Natural abundance of ^{15}N in soil organic matter with special reference to paddy soils in Japan: biogeochemical implications on the nitrogen cycle

EITARO WADA¹, REIKO IMAIZUMI¹ and YASUO TAKAI²

Mitsubishi-Kasei Institute of Life Sciences, Minamiooya 11, Machida, Tokyo 194¹, and
Department of Agricultural Chemistry, University of Tokyo,
Yayoi, Bunkyo-ku, Tokyo 113², Japan

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Isotopic composition of nitrogen in soil organic matters from subalpine forest areas, upland, and paddy fields in Japan was investigated. In the forest areas, ^{15}N abundance in soil organic nitrogen increased with depth irrespective of soil types such as alpine brown forest soil and podzolic soil. Biological nitrogen fixation appear as the main source of soil nitrogen. Higher plants seem to promote the above vertical trend even in a cultivated field. ^{15}N content of soil organic matters in Andosol soils was higher than that of Alluvial soils.

Long-term fertilization experimental rice fields were investigated to elucidate the general principles that govern the variation of ^{15}N content of soil organic nitrogen. In paddies, $\delta^{15}\text{N}$ value in rice plant was variable depending upon the kinds of applied fertilizers. Significant difference of $\delta^{15}\text{N}$ was observed between rice plants and fertilizers, especially in a chemical fertilizer plot and in a green manure plot. Nitrification and denitrification seemed to be responsible for the difference. However, these seem to have a small effect on the variation of soil $\delta^{15}\text{N}$, as rice plants completely assimilate available nitrogen and later are harvested from the paddy fields. The effects of nitrification-denitrification, and of ammonia volatilization were, thus, observed only in biological processes related to relatively short-term phenomena such as the growth of rice plants and hydrophytes.

A long-term addition of a fertilizer clearly increased the amount of soil organic nitrogen in the paddy fields enriched in organic matter. Based on isotope mass balance, nitrogen isotope fractionation factor of 0.9942 was estimated for the process of epidiagenesis, indicating that the heavier isotopic species are preferably decomposed. An addition of ammonium sulfate with a low $\delta^{15}\text{N}$ decreased ^{15}N content of soil nitrogen in a paddy field depleted in soil organic nitrogen.

Org.C/Clay ratio was demonstrated to be an important factor that governs the $\delta^{15}\text{N}$ value in paddy soils. The relation between $\delta^{15}\text{N}$ value in soil organic nitrogen and Org.C/Clay ratio could be presented by a hyperbola for paddy soils so far examined. A simple model is presented to elucidate major factors that govern ^{15}N content of soil organic materials. It is emphasized that $\delta^{15}\text{N}$ of source and plant nitrogen and two isotopic fractionations associated with epidiagenesis of soil organic matters and with adsorption of ammonia by clay minerals, constitute three major factors that determine the $\delta^{15}\text{N}$ of soil organic nitrogen.

INTRODUCTION

Two stable isotopes, ^{14}N and ^{15}N , undergo fractionation in biological and physico-chemical processes. The lighter isotope reacts preferentially over the heavier one, but the extent of isotopic fractionation varies depending upon the type, mode, and mechanism of reactions (WADA, 1980). The ^{15}N abundance ($\delta^{15}\text{N}$) of various

components of an ecosystem may thus reflect events and history of its nitrogen cycle. WADA and HATTORI (1976) have found that $\delta^{15}\text{N}$ values of marine phytoplankton are correlated with the form of nitrogenous compounds used for their growth. PETERS *et al.* (1978) have used $\delta^{15}\text{N}$ data to identify the source of sedimentary nitrogen. A large isotopic fractionation of nitrogen in bacterial denitrification has been reported

(WELLMAN *et al.*, 1968; MIYAKE and WADA, 1971), and this has been applied for the assessment of denitrification in the eastern tropical North Pacific Ocean (CLINE and KAPLAN, 1975) and for global assessment of denitrification (WADA *et al.*, 1975).

The $\delta^{15}\text{N}$ values of soil organic matter have been shown to have a great variation ranging from -3 to $+18\text{‰}$ (CHENG *et al.*, 1964; BREMNER and TABATABAI, 1973; DELWICHE and STEYN, 1970; SWEENEY *et al.*, 1978; KARAMANOS and RENNIE, 1980, WADA and NAKAMURA, 1980). According to JENKINSON and RAYNER (1977), mean turn-over times of organo-mineral compounds and soil humus are 50 and 2000yr, respectively. Accumulation of soil organic matter depends on climate conditions such as effective temperatures, treatments of soils involving water managements, and application of fertilizers and organic materials (DEI and YAMAZAKI, 1979). The $\delta^{15}\text{N}$ data on soil organic nitrogen will undoubtedly provide useful information toward understanding processes of formation and decomposition of soil organics and their temporal and spatial variations. The mechanisms which govern the ^{15}N distribution in soil systems, however, have been largely left unknown.

In this paper, we present $\delta^{15}\text{N}$ data on soil nitrogen from subalpine forests, upland, and paddy fields in Japan. Detailed information was collected from the long-term fertilization experimental paddy fields of the Agricultural Research Center, the Ministry of Agriculture, Forestry and Fisheries, Japan. The records there extended over several decades for fertilizing treatment and other managements. Investigations were performed with an emphasis on how biological processes and a long-term applications of fertilizers and organic materials affect ^{15}N abundance in soil organic matter. Major factors that govern the variation of $\delta^{15}\text{N}$ in soil systems were examined.

