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Simultaneous Determination of Transformation Rates of Nitrate in Soil

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Abstract

We determined the gross rates of nitrification and nitrate immobilization in soil using a ^{15}N-nitrate isotope dilution technique. We also measured the amounts of nitrate immobilization and denitrification simultaneously by combining the acetylene inhibition technique and mass balance of nitrate. The immobilization rate of nitrate was enhanced by about 2 orders of magnitude by the addition of straw, while it did not increase significantly by the addition of manure. The denitrification rate in soil under anaerobic conditions was remarkably enhanced in the presence of straw, and enhancement of denitrification was also observed to some extent by the addition of manure or bark manure. In the soils treated with straw, the immobilization rate of nitrate under aerobic conditions was 24% of the denitrification rate under anaerobic conditions. Effect of water addition on nitrate immobilization and denitrification in soil was investigated in the presence or absence of straw. The amount of nitrate immobilization in the soils with straw did not change drastically with the moisture content, while that of denitrification changed by 2 or 3 orders of magnitude with the moisture content between 54–84% of the maximum water-holding capacity of soil irrespective of straw addition.

Disciplines: Soils, fertilizers and plant nutrition
Additional key words: immobilization, denitrification, ^{15}N isotope dilution

Introduction

Leaching of nitrate from arable land is one of the most serious environmental problems of intensive agriculture where large amounts of fertilizers and organic materials are applied. To reduce the amount of leaching N, some organic materials with a high C/N ratio such as straw, sawdust, etc. have been tentatively applied in the subsurface layers of soils.

Immobilization of nitrate by soil microorganisms is considered to be one of the pathways leading to a decrease of the nitrate content in soils[6, 16]. Powlson et al. (1986)[14] reported that losses of ^{15}N-labelled nitrate applied in the autumn could be reduced from 60 to 47% by the incorporation of 3 t ha^{-1} wheat straw into a silty clay loam soil. However, Recous et al. (1990)[15] showed that immobilization of nitrate did not occur in the soils incubated without glucose, and that whether nitrate immobilization occurred or not depended on the presence of ammonium in soil. Organic matter application to soil and residual ammonium are considered to be 2 important factors that are responsible for the occurrence of nitrate immobilization.

Although denitrification is another process which results in the decrease of the nitrate content in soil, there is a large difference in nitrate consumption by denitrification or immobilization in terms of crop production and/or environmental influence. Denitrified N is lost to the atmosphere, whereas nitrate immobilized by soil microorganisms may be remineralized later, and could provide a nutrient source for crop growth in the later period and/or become the leaching source of N. Nielsen and Jensen (1986)[8], based on an experiment with spring barley, suggested that at least 20–30% of fertilizer N disappeared soon after the application was recovered in the crop after the later period of growth. The estimation of nitrate immobilization and remineralization is of primary importance for assessing the influence on crop production and control of nitrate leaching.

Denitrification and nitrate immobilization are similar in that organic matter (energy source) is necessary for

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their active occurrence. These two processes could simultaneously occur at the same site in soil with easily decomposable organic matter. For example, denitrification was considered to be enhanced in the close proximity of roots by the utilization of root exudates[19], and nitrate immobilization and competition for nitrate between microorganisms and plants were also reported in the rhizosphere where excess C supply and limited N supply occur[12, 16]. However, information on the extent of nitrate consumption in the rhizosphere associated with immobilization or denitrification is limited.

In the present study, we determined the gross rate of nitrate immobilization together with nitrification using a ^{15}N-nitrate isotope dilution method, and investigated the major factors controlling nitrate immobilization in soil. Moreover, we determined the denitrification and nitrate immobilization simultaneously in soil at various moisture levels. Attempts were made to elucidate the effects of environmental factors, mainly organic matter and moisture contents, on the dynamics of nitrate in soil.

