

Microbial Transformation of Nitrogen in Cattle Slurry as Applied to an Andisol Grassland

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Abstract

Organic nitrogen compounds in the animal waste once applied to soil is followed by chemical modification. Microbial transformation of nitrogen in cattle slurry applied to an Andisol grassland with no-cattle grazing over 20 years in northeastern Japan was analyzed with a focus on temporal changes in chemical composition (ammonium- and nitrate-nitrogen) and the natural abundance of nitrogen isotopes, $\delta^{15}\text{N}$. Most urea and related metabolites, which were major organic constituents of nitrogen in cattle excreta, were hydrolyzed into ammonium-nitrogen during storage in an underground closed tank. Nitrification of the ammonium-nitrogen was largely delayed under lower soil temperature conditions with average air temperature (4–14°C), whereas rapid nitrification occurred under higher air temperature conditions (17–27°C). A coupled process of denitrification followed by nitrification was evidenced by a rapid decrease in the nitrate content with a simultaneous increase in the $\delta^{15}\text{N}$ values of the residual. Such a process was facilitated by a combination of the high water retention property of the Andisol with an ample supply of organic matter.

Key words : cattle slurry, volatilization of NH_3 , nitrification, denitrification, fractionation of nitrogen isotopes

Dairy farming is one of the most important agricultural industries in the intra-mountainous regions of northeastern Japan, where Andisol predominates. Cattles have been fed under housing in a narrow space. In order to save costly labor, cattle slurry originating from cattle housing has periodically applied to farmland. Our current concern is the fate of the slurry nitrogen, in particular with respect to the nitrate contamination of local groundwater.

After the application onto the soil surface, urea, organic metabolites and sometimes ammonium, the major constituents of the slurry, are subjected to volatilization, mineralization, nitrification, denitrification and finally leaching, depending on the prevailing soil condi-

tions (Stevenson, 1982 ; Aulakh *et al.*, 1992). Natural variation of $\delta^{15}\text{N}$ values (and $\delta^{18}\text{O}$ of nitrate) in combination with changes in the concentration of mineral nitrogen would provide direct evidence of a relevant process in soil where diverse microorganisms are involved (Robinson and Conroy, 1999). A number of studies have been conducted on searching the origin of nitrate in groundwater using stable isotope technology (Fukada *et al.*, 2003, and herein references). Nevertheless, detailed information on the process of microbial transformation that proceeds the formation of nitrate in soil is very limited. Such data are a prerequisite for elucidating the origin of nitrate in local groundwater.

In humid and cool-to-temperate climatic con-

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ditions, as common in northeastern Japan, the soil surface is subjected to seasonal fluctuations of temperature and moisture. Such fluctuations would control the fate of slurry nitrogen applied to soil. The objective of the present study is to elucidate the detailed mechanisms involved in the microbial transformation of nitrogen in an Andisol field applied with cattle slurry, using stable nitrogen isotopic signatures as a tracer.

1. Materials and Methods

1.1 Experimental design

A trial field (39°48'N, 141°06'E, from 310–320 m above sea level), which had a history of continuous application of cattle slurry (exact rate of nitrogen application could not be estimated due to many variable factors) since 1978, was selected from an Andisol located on the eastern foot slope of Mt. Iwate, Iwate, northeastern Japan. An outline of the field is documented by Noborio *et al.* (2002). The experimental field is a managed pasture. There has been no cattle-grazing for years. From late December to late March the soil and vegetation are covered by deep snow, up to 0.5 m deep.

The soils are derived from weathering of cumulative pyroclastic deposits ejected from Mt. Iwate during the Holocene, and are characterized by the predominance of allophone, imogolite, and aluminum-humus complex.

To separate the coarse fecal fragments, a mixture of urine and dung deposited on the housing floor is mechanically squeezed before transporting to storage. The resulting cattle slurry (separated fluid) collected in the underground tank, together with the relatively fresh urine in the drain channel was periodically sampled and analyzed for both concentration and $\delta^{15}\text{N}$ values of ammonium- and total nitrogen.

Six sampling wells (diameter : 70 mm) were installed by a hand-auger down to the level of groundwater. The geometric layout of the sampling wells is shown in Fig. 1. Water flows constantly from a spring located at the lower end of the trial field. Water samples from the groundwater and from the spring were periodically collected and analyzed. To obtain a reference of natural nitrate derived from precipitation, a sample of spring water virtually free of human-influence was collected on Au-

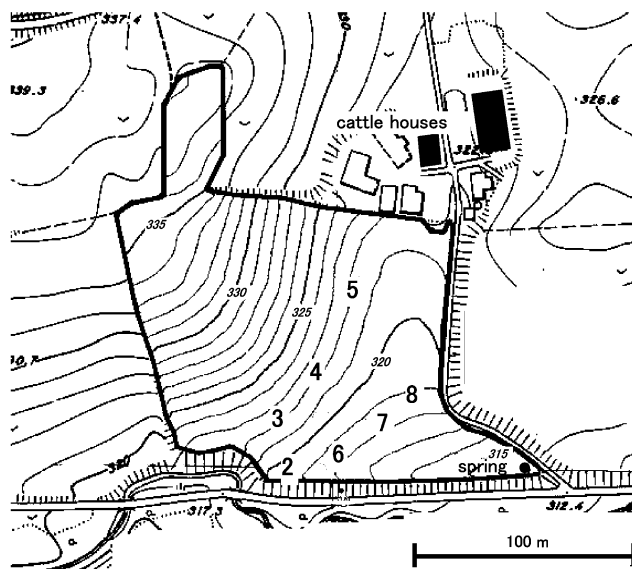


Fig. 1 Location map. Numbers indicate the location of sampling wells.

gust 30, 2004 at the crater rim of Mt. Iwate (elevation : 1,780 m above the sea-level), and similarly analyzed.

