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Studies on Nitrogen Immobilization and Denitrification in Different Soil Types, by Using Labelled Calcium Nitrate, $Ca(^{15}NO_3)_2$

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Abstract. Four different types of soil were studied, namely: calcareous brown, leached brown, andosol and podzol. After addition of ^{15}N in the form of $Ca(^{15}NO_3)_2$ labelled at 33%, the soil samples were incubated for 18 days under controlled conditions of temperature and moisture content. The evolution of different forms of nitrogen and carbon was investigated during the incubation time. The experimental data indicate that, after incubation of the soil samples, the isotopic excess decreased progressively in all samples tested. This was accompanied by a decrease in mineral nitrogen which resulted from either the losses of added ^{15}N by denitrification, or by incorporation of ^{15}N into organic form by microbial synthesis.

Comparison of denitrified and synthesized quantities of nitrogen demonstrated that biodegradation was largely dominant over biosynthesis. Calculations of ^{15}N and P present in organic form showed that organic N was lost more quickly than organic phosphorus in the soils.

Introduction

The equilibrium between inorganic and organic nitrogen represents the balance between immobilization and mineralization process in soil. In general, immobilization processes are fairly rapid and extensive over short periods when conditions are favourable, whereas mineralization reactions tend to proceed more slowly over long periods. This inorganic-organic equilibrium is influenced by a multitude of soil factors [1,2].

Normally the mineralization rate exceeds the immobilization rate in soils, otherwise nitrogen would not be available for the growth of crops.

The major advantage of the use of labelled N in immobilization-mineralization experiments is the ability to distinguish the immobilized nitrogen of the soil organic fraction from nitrogen already present. Since immobilization and mineralization of N take place simultaneously, the ^{15}N tracer technique is the only possible method by which the two opposite processes can be measured simultaneously. This investigation is an extension of an earlier study on kinetics of organic matter bound phosphorus in different types of soils [3,4,5], and is also related to investigations on the kinetics of processes involving nitrogen, using tagged calcium nitrate.

Materials and Methods

A. Investigated soils

Four different types of soil were studied, namely: (a) Calcareous brown called 'grand fraize' and found under *Carpinus betulus*. The soil is a mixture of clay and terra fusca and was formed before the Bajocian age. (b) Leached brown, from under permanent alfalfa with very good absorption of water and saturated with calcium. (c) Andosol: this soil is salty, containing appreciable amount of sodium and aluminium; it is of volcanic, origin and needs much water. (d) Podzol: this soil is organic. All four types of soil were treated with isotope as soon as they had been collected and air-dried. The main physico-chemical characteristics of these soils are presented in Table 1.

B. Soil labelling with ^{15}N

A solution of 8 ml containing 8.32 mg ^{15}N in the form of $Ca(^{15}NO_3)_2$ tagged at 33%, was applied to 30 g of air-dry soil placed in a 125 ml Erlenmeyer flask. The samples were mixed thoroughly and distilled water added to bring the moisture content to approximately 2/3 of field capacity. The flasks were covered with sheets of parafilm and replicated four times. The soil samples were incubated at 28°C for 18 days. The evolution of the forms of nitrogen, carbon and phosphorus was investigated during the incubation period.

C. Analytical methods

a- Analysis of inorganic nitrogen (Hydrosoluble N)

Steam distillation, using magnesium oxide or Devarda's alloy on 0.1 N KCl-soil extract, was employed for the determination of exchangeable and fixed NH_4^+, NO_3^- and NO_2^- [6].

b- Analysis of total and organic nitrogen

As modified version of the method of Bremner [7] and Bremner and Edwards [8] was employed to determine the total nitrogen. The organic nitrogen was estimated by the difference between total and inorganic N.

