博士論文

Study on nitrogen deposition and transformation dynamics along the canopy–soil continuum in a suburban forest near Tokyo metropolitan area

東京首都圏近郊林の樹冠–土壌連続系における窒素の沈着とその形態変化に関する研究

石　　珺

東　京　大　学

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Chapter 1

Introduction

1.1 Global nitrogen cycle

1.1.1 Nitrogen

Nitrogen (N) is a common element in the universe, discovered by Rutherford in 1772 (Galloway et al. 2013). It is the fifth most abundant element in the solar system. It is primarily either tied up in sedimentary rock (−20%) and requires extraction or exists as triple-bonded N in the atmosphere (−78%). Nitrogen is required by all organisms. Although nitrogen is abundant on the earth's surface (there are 5 billion metric tons contained in the atmosphere, oceans, terrestrial and marine biota, soil organic matter and sedimentary rock), only ~2% is available to organisms (Galloway 1998). Nitrogen exists in many different forms, with oxidation states between +5 and −3 (+5: HNO_3; +4: NO_2; +3: HNO_2; +2: NO; +1: N_2O; 0: N_2; −3: NH_3, NH_4^+) (Gu et al. 2013).

Nitrogen is essential for many processes and is crucial to any life on earth. It is a component in all amino acids as incorporated into proteins, and is present in the bases that make up nucleic acids, such as RNA and DNA. In plants, nitrogen is used in chlorophyll molecules, which are essential for photosynthesis and further growth. Although the earth's atmosphere is an abundant source of nitrogen, most is relatively unusable for plants. Chemical processing or natural fixation, which are necessary to convert gaseous N into forms usable by living organisms, make nitrogen a crucial component in food production.

1.1.2 Global nitrogen cycle

Nitrogen can be transformed biochemically or chemically through a number of processes, conceptually summarized as the nitrogen cycle (Nieder and Benbi 2008). Nitrogen is one of the most important nutrients in terrestrial ecosystems. Plants and microbes often compete to acquire bioavailable nitrogen, such as inorganic N and some low-molecule organic N compounds. Fertilizers that include nitrogen stimulate plant growth in most habitats (Albrektson et al. 1977, Tilman 1984), which suggests that the availability of N limits plant production in ecosystems (Ricklefs 1990). Therefore, the nitrogen cycle is one of the most intensively investigated topics in ecosystem ecology. It is also widely known that the N cycle is one of the most complex biogeochemical cycles within terrestrial and aquatic ecosystems (Thamdrup 2012).

The nitrogen cycle is the process by which N is converted among its various chemical forms. This transformation can be carried out through both biological and physical processes. Fig. 1-1 illustrates the mass balance cycle of N in a natural ecosystem.

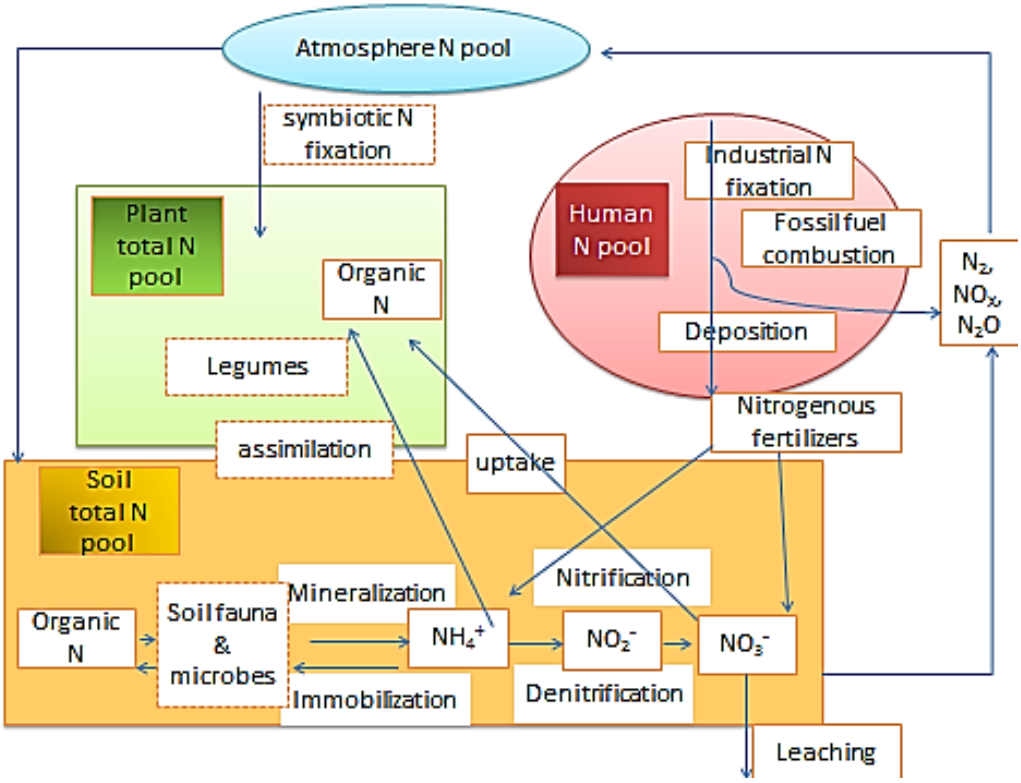

Fig. 1-1 N cycle in a natural ecosystem

Gaseous atmospheric nitrogen (N_2, NO_X, N_2O) is naturally fixated by lightning. In its fixed form, nitrogen is introduced into ecosystems by dry and wet deposition, particularly in the wet tropics. This process accounts for a small portion of all fixated gaseous N. Most fixation is carried out by free-living or symbiotic bacteria. After fixation, gaseous N is changed into ionic nitrogen (e.g., NH_4^+, NO_3^-). It is then taken up by plants and herbivores, thereby entering the food chain. Finally, with decomposition of organic N by microbes, nitrogen is released into the atmosphere through a series of complex soil processes, such as nitrification, denitrification, and mineralization.

1.1.3 Anthropogenic nitrogen deposition impacts on global nitrogen cycle

Natural pre-industrial nitrogen cycles are of similar magnitude on land and in the sea. On the modern earth, rates of net primary production are nearly equally balanced between land and sea at about 4 $\times 10^{15}$ mol yr^{-1} each (Canfield et al. 2010). But with increasing population and rapid industrial development since the advent of industrialized society, especially in the 20th century, human activities have increasingly influenced the global nitrogen cycle. There are now large regions of the world where average N deposition rates exceed 10 kg N ha^{-1} yr^{-1}, more than an order of magnitude increase compared with natural rates (Gu et al. 2013). More than half the reactive nitrogen (Nr) fixed each year is driven by human activity (Mosier 2004). The main impacts of such activity are from agricultural production (Bodirsky et al. 2012), industrial waste and life fertilizers. Although nitrogen is an indispensable nutrient, only a portion of gross Nr is absorbed by plants. For example, only 35% to 65% of Nr applied to croplands is taken up by plants.

Massive acceleration of the nitrogen cycle as a result of the production and industrial use of artificial nitrogen fertilizers worldwide has enabled humankind to substantially increase food production, but has caused a host of environmental problems, ranging from eutrophication of terrestrial and aquatic systems to global acidification (Gruber and Galloway 2008).

Because of human activities, nitrogen may interfere with natural systems. When nitrogen exceeds saturation in a natural system (Aber et al. 1998), the excess N can leach into lakes, streams, groundwater (Van Puijenbroek et al. 2014), or other water bodies. An overabundant availability of N leads to loss of biodiversity and the destruction of balanced ecosystems (Ohte et al. 2010). In nitrous oxide form (N_2O), N contributes to global warming (Gu et al. 2013).

1.2 Nitrogen dynamics in the forest ecosystem

1.2.1 Suburban forest

Suburban forests, especially urban ecosystems, have a unique role in the ecosystem, by carrying out vital ecological functions such as providing wildlife habitat (Adams 2005). The forests also have many positive effects on human health (Tzoulas et al. 2007). Moreover, they have important socioeconomic functions such as stormwater management (Qi and Altinakar 2012). Suburban forests are different from wild, primeval forests since they are more influenced by human activities.

1.2.2 Forest nitrogen dynamics

The main processes of the N cycle in forests include the following: 1) atmospheric N deposition to soil or plant surfaces by rain and wet/dry deposition; 2) partial absorption of atmospheric N by plants and microbes as a result of rainfall—the remaining N reaches the soil in throughfall from plant surfaces; 3) internal N cycle in soil; 4) following complex dynamic soil processes, leaching of nitrogen (NO_3^-) into lakes, streams, groundwater or other water bodies, or as gaseous N (e.g., N_2O, NO_X) back to the atmosphere (Fig. 1-2).

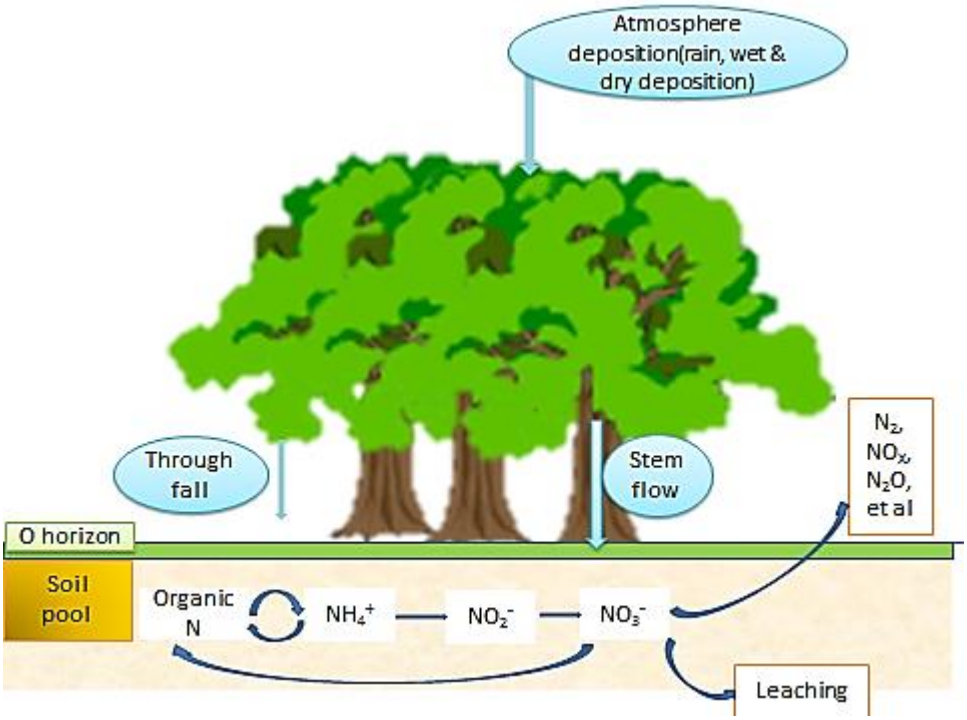

Fig. 1-2 Nitrogen cycle in forest ecosystem

Atmospheric nitrogen compounds, including both inorganic and organic N, are derived from wet and dry deposition. Many studies have indicated that the forest canopy contributes to capturing more aerosol particles by dry deposition compared with surface conditions without a tree canopy, by causing their attachment to plant surfaces. Consequently, higher amounts of nitrogen are incorporated into forest ecosystems with rich canopies, compared with those with poor canopies (Nordén 1991, Freer-Smith et al. 2004, Imamura 2014). In general, leaves have the ability to exchange dissolved N. Moreover, N compounds attached to the canopy on leaf, branch and trunk surfaces can be washed off and transported by throughfall and stemflow during rainfall.

Because organic and inorganic N is continuously supplied to the tree canopy via atmospheric deposition, microbes can utilize those substrates on the surfaces of leaves and trunks (Lindow and Brandl 2003). For example, Gaige et al. (2007) conducted

whole-forest canopy N fertilization and showed that a significant portion of additional N was retained by canopy processes. They speculated that dissolved organic nitrogen (DON) formation by lichens and microbes were dominant processes for effective retention of additional N, rather than plant uptake and assimilation. Lovett (1992) stated that observed canopy NH_4^+ retention at various forest sites in the United States is attributable to plant adsorption and assimilation and not to nitrification processes.

Nitrogen exists in many different forms in soils, plants, animals and atmosphere. Nitrogen dynamics in soil, referred to as the internal N cycle, are complex and diverse, can be affected by many factors including microbe biomass, water content, soil temperature, and soil moisture.

