^{15}N abundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint

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Abstract—Natural abundance of ^{15}N in organic and inorganic nitrogenous substances in land was investigated. Available data on ^{15}N abundance of biogenic nitrogen were summarized.

Denitrification is divided into four types on the basis of the mode of isotope fractionation and environmental conditions. According to the simplified model on nitrogen isotope balance, the contribution of denitrification on land to the global denitrification is twice as much as that in the sea.

The mode of ^{15}N distribution in biogenic substances was discussed along the biogeochemical cycle of nitrogen with special reference to the fractionation of nitrogen isotopes occurring in denitrification and nitrogen fixation.

INTRODUCTION

Since the pioneer work of UREY (1947), the isotope fractionation of nitrogen in biogeochemical systems and its significance have increasingly been recognized. The ratio of $^{15}\text{N}/^{14}\text{N}$ in naturally occurring substances varies between -6 and $+21\text{‰}$ relative to the $^{15}\text{N}/^{14}\text{N}$ ratio of the atmospheric nitrogen (HOERING, 1955; CHENG *et al.*, 1964). According to MIYAKE and WADA (1967), the ratio of $^{15}\text{N}/^{14}\text{N}$ in nitrogenous compounds in marine environments is $+7\text{‰}$ on an average and tends to increase along the trophic level of the food chain.

A variety of nitrogenous compounds such as organic nitrogen, ammonia and nitrate constitute a complicated cycle in nature. The variation of the $^{15}\text{N}/^{14}\text{N}$ ratio observed in the biosphere can be explained by kinetic isotope fractionations in biochemical reactions such as assimilation of inorganic nitrogen, nitrogen fixation, nitrification, ammonification, denitrification and other processes of nitrogen metabolism.

HOERING and FORD (1960) found no isotope fractionation of nitrogen beyond the level of $\pm 1\text{‰}$ in the fixation of atmospheric nitrogen by *Azotobacter* sp. They tried to explain the average high $^{15}\text{N}/^{14}\text{N}$ ratio in the biosphere in terms of the fractionation in the denitrification processes.

The kinetic isotope fractionation factor up to 1.03 was later demonstrated in denitrification process by *Pseudomonas* sp. and marine denitrifying bacteria (WELLMAN *et al.*, 1968; MIYAKE and WADA, 1971) in the presence of excess nitrate.

The variation of $^{15}\text{N}/^{14}\text{N}$ in the oxidation of ammonia to nitrite by a marine nitrifying bacterium was of the same order of magnitude as that found in the denitrification (MIYAKE and WADA, 1971).

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The recent progress in the studies of nitrogen cycle makes it possible to correlate the distribution of ^{15}N in biogenic substances to the naturally occurring isotope fractionation. We present here additional data on the ratio of $^{15}\text{N}/^{14}\text{N}$ in nitrogenous substances occurring especially on land as well as in marine environments and discuss the distribution of ^{15}N in biogenic materials on the basis of the current knowledge of biochemical nitrogen cycle.

MATERIALS AND METHODS

Procedures for the measurement of nitrogen isotope ratios were described in detail elsewhere (MIYAKE and WADA, 1967).

Ammonia was collected in 0.5N sulfuric acid by air distillation and oxidized to nitrogen gas with potassium hypobromite *in vacuo*. The evolved nitrogen gas was purified by passing through a Cu-CuO furnace at 700°C before the mass spectrometric analysis.

Organic materials were digested by a Kjeldahl method using mixed powder of HgCl_2 , Se, CuSO_4 and K_2SO_4 as catalyzers. Nitrate in rain samples was reduced to ammonia with aluminum foil in 1N KOH aqueous solution.

The sampling of Japanese river water was made about 10km up from the river mouth. The river water sample (20l) was acidified with hydrochloric acid (pH 4), evaporated up to 200ml, and subjected to Kjeldahl digestion. The fraction thus obtained, which is defined as "organic nitrogen" contains pre-existing ammonia.

The amount of nitrogen contained in the reagents used was negligibly small ($< 0.02\%$) compared with those of the samples.

The measurement of $^{15}\text{N}/^{14}\text{N}$ ratios was carried out using a Hitachi RMU-6 mass spectrometer equipped with a double collector and a dual inlet system. The atmospheric nitrogen which was supplied by the U.S. National Bureau of Standards was used as the primary standard. Nitrogen isotope ratio is presented in terms of δ expression as follows,

$$\delta^{15}\text{N}(\text{‰}) = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{std.}}}{(^{15}\text{N}/^{14}\text{N})_{\text{std.}}} \times 1,000$$

The precision of the $\delta^{15}\text{N}$ measurement was $\pm 0.5\text{‰}$. The fractionation factor used in the present investigation was defined as the ratio of the rate constant of lighter isotope to that of heavier one assuming a first order reaction with respect to the nitrogen bearing reactant.