Materials and methods

The soils used in the experiments were collected from the surface layer (0–15 cm) in experimental fields of Hokuriku National Agricultural Experiment Station (Takada) and National Agriculture Research Center (Tsukuba), Japan. The Takada soil is classified as a Fine-textured Strong-gley soil (Eutric Gleysols) with a LiC texture, and the Tsukuba soil as a Light-humic Andosol with a L texture. The soils used were abbreviated as FG for the Takada soil and LA for the Tsukuba soil, respectively. Contents of C and N in the soils were 28 and 2.6 g kg^{-1} for FG, and 46 and 3.9 for LA, respectively. The maximum water-holding capacity was 0.387 kg kg^{-1} for FG, and 0.502 for LA on a fresh weight basis. The soils collected were sieved (< 2 mm), and stored at 4ºC until use. Only Exp.1 was conducted by using FG, and the other 3 experiments (Exp.2–4) were conducted by using LA.

1) Exp. 1

Fresh FG samples equivalent to 100 dry wt were placed in 500 mL flasks. Five treatments were set up in duplicate (Fig. 1). To each soil sample, 5 mL of ^{15}N-KNO_3 (equivalent to 50 mg N kg^{-1}dry soil; 31.3 atom % ^{15}N) was added and the moisture content of the soils was adjusted to 28.5% on a fresh weight basis. The soil samples were mixed uniformly, and incubated at 25ºC. Twenty g of soils were taken at appropriate time intervals from each flask, and inorganic N was extracted by a 2 M KCl solution. The extracts were frozen until analysis.

2) Exp. 2

Fresh samples of LA equivalent to 70 g dry wt (moisture content: 0.347 kg kg^{-1}) were placed in 500 mL flasks. In this experiment, 7 treatments shown in Table 1 were set up in duplicate. To each soil sample, 3.5 mL of ^{15}N-KNO_3 (equivalent to 50 mg N kg^{-1}; 31.3 atom % ^{15}N) was added, and additional materials were applied or the incubation temperature was changed (Table 1). The processes of incubation and sampling were the same as in Exp. 1 with minor modifications.

3) Exp. 3

Forty g of field-moistened LA soils were placed in 100 mL wide-mouth bottles. To half of the bottles, 0.4 g of wheat straw cut into fragments less than 1 cm long was added. Moisture content of the soils was adjusted by the addition of distilled water at the beginning of the experiment so that 5 different moisture levels were prepared. To all the soils, 50 mg N kg^{-1} of a KNO_3 solution was added. The moisture contents of the soils after the addition of the solution were 35.4, 38.4, 41.2, 43.8, and 46.1 kg kg^{-1}, which corresponded to a range between 54 and 84% of the maximum water-holding capacity. After the bottles were closed with rubber stoppers that were equipped with a gas injection (sampling) port, 1.5% vol/vol of acetylene was injected to the headspace of the bottles. In a preliminary experiment, we confirmed that 1% vol/vol of acetylene was practically sufficient to inhibit denitrification completely. The soils were incubated at 25ºC, and 20 g of soils were removed for KCl extraction after 2 and 4 days. The gas in the headspace was collected every day to determine the nitrous oxide concentration.

Table 1. Treatments in Exp. 2

Treatment	Materials added	Amount (g)	Temperature (ºC)
1	Wheat straw	1.0	20
2	Wheat straw	1.0	25
3	Wheat straw	1.0	30
4	Manure	3.0	25
5	Bark manure	3.0	25
6	Wheat straw+NH_4^+ (+10 mg N-NH_4^+)	1.0	25
7	No addition		25

Composition(%): Wheat straw C 41.6, N 0.66, C/N 63.5.
Manure C 23.8, N 2.53, C/N 9.41.
Bark manure C 35.4, N 1.80, C/N 19.7.
Water content: Manure 68%, Bark manure 51%.

4) Exp. 4

Forty g of field-moistened soil samples were placed in 100 mL wide-mouth bottles, and the moisture content was adjusted to 38.4 kg kg^{-1}. Duplicate 4 treatments were prepared by the addition of different organic materials. The organic materials consisted of wheat straw, manure and bark manure which were used in Exp. 2. After 5 mg N kg^{-1} of KNO_3 was added, the bottles were stoppered, gas in the headspace was exchanged to helium and acetylene (1.5% vol/vol), and the bottles were incubated at 25ºC. The gas samples were taken from each bottle at appropriate intervals, and the concentration of nitrous oxide was determined by gas chromatography.

