

Nitrogen dynamics in forested ecosystems
elucidated by
 ^{15}N natural abundance method

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SUMMARY

Figure of nitrogen cycle in an intact ecosystem has been obscure because of both its high heterogeneity in time and space and high sensitivity for disturbance. Furthermore, although nitrogen cycle consists of both nitrogen stocks and nitrogen flows, many studies on the nitrogen cycle have focused on nitrogen stocks, while estimations of nitrogen flows have been *budget-oriented* because of difficulty of direct measurements. On account of these features of nitrogen cycle itself, new methodology is needed which can provide us time- and space-integrated information derived from intact ecosystem without any disturbances.

Stable nitrogen isotope distributions in compartments of the nitrogen cycle contain tracer information not available from conventional studies of element standing stocks. These isotope distributions, expressed as the natural abundance of nitrogen isotopes ($\delta^{15}\text{N}$), provide important clues about which sources and sinks are most important in nitrogen budgets. In this thesis I applied the ^{15}N natural abundance method to two forested ecosystems to elucidate the importance of nitrogen flow which has been overlooked.

The ^{15}N natural abundance method was used to assess an intermittent occurrence of denitrification in the Kiryu watershed in Shiga Prefecture, Japan. The concentration and $\delta^{15}\text{N}$ of nitrate (NO_3^- -N), as well as some physical and chemical variables that potentially affect denitrification, were measured along the flow path from precipitation to stream water via soil solution and groundwater. High maximum groundwater level promoted a decrease in NO_3^- -N concentration that was associated with an increase in the $\delta^{15}\text{N}$ of NO_3^- -N with soil depth; i.e., denitrification occurred in the soil due to increasing soil moisture. While the maximum level of groundwater fell, no such changes were observed. Dissolved oxygen and Mn^{2+} concentrations in soil solutions indicated that strong anaerobic condition did not occur during the study period. These results suggested that denitrification was occurring temporarily in anaerobic microsites such as waterlogged soil aggregates. Upward expansion of groundwater zone thus appeared to play an important role in promoting such microanaerobic sites, resulting in the intermittent occurrence of denitrification. Based on these data, a schematic model for assessing denitrification was proposed based on a NO_3^- -N / Cl^- - $\delta^{15}\text{N}$ of NO_3^- -N map for soil-water systems. The data showed that variations of NO_3^- -N concentration and $\delta^{15}\text{N}$ value are useful indicators for elucidating nitrogen dynamics as affected by water mixing, plant uptake, nitrification and denitrification in forested ecosystems.

Because of measurement difficulties, only a few studies on natural ^{15}N abundance ($\delta^{15}\text{N}$) of inorganic nitrogen in forest soil have been pursued despite its importance for interpretations of plant $\delta^{15}\text{N}$ signatures. To investigate stable nitrogen isotope ratios in inorganic nitrogen, the $\delta^{15}\text{N}$ values and concentrations of total N, ammonium (NH_4^+ -N) and NO_3^- -N of forest mineral soils in four profiles were measured along a slope (altitude of 765 ~ 870 m) in a coniferous (Japanese red cedar) forest in Japan. Generally, the $\delta^{15}\text{N}$ values of total N, NH_4^+ -N and NO_3^- -N increased with increasing soil depth. The values of $\delta^{15}\text{N}$ ranged from 1.0 to 6.8, 2.5 to 15.6, and -14.8 to 5.6‰ for total N, NH_4^+ -N and NO_3^- -N,

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respectively. Additionally, the $\delta^{15}\text{N}$ values were different between $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ for each soil depth. Thus it was concluded that the assumptions about inorganic nitrogen used in interpretation of plant $\delta^{15}\text{N}$ values were valid. Moreover, on upper slope sites where soil inorganic nitrogen was predominantly $\text{NH}_4^+\text{-N}$, the order of $\delta^{15}\text{N}$ was generally total N > $\text{NH}_4^+\text{-N}$ > $\text{NO}_3^-\text{-N}$ for each depth, whereas the order of $\delta^{15}\text{N}$ was $\text{NH}_4^+\text{-N}$ > total N > $\text{NO}_3^-\text{-N}$ on lower slope sites where $\text{NH}_4^+\text{-N}$ was less dominant as soil inorganic nitrogen and relatively high net nitrification rates were measured. These results suggested that nitrification played an important role in regulating $\delta^{15}\text{N}$ in forest soil nitrogen.

Recent studies on nitrogen transformation in forest soils have revealed the importance of NO_3^- for soil microorganisms in undisturbed coniferous ecosystems. In order to illustrate availability of NO_3^- , ^{15}N natural abundances ($\delta^{15}\text{N}$) of total N, NH_4^+ and NO_3^- in soils, inorganic nitrogen in precipitation, and plant leaves were studied in a slope in a coniferous (Japanese red cedar) forested ecosystem in Japan. As for the indicators of potential NO_3^- use of plants, nitrate reductase activity (NRA) of leaves and mycorrhizal associations of understory species were examined. Previous studies showed that NH_4^+ was strongly dominated as a soil inorganic nitrogen in upper site of the slope, while less dominated in lower site in this slope. The assumption to be tested is that plants in upper site use NH_4^+ whereas plants use both NH_4^+ and NO_3^- in lower site in accordance with inorganic nitrogen production by soils.

In the lower site, $\delta^{15}\text{N}$ values of plants were similar with those of NO_3^- in soils. This suggested that plants used NO_3^- in soils, which was followed by relatively high NRA. In the upper site, plant $\delta^{15}\text{N}$ values ranged between the values of total N, NH_4^+ and NO_3^- in soils, which suggested availability of NO_3^- was substantial in this site where soil NO_3^- pools were very small. NRA of plants and mycorrhizal associations also supported this suggestion. One plant species (*Pieris japonica*) which showed no NRA and was infected with Ericoid mycorrhizae, also had similar $\delta^{15}\text{N}$ value with other species. Because of the capacity of Ericoid mycorrhizae to assimilate organic nitrogen directly, the $\delta^{15}\text{N}$ values of *Pieris japonica* was considered to imply the use of both NH_4^+ and total N. The ^{15}N distribution in this soil-plant system strongly suggested that NO_3^- availability for plants was substantial even if its pools in soils were small. This implies niche differentiation among plant species in severe condition (low N availability in upper site) where plants should use multiple N sources (total N, NH_4^+ and NO_3^-) because of severe N competition among plant species and soil microorganisms.

By using ^{15}N natural abundance method, I can propose that *invisible flows* such as denitrification in coniferous forested ecosystem and nitrate uptake of plants in the site with low net nitrification rates, the decision on whose importance have been dependent only on stock measurements, *are important*. ^{15}N natural abundance method could allow us to picture the nitrogen cycle in intact systems which has a possibility to be quite different from what is based on conventional budget-oriented data.

I. INTRODUCTION

Nitrogen is a major constituent of the atmosphere (ca. 78%), and is practically inert. Compared with in the atmosphere (3.9×10^{21} g-N), relatively small amount of nitrogen is found in terrestrial biomass (3.5×10^{15} g-N) and in soil organic matter (95 to 140×10^{15} g-N; Post et al. 1985, Batjes 1996). Despite such large abundance of nitrogen in the atmosphere, nitrogen is one of the primary factors that limit the growth of plants in terrestrial, especially forest ecosystems (Vitousek and Howarth 1991) because many plants in forest ecosystems can not utilize nitrogen from the atmosphere directly. Thus many researches have focused on nitrogen dynamics in terrestrial ecosystems.

Nitrogen cycling in a forest ecosystem includes various physicochemical and biological processes. Figure 1 shows a generalized cycle pattern of nitrogen in Hubbard Brook Experimental Forest, one of the best sites providing quantitatively information on nutrient budgets (Bormann et al. 1977). Input of nitrogen to the forest ecosystem is by through nitrogen fixation and precipitation. Through the nitrogen fixation by lightning and/or free-living and symbiotic microbes, N_2 gas is incorporated into the ecosystem. Likewise, precipitation provides the inorganic forms of nitrogen, primarily ammonium (NH_4^+) and nitrate (NO_3^-) into the ecosystem. On the other hand, these inputs are balanced by denitrification and runoff. By denitrification, NO_3^- is converted to N_2 gas, emitted from the soil to the atmosphere, while NO_3^- is exported on streamwater from the forest ecosystem. This cycle of nitrogen between a forest ecosystem and surrounding environment started with nitrogen fixation and precipitation and closed by denitrification and

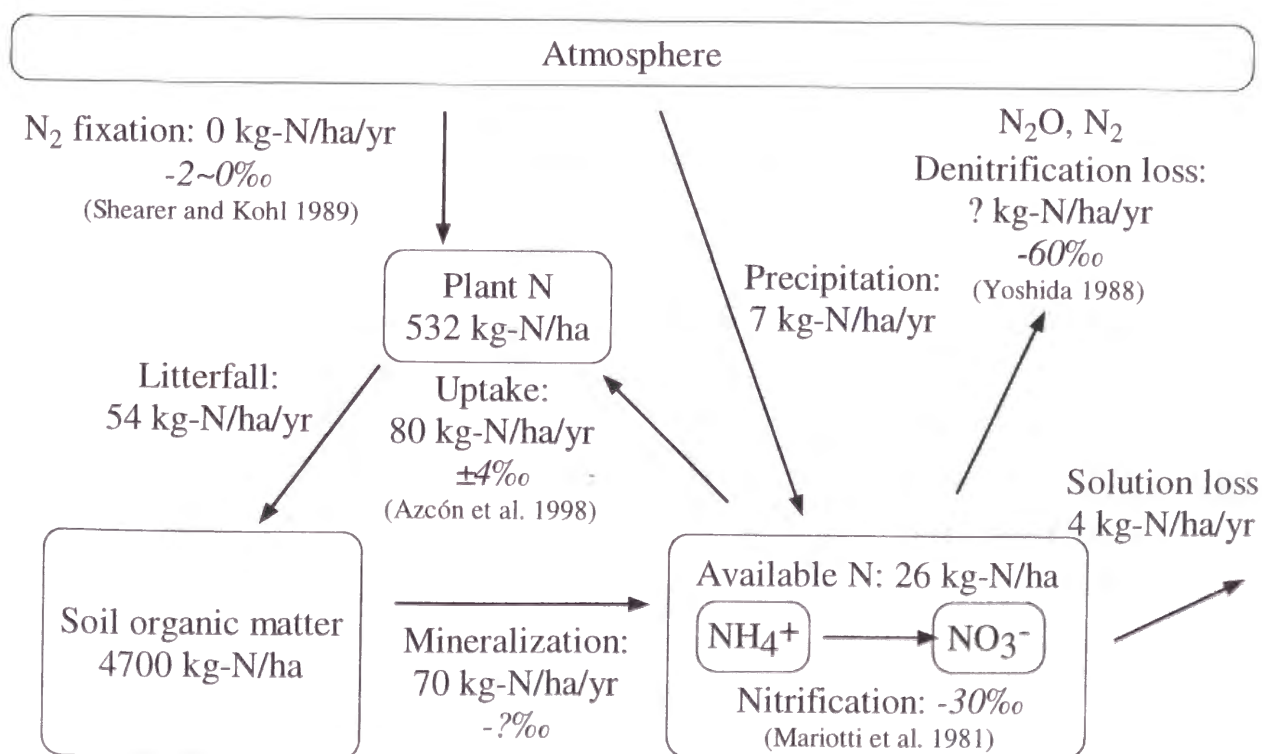


Fig. 1. Nitrogen cycle in a Hubbard Brook Experimental Forest (after Bormann et al. 1977). Figures in *Italic* indicate the apparent isotopic fractionation factors.

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runoff, can be defined as intersystem cycle, while intrasystem cycle is represented by small but fast flow between soil and plants. From organic nitrogen in plant litter, NH_4^+ is produced through heterotrophic microorganisms (mineralization). This NH_4^+ is occasionally converted to NO_3^- by nitrifiers in soils (nitrification). As available nitrogen forms for plants, low molecular organic nitrogen such as amino sugar and amino acid, NH_4^+ and NO_3^- in soils are generally accepted, the production of which are largely dependent on soil microorganism activities.

The occurrence and the extent of these processes in a nitrogen cycle are largely affected by soil properties such as soil pH, redox conditions, water contents, composition of soil biota, which include high heterogeneities. Thus the highly heterogeneous distribution of the soil properties in time and space can cause various patterns of nitrogen cycling at macro- and even microscale levels (e.g. Robertson et al. 1988). This heterogeneity of nitrogen cycling patterns is partly responsible for difficulties in assessing actual nitrogen cycling at a larger scale. Likewise, lack of information on *in situ* processes obscures our understanding of the actual nitrogen cycling in forest ecosystems. While many studies have focused on nitrogen dynamics, many of these studies have been based on laboratory experiments, and field studies were often budget-oriented. Therefore in order to have an in-depth understanding of nitrogen dynamics, it is important to develop an *in situ* monitoring method for assessing the dynamics in heterogeneous systems.

Furthermore, recent impact of human activities on nitrogen cycling in terrestrial ecosystems becomes dramatically large. For instance, an increase in nitrogen deposition through precipitation into a forest ecosystem leads oversupply of nitrogen to the demand of plants and soil microorganisms, followed by significant increase in net nitrification rates in soils, in loss of NO_3^- in streamwaters (Aber 1992). Thus it is a new task for biogeochemists to estimate and predict the effects of environmental change on natural ecosystems. However, despite for the importance of these, it might be difficult to detect the change. From now, many researches have dealt with nitrogen stocks in forest ecosystems because it has been relatively easy to measure the nitrogen stocks as compartments (e.g. soils and plants) of nitrogen cycles. Nitrogen stocks in some compartment are large (which makes the measurement easy), while the nitrogen flows from those are quite small as compared with those. The flow-stock ratio of a compartment in a forest ecosystem is generally quite small. However, it is nitrogen flows that are affected directly by the environmental change such as increase in nitrogen deposition. Thus it is not nitrogen stocks but nitrogen flow that we have to trace their fluctuations caused by environmental change. However, it has been very difficult to trace the nitrogen flows in actual forest ecosystems because of the difficulties for the measurements. Except the measurements of gas emissions from soils and of tracers that is added to natural ecosystems, we do not have any reliable method to trace the nitrogen flows in actual forest ecosystems. What is worse, these two methods occasionally include disturbance of natural ecosystems.

As a first instance to illustrate such a situation, we can take *denitrification process in forest ecosystem*. Although denitrification is one of the gaseous outputs of nitrogen from ecosystems, considerable effort has been made to measure rates of denitrification in forest ecosystems. Denitrification activities,

however, are highly variable in time (Christensen et al. 1990) and space (Parkin 1987, Robertson et al. 1988). In addition, in the field, concentrations of the end-product of denitrification (N_2) are usually insufficient to be measured against its high ambient levels (Ryden and Rolston 1983). As a result, the importance of denitrification in forest ecosystems had often been overlooked, and denitrification had been thought to occur only in flooded, anaerobic soils (Schlesinger 1991). More recently, however, use of microsensors has revealed heterogeneous occurrence of denitrification in aerobic soil samples (Parkin 1987, Højberg et al. 1994). These studies strongly suggest that anaerobic microsites are more common under aerobic conditions than previously expected. Thus, denitrification is now considered to be widespread in terrestrial ecosystems (Schlesinger 1991). Reevaluation of role of denitrification is highly needed for the better understanding of the nitrogen dynamics in forest ecosystems.

To counteract the difficulties associated with studying denitrification, two types of approaches can be used. One is the direct evidence method. This approach provides clear interpretations of heterogeneities in soil conditions at the microscale level. For example, using microscopic instrumentation, Parkin (1987) directly observed the small 'hot-spots' of denitrification activity in soil. However, extension of such measures to large scales such as entire watersheds is quite difficult.

To overcome this problem, another method, the circumstantial evidence method can be used. For example, Ohte et al. (1997) used a *pseudo-in situ* experiment to show that nitrogen dynamics were affected by soil moisture in shallow soil layers. They used a lysimeter approach in which conditions were maintained as close to actual conditions as possible with a relatively large lysimeter system, and observed nitrogen dynamics in soils with altered water conditions. Their results showed that dry conditions in soil restrain nitrification, while water-saturated conditions promote denitrification. This distinct difference in nitrogen dynamics in soil which is dependent on soil moisture condition, implies the possibility of soil moisture condition as an important regulator for nitrogen dynamics, and especially, as an indicator for establishment of anaerobic conditions in soil in a much larger scale. This kind of approach is advantageous in, at least, clearing the problem of soil heterogeneity by dealing with larger soil systems, but in elucidating *in situ* processes, it is still wanting.

As the second example, I can take *the flow of available nitrogen in soils to plants and soil microorganisms* here. It has been recently important to determine which forms of nitrogen are more available for plants and soil microorganisms. This object has been derived from not only ecophysiological interests in niche differentiation in terms of nitrogen into several resources (e.g. Schulze et al. 1994), but also from necessities to estimate the uptake capacity of excess nitrogen by biota (Rothstein et al. 1996). As the available forms of nitrogen for plants, mineral nitrogen (NH_4^+ and NO_3^-) in soils has been widely recognized. Furthermore, nitrification is often restricted and NO_3^- concentration is usually low compared with NH_4^+ in undisturbed coniferous forest soils (Vitousek et al. 1982, Gosz and White 1986). Thus it has been assumed that NH_4^+ is the exclusive available nitrogen for plants in coniferous forest soils (Vitousek et al. 1982). Consequently the importance of NO_3^- in undisturbed coniferous forest ecosystems has not

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been well recognized.

However, two recent ideas in the study on nitrogen cycles require the reexaminations with respect to available N in forest ecosystems. One is about an importance of NO_3^- for soil microorganisms (Davidson et al. 1992, Hart et al. 1994, Stark and Hart 1997). By using ^{15}N -dilution method, their studies clearly indicated that low concentrations of NO_3^- and low net nitrification rates in coniferous forest soils are because of rapid immobilization of NO_3^- by soil microorganisms, not because of low NO_3^- production. The other is about an ability of plants to use organic nitrogen directly (Chapin et al. 1993, Kielland 1994, Näsholm et al. 1998) and through mycorrhizal associations (Stribey and Read 1980, Read and Bajwa 1985, Read et al. 1989). For example, Kielland (1994) showed that the plant in strongly nitrogen-limited ecosystem such as arctic ecosystems, has ability to assimilate organic nitrogen directly. Besides Read et al. (1989) indicated that plant can obtain the ability to use organic nitrogen in soil by an infection of Ericoid or Ectomycorrhiza even under not so severe condition. Thus, these studies indicate the necessity to reevaluate the contributions of each forms of nitrogen to plant demands. Moreover, to elucidate the importance of sources and sinks of nitrogen in actual soil-plant systems, it is necessary to establish methods to observe nitrogen cycles in intact complex ecosystems (Eviner and Chapin 1997).

In the light of these studies, I have thought that we have to admit we are not aware of the importance of nitrogen flow. Nitrogen cycle in a forest ecosystem is consisted of nitrogen stocks and flows, not of only stocks. Because nitrogen cycles are more dynamic than ever thought on the account of highly heterogeneous system and high biological activities, now we are facing the problem of the lack of reliable methods to trace nitrogen flows without any disturbances which can easily alter the nitrogen flows.

The use of natural abundance isotopes has been increased rapidly in many study fields (Wada et al. 1998). Natural abundance of isotopes of a target element is expressed as a difference from international standard,

$$\delta X = (R \text{ sample} / R \text{ standard} - 1) \times 1000 (\text{‰})$$

where X is often hydrogen, carbon, nitrogen, oxygen and sulfur in ecological studies, and R is heavy isotope / light isotope such as $^{15}\text{N}/^{14}\text{N}$ in the case of nitrogen. The primary international standards for isotopic measurements are Pee Dee Belemnite (PDB) for carbon isotope, atmospheric air for nitrogen isotope, Vienna standard mean ocean water (V-SMOW) for hydrogen and oxygen isotopes, and Canyon Diablo meteorite (CDT) for sulfur.

In ecological aspects, carbon natural abundance ratio ($\delta^{13}\text{C}$) has been used intensively for estimation of intrinsic water use efficiency of plants (Farquhar et al. 1982, Ehleringer and Osmond 1989). Hydrogen isotope ratio (δD) and oxygen isotope ratio ($\delta^{18}\text{O}$) of water in plant tissues are useful indicator for identification of water sources of plants in terrestrial ecosystems (White et al. 1985, Dawson and

Ehleringer 1991, Schulze et al. 1996). Sulfur isotope ratio ($\delta^{34}\text{S}$) are often used with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for food web analysis (Peterson and Fry 1987).

Natural abundance of nitrogen isotopes (^{14}N and ^{15}N) has been used as a useful tool for the tracing nitrogen from sources into sinks in many aspects. Nitrogen isotope ratio has been used for many years in the study field of food-web analysis (Minagawa and Wada 1984, Yoshioka et al. 1994, Tayasu et al. 1996, 1998), N_2 -fixing (Shearer and Kohl, 1986, Tayasu et al. 1994), trace gases (Yoshida and Matsuo 1983, Yoshida 1988). Although the use of $\delta^{15}\text{N}$ is a powerful tool for obtaining insights through $\delta^{15}\text{N}$ pattern analysis, and for deriving new questions to be tested, unfortunately it is not a reliable tracer of nitrogen fluxes in soils or in plants growing in soils (Handley and Scrimgeour 1997). This is partly because we have a little knowledge about the isotope fractionation and discrimination during nitrogen transformation processes occurred in soils and plants, and between soils and plants.

