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Field study of nitrification and denitrification in a wet savanna of West Africa (Lamto, Côte d'Ivoire)

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Abstract

Field experiments about denitrification and nitrification processes have been performed in a wet savanna of West Africa (Lamto, Côte d'Ivoire). Two scales have been chosen: (i) a topographic gradient including the main forest and savanna environments; (ii) a soil profile taking into account roots and fauna effects. Denitrifying potential activity was only recorded in the surface soil: it was higher under forest than under savanna. Within the shrub savanna soil profile, denitrification was low in the average soil, but high in the walls of the termite fungus comb chambers, in the soil collected very close to the roots and in the earthworm casts. Nitrifying activity was higher in the grass savanna than in the shrub savanna dominated by the graminaceae *Hyparrhenia diplandra*.

Introduction

The understanding of the mechanisms controlling the processes of nitrification and denitrification in tropical ecosystems is important for two reasons: (i) these processes are known to be two of the major regulators of nitrogen retention in humid tropical ecosystems (Robertson, 1989) and, consequently, to control partly plant nitrogen availability; (ii) they are dominant sources of nitrogen oxides in most ecosystems (Firestone and Davidson, 1989).

Only denitrification is recognized as a significant biological source of N_2O in most natural systems (Firestone and Davidson, 1989). Although nitrification may also produce N_2O (Bremner and Blackmer, 1979; Klemedtsson et al., 1988), its main effect for potential nitrogen losses and N_2O production is probably through its control on NO_3^- availability for denitrifiers.

Lamto (Côte d'Ivoire) is recognized as a reference site of West Africa to assess the importance of the savanna ecosystems in the production of trace gases. N_2O is one of the more studied because of its greenhouse effect (Lacis et al., 1981) and its influence on the stratospheric ozone layer (Crutzen, 1983). Biomass burning is a significant source of N-gas emissions in the site of Lamto (Menaut et al., 1991). If soil biological process must also be considered as important potential sources of N_2O is still unknown. Before any attempt to quantify global emission rates, it appears important to study the factors that underlie the

emission of nitrous oxide and the environment where nitrification and denitrification could take place.

Abbadie and Lensi (1990) observed that forest environment in the Lamto region showed a potential of denitrification 5 to 15 times higher than the savanna and that inside the soil profile of a shrub savanna, roots and soil fauna (earthworms and termites) did not affect the potential of denitrification. These results were obtained from laboratories experiments using dry-stored soil samples: this can explain some unexpected observations, particularly the absence of influence of roots and fauna on the rate of denitrification. Nevertheless, the results obtained have to be confirmed or invalidated on fresh material.

As nitrogen losses in natural ecosystems are directly dependent of the production of nitrate, the occurrence of nitrification in tropical ecosystems must be considered. Although nitrification rates are highly variable (Montagnini and Buschbacher, 1988; Vitousek and Matson, 1988), this process seems to be always present in tropical humid forests. By contrast, little is known about nitrification in the soils of the savanna.

The purpose of this work was to perform field experiments in the Lamto savanna (i) to estimate the potential of denitrification in all the environments of a catena and to assess the influence of roots and soil fauna on this activity (ii) to measure the potential of nitrification in the soils of the savanna and to compare the nitrifying rates of two environments, with and without trees, using a sensitive technique for rapid determination of the nitrification potential (Lensi et al., 1986).

Material and methods

Site description

Lamto is located in Côte d'Ivoire (West Africa) at latitude 6°13′ North and longitude 5°20′ West, at the southern limit of the "V Baoulé", a broad grass expanse of savannas which goes far into the rain forest. The vegetation varies with the topography and five environments can be distinguished, from the downslope to the upslope (Devineau, 1976; Menaut and César, 1979): a riparian forest along the Bandama river, gallery forests in the thalwegs, herbaceous savannas, without trees except 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 semideciduous forests on the plateaus.

