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Organic nitrogen compartments in an alfisol of the western llanos of Venezueladuring three years of maize cultivation

Les compartiments de l'azote organique d'un sol lessivé des llanos de l'Ouest du Vénézuela après trois années de culture de maïs

HETIER Jean-Marie(1) URBINA Pedro (2), GONZALEZ-PRIETO Serafin ARAQUE Yajaira (2), CARBALLAS Tarsy (3)

(1) LCSC ORSTOM 911 Av. Agropolis 34032 Montpellier France
(2) ULA Facultad de Ingenieria Escuela de Quimica Mérida Venezuela
(3) Centro de Agrobiologia de Galicia CISC 15700 Stgo. de Compostela Espana

The increasing agricultural pressure on soils derived of the South American savannas appears to be inevitable. In these soils as in others tropical soils, the main part of the nitrogen azbsorbed by the plants come from the soil and not from the fertilizer. It is thus necessary to examine the short and long term consequences of the intensification of the production on the reserves of organic nitrogen.. The alfisols in the Venezuelan llanos are interesting as they represent some of the best arable acid soils in the Latin American savannas.
A compartimentalization of organic nitrogen was performed for modelling purposes over a three consecutive years period of corn cultivation on an alfisol of Barinas serie after an initial labelling with 15N urea.
The soil was sampled five time per year: before seedling, and four times during crop cycle..The first two years, nitric and ammoniacal nitrogen, microbial biomass, dispersible metabolites (by NaHCO3 after extraction of mineral nitrogen), oxydable nitrogen compounds (by K2MnO4 in acidic medium) or hydrolysable nitrogen compounds (HCL 1M then 3M). Only hydrolyses were performed the third year.
The results were interpreted based on total nitrogen of each fraction, flows through the fractions and fraction labelling compared to that in the plant.
All the fractions studied had nitrogen outflows equal to or higher than the plant's annual requirements: approximately 100 kg N of which 33 fertilizer-derived the first year, 3 the second year and 0,3 the third year. The shortest turnover time for nitrogen was not found in the microbial biomass nitrogen but in the dispersible metabolites. The first fraction of the hydrolisis, (H1 half of total nitrogen) was renewed more rapidly than the second (H2) or the non-hydrolysable residue (R).
It thus appears possible to use this method of fractioning as a basis for defining compartiments which can be used in modelling both nitrogen uptake by the plants and changes in organic nitrogen reserves in the soil.

Keywords : nitrogen, maize, microbial biomass, hydrolisis modelling
Mots clés: azote, maïs, biomasse microbienne, metabolites, hydrolyses, modélisation

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Introduction

The increasing agricultural pressure on soils derived of the South American savannas appears to be inevitable. It is thus necessary to examine the short and long term consequences of intensive production on the reserves of organic nitrogen.. The alfisols of Barinas serie in the Venezuelan llanos are interesting as they represent some of the best arable acid soils in the Latin American savannas (Hétier et al 1992).

The most important scientific basis of the present work was well re-defined in a recent review (Jarvis et al 1996) whose introduction remind us that « the pools defined in models are often conceptual and few can be really measured by physical, chemical or biological methods ». The necessity to find more appropriate definitions has often been pointed out by model designers (Molina et al 1994) and is particulary important for tropical systems where the rapidity of gross mineralization and immobilization processes increase both the spatial and temporal variability of mineral nitrogen.

The physical methods of granulometric fractionation initially seemed a more promising approach (Feller et al. 1983). However, in Barinas, all the crop residues desappeared during the month necessary for soil preparation before seedling and only biological and chemical fractionations could be used to test the turnover time for organic nitrogen fractions. Nevertheless both methods can be complementary. For example, a significative difference between the chemical composition of external and internal nitrogenous compounds of soil aggregates was recently evidenced (Balabane 1996). Thus a pertinent information could also be obtained from chemical fractionation.

The first step was to obtain an experimental definition of some organic nitrogen compartments through in situ labelling and periodical soil sampling of nitrogen in the plants, in the soil mineral fractions, microbial biomass and four organic fractions selected by chemical solvents with increasing aggressivity. The interpretation of the results made

it possible to suggest an alternative to the standard procedures which are generally based on mineral nitrogen and microbial biomass measurements performed on fresh soil and some conceptual compartments in organic nitrogen (de Willingen 1991).