Since the late 1980s, excess N input has been reported to cause increased stream NO_3^- concentrations in forest catchments of the northeastern United States and northern Europe, in what is called "N saturation". A hypothetical scenario of the expected temporal evolution of N saturation, which includes plant decomposition and high N export through hydrological systems, has been proposed. Since then, many studies and discussions have focused on these issues (Aber et al. 1998, Skeffington and Wilson 1988, Aber et al. 1989, Stoddard 1994). To gain a precise understanding of N saturation in forest ecosystems, elucidating the mechanisms of N dynamics in soil and responses to excess N input is essential. In particular, the retention mechanisms of N in soil are the most important factors for understanding N saturation in ecosystems. Mitchell et al. (1997) collected N input-output data from 24 Japanese forest catchments nationwide (43 ℃N to 35 ℃N latitude) to evaluate the status of N saturation. They indicated that forests receiving relatively high inorganic N input (7.6–10.5 kg N ha^{-1} $year^{-1}$) were concentrated on the Kanto Plain and surrounding mountains. The average of all sites was 6.3 kg N ha^{-1} $year^{-1}$, and high N-input sites discharged higher

inorganic N (13.5–28.0 kg N ha^{-1} $year^{-1}$) compared with other sites. The authors interpreted this as one of the symptoms of N saturation in this region of Japan.

In most forest ecosystems, organic matter represents the largest stores of N, and soil organic N can be mineralized and partly nitrified. The produced inorganic N species (NH_4^+ and NO_3^-) can be immobilized by microbes and eventually return to organic forms. Among these multiple transformation processes, the most critical functions controlling retention capability are microbial immobilization.

1.3 Stable isotopes of oxygen and nitrogen

Isotopes are atoms with the same number of protons and electrons but differing numbers of neutrons. Stable isotopes are defined as those that are energetically stable and do not decay (Michener and Lajtha 2008). There are three stable isotopes of oxygen (^{16}O, ^{17}O, ^{18}O) and two of nitrogen (^{15}N, ^{14}N).

Stable nitrogen and oxygen (O) isotopes have been used to identify sources of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) and their relative contributions to the total pool of NO_3^- in an ecosystem (Gruber and Galloway 2008, Aber et al. 1997). Kendall (1998) (Kendall and MacDonnell 1998) showed that $\delta^{18}O$ and $\delta^{15}N$ values of NO_3^- from various sources have distinct isotopic signatures. Since the development of the microbial denitrifier method, which enables higher throughput of samples than previous methods (Sigman et al. 2001), there has been a dramatic increase of data on isotopic compositions of NO_3^- in both marine and terrestrial ecosystems. Especially for the latter, the usefulness of the method has been recognized for its capability to distinguish sources of NO_3^- between atmospheric processes and microbial production in soils (Staelens et al. 2007, Ohte et al. 2004a, Tobari et al. 2010, Sebestyen et al.

2008a). This is because atmospheric NO_3^- has distinctly higher $\delta^{18}O$ values (approximately 60% to 80%) than NO_3^- originating from microbial processing (approximately −15% to 15%) (Kendall et al. 2007).

Nitrification, however, is also a key process responsible for the transformation of ammonia (NH_3) into ammonium (NH_4^+), nitrite (NO_2^-), and finally nitrate (NO_3^-), as mediated by microbes. Because of its high solubility, NO_3^- is a form of nitrogen that is easily used by plants and microbes, and can be leached with water flow. Therefore, the NO_3^- pool size in soil is generally controlled by both biological and hydrological processes in forest ecosystems (Ohte 2012).

As a causal factor, the manner in which N input from atmospheric deposition affects or disturbs the N cycle in forest ecosystems is also critical. Isotopic signatures of NO_3^- can provide useful information in tracing how atmospheric NO_3^- is incorporated into the internal cycling of N in forest soils (Kendall and MacDonnell 1998, Elliott et al. 2007, Ohte 2013). In terrestrial systems, this method has been recognized for its ability to distinguish between atmospheric (Tobari et al. 2010, Ohte et al. 2004b, Sebestyen et al. 2008b) and soil microbial sources of NO_3^-.

Atmospherically deposited NO_3^- is incorporated into the internal N cycle of plants and soils when plant uptake rates and microbial N transformation activities are high. That is, atmospherically derived NO_3^- is replaced by NO_3^- produced by microbes in soils. Changes in the $\delta^{18}O$ value of NO_3^- in soils reflect this NO_3^- replacement (Ohte 2013), which suggests that changes in $\delta^{18}O$ can be used as an indicator to evaluate N transformation activities in soils.

In many studies aimed at quantitatively describing N transformation rates in forest soils, incubation techniques have traditionally been used. However, these conventional methods of measuring net mineralization/nitrification cannot

quantitatively evaluate immobilization. The pool dilution method using ^{15}N tracers proposed by Davidson et al. (1992) allows for measurement of gross rates of mineralization, nitrification, and immobilization. This method has mainly been applied to forest soils, and has revealed that gross mineralization rates are usually much higher than net mineralization (Hart et al. 1994, Gundersen et al. 1998, Tokuchi et al. 2014). Aber et al. (1998) stated that carbon (C) availability is important for the N immobilization potential of microbes. An ^{15}N tracer experiment by Curtis et al. (2011) provided evidence confirming the importance of C availability in microbial immobilization.

Few studies have investigated the nitrogen dynamics of forest soils using the isotopic tracer techniques. Thus, information is still lacking that would permit comprehensive understanding of the soil process mechanisms during the progress of N saturation.

1.4 Objective

To clarify the current N status and elucidate the impact of excess N input on the soil nitrogen cycle, I investigated the transformation dynamics of N on the floor of a suburban forest in Japan that receives heavy atmospheric N deposition.

In this study, the following were examined: 1) deposition and transformation of N in the canopy and soil of a suburban forest; and 2) nitrogen dynamics of organic and mineral soil horizons of forest soil. The objective was to: 1) examine the effects of excess N deposition on the nitrogen cycle in a suburban forest ecosystem; and 2) determine the mechanisms and characteristics of internal nitrogen dynamics of forest soil. This further describes and explains the N dynamics of suburban forest in a high

N deposition area.

I selected two forest stands near the Tokyo metropolitan area for comparison. One stand was a plantation of *Cryptomeria japonica* and the other a natural secondary forest of *Quercus acutissima*, both of which make up typical forest stands in this area. Samples of rainfall, throughfall, stemflow, organic horizon water, and soil water (at 10-, 30-, and 70-cm depths) were collected and analyzed for $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^-, using the microbial denitrifier method. Measurements of NO_3^- isotope composition in rain and soil water were used to evaluate the mixing ratios of atmospheric NO_3^- and microbially generated NO_3^-, which have different stable isotope compositions. Isotopic information was used to elucidate processes involved in NO_3^- transformation under excess N deposition. Organic and mineral horizon forest soil samples were collected and incubated in the laboratory under two conditions: 1) long-term incubation (28 days) to measure net N transformation rates; and 2) short-term incubation (24 h). Then, gross rates of the soil N transformation processes (mineralization, nitrification, and immobilization) were determined using the pool dilution method, and an ^{15}N tracer (Davidson et al. 1992) was used to measure gross rates of N transformation.

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Chapter 2

Study site and Methods

2.1 Study site

Field observations were conducted in The University of Tokyo Tanashi Forest, which is in the city of Nishi-Tokyo, 20 km west of the Tokyo metropolitan area (35°44′N, 139°32′E) (Fig. 2-1).

The total area of the forest is 9.1 ha, and its altitude is about 60 m. Annual mean temperature for 2009–2012 was 14.6 ℃, based on data published by The University of Tokyo Forests (2014). Missing data (March 2012) were filled in using Automated Meteorological Data Acquisition System (AMeDAS) data from Fuchu, approximately 10 km southwest of the study site. AMeDAS data are published by the Japan Meteorological Agency (2014). Average annual precipitation for 2009–2012 was 1,718 mm (Imamura 2014). Annual average relative humidity was 70%. Seasonal changes in meteorological parameters of the canopy at Tanashi from 2009 to 2012 are illustrated in Fig. 2.2. Monthly precipitation from November through February was less than that of other months at both sites. This seasonal precipitation variation is typical of the Asian monsoon climate of the study region.

According to Imamura (2014), average annual inorganic N input was 10.4 kg N ha^{-1} $year^{-1}$ (2009–2012). As mentioned in the introduction section, Mitchell et al. (1997) found that N deposition of the study site was typical of the Kanto Plain, and was

significantly higher than the national average.

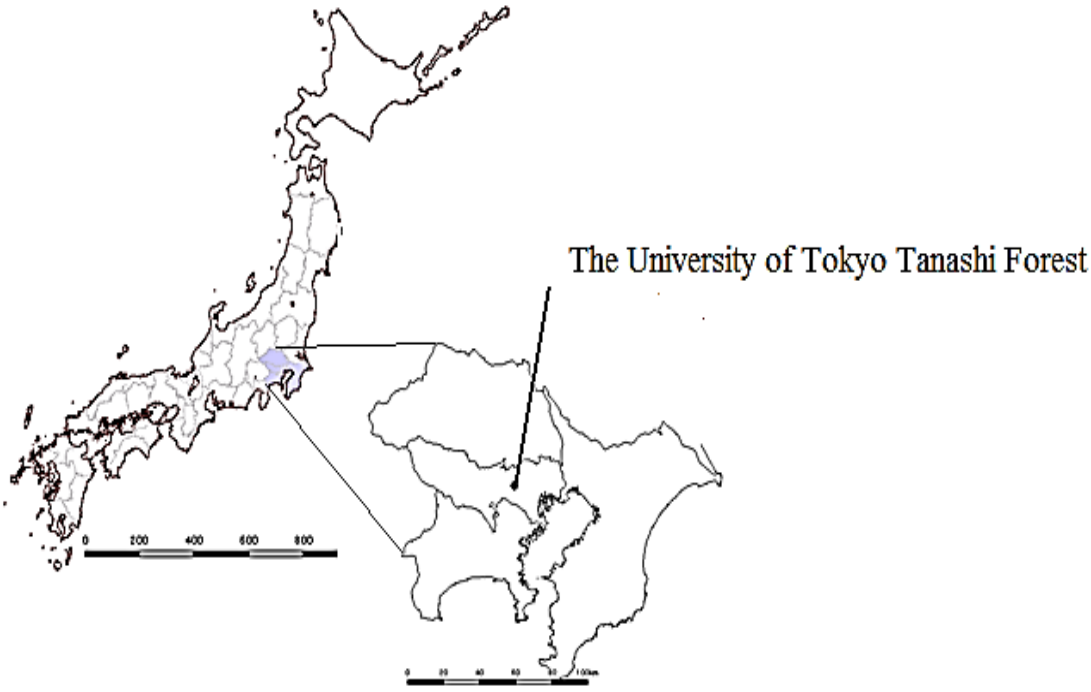

Fig. 2-1 Location of Tanashi Forest

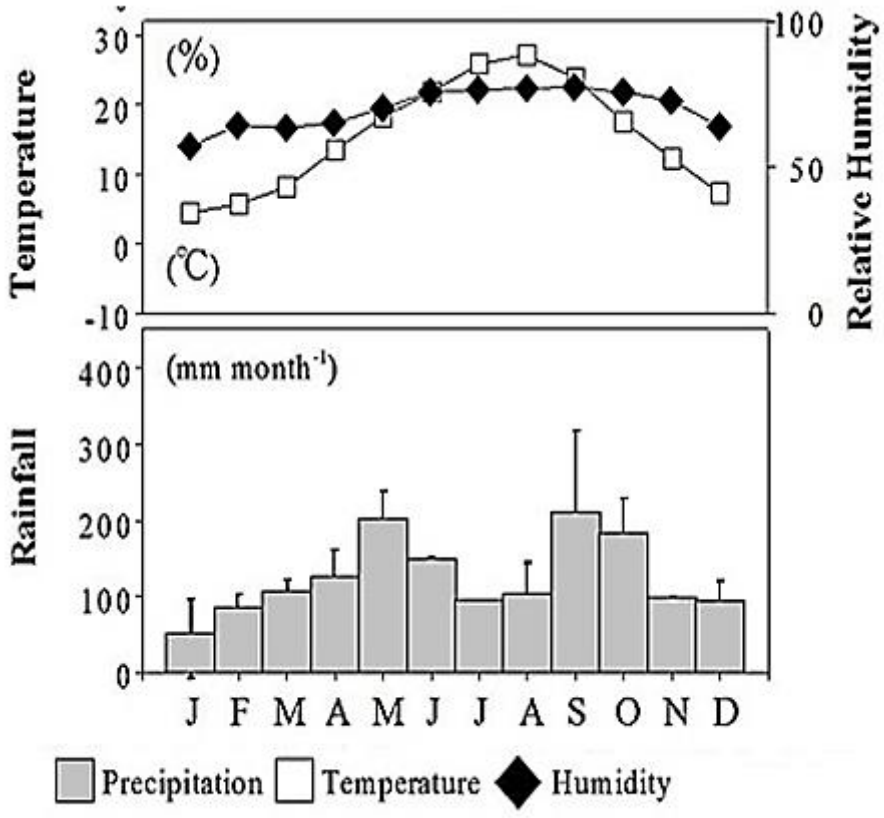

Fig. 2-2 Seasonal changes in meteorological variables of the canopy at Tanashi Forest. Mean monthly air temperature, relative humidity for the period May 2009 through September 2012 at Tanashi are shown. Solid line is 4-year average monthly rainfall for 2009 to 2012 at Tanashi; error bars are 4-year standard deviations. (Imamura 2014)

I selected two different tree stands within the forest, one dominated by *Cryptomeria japonica* and the other by *Quercus acutissima* (Fig. 2-3).

