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Otnitrogen Immobilization and Remineralization in Four Cultivated Soils from Eastern France

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Abstract: Nitrogen immobilization and remineralization from a fertilizer supplied as ^{15}N ammonium sulfate were determined by hydrolytic fractionation of organic nitrogen with HCl 6N into: Acid-Soluble Distillable Nitrogen (NSAD), acid-soluble Non-Distillable nitrogen (NSAnD) and non-hydrolysable nitrogen (Nnh). The study was carried out on Ap horizon of four representative agricultural soils from Eastern France: A rendzina (*Typic Rendoll*), a pelsol (*Vertic Eutrochrept*) and brown leached soil (*Typic Hapludalf*). After one month of incubation under controlled conditions, the immobilization of the fertilizer was higher in the rendzina (24.5%) and pelsol (24.1%) or acid brown soil (16.9%). Conversely, the comparison of immobilized ^{15}Namounts befor and after cultivation, indicated that remineralization in the brown soils (acid brown soil 59.2%, brown leached soil 51.2%) was approximately twice higher as in the rendzina (21.1%) and pelsol (28.7%). These results show that with an Italian rye-grass used as test plant the remineralization was higher in the brown soils than in the tow clayed soils. Although the three NSAD, NSAnD and Nnh fractions participate in both, immobilization and remineralization process, the NSAnD compartment was shown to be the most active. The fact in presence of plants, part of the non-hydrolysable nitrogen fraction was biodegradable, is of particular interest.

Key words: Nitrogen, immobilization, remineralization, cultivated soils, Eastern France

INTRODUCTION

The fate of inorganic and organic fertilizers in the soil-plant system can be determined more accurately using ^{15}N as tracer (Broadbent and Taylor, 1962; John and Lloyd, 1970; Guiraud and Fardeau, 1977a; Marumoto *et al*., 1980). Numerous research studies have shown that 20 to 50% of nitrogen fertilizers added to soil can be recovered in soil at harvest (Kai, 1975; Guiraud, 1984; Vong, 1987; Machete *et al*., 1987; Martinez and Guiraud, 1990). This newly immobilized nitrogen thus forms part of potentially mineralizable reserve whose supply to plants is difficult to estimate due to incomplete knowledge about the structure of nitrogen-containing compounds. Furthermore, nitrogen transfer between inorganic and organic forms operates continuously in both directions (Marumoto *et al*., 1980; Guiraud and Marol, 1982; Nishio *et al*., 1985). This makes it difficult to estimate the proportions of inorganic nitrogen available to plants from soil organic forms and from inorganic fertilizer, unless isotopic tracer methods can be used.

The aim of present research was to quantify the reminerliztion in presence of a plant (Italian rye-grass) of fertilizer-N which had previously immobilized in the soil of 4 cultivated soils. As inorganic nitrogen had been previously removed from the soil before cultivation started, his study was designed to make possible the determination of the extent of each of the main organic nitrogen forms involved in plant nutrition.

MATERIALS AND METHODS

This survey has been achieved in the beginning of the year 1991 and the study samples were taken from Ap horizons in four most representative soils from the Lorraine region (Eastern France); two were calcareous, a rendzina (*Typic Rendoll*) and pelosol (*Vertic Eutrochrept*); the others were acidic soils, an acid brown soil (*Typic Hapludalf*) and a brown leached soil (*Typic Hapludalf*). The main physical and chemical characteristics of these soils are given in Table 1.

Experimental procedure: Air-dried soil sieved at 2 mm was placed in undrained polyethylene pots (8.5 cm high, 9 cm diameter at the top). To each pot containing 300 g of sieved soil, 70 mg of N per Kg of soil were added in the form of $(^{15}NH_4)_2SO_4$ (%^{15}N atom in excess = 50.03%). Soil moisture was adjusted to 80% of field capacity. For each

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Table 1: Main characteristics of the selected soils (Ap horizons)