5) Analytical method

Concentrations of ammonium and nitrate in the KCl extracts were determined colorimetrically with a Technicon autoanalyzer, using the indophenol method for ammonium and the hydrazine reduction method for nitrate, respectively. The ^{15}N ratio of inorganic N was determined by emission spectrometry[3]. Concentration of N_2O in the gas samples was determined using a Shimadzu 14-A gas chromatograph fitted with an electron capture detector (temp. 340ºC) and a 3 mm × 3 m Porapak Q column (temp. 70ºC).

6) Calculation method

Gross rates of nitrate immobilization and nitrification were calculated based on the ^{15}N isotope dilution method[4,5]. The rates were calculated by determining the temporal changes in the pool size and ^{15}N abundance in the ^{15}N-amended nitrate pool of the incubated soil. The equations used were as follows,

$$n = ln\{(p_0/P_0 - k)/(p/P - k)\} \cdot (P_0 - P)/ln(P_0/P)/t$$

$$i = ln\{(p_0 - kP_0)/(p - kP)\} \cdot (P_0 - P)/ln(P_0/P)/t$$

where n is the nitrification rate, i the immobilization rate of nitrate, P the content of nitrate, p the content of ^{15}N-nitrate, P_0 the initial content of nitrate, p_0 the initial content of ^{15}N-nitrate, k the natural abundance of ^{15}N in nitrate produced by nitrification, and t the time.

In the simultaneous determination of nitrate immobilization and denitrification, the amounts of denitrified N were estimated from the N_2O production during soil incubation under acetylene[20] and those of immobilized N were estimated from the difference between the initial and final contents of nitrate in soil, and the amount of denitrified N.

Results

Fig. 1 shows the temporal changes in the contents of nitrate and ^{15}N-nitrate obtained in Exp. 1. Content of ammonium in all the soils was low throughout the experiment (data were not shown). Contents of nitrate and ^{15}N-nitrate in the soils treated with glucose decreased

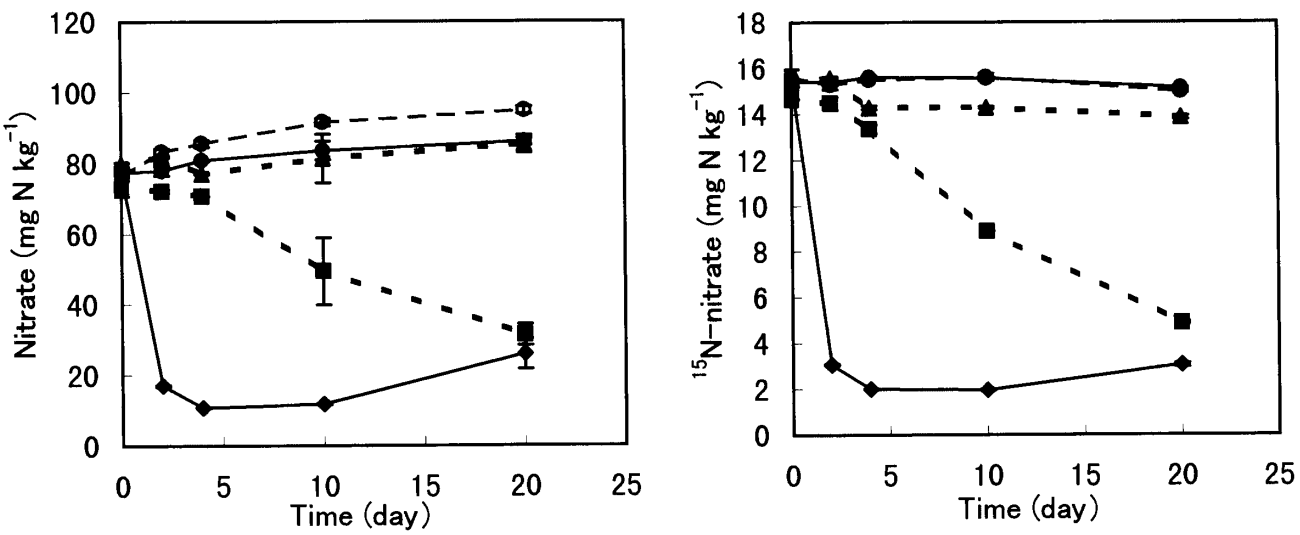

Fig. 1. Changes with time in the contents of nitrate and ^{15}N-nitrate in soil obtained in Exp. 1

