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Changes in the soil organic N fractions of a tropical Alfisol fertilized with ^{15}N-urea and cropped to maize or pasture

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Abstract

Qualitative and quantitative changes in soil and fertilizer-derived organic N fractions were assessed during a cropping season in an intertropical Alfisol, under maize and pasture, fertilized with ^{15}N-urea. Before the sowing, after fertilizing and after the harvest, the organic N of top soil samples was fractionated by a two-step acid hydrolysis under reflux (H1 = 1 *M* HCl for 3 h; H2 = 3 *M* HCl for 3 h). The total hydrolysable N (HN) from H1 decreased significantly during the cropping season in both maize and pasture soils. Contrastingly, the content of HN from H2 and that of non-hydrolysable N did not vary significantly during the cropping season. The easily hydrolysable fractions, especially amino acid N, amino sugar N and amide N, were the most active N pools and the major source of N potentially available for plants. The urea-derived N that remained in the soil was mainly in organic forms at both 7 and 108 d after fertilizing (70–82% and 93–98%, respectively), higher figures being found in pasture than in maize soil. The total amount of urea-derived HN decreased significantly during the crop period in both maize and pasture soils. This decrease was largely due to the decline in HN from H1. The amount of non-hydrolysable urea-derived N was significantly higher in pasture than in maize soil and it decreases in the former and increases in the latter, during the cropping season. During the crop period, the decrease of urea-derived organic N was 4.6 to 9.1 times higher than that of native organic N. Shortly after fertilizing, the proportion of urea-derived N in the easily hydrolysable (H1) organic fractions was higher than that of soil N, whereas the reverse was true for the slowly hydrolysable (H2) or insoluble fractions. These differences were less marked, but still significant, at the end of cropping. The easily hydrolysable organic N fractions were more sensitive than total N to the impact of land use intensification and are, therefore, a more useful index for early detection of soil biological degradation.

Introduction

Nitrogen is the most common limiting nutrient in crop production (Jenkinson, 1981; Ta et al., 1989) and its status in the soil has been proposed as an index of soil biological degradation (Carballas et al., 1986). The N derived from fertilizers is rapidly transformed into those organic forms (Kelley and Stevenson, 1987; Legg et al., 1971; Smith et al., 1993) that dominate soil N (Stevenson, 1982). N fertility is so closely associated with transformations and changes in soil organic N, both endogenous and exogenous, that Peoples et al. (1995) have stated that the key to the long-term sustainability and productivity of soils is organic matter. Jansson (1971) highlighted the need of methods, biologically justified, for the fractionation of the organic matter coupled with experiments on the transformation of labelled known substances. Jenkinson (1971) reported that acid hydrolysis are more efficient than any other method tested to reveal the presence of material biologically stable, and the continuous hydrolysis technique has been used for this purpose by sever-

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al authors (Kelley and Stevenson, 1989; Pal et al., 1989). Nevertheless, continuous hydrolysis provokes both deamination and neoformation of organic compounds, which are prevented by step-wise hydrolysis (Barriuso et al., 1987; González-Prieto and Carballas, 1988), and, moreover, it has severe limitations to differentiate between labile and recalcitrant organic compounds (Campbell et al., 1991; Juma and Paul, 1984). Therefore, to improve the understanding of N cycling in soil there is still a need for development of methods to quantify biologically-meaningful fractions of soil organic matter which turn over in the short or medium term (Magid et al., 1996).

The Orinoco Llanos is a neotropical region covering more than 500,000 km^2 in Northern South America. The original savannas, dominated by perennial bunch grasses of low nutritive value, are being increasingly replaced by annual crops (maize, sorghum, cotton) and improved pastures (Hétier et al., 1989). Alfisols and Vertisols are the soil types more frequently used for crop production.

In contrast to other intertropical regions, soil degradation promoted by land use intensification can still be prevented in the Orinoco Llanos. To achieve this objective it is important to have a better understanding of fertility changes produced after soil cultivation, particularly changes in the organic N pool.