MATERIALS and METHODS

Soil samples Soil materials of various soil

textures were obtained from both forest and cultivated areas in Japan. Forest soils were collected from subalpine areas in the Tokyo University forest in Chichibu, Saitama Prefecture, in July 1976, and from Shiga-yama in Nagano Prefecture with special reference to Podzol and Brown Forest soils. The Chichibu area is characterized for its well developed podzolic soil in Japan and Shiga-yama is one of the typical subalpine forest with brown forest soil where intensive studies on International Biological Programme (IBP) were performed. Soil materials were collected from several layers involving organic and mineral horizons. The organic horizon was divided into three layers. An accumulation of litters was apparent in L-horizon. Decomposition of organic matters proceeded in both F and H-horizons. In the F-horizon, plant residues retain their original forms, while organic substances are decomposed beyond recognition in the H-horizon (Humus horizon). A-horizon is the uppermost part of mineral layer. In the case of podzolic soils, this layer corresponds to elluvial horizon and B-horizon (see Fig. 1) to illuvial one. Chemical compositions and biological characteristics of Shigayama soils were reported by TAKAI *et al.* (1976).

Andosol soil is one of the typical soils originated from volcanic ashes in Japan. The Tokachi plain in Hokkaido provides a representative upland area of this soil type. Four vertical profiles were taken from both Andosol and Alluvial soils in fallowed fields at the Memuro Branch of the National Hokkaido Agricultural Experimental Station on 6 August 1979. Among the upland soils, Andosol brown (dry volcanic soil) and Andosol black soils (wet volcanic soil) originated from the same series of volcanic ashes. A rotation of soybean, sugar beat, corn, wheat and potato is conducted in the upland soils in this district. As a reference, virgin soils were also collected from the site of an oak tree grove where no fertilizer has been applied.

Soil materials were collected from the long-term fertilization experimental paddy fields at the following research stations:

- i) Aizubange branch, Fukushima Prefectural Agricultural Experimental Station, on 6 May, 1976;
- ii) Nagano Agricultural Experimental Station on 26 October, 1976;
- iii) Central Agricultural Experiment Station, Konosu, on 19 April, 1977;
- iv) Anjo branch, Aichi Prefectural Agricultural Experimental Station, on 16 November, 1977; and
- v) Shizuoka Prefectural Agricultural Experimental Station on 22 November, 1978.

In the long-term fertilization experimental paddy fields, rice plants have been grown over several decades continually without any rotation. This means that the same kinds of organic materials have been added to the soils during these years. In an ordinary practice, rice fields are irrigated with surface fresh waters during plantation from May to early October. During summer redox potential of flooded soil goes down to a low level of 0 to -300mV and so-called gleyic soil develops in the plow layer of the paddy soil. Paddy soils were collected mainly from non-fertilized plots (NF-plot) and chemical fertilizer plots (CF-plot) in all the stations during spring or autumn-fallow season. Physicochemical nature and biological characteristics of the paddy soils together with fertilizer treatment were described elsewhere in detail (TAKAI, 1979). Some selected data were given in Table 1.

At Konosu, several kinds of biogenic substances involving soil materials, rice plants, hydrophytes and fertilizers were also collected to make clear the distribution of $\delta^{15}\text{N}$ in paddy fields. There are four kinds of long-term fertilization experimental rice plots at Konosu since 1925. The fertilizer treatments are as follows:

- Non-fertilizer plot (NF-plot): receives no fertilizer.
- Chemical fertilizer plot (CF-plot): receives $10\text{KgN}/10\text{a}/\text{year}$ in the form of ammonium sulfate.
- Green manure plot (GM-plot): receives $10\text{KgN}/10\text{a}/\text{year}$ as Chinese milkvetch.
- Organic manure plot (OM-plot): receives $10\text{KgN}/10\text{a}/\text{year}$ as a mixture of soybean

cake and rice straw compost.

Soil texture at Konosu is a type of heavy clay and the permeability of soil water is, thus, very small as compared with those of other paddies. Both soil organic carbon and nitrogen are increasing linearly with time during recent 55 years except for NF plot where both components have remained constant (NAGANOMA and MOROYU, 1978). Soil materials were dried at room temperature, crushed, and then were sieved to pass a 2mm sieve and homogenized. All biological samples were dried at 4°C and stored until ^{15}N analyses.

Chemical analysis Total nitrogen was determined by a Kjeldahl digestion followed by ammonia measurement using the phenol-hypochlorite method of SOLORZANO (1969), organic carbon by a method of VAN SLYKE and FOLCH (1940) or by using a CHN-analyzer (Yanagimoto) and cation exchange capacity by a Shollenberger's ammonium-acetate method.

$\delta^{15}\text{N}$ assay Organic nitrogen was converted to ammonium by Kjeldahl digestion. Soil materials of two to five grams were digested for 4hr using 3:1:4 mixture in weight of HgO , SeO_2 , and K_2SO_4 as a catalyst (WADA and HATTORI, 1972). The ammonia thus produced was steam-distilled into a 0.5N H_2SO_4 trap and concentrated. It was then converted *in vacuo* to N_2 gas with alkaline hypobromite. The N_2 produced was purified by circulating through CuO furnace with Pt wire (700°C) and Cu furnace (400°C) for 30 minutes. Nitrogen isotope ratio was measured using a Hitachi RMU-6R mass spectrometer installed with a dual inlet system and a double collector for radiometry. Nitrogen isotope ratio was expressed in permil deviations from atmospheric nitrogen as defined by the following equation:

$$\delta^{15}\text{N} (\text{‰}) = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{air}}}{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}} \times 1000$$

Two sets of reagent grade ammonium sulfate solution with $\delta^{15}\text{N}$ of -3.4 and 1.3‰ relative to atmospheric nitrogen were used as running

Table 1. Reproducibility of ^{15}N measurements in a soil sample collected from the CF plot of Konosu paddy field

No.	Org. N %	^{15}N ‰
1	0.253	2.7 ₄
2	0.256	2.9 ₇
3	0.260	3.1 ₄
4	0.246	3.5 ₄
5	0.232	3.6 ₃
6	0.232	2.9 ₈
7	0.237	3.3 ₃
mean	0.245 ± 0.012	$3.19 \pm 0.3_2$

standards. Reproducibility of the measurement was $\pm 0.2\text{‰}$. In case of soil organic nitrogen the reproducibility was $\pm 0.3\text{‰}$, which includes sampling errors (Table 1).