Chemical and physical processes have significant isotopic fractionation, which leads an enrichment or depletion of the heavy isotope. Isotopic fractionation during unidirectional kinetic reactions (not in equilibrium reactions) is expressed as,

$$\alpha = k/k^*$$

where k and k^* are reaction rates of the light and heavy isotopes, respectively. Because lighter isotope generally has higher rate of reaction than heavier one, this factor is more than 1.000, in most cases from 1.000 to 1.060 for nitrogen transformation process (Handley and Raven, 1994). In some cases, the difference between a source and a sink in terms of isotope ratio is appropriate for illustrating a reaction process, as isotopic discrimination (Δ or ϵ),

$$\Delta s/p = \frac{\delta s - \delta p}{1 - \frac{\delta p}{1000}}$$

where subscript S and P are substrate and product, respectively. Generally, $1 + \delta_p/1000$ is approximated by 1,

$$\Delta s/p = \delta s - \delta p$$

It can be another term for discrimination as,

$$\epsilon = (\alpha - 1) \times 1000$$

Using a modification of the classical Rayleigh equation, it is possible to calculate ϵ for a reaction with a finite supply of substrate if the fraction of unreacted substrate, f , and δ_s , and $\delta_{s,0}$, i.e. the δ of the substrate remaining and at time zero, respectively, are known (Mariotti et al. 1981),

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Table 1. Isotopic fractionation factors during biological processes in terrestrial ecosystems.

Biological process in soil	Fractionation factor (ε)	Method	Reference
mineralization	?		
decomposition*	1.0015 ~ 1.0048	soil incubation	Nadelhoffer and Fry (1988)
	1.002	<i>in situ</i>	Evans and Ehleringer (1993)
nitrification	1.035	culture incubation	Mariotti et al. (1981)
denitrification	1.011 ~ 1.017	soil incubation	Blackmer and Bremner (1977)
	1.024 (20°C), 1.029 (30°C)	soil incubation	Mariotti et al. (1981)
	1.011 ~ 1.033	soil incubation	Mariotti et al. (1982)
	1.005	<i>in situ</i>	Mariotti et al. (1988)
	1.004 ~ 1.005	<i>in situ</i>	Fustec et al. (1991)
assimilation	?		

*: calculated from isotopic composition of residual organic matter.

$$\epsilon = \frac{1000 \times \log_e \frac{\frac{1}{1000} \times \delta_s + 1}{\frac{1}{1000} \times \delta_{s,0} + 1}}{\log_e f}$$

Isotopic fractionation factors during nitrogen transformation processes in terrestrial ecosystems are summarized in Table 1. During mineralization process (production of NH_4^+ from organic nitrogen), the extent of nitrogen fractionation might be small because NH_4^+ will be produced from amino group bonding at the edge of carbon chains with no breakage of strong bonds. On the other hand, during nitrification (production of NO_3^- from NH_4^+) and denitrification (production of N_2 gas from NO_3^- or nitrite, NO_2^-), nitrogen isotopes discriminate strongly. This seems because the breakage of N-H bond or N-O bond requires much energy. Unfortunately, there are no reports on isotope fractionation factor in microbial immobilization (assimilation) in soils.

The study using natural abundance of stable isotope ratio, should be started from;

1. determination of isotope fractionation factors of target processes,
2. illustrate the nitrogen dynamics from the isotopic signatures interpreted by the isotopic fractionation factors.

The general purpose of my study is to illustrate the nitrogen dynamics in forest ecosystems by using nitrogen stable isotope ratio. First, it is important to establish the body of knowledge on isotopic signature in forest ecosystems, especially on inorganic nitrogen on account of the lack of information. I studied the effect of denitrification on the enrichment of ^{15}N in NO_3^- in soil solution in Kiryu watershed where groundwater level can be fluctuated according to the precipitation. Further, I studied precisely the isotopic variation of inorganic nitrogen in soil profiles in Ryuoh watershed where soil characterization on nitrogen transformation is quite different between upper and lower part of a mountain slope. Finally I tried to illustrate the flow of soil nitrogen into plants in Ryuoh watershed on the basis of nitrogen isotopic signatures of potential nitrogen sources for plants.

II. INTERMITTENT DENITRIFICATION: THE APPLICATION OF ^{15}N NATURAL ABUNDANCE METHOD TO A FORESTED ECOSYSTEM

INTRODUCTION

Associated with the denitrification process, the large nitrogen isotope fractionation has been well recognized in laboratories (Delwiche and Steyn 1970, Blackmer and Bremner 1977, Chien et al. 1977, Mariotti et al. 1981, 1982a) and *in situ* (Heaton et al. 1983, Mariotti et al. 1988, Fustec et al. 1990). The ^{15}N natural abundance method thus provides an available method to identify the *in situ* occurrence of denitrification by the variation of nitrogen isotope composition of the residual NO_3^- -N without any perturbation in the system (Mariotti et al. 1988, Mariotti 1994). Because variation of $\delta^{15}\text{N}$ in NO_3^- -N reflects the cumulative results of the denitrification process, the ^{15}N natural abundance method provides an *in situ* integrated information on denitrification even in heterogeneous systems. In this paper we will be presenting the ^{15}N natural abundance approach in elucidating the dynamics of denitrification in a temperate forested ecosystem in the Kiryu watershed with emphasis on precipitation and its associated soil conditions.

MATERIALS AND METHODS

The study site is located in the Kiryu experimental watershed in Shiga Prefecture in central Japan (Ohte et al. 1995; Fig. 2). The dominant vegetation is 30-year-old *Chamaecyparis obtusa*.

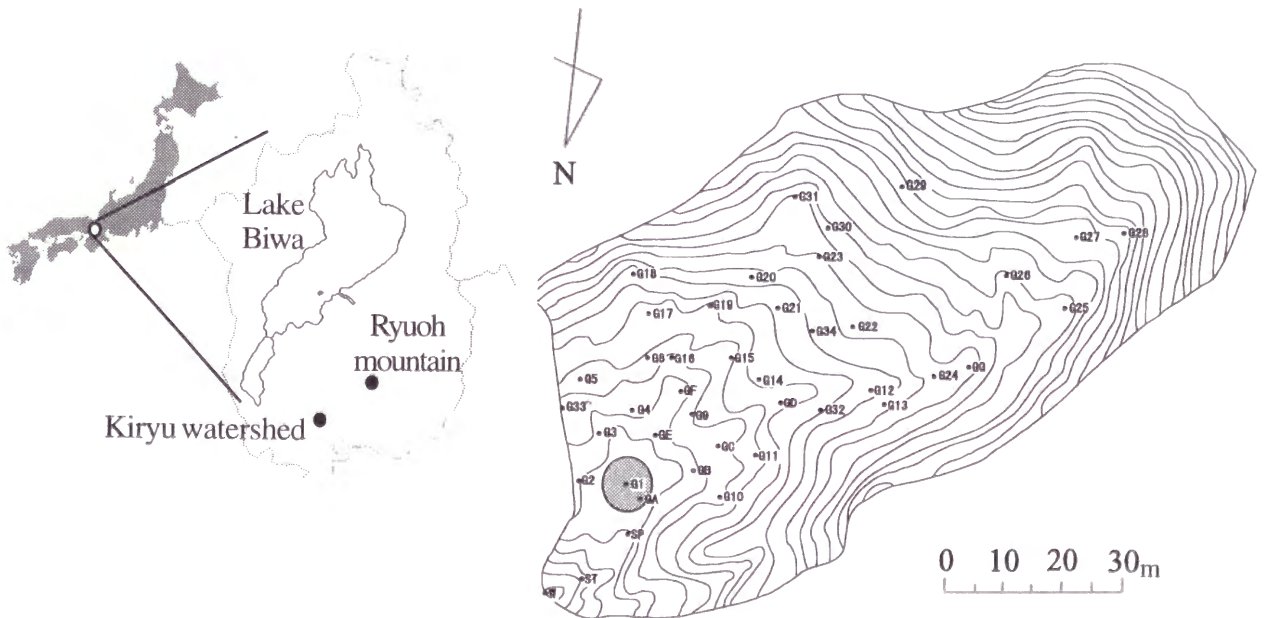


Fig. 2. Map of Kiryu watershed in central Japan. Numbers shown as G** indicate the position of wells in this small catchment. This study was conducted in the lower part of this area illustrate as a shadowed circle.

II. Intermittent denitrification

Chamaecyparis obtusa has shallow root systems, and approximately 80% of the roots are distributed above 30 cm-depth in soil. Soil profiles do not have any clear structures and therefore soil is considered to be immature. Groundwater always existed at the lower part of the slope in this watershed, and under normal precipitation groundwater level was between 0 ~ 100 cm depth. A 2-m × 2-m plot was chosen in the lower part of the slope for soil solution sampling.

Precipitation, throughfall, soil solution, groundwater, spring water and stream water were sampled. Samples were taken during the period of plant growth, 28 May - 14 September in 1993. For nitrogen isotope analysis, considerable amounts of nitrogen (> 0.5 mg) were required. To obtain this quantity of nitrogen, sample sizes of 4 liters of precipitation, throughfall, groundwater, spring water, and stream water, and about 2 liters of the soil solution were required. The ease of collecting water samples depended on soil water content. To overcome this difficulty, the sampling period was divided into 5 periods of unequal length; 28 May to 21 June (period 1), 21 June to 25 June (period 2), 25 June to 9 July (period 3), 9 July to 5 August (period 4), and 5 August to 14 September (period 5), and we could not collect replicate samples.

Bulk precipitation was collected over each sampling period and sampled in an open place near the plot, and throughfall was also under the *Chamaecyparis obtusa* canopy. The amount of precipitation was provided by Japan Weather Association. The soil solutions were collected using tension lysimeters which were set in the plot at 4 depths: 30, 50, 70 and 100 cm (Tokuchi et al. 1993). Soil solution at 100 cm was considered groundwater because the groundwater table fluctuated above this depth. The tension lysimeters were connected to an automatically controlled vacuum system (Ohte et al. 1997). The sucking pressure was set to be weak (0.5 kPa) to collect the solutions continuously over each sampling period. Spring water was sampled at the outlet point and stream water was sampled at the V-notch at the time when other solutions were sampled.

Maximum groundwater level during each period, as an index of expansion and contraction of groundwater zone, was measured in an observation well in the plot basically on solution-sampling day (Ohte et al. 1995), although groundwater level might fluctuated day by day. Dissolved oxygen (DO) concentrations in soil solution, groundwater and spring water were also measured in the field using a DO meter (Horiba, DO-3) on the solution-sampling day. For measurement of DO concentration in soil solution, a water-filled chamber was set at the target depth. We measured O₂ concentrations in the water once it had equilibrated with its environment.

Water samples were analyzed for pH, concentrations of Cl⁻, NO₃⁻-N, NH₄⁺-N, Mn²⁺, dissolved organic carbon (DOC), and δ¹⁵N of NO₃⁻-N. pH was measured with a glass electrode (Horiba D-13). Cl⁻, NO₃⁻-N and NH₄⁺-N concentrations were determined by high-pressure ion chromatography (Shimadzu HIC-6A) and dissolved Mn²⁺ concentration by ICP (Seiko SPS1500VR). Mn can have various ionic forms (+2, +3, +4), but since dissolved Mn in solution is generally Mn²⁺, the Mn concentration analyzed will be referred to Mn²⁺. DOC concentration was determined after the addition of HCl to the sample in order to purge dissolved CO₂ gas (Shimadzu TOC-5000).

The method employed for the conversion of NO_3^- -N to N_2 for isotopic analysis was based on Hauck (1982). Samples were concentrated to about 50 ml with NaOH and NO_3^- -N was reduced to NH_4^+ -N by semi-micro Kjeldahl distillation with Devarda alloy. The distillate was concentrated gently on a hot plate and NH_4^+ -N was converted to N_2 *in vacuo* in a Y-shaped tube with sodium hypobromite through Cu and CuO furnaces for eliminating contaminated gases. Isotope analysis was performed using a Finnigan Mat Delta S mass spectrometer. Isotopic composition is expressed in permil deviation from the atmospheric standard as:

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

The precision of $\delta^{15}\text{N}$ measurements was less than 0.26‰ in this study.

In August of 1996, we collected soil cores (n=4) from the depth of 30 cm in the plot and measured denitrification potentials using the acetylene blockage technique (Payne 1991). Soil cores were sampled in the morning and potentials were measured in the afternoon on the day of sampling. To reflect a situation of a high groundwater table, soil samples were placed in PVC chambers, which were sealed with silicon caps and fitted with butyl septa. Those were then submerged in water containing NO_3^- (1 mg-N/L; the approximate average concentration of the soil solution at the depth of 30 cm). Acetylene was injected into the chambers so as to constitute 10% of the gas volume in each chamber. All samples were incubated at 30°C for 24 hr. The N_2O gas produced was measured by gas chromatography equipped with an electron capture detector (Crill et al. 1995).

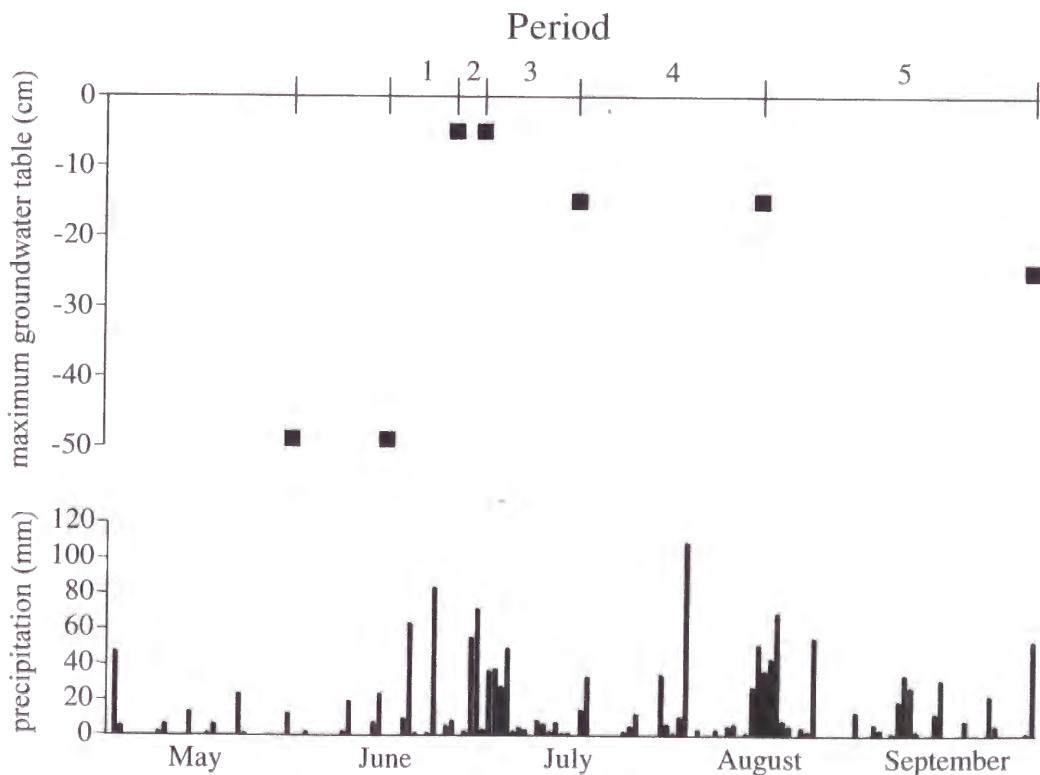


Fig. 3. Fluctuations of precipitation (bars) and level of maximum groundwater level from the soil surface (squares) during study periods.

II. Intermittent denitrification

Table 2. Chemical and isotopic analysis of solutions in Kiryu watershed.

		NH ₄ ⁺ -N	NO ₃ ⁻ -N	δ ¹⁵ N	pH	DOC	Mn ²⁺	NO ₃ ⁻ -N/Cl ⁻
	units	(mg-N/L)	(mg-N/L)	(‰)		(mg-C/L)	(mg/L)	
period 1	precipitation	0.47	0.19	5.6	5.8	2.00	0.02	0.12
	throughfall	0.20	0.59	0.2	5.1	3.59	0.10	0.47
	30cm	0.00	1.53	-5.8	6.4	4.98	0.04	0.53
	50cm	0.00	0.53	-2.3	6.6	2.99	0.03	0.25
	70cm	0.00	0.64	1.5	6.8	1.82	0.02	0.15
	groundwater	0.00	0.73	*	6.5	8.43	0.03	0.13
	spring water	0.00	0.61	0.2	6.1	2.18	0.02	0.14
	stream water	0.00	0.56	-0.2	6.7	1.96	0.02	0.12
period 2	precipitation	0.00	0.18	1.5	5.9	1.65	0.02	0.37
	throughfall	0.00	0.28	-0.3	6.0	2.72	0.03	0.37
	30cm	0.00	1.08	-2.1	6.2	4.56	0.02	0.45
	50cm	0.00	0.90	-1.4	6.3	3.34	0.02	0.25
	70cm	0.00	0.60	5.0	6.1	1.74	0.02	0.14
	groundwater	0.00	0.68	1.7	6.6	1.90	0.02	0.15
	spring water	0.00	0.52	-0.4	6.5	2.52	0.02	0.12
	stream water	0.00	0.58	-0.8	6.4	2.06	0.02	0.14
period 3	precipitation	0.30	0.46	-1.9	4.5	2.13	0.02	0.36
	throughfall	0.51	0.58	-1.7	4.6	3.58	0.08	0.61
	30cm	0.00	1.09	-1.9	6.6	2.80	0.02	0.30
	50cm	0.00	1.02	-1.9	6.8	2.44	0.02	0.27
	70cm	0.00	0.99	-1.9	6.8	2.73	0.02	0.25
	groundwater	0.00	1.01	-1.8	6.8	2.61	0.02	0.27
	spring water	0.00	0.50	-1.9	5.9	2.52	0.02	0.06
	stream water	0.00	0.82	-2.4	6.5	2.23	0.02	0.21
period 4	precipitation	0.16	0.18	-1.5	5.7	1.92	0.02	0.17
	throughfall	0.20	0.27	*	6.2	2.77	0.03	0.32
	30cm	*	*	*	*	*	*	*
	50cm	0.05	0.73	-2.5	6.9	4.03	0.02	0.18
	70cm	0.28	0.72	-2.6	7.3	5.03	0.02	0.18
	groundwater	0.40	0.69	*	6.1	9.07	0.03	0.13
	spring water	0.02	0.73	-2.5	6.2	2.66	0.02	0.18
	stream water	0.00	0.65	*	6.7	2.84	0.02	0.16
period 5	precipitation	0.33	0.13	-2.5	5.4	2.25	0.02	0.13
	throughfall	0.50	0.25	-2.6	6.0	2.81	0.05	0.26
	30cm	0.61	0.82	-1.8	6.5	7.39	0.03	0.23
	50cm	0.33	0.79	0.2	6.8	6.50	0.02	0.19
	70cm	0.34	0.77	0.2	6.9	4.78	0.03	0.19
	groundwater	0.05	0.81	*	6.1	3.15	0.02	0.19
	spring water	0.06	0.74	0.2	6.1	2.19	0.02	0.17
	stream water	0.07	0.61	0.2	6.8	2.41	0.02	0.15

* : not determined

RESULTS

Environmental conditions and denitrification potential

Maximum groundwater level fluctuated throughout the study period (Figure 3). In the beginning of period 1, the level was below about 50-cm depth. At the end of period 1, it was high precipitation. In response, the maximum groundwater level was at 5-cm depth from the soil surface, then precipitation exceeded 400 mm during period 2 and 3. After period 2 groundwater zone gradually contracted, eventually maximum groundwater level reached 25-cm depth in period 5.

DO concentration decreased with depth in the soil (Table 2). Concentrations at 70-cm soil depth were particularly low and similar to concentrations in the groundwater. After seepage, DO concentrations in spring water increased.

Dissolved Mn^{2+} concentrations are good index of redox potential. There was no significant

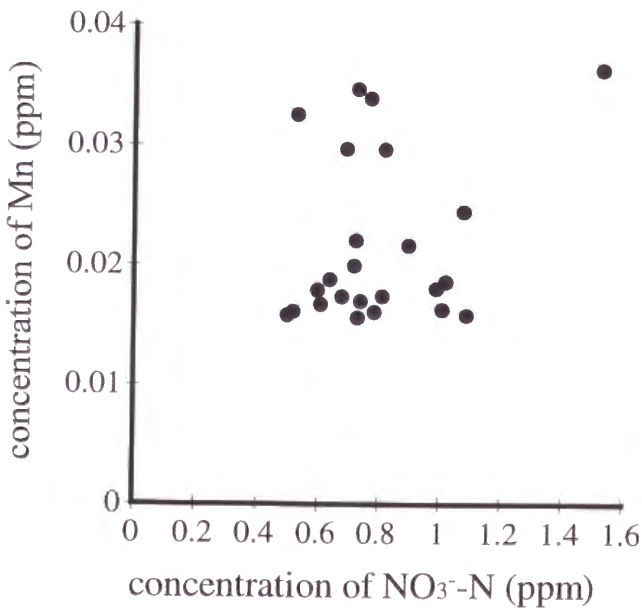


Fig. 4. Relationship between Mn^{2+} and NO_3^- -N concentration during the study periods.

relationship between Mn^{2+} and NO_3^- -N concentrations in our data (Table 2 and Fig. 4).