Reed canary grass (*Phalaris arundinacea*) was the predominant vegetation in the trial field. The matured above-ground parts (leaves together with stem) are cut down three times a year, in June, August and October, and air-dried *in situ* to make hay. In early spring and after harvesting each hay, cattle slurry from the underground storage tank is applied by a manure sprayer, as is common in elsewhere (Sommer and Hutchings, 2001). Since excretion, the cattle slurry has been stored in the underground tanks for at least two months before application. Samples of the slurry to be analyzed for chemical characterization were collected just before application.

Soil temperature and moisture that control the fate of nitrogen applied to the soil flu-

ctuated during the year, since the trial field was located at the mid-latitude of the Northern Hemisphere where slightly cool to temperate climates alternated. The mean daily fluctuation of air temperature and precipitation during the relevant period of the field trial in 2004 is shown in Fig. 2. Soil temperature conditions were categorized into two major groups : a lower soil temperature period in April and October/November, and a higher temperature period in June and August (Fig. 2).

1.2 Sampling surface soils after applying cattle slurry

Seven replicates of soil sampling in the field were made after April 2004, covering middle to lower portions of the slope in the trial field (Fig. 1). After removing the decaying plant residues, surface soil samples including the dense root mat of reed canary grass from 0 to 5 cm deep were collected around the trial wells in the field

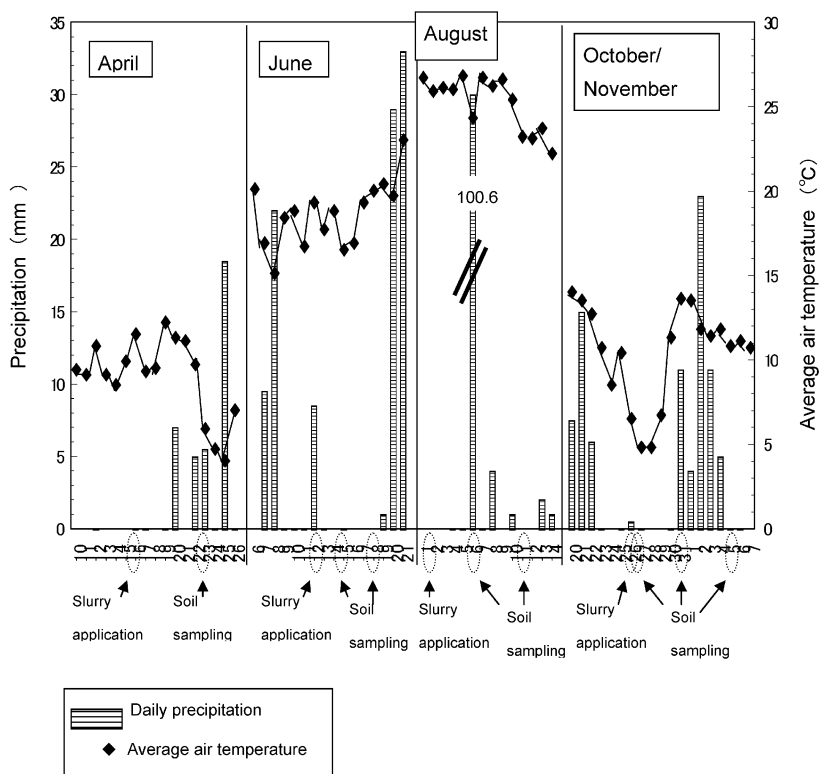


Fig. 2 Daily precipitation and average air temperature in relation to the application of cattle slurry and subsequent sampling of surface soil.

(Fig. 1). Temporal stagnation of the surface water was not observed during soil sampling. The samples were brought back to a laboratory within 1 h of sampling, and passed through a 2-mm sieve to eliminate grass root by gentle crushing and sieving.

1.3 Sampling grain corn and fresh urine

Grain corn, which is imported from the United States of America, constitutes major feed for housing cattle in Japan. A composite sample of dry grain corn imported in 1989 was crush and subjected to $\delta^{15}\text{N}$ analysis. Fresh urine samples were collected by syringe from housing floor approximately within two hours after excrement and placed in a cool container and then quickly brought to laboratory for chemical analysis.