RESULTS

The value of $\delta^{15}\text{N}$ in biogenic nitrogenous compounds collected in land varied from -10 to $+17\text{‰}$ (Table 1). The lowest value of -9.7‰ was obtained for ammonia dissolved in rain water collected in Tokyo, Japan, a value significantly lower than those ($+0.1 \sim +9\text{‰}$) reported by HOERING (1957). The effect of fossil fuel combustion may be responsible for the low value obtained in the present study.

Organic nitrogen in river waters showed a variety of $\delta^{15}\text{N}$ values between $+1.9$ and $+17.1\text{‰}$ with a mean value of $+8.9\text{‰}$. The highest value was found in the sample collected from Asahi River, Okayama Prefecture. The values fell within the range observed in soil nitrogen (CHENG *et al.*, 1964).

Plant materials in land contained less ^{15}N . $\delta^{15}\text{N}$ values of -1.0 to $+5.9\text{‰}$ with an average of $+1.2\text{‰}$ were found.

Crude oil showed higher $\delta^{15}\text{N}$ values than those of organic nitrogen in the underlying oil brine, in which a large amount of ammonium ions was found. This fact is accounted for by isotope fractionation through the decomposition of organic nitrogen to ammonia and subsequent extraction of hydrophilic substances such as ammonia into an aqueous

Table 1. Abundance of nitrogen-15 in nature

				$\delta^{15}\text{N}(\text{‰})$
<i>Plant (land)</i>				
Miscanthus sinensis				-1.0
Wild rose				2.2
Clover				0.0
Dandelion				1.2
Chickweed				5.9
Fern				-0.6
Moss				-0.4
Pine-needles				1.1
-twig				0.4
-board		New Zealand		2.6
-cortical tissue		New Zealand		0.6
Sun tree				2.7
Cryptomeria				1.0
<i>Animal (land)</i>				
Pig-tongue				7.9
-intestines				6.8
-heart				8.2
Milk				7.7
Egg-white				8.8
-yolk				7.0
<i>Soil (land)</i>				
near Lake Yamanaka	1			4.6
	2			4.6
Tokyo	1			4.9
	2			5.8
	3			8.5
<i>River</i>				
Tone				8.6
Yodo				7.6
Asahi				17.3
Yoshino				1.9
<i>Crude oil</i>				
Sarukawa	1	Akita Prefecture		5.1
	2			4.9
	3			3.1
	4			2.3
Barato	1	Hokkaido		3.6
	2			4.6
<i>Oil brine</i>				
Sarukawa		Akita Prefecture		1.1
Barato		Hokkaido		3.4
Seigo		Niigata Prefecture		4.1
<i>Rain</i>				
Ammonia		Tokyo, May, 1966		-9.7
Nitrate				4.3
Ammonia		July, 1966		-8.3
		Sep., 1966		-3.2

phase, ^{15}N rich organic nitrogen being left in the oil phase.

Animals on land and organic materials in the soil always showed $\delta^{15}\text{N}$ values higher than that of atmospheric nitrogen, averaging +6.5 and +6.2‰, respectively (Table 2).

The variation of $\delta^{15}\text{N}$ values in biogenic nitrogen bearing substances is illustrated in Fig. 1. Data given by previous investigators are also included. The average $\delta^{15}\text{N}$ values in specimens collected from the sea were to a significant extent higher than those in the land counterparts. In the case of marine photosynthetic organisms, $\delta^{15}\text{N}$ values higher

Table 2. Summary of the mean values of $\delta^{15}\text{N}$, and standard deviations from the mean for various forms of nitrogenous compounds

	Mean $\delta^{15}\text{N}$ ‰	Standard deviation ‰	Number of samples
<i>Land</i>			
Plant	0.31	2.8	23
Peat and coal	0.77	1.6	21
Soil	6.2	5.0	21
Animal	6.5	1.4	15
Crude oil	3.4	1.6	29
Oil brine	2.9	1.6	3
Mud	6.0	1.2	7
River org. N	8.9	6.4	4
Rain NH_4^+	4.0	2.4	8
NO_3^-	-1.7	3.4	9
<i>Sea</i>			
NO_3^-	5.8	1.6	18
Phytoplankton	4.9	1.8	6
Phytoplankton*	0.4 ₄	0.7 ₂	5
Seaweed	7.5	1.1	16
Zooplankton	7.4	2.7	11
Zooplankton*	2.0	0.4 ₁	3
Fish	14.2	5.4	5
Sediment	6.8	4.1	5

* Samples were collected from an ecosystem where nitrogen was supplied via symbiotic nitrogen fixation. (*Trichodesmium thiebautii*)

Fig. 1. Summary of the data for the distribution of $\delta^{15}\text{N}$ in biogenic nitrogenous compounds in nature.