Soils	Clay %	Silt %	Sand %	C %	N %	pH	W.H.C %
Rendzina	29.7	22.8	47.5	2.83	0.27	7.8	32.0
Pelosol	46.4	39.8	7.8	1.70	0.21	7.6	33.7
Acid brown soil	9.6	23.7	65.3	0.69	0.07	5.3	23.5
Brown leached soil	29.3	52.6	14.7	1.34	0.18	5.9	30.0

soil, 7 pots were used. The pots were then incubated in a dark room at a controlled temperature of 28±2°C and the moisture was checked every tow days. After one month of incubation, inorganic nitrogen was extracted from each pot by stirring the soil for 30 min with 700 mL of 0.01 M $CaCl_2$ to remove the remaining inorganic nitrogen and the final volume of filtrate was made up to 1000 mL with fresh extraction solution. Soil without inorganic nitrogen was then carefully put back into five of the initial 7 pots and the experiments continued immediately in vegetation jars with cultivation of Italian rye-grass (Lolium perenne L. cv Callan, 250 seeds per pot) under controlled conditions in a growth chamber. Soil in the other tow pots was air-dried and stored at 4°C for later chemical analysis. The plants were grown under the following conditions: Day: 14 h at 28°C and 300 $\mu E\ cm^{-2}\ s^{-1}$ of light, night: 10 h at 22°C and 70% air moisture. The pots were watered daily to maintain soil moisture at 80% of field capacity. The rye-grass cultivation lasted 9 weeks and three successive corps were harvested at 3 week growth intervals.

Analytical techniques: Total inorganic nitrogen in soil after incubation and cultivation was extracted with 0.01 M $CaCl_2$. The extract was then distillated in the presence of MgO to displace NH_3^+ ions and then in the presence of Devarda's alloy to reduce NO_2^- and NO_3^- ions (Bremner, 1965). Total organic nitrogen in soil after incubation and in cropped soil separated from roots by shaking with $CaCl_2$ was determined with kjeldahl method, as described by Guiraud and Fardeau (1977b). Organic nitrogen fractions were estimated in an amount of fresh soil equivalent to 10 g of dry soil whose inorganic nitrogen had been removed with 0.01 M Ca Cl_2. This material was hydrolyzed under continues reflux with 100 mL of 6M HCl for 16 h, according to the method proposed by Stewart *et al.* (1963). After cooling, the mixture was passed through a cellulose filter. The residue was rinsed with distilled water and the rinse water was added to the filtrate and made up to final volume of 200 mL. This solution contained the whole hydrolysable nitrogen and the residue (Nnh) non hydrolysable-N was air-dried.

The total amount of hydrolysable nitrogen was obtained by mineralizing an aliquot of the acid hydrolysate, using the kjeldahl method (Guiraud and Fardeau, 1977b). The acid-soluble distillable fraction (NSAD), which corresponded mainly to NH_4^+-N, was obtained by stream distillation of a 10 mL aliquot of filtrate in the presence of 10 mL of 10N NaOH in excess, then trapped in 10 mL of N H_2SO_4 and titrated with 0.01 N NaOH. The acid-soluble non-distillable fraction (NSAnD) was obtained from the difference between total hydrolysable nitrogen and the NSAD fraction. The non-hydrolysable-N fraction (Nnh) and N of soil were obtained by mineralization, using the kjeldahl method (Guiraud and Fardeau, 1977b). Isotopic ^{15}N measurements were run on a GS1 emission spectrometer (Sopra, Bois-Coulombes, France) after reconverting NH_4^+ nitrogen into N_2 by Dumas method (Fiedler and Proksch, 1975). The emission spectrometer was calibrated with a range of known % ^{15}N atom excess of standards. The calibration curves used in this research were as follow: (from 0 to 1% of ^{15}N atom excess: $y = 1.17\times - 0.53$, $r^2 = 0.992$, $n = 5.2$) (from 1 to 10%: $y = 1.15\times - 0.52$, $r^2 = 0.999$, $n = 4.3$) (from 10 to 15%: $y = 0.917\times + 2.19$, $r^2 = 0.999$, $n = 6$; where y = known % ^{15}N atom excess of standards, x = isotopic abundance of samples and n = number of determinations.

RESULTS

General status of ^{15}N before cultivation

Inorganic ^{15}N: After incubation, the amounts of fertilizer added, which remained inorganic were of 13.53 to 16.10 mg of ^{15}N per kg of soil, or 38.63-45.97% of total ^{15}N added. Although this nitrogen had been added in ammoniacal form, it was mostly found as nitrate in all soils, except in the acid brown soil, where it remained mostly in ammoniacal form (Table 2). Approximately 100% of the inorganic v was nitrate ^{15}N in the renzina, 97.6% in the pelosol, 91% in the brown leached soil, but only 39% in the acid brown soil. The lower value observed in the latter soil was due certainly to the effect of acidity on the growth of the nitrifying microflora.

Organic ^{15}N: The sum of total ^{15}N immobilized in each fraction varied with soil type: 24.5% of added ^{15}N was recovred in the soil of rendzina, 24.1% in the pelosol and 21.7% in the brown soil (Table 2). This latter soil, which had both lowest content of organic carbon and clay, presented the lowest nitrogen immobilization.

