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^{15}N-NH_4+ Isotope Dilution Method for Analyzing Nitrogen Transformation in Upland Soils

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Abstract

Utilization of ^{15}N in the studies on N transformation in upland soils was reviewed with emphasis placed on a ^{15}N-NH_4+ isotope dilution method. Although this method is not extensively used at present, it is informative and useful for quantitative investigations on N dynamics in soils, because it is the only means which can determine the gross rates of mineralization and immobilization at the same time. It is of primary importance to examine which kinetics can adequately explain each process of N transformation. Although both the zero-order and the first-order kinetics are applicable, their applicability depends on various factors: e.g. concentration of substrate and time span of incubation. Remineralization of tagged-^{15}N is another critical problem. However, the errors caused by remineralization can be practically avoided by shortening the incubation period.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: gross rate, kinetics, remineralization

Introduction

Utilization of ^{15}N is a promising approach for analyzing quantitatively dynamic aspects of the nitrogen cycle in soils, in which various metabolic processes interact intricately with each other. Although the use of ^{15}N provides a highly reliable means to determine rates of denitrification and nitrogen fixation, the following efficient methods have also been developed for a practical use employing acetylene, comprising an acetylene inhibition method for denitrification and an acetylene reduction method for nitrogen fixation. In recent years, these two methods using acetylene have been adopted more extensively than those with ^{15}N, because they are convenient, rapid and less costly. However, it should be stressed that the use of ^{15}N is still highly recommendable specifically in the studies on mineralization and immobilization in soil N cycle, since it is the only means capable of determining gross rates of those opposite processes simultaneously. The present paper attempts to review the current status of the use of ^{15}N in the studies on N transformation in upland soils, with special emphasis placed on the ^{15}N–NH_4^+ isotope dilution method.

Ammonium in soils is consumed by plants and soil microorganisms, whereas at the same time it is produced by soil microorganisms from organic matter in soils. Apart from fixed ammonium, a pool size of ammonium in soils, which is readily available for microorganisms and plants, is usually small, and turnover of the ammonium contained in the pool is much faster than that of the organic N pool. When an appropriate amount of ^{15}N-tagged ammonium is added to the ammonium pool in soils, the ^{15}N-tagged ammonium is immobilized, and at the same time, ammonium, ^{15}N enrichment of which is nearly at natural abundance, is produced from soil organic matter. The ^{15}N–NH_4^+ isotope dilution method utilizes such characteristics that the ^{15}N-tagged ammonium added to the ammonium pool in soils is diluted within a short period of time (Table 1). Temporal changes in ammonium pool size and its ^{15}N fraction are represented by simultaneous differential equations, which include the processes of mineralization and immobilization. Each of the N transformation rates can be determined by using analytical solutions of the equations or a numerical analysis with the aid of a computer. The ^{15}N isotope

Table 1. Temporal changes in NH_4^+-N and NO_3^--N content during incubation in the presence of 0.5% C_2H_2

(Unit: μgN g^{-1})

Incubation time (hr)	NH_4^+-N		NO_3^--N	Total inorganic N
	$^{14}N+^{15}N$	^{14}N		
0	15.5	7.05	3.80	19.3
42	15.9	5.59	3.86	19.8
96	17.2	5.16	3.81	21.0
142	17.4	4.72	3.86	21.3
192	18.1	4.26	3.90	22.0

Incubation temperature: 20°C. Water content: 55% of the maximum water-holding capacity.
Source: Nishio et al. (1985)[18].

dilution method can also be applied to analyze nitrification and nitrate reduction with the use of $^{15}NO_3^-$ under the same principle with $^{15}NH_4^+$.

The greatest advantage of the ^{15}N isotope dilution method is that the actual heterotrophic activities with regard to N transformation can be determined within a relatively short period of time. Even when net changes in content of inorganic N in soils are hardly detected, rates of mineralization and immobilization could be obtained, if slight changes in ^{15}N enrichment of ammonium pool are detectable. Although the N mineralization rate of the selected organic matter or the microbial biomass in soils can be determined by labelling them with ^{15}N, a gross mineralization rate of native organic matter in soils cannot be determined without the use of the ^{15}N isotope dilution method. It is therefore concluded that the ^{15}N isotope dilution method is an informative and useful tool in analyzing nitrogen dynamics in soils.

History of the ^{15}N isotope dilution method

The phenomenon of ^{15}N isotope dilution has been discussed in relation to the priming effect of fertilizers[6–8,11,20,24,25]. Although an amount of fertilizer N absorbed by crops can be calculated by the following two methods: one is based on the absorption of ^{15}N-tagged fertilizer; and the other is based on the difference in N absorption between the fertilized crops and the non-fertilized control. The amount calculated with the former method is generally smaller than that with the latter. There are three explanations as the cause of this discrepancy: i.e. (1) an fertilizer application results in extention of rhizosphere of the crops and, consequently, increases the absorption of soil N; (2) mineralization of soil N is enhanced by the application of fertilizers; and (3) exchange between the ^{15}N-tagged fertilizer and the non-tagged N mineralized from soil organic matter takes place in the course of mineralization–immobilization processes, and the crops absorb the fertilizers partially substituted by soil N. There might be a question whether the increased effects of fertilizer application on the soil N absorption by crops, as seen in the case (1) and/or (2) above, are significant or not. It is still open to further discussion. In any case, however, in identifying actual effects of fertilizer N, it is prerequisite to quantify the exchange of N between the soil organic matter and the fertilizers applied.