For these reasons, a field experiment was performed during a crop season in the Orinoco Llanos. The objective of this study was to assess the dynamics of the endogenous and the ^{15}N-urea-derived soil organic N fractions in an intertropical Alfisol, under maize or pasture, fertilized with ^{15}N-urea. For this assessment, it was used the step-wise acid hydrolysis method developed by González-Prieto and Carballas (1988), which in a previous study (González-Prieto et al., 1992) has showed its capacity to differentiate between the labile and the recalcitrant organic N in laboratory experiments.

Materials and methods

Study site

The experiment was conducted in the Botanical Garden of the Universidad Nacional Experimental de los Llanos Occidentales Ezequiel Zamora at Barinas (Venezuela, 8°37′ N 70°13′ W, 220 m a.s.l.). The tropical climate of this station shows a sharp contrast between the wet and dry season. During the wet season monthly precipitation can reach as much as 400 mm, the dry season from December to March can be rainless. The mean annual rainfall is 1700 mm (range 1200–2000 mm). The mean annual temperature is 26 °C with only small variations. The soil is a 2.5 m deep Oxic Tropustalf whose main characteristics are listed in Table 1.

Soil texture was determined by the international mechanical analysis method. Soil pH was measured in 1 *M* KCl (soil:solution 1:2.5). Total CEC was determined by extraction with 1 *M* ammonium acetate at pH 7. Organic C content was determined by dry combustion and CO_2 measurement in a Carmhograph 12 (Whostoff, Germany). Total N content was measured by a modified Kjeldahl digestion (Guitián Ojea and Carballas, 1976).

Installation of the plots

Two days before sowing the soil was uniformly fertilized with 100 kg of P ha^{-1} as superphosphate and 100 kg of K ha^{-1} as KCl. *Digitaria decumbens* pasture, which had been established vegetatively three years before by planting sprigs and maintained without fertilization before this experiment, was cut before the experiment began. Maize (PB8 variety) was sowed by hand on 75 rows of 100 m length each with a distance of 0.9 m between rows. Two seeds were spaced every 20 cm along the row. Three weeks after sowing some maize plants were thinned to leave only 5 plants m^{-1}, which represented a nominal density of 55,000 plants ha^{-1}. Twenty-three days after maize sowing (DAS), 5 plots of maize (6 rows of 11 plants each) and 4 plots (0.9 m × 2 m) of pasture were labelled with a solution of ^{15}N urea (3.93 atom % ^{15}N excess) at a rate of 130 kg of N ha^{-1}. Maize was harvested to ground level after maturity (131 DAS) and pasture was cut 42 and 131 DAS.

Fractionation of soil organic N

The top layer (0-20 cm) of maize soil and *Digitaria* soil was sampled 0, 30 and 131 days after sowing (DAS) and sieved (< 2 mm) to remove roots. Two replicates of each soil sample containing 50 mg of N were fractionated by a simple step-wise acid hydrolysis (González-Prieto and Carballas, 1992) (Figure 1). Because of the low N content of the soil, the sample size used for the hydrolysis was 65 g, which is twice the maximum weight of sample used by González-Prieto and Carballas (1991). Therefore, to maintain the soil:acid ratio

Table 1. Main characteristics of the soils studied

Depth (cm)	pH H_2O	Sand (%)	Silt (%)	Clay (%)	C (g kg^{-1})	N (g kg^{-1})	C/N	CEC (cmol kg^{-1})	BS* (%)
0–20	5.2	72	11	17	7.0	0.51	14	11.3	72
20–40	5.2	61	10	29	6.5	0.40	16	11.4	74
40–100	5.7	55	12	33	3.3	0.30	11	12.1	93

*Base saturation of CEC.