RESULTS

Forest soils

$\delta^{15}\text{N}$ of organic nitrogen in subalpine forest soil ranged from -4 to $+5\text{‰}$ relative to atmospheric nitrogen (0.0‰). With increasing soil

Fig. 1. Vertical profiles of soil organic nitrogen (broken line) and $\delta^{15}\text{N}$ (solid line) for the Podzol (Pw) at Jumonji in Chichibu, Saitama Prefecture.

Fig. 2. Vertical profiles of soil organic nitrogen (broken lines) and $\delta^{15}\text{N}$ (solid lines) for a, Podzol (Pwh) and b, Brown Forest soil (Bd), at Mt. Shigayama, Nagano Prefecture.

depth, amounts of organic nitrogen decreased and $\delta^{15}\text{N}$ values increased (Figs. 1 and 2). Low $\delta^{15}\text{N}$ values of -4 to -3‰ were common in the upper layers of the organic horizon (L horizon). A dramatic increase in $\delta^{15}\text{N}$ was found in the lower layers of the organic horizon (F and H horizons), suggesting a progress of decomposition of organic matter in these layers. $\delta^{15}\text{N}$ value was maximal in the B horizon. An exception was found in the podzolic soil at Shigayama, where B_2 horizon exhibited a low $\delta^{15}\text{N}$ value (0.2‰). It was as low as those in A and B horizons in the Brown Forest soil (Fig. 2).

Upland soils

Figure 3 shows vertical profiles of organic nitrogen content and $\delta^{15}\text{N}$ at four sites of the Memuro Experimental Station. The Andosol black soil (Fig. 3b) accumulated more organic matter than did the Andosol brown soil (Fig. 3a), but $\delta^{15}\text{N}$ of the surface organic nitrogen of both soil types was about 5‰ , irrespective of nitrogen content. In Andosol brown soil, the $\delta^{15}\text{N}$ values increased to 10‰ with depth, accompanied by a decrease in nitrogen content. A similar trend was observed in an oak grove preserve located at the center of the Andosol brown farm (Fig. 3c), although ^{15}N abundance increased from 0 to 13‰ with depth. The dis-

Fig. 3. Vertical profiles of soil organic nitrogen (broken lines) and $\delta^{15}\text{N}$ (solid line) for a, Andosol brown; b, Andosol black; c, Andosol brown in an oak grove preserve and d, Alluvial soil at Memuro, National Hokkaido Agricultural Experimental Station.

tribution pattern was almost the same as that observed in the forest soil of Chichibu (Fig. 1). A different distribution pattern was found in the alluvial soil (Fig. 3d). The vertical profile of $\delta^{15}\text{N}$ values of soil nitrogen was relatively flat ($+2$ to $+4\text{‰}$) throughout the soil column down to 100 cm depth, although layered structure was manifested by nitrogen content and soil color.

Paddy fields

1. Konosu paddy field Figure 4 depicts a diversity of $\delta^{15}\text{N}$ values for rice plant, soil organic matter, chemical fertilizer, manure, and other materials from the experimental rice plots at the Konosu paddy fields. $\delta^{15}\text{N}$ values of rice plants ranged from 6 to 11‰ and were invariably higher than those of soil organic nitrogen (3 to 5‰). $\delta^{15}\text{N}$ values of fertilizer (ammonium sulfate), Chinese milkvetch, soybean cake, and

rice straw compost were -0.5 , 1.7 , 0.7 and 15.0‰ , respectively. Differences between $\delta^{15}\text{N}$ values of rice plants and fertilizer-nitrogen were significant at the CF and GM plots. In the NF plot $\delta^{15}\text{N}$ values of suspended particulate organic matter (POM) and microalgae (MP-4) in flood water were 2.9 and 8.1‰ , respectively. Hydrophytes grown in these fields exhibited very high $\delta^{15}\text{N}$ values ranging from 9.3 to 22.8‰ . In the OM plot, the $\delta^{15}\text{N}$ value of soil organic matter was the highest (4.3‰) in the middle of the plow layer ($2\text{--}8\text{ cm}$) and declined down to 2.4‰ in subsurface layers ($26\text{--}31\text{ cm}$). No significant variations with depth were observed in the other plots. Average $\delta^{15}\text{N}$ values in the plow layer of the NF, CF, GM and OM plots were 4.3 , 3.0 , 3.3 and 4.0‰ , respectively (Table 2).

2. Other paddy fields $\delta^{15}\text{N}$ values of soil organic nitrogen in the NF and CF plots of the Aizubange, Nagano, Shizuoka, and Anjo paddy fields are entered in Table 2. The $\delta^{15}\text{N}$ values varied from location to location and extended over a wide range from -0.4 to $+12.3\text{‰}$. In the plow layers at the Aizubange field with or without fertilizer application, $\delta^{15}\text{N}$ values were

Fig. 4. Summary of $\delta^{15}\text{N}$ in the various samples collected from long-term experimental rice fields of Central Agricultural Experimental Station at Konosu. ○: soil, ●: rice plant, PON: particulate organic nitrogen of the irrigating water, MP-4: microalgae grown in the irrigating water during the incubation for a week in natural sunlight. In the incubation, inorganic nitrogenous compounds in the water were completely taken up by the algae. ◎: fertilizer as indicated in the Figure. Mean $\delta^{15}\text{N}$ value of the fertilizers in the OM plot is indicated by an arrow.