Table 1. Physico-chemical characteristics of the soils and the pedological horizons

Soil	Particle sizes %					O.C.	N total	C/N ratio	Exchangeable bases (m.e./100 g)					$100\frac{S}{T}$	% Free elements			pH
	C.S.	F.S.	C.L.	F.L.	Clay	%	%	%	Ca^{++}	Mg^{++}	K^{+}	S	T		Fe	Al	Si	(water)
A_1 Podozol P	64.2	18.7	2.8	4.3	3.7	10.1	0.294	34.2	0.89	0.40	0.08	1.37	12.1	11.3	0.7	0.4	0.4	3.7
A_p Brown calcareous B.C.	2.7	42.0	11.0	12.0	26.0	2.1	0.187	11.3	sat.	–	–	sat.	sat.	sat.	10.2	5.1	0.9	8.1
A_1 Andosol A	36.5	14.5	10.1	24.1	4.7	4.2	0.245	17.0	1.60	0.67	0.37	2.64	19.8	13.3	25.0	26.0	17.0	5.2
A_1 Leached brown L.B.	1.7	2.8	11.0	40.0	22.6	4.1	0.30	13.6	10.00	1.00	0.90	11.9	16.2	73.4	23.0	–	–	6.5

C.S. Coarse sand, F.S. Fine Sand, C.L. Clay loam,
F.L. Fine loam, S = Sum of Ca + Mg + K, T = Total exchangeable bases,

c- Isotopic analysis of ^{15}N

1- *Technique:* The nitrogen isotope ratio was estimated by using an Atlas Mat GD 150 mass spectrometer according to the procedure of Rittenberg [9] as described by Guiraud and Berlier [10] and by Fares [3]. After ammonium titration, an excess of H_2SO_4 (1-2 ml) was added to the sample to prevent loss of nitrogen during concentration. The sample was then evaporated to a volume less than 2 ml. For conversion of the labelled ammonium nitrogen to nitrogen gas, the 2 ml concentrated sample was placed in one arm of a "Y" tube (Rittenberg vessel) while the reagent sodium hypobromite (NaOBr) was placed in the other arm. The "Y" tube was rotated to mix the sample with reagent.

The oxidation reaction converts NH_4^+ to N_2 gas with alkaline hypobromite in the absence of air. The reaction is:

$$3\ NaOBr + 2\ NH_3 \rightarrow N_2 + 3\ NaBr + 3\ H_2O$$

After five minutes, the reaction vessel was submerged in liquid nitrogen, freezing out water and condensable contaminants. The sample was then transferred, using a Toepler pump, into the mass spectrometer inlet system.

The precision of the utilized MAT GD 150 mass spectrometer using the double collector method of analysis is more than 0.1% (1 ppt).

2- ***Calculations:*** The atom percent (%) of ^{15}N, which represents the percent abundance ^{15}N in the sample, is given by the following expression:

$$15 = \frac{\frac{1}{2}(^{14}N\ ^{15}N) + (^{15}N\ ^{15}N)}{(^{14}N\ ^{14}N) + (^{14}N\ ^{15}N) + (^{15}N\ ^{15}N)} \quad (1)$$

$$\text{or} \quad = \frac{29 + 2(30)}{2(28 + 29 + 30)} \quad (2)$$

where 28, 29 and 30 are the level of peaks which are, respectively, proportional to the concentration of the following molecular species $^{14}N\ ^{14}N$, $^{14}N\ ^{15}N$ and $^{15}N\ ^{15}N$.

The isotopic excess was then obtained by subtracting the natural isotopic abundance % ^{15}N (= 0.365), from the percent abundance ^{15}N in the sample.

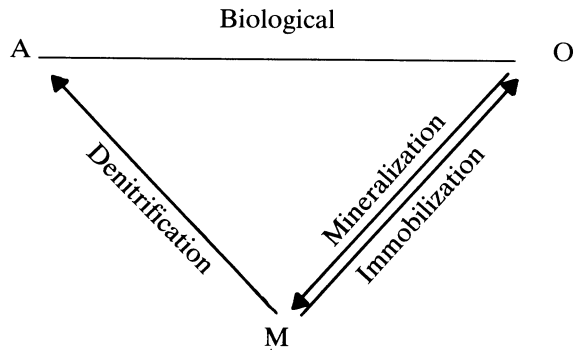
d- Analysis of carbon and phosphorus

The determination of carbon was carried out by the combustion method in the presence of oxygen at 950°C using the "Cormograph 8 wosthoff" [4]. Total and organic phosphorus were determined according to the method of Saunders and Williams [11] and of Bremner and Ho [12], modified by Fares et al. [13].