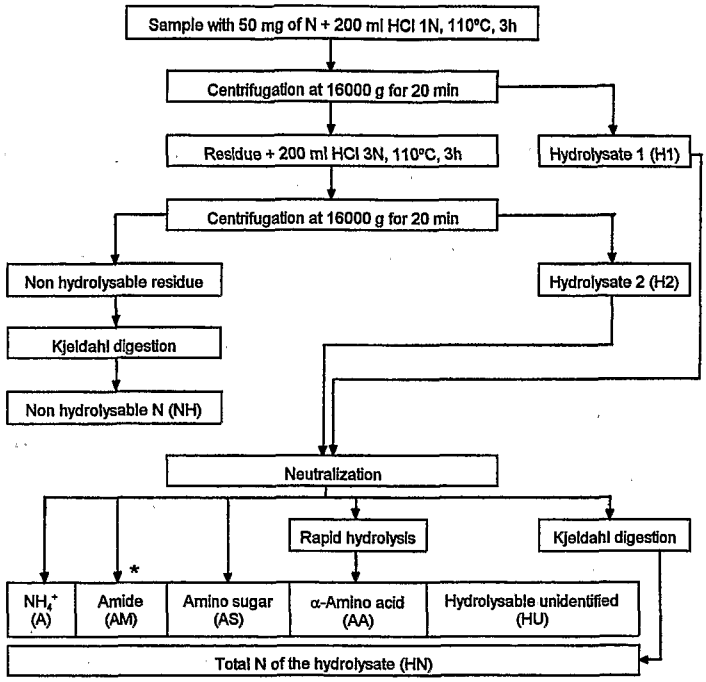

Figure 1. Diagram showing the step-wise acid hydrolysis employed and the organic N fractions obtained. Note: amide N is completely extracted in HI.

below 1:3 as recommended by these authors, the quantity of acid used was increased from 100 to 200 mL. To compensate for the volume increase, the hydrolysates were concentrated to 100 mL by rotary evaporation at 40 °C before neutralization. Both the neutralization of the hydrolysates and the determination of the various forms of organic N were carried out using Yonebayashi and Hattori's (1980) improvements of the original technique by Bremner (1965) and the modifications made by González-Prieto and Carballas (1988).

Under the specific conditions for each form of N (Yonebayashi and Hattori, 1980), the resulting ammonia was steam distilled and collected into 10 mL of 0.005 *M* H_2SO_4 and measured by back titration of the excess of H_2SO_4 with 0.01 *M* NaOH. Two distillates were made and titrated for each form of N. The resulting ammonium sulphate solutions were then acidified with 5 mL of 0.005 *M* H_2SO_4 and oven dried at 80 °C near a vial of 18 *M* H_2SO_4 to prevent possible contamination by atmospheric ammonia.

Nitrogen-15 abundance was determined with a Finnigan MAT delta S mass spectrometer (Finnigan, Bremen, Germany) operating in continuous flow on line with a CN elemental analyser (SCA CNRS, Vernaison, France) as described by Pachiauchi et al. (1991). For isotopic abundance measures the sample size was 50 μg of N and the precision obtained was ± 0.3 δ.

Cross-contamination among samples was prevented by observing the following precautions: glassware was cleaned by immersing it in 18 *M* H_2SO_4 for 1 h (based on Vanden Heuvel and Giamalva, 1988); two

aliquots of each sample were distilled consecutively, discarding the isotopic value of the first distillate if cross contamination with the preceding sample was detected (Mulvaney, 1986); and 25 mL of 95% ethanol were distilled for 3 min between samples (Mulvaney, 1986).

Data were statistically analysed by one-way ANOVA and significant differences were distinguished by the Tukey-HSD test at $p < 0.05$ level.

Results and discussion

Dynamics of unlabelled soil organic N fractions

The evolution of the endogenous N content in the 0–20 cm layer of both the maize and the pasture soils during the crop season is shown in Table 2.

The amide N, which is completely extracted with hydrolyzate 1 (H1) and is calculated by difference between the NH_4^+–N content in H1 and the initial content of exchangeable NH_4^+–N (González-Prieto and Carballas, 1988), decreased significantly during the first month of cropping but not after that (Figure 2).

The concentration of ammonium N in hydrolyzate 2 (H2), which is derived either from decomposition of organic compounds or from fixed NH_4^+, remained unchanged during the first month of cropping (Figure 3); thereafter, ammonium content increased significantly, in both maize and pasture soils, in agreement with the results of Pal et al. (1987). Considering that this ammonium N fraction is positively related to silt content (González-Prieto and Carballas, 1991), its increase between 30 and 131 DAS could be due to an increase of silt-fixed ammonium or of organic compounds decomposable with HCl 3 *M*. This behaviour could be explained by chemical interactions between soil N and urea N (Sen and Chalk, 1993) and/or by the enhancement of the decomposition of the organic matter by living roots through the stimulation of microbial activity (Cheng and Coleman, 1990).