Considerable concentrations of DOC were detected in solution at all soil depths throughout the study period (Table 2). Except for periods 1 and 4, DOC concentration decreased with soil depth. The pH increased with soil depth (Table 2). In spring water pH was lower than soil solutions, and increased again in the stream water.

Soils had relatively high denitrification potentials in spite of being immature. The denitrification potential of soil cores from the depth of 30 cm was 0.53 ± 0.45 kg-N/ha/day (mean \pm s.d. n=4).

Vertical profiles of NO_3^- -N, NH_4^+ -N concentrations and $\delta^{15}N$ of NO_3^- -N

NO_3^- -N concentration generally was higher at 30-cm depth in soil relative to concentrations in the throughfall (Table 2). Vertical changes of NO_3^- -N concentration in soil could be classified into two patterns. One was a decrease in NO_3^- -N concentration from 30 cm to 50 or 70 cm depths (observed in periods 1 and 2), and the other was no concentration depth-related change in NO_3^- -N (periods 3 ~ 5). In periods 1 ~ 3, NH_4^+ -N concentration was below the detection limit in most samples, but increased somewhat during periods 4 and 5.

$\delta^{15}N$ of NO_3^- -N ranged from -5.8 to 5.6‰ (Table 2). In periods 1 and 2, differences in NO_3^- -N $\delta^{15}N$ among various samples were relatively large (approximately 7‰), but the range of values was smaller during periods 3 ~ 5 (0.1 ~ 2‰).

DISCUSSION

To more accurately study nitrogen dynamics in our system, we focussed on the $\text{NO}_3^- \text{-N} / \text{Cl}^-$ ratio rather than $\text{NO}_3^- \text{-N}$ concentration because Cl is biologically and chemically inert and its concentration can change only by dilution (Vitousek 1977, Tokuchi 1993). Consequently, the $\text{NO}_3^- \text{-N} / \text{Cl}^-$ ratio provides information on net biological change in $\text{NO}_3^- \text{-N}$ without any influences of abiotic reactions.

Dynamics of $\text{NO}_3^- \text{-N} / \text{Cl}^-$ ratio and $\delta^{15}\text{N}$ associated with nitrification, denitrification and water mixing

In periods 1 and 2, $\text{NO}_3^- \text{-N} / \text{Cl}^-$ was high and $\delta^{15}\text{N}$ of $\text{NO}_3^- \text{-N}$ was low at depth of 30 cm relative to the throughfall (Fig. 5). In these periods, there was no $\text{NH}_4^+ \text{-N}$ concentrations in soil solutions (Table 2), thus we suggested that nitrification consumes $\text{NH}_4^+ \text{-N}$ in soil solutions produced by mineralization in soil. Because NH_4^+ ion is well absorptive to soil particles and humus, $\text{NH}_4^+ \text{-N}$ in soil solutions was a portion of the entire available $\text{NH}_4^+ \text{-N}$ in soil. Additionally, ^{15}N -depletion of $\text{NH}_4^+ \text{-N}$ in soil solution by preferential sorption of $^{15}\text{NH}_4^+$ on soils have been reported (Delwiche and Steyn, 1970, Karamanos and Rennie 1978, Koba et al. unpublished data) and isotope fractionation occurs during nitrification in soil even *in situ* (Feigin et al. 1974, Shearer et al. 1974, Karamanos and Rennie 1980, Koba et al. 1998). Thus we suggested that $\text{NO}_3^- \text{-N}$ with low $\delta^{15}\text{N}$ was produced because relatively ^{15}N -depleted $\text{NH}_4^+ \text{-N}$ in soil solutions was consumed by nitrification in soil.

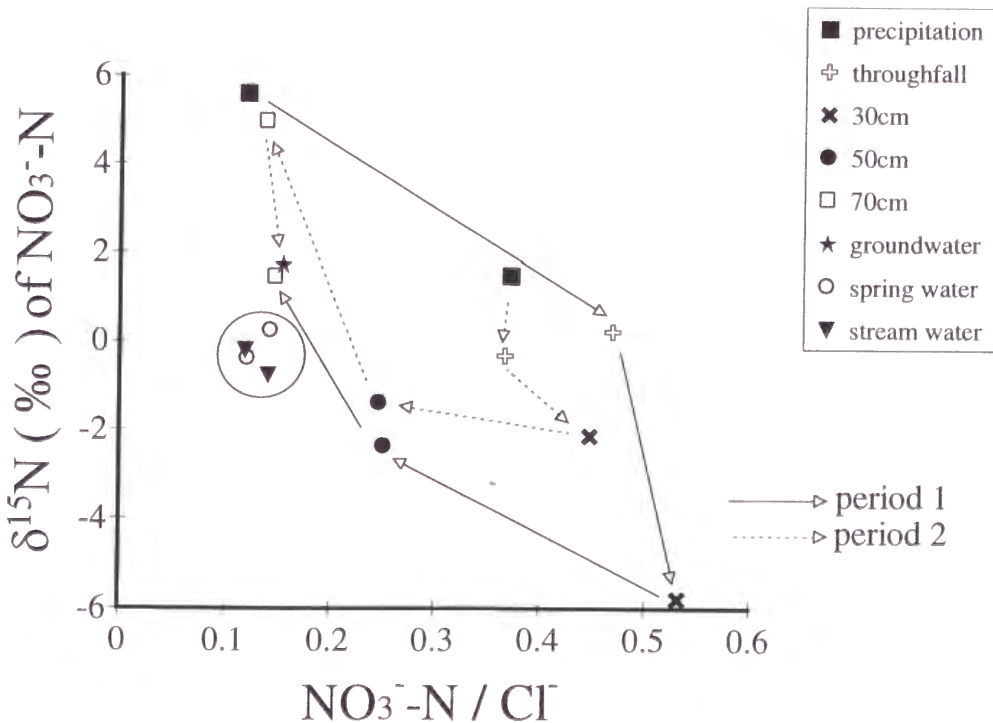


Fig. 5. The dynamics of $\text{NO}_3^- \text{-N} / \text{Cl}^-$ and $\delta^{15}\text{N}$ of $\text{NO}_3^- \text{-N}$. The arrows shows the order of water flow. The data of groundwater in period 1 were not determined.

With the downward movement of soil solution, $\text{NO}_3^- \text{-N} / \text{Cl}^-$ decreased and the $\delta^{15}\text{N}$ values increased. During the denitrification process, the change in $\delta^{15}\text{N}$ values of residual $\text{NO}_3^- \text{-N}$ can be described by the following equation (Mariotti et al. 1981, 1988):

$$\delta^{15}\text{N of } \text{NO}_3^- \text{-N} = C + \epsilon \times \ln [\text{NO}_3^- \text{-N}]$$

where C is a constant and ϵ is the isotopic enrichment factor (‰). The value of the isotopic enrichment factor was -5.6‰ in period 1 and -6.0‰ in period 2 (Fig. 6), similar values to -4.7 ~ -5.0‰ reported in some of the in situ studies by Mariotti et al. (1988), and -4.2 ~ -5.2‰ observed by Fustec et al. (1990). On the other hand, large enrichments in ^{15}N of $\text{NO}_3^- \text{-N}$ have been reported even in *in situ* studies (Vogel et al. 1981, Heaton et al. 1983). This variability in isotope enrichment during denitrification may be explained by a dispersion effect as studied by Kawanishi et al. (1993). In August of 1996, we measured denitrification potentials of soil at the depth of 30 cm. We therefore concluded that denitrification had occurred in these periods.

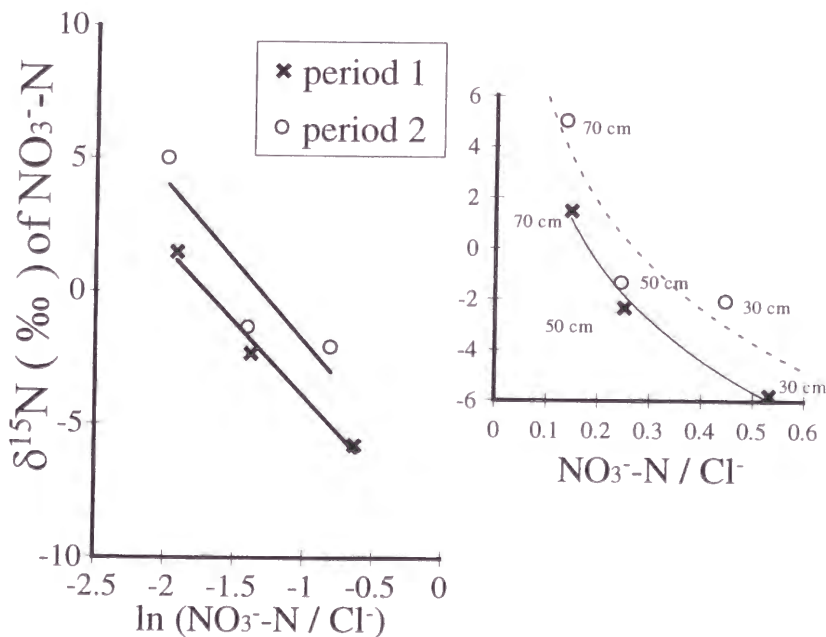


Fig. 6. Relationships between isotopic composition of $\text{NO}_3^- \text{-N}$ and the logarithm of $\text{NO}_3^- \text{-N} / \text{Cl}^-$. The inset shows the non-logarithmic representation of these relationships. Linear relationships existed (period 1: $\delta^{15}\text{N} = -5.57 \times \ln [\text{NO}_3^- \text{-N} / \text{Cl}^-] - 9.52$; $r^2 = 0.98$; period 2: $\delta^{15}\text{N} = -6.00 \times \ln [\text{NO}_3^- \text{-N} / \text{Cl}^-] - 7.88$; $r^2 = 0.82$).

II. Intermittent denitrification

In period 3 and 4, a negative relationship between $\delta^{15}\text{N}$ of NO_3^- -N and NO_3^- -N / Cl^- was not found (Fig. 7). NO_3^- -N / Cl^- values of soil solutions were relatively constant and low compared to throughfall, and differences in $\delta^{15}\text{N}$ among them were small. We concluded that no denitrification occurred during these periods. We suggested that the decrease in NO_3^- -N / Cl^- took place due to plant uptake, as isotope fractionation during plant uptake is weak (Mariotti et al. 1982b) and thus only NO_3^- -N / Cl^- would change.

During period 5, slight differences in $\delta^{15}\text{N}$ of NO_3^- -N were observed among the soil solutions (Fig. 7). Denitrification may also have occurred in this period, but relative to periods 1 and 2, variations of both NO_3^- -N / Cl^- and $\delta^{15}\text{N}$ in this period were not large enough to provide a clear-cut evidence of denitrification (Fig. 7).

Groundwater had similar or somewhat lower $\delta^{15}\text{N}$ values of NO_3^- -N than that of soil solution. Throughout the study period, $\delta^{15}\text{N}$ values and NO_3^- -N / Cl^- were within the range of soil solution (Fig. 5 and 7). This suggested that groundwater at this site was formed by mixing with both denitrified and non-denitrified soil solutions. Stream water, as well as spring water, had similar characteristics to groundwater in the study period.

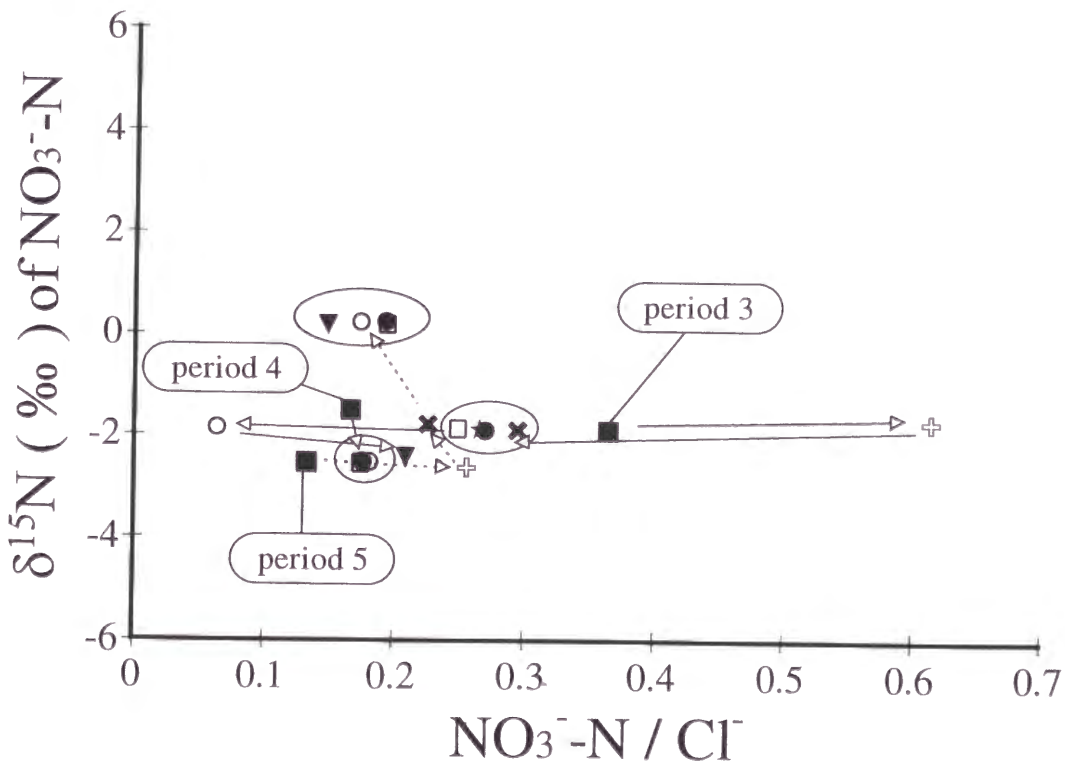


Fig. 7. The dynamics of NO_3^- -N / Cl^- and $\delta^{15}\text{N}$ of NO_3^- -N. Legends are same as those used in Fig. 5. The data following were not available; throughfall, 30-cm depth, groundwater, streamwater in period 4, groundwater in period 5. No distinct relationship as observed in period 1 and 2 existed, suggesting that no occurrence of denitrification during these periods.

Denitrification in unsaturated soil

Four major factors were considered to regulate the occurrence of denitrification in this study: pH, available DOC, NO_3^- -N, and anaerobic condition. At our site, pH was high enough for denitrification activity (Table 2) because denitrification could occur at $\text{pH} > 5$ (Bremner and Shaw 1958). DOC and NO_3^- -N concentrations were also considered to be sufficient for the occurrence of denitrification (Table 2). Conditions sufficiently reducing for denitrification are considered to occur between the disappearance of DO by aerobic respiration ($\text{DO} < 0.12 \text{ mg L}^{-1}$) and the appearance of Mn^{2+} ion in soil solutions (Greenwood 1960, Mariotti et al. 1988). In our study, the fact that decreases in NO_3^- -N concentration were not associated with increases in Mn^{2+} concentration, indicated that anaerobic conditions were not sufficient to promote Mn^{2+} liberation (Fig. 4). Similarly bulk DO concentrations were not low enough for denitrification (Table 2). Denitrification, however, could occur in anaerobic microsites such as in soil aggregates (Parkin 1987, Højberg et al. 1994) since oxygen diffusion into soil aggregates was considered to be slow (Tiedje et al. 1984, Sextone et al. 1985). In this study, redox conditions would be anoxic in the microsites, thus there was a concentration gradient of oxygen between macro- and microsite, due to faster consuming of oxygen in microsites and slow diffusion from macrosites into microsites. Therefore DO concentrations measured here were considered to overestimate the degree of anaerobic conditions at the microsites.

Ohte et al. (1997) have conducted a precise lysimeter experiment with emphasis on soil moisture condition in this study area. Their study showed relationships between soil moisture condition and inorganic nitrogen form in soil solution. Their results indicated that NH_4^+ -N and NO_3^- -N appeared soil in solutions from relatively dry soil, and NO_3^- -N only from relatively wet soil. It showed that soil moisture condition related well to inorganic nitrogen form in soil solution. From their results, we could classify the soil moisture condition in our study periods (Table 3). Soil moisture conditions in period 1 ~ 3 were categorized as “relatively wet”, while the other periods as “relatively dry”. In addition, the maximum groundwater level in period 1 and 2 was different in period 3. Because this change could influence on soil moisture at least, period 1 and 2 were considered wetter than period 3, which led to distinguish period 1 and 2 (“wet”) and period 3 (“relatively wet”). This moisture condition derived from inorganic nitrogen form and fluctuation in groundwater zone were consistent with pattern of the change in NO_3^- -N concentration and $\delta^{15}\text{N}$, i.e., the occurrence of denitrification. Soil moisture condition is considered the most acute

Table 3. The relationship between denitrification and soil water condition derived from inorganic nitrogen in soil solutions. Period 3 was categorized as relatively wet by the contracting of groundwater zone.

Period	Inorganic forms in soil solution	The soil water conditions	Occurrence of denitrification
1	NO_3^- -N	wet	occurred
2	NO_3^- -N	wet	occurred
3	NO_3^- -N	relatively wet	not occurred
4	NO_3^- -N, NH_4^+ -N	relatively dry	not occurred
5	NO_3^- -N, NH_4^+ -N	relatively dry	not occurred

II. Intermittent denitrification

factor controlling denitrification. It also suggests that denitrification occurs intermittently in unsaturated soil in forested ecosystem.

CONCLUSION

In this study, anaerobic conditions were not strong enough to promote denitrification at the macroscopic level. The ^{15}N natural abundance method, however, clearly indicated that denitrification occurred at the depth from 30 cm to 70 cm during periods 1 and 2 when soil moisture conditions were wet, partly indicated by the expansion of groundwater zone to the surface soil. These data thus strongly suggested there was a large potential for denitrification in this forested ecosystem that would not have been appreciated based only on data from the macroscopic level.

These results lead us to propose a schematic model of factors regulating the dynamics of $\text{NO}_3^-/\text{Cl}^-$ and $\delta^{15}\text{N}$ of NO_3^- -N in this watershed (Fig. 8). In the surface soil, ^{15}N -depleted NO_3^- -N is produced by nitrification of NH_4^+ -N in soil solution. $\text{NO}_3^-/\text{Cl}^-$ of the soil solution reflects the balance between nitrification and plant uptake. From 30-cm to 70-cm soil depth, denitrification occurs under anaerobic conditions and NO_3^- -N is partially consumed and $\delta^{15}\text{N}$ increases. Sufficiently anaerobic conditions are provided by rising in soil moisture and/or *in situ* oxygen consumption by intensive nitrification and

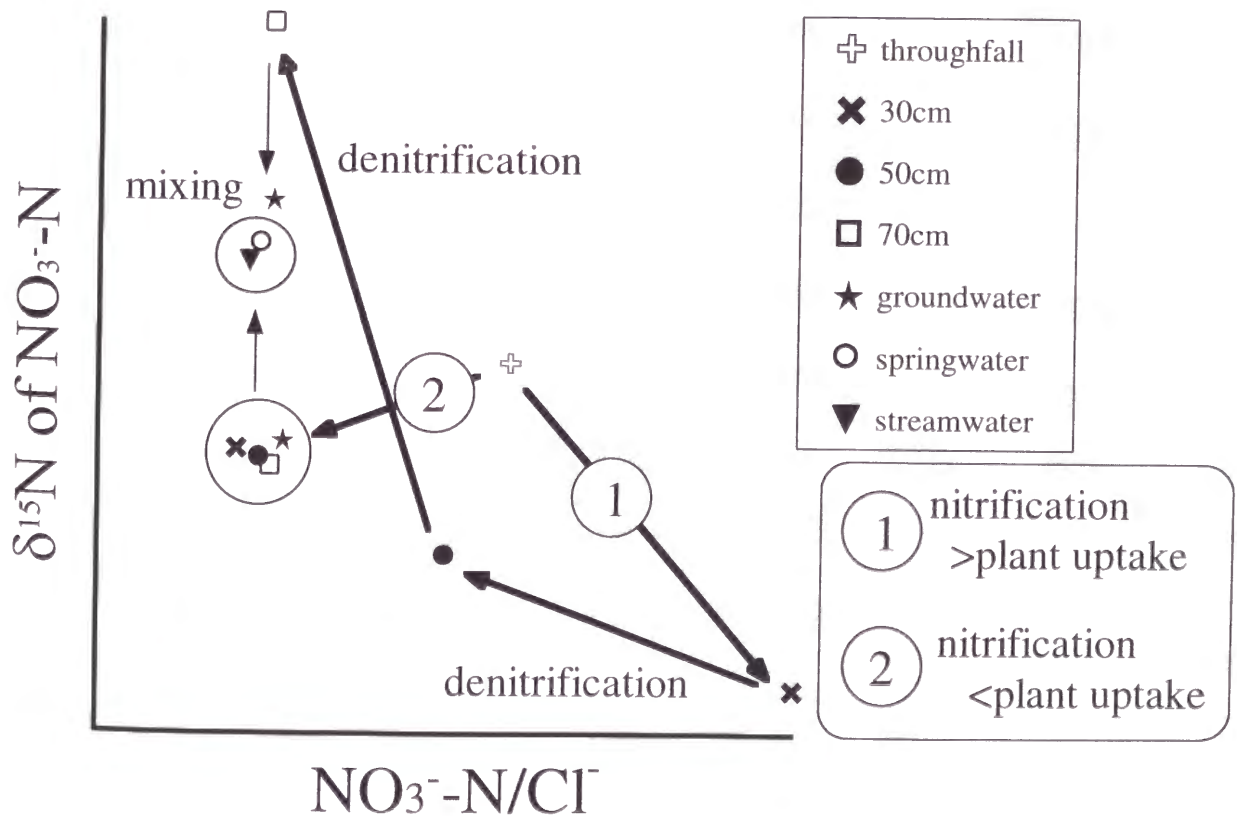


Fig. 8. Schematic model of dynamics of $\text{NO}_3^-/\text{Cl}^-$ and $\delta^{15}\text{N}$ of NO_3^- -N. Legends are same as those used in Fig. 5.

oxidative decomposition of organic matter. When no denitrification occurs in the soil, soil solution just moves downward through the soil column. Groundwater, spring water, and stream water are formed by the mixing of denitrified and non-denitrified soil solutions with $\delta^{15}\text{N}$ values and $\text{NO}_3^- \text{-N/Cl}^-$ in the range of those of soil solutions, with a significant time-lag between soil solution and stream water formations.