Results and Discussion

The main objective of this study is to elucidate and confirm the transformation of inorganic nitrogen into the organic form , and vice versa. Since the C/N, C/P and N/P ratios are related to total microbiological changes, the tracer ^{15}N is one of the best indicators of soil microbiological activity.

The different processes of nitrogen transformations in soil may be shown as follows:



A = atmospheric nitrogen
M = inorganic nitrogen
O = organic nitrogen

A. Inorganic nitrogen

The tagged nitrogen ^{15}N was added to the investigated air-dry soils in the form of calcium nitrate at the rate of 277 ppm of ^{15}N. No ammonium nitrogen was detected in the soil extracts during the incubation period, the extracted nitrogen being only of the nitrate form. After 6 days of soil incubation, it was possible to show, in all tested samples, an increase in the isotopic excess (initial atom % excess) of this nitrate, from which the amount of nitrified nitrogen from soil organic nitrogen may be calculated.

As a numerical example of tested samples such as the leached brown soil (Table 2), it was found that, after 12 days of incubation, the chemical balance of nitrate in this soil could be presented as follows:

345 ppm (QN^+ at the 12th day) – 277 ppm (QN^+ at the 1st day) = 68 ppm.

This means that 68 ppm of soil organic nitrogen were mineralized during 12 days of soil incubation.

Table 2. Evolution of the different forms of nitrogen during the incubation time leached brown soil

Incubation time/days	Inorganic N				Organic N				Total N			Denitrification %
	QN^+	E%	$Q\,^{15}N$	^{15}N%	QN^+	E%	$Q\,^{15}N$	^{15}N %	QN^+	$Q\,^{15}N$	^{15}N %	
1	277	32.90	91.00	100	1720	–	–	–	1997	91.00	100	–
6	324	27.30	88.45	97.2	1382	0.046	0.635	0.692	1706	89.08	97.9	2.10
12	345	22.10	76.24	83.8	1531	0.054	0.826	0.901	1876	77.06	84.7	15.31
18	295	22.60	66.67	73.3	1534	0.150	2.301	2.528	1829	68.97	75.89	24.20

+ = Results in ppm

Q = Quantity in ppm.; $QN^+ = Q\,^{14}N + Q\,^{15}N$

$$E\,\% = \text{Isotopic excess} = \frac{100\,R}{2 + R} - 0.365, \text{ where } R = \frac{29}{28}$$

The utilization of nitrogen tracer ^{15}N shows that more than this quantity was formed, because of the recovered ^{15}N in inorganic form 345 ppm of inorganic N was measured at the 12th day, but only 232 ppm originated from nitrate applied at the beginning of the experiment:

$277 \times 83.8/100$ (^{15}N present on the 12th day as % of added ^{15}N) = 232 ppm of nitrogen,

Consequently, the actual quantity mineralized is:

$345 - 232 = 113$ ppm.

This balance is distinctly different from that obtained by the chemical analysis. Similar results were also found in the other tested soils.

Experimental data concerning the evolution of inorganic (NO_3^-) and organic N are represented in Fig. 1.

It can be seen that there is a progressive drop in isotopic excess and a decrease in tagged ^{15}N in the mineral form.

This decrease was due to one or a combination of the following causes:

– The losses of tagged ^{15}N in gaseous form by denitrification, which is a result of microbial activity of mineralization.

– The incorporation of ^{15}N into the organic fraction, which is due largely to microbial immobilization.