1.4 Analysis of feed, soil, water, and slurry samples

The analytical procedures for determination and preparation for $\delta^{15}\text{N}$ determination for mineral nitrogen (ammonium and nitrate) were developed in the present study. Fifty g of the fresh soil sample was shaken with 125 ml of 2 M KCl solution for 30 min at 15°C and stood overnight at 5°C. Clear supernatant solution was obtained by centrifugation (4,000 rpm, 30 min). The concentration of ammonium- and nitrate-nitrogen in the soil extract was determined by semi-micro Kjeldahl distillation apparatus. Both ammonium- and nitrate-nitrogen were finally converted into ammonium-tetraphenylborate derivatives, and the $^{15}\text{N}/^{14}\text{N}$ ratios were determined using a continuous-flow mass spectrometer (DELTA plus, Thermoquest Ltd.). The nitrogen isotopic composition was shown by common $\delta^{15}\text{N}$ notation, as per mil variation relative to atmospheric dinitrogen ($\delta^{15}\text{N}=0\text{‰}$). Analytical precision during the overall process is better than $\pm 0.2\text{‰}$. The analytical accuracy during the isolation of sample nitrogen and subsequent mass spectrometry was repeatedly monitored using reagent grade NH_4Cl and KNO_3 .

Soil moisture was determined gravimetrically by drying in an oven with constant tem-

perature (105°C) and expressed as a percentage of the maximum water holding capacity (%). Water samples were passed through a membrane filter (Toyo Roshi Co., Ltd.) with a pore diameter of $0.2\mu\text{m}$ and concentrated to about 50 ml on an electric heater. Concentration and preparation of borate derivatives for $\delta^{15}\text{N}$ determinations of the nitrate- and ammonium-nitrogen was carried out in the same way as described above for soil extracts.

Total nitrogen in samples of cattle slurry and relatively fresh urine was converted into ammonium-nitrogen with a procedure of Kjeldahl digestion. Ammonium-nitrogen in the extract, together with that in cattle slurry and urine samples was determined by the same procedure as described above.

2. Results and Discussion

2.1 Concentration and $\delta^{15}\text{N}$ values of nitrogen in cattle slurry

Two fresh urine samples within two h after excrement contained 7,600 to 8,490 mg L^{-1} and 660 to 1,200 mg L^{-1} of total and ammonium-nitrogen (Table 1), respectively. The analytical results indicated that the major form of nitrogen in relatively fresh urine was organic, probably in form of urea (Kreitler, 1975).

The concentration of ammonium-nitrogen in analyzed samples of the cattle slurry ranged from 3,140 to 7,960 mg L^{-1} , whereas that of total-nitrogen ranged from 3,320 to 7,500 mg L^{-1} (Table 1). Urea is the major nitrogen constituent in metabolic excreta of higher animals, including cattle. Except for one slurry sample collected on May 11, 2004, the concentration of ammonium-nitrogen was nearly the same as total nitrogen, indicating microbial hydrolysis of urea excreted from cattle occurred during the storage, as commonly observed in elsewhere (Sommer and Hutchings, 2001). Cattle slurry was stored for two months or more before application and most of the excreted urea would be hydrolyzed to ammonium-nitrogen, since the slurry used in the present study was transparent without virtually solid

Table 1 Concentration and nitrogen isotopic composition of ammonium- and total-nitrogen in samples of cattle slurry and urine

Date of sampling	Concentration		$\delta^{15}\text{N}$	
	NH ₄ -nitrogen	Total nitrogen	NH ₄ -nitrogen	Total nitrogen
	N-mg L ⁻¹		‰	
October 28, 2003	ND [‡]	4,840	ND [‡]	+2.4
May 11, 2004	3,140	7,500	+0.2	+0.1
July 14, 2004	5,380	5,660	ND [‡]	+0.6
July 29, 2004	4,670	4,600	ND [‡]	+1.2
October 13, 2004	3,520	3,320	-0.8	ND [‡]
November 22, 2004	5,960	5,760	ND [‡]	ND [‡]
January 15, 2005	7,160	6,700	ND [‡]	-1.0
February 2, 2005 [†]	1,200	7,600	+1.4	+1.5
February 8, 2005	7,960	6,960	ND [‡]	-1.4
February 8, 2005 [†]	660	8,490	ND [‡]	-2.6

[†]Relatively fresh urine samples collected from a drain gutter.

[‡]Not determined.

pH of the cattle slurry : 9.2~10.1.

constituents. The ammonium-nitrogen content of the slurry samples collected in January, October, and November exceeded that of total-nitrogen, reflecting the analytical errors associated with the heterogeneous nature of the slurry. The low rate of mineralization of urea into ammonium-nitrogen of a sample collected on May 11, 2004 was probably resulted from a relatively lower temperature (from late December to April) during the storage of the cattle slurry in the underground tank.

The $\delta^{15}\text{N}$ values of total nitrogen in relatively fresh urine and cattle slurry fluctuated within fairly narrow ranges from -2.6 to +2.4 ‰ (Table 1). As it is well known, the major part of the current high performance feeds for cattle housing in the Japanese dairy farming system is imported grain corn produced in the central northeast United States. The $\delta^{15}\text{N}$ values of the anhydrous ammonia and urea, which are major chemical forms of nitrogen fertilizers in the area, range from -5 to +3‰ (Panno *et al.*, 2001). The $\delta^{15}\text{N}$ value of a composite grain corn imported from the U.S.A. in 1989 was +4.9‰. Incorporation, digestion, and subsequent excretion of urea and related metabolite nitrogen by cows are accompanied by negative nitrogen

isotopic fractionation of up to 3‰ (Koyama *et al.*, 1985). The observed $\delta^{15}\text{N}$ values of cattle slurry were in reasonable ranges in the view of the isotopic mass balance.