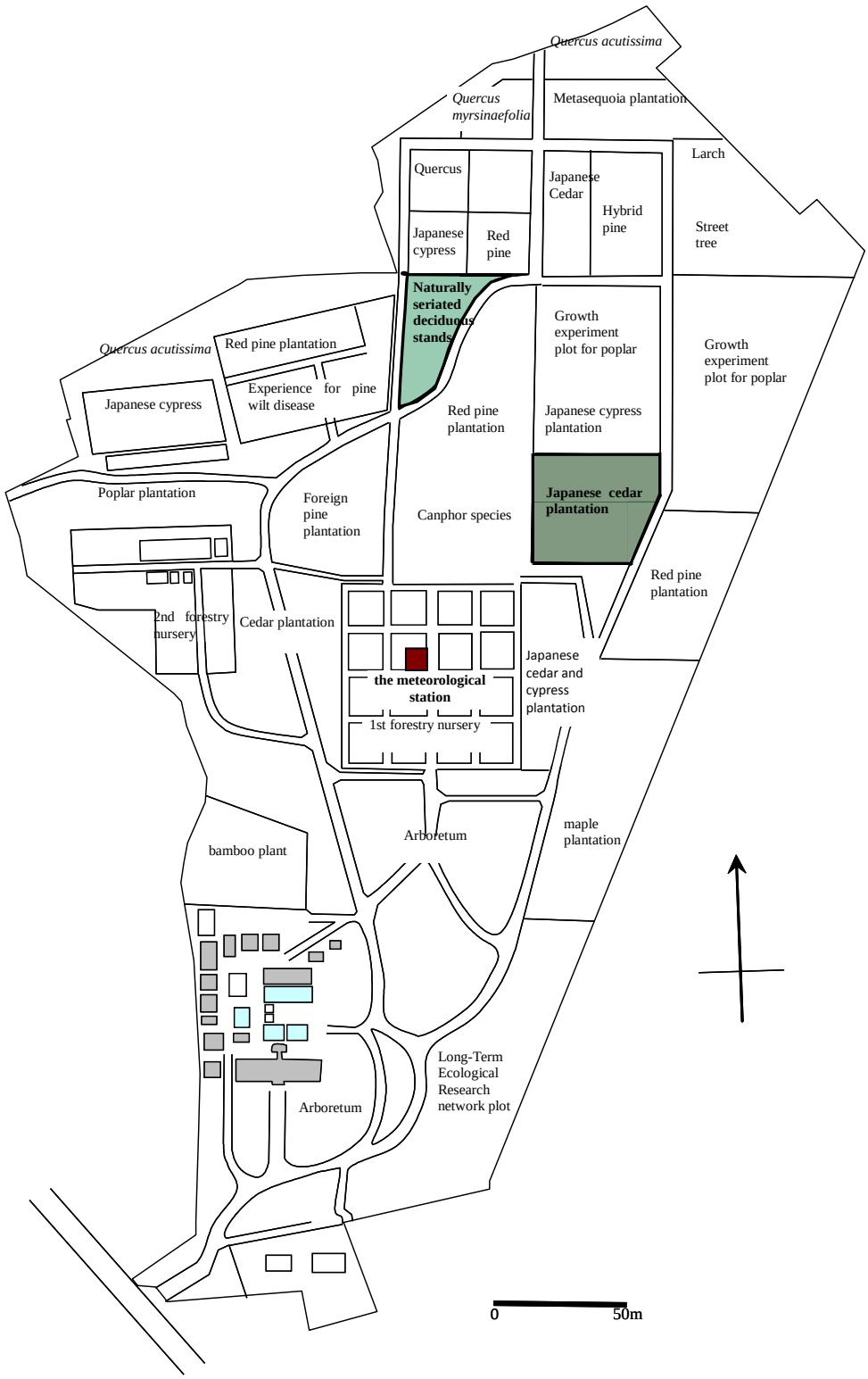

Fig.2.3 Location of *C. japonica* plantation and naturally seriated secondary deciduous stands of *Q. acutissima* at Tanashi Forest. (Imamura 2014)

Cryptomeria japonica (Fig. 2-4) is an evergreen coniferous tree and one of the most popular plantation species for timber production in Japan, and its plantations account for 18% of all forest in Japan. Naturally grown *C. japonica* often forms the upper forest canopy. It has red-brown bark that peels in vertical strips, and its leaves are needlelike (0.5–1 cm in length) and arranged spirally. The *C. japonica* stand of the present study was 52 years old and did not include any other upper-story species. The understory vegetation consisted of dwarf bamboo (*Sasa nipponica Makino et Shibata*) and *Aucuba japonica*.

Fig.2-4 Photo of *C. japonica* (www.cfh.ac.cn)

Quercus acutissima (Fig. 2-5) is an oak native to East Asia. Secondary deciduous forests dominated by *Q. acutissima* are common in a large part of Honshu Island of Japan,

and the species has been used as coppice in agricultural or suburban regions. It is a medium-sized, broadleaf deciduous tree that grows to 25–30 m height, with a trunk that can reach 1.5 m in diameter. The bark is dark gray and deeply furrowed, and leaves are 8–20 cm in length and 3–6 cm in width. The *Q. acutissima* stand in this study included *Quercus variabilis*, and the understory vegetation consisted of *S. nipponica* and *Zanthoxylum piperitum*. The growing season of *Q. acutissima* is April–November, during which leaves are present. December through March is the dormant season, during which leaves are absent.

Fig. 2-4 Photo of *Q. acutissima* (www.cfh.ac.cn)

Soil in the Tanashi Forest is Andisol (i.e., volcanic ash soil), with a relatively thick A horizon (50–55 cm depth from the surface) underlain by B-horizon soil of more than 60-cm depth. Apparent aggregates were observed in the topsoil horizon (< 5 cm depth).

CJ and QA had similar mineral soil horizon profiles. The organic horizon was 6–8 cm in thickness at CJ and 4–7 cm in thickness at QA. Within the organic horizon, the O horizon was thicker at CJ than at QA.

2.2 Sample collection and meteorological observation

2.2.1 Water sampling

Samples of rainfall, throughfall, stemflow, and soil water were collected twice per month from October 2009 through April 2012.(2009.5 ~2010.11 collected by Nagayama) (Nagayama, 2010) Rainfall samples were collected at an unobstructed nursery < 100 m from each site, using a funnel (diameter 21 cm) attached to a polyethylene plastic bottle.

Throughfall samples were collected in polyethylene plastic bottles on the forest floor. Five bulk samplers (Fig. 2-5) were used for collection at each site at one sample time.

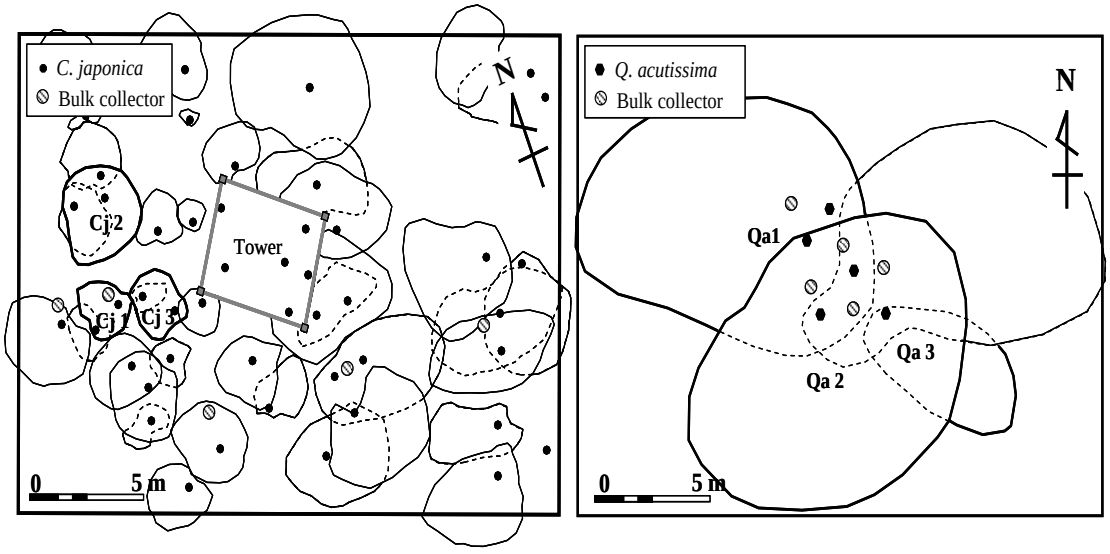

Fig. 2-5 Canopy projection and locations of bulk throughfall samplers at Tanashi observation site. Individual trees of *C. japonica* and *Q. acutissima* are denoted as Cj and Qa, respectively. Stemflow water was collected from Cj1, Cj2 and Cj3, and from Qa1, Qa2 and Qa3. (Imamura 2014)

Stemflow samples were collected by wrapping a hose around a stem, 1.3 m above the ground, and collecting water from the hose in a 70-L polyethylene plastic bottle. Stemflow volumes were calculated by dividing the volume of collected water by canopy projection area at each site (Table 2-1).

Table 2-1 Diameter at breast height (DBH), tree height, canopy projection area, and leaf area index (LAI) of stemflow trees examined in Tanashi Forest. (Imamura 2014)

Tree species	No.	DBH (cm)	Tree height (m)	Canopy projection area (m^2)	LAI (m^2/m^2)
CJ	1	31.0	17.0	4.1	1.92
	2	28.0	17.0	5.6	
	3	22.0	15.0	4.8	
QA	1	43.0	19.7	105.5	1.92
	2	56.0	19.7	114.9	
	3	34.0	16.0	18.2	

I also sampled water percolating through the organic horizon on the forest floor, using zero-tension lysimeters attached to plastic bottles. Soil water was collected using tension lysimeters attached to glass bottles installed at 10-, 30-, and 70-cm depths from the soil surface. These samples were collected in triplicate at each plot.

After water sample collection, each sample was divided into two aliquots. One aliquot was filtered through a 0.25-μv membrane filter and the filtrate was stored at 4 ℃ until measurement of NO_3^- concentration. The second aliquot was filtered through a 0.45-μv membrane and stored in a freezer until measurement of stable NO_3^- isotopes.

2.2.2 Soil sampling

Soil samples for chemical analysis and N transformation experiments were collected in plots CJ and QA during the growing season, June and August 2012 and April and September 2013. Organic soil from the lowest part of the organic horizon was sampled (hereafter, O horizon), as well as mineral soil from 0- to 10-cm depths (hereafter, A horizon). To be precise, the "O horizon" samples included not only organic matter but also a small amount of mineral soil material from the boundary horizon between the accumulation of organic matter and surface of the mineral soil horizon. The boundary horizon consisted of a mixture of well-decomposed organic material and mineral soils, with thickness approximately 3 cm.

Sampling was conducted during mostly fair weather, after a few consecutive fair weather days. Five samples were taken per sampling time, and the corresponding five replicates of fresh soil were used for chemical analysis and measurement of N transformation. The soil samples were stored at 4 ℃ until analysis and used for chemical analysis and net N transformation measurements within 7 days. Gross N transformation measurements using ^{15}N tracers were made within 14 days of sampling.

2.3 Chemical and isotope analysis

Water samples were analyzed for NO_3^- concentration (mg L^{-1}) using an ion chromatograph system (LC-10; Shimadzu, Kyoto, Japan) in the Laboratory of Forest Hydrology and Erosion Control Engineering at The University of Tokyo.

Organic matter and soil samples were sieved using 4- and 2-mm mesh, respectively. Soil water content was measured by drying soil at 105 ℃ in a ventilated oven. Soil samples for total N and C concentrations (hereafter, "total N and C") were ground and measured using an NC analyzer (Sumigraph, Osaka, Japan) at the Field Science Education and Research Center of Kyoto University.