B. Organic nitrogen

In calculating the quantity of tracer N in the organic fraction after the incubation of the samples, it was possible to estimate the quantity of inorganic nitrogen incorporated into the organic fraction, which was largely due to microbial assimilation.

Data of the leached brown soil after 12 days of incubation (Table 2) indicated that:

277 ppm $\times$ $0.901/_{100}$ (^{15}N present on the 12th day as % of added ^{15}N) = 2.50 ppm of immobilized N. A simple comparision between the quantities of mineralized and immobilized nitrogen in all tested soil samples shows clearly that the mineralization rate exceeds, by far, the immobilization rate (Fig. 2).

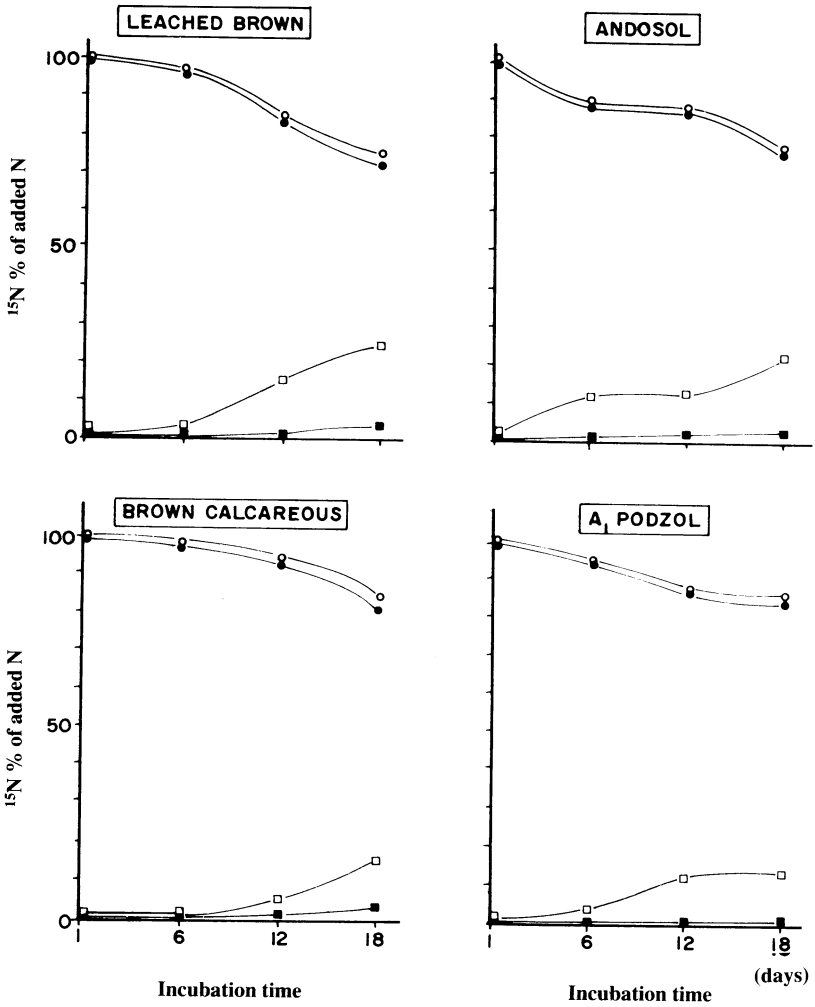


Fig. 1. Evolution of Added N during the incubation time. □ **Lass of ^{15}N** ■ **Organic ^{16}N** • **Inorganic ^{15}N** ○ **Total ^{15}N**