Temperatures are constant throughout the year (average 27°C). Rainfall is more 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. Precipitations average approximately 1,200 mm (statistics from Geophysical Station of Lamto). Granites and derived

Table 1. Mean total organic carbon and nitrogen contents (with standard error), and clay in the soil of the catena between 0 and −10 cm and between −10 and −20 cm depth, relatively to the dry weight of the soil

Location	Riparian forest		Gallery forest		Plateau forest		Shrub savanna		Herbaceous savanna	
Depth (cm)	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20
C (%)	1.95 (0.15)	0.38 (0.02)	1.21 (0.05)	0.02 (0.01)	0.70 (0.01)	0.10 (0.01)	0.79 (0.01)	0.13 (0.02)	0.63 (0.01)	0.11 (0.01)
N (‰)	1.81 (0.02)	0.41 (0.02)	1.35 (0.18)	0.13 (0.01)	0.47 (0.01)	0.13 (0.01)	0.53 (0.02)	0.34 (0.01)	0.32 (0.01)	0.26 ND
clay (%)	25.01 (2.28)	36.36 (0.96)	5.68 (0.42)	4.67 (0.50)	4.88 (0.76)	5.19 (0.41)	7.26 (0.13)	5.95 (0.33)	4.48 (0.83)	3.40 (0.23)

sands have produced tropical ferrugineous soils with a superficial gravelly horizon with pH values around 6; their texture and organic matter content are summarized in the Table 1.

All the experiments were conducted during the long wet season (May 1991).

Denitrification experiments

Potential denitrification was studied at two scales: (i) along a topographic gradient including all the locations described above and (ii) within the shrub savanna through a detailed study taking into account roots and fauna effects.

For the global study, 2 soil cores were collected in each location at two depths (0–10 cm and 40–50 cm) as far as possible from grass tufts and trees to avoid the rhizosphere influence.

For the 'site effect' study, 3 soil cores (controls) were excavated at two depths (0–10 cm and 40–50 cm) avoiding areas with roots and structures built by animals. 6 rhizospheric soil samples were collected by checking 6 grass tufts of the dominant species in this environment (*Hyparrhenia diplandra*). 6 chamber walls samples were obtained from the internal red layer of around 40 mixed fungus comb chambers built by the termite *Ancistrotermes cavithorax*. Fresh earthworm casts (produced during the previous night) were sampled soon in the morning directly on the surface of the soil (n = 6 after mixing). Immediately after sampling, the soils were thoroughly homogenized and 12 g of fresh soil were placed into 150 mL plasma flasks sealed with rubber stoppers. 3 mL of a solution containing sufficient nitrate for zero order kinetics (800 μg NO_3^--N) were added. The air in each flask was removed by inserting a syringe and connecting it to a vacuum pump for 5 min. The flasks were then flushed with helium for 5 min; this procedure was repeated 3 times to ensure a total anaerobiosis. 15 ml helium were then replaced by C_2H_2 to inhibit the N_2O reductase of the soil denitrifying bacteria (Yoshinari et al., 1977). The pressure into the flasks was adjusted to 1 atmosphere. Incubation was carried out at 37°C during 5 hours. Gas samples were stored in evacuated blood collection tubes (Vacutainer™) for subsequent N_2O analysis.

Nitrification experiments

The experiment has been performed in the two savanna types. In each type, 6 soil cores were sampled between 0 and −10 cm as controls. Rhizospheric soils from *Hyparrhenia diplandra* and *Loudetia simplex* (6 from each rhizosphere) were obtained as described above. The nitrification potential was measured according to Lensi et al. (1986): the NO_3^- produced during an aerobic incubation was deduced from N_2O-N measurements after an anaerobic incubation in the presence of C_2H_2.