Approximately 40 years have passed since the ^{15}N isotope dilution method was put to use for the studies on N transformation in soils. However, there have been limited investigations that could confirm an effective application of this method. The reason for the limited use of the ^{15}N isotope dilution method is probably that the determination of ^{15}N enrichment and the mathematical procedures needed to analyze data is too laborious for the practical use in a routine study.

In the 1950s, Hiltbolt et al.[9] evaluated the magnitude of mineralization and immobilization employing a ^{15}N tracer technique. Kilkham and Berthoromew[12,13] formulated a ^{15}N isotope dilution method on a theoretical basis (Fig. 1), and applied it to the experimental data. Jansson[10] investigated the soil N dynamics including remineralization of immobilized N, using also a ^{15}N isotope dilution method. In the following years, the ^{15}N isotope dilution method was applied to the analyses of marine sediment[5], upland field soil[4,15,17,18] and paddy soils[23].

Nishio et al.[18] determined successfully the rates of the N transformation including nitrification by a short-term (within several days) incubation of the soils. Nishio and Fujimoto[17] examined a total amount of the gross N mineralization in maize during its growing period to compare the amount of soil N absorbed by the plants (Table 2). Myrold et al.[15] proposed a more comprehensive model, which included heterotrophic nitrification and denitrification (Fig. 2). The model was applied to the analysis of

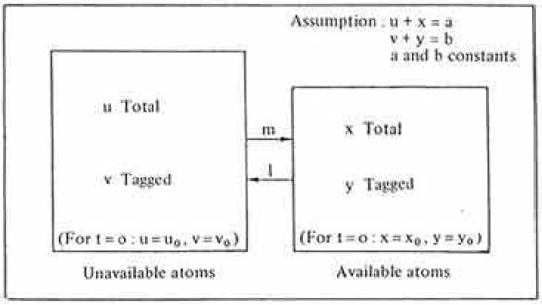

Fig. 1. Schematic diagram of the model used by Kirkham & Bartholomew[13)]

Table 2. Estimates of gross N mineralization in topsoils and N absorption by maize

(Unit: kg/ha)

	Brown Andosol	Ordinary Andosol
Amount of mineralized N[a)] (May 10 – Sept. 20)	521	808
Content of organic N[a)]	6,140	14,000
Absorption by maize		
(Cropped plot 1; N 150 kg/ha)[b)]		
Soil N[c)]	56	–
Fertilizer N[c)]	94	–
(Cropped plot 2; N 0 kg/ha)[b)]		
Soil N[c)]	46	–

a): Calculated by assuming a topsoil depth of 35 cm.
b): Application rate of fertilizer N.
c): Estimated from ^{15}N abundance and total N at harvest time.
Source: Nishio & Fujimoto (1989)[16)].

experimental data with a nonlinear parameter estimation method. They obtained appropriate rate constants of N transformation. However, denitrification parameters were not adequately estimated because they were too low. Bjarnason[4)] examined the validity of the assumptions that were made in the ^{15}N isotope dilution method, advocated the advantages of combined use of his simulation model with an optimization procedure.

Problems in the ^{15}N isotope dilution method

It is of primary importance for the valid use of a kinetic model to identify the kinetics (zero-order, first-order, or Michaelis-Menten kinetics) which the metabolic processes in question are directly associated with. Kirkham and Berthoromew[13)] postulated the following two cases: (1) the mobilization (mineralization) rate m and the immobilization rate i are both constant; and (2) m is proportional to the amount of unavailable materials, i.e. organic matter, while i is proportional to the amount of available materials, i.e. ammonium. They found that the case (2) above was in good agreement with the results obtained in the experiment. However, it was also indicated that the theory for the case (1) could be applicable provided the time span in question is brief. Myrold et al.[15)] showed that the zero-order and the first-order model could both adequately explain the N cycling, noticing that the latter model would be more appropriate for general use. Relationships

