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Title: Ratio of natural isotopes of nitrogen. I. Primary results: Soils of Dombes. (Rapports isotopiques naturels de l'azote I. - Premiers resultats : sols de Dombes.)

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Ratio of natural isotopes of nitrogen. I. Primary results: Soils of Dombes

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Utilization of the heavy isotope of nitrogen as a tracer has found numerous applications in soil biology. It allows better definition of different stages of the nitrogen cycle, in particular the immobilization-mineralization cycle. (JANSSON, 1958).

Recently some nitrogenous products have appeared on the market which are not enriched by ^{15}N but impoverished: the nitrogen in the air naturally contains 0.3663% of nitrogen 15, while the impoverished products only contain 0.001% and thus can serve as tracers. Their gross advantage results from their cost, which is clearly lower than before, permitting a larger utilization.

But use of heavy nitrogen as a tracer, if one wants to follow the added labelled substance for a long time, necessitates knowledge of the natural isotope ratio, which depends on the type of soil, the horizon of the same soil and/or of the nitrogen fraction considered (BREMNER, 1967 - RIGA et col. 1971).

Furthermore, the variations in the ratios of natural isotopes after the elimination of possible errors from dilution, may be able to be used themselves. Several articles have appeared in the last years which attempt to explain the significance of the natural variations but the conclusions which follow are different.

According to KENNIE & PAUL (1974), the values for $\delta 15$ reflect the 'culmulative effects that origin, biological processes and management have all had on the soil'. HAUCK (1973)affirms that the knowledge of variations of natural isotopes can give information on the changes which take place in the level of the soil-plant system. In KOHL, SHEARER, COMMONER (1971) and again LETOLLE & MARIOTTI (1974) the content of

^{15}N in nitrates from surface waters allows the detection of their origin: native organic nitrogen or nitrogenous fertilizers. In comparison EDWARS (1974) found that the isotopic variation does not permit identification of pollution sources of nitrates in the soil-fertilizer system.

In this first work, we report the results of calculations of natural isotope ratios of nitrogen in samples of water, soil and vegetation prevailing in Dombes and discuss the possibilities of errors (BREMNER 1963) and coefficients of fractionation.

Materials and techniques.

The region of Dombes is part of the plateau of Bressan situated to the Northeast of Lyon. It is characterized by the number of ponds; the acid soil (pH 4 to 5), silt-clay, is constituted solely of fine elements, and has been the object of physico-chemical (CHALMET, 1970) and biological studies (BEAUPIED 1969).

Different examples of water, soils and plants prevail in a transect following the water zonation pools; prairie with agrostis, plains (teppes) with sarothammes, coppice to forest of oak or birch. The levels of total nitrogen are calculated in the samples.

To try to envisage at what level the isotopic fractionation took place, we measured the isotopic rates of nitrites produced by Nitrosomonas europaea in culture in the presence of sulfate of ammonium, as well as of the isotopic ratio in the remaining sulfate of ammonium. Nitrosomonas represents an important link in the nitrogen cycle in allowing the transformation of ammonium to nitrite.

The bacteria are innoculated into a buffered medium at pH 7.9 to which has been added 100 ppm of $N\text{-}NH_4^+$. Measurements are taken when approximately half of the ammonium has been transformed to nitrite.

Measurements of the isotopic ratio $^{29}N_2:^{28}N_2$ by mass spectrometry are made as gaseous samples. It is thus necessary to collect the nitrogenous azo-compounds and transform them to N_2.

The preparation is made by the method used by BREMNER, 1965; BREMNER & EDWARDS, 1965) which necessitates converting the nitrogen compounds to ammonium. The ammonium is recovered after distillation of NH_4^+ with the apparatus

from BREMNER.

Nitrates and nitrites are reduced by the Dewarda reaction. The reaction can only be used on samples which have not undergone distillation because it will easilyattack organic matter. Impurities such as amines can also be recovered at the same time as the sulfate of ammonium and can distort the final result.

Transformation of sulfate of ammonium to gaseous nitrogen operates through a reaction with hypobromite of sodium in the Martin-Ross instrument (ROSS-MARTIN, 1970) modified by replacement of the air by helium.

$$2\ NH_4^+ + 3\ BrO^- + 2\ OH^- \rightarrow N_2 + 3\ Br^- + 5\ H_2O$$

According to BREMNER (C.P.), during the reaction of sulfate of ammonium to sulfate hypobromite, nitrogen evolved at the beginning of the reaction contains less nitrogen 15 than that evolved at the end of the reaction. EDWARDS (C.P.) recommends heating and shaking the mixture of ammonium-sodium hypobromite to assure complete reaction and the expulsion of all the N_2 gas in order to prevent fractionation.