Immediately after sampling, the soils were homogenized. From each soil sample, 3 subsamples of 30 g each were placed into 150 mL plasma flasks. The first subsample was immediately submitted to anaerobic conditions in order to measure the initial nitrate content. The second subsample was incubated in aerobiosis, after adding 3 mL distilled water, in order to measure the basic nitrifying activity. The third subsample was submitted also to aerobic conditions, after adding 3 mL of a solution containing 200 μg $(NH_4)_2SO_4$-N g^{-1} wet soil, in order to measure the potential nitrifying activity (potential of nitrification) without ammonium limitation. Then, the nitrifying flasks were sealed with parafilm (which prevents soil from drying but allows gas exchange) and incubated horizontally to ensure homogeneous aeration of the soil. After this aerobic incubation (37°C, 48 hours) which allows nitrate accumulation (Lensi et al., 1986), the soil samples were submitted to anaerobic conditions.

All the anaerobic incubations (for initial NO_3^- content and nitrification measurements) were performed as follows: in order to ensure sufficient number of denitrifiers for a rapid and complete transformation of NO_3^- to N_2O, the soil samples were supplemented with 10 mL of a suspension enriched in denitrifying organisms. This suspension was prepared by inoculating the riparian forest soil (the location of higher potential of denitrification) with a glucose and glutamic acid solution and anaerobically incubating this mixture during 48 hours at 37°C. It was verified that no nitrate remained in this suspension before addition to nitrifying soil samples.

Then, the soil samples were evacuated and

flushed with He in order to perform anaerobiosis and C_2H_2 was added as described above. The incubation was carried out at 37°C during 48 hours (sufficient time to obtain a total conversion of NO_3^- to N_2O). Gas samples were stored in evacuated blood collection tubes (Vacutainer™) for subsequent N_2O analysis.

N_2O analysis

Gas samples (200 μL) were analysed for N_2O on a gas chromatograph equipped with an electron capture detector, using a Porapak Q column. The characteristics of the analysis have been previously described by Lensi and Chalamet (1982). Control flasks without soil but containing distilled water supplemented with N_2O were prepared in order to verify that N_2O solubility had no significant effect on the results.

Results

Denitrifying activity along a transect

The intensity of the N_2O production by the soils sampled between 0 and −10 cm showed a higher denitrifying activity in the forest soils than in the savanna soils. The more active soil were the riparian forest one, followed by the gallery forest one, then the plateau forest one. The less active soil was the herbaceous savanna one, just after the shrub savanna one (Fig. 1).

In the forest soils, the denitrifying activity was much higher in the 0–10 cm layer than in the 40–50 cm layer. In the savanna soils, the difference was not so important and, in the case of the grass savanna, it did not exist.

Study of denitrification within the shrub savanna soil

As expected, denitrification potentials were very low in the average soil sampled between 0 and −10 cm and between −10 and −20 cm. On the contrary, in the soils where a recent biological activity has occurred, denitrification was very active. Compared to 0–10 cm control soils, it was 14.3 times higher in the walls of the termite fungus-comb chambers, 5 times higher in the samples collected very close to the roots and 4.3 times higher in the earthworm casts (Fig. 2).

Nitrifying activity

In all the soil samples, the initial nitrate contents, as measured by N_2O accumulation, was very low. On the contrary, under optimal conditions, the soil samples from *Loudetia* savanna exhibited a potential of nitrification, even without ammonium addition. The measured nitrify-