Material and Method

Site, Climate and soil

The study was performed in the Botanical Garden of the Experimental University of Llanos, Unellez at Barinas (8°37'N, 70°12'W, altitude: 180m). The climate was typical of seasonal savannas i.e. mean annual temperature of 26°C, a four month dry season (about 300mm), a rainy season (P:about 1300 mm) with heavy initial precipitation .

The soil on which the maize was grown was an alfisol (Kandic Paleustalf isohyperthermic with a well-balanced texture), low in organic matter (C: 8 mg kg^{-1} N: 0.55 mg kg^{-1}) The whole experimental field was first tilled and fertilized by 100 kg P ($Ca_3(PO_4)_2$) and K (KCl) then fertilized with 132 kg N ha^{-1} (Urea).

Sampling and analysis

Sufficiently large and homogenous labelled plots made it possible to obtain representative plant and soil sampling without overly disrupting normal crop cycles. After soil preparation and fertilization maize was sowed at a initial density of 55 000 plants ha^{-1} . Three weeks after sowing, labelled urea was applied as uniformly as possible to the 4 microplots (2 x 6 m) between the 0,9m spaced rows at the same rate of 132 kg N ha^{-1}. The isotopic excess of nitrogen was 3,85 % .

For all years, the first samples, PO, were taken at sowing, P1: 30 days later, i.e. 8 days after urea application, P2: one month afterwards (60 days after planting (« DAP »), P3: 101 DAP and P4: at final harvest, i.e. 130 DAP.

During the crop cycle, 3 plant samples were analysed in each of the 5 labelled microplots. Three soil samples of approximately 5 kg of fresh soil were taken using an auger in the labelled microplots. A composite sample of each labeled microplot was analysed for mineral nitrogen using 2M KCl with a soil/solution ratio of 1/3 .

Four additional analysis were performed during 93 and 94: microbial biomass (Amato & Ladd 1988), dispersible metabolites (by NaHCO3 after extraction of mineral nitrogen (Fox & Piekilek 1978)), oxydizable nitrogen compounds (by K2MnO4 in acidic medium (Juma & Paul 1984)) or hydrolysable nitrogen compounds (1M HCl then 3M (Gonzalez-Prieto & Carballas 1992)). Only hydrolyses were performed in 95. The isotopic excess of the labelled samples was measured by optical spectrometry above 0,1% excess values and by high resolution mass spectrometer below these values.

Spatial variability was measured using twelve samples taken off the same day from plots used for related experiments (C.V. about 15% for N total, and 30% for $N\text{-}NH_4^+$, 40% for $N\text{-}NO_3^-$ above of 5 mg kg^{-1}). The analytical variability decreased from microbial biomass (from 10 to 45%) to metabolites (5 to 15%) oxydizable compounds (3 to 10%) and hydrolysable fractions H1, H2 (3 to 10%). The variability of isotopic excess below 0,1 % was similar to that of N and was two times lower above this threshold.

Results and discussion

Plant nitrogen derived from the fertilizer during the three years (Fig 1)
At P1, nearly half of the fertilizer had already been lost. The immobilization of Ndff was also nearly complete. When the fertilizer was added, the nitrogen content of above ground parts of maize was approximately 8 kg Ndfs (Nitrogen derived from the soil). During the week following the fertilization in the first year, about 6 kg Ndff (Nitrogen derived from the fertilizer) and 1 kg Ndfs were absorbed from a soil solution whose N content decreased from 145 to 60 kg while Ndff % decreased from 90 to 60% yielding a total of 14 kg N (including the 7 kg Ndfs previously absorbed). The following samples demonstrated losses of part of the absorbed Ndff and illustrated the need to include mineralization of organic reserves to explain the nitrogen nutrition between P3 and P4.

To extend the interpretation further, a Ndff balance was designed based on three hypothesis:
- the grain does not lose but only accumulates nitrogen mainly coming from translocation
- the translocated nitrogen reaches the grain with the isotopic excess of the green parts.
- the same isotopic excess was attributed to the possible losses from the plant.