For one slurry sample collected on May 11, 2004, there was no discernible difference in the $\delta^{15}\text{N}$ value between ammonium- and total-nitrogen. It suggests a very limited nitrogen isotopic fractionation associated with the hydrolysis of urea in cattle excreta, as in the cases of NH₄⁺ production from organic matter decomposition (ammonification) (0 to 5‰) as reviewed by Robinson (2001).

Reflecting the high proportion of ammonium-nitrogen in the cattle slurry, the pH values were high, ranging from 9.0 to 9.6. Applying the slurry with such high pH values would be expected to cause marked volatilization. Yang *et al.* (2003) observed an emission loss of up to 60% of the applied slurry nitrogen during five-day periods after application on an Andisol in southern Kyushu, Japan. Volatilization of NH₃ into the free atmosphere could be confirmed by distinct $\delta^{15}\text{N}$ values of residual ammonium-nitrogen in soils, as described later.

Table 2 Temporal variation of moisture content of surface soils from the trial field

Date of sampling	Moisture content relative to the maximum water holding capacity						
	Sampling site No.						
	2	3	4	5	6	7	8
	———— % ————						
April 23, 2004	72	66	63	ND [†]	ND	69	ND [†]
June 15, 2004	82	85	97	89	102	86	ND [†]
June 18, 2004	77	77	86	74	83	92	ND [†]
August 6, 2004	89	97	103	109	89	98	ND [†]
August 11, 2004	72	75	86	83	94	82	ND [†]
October 27, 2004	97	98	106	102	112	112	102
October 31, 2004	97	100	105	106	103	100	106
November 5, 2004	89	89	102	103	106	98	91

[†]Not determined.

2.2 Soil moisture status during the field trial

Ammonium-nitrogen together with urea once applied to soil is subjected to volatilization, nitrification, denitrification, and leaching, according to the relevant soil conditions. Table 2 shows the moisture content of the surface soil during the present field trial in 2004. All the soil samples were characterized by fairly high moisture content in the field conditions, as shown by relative values with the maximum water holding capacity, ranging from 63 to 106% (Table 2). Such high water retention may have been attributed to the presence of allophane, imogolite, and aluminum-humus complex, by which the unique physical property of Andisols is imparted (Maeda *et al.*, 1977). Aulakh *et al.* (1992) reviewed the environmental conditions that control the fate of mineral nitrogen applied to common soils, and emphasized the primary importance of the soil moisture status, ambient temperature, and concentration of NH_4^+ in the soil solution. Depending on the water holding capacity of soils, the common optimal status for nitrification is in a range from 60 to 75%, under which the nitrification is retarded, whereas marked denitrification prevails over the range of 65 to 75%. According to the criteria, the soil examined in the present study was in an optimal range for

denitrification, but not for nitrification.

2.3 Temporal changes in the chemical forms and $\delta^{15}\text{N}$ values of nitrogen derived from cattle slurry as applied to soil

Surface application of cattle slurry results in a large kinetic nitrogen isotope fractionation that is often associated with the microbial transformation of the chemical species involved in the slurry. The relevant nitrogen isotope fractionation factors are summarized in Table 3. Normally a heavy isotope, ^{15}N tends to enrich in a residual phase.

Temporal changes in the chemical forms together with $\delta^{15}\text{N}$ values of mineral nitrogen derived from cattle slurry as applied to soils are shown in Figs. 3a, and 3b. Fairly high spatial variations could be seen in both concentration and $\delta^{15}\text{N}$ values of ammonium- and nitrate-nitrogen. It is evident that the chemical status of the mineral nitrogen in soil as applied after cattle slurry is strongly affected by soil temperatures. As a whole, the concentration of ammonium-nitrogen in soil at a certain duration after slurry application is higher than that of nitrate during the trial periods of April (mean air temperature : 9 to 12°C) and October/November (mean air temperature : 5 to 14°C) when cooler air temperatures prevail. As with most biological processes, the nitrification rate

Table 3 Estimates of nitrogen isotope enrichment factors (ϵ_N) for major nitrogen cycle processes in soils