○: HOERING (1955), : CHENG *et al.* (1964), ~: PARWEL *et al.* (1957), ×: SAKATA and MATSUO (1972), ⊙: HOERING (1957), : HOERING *et al.* (1958), ○: MIYAKE and WADA (1967), △: WADA and HATTORI (in preparation), ∅: WADA (unpublished data), ●: present data.

than +4‰ were always obtained except for pelagic blue-green algae, *Trichodesmium* sp. (+4‰) collected in the Pacific to the east of Taiwan, where nitrogen fixation is quite active (WADA and HATTORI, manuscript in preparation).

Nitrogen in land soils and marine sediments shows a wide range of $\delta^{15}\text{N}$ values, suggest-

ing the difference in the source materials and an involvement of complicated biochemical reactions. Their averages are +6.2 and +6.8‰, respectively (Table 2). This is very important, because of their great weight in the inventory of biogenic nitrogen on the earth's surface (EMERY *et al.*, 1955). The high $\delta^{15}\text{N}$ value of the biogenic nitrogen (around +6‰) is, thus, well established.

DISCUSSION

The kinetic isotope effect on biogeochemical oxidation-reduction reactions and the geochemical transport of various kinds of nitrogenous compounds are the two major factors that determine the variation of $\delta^{15}\text{N}$ values in nature. Isotope fractionation of nitrogen in laboratory experiments (Table 3) gave us information on the maximum extent of fractionation that could take place in individual metabolic steps in the nitrogen cycle. The magnitude of the fractionation depends upon whether or not the step of an N-X bond cleavage is rate determining in the whole sequence of reaction (BIGEISEN and WOLFSBERG, 1958). For example, in the case of molecular nitrogen fixation, the rate determining step does not involve a change in bonding of nitrogen (HOERING and FORD, 1960), while in nitrate reducing process, N-O bond rupture is a slow step which regulates the overall reaction rate (BROWN and DRURY, 1967).

Molecular nitrogen, organic nitrogen and nitrate are the three major forms of nitrogen in the inventory of biogeochemical circumstances. Isotope fractionations between these three forms thus, play an important role in controlling the pattern of ^{15}N distribution in the biosphere. The positive $\delta^{15}\text{N}$ value of biogenic nitrogen especially correlates to the isotope fractionation occurring in both fixation ($\text{N}_2 \rightarrow$ organic nitrogen) and denitrification ($\text{NO}_3^- \rightarrow \text{N}_2$).

Nitrogen fixation is the major pathway through which atmospheric nitrogen enters into the biosphere. The isotope fractionation factor of nitrogen in the fixation process ranging from 0.996 to 1.009, has been demonstrated in the laboratory culture of two representative nitrogen fixers, *Azotobacter* sp. and *Anabaena cylindrica* (Table 3).

Table 3. Nitrogen isotope fractionation factors in various biochemical reactions found in laboratory experiments

$\delta^{15}\text{N}\text{‰}$ of product ($\delta^{15}\text{N}$ substrate = 0.0)		
<i>Assimilation</i>		
Nitrate	-0.9 - -18.5	<i>Phaeodactylum</i>
Nitrite	-1.4 - - 4.0	<i>Tricornutum</i>
Ammonia	-9.6 - + 7.3	<i>Chaetoceros</i> sp.
<i>Nitrification</i>		
Ammonia to nitrite	-5.4 - -21.1	Marine nitrifier collected from Maizuru Bay*
<i>Decomposition of org. N</i>	0.0 - - 2.3	<i>Scenedesmus</i> sp.*
<i>Nitrate reduction to nitrite</i>	-5.0 - -29.0	<i>Serratia marinorubra</i> ****
<i>Denitrification</i>		
Nitrate to nitrogen gas	-30	Denitrifier***
	-14.4 - -20.7	Denitrifier collected from Maizuru Bay, Japan*
	-1.9 - -12.3	Denitrifier collected from Lake Hamana, Japan
<i>N₂-fixation</i>		
Nitrogen gas to org. N	-9.0 - + 4.0	<i>Azotobacter</i> **
	-1 - - 3	<i>Anabaena cylindrica</i> *****

* MIYAKE and WADA (1971). ** HOERING and FORD (1960). *** WELLMAN *et al.*, (1968).