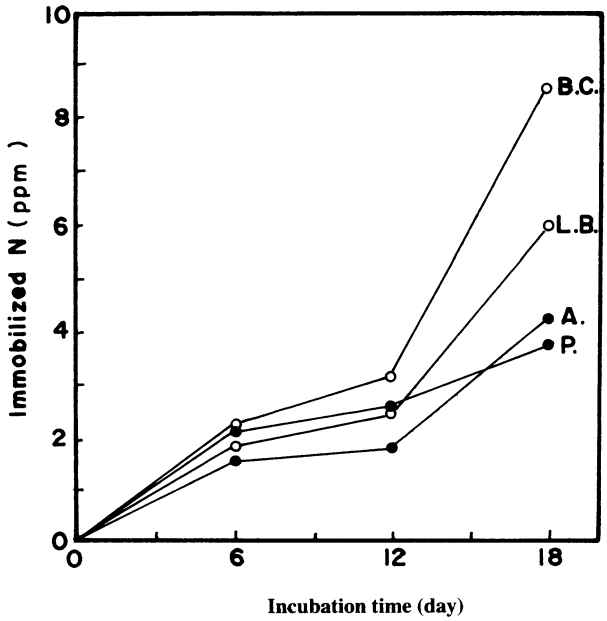


Fig. 2. Rate of nitrogen immobilization during the incubation time.

Broadbent [2] has reported an immobilization rate of an order of 10-15 ppm N per day after addition of mature plant residues at a 1% level. This level declined to a very low value after 12 days or less.

Calculations for the rates operative in this experiment, without adding any organic or energetic material, show lower values than those reported by Broadbent. This rate was about 0.25 ppm N/day for the podzolic and andosolic soils, which were characterized by high values of C/N ratio (34 and 17 respectively), and with an unsaturated sorbing complex, whereas this rate was 0.45 ppm N/day for the brown soils (B.C. and L.B.) having a C/N ratio ranging between 11 to 13 respectively.

The relationships between the nitrogen incorporated into the organic fraction during the incubation time and the C/N ratio of the tested soils are shown in Fig. 3.

It seems that the lower the C/N ratio, the higher the incorporation of N into the organic fraction.