Three kinds of organic materials were added in treatments 1–3, respectively: 0.1 g-C of glucose was added in treatment 1; 1 g of rice straw (C 36.5%, N 0.58%) in treatment 2; 3 g of manure (C 22.5%, N 1.26%, water content 60.0%) in treatment 3. The rice straw was cut into fragments less than 1 cm long before the addition. In treatment 4, the soil was air-dried to the moisture content of 0.05 kg kg^{-1} at 25ºC and remoistened to the original moisture level at the beginning of the experiment.

◆ : Glucose, ■ : Straw, ▲ : Mmanure, ○ : Air-drying, ● : No addition. Bars on this figure show standard errors.

Table 2. Rates of nitrification and immobilization obtained in Exp. 1 (mg N $kg^{-1}d^{-1}$)

Treatment	Nitrification	Immobilization
1 Glucose	2.22	32.30
2 Rice straw	0.37	2.63
3 Manure	0.70	0.41
4 Air-drying	0.89	0.14
5 No addition	0.52	0.08

Table 3. Rates of nitrification and immobilization obtained in Exp. 2 (mg N $kg^{-1}d^{-1}$)

Treatment	Nitrification	Immobilization
1 Straw 20ºC*	0.10	3.66
2 Straw 25ºC*	0.74	5.47
3 Straw 30ºC*	0.88	6.93
4 Manure	0.47	0.02
5 Bark manure	1.27	0.56
6 Straw+NH_4*	9.18	3.74
7 No addition	0.31	0.02

* The values in the initial 7 day-period were used to calculate the rates.

most rapidly at the beginning, and increased after 10 days. Considerable decrease in the nitrate and ^{15}N-nitrate contents was observed in the soils amended with rice straw. In the ot… increased slowly, … decreased. The gro… zation were calculated from the data shown in Fig.1 assuming that these rates followed a zero-order kinetics (Table 2). Although the immobilization rate of nitrate was enhanced by the addition of rice straw, the rate was one order of magnitude lower than that in the glucose-amended soil. The immobilization rate of nitrate in the other treatments was in the same order as in the control.

Similar results were obtained in Exp. 2 (Fig. 2). Changes with time in the contents of nitrate and ^{15}N-nitrate were very fast in the soils treated with wheat straw. Content of ^{15}N-nitrate in the soils in which both wheat straw and ammonium were applied decreased too, but the rate was not as fast as that without ammonium addition. Since the bark manure contained a high concentration of ammonium, the nitrate content in the soils amended with bark manure increased with time. How-… zation of ^{15}N-nitrate was observed. The rates of immobilization and nitrification calculated based on the data in Fig. 2 are shown in Table 3. The trend obtained in the LA soil was nearly the same as that obtained in FG soil.

Table 4 shows the amounts of immobilized N and denitrified N during 4 days after water was added to change the moisture content of the soils. The amounts of nitrate immobilization and denitrification were represented by the cumulative amounts during 4 days, because the rate of denitrification could not be calculated due to the lag time until denitrification occurred[11]. The amount of denitrified N at a high moisture content was 2 or 3 orders of magnitude higher than that at a low moisture content irrespective of the addition of wheat straw. However, the rate of immobilization did not change drastically with the moisture content of the soil. At a high moisture