**** MIYAZAKI (1971). ***** OHMORI, M. (person. com)

The value of $\delta^{15}\text{N}$ for the substrate N_2 in nature is 0 to +1‰ (BENSON and PARKER, 1961; KLOTS and BENSON, 1963; MIYAKE and WADA, 1967). A small fractionation is also seen in the low $\delta^{15}\text{N}$ values of clover and *Trichodesmium* sp. of which nitrogen would be provided *via* nitrogen fixation (Tables 1 and 4). The *in situ* fractionation factor in the fixation system can then be roughly postulated to be 1.000 ± 0.001 on an average in the discussion.

Table 4. Apparent isotope fractionation of nitrogen estimated from the natural abundance of ^{15}N

Reaction	Temp. °C	$\delta^{15}\text{N}\text{‰}$ (N_2air , 0.0‰)	Fractionation factor*
Soil-org. N ——— NH_4^+	30		1.003 CHENG <i>et al.</i> , (1964)
Sediment-org. N — NH_4^+	25		1.001 ₃ MIYAKE and WADA (1967)
Denitrified N_2			
Lake Hamana, Japan	20	2.9 (6)**	HATTORI <i>et al.</i> , (1970)
Cariaco Trench	16	44 (10)**	RICHARDS and BENSON (1961)
Dramsford	6	24 (6)**	
Nitrate-assimilation 44°N, 154°E	2 – 8		1.005 WADA, unpublished data
N_2 fixation	22 – 27	(5)**	1.0003 WADA and HATTORI (1973)

* Mean value of $^{14}\text{K}/^{15}\text{K}$.

** Numbers of samples

On the other hand, the fractionation factor up to 1.03 is observed in denitrification (Table 3). The magnitude of the fractionation in nature depends upon the concentration of nitrate, the turn-over rate of reducing enzyme, partial pressure of oxygen and *in situ* temperatures. When N-O bond cleavage is the rate determining step, the temperature exerts primary effect on the fractionation. The amount of nitrate regulates denitrification or nitrate reduction in the following dual ways. Nitrate determines the rate of denitrification as a terminal electron acceptor and also controls the activity level of the enzyme of denitrifying bacteria in a denitrification system (KOIKE *et al.*, 1972). The supply of energy source such as organic carbon for heterotrophic organisms and of light for photosynthetic organisms, controls the intercellular concentration level of reductant in the electron transport system, e.g. NADA or NADPH and in turn regulates the balance between the *in vivo* concentration of nitrate and the turnover rate of nitrate reductase (WADA and HATTORI, 1973). From these considerations described above, denitrification is divided into four types on the basis of the mode of isotope fractionation and the change in environmental conditions.

Case 1: When denitrification proceeds under completely anaerobic conditions, all of nitrate in the system is converted into nitrogen gas and the $\delta^{15}\text{N}$ value of the product is identical with that of the nitrate metabolized. A $\delta^{15}\text{N}$ value of +2.9‰ (Table 4) was reported for nitrogen gas evolved in anaerobic layers of a brackish lake Hamana (HATTORI *et al.*, 1970). This value is regarded as the $\delta^{15}\text{N}$ value of the original nitrate.

Case 2: Denitrification in a large volume of oxygen deficient water of the eastern tropical Pacific Ocean has been substantiated by extremely low concentrations of oxygen (often < 0.02 ml/l), high concentrations of nitrite (2.5 μg at.N/l), and nitrate anomaly (FIADEIRO and STRICKLAND, 1968; CLINE and RICHARDS, 1972; GOERING *et al.*, 1973). GOERING (1968) demonstrated a close correlation between nitrite concentration in deep oxygen-deficient layers and denitrification activity. High nitrate concentrations (25 ~ 40 μg at.N/l) and slow reaction rate give, in nature, an isotope fractionation factor up to ca. 1.020, a mean of the maximum values of fractionation factors which have been obtained in culture experiments with various organisms grown at around 25°C. In the later discussion, we assigned a fractionation factor of 1.021 (10°C) by considering a small temperature

dependence of the fractionation factor.