The ^{15}N natural abundance method detected the intermittent denitrification in the Kiryu watershed. To accurately estimate nitrogen loss from forest ecosystems, it is important to take into account the intermittency of denitrification and its large contribution to total nitrogen loss (Tietema et al. 1991). The natural ^{15}N abundance method may well make it possible to detect this intermittency and thus to better elucidate the dynamics of the nitrogen cycling.

III. NATURAL ABUNDANCE OF ^{15}N IN FOREST SOILS

INTRODUCTION

The natural abundance of stable nitrogen isotopes ($\delta^{15}\text{N}$) has been used for interpreting nitrogen cycles in forest ecosystems. For example, natural abundance studies have been used to quantify nitrogen fixation *in situ* (Virginia and Delwiche, 1982; Shearer and Kohl, 1986; Kohl and Shearer, 1995). Recently, foliar $\delta^{15}\text{N}$ values have been used to explain relationships between plants and available nitrogen sources (Garten, 1993; Evans and Ehleringer, 1994; Garten and Miegroet, 1994; Schulze et al., 1994; Page, 1995; Michelsen et al., 1996; Nadelhoffer et al., 1996).

In order to interpret the $\delta^{15}\text{N}$ values of plants, it is necessary to know the $\delta^{15}\text{N}$ values of the possible nitrogen sources (Handley and Raven, 1992; Nadelhoffer and Fry, 1994; Handley and Scrimgeour, 1997). Thus, it is important to investigate the variability in $\delta^{15}\text{N}$ of organic and inorganic nitrogen. Although the measurement of $\delta^{15}\text{N}$ requires a relatively large amount of nitrogen (> 0.1 mg-N in this study), it is relatively easy to measure $\delta^{15}\text{N}$ values of the bulk soils. Therefore, many studies have dealt with organic (total) nitrogen in soil (Mariotti et al., 1980; Ledgard et al., 1984; Wada et al., 1984; Nadelhoffer and Fry, 1988; Vitousek et al., 1989; Sutherland et al., 1993). On the other hand, for inorganic nitrogen isotopic measurement in forest soils, a large amount of soil must be processed (200 g soil in this study) because of the low concentration of inorganic nitrogen. Therefore, only a few studies have been reported on $\delta^{15}\text{N}$ values of inorganic nitrogen in forest soils (Binkley et al., 1985; Herman and Rundel, 1989; Garten, 1993). However, it is insufficient for interpretations of plant $\delta^{15}\text{N}$ values to use $\delta^{15}\text{N}$ data of only organic nitrogen because organic nitrogen is considered to be less available for plants than inorganic nitrogen. As a result, some assumptions about the $\delta^{15}\text{N}$ of inorganic nitrogen in soils have frequently been employed, fundamentally on the basis that ^{14}N reacts faster than ^{15}N in various processes (Nadelhoffer and Fry, 1994; Schulze et al., 1994; Michelsen et al., 1996; Nadelhoffer et al., 1996). These assumptions include, (1) the $\delta^{15}\text{N}$ values of inorganic nitrogen probably increase with depth, which is due to increases in the $\delta^{15}\text{N}$ content of the organic nitrogen from which inorganic nitrogen is derived, and (2) different forms of nitrogen at any given soil depth could show different $\delta^{15}\text{N}$ values due to mineralization and nitrification ($\delta^{15}\text{N}$ of total N $>$ $\text{NH}_4^+\text{-N}$ $>$ $\text{NO}_3^-\text{-N}$) because the substrate could be enriched compared to product. The purpose of this study was to test the validity of these assumptions.

We sampled mineral soils along a slope in a coniferous forest, which were generally of similar origin but showed different nitrogen dynamics due to topographic influences. We examined $\delta^{15}\text{N}$ values of different nitrogen forms in soil profiles, and used these data to elucidate the relationship between variations in $\delta^{15}\text{N}$ and nitrogen dynamics in soil.

METHODS

This study was carried out at Mt. Ryuoh, Shiga Prefecture, Japan ($35^{\circ} 1' N$, $136^{\circ} 20' E$; Fig. 9). Mean annual precipitation and temperature from 1986 to 1991 were 2050 mm and $10^{\circ}C$ respectively. A forested area, with a mean slope of 38.5° was chosen for the study site (Tokuchi et al., 1993). The site had a southwest aspect at the summit (altitude 870 m) and a south aspect at the base (altitude 670 m). This site was on alluvium and colluvium, composed predominantly cherty limestone. The soil was a silty loam Dystrochrept and the dominant overstory vegetation on the slope was a 45-year-old *Cryptomeria japonica* D. Don (Japanese red cedar) plantation, which had reached canopy closure.

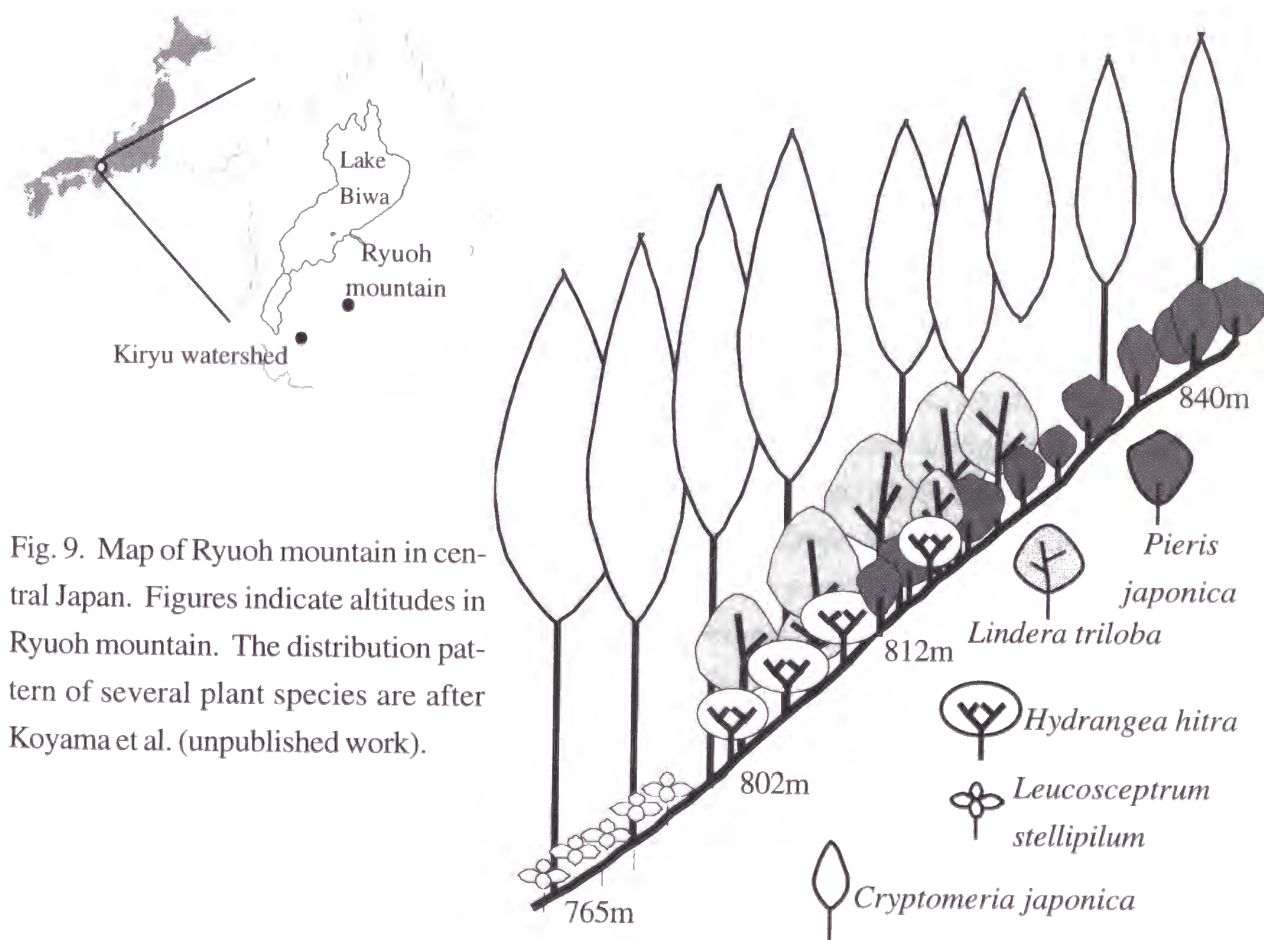


Fig. 9. Map of Ryuoh mountain in central Japan. Figures indicate altitudes in Ryuoh mountain. The distribution pattern of several plant species are after Koyama et al. (unpublished work).

A transect on the slope (5 m wide \times 135 m long) was established at an elevation of 765 m. Soil was mor-type at the upper part of the slope, mull-type at the lower part. The soil pH (H_2O) of mineral soils ranged from 3.4 to 4.9 and carbon content ranged from 8.6 to 18.7%. Two subplots (5 m \times 15 m) were established on both the upper part of the slope (U) and lower part of the slope (L); the elevation was 842 ~ 853 m (U-1) and 831 ~ 842 m (U-2) for upper sites, and 798 ~ 809 m (L-1) and 776 ~ 787 m (L-2) for lower sites.

Mineral soil was collected along the transect at sites U-1, U-2, L-1 and L-2 in October 1994. The mineral soils (about 1 kg wet soil each) were sampled by depth (0-5, 5-10, 10-20, 20-30, and 30-50

III. Nitrogen-15 in a forest soil

cm). To reduce soil heterogeneities, we collected soils from a whole profile (3 m in width) at each depth. Mineral soil was sieved through a 2-mm mesh to remove coarse fragments and then was homogenized by hand. About 10 g of each soil sample were ground using a ball mill for organic nitrogen measurement, and about 200 g of each soil sample were incubated for 35 days at 30 °C, while maintaining a constant water content. Part of the remainder of the soil (about 200 g) was used to measure the concentration and isotopic composition of inorganic nitrogen.

The total N concentration of soil samples was measured using the combustion method (Bremner, 1996) with a CN analyzer (Yanako MT-600). Both unincubated and incubated soils (about 200 g wet soil each) were extracted with 500 mL of 2 M KCl. By extracting relatively large amounts of soil, we could avoid problem of soil heterogeneity to some extent. Ammonium concentrations were determined by the indophenol blue method (Keeney and Nelson, 1982) and NO_3^- -N concentrations were determined colorimetrically (Keeney and Nelson, 1982) after Zn reduction instead of Cd reduction. Net mineralization rates were calculated by subtracting initial inorganic nitrogen (NH_4^+ -N + NO_3^- -N) concentrations from final concentrations. Net nitrification rates were calculated by subtracting initial NO_3^- -N concentrations from final NO_3^- -N concentrations.

The method employed for the conversion of NH_4^+ -N and NO_3^- -N to N_2 for isotopic analysis was based on Hauck (1982). Isotopic analysis was only done on the extract from unincubated soils with no replicates. For the large volumes of extract used, we modified the apparatus and operations of Kjeldahl distillation. It took about 45 minutes to liberate NH_4^+ -N by MgO completely from the 500 mL KCl extract in 1 L Kjeldahl flask. Nitrate was reduced to ammonia by Devarda's alloy and it took about 75 minutes for complete reduction and liberation. The distillate was concentrated gently on a hot plate and NH_4^+ -N was converted to N_2 *in vacuo* by reaction with hypobromite in a Y-shaped tube (Hauck, 1982; Mulvaney, 1993). Samples were analyzed for $\delta^{15}\text{N}$ on a Finnigan Mat Delta S and MAT 252 mass spectrometer. Isotopic composition is expressed in per mil deviation from the atmospheric nitrogen as defined by a following equation:

$$\delta^{15}\text{N} (\text{‰}) = \left(\frac{{}^{15}\text{N}/{}^{14}\text{N} \text{ sample}}{{}^{15}\text{N}/{}^{14}\text{N} \text{ standard}} - 1 \right) \times 1000.$$

The precision for $\delta^{15}\text{N}$ measurements (standard deviations) was respectively 0.14, 0.11, 0.07‰ for total N (dl-alanine by combustion method, n=10), $(\text{NH}_4)_2\text{SO}_4$ (by Kjeldahl method, n=7) and KNO_3 (by Kjeldahl method, n=3). The spatial variability of $\delta^{15}\text{N}$ (standard deviations) of total N (0-5 cm of mineral soil) in this slope was 0.91‰ for the lower part (n=5) and 1.95‰ for the upper part (n=4).

RESULTS

The concentration of total N in the mineral soil ranged from 0.1 to 0.7% (Table 4). Except at U-1, total N concentrations were greatest at the surface (0-5 cm) layer and gradually decreased with depth. On upper sites (U-1 and U-2), NH_4^+ -N was the dominant inorganic form of nitrogen in the soil, comprising more than 90% of total inorganic nitrogen concentration at each depth (Table 4). The NH_4^+ -N concentrations decreased with soil depth. Nitrate concentrations were < 1.2 mg kg⁻¹ soil, and the decrease

Table 4. Nitrogen concentrations and net rates in forest soils in four profiles of a Japanese red cedar stand

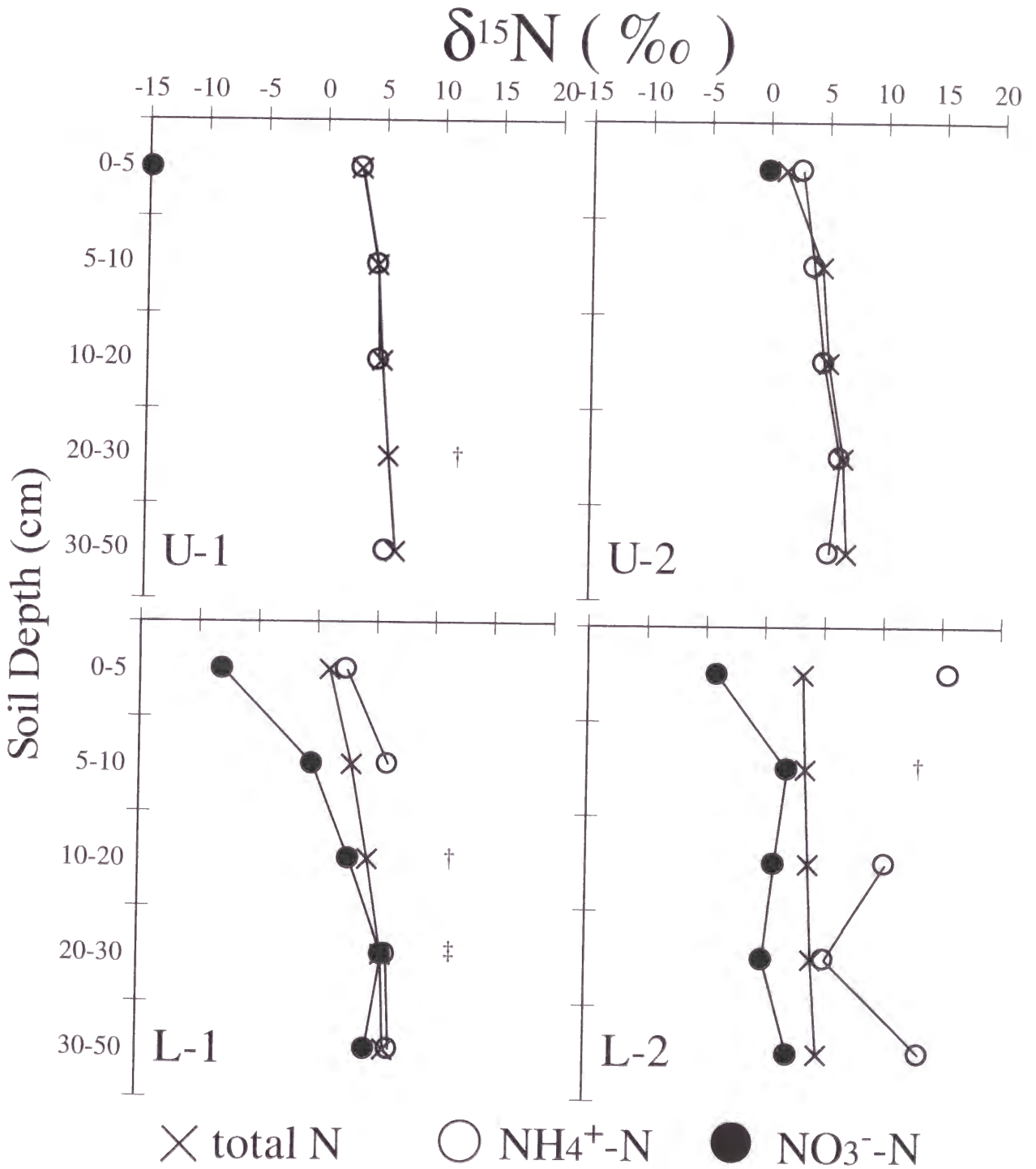
site	depth cm	Total N %	NH ₄ ⁺ -N -- mg-N kg ⁻¹ soil --	NO ₃ ⁻ -N -- mg-N kg ⁻¹ soil --	Net Rates	
					Mineralization -- mg-N kg ⁻¹ soil 35 days ⁻¹ --	Nitrification
Upper						
U-1	0-5	0.3	30.4	1.0	51.0	0.1
	5-10	0.4	21.3	0.7	26.6	0.2
	10-20	0.3	18.7	0.7	23.1	0.6
	20-30	0.2	18.4	0.8	19.0	-0.5
	30-50	0.1	9.3	0.8	17.4	-0.8
U-2	0-5	0.7	15.2	1.2	43.6	0.6
	5-10	0.2	13.4	0.8	8.8	0.5
	10-20	0.1	9.7	0.8	10.8	0.0
	20-30	0.1	8.9	0.7	6.2	0.0
	30-50	0.1	4.2	0.7	8.2	-0.7
Lower						
L-1	0-5	0.7	28.0	7.7	59.2	86.2
	5-10	0.3	15.0	3.8	3.8	18.0
	10-20	0.3	13.6	1.7	-3.2	6.3
	20-30	0.3	7.2	1.4	2.4	-1.4
	30-50	0.2	6.1	1.6	4.6	-1.3
L-2	0-5	0.6	5.4	5.8	29.8	34.4
	5-10	0.4	1.5	6.6	18.6	19.3
	10-20	0.4	3.3	5.1	1.3	3.6
	20-30	0.3	1.7	2.9	6.6	7.6
	30-50	0.3	4.6	4.3	0.7	4.6

with soil depth was less pronounced than for NH₄⁺-N. In accordance with this characteristic of inorganic nitrogen concentrations, net mineralization rates were much greater than net nitrification rates at every depth and decreased with increasing soil depth (Table 4).

On lower sites (L-1 and L-2), the dominance of NH₄⁺-N in total inorganic nitrogen was less distinct (Table 4). Ammonium comprised more than 80% of total inorganic nitrogen at L-1, whereas NO₃⁻-N comprised more than 50% at L-2. The concentration of both inorganic nitrogen forms decreased with soil depth at L-1, but that pattern was not distinct at L-2. Large net nitrification rates were found in each soil depth at L-2, and at shallower layers at L-1 (Table 4), where most of mineralized nitrogen was nitrified. Net nitrification rates decreased with increasing soil depth.

On upper sites, the δ¹⁵N values of total N ranged from 2.9 to 6.2‰ at U-1, from 1.4 to 6.8‰ at U-2; both increased with soil depth (Fig. 10). The δ¹⁵N values of NH₄⁺-N were similar to or slightly smaller than those of total N at each depth except at 0-5 cm at U-2. They increased with soil depth and ranged from 3.0 to 5.4‰ at U-1, and from 2.7 to 6.2‰ at U-2. The δ¹⁵N value of NO₃⁻-N was not determined except for the 0-5 cm layer because of the low NO₃⁻-N concentrations in the U-1 and U-2 soils. The δ¹⁵N value of NO₃⁻-N was -14.8‰ for U-1 and 0.0‰ for U-2.

