accepted 30 March 2004