Table 1 was generated to provide an example of probable repartition of translocation, losses and absorption during the last two months of maize cultivation. At P2, only 6% of mineral nitrogen was derived from the fertilizer and its level was already back close to the initial level of 20 kg N. At P3 and P4 time, a net increase of 24 kg N in the plant resulted of the active absorption of 30 + 14 kg N by the plant (1,2 then 0,3 of which were derived from the fertilizer) and of losses of 8 + 11kg N (4 then 3 of them were derived from the fertilizer). At the end of the first crop cycle, the nitrogen absorbed by the plant was approximately twice as labelled as total nitrogen in the soil.
The main conclusion whch can be drawn from all these results, is that net mineralisation during the second part of maize cultivation decreased from 1 to 0,5 kgN day^{-1} and was almost (96 to 98%) derived from the soil.

Nitrogen fractionation during the three years (Fig. 2 & 3)
The interpretation of the results presented in Fig.2 was based on quantity of nitrogen in each fraction, outflow when it could be assimilated to a first order reaction, and its Ndff % compared to that in the grain at final harvest of each year.
Mineral nitrogen and three organic fractions have nitrogen outflows which could considered to be a first order reaction: dispersible metabolites, microbial biomass and H1 fraction of hydrolisis. A combination of these organic fractions may be used to run models. Metabolites and microbial biomass look like more adequate for mechanistic models. But both fractions show high spatial and analytical variability and must be measured on fresh soil samples. On the contrary, hydrolisis may be performend on dry samples and repeated up to the required precision. The H1 fraction associated with a sink has a volume and outflow suficient to simulate all the three main processes: nutrition of the plant, leaching and gaseous losses.
Fig 3 shows that nitrogen of the plant and of H1 were isotopically quite identical during the second year. During the third year, the grain was less labelled than the H1 fraction and than total nitrogen due to two successive unlabelled fertilization. Thus the H1

fraction is at least partially renewed each year and implied in plant nutrition. During this same third year, hydrolisis was an appropriate discriminating method, as the second fraction and the non-hydrolysable residue were less labelled than the plant and total nitrogen

It thus appears possible to use this method of fractioning to define compartments which can be used to design models both of nitrogen uptake and of the evolution of organic nitrogen reserves in the soil.

Hydrolisis offer also a way of extending more to mechanistic modelling because it was possible to separate organic compounds in the hydrolysate such as amides, osamins, amino acids. Stability indexes (S.I.)for Ndfs and Ndff of each group of compounds in Tab. 2 correspond to the final nitrogen content of each fraction as % of the initial content.

Newly formed amides look very unestable probably because they were including urea. Newly formed osamines represented a small fraction which closely corresponded to the definition of a labile pool. Amino acid form a heterogeneous set in which a sub-groups of decreasing stability remain to be separated.

Conclusion

For fractions of organic nitrogen as metabolites or microbial biomass, a rather high analitical variability added to the already high field variability will always limit the accuracy of mechanistic models.

When crop residues represent less than 5% of total nitrogen in the soil granulometric fractioning is useless, thus hydrolisis still may be an interesting way of fractioning organic nitrogen to define labile nitrogen. The H1 fraction may already be used in empirical models such as labile nitrogen compartment.

Within this first H1 fraction, the Osamine group looks like a labile pool. Within the large amino-acid group, sub-sets of decreasing lability still remain to be fractionated by apropriate methods.

Litterature

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Fig 1 Evolution of mineral, absorbed and immobilized nitrogen during two successive years of maize cultivation.