The ^{15}N repartition showed that only 60.3-70.5% of the fertilizers added were recovered in the 4 soils. The losses may have may have occurred in gaseous form

Table 2: Total N and ^{15}N distribution after one month of incubation

	Inorganic N						Organic N											
	$N\text{-}NH_4^+$			$N\text{-}NO_3^-$			NSAD			NSAnD			Nnh			N recovery		
Soils	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$
Rendzina	9.7	0.05	0.14	123.4	16.05	45.83	696	1.88	5.37	1537	4.30	12.28	924	2.40	6.85	3290	24.68	70.47
Pelosol	11.9	0.38	1.09	106.2	15.34	43.80	858	3.00	8.57	1180	4.01	11.45	893	1.43	4.08	3049	24.16	68.99
Acid brown soil	53.5	9.47	27.04	40.2	6.14	17.53	266	0.90	2.57	535	3.85	10.99	402	1.17	3.34	1297	21.53	61.48
Brown leached soil	20.3	1.22	3.48	75.0	12.31	35.15	455	1.59	4.54	857	3.69	10.53	678	2.31	6.60	2085	21.12	60.31

With QN = mg total N kg^{-1} soil, $Q^{15}N$ = mg labeled N kg^{-1} soil,$\%^{15}N$ = labeled N in% of 15N input

Table 3: Total N and ^{15}N distribution after 9 weeks of incubation

				Soil organic N											
	Plant N			NSAD			NSAnD			Nnh			N recovery		
Soils	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$	QN	$Q^{15}N$	$\%^{15}N$
Rendzina	124.9	0.90	2.57	713	1.71	4.88	1555	3.42	9.77	959	1.63	4.65	3352	7.66	21.87
Pelosol	145.0	1.22	3.48	858	1.40	6.85	1082	2.49	7.11	873	1.13	3.23	2958	7.24	20.67
Acid brown soil	103.4	3.41	9.74	259	0.60	1.91	505	1.11	3.17	372	0.63	1.80	1239	5.82	16.62
Brown leached soil	138.2	3.69	10.53	433	1.21	3.46	854	1.54	1.54	636	0.95	2.71	2061	7.39	21.10

With QN = mg total N kg^{-1} soil, $Q^{15}N$ = mg labeled N kg^{-1} soil,$\%^{15}N$ = labeled N in% of 15N input

through denitrification or volatilization. This is supported by the fact that during the first month of incubation, most nitrogen in the soil was inorganic.

Forms of immobilized nitrogen: Hydraulic fraction of organic nitrogen lead to determination of ^{15}N distribution among the three soil biochemical fractions distinguished according to their solubility in acid and volatility (Table 2 and 3).

Our previous research (Vong *et al*., 1990) found that under monoculture of maize and under oak forest the seasonal variations of the NSAnD fraction was signicantly correlated to those of microbial biomass-C. Thus we consider that this fraction is mainly made up of amino acids. According to Kai (1975), Guiraud (1984), Jacquin *et al*. (1985) and Vong (1987) this nitrogen is the seat of biological immobilization and remineralization processes. The present findings clearly confirm the predominance in this fraction of immobilized nitrogen from fertilizer (Fig. 1). When expressed in percentage of ^{15}N present in soil after one month of incubation, the highest values were observed in the acid soil (65%), whereas lower values were found in the rendzina (50%), the brown leached soil (48.5%) and the pelosol (47.5%). The NSAD fraction, which contained mostly NH_4^+ -N resulting from the hydrolysis of various sources, is considered according to Vong (1987) as an intermediate form. In percentage of the ^{15}N present in the soil after one month of incubation it was particularly abundant in the pelosol (35.5%) and relatively scarce in the acid brown soil (15.2%). The rendzina (22%) and the brown leached soil (21%) had intermediate values with respect to the first two soils. It should be noted that these variation in the NSAD fraction were similar to corresponded to hydrolysis-resistant nitrogen, sometimes referred to as heterocyclic (Flaig, 1971) or aromatic ring-bound nitrogen in humic polycondensates (Andreux, 1981). In percentage of the ^{15}N remaining in the soil after incubation, greater ^{15}N immobilization was found in the brown leached soil (30.5%) and the rendzina (28%) than acid brown soil (19.8%) and pelosol (17%).