Case 3: Oxygen apparently inhibits denitrification. However, the existence of organic aggregates on which heterotrophic bacteria are attached makes it possible, even in oxygenated environments, to produce a microcosm where anaerobic condition is established (SKERMAN *et al.*, 1958). This indicates the existence of a transient region, where both nitrification and denitrification can proceed simultaneously owing to the presence of microcosm (WADA *et al.*, 1970). YOSHINARI (1973) clearly demonstrated that N_2O is inversely correlated with the concentration of dissolved oxygen. This is supported also by the occurrence of obligate anaerobic bacteria in oxygenated waters (SEKI, 1972). In a microcosm, almost all the nitrate entered into the microcosm would be converted into molecular nitrogen through denitrification. The fractionation of nitrogen should not be expected in this case. On land, the $\delta^{15}\text{N}$ value of evolved N_2 is the same as that of nitrate in rain water (-1.7‰) which comes mainly from nitrogen oxides in the soil (KAWAMURA, 1964). In the sea, the $\delta^{15}\text{N}$ value of denitrified nitrogen is the same as that of ambient nitrogen bearing compounds in seawater ($+5.8\text{‰}$, Table 2) (case 3').

Case 4: The processes occurring in the Cariaco Trench and Norwegian fjords yield nitrogen with high $\delta^{15}\text{N}$ values ($+34\text{‰}$, Table 4) (RICHARDS and BENSON, 1961). The reason for resulting in these high $\delta^{15}\text{N}$ values is obscure but this fact is taken into consideration.

For the purpose of a semi-quantitative treatment of global denitrification, a simplified model for nitrogen cycle is postulated as follows:

where k_+ and k_- are rate constants in the indicated steps, α and β are ratios of rate constants for light (^{14}N) and heavy (^{15}N) species in the respective steps ($\alpha = {}^{14}k_+ / {}^{15}k_+$, $\beta = {}^{14}k_- / {}^{15}k_-$). Here, each amount of organic nitrogen, molecular nitrogen and nitrate ions on the earth's surface is assumed to be constant (a stationary state). The ^{15}N distribution among these components is then also in a stationary state. Then,

$$R_{\text{biogenic-N}} = \text{constant} \quad (1)$$

$$\frac{\beta}{\alpha} = \frac{R_{\text{NO}_3^-}}{R_{\text{N}_2}} = \frac{1 + 10^{-3}(\delta^{15}\text{N})_{\text{NO}_3^-}}{1 + 10^{-3}(\delta^{15}\text{N})_{\text{N}_2}} \quad (2)$$

where R denotes $^{15}\text{N}/^{14}\text{N}$ ratio. As described previously, $(\delta^{15}\text{N})_{\text{atmosph. N}_2} = 0.0\text{‰}$ and $\alpha = 1.000$. Then, eq. (2) can be rewritten:

$$\delta_{\text{NO}_3^-} = 1,000\Delta\beta, \quad (3)$$

where $\Delta\beta = \beta - 1$.

In this study, the denitrification process in nature is divided into four cases as mentioned above. The $\delta^{15}\text{N}$ value of nitrate and the contribution to the global denitrification are different for each case. (Table 5). Then, the following two material balance equations hold for both bulk nitrogen and ^{15}N .

$$\sum X_i = 1 \quad (4)$$

$$\sum X_i (\delta_{\text{NO}_3^-} - 1,000\Delta\beta)_i = 0 \quad (5)$$

where suffix, i refers to each of the four different denitrification cases, and X_i 's, the fraction of the contribution of each process.

Table 5. Scheme of denitrification in nature

	$\delta^{15}\text{N NO}_3^-$ ‰	Fractionation factor	$\delta^{15}\text{N N}_2$ ‰
Land			
(a) anaerobic condition	2.9	1.000	2.9
(c) microcosm	2.9	ca. 1.005	-1.7
Sea			
(b) oxygen deficient water	6.0	1.021	-15
(d) microcosm	5.8	1.000	5.8
(e) anaerobic basin	—	—	34