Process	Setting	Temperature	ϵ_N	Reference
		°C	‰	
Mineralization of organic nitrogen	Microbial transformation under laboratory condition	Undescribed	-5 to 0	Handley <i>et al.</i> (1999)
NH ₃ volatilization	Theoretical calculation for equilibrium fractionation	25.1	-35	Urey (1947)
	Experimental	25	-34	Kirshenbaum <i>et al.</i> (1947)
	Experimental : barnyard soils	Undescribed	-21.6 to -21.0	Kreitler (1975)
	Experimental : decay of agrocybe	Undescribed	-40 to -30	Handley <i>et al.</i> (1996)
Nitrification	Pure culture of <i>Nitrosomonas europaea</i>	Undescribed	-26.0	Delwiche and Steyn (1970)
	Soils : central Illinois	Undescribed	-15	Feigin <i>et al.</i> (1974)
	Pure culture of <i>Nitrosomonas europaea</i>	28	-34.7±2.5	Mariotti <i>et al.</i> (1981)
	Pure culture of <i>Nitrosomonas europaea</i>	28	-33 to -25	Yoshida (1988)
Denitrification	Pure culture of <i>Pseudomonas denitrificans</i>	Undescribed	-17.3	Delwiche and Steyn (1970)
	Soil culture	20	-29.4±2.4	Mariotti <i>et al.</i> (1981)
		30	-24.6±0.9	
	Submerged soils	10	-25.4	Mariotti <i>et al.</i> (1982)
		20	-20.5, -19.0	
		30	-15	
	Diverse	Diverse	-40 to -5	Lehmann <i>et al.</i> (2003)

increases with increasing temperature until an optimum is reached above which it decreases (Malhi and McGill, 1982).

Soil moisture status is another important factor for controlling the transformation of mineral nitrogen in soils. Optimum moisture conditions for nitrification and denitrification are known to be around 60% and more than 80%, relative to maximum water-holding capacity of soils, respectively (Aulakh *et al.*, 1992).

2.4 Chemical and isotopic status of mineral nitrogen in soil under lower temperature conditions (April and October/November)

The concentration of ammonium-nitrogen in samples collected from sites 2 and 3 (83 and 135 mg-N kg⁻¹ (dry soil)) on six days after slurry application in April was evidently higher than that in samples from sites 4 and 7 (Fig. 3a).

Very low concentrations of ammonium-nitrogen in soil from sites 4 and 7 indicate that the application of cattle slurry may not be uniformly applied around the two sample sites. The $\delta^{15}N$ values of ammonium-nitrogen in soil ranged from +16.1 to +21.7‰. These values were clearly higher than those obtained for nitrogen in the cattle slurry ($\delta^{15}N = -0.8$ to +2.4‰, Table 1) and even common organic nitrogen in Japanese arable Andisols ($\delta^{15}N = +4.7$ to +8.4‰, Yoneyama, 1987). Such an increase in the $\delta^{15}N$ values as observed here is interpreted as indicating a nitrogen isotopic fractionation associated with the prompt volatilization of NH₃, i.e. selective removal of light ¹⁴N relative to heavy ¹⁵N during the application and subsequent deposition of nitrogen onto soil. The fractionation factors for volatilization of NH₃ obtained under the laboratory conditions are

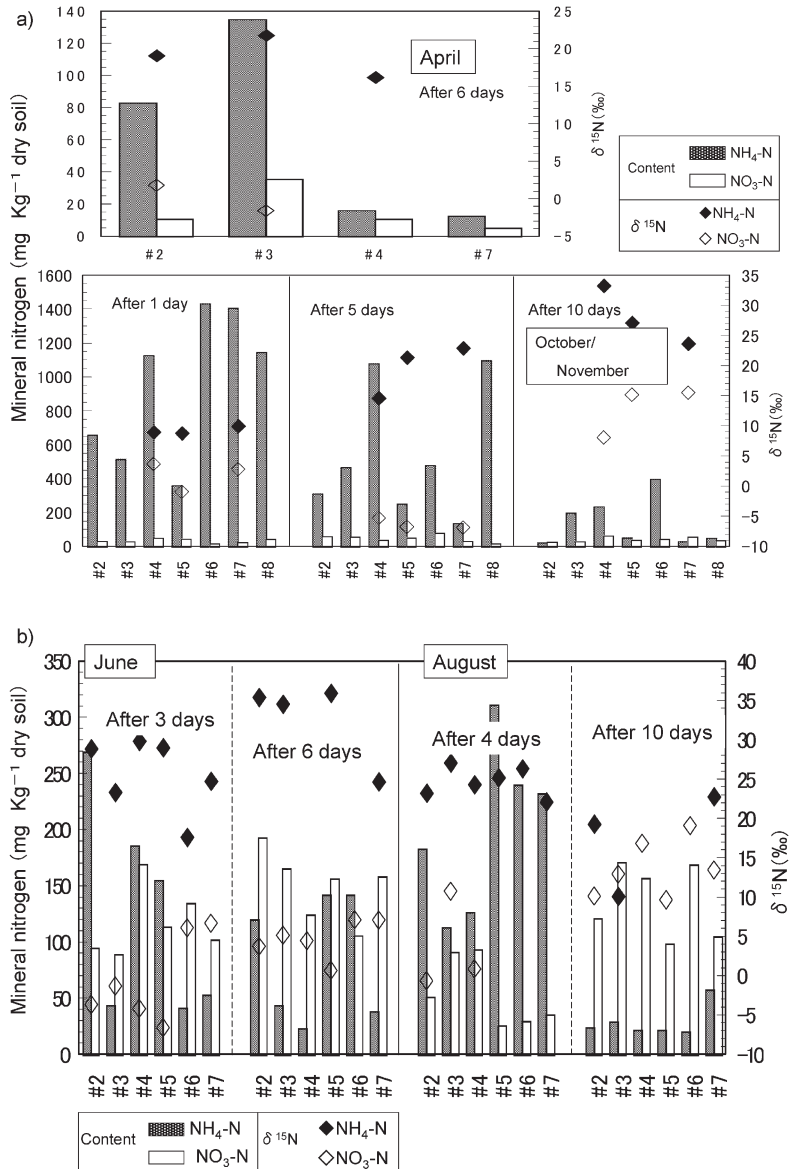


Fig. 3 Temporal changes in the concentration and the $\delta^{15}\text{N}$ values of mineral nitrogen soil as affected by application of cattle slurry : a) for April and October/November period under lower air temperature conditions, b) for June and August under higher air temperature conditions.