Frozen aliquots were analyzed for $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ using the microbial denitrifier method (Sigman et al. 2001) at the stable isotope laboratory of the Center for Ecological Research, Kyoto University, and the International Research Center for River Basin Environment, University of Yamanashi. Cultured denitrifying bacteria (*Pseudomonas chlororaphis f. sp. aureofaciens* (ATCC 13985)) were used to convert NO_3^- to N_2O for measuring isotopic composition. Water samples containing 30 nmol NO_3^- were injected into vials of nitrifying bacteria. The produced N_2O was stripped from the sample vials using He as the carrier gas, purified using cryogenic trapping, separated chromatographically (CryoPrep; Sercon Limited, Cheshire, UK) and analyzed via mass spectrometry (Hydra20-20; Sercon Limited, Cheshire, UK). N and O stable isotope ratios are expressed in the following generally accepted delta notation as values in parts per thousand (‰):

$$\delta^{15}N,\ \delta^{18}O = \left(\frac{R_{(sample)}}{R_{(standard)}} - 1\right) 1000, \quad (1)$$

where R is the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio of NO_3^- in a sample or standard. The $\delta^{15}N$ values of NO_3^- are reported relative to atmospheric N, and $\delta^{18}O$ values of NO_3^- are reported relative to the Vienna Standard Mean Ocean Water (VSMOW). Isotope values of NO_3^-

were calibrated using IAEA-N3, USGS34, and USGS35 purchased from the International Atomic Energy Agency, Vienna, as reference materials. Reference materials were also measured with each batch of samples. Standard deviations for isotope analyses were ±0.2‰ and ±0.3‰ for $\delta^{15}N$ and $\delta^{18}O$ measurements, respectively. In this paper, I used only the $\delta^{18}O$ values of NO_3^- because they were useful in distinguishing between different sources of NO_3^-, such as atmospheric deposition or microbial production.

2.4 Measurements of net and gross N transformation rates

2.4.1 Net N transformation rates

The equivalent of 20 g soil was placed into two vials as incubation and initial samples. The initial samples were extracted with 50 ml 2 M KCl, then filtered after 1 hour of shocking, and stored at 4 ℃ for soil NH_4^+ and NO_3^- concentration analyses. The incubation samples were incubated at 25 ℃ for 4 weeks, maintaining soil moisture by adding distilled water. After this incubation, the samples were extracted with 50 ml 2M KCl, filtered, and then stored at 4 ℃ for soil NH_4^+ and NO_3^- concentration analyses.

Net mineralization and nitrification was determined from changes of NH_4^+ and NO_3^- concentrations of pre- and post-incubated soils (Tokuchi et al. 2014).

Net mineralization and nitrification rates (NMR, NNR)were calculated using the following:

$$\mathrm{NMR} = (\mathrm{C} - C_0)/(\mathrm{T} - T_0) \qquad (2)$$

$$\mathrm{NNR} = (\mathrm{C}' - C'_0)/(\mathrm{T} - T_0), \qquad (3)$$

where NMR is net mineralization rate and NNR net nitrification rate; C is NH_4^+ +NO_3^-

concentration; C' is NO_3^- concentration; and T_0 and T are the times before and after incubation. NO_2^- concentration was negligible during the analysis.

2.4.2 Gross N transformation rates

Gross NH_4^+ production (mineralization) and nitrification rates were determined using the following measurements and calculations. Gross NH_4^+ production rates were ascertained using the $^{15}NO_3^-$ isotope dilution method (Hart et al. 1994). For each replicated sample, two 7.0-g subsamples of fresh soil, equivalent to 3.5 g dry soil, were labeled with ^{15}N using small-volume injections made with a needle and syringe. Then, 1.0 mL of N solution (1 mmol L^{-1}; 99 atom % ^{15}N in excess) was added to each subsample. Within 2 h after ^{15}N addition, one vial of soil from each sample was extracted using 35 mL of 2 M KCl for the initial measurements. The other was incubated in the dark at 25 ℃ for 26 h, and then extracted using 50 mL of 2 M KCl to obtain the 24h incubation measurement. Moisture content of each incubation sample was retained as that at sampling time.

NH_4^+ in the extracts was recovered as $(NH_4)_2SO_4$ using the NH_3 diffusion method, and then converted into NO_3^- by persulfate oxidation to apply the denitrifier method as sample preparation for ^{15}N isotopic measurement. Details of these procedures are described in Isobe et al. (2011).

NO_3^- in samples prepared by the above procedures for ^{15}N isotopic analysis was converted into N_2O, using the same procedure for measurement of $\delta^{18}O$ from NO_3^- as described above. Isotopic measurement of N_2O was conducted using a gas chromatography mass spectrometer (GCMS-QP2010 Ultra; Shimadzu). Then, the ^{15}N

atom % in the N_2O was determined using the equations in Isobe et al. (2011). NH_4^+ and NO_3^- production rates, as well as NH_4^+ and NO_3^- consumption rates, were determined from changes of fractional abundance above natural abundance in the soils and N concentrations of pre- and post-incubated soils. Gross NH_4^+ and NO_3^- production rates as well as NH_4^+ and NO_3^- consumption rates were calculated using the isotope dilution model of Kirkham et al. (Kirkham And Bartholomew 1954):

$$m = \frac{M_0 - M_t}{t} \times \frac{\log(H_0 H_t / H_t M_0)}{\log(M_0 / M_t)} \quad (4)$$

$$c = \frac{M_0 - M_t}{t} \times \frac{\log(H_0 / H_t)}{\log(M_0 / M_t)}, \quad (5)$$

where M_0 = initial $^{14+15}N$ pool (μmol N (g dry soil)$^{-1}$), M_t = post-incubation $^{14+15}N$ pool (μmol N (g dry soil)$^{-1}$), H_0 = initial ^{15}N pool (μmol N (g dry soil)$^{-1}$), H_t = post-incubation ^{15}N pool (μmol N (g dry soil)$^{-1}$), m = mineralization rate (μmol N (g dry soil)$^{-1}$), c = consumption rate (μmol N (g dry soil)$^{-1}$), t = time (26 h in this study), and 0 = initial time (2 h after ^{15}N addition in this study). The NH_4^+ consumption rate includes the gross nitrification and NH_4^+ immobilization rates. The NO_3^- consumption rate is equivalent to the gross NO_3^- immobilization rate.

2.5 Statistical analysis

I used a nonparametric one-way analysis of variance (Kruskal-Wallis test) to test for differences among samples in terms of N transformation and chemical characteristics

(total C, total N, total C/N) and soil water content in each horizon. I also analyzed relationships between soil properties and gross N transformation rates using linear regression models. All analyses were performed using SPSS 13.0.1 software (SPSS Inc., Chicago, IL, USA).

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Chapter 3

Nitrogen deposition and transformation dynamics along the canopy-soil continuum of a suburban forest

Currently, there are large regions of the world where average nitrogen (N) deposition rates exceed 10 kg N ha^{-1} yr^{-1}, more than 10 times the natural rate. I examined the effects of excess nitrogen deposition on the N cycle in a suburban forest in Japan. In this chapter, I used stable isotopes of nitrogen (N) and oxygen (O) to trace the source and transformation dynamics of nitrate (NO_3^-) in two forest stands: a plantation of *Cryptomeria japonica* (coniferous tree; CJ) and a natural secondary forest of *Quercus acutissima* (broadleaf deciduous tree; QA). I measured NO_3^- and ammonium (NH_4^+) concentrations as well as $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in rainfall, throughfall, stemflow, the litter layer, and soil water (at 10-, 30-, and 70-cm depths).

3.1 Results

3.1.1 Inorganic nitrogen deposition

Annual fluxes in deposition of atmospheric NH_4^+ and NO_3^- are summarized in Table 3-1. Although the difference of annual throughfall between the CJ and QA sites was small, stemflow at the CJ site was significantly greater than at the QA site. NH_4^+ and NO_3^- flux associated with throughfall was greater at the CJ site than at QA. Specifically, the NO_3^-

flux at the CJ site was more than twice that at the QA site. NH_4^+ and NO_3^- fluxes associated with stemflow were also significantly greater at CJ than QA.

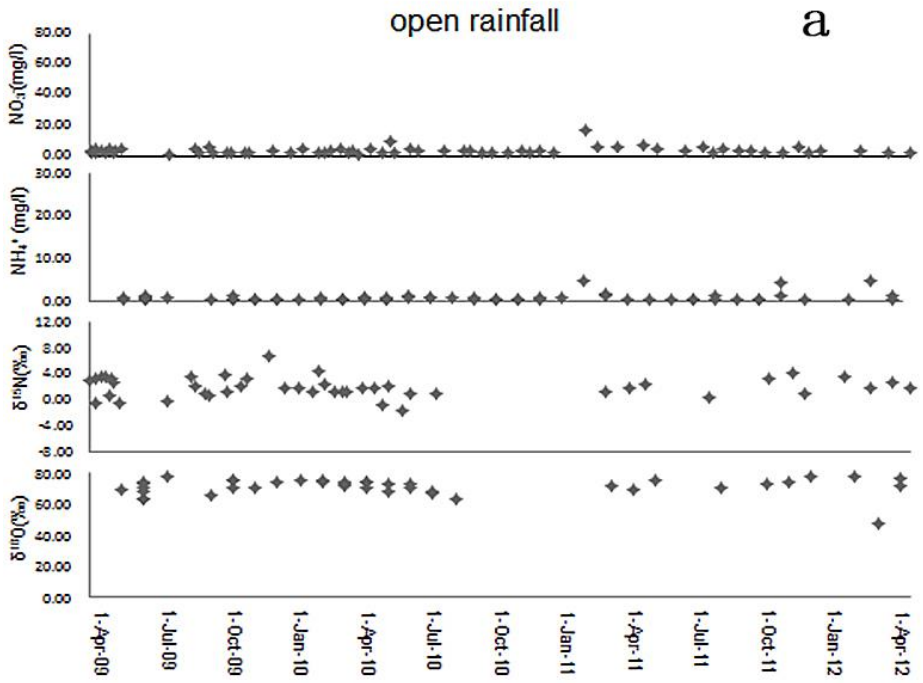
Table 3-1 Annual fluxes of inorganic nitrogen (NH4+ and NO3-) by atmospheric deposition

	CJ			QA		
	Water amount	NH_4^+ flux	NO_3^- flux	Water amount	NH_4^+ flux	NO_3^- flux
	(mmyear^{-1})	(kgha^{-1}year^{-1})	(kgha^{-1}year^{-1})	(mm year^{-1})	(kg ha^{-1} year^{-1})	(kg ha^{-1} year^{-1})
Open rainfall	1570.9	6.4	23.8	1570.9	6.4	23.8
Throughfall	1360.1	21.1	71.5	1310.7	12.4	32.8
Stem flow	132.5	3.6	16.4	20.4	0.1	0.5

Seasonal variations in NO_3^- and NH_4^+ concentrations as well as $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of rainfall, throughfall, and stemflow are presented (Fig. 3-1a, b and c) for both CJ and QA sites from October 2009 through April 2012. There were no significant seasonal variations in rainfall NO_3^- concentrations at either site, and rainfall had lower NO_3^- concentrations than either throughfall or stemflow. At the CJ site, NO_3^- concentrations were lower in throughfall than in stemflow. I found no significant difference between throughfall and stemflow NO_3^- concentrations at site QA. There were no significant seasonal variations of rainfall NH_4^+ concentrations, which were lower (near zero) than those of throughfall and stemflow. There were no significant differences between throughfall and stemflow NH_4^+ concentrations at either CJ or QA.

The range of $\delta^{15}N_{NO3}$ values for rainfall was similar at CJ and QA (−10‰ to 10‰) and fluctuated seasonally, with smaller values in summer and higher ones in winter. The range of $\delta^{15}N_{NO3}$ values for stemflow was smaller than that of $\delta^{15}N_{NO3}$ values for throughfall at

both sites. Although I observed no significant seasonal variation in $\delta^{18}O_{NO3}$ values of rainfall or throughfall at either site, there were some notable fluctuations throughout the observation period. Most $\delta^{18}O_{NO3}$ values for rainfall and throughfall were in the 60%–80% range. The range of $\delta^{18}O_{NO3}$ values for stemflow was smaller than that for throughfall at both sites. This was especially so at the QA site in summer, where $\delta^{18}O_{NO3}$ values of stemflow decreased significantly, to < 40‰.