Table 2. ^{15}N values of soil organic matter in long-term fertilization experimental paddy fields

Horizon	Depth cm	Texture	Mansell color	pH (H ₂ O)	CEC me/100g	Org.C %	Org.N %	Clay %	$\delta^{15}\text{N}$ ‰
Konosu, 19 April 1977									
NF									
I	0-14	LiC	10YR4/3	6.2	18.6	2.07	0.187	43.2	4.3
II	14-18.5	LiC	2.5Y4/2	7.0	23.5	1.50	0.137	43.6	3.6
III	18.5-32.6	HC	2.5Y3.5/1	7.0	25.6	2.13	0.140	49.9	5.0
CF									
I	0-15	LiC	2.5Y3.5/2	5.4	19.8	2.73	0.239	44.0	3.0
II	15-20	LiC	2.5Y4/2	5.4	19.7	2.41	0.192	41.2	3.7
III	20-30	HC	2.5Y4/2	6.7	21.6	2.24	0.195	52.5	3.9
GM									
I	0-15	LiC	5Y4/2	6.5	18.4	2.75	0.229	30.4	3.3
II	15-19	LiC	2.5Y3.5/1	7.3	15.2	1.34	0.142	39.2	3.0
III	19-22	LiC	nd	7.4	12.0	0.53	0.050	38.1	4.4
IV	22-30	LiC	nd	7.4	20.5	nd	0.083	nd	4.4
OM									
I	0-13	LiC	10YR4/1.5	5.6	20.2	2.39	0.329	38.8	4.0
II	13-17	LiC	2.5Y4/2	7.0	18.4	2.00	0.128	40.3	3.8
III	17-30	LiC	2.5Y4.5/1	7.3	17.6	1.09	0.104	42.2	2.7
Aizubange, Fukushima, 6 May 1976									
NF plot									
I	0-14	CL	7.5YR4/2	5.39	12.8	1.96	0.182	17.3	0.1
II	15-18	SL	2.5Y3/1	5.34	11.8	1.76	0.140	14.5	-0.4
III	18-30	CoS	5YR4/8	6.18	5.7	0.22	0.170	3.6	2.1
IV	30-40	S	1.5GY3/1	nd	nd	nd	nd	nd	nd
CF plot									
I	0-15	CL	10Y4/1	5.01	13.4	2.46	0.272	18.3	0.1
II	15-19	SCL	10YR3.5/1	5.02	14.3	2.13	0.236	15.7	-0.2
III	19-30	SL	5Y2/1	5.22	11.9	1.20	0.089	13.9	4.5
IV	30-41	SC	5Y2.5/1	nd	—	1.22	0.086	—	4.2
Nagano, Oct. 26, 1976									
PK plot*									
I	0-14	LiC	10YR5/1	6.3	18.4	1.08	0.111	26.1	7.2
II	14-21	CL	2.5Y5/1	6.5	19.3	0.81	0.105	19.1	6.2
III	21-29	CL	10YR4/1	6.5	20.4	0.80	0.103	23.9	4.3
IV	29-40	CL	10YR4/1	6.3	20.8	—	0.104	23.6	5.0
CF plot									
I	0-14	LiC	2.5Y4/2	5.9	18.8	1.18	0.129	25.2	3.4
II	14-17	CL	10YR4.5/1	5.9	18.0	0.92	0.115	22.5	4.7
III	17-27	CL	10YR5/1	6.1	19.4	0.81	0.101	19.4	7.1
IV	27-37	CL	2.5Y5/1	6.1	19.6	—	0.090	20.0	6.7
Shizuoka, Nov. 22, 1978									
PK** plot									
I	0-13	LiC	nd	6.19	8.7	1.49	0.173	27.8	2.7
II	13-15	LiC	nd	6.68	8.9	1.24	0.141	28.3	3.6
III	15-21	CL	nd	7.14	8.5	0.74	0.108	22.8	3.9
IV	21-30	SiC	nd	7.05	9.5	0.94	0.117	30.2	3.8
CF plot									
I	0-12	LiC	nd	6.00	9.4	1.65	0.183	31.9	4.1
II	12-18	LiC	nd	6.40	9.9	1.67	0.175	31.6	3.4
III	18-24	LiC	nd	6.55	13.1	2.38	0.211	32.1	3.6
IV	24-30	LiC	nd	6.51	11.2	1.79	0.172	29.8	2.9
Anjo, Oct. 11, 1977									
NF plot									
I	0-13	LiC	10YR4.5/3.5	5.33	7.6	1.01	0.098	30.6	4.4
II	13-16	LiC	10YR4/3.5	5.68	7.7	0.88	0.094	32.4	5.4
III	16-21	LiC	7.5YR5/7	6.22	12.1	0.51	0.059	54*	8.4
IV	21-39	HC	7.5YR4.5/5.7	5.85	12.0	0.44	0.058	53.4	12.3
NPK plot									
I	0-15	LiC	10YR4.5/4	5.07	9.5	1.14	0.117	32.2	0.5
II	15-21	LiC	10YR5/4	5.30	8.5	0.86	0.083	32.9	4.8
III	21-25	HC	7.5YR4.5/7	5.60	9.7	0.51	0.061	42*	7.1
IV	25-39	HC	7.5YR4.5/7	5.61	10.7	0.36	0.041	47.9	7.8

*Clay content was calculated by using equation (8).

**PK plot stands for a chemical fertilizer plot which receives phosphate and potassium.

close to that of atmospheric nitrogen. This probably resulted from slow decomposition rate of organic matter at this site owing to low temperature.

The highest value of 12.3‰ was found in the clayey soil at the Anjo field where organic carbon content was extremely low. $\delta^{15}\text{N}$ values at the Anjo field increased and nitrogen content decreased with increasing depth in both the NF and CF plots. Furthermore, the $\delta^{15}\text{N}$ values of the NF plot were invariably higher than those of the CF plot throughout the soil column. A similar trend was observed in the plow layer (0–14 cm) of the Nagano field where the amount of organic matter was also low. At the Shizuoka field, $\delta^{15}\text{N}$ values ranged from 2.7 to 4.1‰. Both the $\delta^{15}\text{N}$ values and organic nitrogen content were similar to those at the Konosu field.

DISCUSSION

Variation of $\delta^{15}\text{N}$ in soil ecosystems In general, organic fraction accounts for most of the total soil nitrogen; the concentration of mineralized nitrogen is less than 7% of the total. This was also the case with most of soil materials studied in the present work (TAKAI, 1979). The $\delta^{15}\text{N}$ values reported here thus approximate the $\delta^{15}\text{N}$ values of total organic nitrogen.