reported to be around 40 to 60‰ (Handley *et al.*, 1999). Similarly high $\delta^{15}\text{N}$ signatures of ammonium-nitrogen in soil, which are resulted from the emission of NH_3 , have been documented for soil during the microbial decomposition of uric acid deposits derived from seabird's excreta

(Mizutani *et al.*, 1985, 1986).

The $\delta^{15}\text{N}$ signatures of two soil nitrates (sites 2 and 3) collected on six days after slurry application in April showed an identical value of -1.7‰ . The apparent nitrogen isotopic fractionations during the nitrification (nitrogen

isotopic difference between ammonium- and associated nitrate-nitrogen) observed in the present trial field was lower than those of previous measurements (35 to 60‰, Handley *et al.*, 1999). The sum total of mineral nitrogen decreased to less than 10 mg-N kg-dry-soil⁻¹ in less than two weeks after slurry application (data not shown).

A comparable trend had also been observed for the status of mineral nitrogen in soil collected in October-November with similarly low soil temperature regimes (5 to 14°C, Figs. 2, and 3a). The concentration of ammonium-nitrogen in soils was higher, relative to those collected on the other days. Soil samples collected from sites 4, 6, 7, and 8 one day after slurry application showed very high concentration of ammonium-nitrogen (1,130 to 1,430 mg kg⁻¹ (dry soil)), which was one order higher than the other samples. This may be resulted from the lower air temperature (9°C) at the time of applying cattle slurry and hence limited volatilization of the applied ammonium-nitrogen, or heterogeneous nature of the applied slurry. It has been well documented that higher air temperatures promote greater ammonia volatilization (Sommer and Hutchings, 2001 ; Huijsmans *et al.*, 2003).

The rate of nitrification of ammonium-nitrogen applied in October-November under lower air temperature was slow, as seen from low content of nitrate-nitrogen, even ten days after slurry application. The trend was comparable to that of April (Fig. 3a). Appreciable amounts of precipitation (11 mm and 43 mm) occurred on five and ten days after slurry application, respectively. Lower concentrations of mineral nitrogen as observed for soil samples taken ten days after slurry application may be due to leaching from the soil system.

Very high concentration of added ammonium-nitrogen in soil as observed for sites 4, 6, 7, and 8 would suppress subsequent nitrification (Harada and Kai, 1968). The calculated concentration of the ammonium-nitrogen in soil solution of the soil ranged from 503 mg L⁻¹

for site 4 soil to 594 mg L⁻¹ for site 8, which is higher than those reported as the critical concentration of the inhibitory effect for nitrification, more than 200 mg L⁻¹ of ammonium-nitrogen (Harada and Kai, 1968). A lower rate of nitrification as observed in October would be resulted from such an inhibitory effect, in addition to lower ambient temperature.

The $\delta^{15}\text{N}$ values of ammonium in soil samples after slurry application on October-November apparently increased from one day after application (+8.7 to +9.9‰) to five days after (+14.5 to +22.8‰) and then ten days after (+23.6 to +33.2‰), reflecting the advance of nitrification (Fig. 3a). The $\delta^{15}\text{N}$ values of the associated nitrate in soil samples ranged from -1.1 to +3.6‰ on one day, and then decreased to -7.0 to -5.4‰ on five days after slurry application. Ten days after slurry application, the $\delta^{15}\text{N}$ values of soil nitrate had increased to +8.0 to +15.4‰. Since there was no discernible increase in soil nitrate after application of cattle slurry in October/November, such a nitrogen isotopic trend indicates coupling of nitrification and subsequent denitrification in soil. The effects of ambient temperature on the nitrogen isotopic fractionation associated with mineralization, nitrification, and denitrification of urea and potassium nitrate added to an Andisol under laboratory conditions will be reported elsewhere.

2.5 Chemical and isotopic status of mineral nitrogen in soil under higher temperature conditions (June and August)

Relative to April and October-November, the period of lower air temperature conditions, chemical processes involving the transformation of mineral nitrogen in soil applied in June gave unique trends (Fig. 3b). Concentration of nitrate-nitrogen of the soil clearly increased in comparison with the soil obtained in April and October-November. There was no precipitation during the field trial in June. The marked trend observed here was that nitrification in soil from June onwards was accelerated by the rise in soil temperatures (average air tempera-