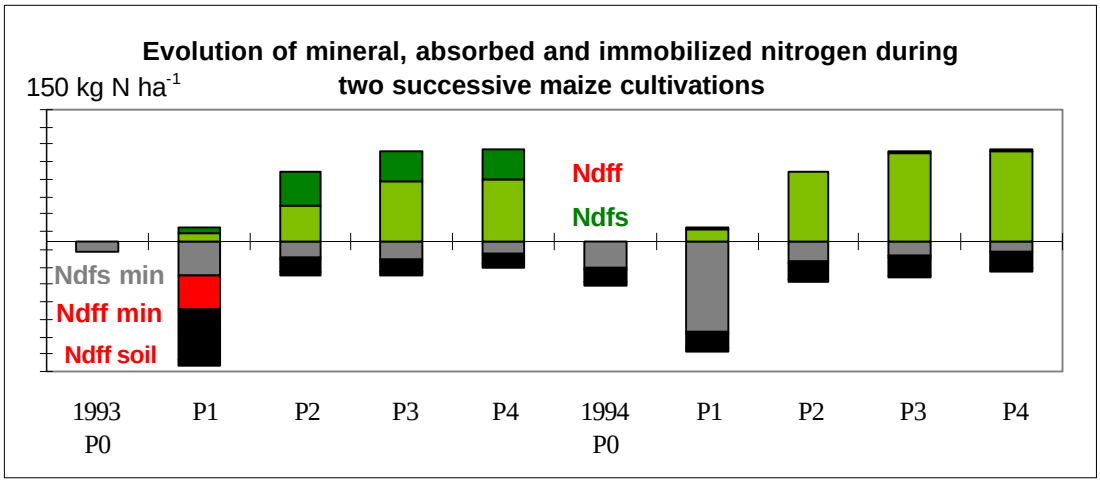
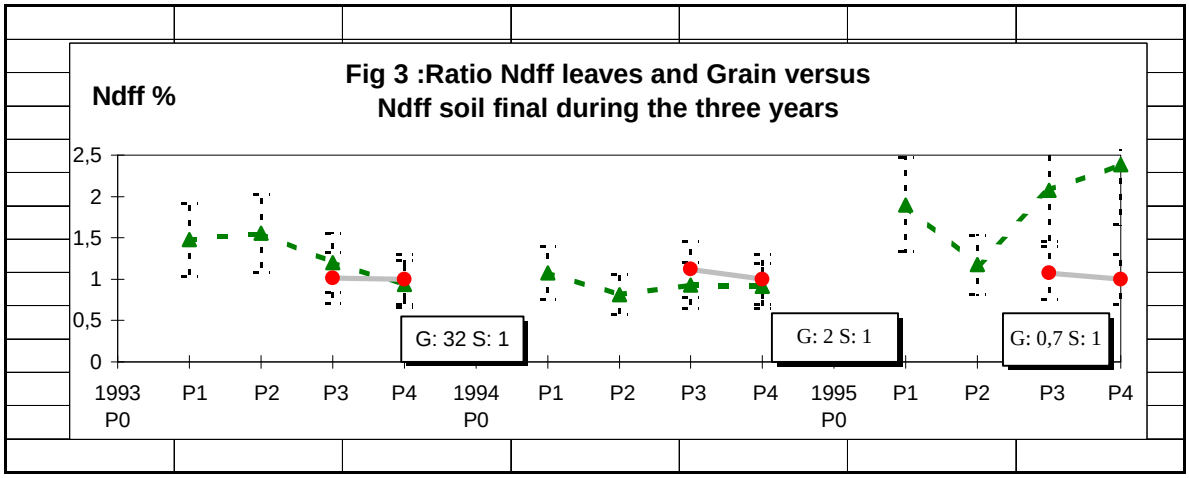


Fig3 Relationships between Ndff of green parts, grain and N total of the soil (0-20cm) during the three years of maize cultivation