Low $\delta^{15}\text{N}$ values (–2.3 to +2.8‰) were commonly found in the surface layer (the organic horizon) of the subalpine forest soils in Chichibu and Shigayama (Figs. 1 and 2). This is in accord with the observations at a Belgian forest (RIGA *et al.*, 1971), a Canadian Grey forest (RENNIE *et al.*, 1976) and in the French Alps (MARIOTTI *et al.*, 1980). Low ^{15}N abundances of organic nitrogen in the organic horizon can be understood, because the organic horizon consists mainly of litters originating from higher plants which generally have lower $\delta^{15}\text{N}$ values than that of atmospheric nitrogen (HOERING, 1955; MIYAKE and WADA, 1967). In mountain areas, nitrogen is supplied mainly by biological nitrogen fixation and precipitation. According to BORMANN *et al.* (1977), about 70% of the

annual nitrogen input at the Hubbard Brook Experimental Forest is from biological nitrogen fixation, and the rest from bulk precipitation. Nitrogen isotopic fractionation associated with nitrogen fixation is small (HOERING and FORD, 1960; DELWICHE and STEYN, 1970). A fractionation factor of 1.002 has been found by M. MINAGAWA and E. WADA (unpublished data). The ^{15}N abundances of ammonium and of nitrate in rain waters are also low; average values of –1.4 and –6.6‰ were given, respectively (MOORE, 1977; WADA and NAKAMURA, 1980). In a forest ecosystem, most of inorganic nitrogenous compounds are taken up by vegetation. Ammonification, which has a little nitrogen isotopic fractionation, is probably limiting overall process in mineralization of organic nitrogen. Soil pH is so low (3.6 to 4.8) as to prevent the nitrification. Denitrification, which occurs without any substantial isotopic fractionation, proceeds only in anaerobic microsites, if it ever occurs. Under these circumstances, therefore, the apparent isotopic fractionation of nitrogen would be minimal. RENNE *et al.* (1976) have found that $\delta^{15}\text{N}$ of nitrate in forest soils is almost identical with that of organic nitrogen. The low $\delta^{15}\text{N}$ values of the surface soils obtained in the present investigation probably reflect the low ^{15}N abundance of the source nitrogen.

In forest soils, the increase in ^{15}N abundance with depth was found to be common (Figs. 1 and 2). The same trend was clearly observed in French forest soils such as brown and podzolic soils (MARIOTTI *et al.*, 1980). Organic substances are transported from the surface to deeper layers by a downward water flow and also by the movement of soil fauna. KANAZAWA *et al.* (1976, 1977) have noted a high C/N ratio (35.3) and an accumulation of fulvic substances in the B₂ layers of the podzolic soil at Shigayama, a finding which provides evidence for the vertical transportation of organic matter. During the course of the vertical transport, organic matter is decomposed with resultant nitrogen isotopic fractionation. This may produce ^{15}N enrichment in deeper layers. Nitrogen isotopic fractionation associated with ammonium exchange between

liquid and solid phases in soil should be considered as another possible mechanism for the ^{15}N enrichment (DELWICHE and STEYN, 1970). Enrichment of ^{15}N in exchangeable sites of clay minerals has been reported by KARAMANOS and RENNIE (1978). The effect of these fractionation will be discussed later.

The three Andosol soils at the Memuro Station generate from the same series of volcanic ashes, but different organic contents (Fig. 3) suggest that organic material must turnover differently in time. In the Andosol black soil, the $\delta^{15}\text{N}$ values of soil nitrate, soybean and wheat are similar to that of soil organic nitrogen in surface soil ($\sim +5\text{‰}$). On the other hand, the $\delta^{15}\text{N}$ values of the plants grown on the Andosol brown soil (-2 to $+5\text{‰}$) are lower and sometimes in the range of fertilizer nitrogen (-1.2 to 1.7‰) (WADA, unpublished data). Nevertheless, similar values were found among ^{15}N abundances of organic nitrogen in the Ap layer of the two Andosol soils and in the IIAC layer of the oak tree grove (Fig. 3). The observed $\delta^{15}\text{N}$ value of 5‰ of the soil organic nitrogen seems to be an inherent value of Andosol soils, and their ^{15}N abundance is primarily determined by physico-chemical nature of the soils. The situation is probably the same with respect to the mineral substrata where $\delta^{15}\text{N}$ values of about 10‰ were commonly obtained for soils (Fig. 3). The low ^{15}N abundances of the alluvial soil may reflect a contribution of river-derived nitrogenous compounds with a low ^{15}N content. The nitrogenous compounds coming from forest areas had lower $\delta^{15}\text{N}$ than those from cultivated areas (MARIOTTI *et al.*, 1976, WADA *et al.*, 1983). Low $\delta^{15}\text{N}$ values of 0.3 to 3‰ have been also reported for kerogen extracted from river sediments (PETERS *et al.*, 1978).

Ammonium concentration in interstitial water of paddy soils is high during an early period of flooding (May through June). After transplantation of rice seedlings, it declines and becomes undetectable by the middle of the flooding period (July). During this period a part of the ammonium is oxidized to nitrate and the nitrate is then converted near the soil surface to

N_2 by the action of denitrifying bacteria. Nitrogen isotopic fractionation associated with these reactions would be substantial because relatively large amounts of ammonium might be initially present. Then, the residual nitrogenous compounds enriched in ^{15}N would be taken up by rice plants. The $\delta^{15}\text{N}$ values of rice plants higher than those of chemical fertilizer and green manure appear to support this inference (Fig. 4). During the preparation of farmyard manure, ammonia is often volatilized. Nitrogen isotopic fractionation associated with the ammonia volatilization may result in the ^{15}N enrichment in ammonia and microorganisms grown in farmyard manure (KREITLER, 1975). The high $\delta^{15}\text{N}$ values of rice plants grown in the OM plot (Fig. 4) can be explained by this mechanism. The high $\delta^{15}\text{N}$ values (9.3 to 22.8‰) of hydrophytes at the Konosu field may also result from ammonia volatilization. Actually, pH values higher than 9 were often recorded in the flood water during the summer season because of high photosynthetic activities of microalgae and hydrophytes.