On lower sites, the δ¹⁵N variability in total N was similar to that observed on upper sites (Fig. 10). The δ¹⁵N values of total N ranged from 1.0 to 5.9‰ at L-1, and from 3.2 to 4.7‰ at L-2, increasing



† $\text{NH}_4^+\text{-N}$ $\delta^{15}\text{N}$ value was not determined due to incomplete processing during distillation or cryogenic treatment.

‡ 5.6, 6.4 and 5.6 ‰ for total N, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, respectively.

Fig. 10. $\delta^{15}\text{N}$ by soil depth in the four profiles of a Japanese cedar stand. In U-1 and 2, $\delta^{15}\text{N}$ of $\text{NO}_3^-\text{-N}$ was not determined because of low concentration of $\text{NO}_3^-\text{-N}$ except at the 0-5 cm depth.

with soil depth. At L-1 the $\delta^{15}\text{N}$ values of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ increased generally with soil depth. Ammonium $\delta^{15}\text{N}$ ranged from 2.5 to 6.4, whereas $\text{NO}_3^-\text{-N}$ $\delta^{15}\text{N}$ ranged from -8.1 to +5.6‰ (Fig. 1). At L-2 as well as at L-1, $\delta^{15}\text{N}$ of $\text{NO}_3^-\text{-N}$ (from -4.1 to +2.2‰) generally increased with soil depth, whereas the $\delta^{15}\text{N}$ of $\text{NH}_4^+\text{-N}$ had no clear trend (from 5.3 to 15.6‰).

DISCUSSION

The $\delta^{15}\text{N}$ values of total N ranged from 1.0 to 6.8‰ and increased with depth (Fig. 10) corresponding to a decrease in nitrogen concentration (Table 4). This increase suggested that isotope fractionation occurred during nitrogen loss due to a faster reaction rate of ^{14}N than ^{15}N . Assuming the soil at each depth to be a closed system with respect to inputs of total N, this relationship should follow Rayleigh distillation kinetics (Mariotti et al., 1981; Nadelhoffer and Fry, 1988) and can be described by

$$\delta^{15}\text{N}_{(\text{total Nt})} = \delta^{15}\text{N}_{(\text{total N0})} - \epsilon \times \ln ([\text{N}]_t / [\text{N}]_0) \quad (1)$$

where $\delta^{15}\text{N}_{(\text{total Nt})}$ is the isotopic composition of total N at time t, $\delta^{15}\text{N}_{(\text{total N0})}$ the isotopic composition at the start of nitrogen loss, ϵ , the isotopic enrichment factor associated with loss of total N, and $[\text{N}]_t / [\text{N}]_0$ the fraction of total N remaining at time t, respectively. Because $\delta^{15}\text{N}_{(\text{total N0})}$ and $[\text{N}]_0$ are rarely known, equation (1) is rewritten as

$$\delta^{15}\text{N}_{(\text{total Nt})} = k - \epsilon \times \ln ([\text{N}]) \quad (2)$$

where k is a constant and $[\text{N}]$ is the nitrogen concentration, respectively (Fustec et al., 1990; Evans and Ehleringer, 1993). The calculated isotopic enrichment factors during nitrogen loss ranged from 1.7 to

3.3‰ ($r > 0.71$; Fig. 11), similar to the range of 1.5 to 4.8‰ summarized in Nadelhoffer and Fry (1988). This enrichment of $\delta^{15}\text{N}$ in residual nitrogen is presumably the sum of many processes including the differential preservation of ^{15}N -enriched materials, illuviation of ^{15}N -enriched materials from shallower to deeper soil layers, and decomposition (Nadelhoffer and Fry, 1988).

The $\delta^{15}\text{N}$ values of total and inorganic nitrogen generally increased with soil depth, and moreover $\delta^{15}\text{N}$ values were different between $\text{NH}_4^+\text{-N}$

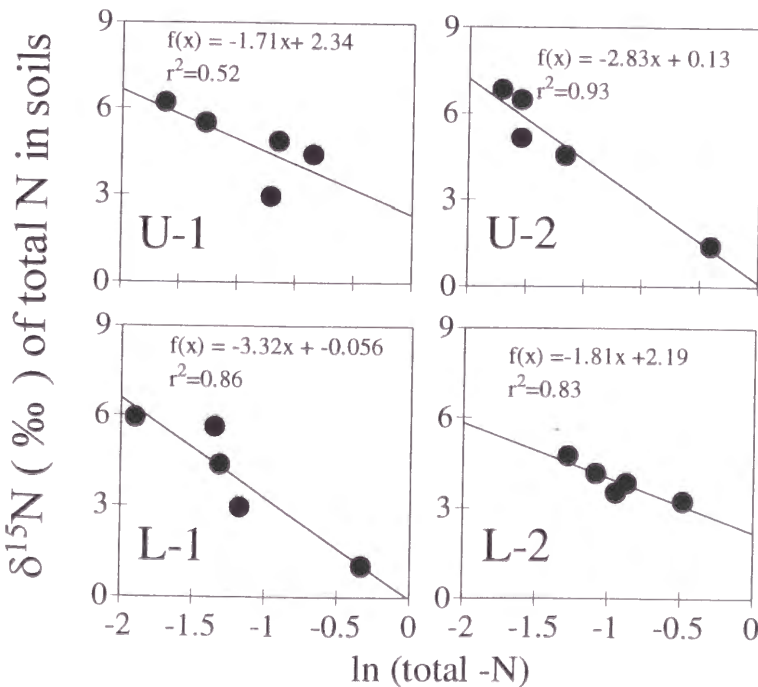


Fig. 11. The relationship between total N concentration and $\delta^{15}\text{N}$. The equation for all soil samples complied is, $f(x) = -2.37x + 1.26$ ($r^2 = 0.77$, $p < 0.001$).

III. Nitrogen-15 in a forest soil

and NO_3^- -N (Fig. 10), supporting assumptions (see INTRODUCTION) found in previous studies. Thus it was shown here that the assumptions used for interpretation of plant $\delta^{15}\text{N}$ were generally valid. In detail, however, the order of ^{15}N -depletion among different forms of nitrogen was not always the same as expected (i.e., total N > NH_4^+ -N > NO_3^- -N). Generally, the order of $\delta^{15}\text{N}$ was total N > NH_4^+ -N > NO_3^- -N on upper sites but NH_4^+ -N > total N > NO_3^- -N on lower sites (Fig. 10). Differences in $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}_{\text{total N}} - \delta^{15}\text{N}_{\text{NH}_4^+}$) showed that NH_4^+ -N was remarkably enriched in $\delta^{15}\text{N}$ relative to total N on lower sites ($-4.3 \pm 1.1\%$, $n=8$, mean \pm S.E.), but not on upper sites ($0.3 \pm 1.0\%$, $n=9$). The large net nitrification rates and high NO_3^- -N concentrations at lower sites (Table 4) coupled with the large isotopic fractionation that has been measured during nitrification in the laboratory (Mariotti et al., 1981), suggested that isotopic enrichment of NH_4^+ -N during nitrification in forest soils accounted for the isotopic pattern observed on lower sites. This enrichment in $\delta^{15}\text{N}$ of NH_4^+ -N was also observed on a upper site at 0-5 cm depth at U-2 (Fig. 10), where a relatively large nitrification rate and concentration of NO_3^- -N were determined (Table 4). The large isotopic differences observed between NH_4^+ -N and NO_3^- -N ($\delta^{15}\text{N}_{\text{NH}_4^+} - \delta^{15}\text{N}_{\text{NO}_3^-}$) further supported this interpretation ($10.2 \pm 4.8\%$; $n=2$, and $8.1 \pm 2.4\%$; $n=8$ for upper and lower sites, respectively). In an incubation study, Herman and Rundel (1989) reported a strong ^{15}N -enrichment of NH_4^+ -N during nitrification, which further supported the interpretation offered here. Thus we could conclude that the isotopic fractionation associated with net nitrification strongly regulated the $\delta^{15}\text{N}$ values of inorganic nitrogen in this study. For a more quantitative interpretation of the effects of isotope discriminations during nitrification and other processes, more detail on denitrification (Mariotti et al., 1988; Fustec et al., 1990; Koba et al., 1997), NH_4^+ -fixing to soil (Delwiche and Steyn, 1970), microbial immobilization, and mixing among soil layers, must be considered as important determinants for $\delta^{15}\text{N}$.

Isotope fractionation during nitrification calculated from these data

In forest soil, the concentration of organic nitrogen is generally much higher than that of inorganic nitrogen. Therefore, the mineralization process is often considered to exhibit zero-order reaction kinetics, i.e. the process rate is independent of the amount (or concentration) of reactants. In this study, however, total nitrogen contents and net mineralization (ammonification + nitrification) rates were positively correlated ($r=0.65$ $p<0.005$; Fig. 12). This showed that mineralization is a first-order process. Because the significance of heterotrophic nitrification process is controversial as yet (e.g. Killham 1994), we consider the ammonifica-

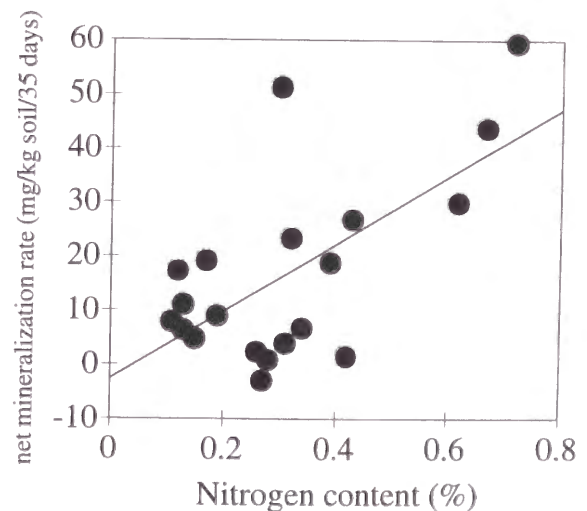


Fig. 12. The relationship between total N concentration and net mineralization rate.

$$f(x) = 6.2x - 0.3; r^2 = 0.42, p < 0.005$$

tion itself is a first order process.

As inorganic nitrogen can be regarded as the instantaneous product from a large amount of organic nitrogen (reactant), the $\delta^{15}\text{N}$ value of NH_4^+ produced from organic nitrogen by ammonification, was expected by using ϵ_{min} as isotope enrichment factor during ammonification as (Mariotti et al, 1981)

$$\delta^{15}\text{N}_{\text{NH}_4^+ (t=0)} = \delta^{15}\text{N}_{(\text{total Nt})} + \epsilon_{\text{min}} \quad (3)$$

Isotope enrichment of ammonium during nitrification

If we assume that the inorganic nitrogen pool in soil is a closed system and that the ratio of NO_3^- to total inorganic nitrogen is considered to be the fraction of mineral nitrogen that is nitrified, f , we obtain $\delta^{15}\text{N}$ value of NH_4^+ enriched by nitrification process as follows

$$\delta^{15}\text{N}_{\text{NH}_4^+ (t=t)} - \delta^{15}\text{N}_{\text{NH}_4^+ (t=0)} = \epsilon_{\text{nit}} \times \ln (1-f) \quad (4)$$

where ϵ_{nit} means the apparent enrichment factor during nitrification for NH_4^+ . Combining (4) with (3) gives

$$\delta^{15}\text{N}_{\text{NH}_4^+ (t=t)} - (\delta^{15}\text{N}_{(\text{total Nt})} + \epsilon_{\text{min}}) = \epsilon_{\text{nit}} \times \ln (1-f) \quad (5)$$

Figure 13 shows the change in isotopic composition of substrate NH_4^+ during nitrification, from which we obtain the apparent enrichment factor for NH_4^+ of -11.15% during nitrification ($r^2=0.72$; $p<0.001$), where ϵ_{min} is determined as -2.37% from the regression between $\delta^{15}\text{N}_{(\text{total N})}$ and $\ln (N)$ ($r^2=0.77$; $p<0.001$). This value is different from the isotopic enrichment factor during nitrification ($-34.7 \pm 2.5\%$; mean \pm s.d.) reported by Mariotti et al. (1981). In this study, only the apparent isotopic enrichment factor is obtained. This factor is not independent of the effects from the processes associated with NO_3^- . For example,

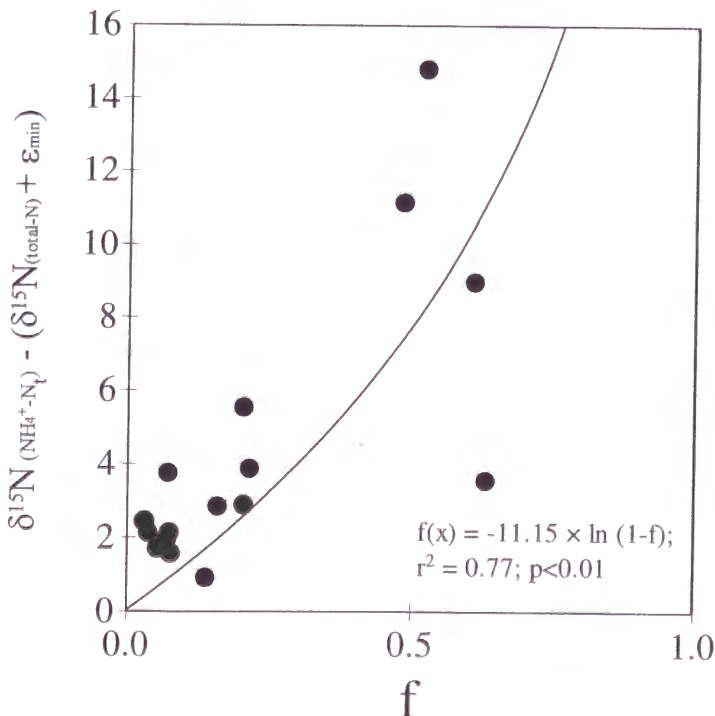


Fig. 13. The relationship between ^{15}N -enrichment of residual NH_4^+-N , and the fraction of mineral nitrogen nitrified, f .

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denitrification process could obscure the nitrification signal since denitrification process is known to discriminate against ^{15}N strongly and to produce ^{15}N -enriched NO_3^- (Mariotti et al. 1981, 1988, Fustec et al. 1990). The isotopic noise by denitrification might appear in ^{15}N profiles, especially at the lowest subplot L-2, where anaerobic conditions might be favored in soil. This kind of processes diminishes the extent of apparent enrichment by nitrification *in situ*, which results in the difference between apparent and actual isotopic enrichment factor of nitrification.

IV. NITRATE AVAILABILITY FOR PLANTS IN A CONIFEROUS FORESTED ECOSYSTEM IN JAPAN

INTRODUCTION

Nitrogen is an essential element for plants, and plant growth is restricted by low supply rates of available nitrogen in forest ecosystems (Vitousek and Howarth 1991). Recently, many forest ecosystems have experienced anthropogenic disturbance such as increased nitrogen inputs (e.g. Aber et al. 1989). Elevated level of anthropogenic nitrogen input into forest ecosystems has very significant impacts on nitrogen cycling and ecosystem functions such as declines in plant productivity, depletions in soil base cations, and increased rates of nitrogen leaching to streamwaters (Reuss and Johnson 1986, Aber et al. 1989, Aber 1992 and references therein). To predict these changes and conserve forest ecosystems, much interest has developed in understanding the processes that regulate nitrogen cycling in forest ecosystems.

Stable nitrogen isotope distributions in compartments of nitrogen cycles contain information not available from conventional studies of element standing stocks and fluxes. These isotope distributions, expressed as the natural abundance of nitrogen isotopes ($\delta^{15}\text{N}$), provide important clues about which sources and sinks are most important in nitrogen budgets (Fry et al. 1995). Unfortunately, many processes can fractionate nitrogen isotopes, and care should therefore be taken when plant $\delta^{15}\text{N}$ data are evaluated (Handley and Raven, 1992; Handley and Scrimgeour, 1997). Nevertheless, the natural abundance method is a powerful tool for studying intact nitrogen dynamics because the isotope distributions are established over long periods of time by natural processes, and thus reflect undisturbed *in situ* processes (Högberg 1997).

Here we present the ^{15}N isotopic signatures of nitrogen sinks (plants) and sources (inorganic nitrogen in soil and precipitation, and total nitrogen in soil) to illustrate the relative importance of NO_3^- in the nitrogen cycle in a mountain slope of a coniferous forested ecosystem in central Japan. In this mountain slope, it was reported that there were distinct differences in nitrogen cycling patterns in soils between upper and lower part of the slope (Hobara and Tokuchi 1998, Koba et al. 1998, Hirobe et al. 1999) such as proposed by Read et al. (1989) and observed by Giesler et al. (1998). The characteristics of nitrogen dynamics in this mountain slope are summarized in Table 5. The most distinctive character in this slope is that soils from an upper part of a slope had both low net nitrification potentials and small pools of NO_3^- , while high net nitrification rates and large NO_3^- pools are observed in soils from lower part of the slope (Hobara and Tokuchi 1998, Koba et al. 1998, Hirobe et al. 1999). This difference in nitrogen dynamics in soils leads to a simple hypothesis; plants in the upper part of this slope take up NH_4^+ , while in the lower part they take up NH_4^+ and NO_3^- . To test the validity of this assumption, we first examined the potentiality for plant use of NO_3^- by measuring nitrate reductase activity (NRA) of plant leaves and mycorrhizal associations. In addition we applied the ^{15}N natural abundance method to obtain the in-situ evidence of NO_3^- use of plants and niche differentiation for N acquisition.

Table 5. General description of Ryuoh mountain slope. 'Upper Site' and 'Lower Site' are defined according to the results on different nitrogen transformation patterns by Hirobe et al. (1999).

Variable	Position in a mountain slope		Comments	Data Source
	Upper Site	Lower Site		
Plant species composition	<i>Cryptomeria japonica</i> <i>Hydrangea hirta</i> <i>Lindera triloba</i> <i>Pieris japonica</i>	<i>Cryptomeria japonica</i> <i>Leucosceptrum stellipilum</i> <i>Hydrangea hirta</i> <i>Lindera triloba</i>		
DBH of <i>Cryptomeria japonica</i> (cm)	9.4 - 25.0	12.4 - 38.2	Nov. 1997	
Intensive root distribution	in forest floor	in mineral soil	<i>Cryptomeria japonica</i>	Kasuya and Shimada (1996)
Net mineralization rates (mg-N kg soil ⁻¹ d ⁻¹)	0.18 - 1.46	0.00 - 1.69	<50-cm depth; Oct. 1994	Koba et al. (1998)
	0.03 - 0.25	0.19 - 0.45	<10-cm depth; Aug. 1995	Hirobe et al. (1999)
	1.82 - 2.65	1.95 - 4.48	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)
	6.77 - 15.91	5.77 - 12.41	FH layer; Nov. 1996	Hobara and Tokuchi (1998)
Net nitrification rates (mg-N kg soil ⁻¹ d ⁻¹)	0.00 - 0.02	0.00 - 2.46	<50-cm depth; Oct. 1994	Koba et al. (1998)
	0.00 - 0.09	0.04 - 0.44	<10-cm depth; Aug. 1995	Hirobe et al. (1999)
	0.16 - 0.94	1.85 - 4.36	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)
	0.20 - 3.03	1.66 - 12.04	FH layer; Nov. 1996	Hobara and Tokuchi (1998)
Gross mineralization rates (mg-N kg soil ⁻¹ d ⁻¹)	3.13 - 8.39	8.45 - 49.28	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)
Gross nitrification rates (mg-N kg soil ⁻¹ d ⁻¹)	0.01 - 0.04	0.04 - 11.07	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)
C/N ratio	20.5 - 21.6	17.5 - 21.2	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)
DOC concentration (mg-C kg soil ⁻¹)	536.4 - 760.6	478.9 - 861.5	<10-cm depth; Jun. 1996	Tokuchi et al. (1998)

MATERIALS AND METHODS

Site description

This study was carried out at Mt. Ryuoh, Shiga Prefecture, Japan (35° 1' N, 136° 20' E; Fig. 9). Watershed had low annual nitrogen input (3.3 kg-N ha⁻¹ yr⁻¹; by bulk precipitation) and low nitrogen drainage water loss (0.6 kg-N ha⁻¹ yr⁻¹; Tokuchi and Iwatsubo 1992, Ohrui and Mitchell 1997).

A transect in a slope (5-m wide and 135-m long) was established at an elevation of 765 m to 851 m. As mentioned above, there was a distinctly different pattern in nitrogen dynamics between different slope positions (Hobara and Tokuchi 1998, Koba et al. 1998). Hirobe et al. (1999) reported that mineral soils (0- to 10-cm depth) showed (i) low to zero net nitrification rates in the upper part (812- to 851-m elevation), (ii) large net nitrification rates in the lower part (765- to 802-m elevation), and (iii) variable net nitrification rates in the transition zone (802- to 812-m elevation). The pattern of increasing with respect to net nitrification rate was the same at all depths (Hobara and Tokuchi 1998, Koba et al. 1998). Accordingly, we divided the transect into two sites with; Upper Site (812- to 851-m elevation) with a low NO₃⁻ supply rate, and Lower Site (765- to 812-m elevation) with a high NO₃⁻ supply rate.