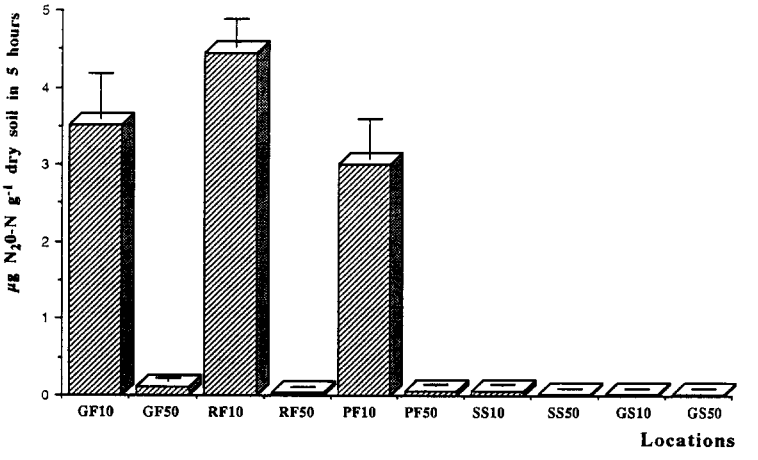

Fig. 1. Denitrifying activity according to the topographic gradient as μg N_2O-N g^{-1} dry soil after 5 hours incubation (GF: gallery forest soil; RF: riparian forest soil; PF: plateau forest soil; SS: shrub savanna soil; GS: grass savanna soil; 10: sampling between 0 and −10 cm; 50: sampling between −40 and −50 cm).

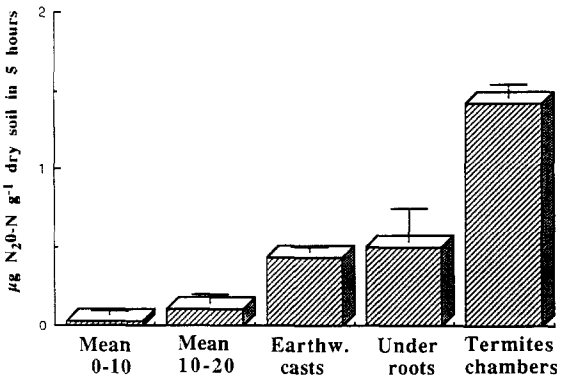

Fig. 2. Denitrifying activity within the shrub savanna soil as $\mu g\, N_2O\text{-}N\, g^{-1}$ dry soil after 5 hours incubation.

ing activities were similar under and between the tufts. (Fig. 3).

The soil samples from the shrub *Hyparrhenia* savanna exhibited a low as negligible potential of nitrification even after ammonium addition. The NO_3^- accumulation was higher between the tufts than under the tufts.

Discussion

The Lamto savanna is a heterogeneous ecosystem where different environments are easily distinguished. Tree density, soil texture, specific composition of the grass layer and soil fauna activity are the main components of this heterogeneity. Therefore, any attempt to quantify the processes controlling nitrogen oxides emissions will be fruitful only if this heterogeneity is taken into account.

Up to date, the results available about denitrifying activity in the Lamto savanna soils have been obtained on air-dried soil samples (Abbadie and Lensi, 1990). It was necessary, whatever might be the difficulties, to check if these previous observations are reliable using fresh material directly in the field. Globally and qualitatively, field measurements meet the results obtained in the laboratory, except for soil samples submitted to recent biological activity.

Denitrifying activity along a transect

The deep soil layers of all the environments exhibited a very low denitrifying potential. Only the surface layers, between 0 and −10 cm show a significant activity and the different environments can be arranged as follows: riparian forest > gallery forest > plateau forest > shrub savanna > grass savanna.

These results corroborate previous data by Abbadie and Lensi (1990) obtained on stored and air dried soil samples. From a qualitative viewpoint, air drying and storing have no effect on the potential of denitrification of the soils exempt of recent biological activity. That means likely that the differences observed between the locations are probably due to important and permanent differences of the soil microbial communities.