Based on the discussion given earlier with reference to Tables 2 and 3, we obtain $\delta_{\text{NO}_3^-}$: $-1.000\Delta\beta_1 = 2.9 - 0 = 2.9\text{‰}$ for case 1, $5.8 - 21 = -15\text{‰}$ for case 2, $-1.7 - 0 = -1.7\text{‰}$ for case 3, $5.8 - 0 = 5.8\text{‰}$ for case 3', and $34 - 0 = 34\text{‰}$ for case 4. Then eqs. (4) and (5) are presented as follows:

$$X_1 + X_2 + X_3 + X_3' + X_4 = 1 \quad (6a)$$

$$2.9 X_1 - 15 X_2 - 1.7 X_3 + 5.8 X_3' + 34 X_4 = 0 \quad (6b)$$

In the sea, nitrogen is supplied mainly by precipitation ($\delta^{15}\text{N} = +1.5\text{‰}$, $\text{NO}_3^-/\text{NH}_4^+ \sim 1$) and river run off ($\delta^{15}\text{N} = +7.2\text{‰}$, $[\text{organic nitrogen} + \text{NH}_4^+]/\text{NO}_3^- \sim 2.5$). The weighted $\delta^{15}\text{N}$ value of the total input nitrogen is estimated to be $+3.3\text{‰}$ using the relative contribution of two types of input, 68 and 32%, respectively given by TSUNOGAI (1971). Main processes of the output of nitrogen from the sea consist of denitrification (97%) and sedimentation (3%) according to TSUNOGAI (1971). Then, the average $\delta^{15}\text{N}$ value for denitrified nitrogen gas in the sea is calculated to be 3.2‰ on the basis of the steady state assumption.

Equation (6) turns out to be as follows, when a steady state is established in the sea,

$$X_{1 \text{ sea}} + X_{2 \text{ sea}} + X_{3 \text{ sea}} + X_{4 \text{ sea}} = 1 \quad (7a)$$

$$2.9X_{1 \text{ sea}} - 15X_{2 \text{ sea}} + 5.8X_{3' \text{ sea}} + 34X_{4 \text{ sea}} = 3.2 \quad (7b)$$

According to GOERING *et al.*, (1973), areas where denitrification of case 1 prevails are probably very small. The Black Sea is a representative area where the maximum denitrification rate is estimated to be $2 \times 10^{11} \text{gN/y}$. They also estimated the annual loss of combined nitrogen in the Cariaco Trench would be $1 \times 10^{10} \text{gN/y}$. These figures give the values for $X_{1 \text{ sea}}$ and $X_{4 \text{ sea}}$ to be 0.01 and 0.0004, respectively, when we adopt $2.5 \times 10^{13} \text{gN/y}$ as the annual excess of nitrogen in the sea (TSUNOGAI, 1971). From eqs. (7a) and (7b), we obtain $X_{2 \text{ sea}} = 0.12$ and $X_{3' \text{ sea}} = 0.88$. From these figures with the help of eqs. (6a) and (6b), we finally obtain $X_1 = 0.003$, $X_2 = 0.04$, $X_3 = 0.66$, $X_{3'} = 0.30$, and $X_4 = 0.0001$.

The figures obtained here are based on only limited data, and may be premature for further discussions. It should be noted, however, that the contribution of denitrification on land (X_3) to the global denitrification is twice as much as that in the sea. Furthermore, the fraction of denitrification in specified sea areas where intensive denitrification has been expected on the basis of the distribution of nitrate and oxygen (case 2) accounts for only 12% of the denitrification in the sea against 100% as was urged by CLINE and RICHARDS (1972).

The mode of differentiation of nitrogen isotopes in biogenic nitrogen is illustrated

Fig. 2. Mode of ^{15}N distribution and the fractionation of nitrogen in the biogeochemical nitrogen cycle.

with special reference to the possible fractionations in biogeochemical processes (Fig. 2). The lower $\delta^{15}\text{N}$ values of higher plants on land probably result from the active recycling of nitrogen. The lighter isotope is preferentially assimilated by plants, but inorganic nitrogen rich in lighter isotope becomes again available through the decomposition of organic nitrogenous compounds. Symbiotic nitrogen fixation is another factor which serves to lower $\delta^{15}\text{N}$ value for higher plants. Some of organic nitrogenous compounds which are resistant to bacterial attack are left in the soil. This results in the enrichment of ^{15}N in soil. Nitrogen in sedimentary rocks might have contributed to increase the ^{15}N level on land in a geological cycle.

The higher $\delta^{15}\text{N}$ values of nitrogenous compounds in the sea than those on land can be explained by the occurrence of denitrification in the sea associated with a high isotope fractionation factor (1.021) and continuous inflow of organic materials rich in ^{15}N (+3.3‰). The high $\delta^{15}\text{N}$ values for animal and fish can at least partly be attributed to the isotope effects in amino-acid metabolism. GAEBLER *et al.*, (1966) have shown that ^{15}N is concentrated in newly formed amino-acids through the process of transamination.

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