Mineralization of ^{15}N immobilized

N recovered in plant: At harvest, the amounts to inorganic N in the 4 soils were not measurable. After 9 weeks of cultivation, the amounts of ^{15}N in the shoots and roots expressed in percentage of ^{15}N added were low (Table 3): Values were higher in the brown leached soil (10.5%) and acid brown soil (9.7%) than in the pelosol (3.5%) or the rendzina (2.6%).

In the presence of plant, the remineralized nitrogen, (expressed in percentage of v present in soil after incubation and represented by the difference between the sum of total ^{15}N immobilized in each fraction before and after rye-grass cultivation) was higher in the acid brown soil (59.2%) and the brown leached soil (51.2%), but was approximately twice lower in the rendzina (21.1%) and the pelosol (28.7%). The remineralized nitrogen was not totally recovered in the plant biomass in all soil types. It was either absorbed by the plant or lost in gaseous form, as already stated above. The losses recorded depend on the type of soil, ranging from 1.7% of ^{15}N present in the soil after one month of incubation for the sandy acid brown soil to 14.2% for the clayed pelosol.

Sources of remineralized ^{15}N: The organic status of ^{15}N before and after rye-grass cultivation is compared in Fig. 1. The rendzina, despite its good texture, had a lower

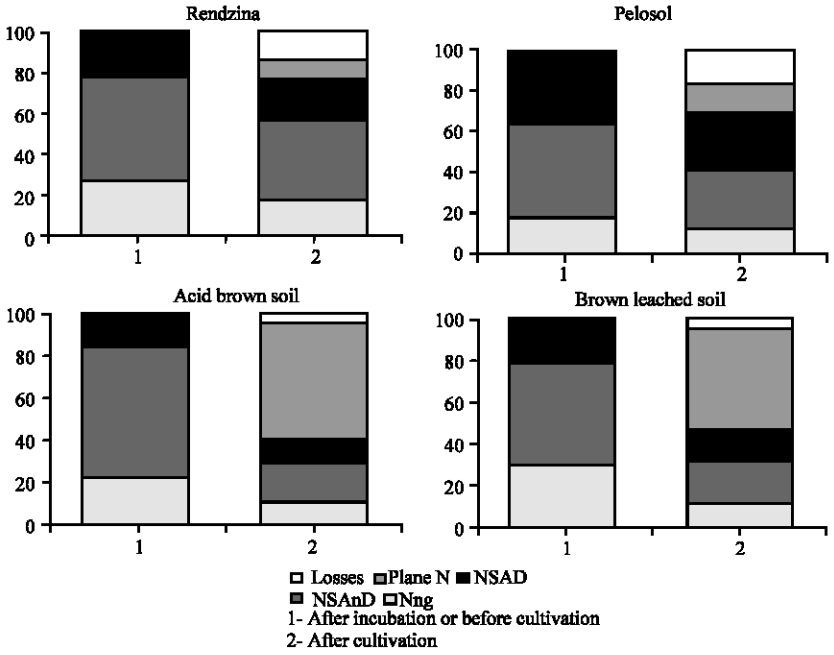

Fig. 1: ^{15}N Status in the four studied soils before and after cultivation (Results are expressed in% of ^{15}N recovered in soils after one month of incubation)

remineralization rate than other soils. Such low remineralization was probably due to sequestration of organic matter by finely crystallized calcium carbonate, as shown by Jacquin *et al.* (1980) and Duchaufour (1988). The 21.1% remineralized rate of immobilized ^{15}N before cultivation was mostly at expenses of the NSAnD (-10.1%) and Nnh (-9%) fraction, with low variation in the NSAD fraction (-2%).

In the pelosol, ^{15}N remineralization would hindered by clay minerals which adsorb amino acids, thus limiting their biodegradation (Andreux and Jocteur Monrozier, 1981; Sorensen, 1981; Vong *et al.*, 1989). Remineralization of the 28.7% of immobilized ^{15}N mostly was due to the NSAnD fraction (-18.0%), followed by the NSAD (-7.1%) and Nnh (-3.6%) fractions.

In the acid brown soil, the remineralization of 59.2% of immobilized fertilizer was due to the greater lability of nitrogen recently immobilized by the microbial biomass. Remineralization of ^{15}N in the presence of the culture resulted in a substantial decrease in the NSAnD fraction (-42.2%) followed by the Nnh fraction (-9.1%), but only very little change in the NSAD fraction (-3.9%).

In the brown leached soil, the remineralization rate (51.2%) for immobilized ^{15}N was similar to that in the acid brown soil. This highly active turnover of ^{15}N confirms the observation of Jacquin (1985, 1991) about the intense mineralization in medium-textured acid mull media. As in other types of soils, most of the remineralized ^{15}N came from the NSAnD fraction (-28.2%).