Aerial part of rice plants is harvested in early October. Therefore, most part of rice plants with high $\delta^{15}\text{N}$ is removed and only plant roots are left behind. According to KIMURA *et al.* (1980), root to aerial part ratio at Konosu ranges from 0.07 to 0.11 by dry weight base and more than 50% of plant remains incorporated into paddy soil is decomposed within a year. A yearly accumulation of soil organic matter, thus, becomes small compared with total soil nitrogen. However, increases in soil organics are clearly observed for the OM, GM and CF plots (Table 2). In the present investigation, $\delta^{15}\text{N}$ of the root was not measured because of a possible contamination by soil. Since variation of $\delta^{15}\text{N}$ among plant tissues was reported to be $\pm 0.99\text{‰}$ (KOHL *et al.*, 1979; YONEYAMA, unpublished), $\delta^{15}\text{N}$ of the root may be as high as that of the aerial part which is higher than that of soil (Fig. 4). Its annual addition, however, did not change the ^{15}N of soil organics (Table 1). This was the case irrespective of whether or not soil organic nitrogen was accumulated. A significant isotope discrimina-

tion, therefore, must take place during the epidiagenesis of plant remains. This will be discussed later.

The average $\delta^{15}\text{N}$ value of chemical fertilizer (ammonium sulfate) is estimated to be -0.4‰ (range: $-3.5 \sim 4.8\text{‰}$) (WADA and NAKAMURA, 1980). The effect of the long-term application of ammonium sulfate can be evaluated by comparing $\delta^{15}\text{N}$ values of soil organic nitrogen in the NF and CF plots. At Anjo and Nagano, soil from plow layers of NF plots appeared to have higher $\delta^{15}\text{N}$ values than that from the CF plots. At both experimental stations concentrations of organic matter in soil are small. According to TAKAI (1979), the decomposition of organic matter at these paddy fields is relatively rapid because of the high permeability of soil water. At the Anjo field, the difference in $\delta^{15}\text{N}$ between the NF and CF plots is significant irrespective of soil depth. For both the CF and NF plots, a portion of the fertilizer may go down along with water movement realizing some substantial isotope fractionation so as to yield similar shift in $\delta^{15}\text{N}$ values of soil organic materials through soil columns.

The effect of long-term application of fertilizer on the $\delta^{15}\text{N}$ of soils seems to be related to the soil nitrogen content (Fig. 5a). The variations of the $\delta^{15}\text{N}$ in plow layers between the NF and the CF plots during the indicated years become significant if soil nitrogen content is relatively low (Anjo and Nagano), while the reverse is the case for soils with large amounts of organic nitrogen (Aizubange, Konosu, and Shizuoka). Fertilizer treatments increased the soil organic nitrogen in plow layers more than 30% relative to the NF plot in the GM and OM plots at Konosu and the CF plots at Aizubange. Assuming that both soil organic nitrogen content and $\delta^{15}\text{N}$ value in the NF plot have been kept constant over the period of the long-term experiments, we can calculate the $\delta^{15}\text{N}$ values of soil organic nitrogen accumulated during the experiments. $\delta^{15}\text{N}$ of organic nitrogen accumulated in the GM and OM plots at Konosu, and in the CF plots at Aizubange were estimated to be 0.1, 4.0 and -0.7‰ , respectively, on the basis

Fig. 5a

Fig. 5b

Fig. 5. Relation between soil organic nitrogen and $\delta^{15}\text{N}$. (a) Data from the long-term fertilization experimental rice fields. AB: Aizubange, K: Konosu, S: Shizuoka, N: Nagano, and A: Anjo. Data from the non-fertilized plots are shown by circles and those from the chemical fertilizer plots by squares. Arrow represents the difference between NF and CF plots during the indicated years in each plow layer (Horizon I). (b) Data from various terrestrial ecosystems. $\square$: forest soil, $\circ$: paddy fields, $\triangle$: upland fields, $\times$: upland fields after RENNIE et al. (1976) and MEINTS et al. (1975).

of on isotope mass balance calculations. From these results, we can estimate the nitrogen isotope discrimination (Δ_E) during the epidiagenesis by the following equation.

$$\Delta_E = \delta_{\text{rice plant}} - \delta_{\text{accumulated}}$$

At Konosu, Δ_E value of 5.8‰ was obtained for

OM plots. This value is very close to the reported average difference in $\delta^{15}\text{N}$ value between total organic nitrogen and coexisting kerogen in river sediments ($4.6 \pm 2.0\text{‰}$, PETERS *et al.*, (1978). A significant differences was also found by NAKAMURA (1982) with respect to ^{13}C abundance in soil organic carbon. According to CHENG *et al.*, (1964), hydrolyzable substances such as amino acids and amides are enriched in ^{15}N as compared with non-hydrolyzable organic nitrogen in soils. This fact supports the view that a part of easily decomposable nitrogen compounds with high ^{15}N content is lost more rapidly and soil humus should be less enriched in ^{15}N .

The $\delta^{15}\text{N}$ values of soil organic materials appear to be inversely correlated with nitrogen content of soil (Fig. 5b). The $\delta^{15}\text{N}$ also depend upon soil texture. The highest values are found in subsurface soils rich in clay minerals (Table 2). The process of cation exchange between clay colloids and interstitial waters probably plays a primary role in determining ^{15}N abundance in soil systems as suggested by DELWICHE and STEYN (1970). The heavier isotopic species ($^{15}\text{NH}_4^+$) is preferably adsorbed on clay colloids to the lighter one ($^{14}\text{NH}_4^+$), but the extent of nitrogen isotopic fractionation varies depending upon the kind of cations at the reaction sites. A variation of 1.003 to 1.011 has been reported with respect to the isotopic equilibrium constant (α_K) of the ammonium adsorption on clay colloids (KARAMANOS and RENNIE, 1978).