The predominant understory species are *Leucosceptrum stellipilum* (LABIATAE; short herb), *Hydrangea hirta* (SAXIFRAGACEAE; deciduous tree), *Lindera triloba* (LAURACEAE; deciduous tree) and *Pieris japonica* (ERICACEAE; evergreen tree). *Pieris japonica* was observed mostly in Upper Site and *Leucosceptrum stellipilum* only was observed only in Lower Site, while *Hydrangea hirta* and *Lindera triloba* distributed both in Upper and Lower Sites (Koyama et al. unpublished work).

Fine root distributions of *Cryptomeria japonica* in this slope were concentrated in the forest floor in Upper Site, and in shallow mineral soils (0 - 30-cm depth) in Lower Site (Kasuya and Shimada 1996).

Plant and soil sampling

Collection of tree leaves began in 1994. Preliminary leaves of *Cryptomeria japonica* were collected in June 1993, but the canopy of this species was too high to obtain leaves from entire canopy especially in Lower Site. Thus we collected the samples from lower part in a canopy in Lower Site (June 1994), and from several positions in a canopy in Upper Site (June 1994 and 1995).

Leaves of understory species were collected during three successive years (June 1995, July and October 1996, and August 1997) from Upper and Lower Site. Foliar samples in Lower Site for δ¹⁵N and NRA measurements were not taken from the intermediate zone (802 to 812 m) in August 1997. Care was taken to collect the leaves from the entire canopy to eliminate inter-canopy differences in δ¹⁵N and NRA, and collection of samples for NRA should be finished within 2 h of solar noon on sunny days. Each sample was a composite of at least 10 leaf samples from a single plant with exception of *Leucosceptrum stellipilum* which had large leaves (2 - 4 leaves for one composite). Composite leaf samples were obtained from *Leucosceptrum stellipilum*, *Hydrangea hirta*, *Lindera triloba*, *Pieris japonica*.

Whole plants were collected from both Upper Site and Lower Site except *Leucosceptrum*

IV. In situ nitrate availability for plants

stellipilum which was present only in Lower Site, in June (for *Pieris japonica*, *Hydrangea hirta* and *Leucosceptrum stellipilum*) and in September (for *Lindera triloba*) 1995 to determine the internal variations of $\delta^{15}\text{N}$. The plants were divided into several organs according to each life forms or morphological features. Whole plant $\delta^{15}\text{N}$ was calculated as the weighted average using the data of nitrogen contents (L. Koyama, unpublished work),

$$\text{Whole plant } \delta^{15}\text{N} = (\delta^{15}\text{N organ} \times \text{mg-N organ}) / \text{mg-N whole plant}$$

Forest floor was collected in September 1995 and October 1997. From Lower Site, only the L layer was sampled because the FH layer was so thin (Hobara and Tokuchi 1998). Samples of leaves and organic soils were dried and finely ground in a mill for $\delta^{15}\text{N}$ measurements.

Nitrate Reductase assays

Nitrate reductase activity (NRA) was measured as in Gebauer et al. (1984) in order to indicate relative levels of NRA of already endogenous enzyme under the condition of non-limiting amounts of substrate. NRA was measured in August 1997. Leaves were cut into 5-mm circles and incubated in glass vials with 5 ml of 0.1 M KNO_3 , 0.1 M KH_2PO_4 and 3% propanol. Samples were vacuum infiltrated (6 mm Hg; twice for 30 s each) and incubated for 1 h at 30 °C. Enzyme activity was stopped by placing vials in boiling water. Aliquots of the incubation buffer were analyzed for NO_2^- by adding sulfanilamide in HCl, and N-naphtylethylene diamine dihydrochloride. The formed NO_2^- was measured as absorbance at 545 nm including a correction for absorption by plant pigments which interfered. The incubated leaf samples were oven-dried and their dry-weights were determined to convert from fresh to dry weights.

Mycorrhizal associations

The roots were analyzed for mycorrhizal fungi. We collected the fine root samples and brought them in 80 % ethanol-formalin solution to laboratory. The infection of Vesicular-Arbuscular mycorrhiza (VAM) was observed by staining trypan-blue after cleaning of the roots with KOH solutions. Any infections of ectomycorrhiza to plant roots were not observed on the basis of visual appearance of Hartig nets and fungal sheath around the roots.

Dissolved and exchangeable mineral nitrogen

The resin bag technique (Binkley and Matson 1983, Pate et al. 1993) was used to obtain the average $\delta^{15}\text{N}$ values of NO_3^- in soils during 1 year. Each 80 g of cation and anion exchange resins were put into a cylinder-bag (PVC ring with nylon mesh). At 831- and 840-m elevation in Upper Site, the bags were buried to a 10-cm depth (M. Hirobe, unpublished work) and beneath the organic soil (N. Tokuchi, unpublished work) from October 1994 to October 1995. About 10 g of resins were extracted by 100 ml of 2 M KCl. This extraction was repeated for three times to prevent potential isotopic fractionation during extraction.

In October 1997, for $\delta^{15}\text{N}$ analysis of NH_4^+ and NO_3^- , 100:700 (w/v) 2 M KCl extracts of field collected forest floor (FH layer from Upper Site) were prepared.

Throughfall was collected from April to December in 1995. The solutions were filtered and concentrated by rotary-evaporator with checking pH.

From extracts of organic soils, and concentrated precipitations, NH_4^+ and NO_3^- were collected separately through semi-micro Kjeldahl distillation with MgO and Devarda's alloy, whereas only NO_3^- was collected for IER extracts (Mulvaney 1993, Koba et al. 1997, 1998). Isotopic analysis was conducted on N_2 gas following combustion of the NH_4^+ absorbed onto cation exchange resin (Garten 1992).

Nitrogen isotope analysis

Nitrogen isotope compositions of plant tissues, soils, and NH_4^+ absorbed onto the cation exchange resin were measured on Finnigan Mat Delta-S and 252 (Finnigan MAT, Bremen, Germany), followed by a the manual cryo-purification of combustion products in a vacuum system (Minagawa et al. 1984) or coupled with an elemental analyzer including measurement of total C and N concentration (Carlo Erba, Italy).

Results of ^{15}N natural abundance are expressed as

$$\delta^{15}\text{N} (\text{‰}) = (\text{R}_{\text{sample}} / \text{R}_{\text{standard}} - 1) \times 1000$$

where R = mass 29 / mass 28 and the standard is the atmospheric nitrogen ($\delta^{15}\text{N} = 0\text{‰}$).

The difference between manual and automatic procedures based on measurements of *Lindera triloba* leaves (n=22) was $0.06 \pm 0.11\text{‰}$ (mean \pm standard deviation; S.D.). The range of the precision based on multiple analysis of a laboratory standard was $\pm 0.18\text{‰}$ (as S.D.).

Statistics and calculations

Data were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD; SYSTAT 1992). Differences were considered significant at $P < 0.05$.

Table 6. Nitrate reductase activity of plant leaves in different sites.
Data are means \pm 1 S.E. Number of samples is given in parentheses.

Plant species	NRA ($\text{N } \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)	
	Upper site	Lower site
<i>Leucosceptrum stellipilum</i>		2.17 ± 0.24 (10)
<i>Hydrangea hirta</i>	0.04 ± 0.02 (9)	0.06 ± 0.01 (9)
<i>Lindera triloba</i>	0.02 ± 0.02 (9)	0.08 ± 0.01 (10)
<i>Pieris japonica</i>	not detected	

IV. In situ nitrate availability for plants

RESULTS

Nitrate Reductase Activity

All understory species had NRA both in Upper and Lower Site with the exception of *Pieris japonica* (Table 6). Both *Hydrangea hirta* and *Lindera triloba* in Lower Site had significantly higher NRA than those in Upper Site ($F_{1,16} = 5.700$, $P < 0.030$ for *Hydrangea hirta* and $F_{1,17} = 31.693$, $P < 0.001$ for *Lindera triloba*). In Upper Site, *Hydrangea hirta* had higher NRA than *Lindera triloba* ($F_{1,16} = 4.496$, $P < 0.050$), and *Leucosceptrum stellipilum* had highest NRA in Lower Site ($F_{2,26} = 65.305$, $P < 0.001$).

Mycorrhizal associations

In both Upper and Lower Site, *Cryptomeria japonica* was infected by VAM mycorrhizae, whereas we did not observe any mycorrhizal associations for *Lindera triloba* and *Hydrangea hirta*. *Pieris japonica* was associated with Ericoid mycorrhizae and *Leucosceptrum stellipilum* was infected by VAM.

Plant ^{15}N natural abundances

The overstory species in this mountain slope, *Cryptomeria japonica*, had negative values both in Upper Site ($-2.07 \pm 0.27\text{‰}$, $n=5$, mean \pm standard error; S.E.) and in Lower Site ($-2.79 \pm 0.24\text{‰}$, $n=4$) in June 1994. Variations in ^{15}N abundances among species were narrow, with $\delta^{15}\text{N}$ values ranging from -2.97‰ to 0.36‰ in Upper Site and from -3.74‰ to 0.23‰ in Lower Site (Table 7). In Upper Site, *Pieris japonica* had lowest $\delta^{15}\text{N}$ values over the entire sampling periods, and the ranking in $\delta^{15}\text{N}$ was generally *Pieris japonica* < *Lindera triloba* \leq *Hydrangea hirta* (Table 7). In Lower Site, the ranking in $\delta^{15}\text{N}$ fluctuated over the sampling periods (Table 7).

The internal variations in $\delta^{15}\text{N}$ (expressed by $\delta^{15}\text{N}$ of current leaves - $\delta^{15}\text{N}$ of each plant organ) were small in all understory species, except there was a large difference between leaves and roots of *Leucosceptrum stellipilum* (Table 8). Generally, the differences between current leaves and whole plants were small (Table 8).

^{15}N natural abundances of nitrogen sources

The $\delta^{15}\text{N}$ values of total N in forest floor (L layer) had the similar values of *Cryptomeria japonica* leaves, whereas the value of FH layer was higher than that of L layer in Upper Site (Table 5).

The exchangeable NH_4^+ in FH layer had lower $\delta^{15}\text{N}$ values compared with total N (Table 9). The average values of $\delta^{15}\text{N}$ NO_3^- derived from IER buried in soil during 1994-1995 were all negative. The NO_3^- in mineral soils had more negative $\delta^{15}\text{N}$ values than NO_3^- in forest floor (Table 9). The $\delta^{15}\text{N}$ values of NH_4^+ and NO_3^- were $-0.33 \pm 0.57\text{‰}$ ($n=3$) and $-2.09 \pm 0.36\text{‰}$ ($n=2$) during 1995.

Table 7. Natural abundance of $\delta^{15}\text{N}$ of different plants. Data are means \pm S.E. The number of samples is given in parentheses. Results tagged with the same letter within a sampling period in a site are not significantly different at the 0.05 probability level by ANOVA with LSD test.

Position	Plant species	Variation of $\delta^{15}\text{N}$ (‰) among plant species			
		Jun-95	Jul-96	Oct-96	Aug-97
Upper Site	<i>Hydrangea hirta</i>	0.36 \pm 0.20 (4) ^b	-1.38 (1)	-0.11 \pm 0.35 (3) ^c	-0.85 \pm 0.23 (10) ^b
	<i>Lindera triloba</i>	-0.38 \pm 0.32 (11) ^b	-1.08 \pm 0.18 (20) ^b	-1.71 \pm 0.25 (15) ^b	-1.48 \pm 0.18 (10) ^b
	<i>Pieris japonica</i>	-2.63 \pm 0.31 (13) ^a	-2.63 \pm 0.29 (21) ^a	-2.97 \pm 0.22 (20) ^a	-2.72 \pm 0.24 (10) ^a
	<i>Cryptomeria japonica</i>	-2.48 \pm 0.13 (3) ^a	no data	no data	no data
Lower Site	<i>Leucosceptrum stellipilum</i>	-3.74 \pm 0.29 (5) ^a	-3.09 \pm 0.14 (16) ^a	-1.23 \pm 0.21 (10) ^{bc}	-1.94 \pm 0.26 (10) ^a
	<i>Hydrangea hirta</i>	0.23 \pm 0.50 (9) ^c	-0.47 \pm 0.24 (21) ^c	-0.63 \pm 0.19 (14) ^c	-1.16 \pm 0.25 (10) ^b
	<i>Lindera triloba</i>	-1.26 \pm 0.21 (8) ^b	-1.22 \pm 0.14 (23) ^b	-1.69 \pm 0.20 (10) ^{ab}	-2.04 \pm 0.15 (10) ^a
	<i>Pieris japonica</i>	0.18 \pm 0.50 (5) ^c	-2.21 \pm 0.61 (6) ^a	-2.17 \pm 0.58 (5) ^a	no data

Table 8. Internal variation of $\delta^{15}\text{N}$ for each plant species. The difference in $\delta^{15}\text{N}$ between current leaf and each organ or calculated whole plant body are expressed. Data are means \pm 1 S.E.

Plant species	N†	$\delta^{15}\text{N}$ current leaf - $\delta^{15}\text{N}$ each organ (‰)					
		Whole plant‡	Root	Current shoot	Branch	Below ground shoot	Old leaf
<i>Leucosceptrum stellipilum</i>	4	0.47 \pm 0.23	1.54 \pm 0.48	0.12 \pm 0.20		0.14 \pm 0.46	
<i>Hydrangea hirta</i>	4	-0.35 \pm 0.32	-0.61 \pm 0.24	-0.27 \pm 0.10	0.26 \pm 0.10		
<i>Lindera triloba</i>	6	-0.27 \pm 0.25	-0.37 \pm 0.36	-0.04 \pm 0.07	-0.47 \pm 0.20		
<i>Pieris japonica</i>	6	0.43 \pm 0.21	0.25 \pm 0.29	0.53 \pm 0.21	0.91 \pm 0.18		0.43 \pm 0.19

† N = number of individuals

‡ Whole plant $\delta^{15}\text{N}$ was calculated by data on N content from unpublished data of L. Koyama.

IV. *In situ* nitrate availability for plants

Table 9. The natural abundance of ^{15}N of N sources in different compartments for soils in a mountain slope. Data are means \pm 1 S.E., and number of samples is given in parentheses.

N forms	Sampling point	Upper Site	Lower Site
Total N	F layer	$-3.04 \pm 0.10\text{‰}$ (4)	$-2.31 \pm 0.06\text{‰}$ (7)
	FH layer	$1.73 \pm 0.24\text{‰}$ (9)	no data
Exchangeable NH_4^+	FH layer	$-3.55 \pm 1.24\text{‰}$ (3)	no data
NO_3^- absorbed onto IER	beneath forest floor	$-2.66 \pm 0.86\text{‰}$ (6)	no data
	beneath the surface mineral soil*	$-4.56 \pm 0.56\text{‰}$ (6)	no data

*: at 10-cm depth in mineral soil

DISCUSSION

Available nitrogen forms for plants suggested by NRA and mycorrhizal associations

As for NH_4^+ and organic nitrogen uptake of plants, we did not done any substitute measurements for foliar NRA and NO_3^- pool measurements. Furthermore it was unlikely in this study that the concentration of NH_4^+ in soil solutions was high enough to prevent NH_4^+ uptake (Nye and Tinker 1977). Thus, it is likely that all plants observed in this study can use NH_4^+ .

Pieris japonica was infected by Ericoid mycorrhizae. This suggests that *Pieris japonica* can use organic nitrogen as well as NH_4^+ because Ericoid mycorrhizae can assimilate organic nitrogen directly (Stribley and Read 1980, Read and Bajwa 1985, Smith and Read 1997). As for NO_3^- uptake, the lack of detectable NRA of *Pieris japonica* (Table 6) indicated that the ability to use NO_3^- is very low, as reported in many studies on Ericaceous plants (Ingestad 1973, Haynes and Goh 1978, Nadelhoffer et al. 1996). The low to undetectable NO_3^- pool size in *Pieris japonica* leaf in this mountain slope supports this interpretation (L. Koyama, unpublished work).

Hydrangea hirta and *Lindera triloba* were not infected by any mycorrhizae. This implies that these species cannot use organic nitrogen because it is unknown that plants could use organic nitrogen directly without mycorrhizal association in a less nutrient-limited site such as our temperate site, although it has been shown that some non-mycorrhizal plants have the capacity to take up organic nitrogen in nutrient-limited sites (Chapin et al. 1993, Raab et al. 1996). Assumed *Cryptomeria japonica* and *Leucosceptrum stellipilum* also use NH_4^+ and NO_3^- because VAM mycorrhizae do not assimilate organic nitrogen (Smith and Read 1997). This interpretation of available nitrogen forms is supported for *Leucosceptrum stellipilum* with regard to NO_3^- by positive NRA in both sites (Table 6).

^{15}N signature; its assumptions, limitations and interpretations

Available data on ^{15}N abundance of plants and nitrogen sources on Ryuoh mountain slope from 1994 to 1997 are summarized in Fig. 14 and 15. Details about the isotopic composition of total N, NH_4^+ and NO_3^- in mineral soils (0 - 50-cm depth) were presented elsewhere (Koba et al. 1998). The trend in $\delta^{15}\text{N}$ distributions was that plants had lower $\delta^{15}\text{N}$ values than mineral soils (Figs. 14 and 15), which is consistent with many other studies (Gebauer and Schulze 1991, Garten 1993, Garten and Miegroet 1994,

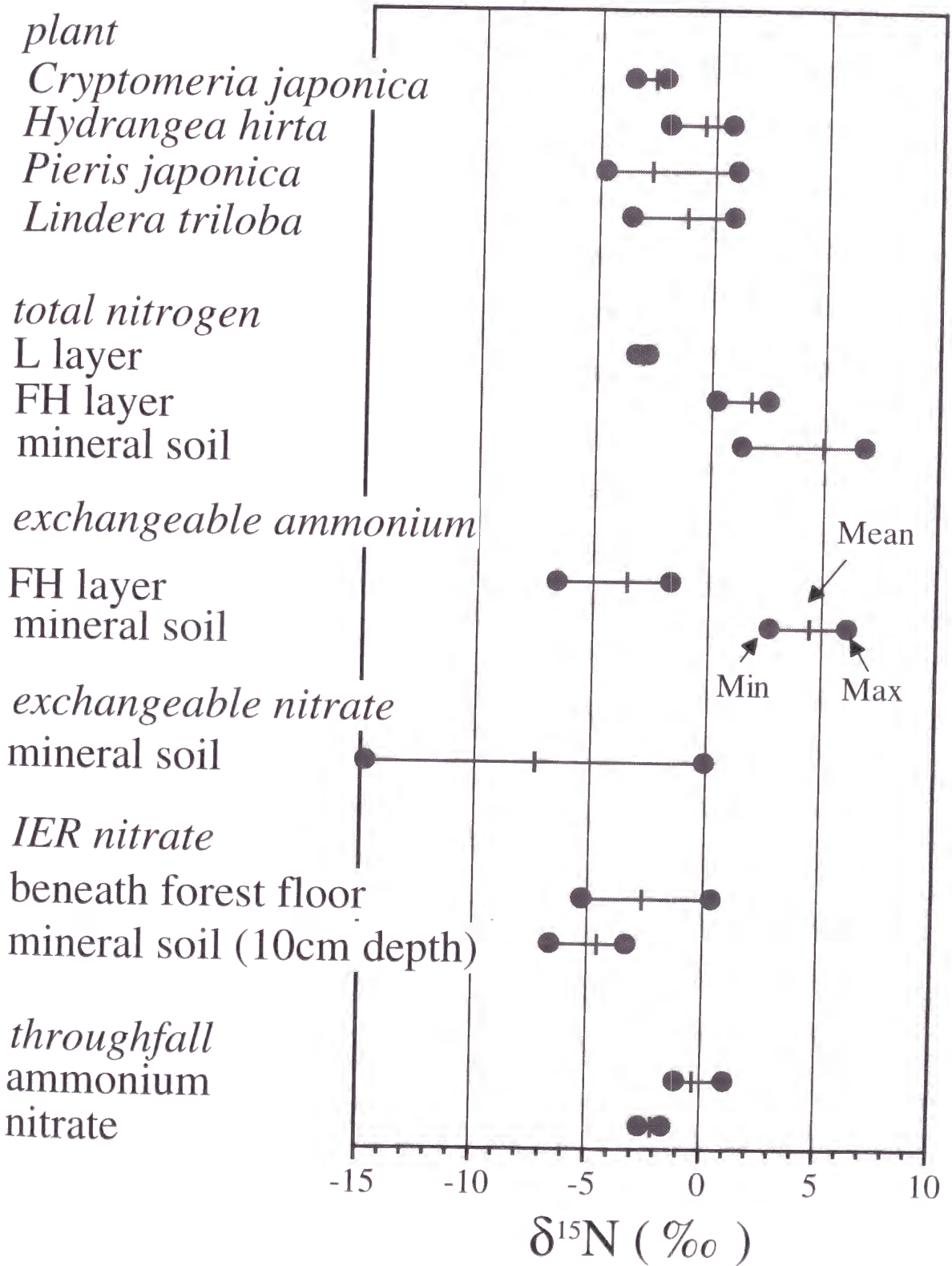


Fig. 14. Isotopic compositions of several components in Upper Site in Ryuoh watershed in Japan.