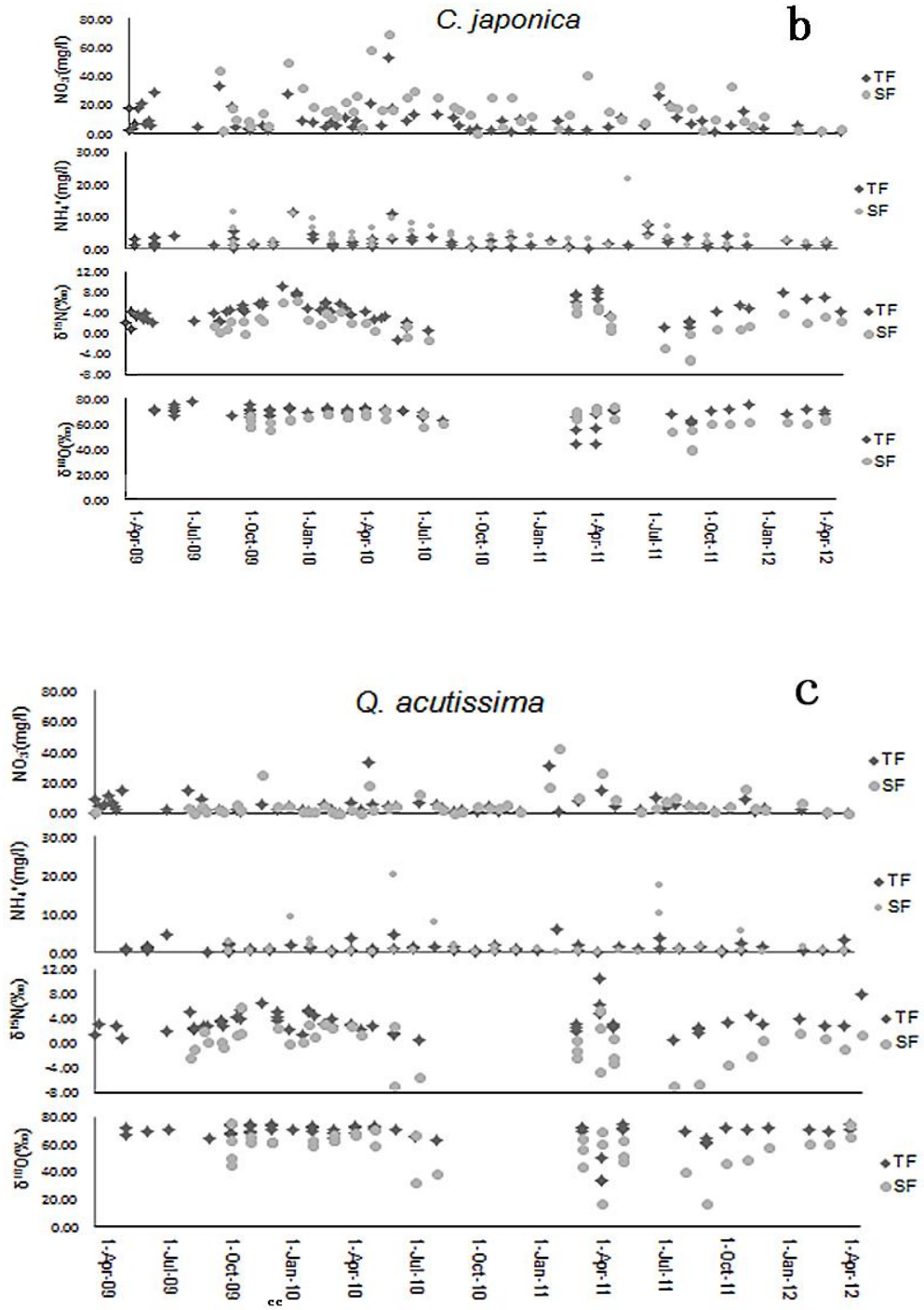


Fig. 3-1 Seasonal variations of nitrate (NO_3^-) and ammonium (NH_4^+) concentrations, and $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in (a) rainfall for both *C. japonica* (CJ) and *Q. acutissima* (QA) sites; (b) throughfall (TF) and stemflow (SF) at CJ site; (c) TF and SF at QA site, from October 2009 through April 2012.

3.1.2 Changes of NO_3^- concentration, $\delta^{15}N_{NO3}$, and $\delta^{18}O_{NO3}$ along canopy-soil continuum

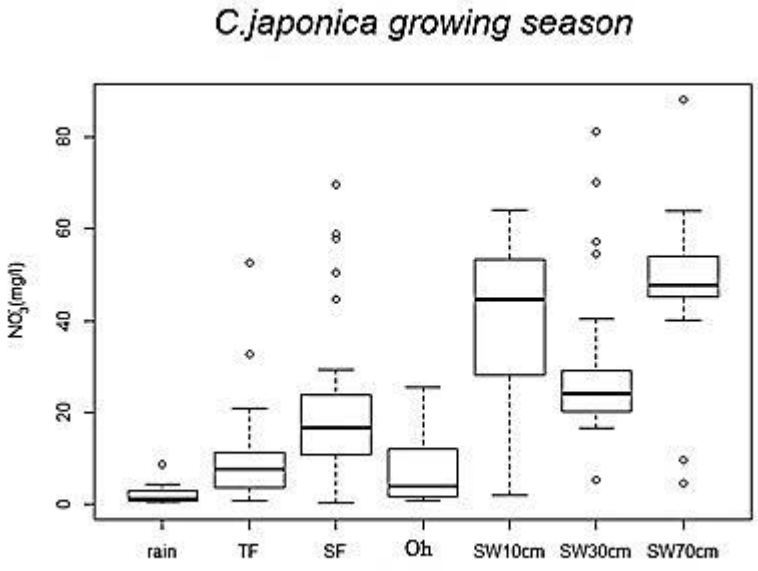
I found seasonal variation in $\delta^{15}N_{NO3}$ values of throughfall and stemflow at both sites, and in $\delta^{18}O_{NO3}$ values of throughfall and stemflow at QA. *Quercus acutissima* is a broadleaf, deciduous species, whose juvenile leaves expand in early to mid April and fall in mid December. To elucidate differences in throughfall, stemflow, and soil water NO_3^- between the growing and dormant seasons, I addressed changes in NO_3^- concentration, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values based on a growing season of April–November and dormant season of December –March.

NO_3^- concentrations

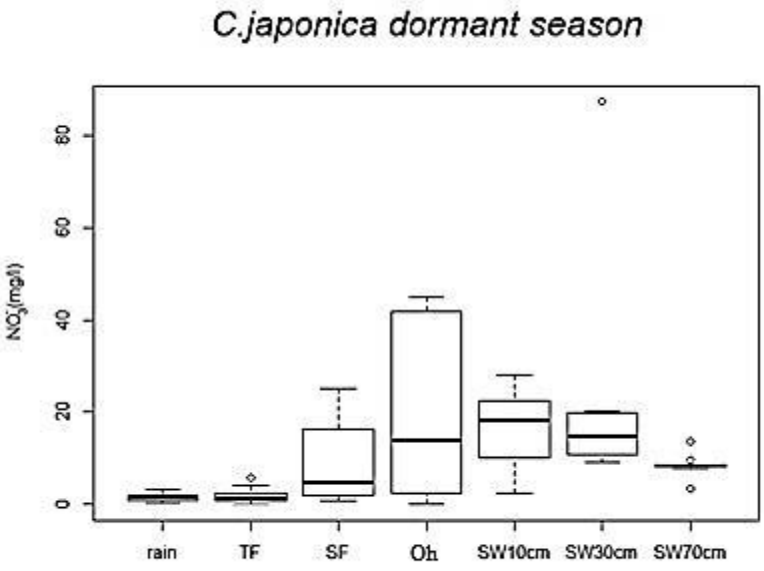
Box plot diagrams of rainfall, throughfall, stemflow, and soil water NO_3^- concentrations are presented in Fig. 3-2. Data were plotted separately based on the growing (April–November; Fig. 3-2a and c) and dormant (December–March; Fig. 3-2b and d) seasons. At the CJ site, NO_3^- concentrations in soil water were significantly higher during the growing season than in the dormant season. Specifically, this concentration in surface soil water (0–10 cm depths) was higher (median > 40 mg N/L) in the growing season than in the dormant season (~20 mg N/L). No significant seasonal differences were observed in soil water NO_3^- concentrations at the QA site. At that site, the highest NO_3^- concentration was observed in soil water from the organic horizon, which was

higher than those of NO_3^- in rainfall, throughfall, and stemflow.

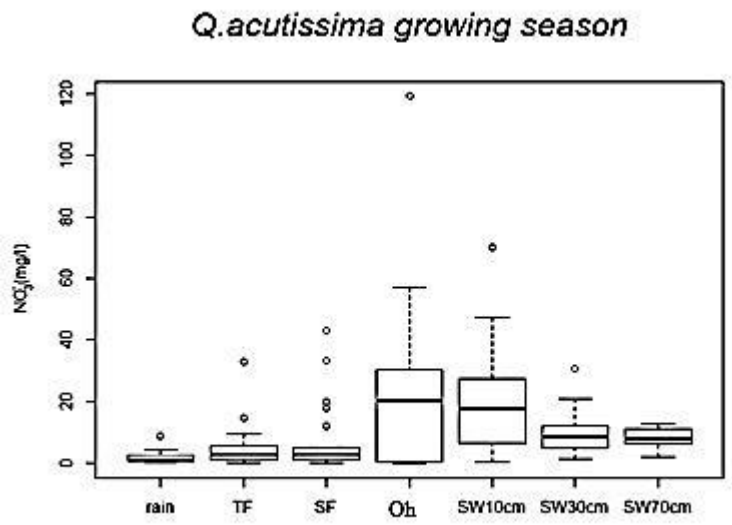
3-2a



3-2b



3-2c



3-2d

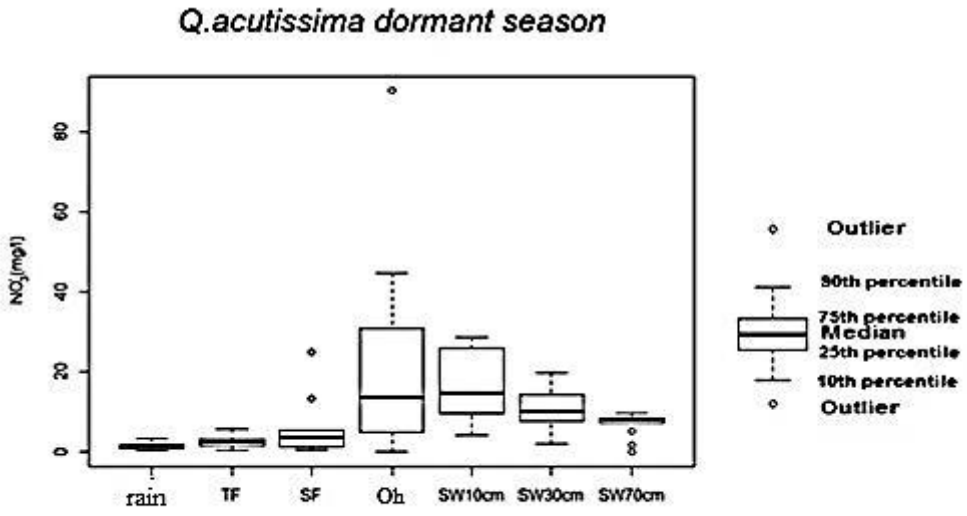
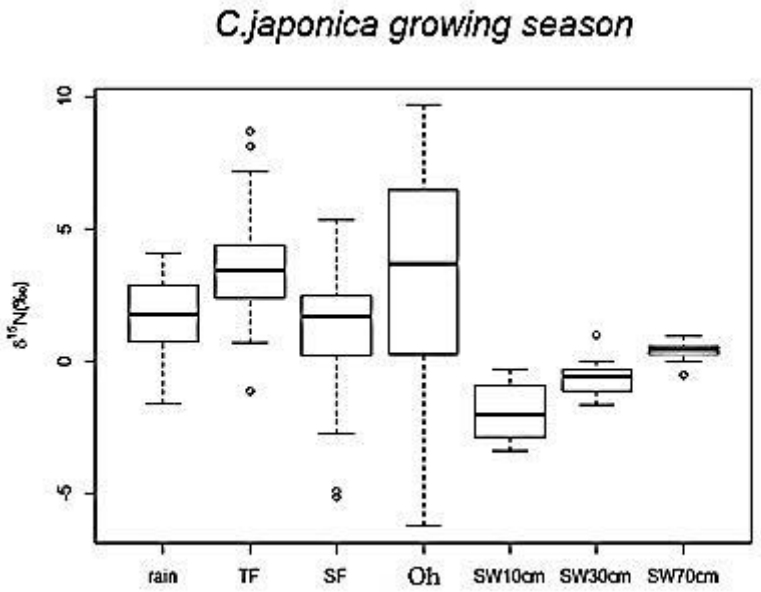


Fig.3-2 NO_3^- concentrations in rainfall (rain), throughfall (TF), stemflow (SF), the organic horizon (Oh), and soil water (SW; 10-, 30-, and 70-cm depths) during (a) growing and (b) dormant seasons at *C. japonica* (CJ) site, and (c) growing and (d) dormant seasons at *Q. acutissima* (QA) site. Box plots with different small letters indicate significant differences among sample types.