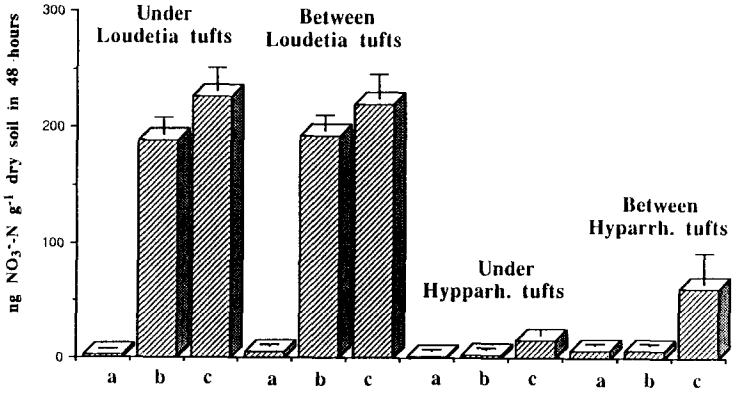

Fig. 3. Nitrifying activity in the shrub savanna surface soil (under and between *Hyparrhenia diplandra* tufts) and the grass savanna surface soil (under and between *Loudetia simplex* tufts) as $\mu g\, NO_3^-\text{-}N\, g^{-1}$ dry soil after 48 hours incubation (a: initial nitrate concentration; b: NO_3^- concentration at the end of the incubation period after water addition; c: NO_3^- concentration at the end of the incubation period after ammonium supply).

Detailed study of denitrification within the shrub savanna soil

A relatively high potential of denitrification has been measured on the fresh soils submitted to recent biological activity: walls of the fungus comb chambers of termites, earthworm casts and soil collected very near the roots of *Hyparrhenia diplandra*. On the contrary of what we observed at the scale of the transect, this higher denitrifying activity is likely due to transient modifications of the denitrifiers physiology. These modifications could imply labile chemical compounds produced by soil fauna or plants, like available carbon, quickly mineralized during drying.

However that may be, our field data show that particular sites influenced by biological activity may be active points of denitrification in bulk control soil which is rather inactive. Quantitatively, the impact of this fact on the global rate of denitrification in the savanna is still unclear. It depends mainly on the duration of the phenomenon (throughout the whole rainy season?), the volume of the soil modified by the underground biomass and the soil fauna, and the nitrate availability.

Nitrifying activity

The use of the transformation of NO_3^- to N_2O is a very sensitive method for measuring the native nitrate content of the soils or the level of the nitrification process. It allowed us to demonstrate that a non-negligible nitrifying activity exists in the Lamto savanna, in spite of its low intensity already underlined by De Rham (1973).

In the grass savanna, a potential of nitrifying activity has been observed under the tufts as well as between the tufts. The supply of ammonium had few effect, suggesting that in our experimental conditions, ammonium did not greatly limit nitrifying activity, at least during the wet season.

In the shrub savanna, the potential of nitrification is much lower, and clearly ammonium limited. More, nitrifying activity is strongly lowered under the tufts of *Hyparrhenia diplandra* compared to that between the tufts.

These results meet previous observations in Ghana (Meiklejohn, 1962) and Zimbabwe (Meiklejohn, 1968; Munro, 1966a) showing that several perennial African grasses, especially *Hyparrhenia* spp., strongly decrease the number of nitrite-oxidizers. Munro (1966b) has shown that roots of *Hyparrhenia filipendula* contained a water soluble substance which inhibits very efficiently nitrifying bacteria. This could explain why in humid savannas, whose grass layer is very often dominated by *Hyparrhenia* spp., rather all mineral nitrogen is under the form of ammonium. As nitrate is a more labile form than ammonium, very susceptible to leaching, this fact results likely in a better conservation of nitrogen within the ecosystem.

Nitrification is extremely low not only in the soil near the roots of *Hyparrhenia diplandra*, but in all the shrub savanna soil. Probably, the actual nitrification is rarely active in this location. Nevertheless, a potential does exist and nitrifying bacteria can contribute to plant nutrition, even at a low level. The nitrate produced can also be transformed in nitrogen oxides by the microbial denitrification process. Further research are needed on these points. Particularly, it is necessary to identify the ecological conditions which allow the simultaneous or successive expression of the nitrification and denitrification processes: soil water content, soil carbon content, relative specific composition of the grass layer.

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