In addition to that transformation of nitrogen, a secondary reaction of oxydation to N_2O can take place. But BREMNER concludes that this is not a difficulty in the spectrophotometric measurement, the gas being trapped by the liquid nitrogen. Indeed, we think, as does BREMNER, that there is no contamination of the nitrogen portion, but that variations observed in measurements of the same sample can be explained by the intervention of a lighter isotope fraction in the course of the reaction. Unhappily, we can do very little about it.

The nitrogen thus formed is introduced directly into the mass spectrometer in order to isotopically analyze it.

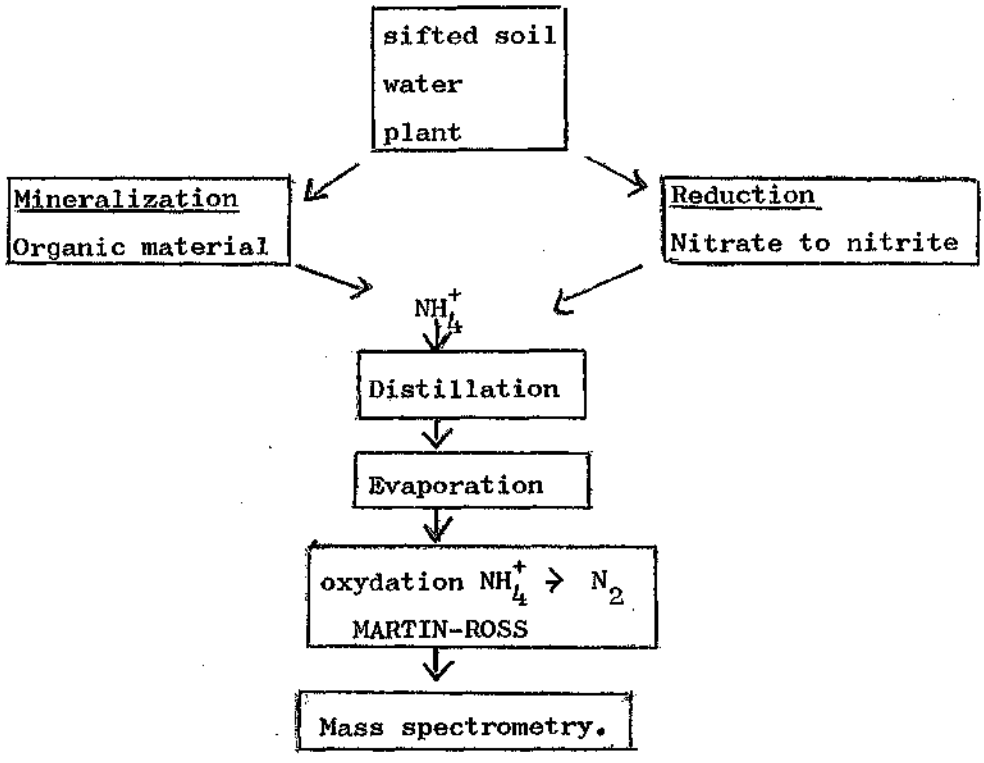
The mass spectrometer used is a AEI MS 20 with dual introduction: one for the sample to be analyzed, the other for a very pure (N 48) nitrogen gas standard. The gas standard allows comparison between measurements. The isotopic value in relation to air can be measured owing to a reaction of pyrogallol which retains the oxygen in the air. The percentage of nitrogen 15 in the standard is 0.3651%. The spectrometer is furnished with mechanisms allowing a vacuum extending into all the tubing connecting the

sample with the instrument. This assemblage follows the design given by VAN PRAAG (1971). Dead spaces are at a minimum and allow work on small quantities of sample (0.5 to 1 mgN). Results are given in $\delta^{15}N$ where:

$$\delta^{15}N = \frac{^{15}N/^{14}N \text{ sample} - {}^{15}N/^{14}N \text{ standard}}{^{15}N/^{14}N \text{ standard}} \times 100$$

The different steps of the method used are shown schematically in figure 1.

Fig. 1 - The different steps are:



Results and conclusions.

1) Dombes.

The number of samples varied from 3 - 6. Values are given with a maximum variation of 2%.

Table 1

Sample	Forest soil	Teppes soil	Vegetation	Prairie soil	Pool water
Mean of $\delta^{15}N$	-5.2	+5.5	+5.2	+9.9	+9.1

2) Nitrosomonas E.

We considered the values of $\delta^{15}N$ and the percentage of Nitrogen 15 from:

- the nitrite formed,
- the sulfate of ammonium which remained,
- the control sulfate of ammonium (half had not been reacted),

The fraction f having reacted:

$$f = \frac{\text{initial ammonium - remaining ammonium}}{\text{initial ammonium}}$$

Table 2.