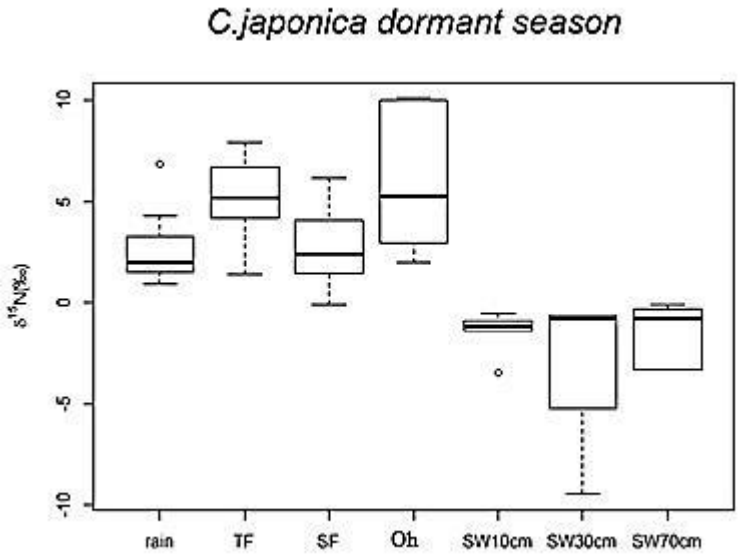
$\delta^{15}N_{NO3}$

Variations of $\delta^{15}N_{NO3}$ values are presented in Fig. 3-3. For both CJ and QA sites, the range of $\delta^{15}N_{NO3}$ values of soil water was slightly smaller (−4‰ to 0‰) than those of rainfall, throughfall, or stemflow (0‰–4‰). Although there was marked variation of $\delta^{15}N_{NO3}$ values of the organic horizon during the growing season at both sites, the median value of $\delta^{15}N_{NO3}$ at QA was lower during the growing season (−5‰) than during the dormant season (0‰), and was similar to $\delta^{15}N_{NO3}$ values for soil water. However, there was no significant seasonal difference in $\delta^{15}N_{NO3}$ of the organic horizon at site CJ.

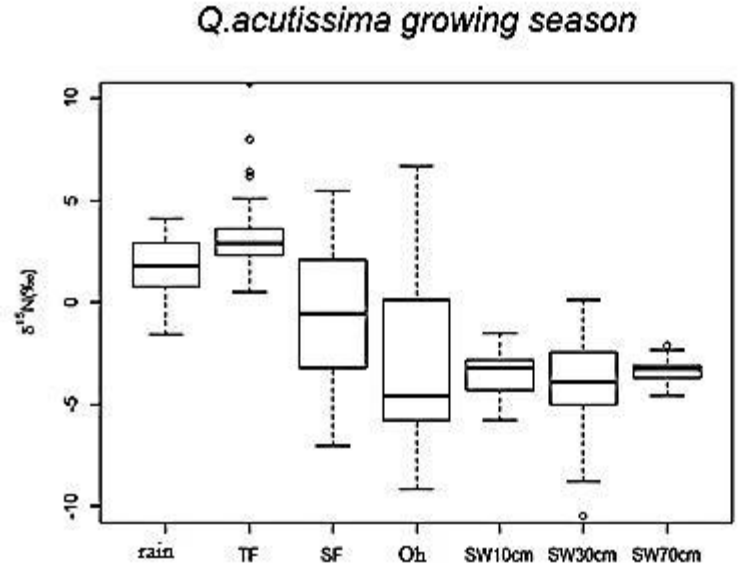
3-3a



3-3b


C.japonica dormant season
10
5
0
-5
-10
rain
TF
SF
Oh
SW10cm
SW30cm
SW70cm

3-3c


Q.acutissima growing season
10
5
0
-5
-10
rain
TF
SF
Oh
SW10cm
SW30cm
SW70cm

3-3d

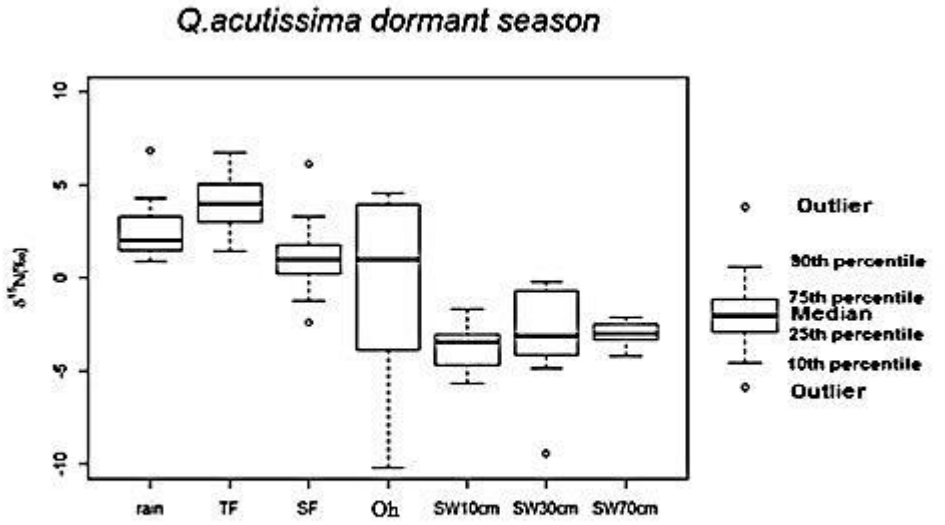


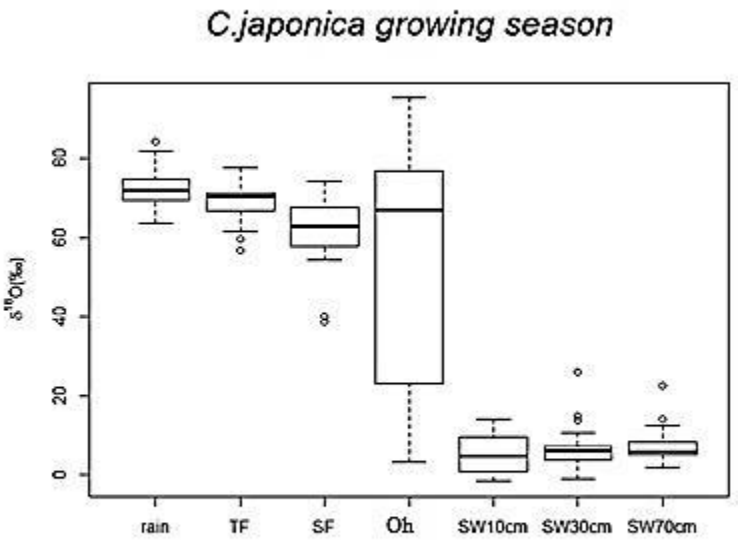
Fig. 3-3 $\delta^{15}N$ values of NO_3^- in rainfall (rain), throughfall (TF), stemflow (SF), the organic horizon (OH), and soil water (SW; 10-, 30-, and 70-cm depths) during (a) growing and (b) dormant seasons at *C. japonica* (CJ) site, and (c) growing and (d) dormant seasons at *Q. acutissima* (QA) site. Box plots with different small letters indicate significant differences among sample types.

$\delta^{18}O_{NO3}$

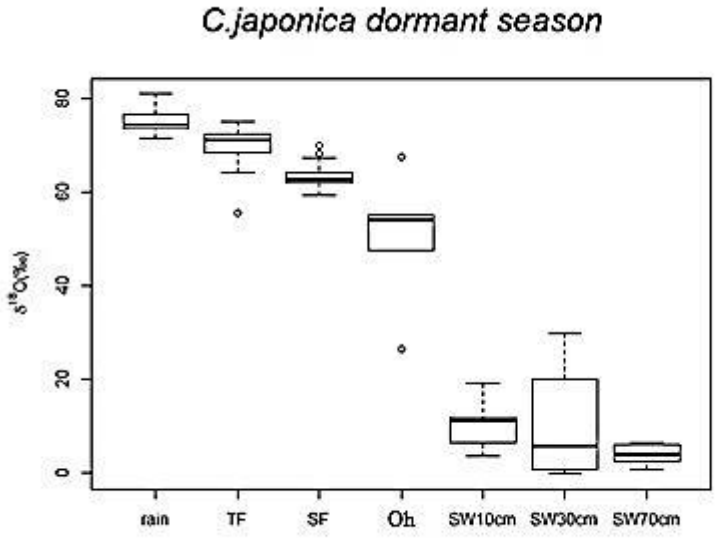
Variations of $\delta^{18}O_{NO3}$ values are presented in Fig. 3-4. For both sites and seasons, the range of soil water $\delta^{18}O_{NO3}$ was significantly lower (0–10‰) than those of rainfall, throughfall, or stemflow (60%–70‰). The $\delta^{18}O_{NO3}$ value of the organic horizon was

variable at both sites; however, the median value was significantly lower at the QA site (19‰) than at the CJ site (63‰). $\delta^{18}O_{NO3}$ values of stemflow (60‰) were smaller than those of rainfall (75‰) for both seasons and sites.

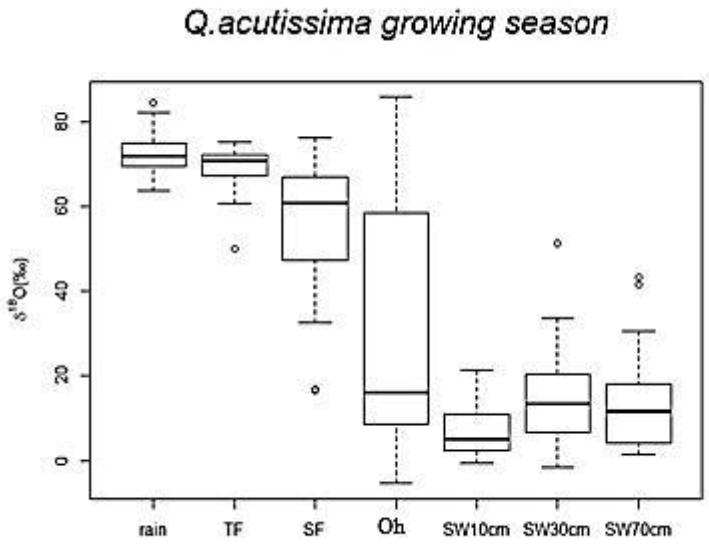
3-4a



3-4b


C.japonica dormant season
δ18O(‰)
0
20
40
60
80
rain
TF
SF
Oh
SW10cm
SW30cm
SW70cm

3-4c


Q.acutissima growing season
δ18O(‰)
0
20
40
60
80
rain
TF
SF
Oh
SW10cm
SW30cm
SW70cm

3-4d

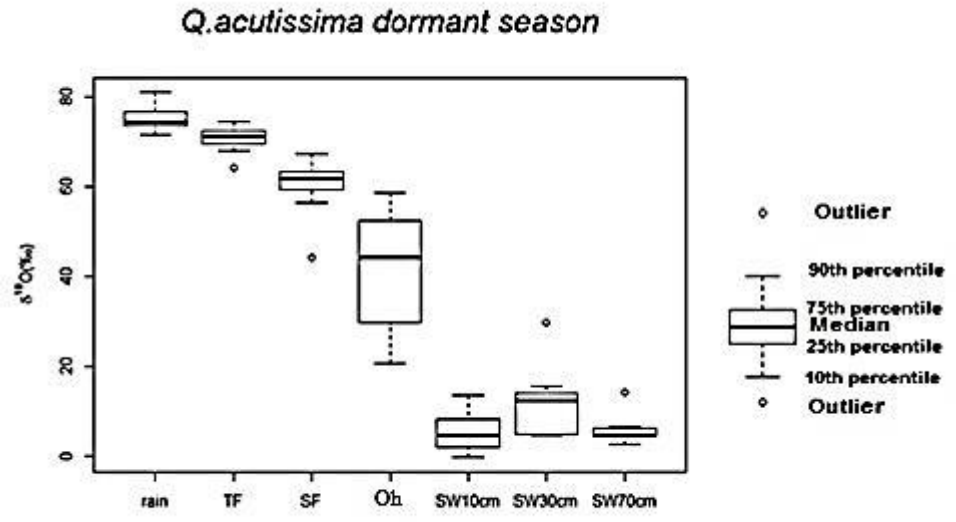


Fig. 3-4 $\delta^{18}O$ values of NO_3^- in rainfall (rain), throughfall (TF), stemflow (SF), the organic horizon (OH), and soil water (SW; 10-, 30-, and 70-cm depths) during (a) growing and (b) dormant seasons at *C. japonica* (CJ) site, and (c) growing and (d) dormant seasons at *Q. acutissima* (QA) site. Box plots with different small letters indicate significant differences among sample types.

3.2 Discussion

3.2.1 Changes of NO_3^- source

The $\delta^{18}O_{NO3}$ values decreased dramatically from deposition (rainfall, throughfall, and stemflow) to soil water at both CJ and QA sites (Fig. 3-4). This implies that forest NO_3^- received from atmospheric deposition was effectively replaced by microbially generated NO_3^-. Replacement was nearly complete before water reached the mineral soil horizon (10 cm), which suggests significant consumption and production of NO_3^- in the organic horizon and at the surface of the mineral horizon.