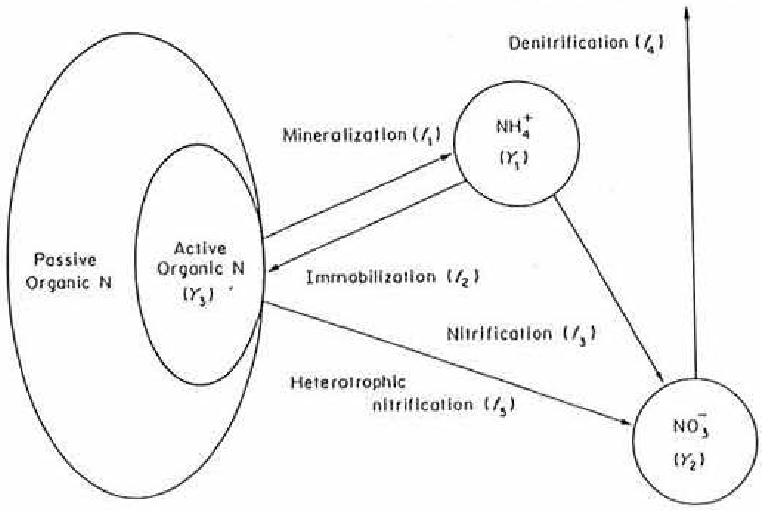

Fig. 2. Schematic diagram of the compartmental model used by Myrold et al. (1986)[15)]

Table 3. Correlation between ammonium concentration and rates of N transformation

Initial NH_4^+ concentration (μgN g^{-1})	Rates (μgN g^{-1} day^{-1})		
	Minerali.	Immobili.	Nitrifi.
2.5	1.82	1.40	2.01
5.5	2.10	1.87	2.25
10.2	1.89	1.96	2.80
51.2	2.16	2.75	5.73

Source: Nishio et al. (unpublished data).

between the ammonium concentration in soils and the N transformation rates are shown in Table 3[18]. It is indicated that the mineralization rate is almost constant irrespective of the ammonium concentrations in soils, providing that the ammonium concentration in soils is less than 50 μgN g^{-1}. However, the rates of immobilization and nitrification increase with higher ammonium concentrations.

It might be expected that better results could be obtained by assuming that each N transformation rate is represented by the function of pool size of the substrate of each process. However, it is difficult to explain fully each of those processes with a simple kinetics. Okereke & Meints[19] demonstrate that the instant uptake of ammonium takes place immediately after the addition of fertilizer ammonium. The nitrification rate increases with time after the addition of ammonium because of proliferation of nitrifiers. The denitrification rate is drastically enhanced, when anoxic microsites are developed with an increased consumption of O_2 by soil microorganisms. In case of mineralization, the determination of pool size of active (easily decomposable) organic matter is very difficult. As a consequence, any precise description on those processes is practically not possible on the basis of a simple kinetics. However, some approximations could be made on a simple kinetics to explain N transformation processes, unless the soil incubation is prolonged under an artificial condition.

Remineralization of tagged-^{15}N is another critical problem for the quantitative determination of N transformation rates. It is demonstrated that the newly immobilized N is mineralized faster than the indigenous soil organic N[2,10]. It is therefore likely that remineralization of tagged-^{15}N results in underestimation of N transformation rates. Bjarnason[4] examined the errors which might be caused by the assumption that no remineralization of tagged-N occurred. He concludes that the possible errors caused by omitting remineralization are not large enough during the first week, but very significant in 2 weeks or beyond. Since the apparent turnover times of N in the active organic pool are estimated to be 63–114 days[15], and 80–130 days[17], influence of the remineralization may probably be negligible in a short-term (within several days) incubation of soils.

Stanford & Smith[21] and Stanford et al.[22] developed an incubation procedure to estimate N availability in soils. Although a series of time-related data on inorganic N production in soils are obtained by that method, the outputs are related to determination of the pool size of easily decomposable organic matter. On the other hand, the aim of the ^{15}N isotope dilution method refers mainly to determination of the activities of soil microorganisms. In this respect, it is necessary to take into account that biological activities are liable to change with various changes in soil conditions such as period and way of soil preservation, experimental treatments for soils. For example, when the ^{15}N-tagged ammonium solution is uniformly added to the soil, an increase in moisture content of the soils may affect the microbial activity to some extent. In addition, mixing of the solution with soils might physically disrupt the soil aggregate and as a consequence, accessibility of the microorganisms to soil organic matter might be increased[1], still subject to a further study.

Perspectives on the ^{15}N isotope dilution method

It is demonstrated that dead microbial biomass plays an important role as a substrate of N mineralization[3,14]. It is recognized that considerable portions of the N absorbed by plants are produced by the decomposition of the dead microbial biomass. Therefore, in analyzing N dynamics, it is useful to identify likely correlations between the fluctuations in microbial biomass (or active organic N) and the changes in gross rates of N transformation. Since characterization and quantification of the active pool of soil organic matter still remians unexplained, N transformation processes should be pursued in close association with C metabolism in soils. In the plant–soil system, competition for inorganic N between plants and soil microorganisms is another subject that requires further studies. Partitioning of inorganic N, including fertilizer, into microbial recycling and plants absorption would probably be clarified by the aid of the ^{15}N isotope dilution method and/or the ^{15}N tracer technique.

In conclusion, pool size of the components in soil nitrogen cycle and transformation rates among those components are indispensable factors for quantitatively analyzing the N dynamics in soils. Far more data on N transformation in soils should be collected in using the ^{15}N isotope dilution method so that a wide range of conditions and various types of soils could be adequately covered.

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