DISCUSSION

The use of low concentration of $CaCl_2$ (0.01M) as extractant might raise the question of non total extraction of exchangeable ammonium in the soil solution which will be left on the exchange complex. The same question might also be raised relative to the use of relatively concentrated salt solution (1 M to 2M KCl). Such extracts often contain more appreciable amounts of labile organic matter, a portion of which may be converted to ammonium during alkaline distillation (Stanford and Smith, 1972). In addition, these concentrated extractants do enrich the soil submitted immediately to cultivation. As to the soil structure which may have disturbed by the extraction procedure we have adopted, it is widely compensated by using the resistant-high developing plant such as the rye-grass.

Another suggestion is to immobilize sufficient ^{15}N and label the SOM by adding, at the beginning of incubation, amounts of labile organic C such as the glucose. In this way, a preliminary study is necessary to determine, for each soil, the time required for complete immobilization of N added. Such a study would be difficult when different soils are conducted together, because: The complete immobilization time is different from one soil to another and subsequently the onset of remineralization is also different and soil dependent.

For a silt loam soil maintained at 30°C with increasing amounts of ^{15}N-labelled $(NH_4)_2SO_4$ and glucose

(C-to-N ratio of 30 for all additions) Azam *et al.* (1989) found that the immobilization was maximum and constant after 106 h and remineralization began after 250 h of incubation. Furthermore, the important gas-N losses in such study could not be avoided according to the results of Germon *et al.* (1981), Batonda and Woring (1984) showing the enhancing role of labile carbon substrates in denitrification.

The choice of mild extractant (0.01M $CaCl_2$) was somethat of a compromise, adopted in order to avoid or minimize some undesirable effects accompanying the use of either distillated water or concentrated salt solution. In addition, Bottner (1985) working on alternate moist and dry condition effects on soil microbial biomass showed that the intra-aggregates biomass non killed by drying could completely established within one month under controlled conditions. If the incomplete NH_4^+ exchangeable extraction by 0.01M $CaCl_2$ is questionable, this fraction will be in majority recovered in the NSAD obtained by direct distillation of acid hydrolysate. Therefore, the value of the tow others fractions: NSAnD and Nnh remain valid in our interpretation. The ^{15}N immobilization, which varies with the type of soil, underlined the physical-chemical properties on these processes.

Nitrogen fertilizer immobilized in the soil was incorporated into the three organic fractions distinguished by acid hydrolysis, with a preferential immobilization in the NSAnD fraction. This result underlines the major role of soil microorganisms in the storage of nitrogen in soils (Kai, 1975; Söchting, 1980; Berg and Ekhbohm, 1983; Guiraud, 1984; Vong, 1987). The immobilization of ^{15}N in the NSAD fraction appeared to be in relation with clay contents of the four studied soils. This result verifies the observation done by Egoumeniedes *et al.* (1987) and illustrates the previous observation as a consequence of incomplete extraction of exchangeable ammonium by 0.01M $CaCl_2$.

The intensity of remineralization of immobilized nitrogen depended on soil type and also the capacity of root development. It was higher in the tow brown soils in comparison to the pelosol with high clay content. The first explanation is a possible retention of some nitrogen compounds inside the aggregates and the physical protection of clay present in this soil. These results agree with those of Chaussod *et al.* (1986) showing an important role of soil physical and chemical characteristics on the rates of nitrogen mineralization. The second explanation corresponds to a mechanical consequence on the soil structure resulting from the soil $CaCl_2$-extraction procedure. This fact limits the ability of root exploration and therefore its activity in soil. Expressed in percentage of ^{15}N added, we observed higher values of ^{15}N recovered in the roots developed in the brown leached soil (1.23%) and in the acid brown soil (1.03%) against those developed in the rendzina (0.23%) and the pelosol (0.31%). These results clearly illustrate the higher remineralization of ^{15}N observed in the tow bron soils.

The evidence of significant remineralization from the Nnh fraction suggested that structures, resistant to the hydraulic action of 6N Hcl, did not exclusively correspond to the commonly admitted irreversible incorporation of nitrogen in humic polycondensates (Andreux *et al.*, 1977; Andreux, 1981; Andreux and Jocteur Monroeier, 1981; Schnitzer and Hindel, 1981; Vong *et al.*, 1990). Finally it can be underlined as previously discussed that soil characteristics influence strongly the biochemical mechanisms of nitrogen storage in cultivated soils after inorganic fertilizer application.

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