The amount of amino sugars extracted with H1 in the maize soil remained unchanged during the first month of culture, but there was a significant decrease at harvest (Figure 2); the amino sugars extracted with H2 decreased continuously during the cropping period (Figure 3). The pasture soil exhibited a significant increase in the amino sugar fraction during the first month of cropping; thereafter it decreased almost to the original levels (Figures 2 and 3). This temporal increase of amino sugars from H1 and H2 could be related to a transient increase in the soil microbial population, with the consequent formation of bacterial cell-walls which contain N-acetyl-glucosamine (Parsons, 1981). The significant diminution of the amino sugars content from 30 to 131 DAS, in both the maize and the pasture soils, suggests that these compounds were part of an active pool of mineralizable N. Although these results contrast with the lack of change found by Pal et al. (1987), they are similar to those reported by Parsons (1981) who found rapid mineralization in the absence of stabilizing agents in the soil.

The α-amino acid fraction from H1 decreased significant and continously in both soils during the cropping season (Figure 2), whereas that from H2 showed a transient increase 30 DAS, which was only significant for the pasture soil, and a significant decrease after that (Figure 3). A diminution of α-amino acids derived from both recently immobilized fertilizer N and endogenous organic matter during the cropping season was also reported by Kelley and Stevenson (1987) and Pal et al. (1987). The greater depletion of α-amino acid N in cultivated soils compared to adjacent virgin soils was also reported by González-Prieto and Carballas (1991) and Sowden (1956). These results suggest that the α-amino acid N could be considered as an active N pool in the soil and a major source of potentially available N for plants.

In the maize soil, the amount of hydrolysable unidentified N (HU N) extracted with H1 was similar at 0 and 131 DAS, showing a transient and significant decrease 30 DAS, but that from H2 increased continuously. In the pasture soil, the evolution of HU N during the crop season showed a transient and significant decrease from 0 to 30 DAS, the initial content being almost completely regained by 131 DAS (Figures 2 and 3). The dynamics of HU N in the maize soil agrees with the increase of HU N during the crop season in cultivated soils reported by Kelley and Stevenson (1987) and Pal et al. (1987). The different behaviour of HU N in the pasture soil, which did not show a net increase at harvest compared to the levels at sowing, is difficult to explain due to the heterogeneus nature of this N fraction but it could be related to the different rhizosphere conditions between pasture and cultivated soils.

The total organic N hydrolysable with H1 (i.e. all the hydrolysable forms considered as a whole) decreased significant and continuously during the crop season in both maize and pasture soils (Figure 2), in agreement with the findings of Pal et al. (1987). This

Table 2. Evolution of the endogenous and urea-derived N content in the 0-20 cm layer of the maize and the pasture soils during the crop season

Soil	Endogenous soil N (mg N kg^{-1} soil)			Urea-derived N (mg N kg^{-1} soil)		Urea-derived N (% applied urea)	
	0 DAS*	30 DAS	131 DAS	30 DAS	131 DAS	30 DAS	131 DAS
Maize	565.4 ± 7.8	533.0 ± 6.6	528.2 ± 7.1	12.91 ± 0.13	3.7 ± 0.05	29.8	8.5
Pasture	602.1 ± 8.1	537.9 ± 4.7	535.9 ± 5.4	15.13 ± 0.06	6.01 ± 0.03	34.9	13.9

*DAS–days after maize sowing.