Osaka et al. (2010) conducted a similar study in a stand of coniferous Japanese cypress trees (*Chamaecyparis obtusa*) within the Kiryu Experimental Watershed (KEW) in central Japan. They found that $\delta^{18}O_{NO3}$ decreased gradually along the soil profile through depth 50 cm, and that there was substantial replacement of atmospheric NO_3^- by microbially generated NO_3^- in mineral soils. Possible explanations for the differences between their study and the present work include differences in richness of the organic horizon and hydraulic properties of the mineral soils. The organic horizon at both the present study sites was significantly thicker than that at KEW (Osaka et al. 2010, Ohte et al. 1995), which provides more favorable substrate and moisture conditions for microbial communities. The soil at KEW is sandy and has a higher permeability compared with the soil at our sites, which allows a greater portion of atmospheric NO_3^- to reach the forest floor, to then be transported to deeper soil horizons.

$\delta^{18}O_{NO3}$ values of soil water at depths 10–70 cm ranged from 0‰ to 10% at both sites, indicating that most NO_3^- in mineral soils was produced by microbes. Additionally, $\delta^{15}N_{NO3}$ values in water from mineral soil horizons were slightly smaller than those in deposition water (rainfall, throughfall, and stemflow; Fig. 3-3). This suggests that the source of N atoms in NO_3^- was not currently or recently derived N compounds, such as atmospheric NH_4^+ and NO_3^-.

3.2.2 Differences of NO_3^- dynamics between CJ and QA sites

Along the atmospheric deposition sequence from the canopy to organic horizon, $\delta^{18}O_{NO3}$ values decreased in the following order at both sites: rainfall, throughfall,

stemflow, and organic horizon. At the CJ site, there was no significant seasonal difference in this trend; the median $\delta^{18}O_{NO3}$ value in the organic horizon was ~50‰ and consistent between seasons. In contrast, at the QA site there was a dramatic decrease in the $\delta^{18}O_{NO3}$ value of stemflow to < 30% during the growing season. The median $\delta^{18}O_{NO3}$ value in the organic horizon was also significantly smaller during the growing season than during the dormant season, and the difference was more significant at QA than at CJ.

There are two potential explanations for the smaller $\delta^{18}O_{NO3}$ values of stemflow and throughfall water. One concerns the mixing of NO_3^- produced by microbial activity (nitrification) on tree surfaces, including leaves, branches, and trunks; the other involves mixing of NO_3^- leached from living tree tissues. However, a previous study of stemflow and throughfall chemistry at the same sites found no significant NO_3^- leaching in either the CJ or QA stands (Imamura 2014). Therefore, it is more likely that the decrease of $\delta^{18}O$ values of stemflow and throughfall water, especially for the QA stands, was caused by nitrification in the canopy, as discussed by Chen et al. (1983). Papen et al. (2002) and Teuber et al. (2007) reported the presence of chemolithoautotrophic nitrifiers on conifer leaf surfaces. However, prior to that study, there were no reports supporting the presence of microbial nitrifying activity using isotopic signatures.

The present result suggests that the broad canopy leaves of the QA site provide more favorable conditions for microbial nitrification than do the needle-like canopy leaves of the CJ site.

The canopy of both the CJ and QA sites received sufficient amounts of NH_4^+, one of the intermediary substrates for NO_3^- production by nitrification, from atmospheric deposition (Table 3-1). Imamura (2014) reported that a substantial portion of atmospheric

NH_4^+ was consumed by the canopy at both CJ and QA. This finding also supports the idea that marked microbial nitrification occurs on the surface of canopy leaves and bark of stems. The lack of a significant decrease in $\delta^{18}O_{NO3}$ values of stemflow at CJ can be attributed to the fact that atmospheric NO_3^- made up a greater portion of overall NO_3^- at that site than at QA. This is further supported by the fact that throughfall and stemflow NO_3^- flux were significantly greater at CJ than at QA (Table 3-1). Furthermore, an earlier study found that the pH of stemflow was lower at CJ than at QA (Imamura 2014), which suggests that the surface conditions of *C. japonica* are relatively unfavorable for microbial nitrification relative to *Q. acutissima*. This is because pH favorable for microbial nitrification in forest ecosystems is generally > 4.0 (Toda 1994, Yoshida et al. 1979, Kai 1981).

3.3 Conclusion

NO_3^- supplied by atmospheric deposition to the forest sites evaluated in the present study was effectively replaced by microbially generated NO_3^- in the organic horizon and surface part of the mineral soil. Overall, $\delta^{18}O_{NO3}$ values at both sites decreased in the following sequence: rainfall, throughfall, stemflow, and organic horizon. There was no significant seasonal variation in this trend at the CJ site. However, the trend did vary seasonally at the QA site, where there was a dramatic decrease in $\delta^{18}O_{NO3}$ values of stemflow to < 30‰ during the growing season, and the median value in the organic horizon was significantly smaller during the growing season than during the dormant season. This suggests that microbial nitrification on the surface of leaves and bark of

branches and stems contributed a significant portion of the NO_3^- present in throughfall and stemflow, in addition to atmospheric NO_3^-. Differences between the QA and CJ sites regarding the decreasing trend in $\delta^{18}O_{NO3}$ values of stemflow may be attributed to more favorable conditions for microbial nitrification on plant surfaces at QA; this may be associated with characteristics specific to the plant species present.

Nitrogen dynamics on plant surfaces with respect to the pathways responsible for producing available nitrogen in forest ecosystems have not been thoroughly investigated. The results of the present study will facilitate further investigation into the role of plant surfaces in the nitrogen cycle of forest ecosystems.

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Chapter 4

Soil N transformation dynamics in suburban forest

Nitrogen deposition and dynamics along the canopy-soil continuum of a suburban forest were analyzed in Chapter 3. As a result, NO_3^- from atmospheric deposition was replaced by microbial-generated NO_3^-, mainly in the O horizon and surface part of mineral soil in this forest under excess nitrogen deposition. Microbial activities including both immobilization and nitrification in organic-rich horizons of near-surface soils contributed to incorporating the atmospheric NO_3^- rapidly into the internal microbial nitrogen cycle. The internal nitrogen cycle includes nitrification, denitrification and immobilization, and thus it is a series of reactions. These reactions, particularly their gross rates, were complex and not easily determined. In this chapter, I determined the gross rates of N transformation processes (mineralization, nitrification, and immobilization) in soils using isotope tracer techniques, and natural abundances of NO_3^- isotopes were measured. The aim was to investigate the transformation dynamics of N on the forest floor of the studied forest receiving heavy atmospheric N deposition and to address the effects of excess N deposition on the N cycle.

4.1 Results

Fig. 4-1 shows changes of monthly mean air temperature observed at the meteorological station of the Tanashi Forest, 100 m southwest of the CJ site. Seasonal patterns of air temperature in 2012 and 2013 were almost identical. Thus, I present results of experiments in the growing season from April through September (sampling was in June and August 2012, and April and September 2013).

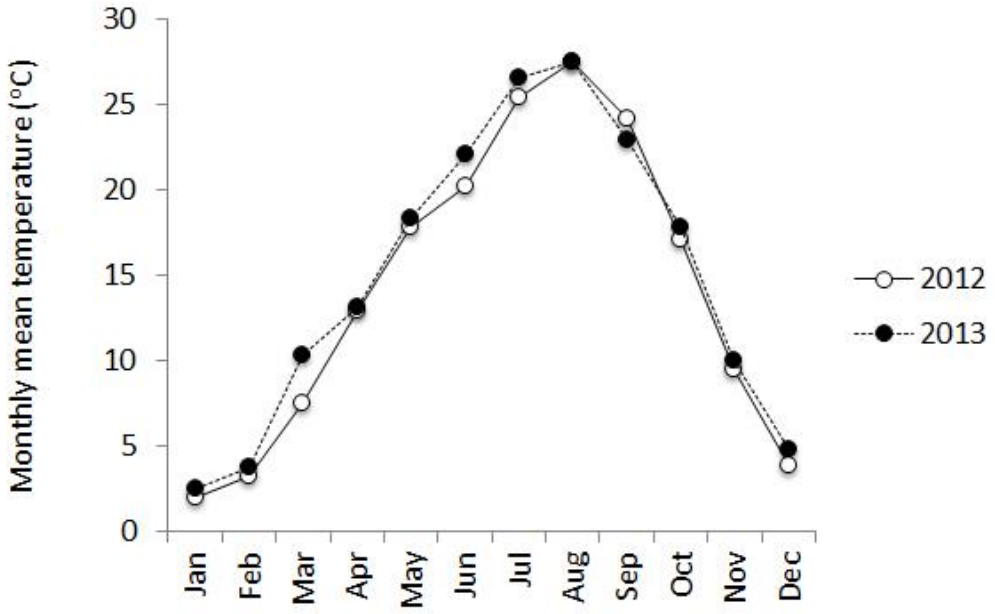

Fig 4-1 Monthly mean temperature in 2012 and 2013 (source: data published by The University of Tokyo Forests (2014). Data gaps (March 2012, June 2013) were filled with Automated Meteorological Data Acquisition System (AMeDAS) data at Fuchu, approximately 10 km southwest of the study site, published by Japan Meteorological Agency (2014).

4.1.1 Total C and N concentrations and NH_4^+ and NO_3^- concentrations in

soils

Although statistical differences were found among the sample times for several variables (total N concentration, C/N ratio, and gravimetric water content), clear seasonal variations were not identified (Table 4-1). Significant differences between Plots CJ and QA were detected only for total N concentration of the O horizon ($P < 0.05$). NH_4^+ and NO_3^- concentrations of extracted samples from each sampling time are shown in Fig. 4-2. Although NH_4^+ and NO_3^- concentrations were higher in the A horizon than O horizon at a few sample times, the opposite was the case at most sampling times. Although there were statistically significant differences among the sample times in all the A horizon samples and in NO_3^- concentrations of the O horizon at Plot CJ, consistent seasonal patterns were not identified.

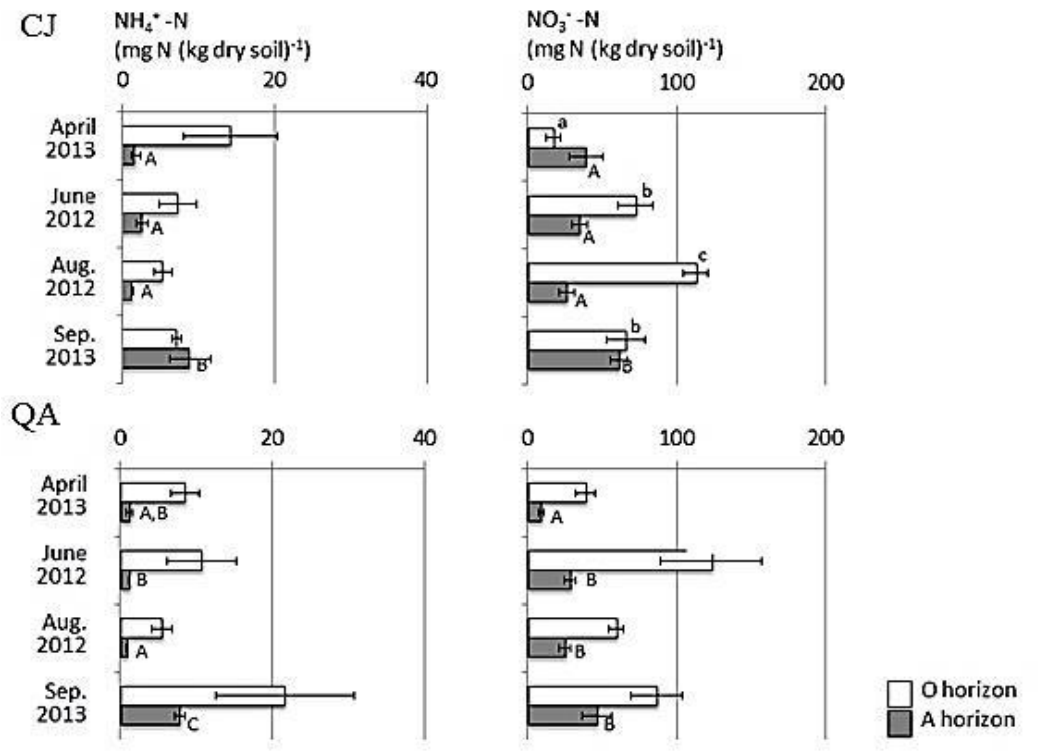

Fig. 4-2 Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations at each sampling time in the O and A horizons in the *Cryptomeria japonica* (CJ) and *Quercus acutissima* (QA) stands. Bars with different lower-case and capital letters indicate significant differences among each sample series.