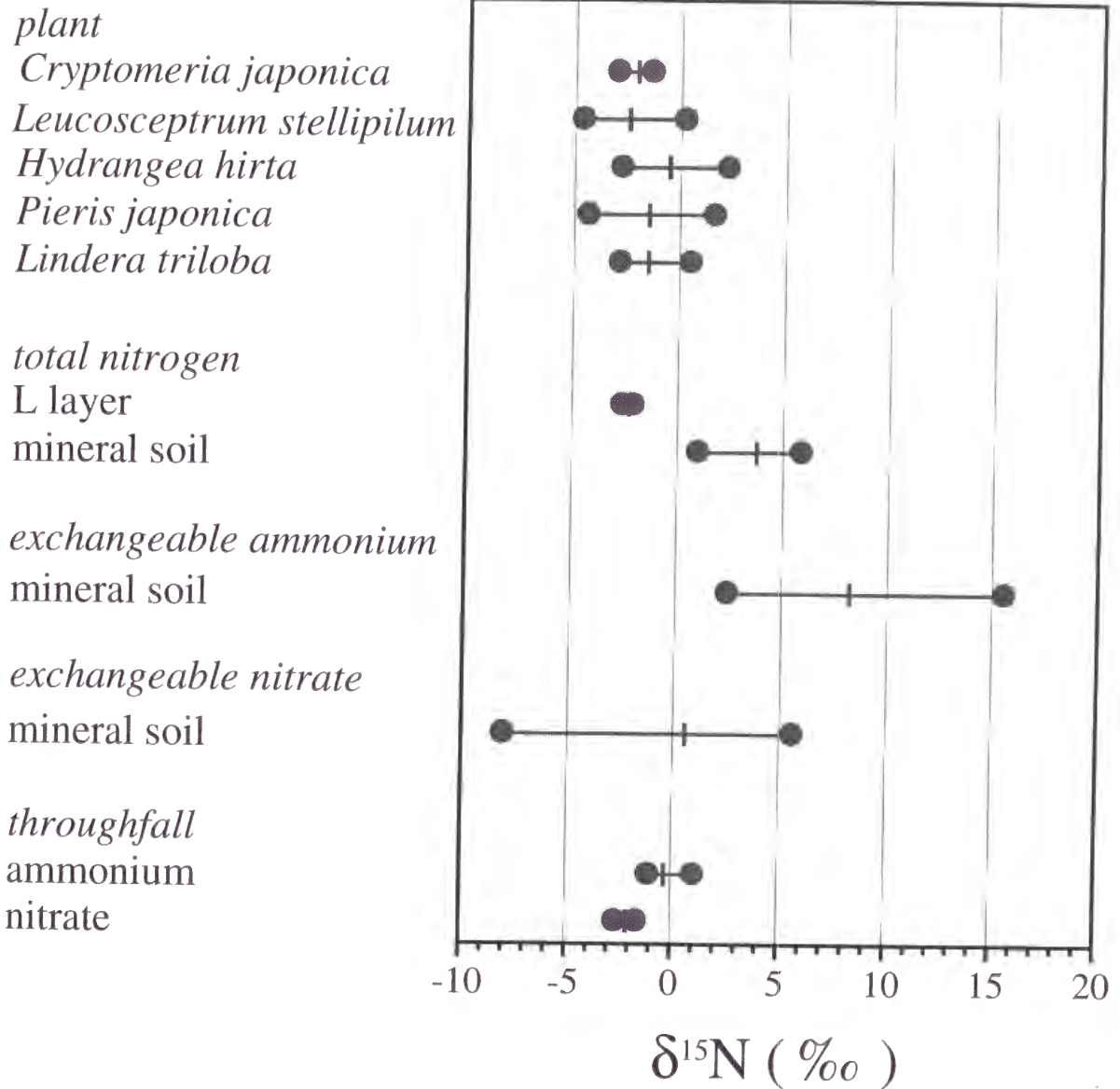


Fig. 15. Isotopic compositions of several components in Lower Site in Ryuoh watershed in Japan.

Nadelhoffer et al. 1996, Koopmans et al. 1997).

For an interpretation of $\delta^{15}\text{N}$ data, it is desirable to first examine the assumptions for a natural ^{15}N abundance study. The general assumptions used in natural abundance studies of foliar $\delta^{15}\text{N}$ are the following: (1) foliar $\delta^{15}\text{N}$ values represent the $\delta^{15}\text{N}$ values of the whole plant; (2) different nitrogen sources (e.g. NH_4^+ and NO_3^- in soils) have different $\delta^{15}\text{N}$ signatures; (3) no isotopic fractionation occurs during plant uptake of nitrogen.

We examined a validity of the assumption (1) by $\delta^{15}\text{N}$ measurements of different tissues of a plant for each understory species (Table 8). The differences in $\delta^{15}\text{N}$ between current leaves and whole

plants were $< 1\%$ for all species observed (Table 4). Thus, taking the measurement precision ($\pm 0.18\%$) into account, it is concluded that foliar $\delta^{15}\text{N}$ represented the plant $\delta^{15}\text{N}$ because of these small internal variations of plant $\delta^{15}\text{N}$ in this study.

As far as assumption (2), a preliminary work illustrated that there were differences in $\delta^{15}\text{N}$ among total N, NH_4^+ and NO_3^- in this mountain slope site because of large isotopic fractionation during nitrification (Koba et al. 1998). However, the range of $\delta^{15}\text{N}$ of total N, NH_4^+ and NO_3^- overlapped when the data from different soil depths (from forest floor to 50-cm depth of mineral soil) were compiled (Figs. 14 and 15). In these sites, fine roots of *Cryptomeria japonica* were distributed from the forest floor to the 80-cm depth of mineral soils (Kasuya and Shimada 1996). Thus, at least, we should apply all $\delta^{15}\text{N}$ data (i.e. from forest floor to 50-cm soil depth) to exploitable nitrogen sources for plants even though net mineralization rate of deeper soil were low (Koba et al. 1998). For this reason, it is impossible here to discuss the importance of each nitrogen source quantitatively by direct comparison of $\delta^{15}\text{N}$ signatures.

Many studies have focused on the validity of assumption (3). Mariotti et al. (1982) showed that there is no isotopic fractionation during NO_3^- uptake of plants. Yoneyama and Kaneko (1989) and Yoneyama et al. (1991) also indicated no isotopic fractionation during NO_3^- uptake, while there was a large isotopic fractionation ($\delta^{15}\text{N}$ of plant $< \delta^{15}\text{N}$ of NH_4^+) associated with NH_4^+ uptake. Evans et al. (1996), however, showed no isotopic fractionation during both NH_4^+ and NO_3^- uptake of a plant under low (i.e. field) levels of nitrogen supply. Although these hydroponic studies were able to determine the $\delta^{15}\text{N}$ of inorganic nitrogen, they were somewhat unrealistic in a lack of mycorrhizal infections. Handley et al. (1993) indicated no effect of ectomycorrhizal infection on the $\delta^{15}\text{N}$ of Eucalyptus seedlings when only organic nitrogen was supplied, whereas VAM infection on Ricinus influenced the whole plant $\delta^{15}\text{N}$. Michelsen and Sprent (1994) showed the opposite results on VAM-infected plants. However, a recent study observed that mean $\delta^{15}\text{N}$ of whole plants of lettuce and barley can vary up to 3.5‰ at the maximum when given a uniform nitrogen source (Azcón et al. 1998), but they could not calculate the direct isotopic fractionation between nitrogen source and plants. Thus, as of now, there is no consensus on this matter, and isotopic fractionation during nitrogen uptake by plants is considered to be unlikely under conditions of plant nitrogen limitations in well-drained soils (Nadelhoffer and Fry 1994).

In the light of these studies, we assume that (i) there is no isotopic fractionation during inorganic nitrogen uptake without mycorrhizal associations (after Evans et al. 1996), and (ii) there should be an isotopic fractionation between nitrogen source and plant leaves during assimilation of nitrogen through mycorrhizal associations (but it should be smaller than 3.5‰ after Handley et al. (1993) and Azcón et al. (1998)). Although it might be possible to interpret the ranking for $\delta^{15}\text{N}$ in each sampling period (Table 7), we think that our limited $\delta^{15}\text{N}$ data on nitrogen sources and the potential for isotopic fractionations do not allow us to discuss the small $\delta^{15}\text{N}$ differences among nitrogen compartments.

In Upper Site where the NO_3^- supply rate is considered to be low (Table 5), $\delta^{15}\text{N}$ values of plants overlap the range of total (organic) N, NH_4^+ and NO_3^- in forest floor and NO_3^- in surface mineral soils (Fig. 14). Therefore, we cannot derive direct evidence about the quantitative importance of a certain

IV. In situ nitrate availability for plants

nitrogen source. However, the $\delta^{15}\text{N}$ distribution, at least, does not contradict the interpretation based on NRA measurements and other physiological results. The $\delta^{15}\text{N}$ distributions of *Hydrangea hirta* and *Lindera triloba* which overlapped with nitrogen sources (Fig. 14), complement the interpretation supported by other results that both species used both NH_4^+ and NO_3^- . Similarly, the $\delta^{15}\text{N}$ distribution of *Cryptomeria japonica* with VAM, allows us to interpret that *Cryptomeria japonica* used both NH_4^+ and NO_3^- in soils (Fig. 14). NRA was not measured for *Cryptomeria japonica* in this study, but it was reported that this species can use NO_3^- (Matsui 1995, Akama 1996). As well as other species, the distribution of $\delta^{15}\text{N}$ values of *Pieris japonica* associated with Ericoid, implies that *Pieris japonica* used all nitrogen sources (Fig. 14). However, from the results of extremely low NRA (Table 6) we conclude that *Pieris japonica* did not use NO_3^- . Thus its $\delta^{15}\text{N}$ distribution indicates the uptake of both organic nitrogen and NH_4^+ in soils through Ericoid mycorrhizal associations. Of course we should take the isotopic fractionation into account for *Cryptomeria japonica* and *Pieris japonica*, but the wide $\delta^{15}\text{N}$ ranges of nitrogen sources in soil might mask the effect of isotopic fractionations in this site.

Furthermore, isotopic signatures implied that forest floor is important nitrogen source for all plant species, which is also supported partly by the root distribution in Upper site (Kasuya and Shimada 1996). This means that in Upper site where nitrogen availability is low, plants select forest floor as nitrogen supply zone where net mineralization is much higher than mineral soils (Table 5; Hobara and Tokuchi 1998), and in forest floor, plants use multiple nitrogen sources possibly because of severe competition for nitrogen acquisition among plants and soil microorganisms.

In Lower Site where NO_3^- availability is high (Table 5), $\delta^{15}\text{N}$ values of plants were similar to those of total soil N and soil NO_3^- (Fig. 15). As for non-mycorrhizal species (*Hydrangea hirta* and *Lindera triloba*), we conclude here that these two species used both NH_4^+ and NO_3^- both from $\delta^{15}\text{N}$ data strongly suggesting the use of soil NO_3^- (Fig. 15) and from NRA data (Table 6). With regard to VAM-infected species (*Cryptomeria japonica* and *Leucosceptrum stellipilum*), we can reach the same conclusion that these species also took up both NH_4^+ and NO_3^- supported by positive NRA (for *Leucosceptrum stellipilum*; Table 6) and $\delta^{15}\text{N}$ signature (Fig. 15), despite any isotopic fractionation during nitrogen uptake ($\pm 3.5\%$). From the $\delta^{15}\text{N}$ signature, *Pieris japonica* can also be considered to use total N and NO_3^- (Fig. 15). However, the NRA of this species might be quite low in Lower Site as observed in this study in Upper Site (Table 6), and many studies on Ericaceous plants (Ingestad 1973, Haynes and Goh 1978, Nadelhoffer et al. 1996). Thus we conclude that the $\delta^{15}\text{N}$ signature of *Pieris japonica* probably reflects both organic nitrogen and NH_4^+ uptake with an isotopic fractionation. Ericoid-associated plants have shown quite low $\delta^{15}\text{N}$ values all over the world (Gebauer and Dietrich 1993, Schulze et al. 1994, Högberg et al. 1996, Nadelhoffer et al. 1996). This is due the isotopic fractionation during the transfer of nitrogen through Ericoid mycorrhizae, and probably to shallow rooting system concentrated into FH layer which is typical for Ericaceous species, *Pieris japonica* might have relatively low $\delta^{15}\text{N}$ values in both sites in spite of low NO_3^- use.

The coincidence between $\delta^{15}\text{N}$ of plants and soil NO_3^- was clear in Lower Site (Fig. 15), whereas

the distribution of plant $\delta^{15}\text{N}$ was skewed towards that of NH_4^+ in Upper site (Fig. 14). Taken together, this difference in $\delta^{15}\text{N}$ distribution indicated well the different availability of NO_3^- for plants in these sites.

Availability of NO_3^- in undisturbed coniferous forest soils

In this study, we can not distinguish among preferential NH_4^+ , NO_3^- or total N uptake by vegetation. However our NRA measurements and $\delta^{15}\text{N}$ signatures in this ecosystem showed, at least, that plants used NO_3^- even in Upper Site where NO_3^- availability is considered to be quite low. We can conclude that plants used both NH_4^+ and NO_3^- except *Pieris japonica* (which used organic nitrogen and NH_4^+) in Upper and Lower Sites.

It is well known that NO_3^- can move much faster than NH_4^+ in forest soils because of the positive charge of NH_4^+ (Killham 1994). However, NH_4^+ assimilation is energetically less costly than NO_3^- assimilation (Raven et al. 1992). The availability of each nitrogen form for a plant in an intact soil-plant system is considered to be a consequence of several advantages and disadvantages for plants against competitors (e.g. soil microorganisms) affected by root morphology, mycorrhizal association, other nutrient status of the plant, energy cost to assimilate, accessibility and so forth. However, it is quite noteworthy here that our results illustrated that NO_3^- uptake of plants is substantial even in Upper Site where net nitrification rate is quite low. This availability of NO_3^- for plants in an undisturbed coniferous forest ecosystem strongly complements the fact that NO_3^- availability for soil microorganism is relatively larger than expected (Davidson et al. 1992, Hart et al. 1994, Stark and Hart, 1997). It is likely that plants can compete efficiently against soil microorganisms with regard to NO_3^- use because plants can afford the energy cost of NO_3^- reduction. Or the availability of NO_3^- for plants might become relatively high because soil microorganisms are considered to be much stronger competitors for NH_4^+ than plants. Thus, it is not surprising that availability of NO_3^- for plants is higher than expected on the basis of net nitrification rates and NO_3^- pool sizes in forest soils. This kind of high availability of NO_3^- for plants compared to that of NH_4^+ was reported in tracer experiments (Jackson et al. 1989, Schimel et al. 1989, Norton and Firestone 1996). The consistent pattern of lower $\delta^{15}\text{N}$ values of plant than in soils observed all over the world (e.g. Fry 1991) might partly reflect the plant uptake of NO_3^- which has lower $\delta^{15}\text{N}$ than total soil nitrogen (Koba et al. 1998).

It is concluded that plants utilized NO_3^- even under the condition of low NO_3^- supply in an intact soil-plant system, which can be considered as a consequence of niche differentiation for nitrogen acquisition of plants (Fig. 16). In a site with high nitrogen availability (Lower Site) with high gross mineralization rates (Tokuchi et al. 1998), NO_3^- uptake would be advantageous for plants because of high mobility of NO_3^- , and possibly because plants can earn energy for NO_3^- assimilation through photosynthesis by themselves. Thus plants would depend highly on NO_3^- in Lower site. On the other hand, in Upper site where NH_4^+ was accumulated because gross mineralization rate was much higher than immobilization of NH_4^+ , and NO_3^- pool was very small because of low gross nitrification rate, severe competition for N between soil microorganisms and plants would occur as proposed by Kaye and Hart (1997). This severe competition for N uptake would urge the plants to take several N sources.

IV. In situ nitrate availability for plants

Upper site (low N availability)

Lower site (high N availability)

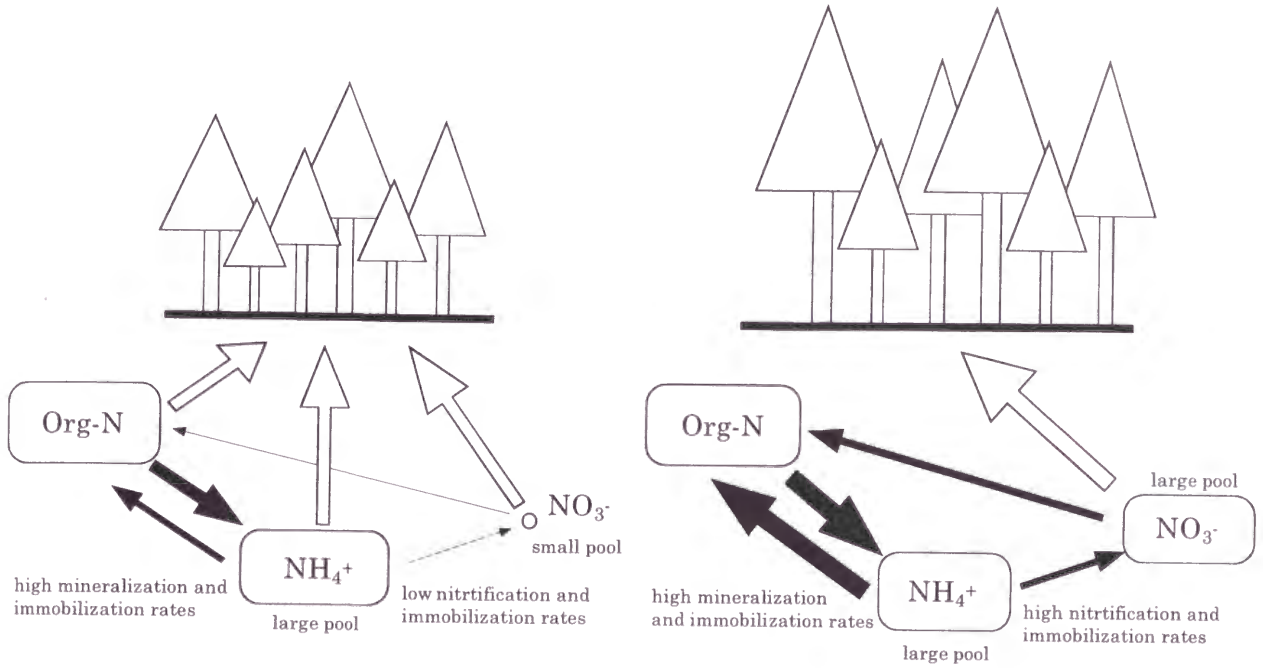


Fig. 16. Schematic model of N dynamics in two sites in one mountain slope. N sources for plants are diverse in low N availability site (Upper site), while plants largely rely on NO_3^- in high N availability site (Lower site).

In this study, the natural abundance method could derive the in-situ availability of NO_3^- for plants, although more precise and detailed information about d^{15}N signatures of nitrogen sources and plants are strongly required for further discussion on relative importance of each nitrogen sources. This importance of NO_3^- in the soil-plant system suggests the strongly tight nitrogen cycle in a forest ecosystem which should be characterized by small loss of nitrogen from the ecosystem.

V. GENERAL DISCUSSION

Nitrogen isotope fractionation factor as an essential for tracing nitrogen flows

Natural abundance of nitrogen isotopes can reveal the importance of nitrogen flow; denitrification and plant uptake of NO_3^- in forest soils. About denitrification, as emphasized in Kellman and Marcel (1998), it is still difficult to determine the extent of denitrification in intact ecosystem. The results in Kiryu watershed can provide the possibility for the natural abundance method to be an important method to, at least, obtain an insight for nitrogen cycling in an intact system. For example, Böhlke and Denver (1995), Komor and Magner (1996) and Hedin et al. (1998) used $\delta^{15}\text{N}$ of inorganic nitrogen to get insight of the occurrence of denitrification.