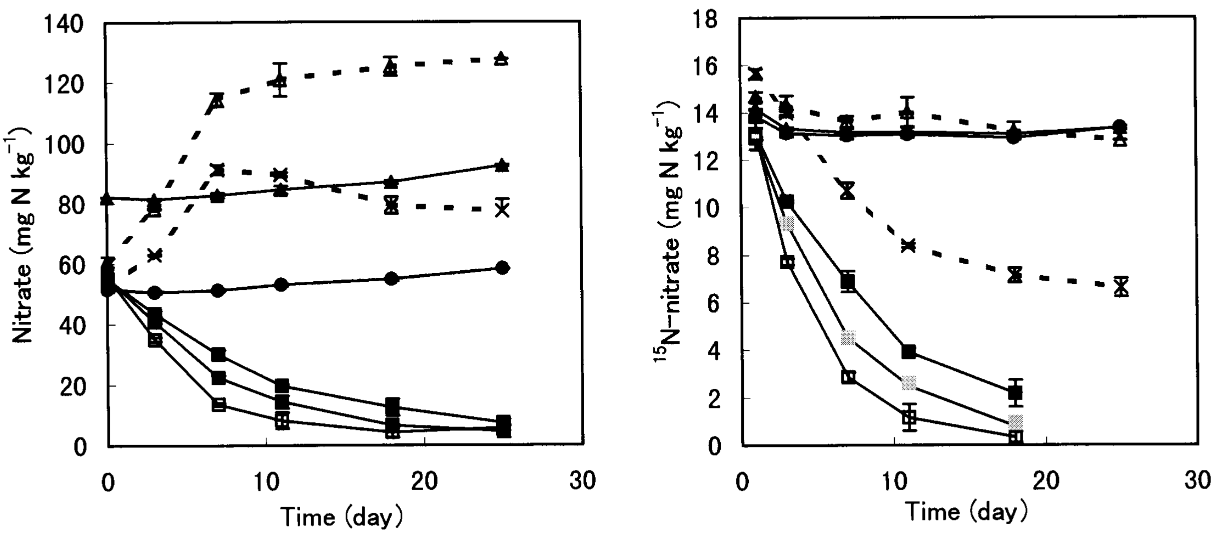

Fig. 2. Changes with time in the contents of nitrate and ^{15}N-nitrate in soil obtained in Exp. 2
■ : Wheat straw 20ºC, ■ : Wheat straw 25ºC, □ : Wheat straw 30ºC, × : Wheat straw+ammonium, ▲ : Manure, △ : Bark manure, ● : No addition. Bars on this figure show standard errors.

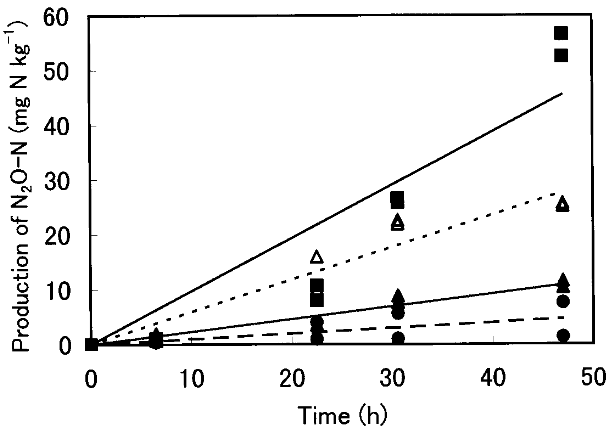

Fig. 3. Changes with time in the production of nitrous oxide from soil (Exp. 4)

Straight lines were obtained by linear regression.
■ : Wheat straw, ▲ : Manure, △ : Bark manure,
● : No addition.

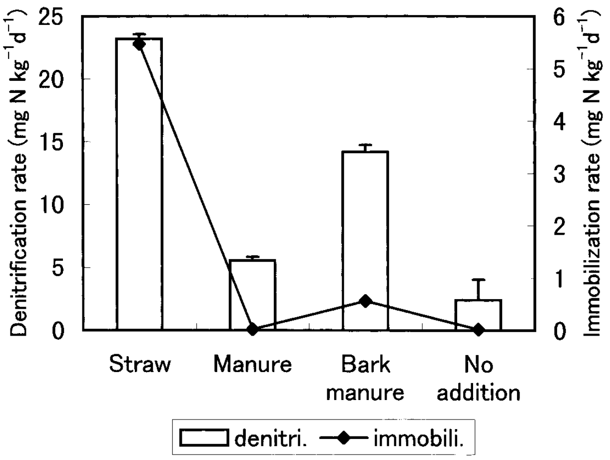

Fig. 4. Rates of denitrification and nitrate immobilization in the soils amended with organic materials

The rates of denitrification were obtained in Exp. 4, and those of nitrate immobilization are the same as those shown in Table 4.

content, the amount of denitrified N was in the same order of magnitude as that of immobilized N.