ture : 17 to 19°C). The concentration of ammonium-nitrogen in soil three days after application of cattle slurry ranged from 40 to 270 mg-N kg⁻¹ (dry soil), whereas those of the associated nitrate were from 90 to 170 mg-N kg⁻¹ (dry soil). The concentrations of nitrate in three soil samples collected from sites 3, 6, and 7 were higher than those of the associated ammonium-nitrogen. The $\delta^{15}\text{N}$ values of ammonium-nitrogen were high, ranging from +18 to +30‰. The observed high $\delta^{15}\text{N}$ values were resulted from the marked nitrogen isotopic fractionation associated with the prompt volatilization of ammonium-nitrogen under higher air temperature at the time of slurry application (air temperature : 20°C), in addition to subsequent active nitrification. The $\delta^{15}\text{N}$ values of nitrate associated with the ammonium-nitrogen were classified into two major groups, according to the relevant nitrogen isotopic signatures. The first group is characterized by distinctly lower isotopic values with negative signature ($\delta^{15}\text{N} = -7$ to -2‰) for four sample soils (sites 2, 3, 4, and 5), and the second group by those with higher, positive values ($\delta^{15}\text{N} = +6$ to $+7\text{‰}$) for two other soil samples (sites 6 and 7). Those differences relate to the relative proportion of mineral nitrogen in soil samples. The rate of nitrification in soil from sites 2, 3, 4 and 5 was slower than the other two soils (sites 6 and 7), probably reflecting the micro-scale difference in the soil environments.

The concentration of ammonium-nitrogen in soils collected after six days of application on June decreased relative to that after three days. The decrease reflected the continued nitrification. As ammonium-nitrogen content decreased, the $\delta^{15}\text{N}$ values increased up to +36‰, which reflected nitrogen isotopic fractionation due to ¹⁵N enrichment in the residual ammonium-nitrogen during the nitrification. The $\delta^{15}\text{N}$ values of the nitrate ($\delta^{15}\text{N} = +1$ to $+7\text{‰}$) increased compared with those collected after three days of slurry application ($\delta^{15}\text{N} = -6.7$ to $+7\text{‰}$), reflecting the simultaneous denitrification of the nitrate formed under high

soil moisture conditions (Table 2). The coupled occurrence of nitrification and denitrification in soil associated with nitrogen fertilization has been suggested for soils with stagnogley features in the United Kingdom (Nielsen *et al.*, 1996 ; Abbasi and Adams, 1998).

Denitrification—an anaerobic process—is stimulated by hypoxia. Denitrification in the field is episodic and localized, being restricted to times when and places where NO₃ and carbon are readily available and O₂ is not (Robinson and Conroy, 1999). The soil conditions under dense rhizome from reed canary grass (a carbon source), and ample supply of nitrate derived from repeated application of cattle slurry (nitrate supply), in addition to very high retention of water (oxygen deficiency), provide the conditions for active denitrification.

Denitrification of nitrate results in gas formation of dinitrogen together with nitrous oxide, which escapes from the soil system, causing marked nitrogen isotopic fractionation. The nitrogen isotopic fractionation during denitrification is reported to be 28 to 33‰ (Handley and Raven, 1992). The overall process indicated rapid nitrification followed by active denitrification under higher soil temperature regimes as observed in the present study.

Nearing August, when the average air temperature rose to 27°C (Fig. 2), the comparable figure for microbial transformation of slurry nitrogen in soils was observed (Fig. 3b). There was no precipitation for four days after slurry application. The concentration of ammonium-nitrogen varied from 110 to 310 mg-N kg⁻¹ (dry soil), whereas that of the associated nitrate ranged from 30 to 90 mg-N kg⁻¹ (dry soil). The $\delta^{15}\text{N}$ values of ammonium-nitrogen were again high, in a range from +22 to +31‰. The $\delta^{15}\text{N}$ values of the associated nitrate showed values of -1 to $+11\text{‰}$.

Very heavy daily precipitation, amounting to 106 mm, occurred just after the first soil sample collection (four days after slurry application) in August. Nitrogen loss may be caused

by surface run off together with percolating water. The concentrations of both ammonium- and nitrate-nitrogen in soil ten days after slurry application were significantly low, showing fairly homogeneous values of 20 to 60 mg and 100 to 170 mg-N kg⁻¹ (dry soil), respectively, that reflected the advanced stage of nitrification under higher soil temperature conditions. The $\delta^{15}\text{N}$ values of mineral nitrogen were in a range from +10 to +23‰ that tended to be lower than those four days after the application. A somewhat lower $\delta^{15}\text{N}$ value of ammonium-nitrogen observed for soil sample 3 (+10.0‰) may be derived from the mineralization of intrinsic organic nitrogen under a higher temperature condition.

2.6 Temporal changes in concentration and $\delta^{15}\text{N}$ values of ammonium- and nitrate-nitrogen in groundwater

The concentration and $\delta^{15}\text{N}$ values of miner-

al nitrogen in groundwater samples collected from five sampling wells and one spring were periodically monitored from October to December, 2004 (Fig. 4). The figure contrasts with a distinctly low concentration of nitrate-nitrogen (0.69 mg L⁻¹) in addition to negative $\delta^{15}\text{N}$ value (-8.3‰) for nitrate in one spring at an area of high elevation (Mt. Iwate) where the only possible source of nitrate is meteoric in origin. Major mineral nitrogen in the trial wells would be derived from local sources, probably from migration of slurry-derived nitrogen.