Summing up results presented here, we conclude that $\delta^{15}\text{N}$ of primary producer such as rice plants and hydrophytes is affected mainly by the nitrogen isotope fractionation during the denitrification, nitrification, ammonia volatilization, and nitrogen fixation, and by $\delta^{15}\text{N}$ of source nitrogen. Most of soil organic nitrogen quite likely keep their inherent $\delta^{15}\text{N}$ value reflecting the long-term history of individual soil over more than hundreds of years (JENKINSON and RAYNER, 1977). The $\delta^{15}\text{N}$ of soil organic nitrogen thus changes very slowly depending upon the mode of epidiagenesis involving kinds and ^{15}N content of primary producers, soil texture,

and climate conditions.

A simple model for isotopic fraction in soil

With these considerations given above in mind, we present a simple model to explain ^{15}N abundance in soil systems with emphasis on paddy fields. Most of paddy fields in Japan are located in alluvial areas where source nitrogen ($0\text{--}2\text{‰}$) have been transported from mountain areas. Since flushing time of river water in Japan is short, $\delta^{15}\text{N}$ of the source nitrogen in alluvial areas is nearly equal to that in mountain areas and is homogeneous all over the alluvial areas in question. Permeability of soil water is also sufficiently high to eliminate soluble substances from the present system. In the model (Fig. 6), we assume:

(i) $\delta^{15}\text{N}$ of fertilizer nitrogen (ammonium sulfate) is nearly equal to $\delta^{15}\text{N}$ of source nitrogen in the alluvial areas. Newly added soil organic matter (BIO) exhibits almost the same $\delta^{15}\text{N}$ values in the alluvial areas on long time scale of

Fig. 6. Schematic model for nitrogen isotope fractionation in soil organic matter. BIO: soil biota, SH: soil humus, CM: Ca (Mg) Organo-Clay mineral, DON: dissolved organic nitrogen in interstitial water, and Δ : nitrogen isotope discrimination.

over hundreds of years. Consequently, the nitrogen isotopic fractionation associated with assimilation occurs with a constant fractionation factor in alluvial soil, and can be given by the following equation.

$$\Delta_A = \delta^{15}\text{N}_{\text{BIO}} - \delta^{15}\text{N}_{\text{source nitrogen}} \quad (1)$$

(ii) Nitrogen isotope fractionation factor (α) is 0.9942 ($\Delta_E = 5.8$) during epidiagenesis. The heavier isotopic species are preferably decomposed to available nitrogen, but the fractionation takes place only at the site of organic matter (BIO); no fractionation occurs at the site of clay minerals (CM), which adsorb organic matter very tightly. Numbers of these sites in soil are further assumed to be proportional to the cation exchange capacity (CEC) of each component in soil. Then, $\delta^{15}\text{N}$ soil at the site of

$$\text{BIO} = \delta^{15}\text{N}_{\text{BIO}} - \Delta_E \quad (2)$$

under steady state condition of epidiagenesis.

(iii) Isotopic fractionation is also considered in the process of ammonium adsorption at the site of clay minerals (CM). We consider the following isotopic exchange reaction:

and the isotope fractionation factor (α) becomes $1 + \Delta_C/1000$. In this reaction, $\delta^{15}\text{N}$ of ammonium in water moving downward is the same as that of BIO throughout whole soil column in paddy field areas. Then, we obtain

$$\begin{aligned} \delta^{15}\text{N}_{\text{soil at the site of CM}} &= \delta^{15}\text{N}_{\text{BIO}} \\ &+ \Delta_C. \end{aligned} \quad (4)$$

It is generally accepted that the cation exchange capacity (CEC, me/100g soil) of paddy soil is determined mainly by the concentration of clay mineral (CM) and soil humus (SH), and can be approximated by the equation:

$$[\text{CEC}] = \sum_i \gamma_i [\text{CM}_i] + \gamma_{\text{SH}} [\text{SH}], \quad (5)$$

where γ 's are proportionality constant (me/100g), the subscript i refers to species of clay minerals, and the brackets indicate the concentration (%) of clay minerals of soil humus on dry weight basis. Equation (5) can be rewritten by using the total clay content [CM] and the weighted average proportionality constant for clay minerals [$\bar{\gamma}_{\text{CM}}$] and by substituting organic carbon content [OC] for [SH] as:

$$[\text{CEC}] = \bar{\gamma}_{\text{CM}} [\text{CM}] + \gamma_{\text{OC}} [\text{OC}] \quad (5')$$

Based on a simple mass balance for the model, the following relation can be applied to the isotopic fractionation for overall processes:

$$\begin{aligned} \delta^{15}\text{N}_{\text{soil}} &= \delta^{15}\text{N}_{\text{BIO}} - \Delta_E \frac{\gamma_{\text{OC}} [\text{OC}]}{\text{CEC}} \\ &+ \Delta_C \frac{\bar{\gamma}_{\text{CM}} [\text{CM}]}{\text{CEC}} \end{aligned} \quad (6)$$

From equations (5') and (6), we obtain the following equation:

$$\begin{aligned} \delta^{15}\text{N}_{\text{soil}} &= \Delta_E + C \frac{\frac{[\text{CM}]}{[\text{OC}]}}{\frac{[\text{CM}]}{[\text{OC}]} + \frac{\gamma_{\text{OC}}}{\bar{\gamma}_{\text{CM}}}} \\ &+ (\delta^{15}\text{N}_{\text{BIO}} - \Delta_E), \end{aligned} \quad (7)$$

where $\Delta_{E+C} = \Delta_E + \Delta_C$.