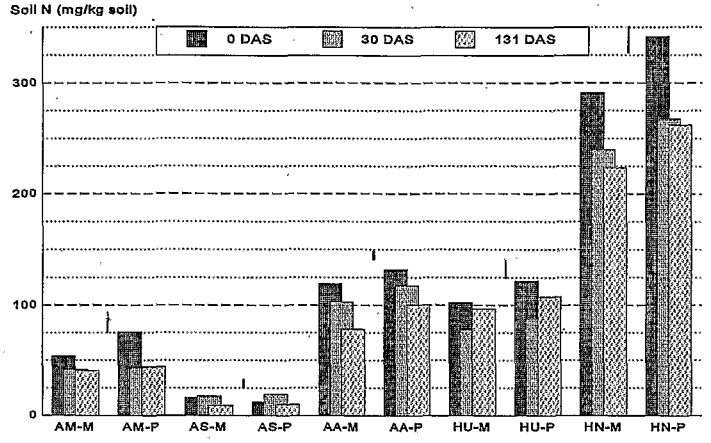

Figure 2. Soil organic N hydrolysed in H1. Quantitative changes in the fractions during a crop season in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS – days after sowing, AM–amide N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN−(AM+AS+AA)), HN–total N of the hydrolysate.

decrease was higher during the first month of the growing period. Contrastingly, the total organic N hydrolysed with H2 did not vary during the cropping season in pasture soil and it increased slightly in maize soil (Figure 3).

These results showed that the most easily hydrolysable fractions, which include the more recently incorporated N (González-Prieto et al., 1992), were the most active N pools in the soil and the major source of potentially available N for plants. The step-wise hydrolytic method overcomes some of the limitations of continuous hydrolysis to differentiate between N compounds from the active and recalcitrant organic phases reported by Campbell et al. (1991) and Juma and Paul (1984). The present results suggest that the mechanisms leading to loss of soil organic material are not independent of the chemical properties of the organic fractions, contrastingly with the statement of Capriel et al. (1992).

The content of non-hydrolysable organic N tended to increase slightly during the crop season, but the difference between 30 DAS and 131 DAS was only significant in the maize soil (Figure 3). This result agrees with the greater crop availability of the labile hydrolytic N fractions discussed above.

Dynamic of organic N derived from ^{15}N labelled urea

The evolution of the urea-derived N content in the 0–20 cm layer of both the maize and the pasture soils during the crop season is shown in Table 2.

Organic N accounted for 70% (maize) and 82% (pasture) of the total urea-derived N that remained in the soil 30 DAS. These percentages increased to 98 and 93%, respectively, after the harvesting in agreement with the findings of Reddy and Reddy (1993) and Timmons and Cruse (1990) who reported that most of the fertilizer remaining in the soil at the end of the growing season was in the organic fraction.

The amide N accounted for important and similar amounts of urea-derived N in both maize and pasture soils at 30 DAS (11.5–13.2% of the applied urea). A significant high net decrease was observed in this fraction at the harvest (Figure 4).

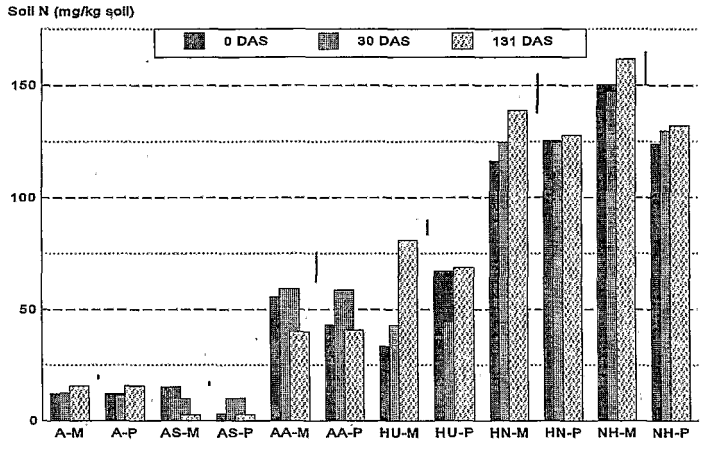

Figure 3. Soil organic N hydrolysed in H2 and non-hydrolysable N. Quantitative changes in the fractions during a crop season in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS–days after sowing, A–NH_4^+-N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN–(AM+AS+AA)), HN–total N of the hydrolysate, NH–non-hydrolysable N.