Sample		Proportion f	$\delta^{15}N$	% ^{15}N
Control	1		10	0.3687
	2		10.8	0.3690
	3		10.5	0.3689
NH_4 remaining	1	0.66	54.4	0.3850
	2	0.64	46.6	0.3821
	3	0.47	34.6	0.3778
NO_2^- formed	1		-18.6	0.3583
	2		-19.1	0.3581
	3		-16.6	0.3590

The results presented in table 1 show that the forest soil has a $\delta^{15}N$ equal to -5.2 while that of the prairie soil is +9.9.

The soil of the forest has a smaller isotopic ratio than that of the air. This agrees with the results found by RIGA and colleagues (1971) as well as those of CHENG and colleagues (1964) who also found a negative $\delta^{15}N$ for forest soils. The explanation of RIGA et al is that a large quantity of relatively transformed vegetable debris returns to the soil. This debris is subjected to impoverishment in living tissues.

The soil under Sarothamme, and Sarothamme have similar $\delta^{15}N$ (5.5 and 5.2, respectively), lower than that of the prairie beside which they are found: ($\delta^{15}N$=9.9). This seems to confirm the influence that vegetation, in this case Sarothamme, has on the supporting soil.

The decrease in $\delta^{15}N$ can be explained by preferential assimilation of ^{14}N from the air by the plants. HOERING (1955) found negative values of $\delta^{15}N$ for leaves of clover. RIGA et al (1971) compared the isotope ratio of a field cultivated with vetch and that of the same field without vetch and established a decrease in the value of $\delta^{15}N$ for the field with vetch.

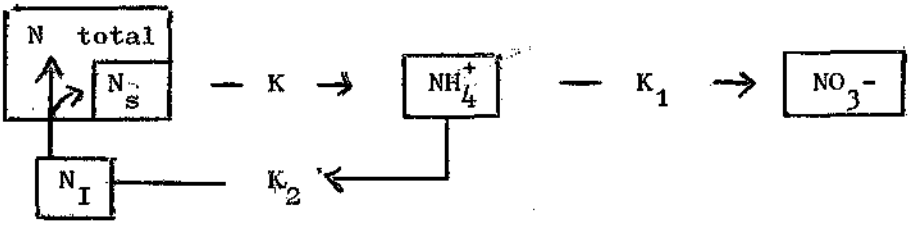
FREYER and ALY (1974) are equally interested in the isotopic ratio

of water: water recovered fron the soils of prairies, fields, forests. The values of ^{15}N of water reflect that of the terrain on which they are found. For example they are very low in a forest.

In the Dombe, the isotope ration of the water of the pond: $\delta^{15}N=9.1$, is very close to that of the prairie: $\delta^{15}N=9.9$. Is it possible then, as suggested by FREYER & ALY (1975) , to establish a relation between the soil nitrogen of the prairie and that of the pond water?

SHEARER and colleagues (1974) tried to analyze the fractions that appeared with a methematical model which schematically represents the transformation of nitrogen including ammonification, nitrification, denitrification.

Table III (from SHEARER et al).



where N_s is mineralized nitrogen.
N_I is immobilized nitrogen
K, K_1 ... K_4 are the equilibrium constants of the different processes.

Given the knowledge of the constants of fractionation: $\beta_1 = \frac{^{15}K_1}{^{14}K_1}$: nitrification constant & $\beta_2 = \frac{^{14}K_2}{^{15}K_2}$: for the immobilized ammonium, SHEARER et al have reached the following mathematical conclusions: if the immobilization increases, $\delta^{15}N$ decreases and the nitrogen returning to an organic pool is more rich in ^{15}N than the nitrogen which disappears by ammonification. The organic nitrogen is enriched in ^{15}N while the nitrate nitrogen is impoverished.

If one considers the ammonium - nitrite transformation which is effected by Nitrosomonas (Table II), one can see an important depletion of ^{15}N from

nitrite while the ammonium remaining is enriched in ^{15}N. Nitrosomonas utilizes essentially the ^{14}N. A part of the isotopic fractionation takes place at the level of the microorganisms. Given the values found, it is possible to calculate the fractionation constant β_0.

If one allows that the ammonium-nitrite is a first order reaction as SHEARER et al found in accordance with other authors, (TONG & YANKWITCH, 1957; DELWICHE & STEYN, 1970), the value of the factor of fractionation of nitrification (β_0) is found by applying the formula of TONG & YANKWITCH.

$$\beta_0 = \frac{\mathrm{Ln}\left[\frac{1}{(1-f)} \quad \frac{f\,(Rbt - Rat)}{(1-f)\,(Rbt + 1)}\right]}{\mathrm{Ln}\left[\frac{1}{(1-f)} \quad \frac{f\,(Rbt - Rat)}{(1-f)\,Rat\,(Rbt + 1)}\right]}$$

where f is the proportion of transformation.