The validity and applicability of the proposed model was examined by using the data obtained from the NF and CF plots of the long-term fertilization experimental rice fields (Table 2). Figure 7 illustrates the relationship given by equation (5'). Cation exchange capacity of paddy soil ranges from 5.7 to 26 me/100g dry soil and calcium ions are major exchangeable cations. Although differences among stations are substantial, CEC/(OC) ratios are linearly correlated with (CM)/(OC) ratios on a first

Fig. 7. Evaluation of equation (1) by using data obtained from the present rice fields. Data obtained at the other chemical fertilizer plots of Nagano, Anjo and Shizuoka stations are included.

approximation. The applicability of the equation (5') is, thus, verified. For the paddy soils from Anjo and Shizuoka stations, the following empirical equation is obtained by regression analysis:

$$\text{CEC} = 0.20 [\text{CM}] + 2.4 [\text{OC}] \quad (8)$$

with $\gamma_{\text{OC}}/\bar{\gamma}_{\text{CM}}$ ratio of 12 and with a correlation coefficient of 0.98. For the soils from Aizu and Nagano stations, we get the equation:

$$\text{CEC} = 0.80 [\text{CM}] + 1.1 [\text{OC}] \quad (9)$$

with $\gamma_{\text{OC}}/\bar{\gamma}_{\text{CM}}$ ratio of 1.4 and with correlation coefficient of 0.64. The data from Konosu station deviate from equation (9). As described previously characteristics in soil texture and small permeability of soil water may constitute one of the possible cause for the deviation. High $\bar{\gamma}_{\text{CM}}$ value of 0.80 me/100g for Nagano soil probably results from the high montmorillonite content.

The relationship between the ^{15}N abundance of soil organics and the variation of $[\text{CM}]/[\text{OC}]$ ratio in paddy soils can be described by a hyperbola (Fig. 8) as expected from equation (7). For the purposes of calculation by regression analysis, we assign 12 for $\gamma_{\text{OC}}/\bar{\gamma}_{\text{CM}}$. The values for $\Delta_{\text{E+C}}$ and $(\delta^{15}\text{N}_{\text{BIO}} - \Delta_{\text{E}})$ which fit best the most of observed data from Anjo and Shizuoka

Fig. 8. Relation between clay to organic carbon ratio and $\delta^{15}\text{N}$ in the long-term fertilization experimental paddy fields. Open symbol stands for NF plots and closed one stands for CF plots. The solid line is the best fit curve excluding samples marked with asterisks for soils collected from Anjo, and Shizuoka. For Nagano and Aizubange soils $\gamma_{\text{OC}}/\bar{\gamma}_{\text{CM}}$ value of 1.4 was adopted as indicated by the dashed line.

stations are 13.9 and -5.1‰ , respectively, with a correlation coefficient of 0.77 (solid line in Fig. 8). Δ_{E} value of 5.8‰ was then used to estimate Δ_{C} and $\delta^{15}\text{N}_{\text{BIO}}$ at 8.1 and 0.7‰ , respectively. The former value of 8.1‰ ($\alpha = 1.008$) is very close to 1.011 for Ca^{2+} -clay reported by KRAMANOS and RENNIE (1978). $\delta^{15}\text{N}$ value of BIO is also in the range of $\delta^{15}\text{N}$ value in nitrate and suspended particles in the middle reach of Otsuchi River ($0.7\text{--}2.3\text{‰}$) where human impact is negligibly small. Dashed line in Fig. 8 was tentatively obtained for Nagano and Aizubange soil by using $\gamma_{\text{OC}}/\bar{\gamma}_{\text{CM}}$ of 1.4 instead of 12. Since the data comprise small numbers, the value of 1.4 seems to have a large error as indicated by a small correlation coefficient of 0.64 in equ. (9). As indicated in Fig. 8, most data are distributed in the expected areas, suggesting the applicability of the present model to alluvial soils. Two exceptional data from Anjo station (marked by *) may result from the discrepancy in $\delta^{15}\text{N}$ value for BIO and NH_4^+ in soil water. Namely, the low $\delta^{15}\text{N}$ value of 0.5‰ in the plow layer of the CF plot of Anjo resulted

Fig. 9. Factors controlling ^{15}N in soil organic nitrogen with emphasis on paddy fields. Solid line represents soil $\delta^{15}\text{N}$ expected from the present model. In this figure $\delta^{15}\text{N}$ of source nitrogen is assumed to be 0.0‰ . Open arrow gives the direction of the variation of $\delta^{15}\text{N}$ in the case the indicated parameter changes. Solid arrows indicate the effects of chemical and biochemical processes on the $\delta^{15}\text{N}$ value of plants as well as the magnitude of $\Delta\epsilon$. Soil samples studied in the present work occupy the circled areas as indicated.

from the low $\delta^{15}\text{N}$ values of BIO by the long-term application of ammonium sulfate, as a high permeability of soil water might restrict the progress of denitrification with a large isotopic fractionation. While, the high $\delta^{15}\text{N}$ value of 12.3‰ in the third layer of the NF plot may result from high $\delta^{15}\text{N}$ value of NH_4^+ in soil water. High ΔC value of clay mineral in this layer can be another possible cause.

The result of the model analysis supports the inference that ^{15}N abundance of source nitrogen, nitrogen isotopic fraction associated with assimilation, epidiagenesis, and adsorption of ammonia on clay mineral form major factors for the determination of ^{15}N abundance in soil organic nitrogen as summarized schematically in Fig. 9. Variation of $\delta^{15}\text{N}$ appeared in Fig. 8 can, thus, be explained by this model qualitatively along with the physico-chemical nature of each soil. High $\delta^{15}\text{N}$ value for Nagano soil resulted from its low $\gamma_{\text{OM}}/\bar{\gamma}_{\text{CM}}$ ratios. On the other hand, gleyic soils have accumulated a large amount of soil organic matter under a cold climate condition at Aizubange, with a lower $\delta^{15}\text{N}$ of source nitrogen than 0.7‰ in this inland area. Cold climate

may also decrease $\Delta\epsilon$ at Aizubange. Further, volcanic soils contain a large amount of allophane of which $\bar{\gamma}_{\text{CM}}$ value is as high as 2.8me/g . Very low value of $\gamma_{\text{OC}}/\gamma_{\text{CM}}$ in equation (7) can provide the inherent $\delta^{15}\text{N}$ value of 5‰ for Andosol soil over the wide range of (CM/OC) ratios. L-horizon of forest soils can give the lowest $\delta^{15}\text{N}$ value because of the accumulation of large amounts of litters. $\delta^{15}\text{N}$ in soil organic nitrogen can, thus, be an useful parameter for time-integrated nitrogen cycling.

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