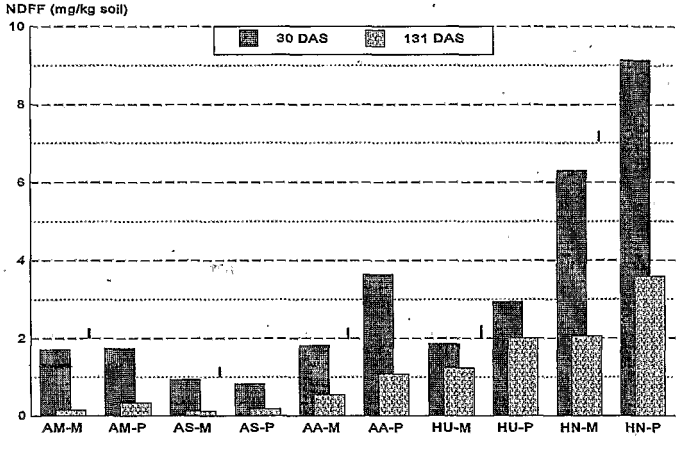

Figure 4. N derived from the fertilizer (NDFF) hydrolysed in H1. Quantitative changes in the organic fractions during a crop season in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS–days after sowing, AM–amide N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN–(AM+AS+AA)), HN–total N of the hydrolysate.

The ammonium N was one of the urea-derived N fractions of lesser importance in the two soils both at 30 and 131 DAS, accounting for 3.3–5.4% and 1.6–1.8% of the applied urea, respectively. Ammonium N was significantly higher in the maize soil than in the pasture soil a week after fertilization (30 DAS), but not at harvesting because of the higher decrease of this N fraction during the crop season in the maize soil (Figure 5).

The evolution of the urea-derived amino sugar N was the same in both maize and pasture soils and for both hydrolysates: at 30 DAS they had similar contents which decreased significantly and similarly thereafter (Figures 4 and 5).

The fertilizer-derived amino acid N decreased significantly during the crop season as was reported by Kelley and Stevenson (1987). At each time, the amino acid N content was higher in the pasture than in the maize soil. As for amino sugar N, this pattern was similar for both hydrolysates although the net decrease from 30 to 131 DAS was higher for the amino acid N extracted with H1 (Figures 4 and 5).

The dynamic of the urea-derived hydrolysable unidentified N (HU N) was more complex than that of the other fractions, probably due to the heterogene-

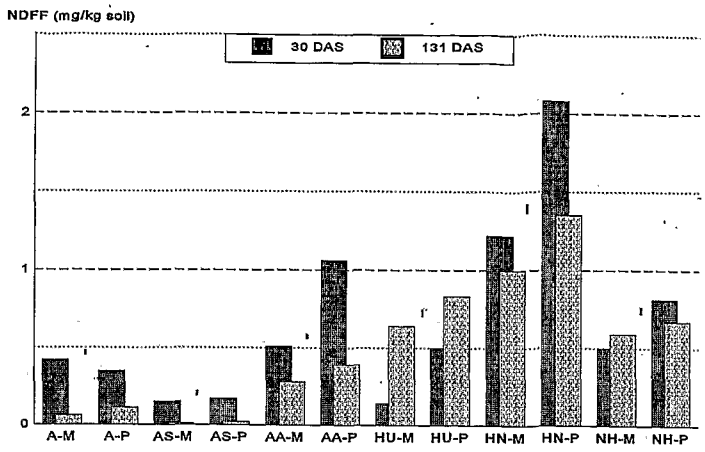

Figure 5. N derived from the fertilizer (NDFF) hydrolysed in H2 and non-hydrolysable NDFF. Quantitative changes in the organic fractions during a crop season in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS–days after sowing, A–NH_4^+–N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN–(AM+AS+AA)), HN–total N of the hydrolysate, NH–non-hydrolysable N.

ity of this N pool. In both maize and pasture soil, HU N of H1 decreased significantly from 30 to 131 DAS whereas the reverse was true for HU N of H2 (Figures 4 and 5). As with the urea-derived amino acid N, at each time the amount of the HU N was higher in the pasture than in the maize soil.

Reflecting the evolution of the major urea-derived organic N fractions, the total hydrolysable N (HN) decreased significantly during the crop season in both maize and pasture soils (Figures 4 and 5). The decrease of HN from H1 was higher than that from H2. At each time, the amount of HN in H1 and H2 was significantly higher in the pasture soil than in the maize soil.