Nitrate was a major form of mineral nitrogen in the groundwater from the trial wells, whereas two wells (sites 4 and 6) contained appreciable amounts of ammonium-nitrogen. It is evident that each well has its own chemical together with isotopic characteristics. Well sites 2 and 3 and the spring were characterized by

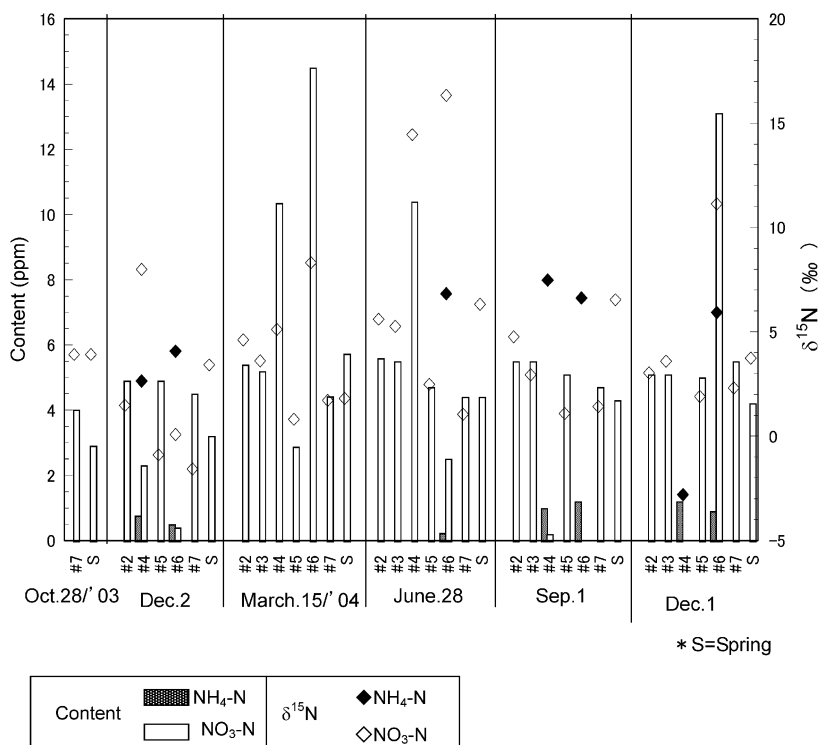


Fig. 4 Temporal changes in the concentration and the $\delta^{15}\text{N}$ values of mineral nitrogen in groundwater samples.

an almost constant concentration of nitrate and $\delta^{15}\text{N}$ values. Two additional samples of the spring water collected on May 11 and June 1, 2004 showed nearly comparable values (concentration of nitrate-N ; 4.6 and 4.7 mg L⁻¹, $\delta^{15}\text{N}$ = +5.8 and +7.3‰, respectively). Well sites 4 and 6 often contained nitrate-nitrogen characterized by high $\delta^{15}\text{N}$ values of +14 and +16‰ which were collected on 28 June. Such high $\delta^{15}\text{N}$ values were derived from residual nitrate after denitrification, as commonly observed for animal excreta (Wassenaar, 1995). Well 7 was characterized by an almost constant concentration of nitrate (4.2 to 4.7 mg L⁻¹) and low $\delta^{15}\text{N}$ values (-1.6 to +1.7‰). A comparable $\delta^{15}\text{N}$ value between residual soil nitrate formed after certain periods (more than two weeks) of slurry application and those in the current groundwater may indicate possible migration of the local origin of nitrate. For more information on the origin of nitrate in the groundwater, measurement of oxygen isotopic composition ($\delta^{18}\text{O}$) of nitrate is required (Silva *et al.*, 2002). A technical problem would be the isolation of valid nitrate from Andisols where the concentration of humic substances together with soluble silica is often high. No successful protocols are available for the $\delta^{18}\text{O}$ value measurement of nitrate in such type of soils.

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黒ボク土草地に散布された乳牛尿スラリーに含まれる窒素の微生物学的変換

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要 旨

家畜排泄物に含まれる有機窒素成分は、土壌に散布されると土壌生物の作用によって化学変化を受ける。東北地方の黒ボク土採草地に散布された乳牛尿スラリーに含まれる窒素の微生物学的変換を化学組成（アンモニア態と硝酸態窒素）と窒素同位体自然存在比（ $\delta^{15}\text{N}$ ）の経時的変化に注目して解析した。乳牛糞尿の主な有機態窒素である尿素とその代謝物のほとんどは、密閉式地下タンクに貯蔵中にアンモニア態窒素に加水分解された。平均気温が低く地温が低い条件下では、アンモニア態窒素の硝化が大きく遅れた。一方、平均気温が高い条件下では、硝化は促進された。硝化に続く脱窒過程は、硝酸態窒素含量の急激な減少と同時に起こった残存硝酸態窒素中の $\delta^{15}\text{N}$ 値の上昇によって示された。このような過程は黒ボク土の高水分保持特性と草本植生からの有機物の十分な供給が組み合わされることによって促進されると推定された。

キーワード：乳牛尿スラリー，アンモニア揮散，硝化，脱窒，窒素同位体分別

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