In Exp. 4, the denitrification rate in the soils treated with wheat straw was highest, followed by bark manure (Fig. 3). Fig. 4 shows the rate of denitrification calculated based on the data shown in Fig. 3. The immobilization rate shown in Table 3 was also plotted in Fig. 4 to compare both rates, indicating that the consumption rate of nitrate in an anaerobic soil was more than 4 times higher than the immobilization rate in an aerobic soil. In the soils treated with manure or bark manure, the rate of immobilization was more than one order of magnitude lower than that of denitrification.

Discussion

In this study, the rates of nitrification and nitrate immobilization were estimated simultaneously by using a ^{15}N-nitrate isotope dilution method. The ^{15}N isotope dilution method is extensively used to estimate the rates of N transformation by applying ^{15}N-ammonium [2,10], and both ^{15}N-ammonium and ^{15}N-nitrate[7,17]. The ^{15}N-nitrate iso-

Table 4. Effect of addition of straw and water on the amounts of immobilization and denitrification[a)]

(Unit: mg N kg^{-1})

Moisture content(%)	Straw	Immobilization[b)]	Denitrification	Total
35.4 (54)[c)]	–	ND	ND	ND
38.4 (62)	–	ND	0.01 ± 0.00	0.01 ± 0.00
41.2 (69)	–	0.80	0.03 ± 0.01	0.83 ± 0.63
43.8 (77)	–	1.95	1.03 ± 0.73	2.98 ± 0.74
46.1 (84)	–	0.73	2.26 ± 0.22	2.99 ± 0.92
35.4 (54)	+	31.54	0.01 ± 0.00	31.55 ± 0.72
38.4 (62)	+	34.16	0.12 ± 0.09	34.28 ± 2.27
41.2 (69)	+	35.74	9.22 ± 1.15	44.96 ± 4.68
43.8 (77)	+	33.47	29.51 ± 1.42	62.98 ± 0.24[d)]
46.1 (84)	+	34.73	28.85 ± 1.62	63.58 ± 0.04[d)]

a): Total amount during 4 days.
b): (Immobilization) = (Total) – (Denitrification) (see text).
c): The values in parenthesis represent the percentage of the maximum water-holding capacity.
d): Nitrate in the soils was consumed completely during 4 days.

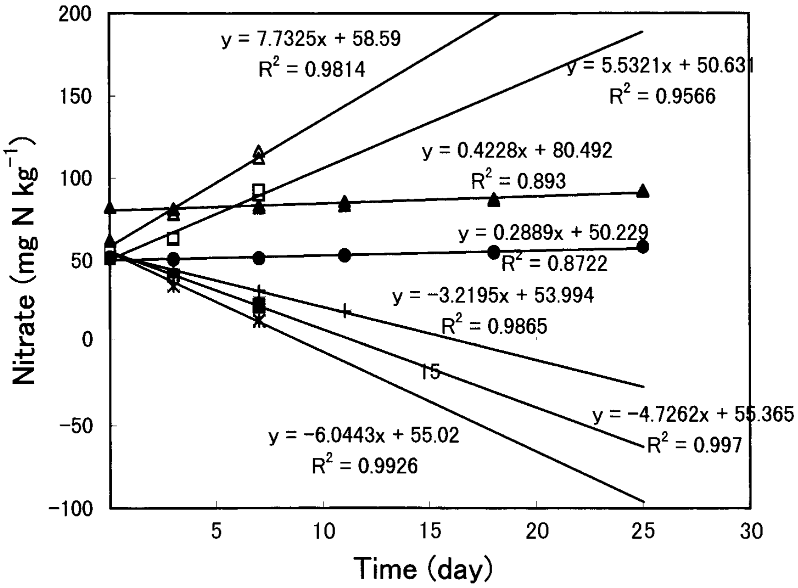

Fig. 5. Linear regression lines of the time course of nitrate content in Exp. 2
＋ : Wheat straw 20ºC, ■ : Wheat straw 25ºC, × : Wheat straw 30ºC,
▲ : Manure, △ : Bark manure, □ : Wheat straw+NH4, ● : No addition.