The non-hydrolysable urea-derived N increased during the crop season in the maize soil and decreased in the pasture soil (Figure 5). These variations were small but significant. As with hydrolysable N, at each time the amount of non-hydrolysable N was significantly higher in the pasture than in the maize soil. These results showed a higher immobilization of the fertilizer-derived N as organic N in the pasture soil than in the maize soil, i.e. the impact of maize crop on the soil organic N pool derived from the fertilizer was significantly higher than that of pasture crop.

During the cropping period, the decrease of urea-derived N was 4.6 to 9.1 times higher than that of native organic N, figures similar to those reported by Kelley and Stevenson (1987) and Clay et al. (1990).

Distribution of organic ^{14}N and ^{15}N

The amide fraction accounted for similar percentages of soil organic N in both maize and pasture soils and did not vary significantly during the crop season (Figure 6). In both soils, amide N was a more important fraction for the urea-derived N than for the soil N at 30 DAS, but the reverse was true at 131 DAS. The high proportion of urea-derived N in the amide N fraction 30 DAS (a week after fertilization), and the rapid decrease during the crop season, suggested the presence of unhydrolysed urea in soil 30 DAS. Clay et al. (1990) and Sahrawat (1992) have also reported incomplete urea hydrolysis in acid soils even 10–18 days after urea addition.

The ammonium N was one of the less important fractions of soil and urea-derived N, in both maize and pasture soils (Figure 7). This result contrasts with the high proportion of urea-derived ammonium N (up to 20% of immobilized urea-N) found by Clay et al. (1990), but this difference could be due to the greater destruction of organic compounds produced by the continuous hydrolysis method that they employed (González-Prieto and Carballas, 1988). In the two soils studied, endogenous and urea-derived ammonium N followed opposite trends during the crop season: the former increased and the latter decreased significantly. According to this result and those discussed in preceding sections, it seems probable that the endogenous ammonium N fraction was a sink of N (i.e. fixed on clays), while the urea-derived one was a source of potentially available N for plants.

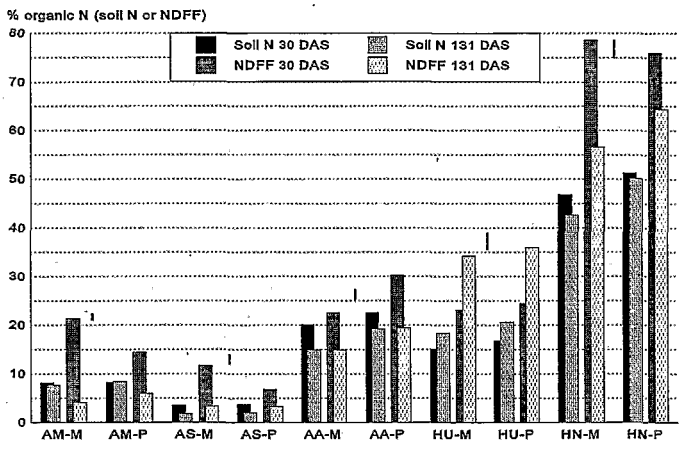

Figure 6. Organic N from H1; distribution of soil N and N derived from fertilizer (NDFF) in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS–days after sowing, AM–amide N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN–(AM+AS+AA)), HN–total N of the hydrolysate.