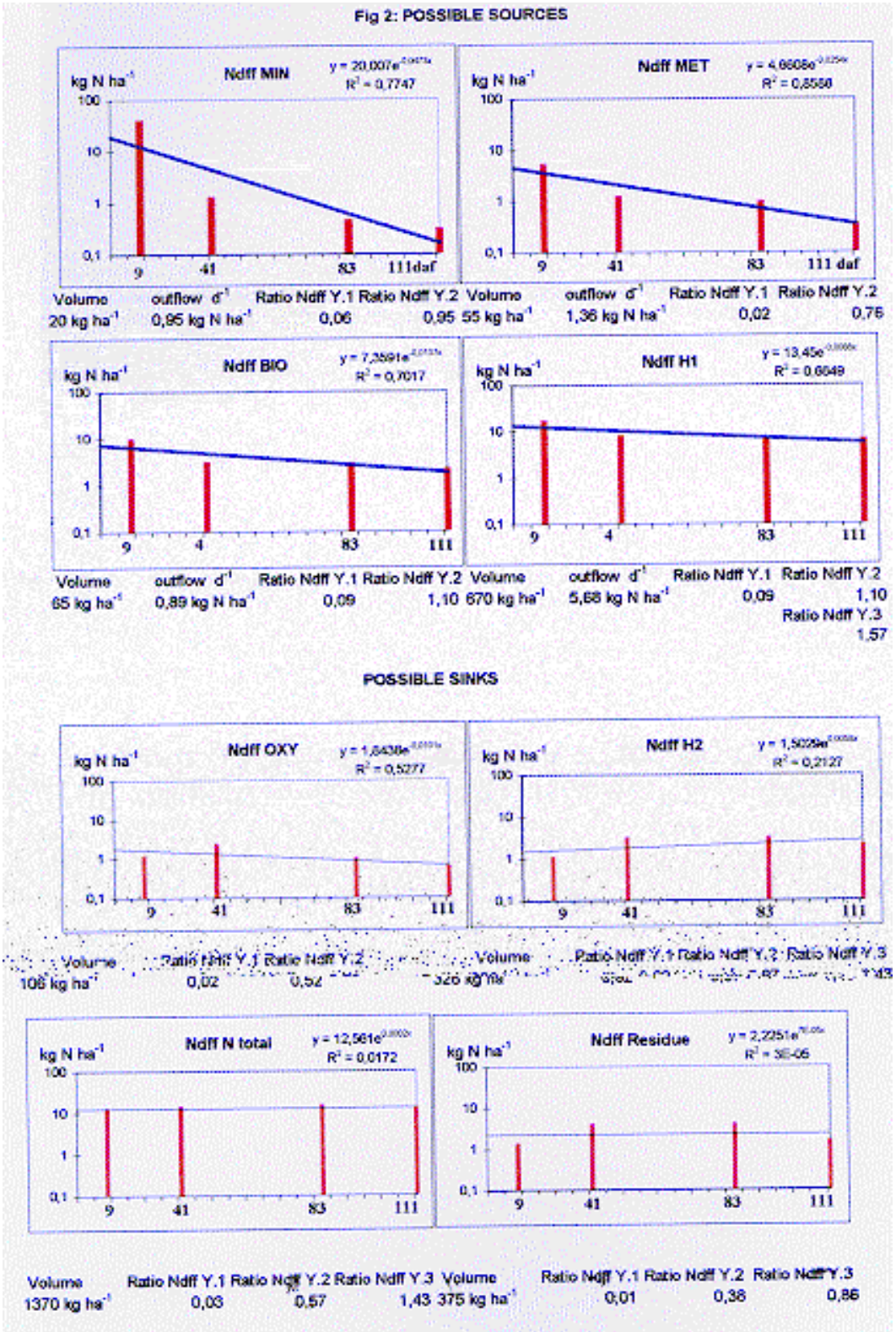


Fig 2: Nitrogen fractions as possible source and sinks compartments.

(The annual nitrogen extraction by plants was and the amounts of nitrogen in each organic fraction were considered constant during the three years.)

Table 1:Translocated and absorbed nitrogen during maize cultivation

Process	Sampling	Ndff	Ndfs kg ha^{-1}	Total
	P2 Nitrogen content of the plant			
Total P2	**Green Parts**	**40**	**39**	**79**
P3	Nitrogen content of the plant			
	Green Parts	17	26	43
	Grain	19	39	58
Total P3		**36**	**65**	**101**
Translocation		18	18	36
Losses		4	4	8
Absorption		**1,2**	**28,8**	**30**
P4	Nitrogen content of the plant			
	Green Parts	11	25	36
	Grain	22	46	68
Total P4		**33**	**71**	**104**
Translocation		2,5	6,5	9
Losses		3	8	11
Absorption		**0,3**	**13,7**	**14**

Tab. 2 Ndfs and Ndff stability indexes during the first cultivation

Fractions	P1 Ndfs kg ha^{-1}	***Ndff kg ha^{-1}***	P4 Ndfs kg ha^{-1}	***Ndff kg ha^{-1}***	S.I. Ndfs (P4 - P1)/P1 %	**S.I. Ndff (P4 - P1)/P1 %**
Amides	107	***4***	106	***0,4***	1	***91***
Osamins	47	***3***	23	***0,3***	51	***88***
Amino acids	268	***5***	204	***1,4***	24	***70***
H1	623	***17***	582	***5,4***	7	***681***

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