tope dilution method was also used as a short-term nitrification assay[18].

In calculating the gross rates, two aspects must be considered. The first one is to confirm that the rates of nitrification (nitrate production) and nitrate immobilization follow a zero-order kinetics. Strictly speaking, the rates vary with various environmental factors. Nitrate content in soil rapidly increased following a logistic curve after application of ammonia or urea[9], and the immobilization rate of inorganic N immediately after the addition of ammonium sulfate was much higher than the rate obtained on a long-term basis[13]. However, in our experiments, since the ammonium content in the original soil was very low, the nitrification rate was limited by the production of ammonium from organic matter and remained almost constant during the short period of incubation. On the other hand, when a certain amount of ammonium was added at the beginning of soil incubation, the temporal changes in the content of nitrate in the initial phase could be approximated by a straight line. Therefore, the rates could be practically calculated for a 7-day period (Fig. 5).

Secondly, it is possible that remineralization of ^{15}N immobilized in the earlier phase of the experiment resulted in the underestimation of the rates. However, Wessel & Tietema (1992)[17] concluded that recycling of ^{15}N back to the enriched ^{15}N-nitrate pool did not play any role when nitrate immobilization was not activated by some special means. We neglected the recycling of ^{15}N when the decrease of the ^{15}N-nitrate content was very slow. When a considerable amount of ^{15}N-nitrate was immobilized, the immobilization rates were calculated using the values during the initial 7-day period or less of the experiments.

Three kinds of manure that were used in Exps. 1 & 2 hardly promoted the immobilization of nitrate. As these materials were considered to have already become mature enough at this time, these results suggested that the application of mature manure did not practically affect the capacity of soil to immobilize nitrate-N.

The extent of gaseous loss by denitrification under the aerobic atmosphere depended on the moisture content in soil, whereas nitrate immobilization did not vary appreciably with the moisture content. According to our data, the disappearance of nitrate was attributed to immobilization when the moisture content of the soils did not exceed about 70% of the maximum water-holding capacity, and denitrification was comparable to immobilization when the moisture content was over 70%.

In our experiments, the denitrification rate under anaerobic conditions was much higher than the immobilization rate under aerobic conditions. We had observed in a preliminary experiment that the immobilization rate under anaerobic conditions was 26% of that under aerobic conditions. This indicates that the major part of the nitrate consumed in soil is associated with denitrification if O^2 is completely deficient in soil. However, complete anaerobic conditions seldom occur in well-aerated arable soils. Azam et al. (1988)[1] reported that in an experiment using soil amended with glucose, sucrose and cellulose, the main cause of nitrate elimination was microbial immobilization except for 100% WHC conditions. The relative ratio of immobilized N to denitrified N may depend on the development of anoxic sites in soil, and various factors, e.g. water movement, easily decomposable organic matter, soil structure and temperature, etc. may affect the formation of the anoxic sites in soil.

The difference in the denitrification rate among the treatments with three kinds of organic materials was not appreciable compared to that in the immobilization rate (Fig. 4), presumably because of the C/N ratio of the organic matter (see Table 1), which may affect the immobilization more than the denitrification. Since ammonium is continuously supplied by the decomposition of soil organic matter, deficiency of nitrogen is presumably not the major factor for soil microorganisms in the soils amended with organic matter with a low C/N ratio.

In our study, although the effects of organic matter and moisture content were investigated by using a sieved soil, it is necessary to use intact soil systems where the soil structure and environmental conditions remain under the same conditions as in *in situ* field to reveal the real processes quantitatively.

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