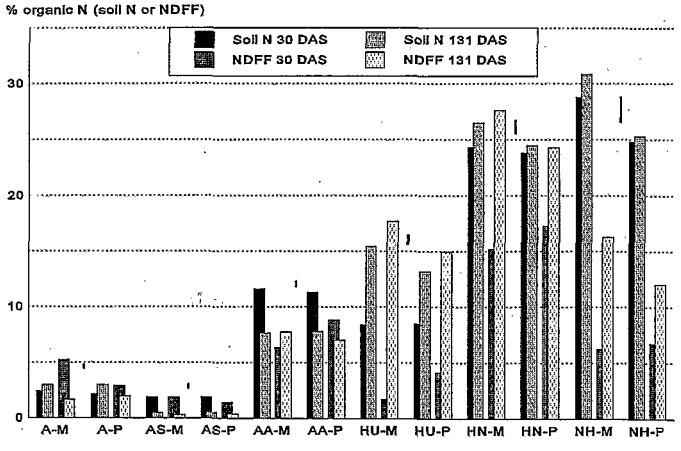

Figure 7. Organic N from H2 and non-hydrolysable residue; distribution of soil N and N derived from fertilizer (NDFF) in the maize (M) and the pasture (P) soils. Bars show significant differences for each N fraction (Tukey's test, $p < 0.05$). DAS–days after sowing, A–NH_4^+–N, AS–aminosugar N, AA–aminoacid N, HU–hydrolysable unidentified N (HU=HN–(AM+AS+AA)), HN–total N of the hydrolysate, NH–non-hydrolysable N.

The proportion of soil organic N as amino sugars decreased significantly during the crop season, in both soils (Figures 6 and 7). Whether 30 or 131 DAS, the amino sugar N was a more important fraction for the urea-derived N than for the soil N. In both hydrolysates, at 30 DAS the proportion of urea-derived N as amino sugar N was significantly higher in the maize than in the pasture soil and it decreased significantly at harvest in both crops.

With one exception, the α-amino acid N fraction was the largest for both soil and urea-derived organic N in both soils at 30 DAS (Figures 6 and 7). The α-amino acid N fraction from H1 was significantly more important for pasture soil than for maize soil and for urea-derived N than for soil N. In both soils, the importance of the α-amino acid N fraction for both soil and urea-derived N decreased significantly during the crop season. The results showed that, at the point of maximum immobilization of applied N, higher proportions of the urea-derived N occurred as amino acids compared to the native N, as also reported Kelley and Stevenson (1987). This was due to the higher proportion of urea-derived N in the most labile α-amino acid fraction (i.e. that of H1).

In both hydrolysates, the HU N was an important N fraction for both soil and urea-derived N, in both

soils, and indeed was the largest fraction after harvest (Figures 6 and 7). The proportion of N as HU N was significantly higher for urea-derived N than for soil N in agreement with the results of Kelley and Stevenson (1987), except for H2 at 30 DAS. According to these authors, this result, together with the similar one obtained for α-amino acid N, suggests that much of the fertilizer-derived organic N at the point of maximum immobilization was in the microbial biomass or related products.

According to the previous results, the total hydrolysable N of H1 tended to decrease during the crop season and that of H2 tended to increase for both soil and urea-derived N; these variations were important and significant for the urea-derived N but for the soil N significant differences were found only for the maize soil (Figures 6 and 7). The percentage of N as HN was significantly higher for urea-derived N than for soil N in H1; in H2 the reverse was true at 30 DAS and the differences were not significant at 131 DAS.

The proportion of soil N as non-hydrolysable N did not vary during the crop season in both soils, but was significantly higher for maize than for pasture (Figure 7). The percentage of non-hydrolysable urea-derived N was similar in both soils at 30 DAS and it increased significantly during the rest of the cropping period. Similar results were reported by Kelley and Stevenson (1987). These authors suggested that the process whereby immobilized N is converted to relatively unavailable organic forms begins within days after immobilization and continues over a period of years. The increase of non-hydrolysable urea-derived N during the crop period was more important in the maize than in the pasture soil. Nevertheless, the proportion of non-hydrolysable N was significantly more important for soil N than for urea-derived N, as also reported Kelley and Stevenson (1987), both at 30 and 131 DAS.

All these results show that the distribution of urea-derived N was markedly different from that of soil N shortly after fertilization (7 d, i.e. 30 DAS). The main differences found were the higher proportion of urea-derived N in all the easily hydrolysable organic N fractions (those of H1) and the lower proportion in the difficultly hydrolysable (H2) or insoluble fractions. These differences became less important, but were still significant after a cropping period. It should be highlighted that the results showed that the easily hydrolysable organic N fractions are more sensitive than total N to the impact of land use intensification and are, therefore, a more useful index for the early detection of soil biological degradation.

Acknowledgements

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