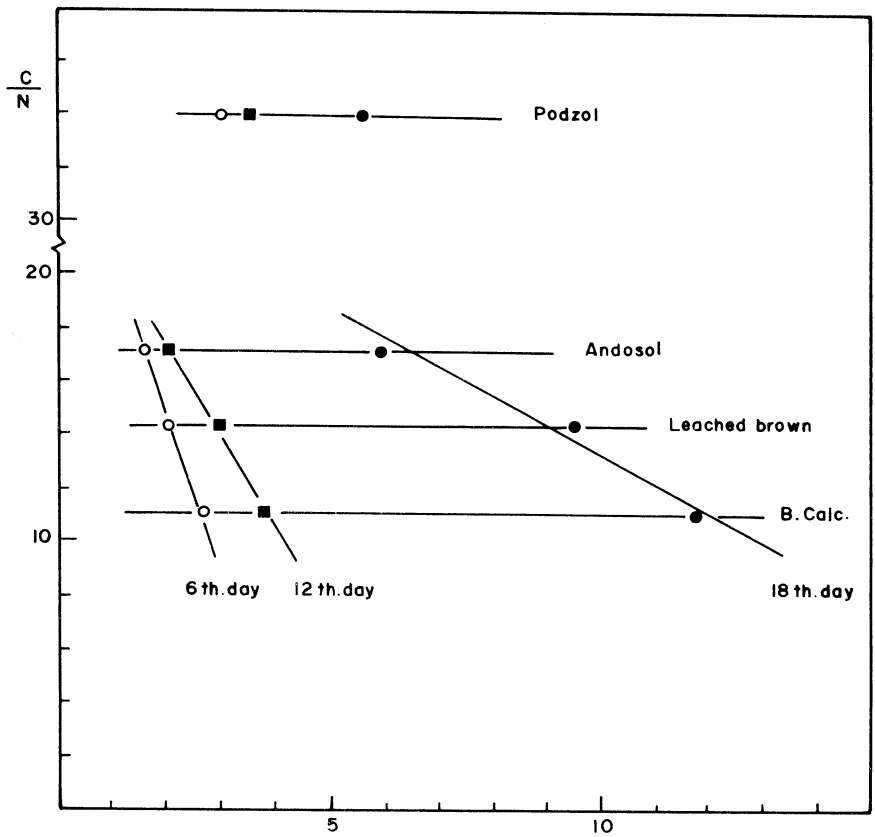


Fig. 3. Relationship between N incorporated in organic fraction and C/N ratio

C. Loss of ^{15}N by denitrification

Calculation of nitrogen balance at the end of the incubation period gives the quantity of denitrified N [4]. The mean loss of N by denitrification ranges between 12 to 24% of added ^{15}N at the beginning of this experiment. This means that the rate of denitrification in the tested samples is between 1.8-3.6 ppm of N/day.

These quantities calculated on the bases of total added nitrogen (e.g. 277 + 12/100 = 33/18 = 1.8 ppm), demonstrate a slight difference between the leached brown and andosol as compared with brown calcareous and podzolic soil. The foregoing results indicate that part of the added nitrogen (N) was incorporated into the organic

fraction by immobilization, and because of that it became insoluble; the rest was volatilized in the form of gaseous configurations N_2 or/and N_2O by denitrification.

The kinetics of ^{15}N suggest the presence of two opposite processes occurring simultaneously. Furthermore, it should be noted that the direction of the change confirms the same dynamics of phosphorus in these samples by using ^{32}p [5]. In fact, the rate of mineralization exceeds and is predominant to the rate of biosynthesis of nitrogen and/or phosphorus.

D. Relationship between kinetics of nitrogen and phosphorus

Data reported in Table 3 show the N/P ratios in plants, tested samples and the organic substances which were synthesized during the incubation period. The synthesized amounts of organic P and N in the same soils were determined by using ^{32}p and ^{15}N isotopes.

Table 3. A comparison between N/P ratio in plants, soil and synthesized substances.

N/P			
Plants	**Soil**	**18th day**	**Synthesized substances**
≃4	A	20.00	0.76
	L.B	8.46	0.73
	B.C	17.64	2.06
	P	29.00	1.10

The amounts of synthesized organic P were counted as the sum of P in both fulvic and humic acid; they were also calculated by the presence of ^{32}p [5].

The calculations of nitrogen were based on measurements by ^{15}N. The soil organic N was lost faster than the soil organic P. On the other hand, it was possible to observe the presence of ^{32}p in the organic fraction in the first extraction which did not contain any ^{15}N. It is therefore concluded that the ^{32}p was retained by the organic matter by another mechanism rather than a biosynthesis, which should have consumed some part of the added ^{15}N.

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دراسات على إطلاق وفقد النيتروجين في أنواع مختلفة من التربة بواسطة استعمال نترات الكالسيوم الموسومة

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ملخص البحث. استخدمت في هذه الدراسة أربعة أنواع من التربة هي التربة الكلسية والتربة البنية المغسولة وتربة الأندوسول وتربة البودزول بعد إضافة نظير النيتروجين في صورة نترات الكالسيوم الموسومة بحوالي ٣٣٪، حضنت التربة لحوالي ١٨ يوماً تحت درجة حرارة ومحتوى رطوبة ثابتين. درست قيم صور النيتروجين المختلفة والكربون خلال فترة التحضين. ويمكن إيجاز النتائج على النحو التالي:

أي زيادة في النظائر يصاحبها نقص في النيتروجين المعدني وهذه الملاحظة ترجع إما إلى فقد نظير النيتروجين المضاف على صورة غازية بواسطة انتزاع النيتروجين أو بواسطة اتحاد نظير النيتروجين في صورة مادة عضوية بواسطة التمثيل الميكروبي.

المقارنة بين كميات النيتروجين المفقود والممثل تثبت أن عمليات التحلل والفقد أكبر من عملية التمثيل.

ولحساب نظير النيتروجين والفسفور الموجودين في صورة المادة العضوية أثبت أن النيتروجين العضوي كان أسرع فقدًا من الفسفور العضوي.