In Fig. 17, isotopic data in Ryuoh watershed were summarized together with apparent fractionations during mineralization and nitrification in mineral soils. $\delta^{15}\text{N}$ of total nitrogen in L layer was similar to that of *Cryptomeria japonica* which was the dominant species in Ryuoh watershed (Fig. 17). Between L and FH layers, large difference in $\delta^{15}\text{N}$ was observed (4.31‰) which would be caused by rapid decomposition of organic nitrogen in forest floor. To determine a fractionation factor during decomposition of organic nitrogen in forest floor, litter-bag samples of *Cryptomeria japonica* in Hiei mountain in Shiga prefecture were analyzed (gifted from Mr. Yamashita, Shimane University; Fig. 18). In spite of large change in nitrogen concentration, $\delta^{15}\text{N}$ values of litters decomposed were quite stable, and apparent fractionation factor was estimated as -0.64‰ (Fig. 18), very small compared with that of mineral soils (-

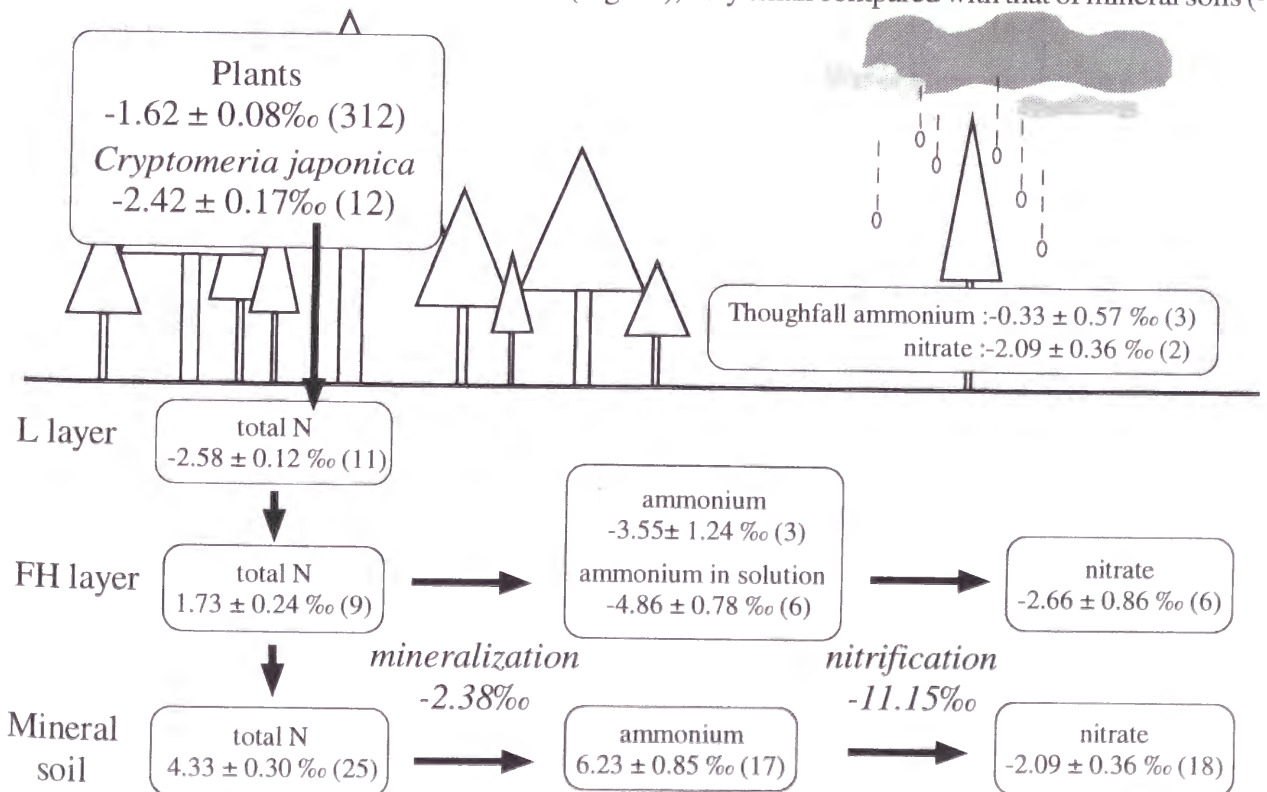


Fig. 17. Isotopic map in Ryuoh watershed with emphasis on isotopic compositions of inorganic nitrogen in soils.

V. General Discussion

2.38‰ in average; Fig. 11). As far as I know, the study on the relationship between decomposition and nitrogen isotope ratio using litter bags, was that of Melillo et al. (1989), which showed complex fluctuation along the decay continuum. Furthermore, the pattern of $\delta^{15}\text{N}$ variability of inorganic nitrogen in forest floor

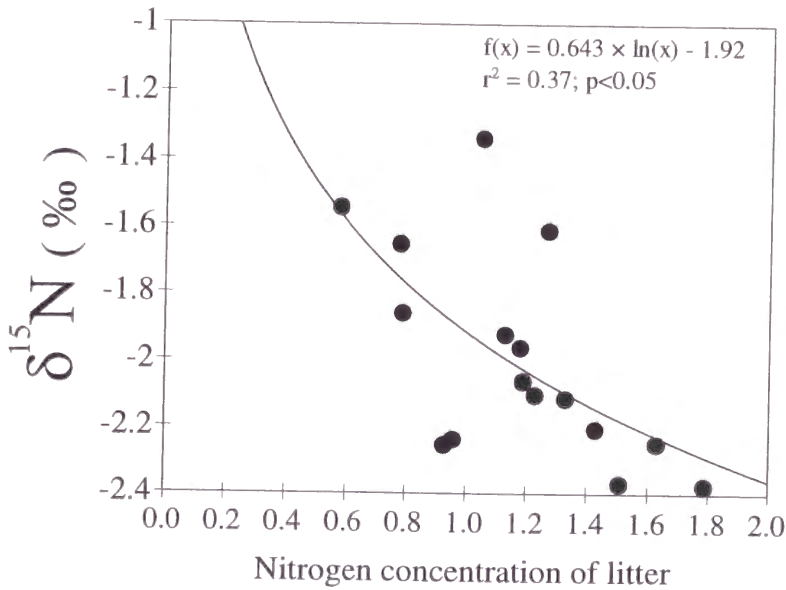


Fig. 18. Isotopic fractionation during decomposition of *Cryptomeria japonica* litter in Mt. Hiei (gifted from T. Yamashita).

(FH layer), was seemed different from that in mineral soils, as well as total N. $\delta^{15}\text{N}$ value of NH_4^+ was lower than that of total nitrogen and NO_3^- in FH layer (Fig. 17). This was considered to be affected partly by strong cation exchange of NH_4^+ by organic matter (Tokuchi et al. *submitted*) and partly by strong immobilization of NH_4^+ and NO_3^- by high C/N of soils. Exchangeable NH_4^+ had higher $\delta^{15}\text{N}$ value than NH_4^+ in soil solutions captured by IER (Fig. 17). This suggested that heavy NH_4^+ was fixed into exchangeable sites of soils as shown by Delwiche and Steyn (1970) and Karamanos and

Rennie (1978). On the effect of immobilization on isotopic compositions of inorganic nitrogen in soil, Shearer et al. (1974) reported mathematically that immobilization rate could be an important factor to determine $\delta^{15}\text{N}$ values of inorganic and organic nitrogen. Although we can't explain the isotopic signature in forest floor, the fact that isotopic signature of N in forest floor is quite difficult from that in mineral soil which is relatively easy to predict, is quite important to trace N flow in forest ecosystems.

We can show now here that natural abundance of inorganic nitrogen can easily fluctuate largely depending on environment (redox condition for denitrification in Kiryu watershed, soil pH and/or C/N ratio for nitrification in Ryuoh watershed), as well as observed in Jordan et al. (1997). From the data on inorganic nitrogen in soils and soil solutions, we could calculate the apparent isotopic fractionation factors for denitrification (-5.57 and -6.00‰ in Kiryu watershed; Fig. 6), mineralization (-1.17 to -3.32‰ for mineral soils in Ryuoh watershed; Fig. 11) and nitrification (-11.15‰ for mineral soils in Ryuoh watershed; Fig. 13). These were rough estimations because of the small number of samples. However, it was likely that NO_3^- dynamics and nitrification are the determinant of great importance for isotopic compositions of inorganic nitrogen, which would affect those of plants, on account of its large fractionations during nitrification and denitrification, and of its high mobility in forest soils. Additionally, $\delta^{15}\text{N}$ of NO_3^- in soil was increased during 35-day incubation in Ryuoh watershed (Fig. 19). Of course this increase in $\delta^{15}\text{N}$ of NO_3^- was partly denitrification during incubation, it was likely that immobilization of inorganic nitrogen enhanced

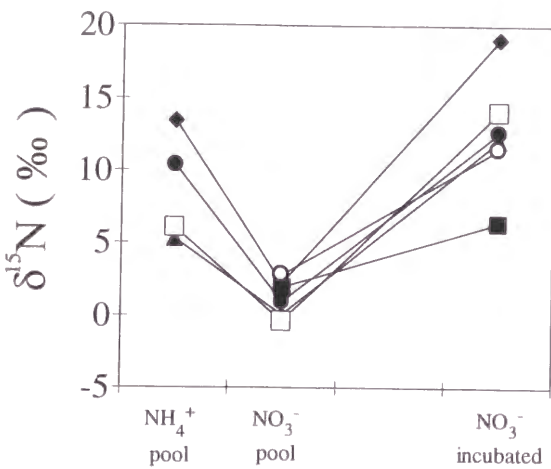


Fig. 19. Isotopic enrichment of inorganic nitrogen during incubation (35 days) of forest soils collected from Lower Site in Ryuoh watershed. Because of high net nitrification rates, no data was obtained on NH₄⁺-N after incubation.

nitrogen cycle in a soil, which led that increase.

What we can derive from field and incubation data on isotopic signatures as well as calculated apparent isotope fractionation factors, is that inorganic N can have wide range of δ¹⁵N, but both plants and inorganic N in soil have narrower range of δ¹⁵N in a forest ecosystem than expected on the basis of isotope fractionation. I think that this skewness of δ¹⁵N in a forest ecosystem would imply an intense N dynamics among soil, soil microorganisms and plants. Inorganic pools cannot be enriched as expected by apparent isotopic fractionation in natural condition (field), but in an incubation study, they can be easily enriched (Fig. 19), which can be explained that δ¹⁵N of inorganic N was controlled by plant uptake. Taking an

isotope fluctuation of inorganic N transformation (mineralization and nitrification), we should be able to observe large isotopic fluctuation of inorganic N in an intact forest ecosystem such as observed by Jordan et al. (1997), because there must be a chance for inorganic N to be enriched by nearly complete nitrification (for NH₄⁺) and denitrification (for NO₃⁻). However, δ¹⁵N data of inorganic N didn't show such large range of δ¹⁵N of inorganic N (Fig. 13) as compared with Jordan et al. (1997). The significant difference between two site is N supply; one is Wastewater Treatment Plant with high inorganic N concentration in water, and the other is the forest ecosystem considered as N-limited site. Thus, isotopic signature of inorganic N is probably strongly regulated by plant uptake which may not affect isotopic signature of inorganic N itself (Evans et al. 1996), but do inorganic pool size directly (Fig. 20). This indicates a subtle balance among N pools and N flows to plants, soil and soil microorganisms that determine

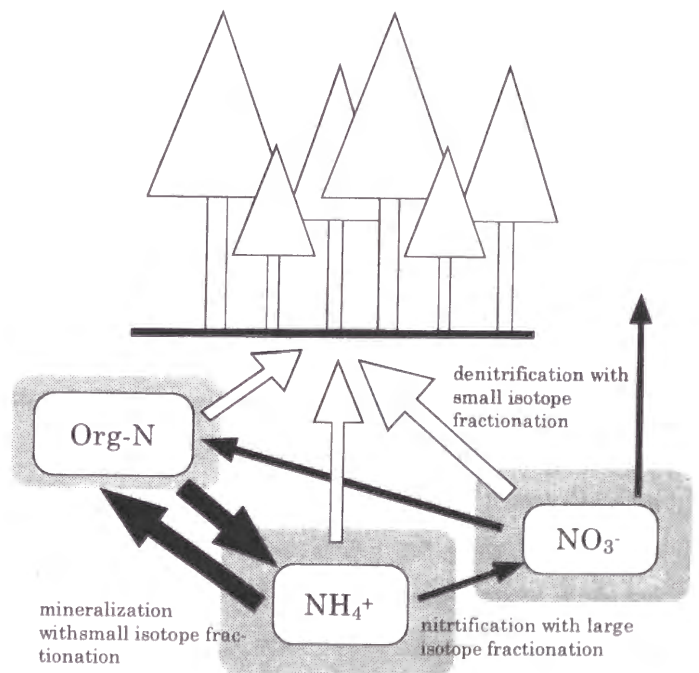


Fig. 20. Schematic model of N dynamics affected by plant uptake. Plant uptake can affect inorganic N pool more than expected, which results in small pool size of inorganic N with moderate isotopic signature in a N-limited ecosystem. If plant uptake is negligible, pools are larger (as shadowed) and isotopic signatures of them would have much wider range according to isotopic fractionation, suggested by incubation data.

V. General Discussion

pool and isotopic signature of inorganic N in the forest ecosystem; N cycle system which can be driven easily in incubation (e.g. complete consumption of NH_4^+ by nitrification) cannot in an intact ecosystem. Have we been able to observe well such subtle, and probably delicate feature of N dynamics in an intact ecosystem by using incubation and tracer experiments? The picture drawn here by natural abundance of N isotopes tells us some dynamic and subtle features of N dynamics in intact ecosystems and thus, natural abundance method can be a great tool to connect laboratory and field.

In spite of these large fractionations for nitrogen transformation processes, from which we can easily expect the large fluctuations of plant $\delta^{15}\text{N}$ values according to the fluctuations of isotope signatures of nitrogen sources, plant $\delta^{15}\text{N}$ value was negative and quite constant (Fig. 17). Such lower values of plants compared with nitrogen sources (inorganic nitrogen in soils) was similar to the results of Jordan et al. (1997). Their data indicated that $\delta^{15}\text{N}$ values of available nitrogen for plants were higher than 11‰, while those of plants were 1~8‰ lower. They interpreted that excess provide of nitrogen compared with plant demand in their field (Wastewater Treatment Plant), which should lead an isotopic fractionation during plant uptake of nitrogen, could be responsible for this disagreement between nitrogen sources and plants regarding to nitrogen isotope ratio. Such disagreement in $\delta^{15}\text{N}$ values between plants and nitrogen sources was observed in Stewart et al. (1997) where most of plants had negative $\delta^{15}\text{N}$ values while total nitrogen, NH_4^+ and NO_3^- had positive values.

From the data in Ryuoh watershed, two subjects could be suggested to explain those lower $\delta^{15}\text{N}$ values of plants compared to those of inorganic nitrogen;

1. plant uptake of NO_3^- which had small pools and high mobility in forest soils
2. plant uptake of inorganic nitrogen from forest floor.

As emphasized throughout this study, the importance of NO_3^- dynamics had been underestimated because of its small pool sizes. Furthermore, as far as I know, there are no studies that dealt with $\delta^{15}\text{N}$ of inorganic nitrogen in forest floor. Uneven distribution of $\delta^{15}\text{N}$ values of plants in the world (especially from temperate to subarctic ecosystems) toward negative value, was now considered to be responsible for these two factors which one should consider in the study on nitrogen source for plants by using natural abundance method. This consequence derived from natural abundance method which could illustrate the *in situ* processes in intact ecosystems, might imply the importance of NO_3^- and forest floor for plants. On the other hand, plants seem to have relatively higher $\delta^{15}\text{N}$ values in tropical ecosystems than in other ecosystems such as temperate ecosystems (Yoneyama et al. 1993). Partly this enrichment is caused by high rates of nitrogen cycling in forest soil in tropical ecosystems, which is expected from high $\delta^{15}\text{N}$ value of ploughed agricultural soil (Mariotti et al. 1980). However, expected high denitrification rates of tropical forest soils might be a significant factor for the enrichment in ^{15}N of plants (and soils) because isotopic fractionation during mineralization was not so large that only internal cycle of nitrogen in forest soil (from mineralization to immobilization) could not explain completely that enrichment. Thus, I could now refine the first topic as

- 1' plant uptake of NO_3^- which had small pools and high mobility in forest soils *with high vari-*

ability in $\delta^{15}\text{N}$ dependent of nitrification (leading low $\delta^{15}\text{N}$ value) and/or denitrification (leading high $\delta^{15}\text{N}$ value).

^{15}N natural abundance method: beyond its limitations

In this study, natural abundance of N can give us useful information on N flow in intact ecosystems. However, either on denitrification or on plant preference for different nitrogen forms, it is the fundamental isotopic information that we have to consider and obtain to make natural abundance method a reliable tool. In an excellent review by Handley and Scrimgeour (1997), they suggested several points to which we have to pay attentions in natural abundance study as;

- (1) the possible limits of interpretation of natural abundance method
- (2) the existing knowledge in isotope chemistry without extrapolating field data
- (3) detailed background knowledge of the study subject
- (4) s/n of isotopic signatures
- (5) data of sources, sinks and fractionations as many as possible
- (6) careful experimental design and combined this with use of statistical analyses
- (7) misusing the term 'qualitative' as 'quantitative'.

In the light of this comment, what kind of approaches can we take in the study on nitrogen dynamics? My approach has been especially to establish firm background on isotopic signatures to interpret the ones of target materials accurately. Although our present analytical method, especially for inorganic nitrogen measurements (e.g. Kjeldahl distillation + Rittenberg reaction for NH_4^+ and NO_3^- conversion into N_2 gas) is not a highly reliable one, and it is required to establish new methodology (Handley and Scrimgeour 1997), we have been challenging to measure what they have resigned themselves to measurement by using and refining present (traditional) methods. According to the $\delta^{15}\text{N}$ data of inorganic nitrogen, it might be suggested that ^{15}N natural abundance method should be applied to the ecosystem which has a unique feature on nitrogen cycle in the view of $\delta^{15}\text{N}$ signatures. For example, we have measured plants $\delta^{15}\text{N}$ values in forested ecosystem with additional nutrient input by cormorants in Shiga prefecture, Japan. We observed high $\delta^{15}\text{N}$ values of plants (up to 19‰) in the site where cormorants nest and their droppings have also high $\delta^{15}\text{N}$ (15‰), while 0‰ where cormorants have not nested yet (Kameda and Koba, unpublished data). Unfortunately, anthropogenic nitrogen inputs might affect little on $\delta^{15}\text{N}$ of plants (Garten and Van Miegroet 1994, Koopmans et al. 1997), the ^{15}N -enriched provided from nitrogen sources with high trophic level (birds, fish, human etc.) could establish the suitable situation for ^{15}N natural abundance method (Ben-David et al. 1998, Erskine et al. 1998, Wainright et al. 1998) as well as slight-enriched tracer experiment (Fry et al. 1995).

Another conclusion for appropriate topic for ^{15}N natural abundance method is to determine the emission processes of trace gases, especially N_2O . Because N_2O during nitrification (Yoshida 1988) and denitrification (Mariotti et al. 1981), isotopic fractionations must be large enough to trace the nitrogen flows by using present methodology. ^{15}N natural abundance method seems appropriate in this study field

V. General Discussion

and provides significant insights in N_2O global budgets (Yoshida and Matsuo 1983, Yoshida et al. 1984, 1989, Wada et al. 1996). Recently double isotope tracing of N_2O using oxygen and nitrogen isotope ratios successfully applied (Yoshinari 1990, Kim and Craig 1993, Yoshinari and Koike 1994, Rahn and Wahlen 1997, Yung and Miller 1997, Yoshinari et al. 1997, Dore et al. 1998, Naqvi et al. 1998). Such isotopic signatures, combined with nitrogen and oxygen isotope compositions of NO_3^- (Amberger and Schmidt 1987, Böttcher et al. 1990, Durka et al. 1994, Kendall et al. 1995, Wassenaar 1995, Révész et al. 1997, Silva et al. in press), will provide the information in high quality on NO_3^- dynamics in complex systems like forest soils.

Although there are many problems based on the lack of information on isotope fractionation during N transformations and small numbers of isotopic data, I present natural abundance method as a promised methodology to trace N flows in intact ecosystems and bridge between laboratory and field.

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Synopsis (In Japanese)

森林生態系の窒素循環はこれまで様々に研究されてきたが、現在まで用いられている手法では、野外での窒素循環の実態を捉えることが困難である。そのため、現実の循環系で生じている様々な過程を捉える手法の確立が急務となっている。申請者は、物質中の窒素自然安定同位体比を1つの指標とし、この値の変動を追跡することにより、複雑かつ不均一な森林生態系での窒素循環の実態を明らかにすることを目的として研究を行った。

得られた主要な結論は以下の通りである。

1) 滋賀県南部のヒノキ人工林において、森林土壌溶液中の硝酸についてその濃度、窒素安定同位体比の変動を追跡した。同位体比の変動から、土壌水分条件の変動によって硝酸が脱窒過程を受け、消失していることを確認し、普通の森林においても、土壌水分が上昇することで脱窒過程が生じていることが明らかになった。

2) 滋賀県東南部のスギ人工林において、森林での植物、土壌中の窒素の安定同位体比を3年間にわたって追跡した。まず、土壌中の無機態窒素についての同位体比測定を行い、アンモニアと硝酸が異なる同位体比を取ることを明らかにした。この結果に基づき、植物の同位体比がどちらの窒素の同位体比に近いか調べることで、植物の窒素源が判定できるという可能性を示した。そこで、植物と土壌中のアンモニア、硝酸について窒素同位体比を比較し、硝酸と植物の同位体比が近かったことから、植物にとって硝酸が重要な窒素源であることを明らかにし、養分供給の少ない斜面上部では、植物が複数の窒素源を利用していることを示唆した。

これら安定同位体を用いた森林生態系の窒素循環に関する研究の一連の成果から、実際の森林における脱窒過程および植物-土壌間の窒素循環の実態を明らかにした。同時に窒素安定同位体比というパラメーターを用いることが、森林生態系をほとんど攪乱することなく、窒素循環の実態を明らかにする有効な手法であることを示した。

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