R_{bt}: percentage of ^{15}N from the accumulated product, in this case, nitrite, in time t.

R_{at}: percentage of ^{15}N from the remaining product, in this case, ammonium, in time t.

Table IV

After 4 days of incubation, the values β_0 and f are respectively:

Sample	β_0	f
1	1.044	0.66
2	1.042	0.64
3	1.038	0.47

In the same way, DELWICHE & STEYN (1970) found a median value of: β_0=1.026 for a variation of f from 0.54 to 0.72.

FREYER & ALY (1975) calculated the fractionation constant during the whole of nitrification (nitritation and nitratation). This constant has a slightly smaller value:1.017.

This method will only give a value approaching the constant of fractionation because the initial quantities are given by the control samples without Nitrosomonas europaea and can only be an approximation of the value from the

initial quantities of ammonium disseminated from the samples.

Furthermore, one cannot measure the quantity of ammonium which is immobilized by the micro-organisms themselves or that which has volatilized in the course of the incubation. A raised pH (pH 7.9) favors this volatilization.

The degree of fractionation seems to vary as a function of the quantity of transformation is larger.

In the soil, it can be said that the quantity of ammonium stays constant (SHEARER, 1974) because it is produced in proportion to its utilization. In that case, the degree of fractionation is constant and can have a different value from that found in culture media.

The constant of fractionation of nitritation which was used by SHEARER et al (1974) only corresponds to that of nitrification. It remains to be proved whether biological transformation of nitrites to nitrates is not accompanied by any isotopic fractionation.

The problem remaining is to find if fractionation plays a role when using depleted or enriched nitrogen as a marker.

For this we have innoculated a medium containing labelled sulfate of ammonium with Nitrosomonas europaea.

Table V

% ^{15}N	Control	NH_4^+ remaining	NO_2^-
Nitrogen impoverished	0.018	0.019	0.018
		0.029	0.026
		0.022	0.018
Nitrogen enriched	4.60	4.86	4.49
		4.83	4.11
		4.65	4.19

We noticed a small difference between NH_4^+ and NO_2^-: ammonium has a higher isotopic value (0.1 to 0.7%) than that of nitrite.

In reality, these differences are of little significance because they are of the same order of magnitude as those found between different experiments. They will arise from the variations which one observes in all biological processes, and also from the precision of the measurements. Mass spectrometry gives less precise meaasurements on samples containing a large quantity of labelled product than on those containing nitrogen impoverished in N. One has to work within very sensitive limits and the influence of the standard of reference on the sample is of great importance. The values obtained seem less stable and less precise.

If labelled nitrogen is used for the study of different stages of the nitrogen cycle, the value of the ratio of natural isotopes occurring in the dilution of the label becomes very important. On the other hand, it can be considered negligible compared to the values of ^{15}N of the products that we have utilized, which are of the order $\pm$ 10.000, soils themselves having a $\delta^{15}N$ of $\pm$ 30.

Conclusions.

The content of ^{15}N measured in certain natural constituents is sufficiently small to be ignored during the use of substances labelled by enrichment or depletion of the heavy isotope; at the most, it will have to be taken into account at large dilutions of the labelled substances.

The impoverished nitrogen used corresponds to a $\delta^{15}N$ of about 10 000 and the maximum difference between the $\delta^{15}N$ of non-labelled samples is 70 for Nitrosomonas europaea. When calculating the possibility of error (one must remember), the substance containing nitrogen impoverished in ^{15}N can be diluted about a hundred times in the course of the diverse transformation to which it is subject. This allows great possibilities of utilization in the field.

By contrast, it allows emphasis on the natural variations of nitrogen 15 in different soils: $\delta^{15}N$ of the forest -4.5, of the teppes +5.2 and of the prairie +9.9 as well as the role of microorganisms in isotope fractionation; the coefficient fractionation by Nitrosomonas europaea is 1.04

Without entering into a discussion per se, we would agree with the sentiments of RENNIE & PAUL (1975) (it is necessary) "to know and understand the processes,

biological and other, which control the isotopic concentrations of nitrogen in the soil before being able eventually to use and interpret them".

It appears from the model of SHEARER et al (1974), that laboratory studies allow us to better understand the diverse fractionation in the course of the nitrogen cycle. In addition, the prairie of the Dombes encompasses ponds fortified only by ground water and leached water seems to be an interesting field for following labelled ^{15}N.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.