	Total C (mg C (g dry soil)$^{-1}$)		Total N (mg N (g dry soil)$^{-1}$)		C/N		Gravimetric water content (%)	
Cryptomeria japonica	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon
April, 2013	199.5 (23.8)	a 98.5 (4.2)	10.5 (0.8)	6.8 (0.2)	a 18.7 (0.8)	a 14.4 (0.3)	a,c 53.8 (1.6)	49.6 (1.1)
June, 2012	183.1 (19.2)	a,b 106.9 (8.4)	11.5 (1.1)	7.8 (0.6)	b 15.9 (0.4)	b 13.7 (0.1)	a,b 57.5 (0.9)	49.9 (1.0)
August, 2012	213.3 (32.2)	a,b 116.5 (6.2)	10.6 (0.9)	7.4 (0.4)	a 19.7 (1.4)	c 15.8 (0.2)	b 62.1 (2.2)	52.2 (1.5)
September, 2013	206.0 (18.5)	b 133.3 (2.0)	11.0 (0.8)	8.3 (0.2)	a 18.7 (0.4)	c 16.1 (0.3)	c 50.4 (1.0)	46.6 (1.2)
Average	199.9 (12.0)	111.6 (4.1)	10.9 (0.4)	7.5 (0.2)	18.2 (0.6)	14.9 (0.3)	56.0 (1.2)	49.6 (0.7)
Quercus acutissima	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon
April, 2013	187.0 (16.0)	106.2 (9.1)	12.2 (0.9)	a,b 7.8 (0.7)	a 15.3 (0.3)	a 13.5 (0.1)	54.2 (0.8)	48.3 (0.7)
June, 2012	223.6 (16.6)	117.9 (5.1)	15.7 (0.8)	b 9.2 (0.4)	a 14.2 (0.3)	b 12.9 (0.1)	60.2 (1.4)	50.2 (1.0)
August, 2012	235.4 (20.4)	106.0 (5.2)	13.7 (1.0)	a 7.2 (0.2)	b 17.1 (0.5)	c 14.6 (0.3)	64.0 (1.1)	49.4 (0.4)
September, 2013	233.1 (15.5)	143.0 (12.9)	14.0 (1.0)	a,b 9.3 (1.0)	b 16.7 (0.1)	c 15.4 (0.3)	46.4 (1.1)	40.8 (1.3)
Average	218.3 (9.5)	115.5 (4.7)	13.9 (0.5)	8.3 (0.3)	15.7 (0.3)	14.0 (0.2)	56.2 (1.6)	47.2 (0.9)

Table 4-1. Total carbon and nitrogen concentrations and gravimetric water content of soil samples. Values in parentheses indicate standard errors. Values with different small letters indicate significant differences among each sample series.

4.1.2 Gross rates of NH_4^+ and NO_3^- production and consumption

Gross rates of $NH_4^+$and NO_3^- production and consumption are presented in Figs. 4-3 and 4-4, respectively. In most cases, the gross transformation rate of NO_3^- was an order of magnitude less than that of NH_4^+. Although an extremely high gross NO_3^- consumption rate was observed in the O and A horizons of Plot QA site in June, consistent seasonal patterns were not identified. Gross NO_3^- consumption rates in Plot QA from June through September were slightly higher than those in Plot CJ, especially in the O horizon.

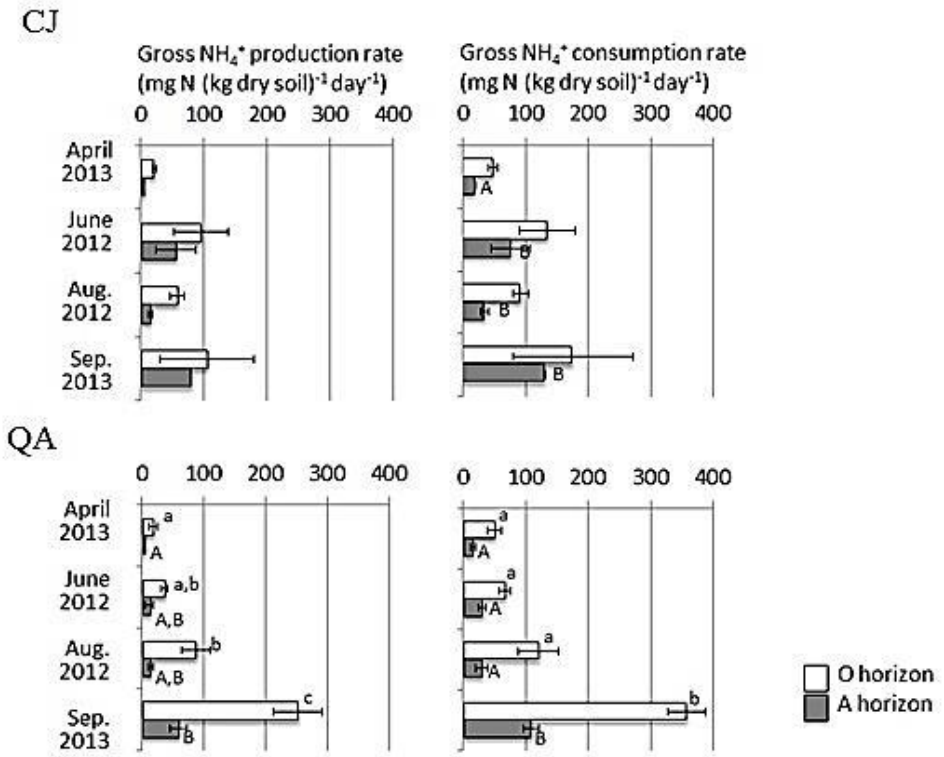

Fig. 4-3 Gross ammonium (NH_4^+) production and consumption rates of each sampling time in the O and A horizons in the *Cryptomeria japonica* (CJ) and *Quercus acutissima* (QA) stands. Bars with different lower-case and capital letters indicate significant differences among sample series.

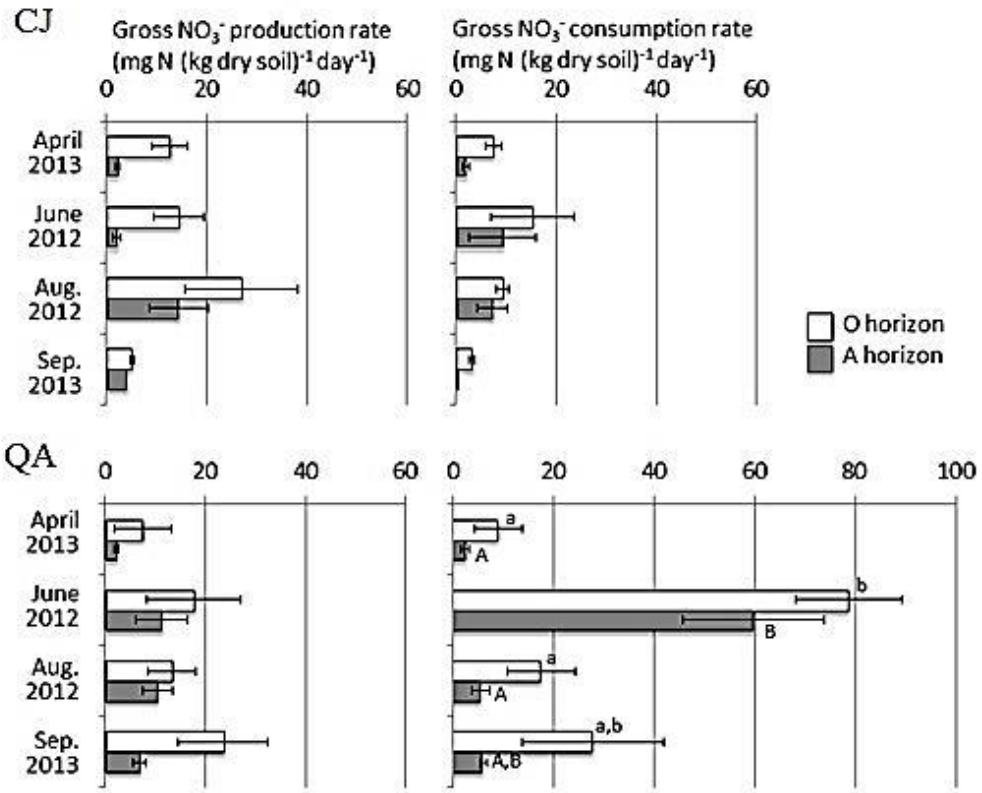

Fig.4-4 Gross nitrate (NO_3^-) production and consumption rates during each sampling time in the O and A horizons at the *Cryptomeria japonica* (CJ) and *Quercus acutissima* (QA) stands. Bars with different lower-case and capital letters indicate significant differences among sample series.

4.2 Discussion

4.2.1 NO_3^- concentration and NO_3^- production and consumption

To compare the gross rates of NH_4^+ and NO_3^- transformation between Plots CJ and QA, averages of all four sampling times at each site are presented in Fig. 4-5. For both NH_4^+ and NO_3^- in the O and A horizons, no statistically significant differences were found between Plots CJ and QA. Gross production rates of both NH_4^+ and NO_3^- were higher in the O horizon than the A horizon at both plots, although the gross NO_3^-

consumption rate at Plot QA was significantly higher than that of Plot CJ (Fig. 4-5).

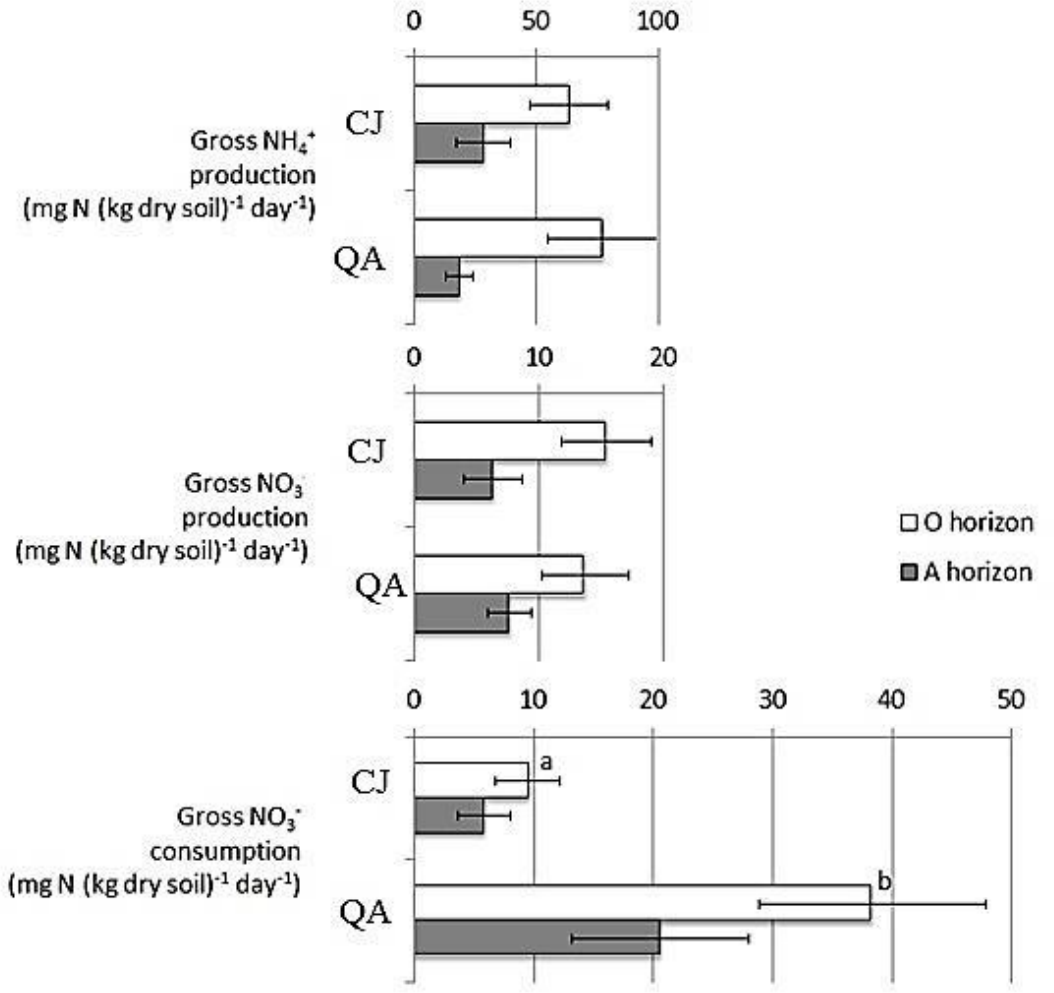

Fig.4-5 Averages of gross ammonium (NH_4^+) production rate, nitrate (NO_3^-) production, and consumption rates for all four sampling times in the *Cryptomeria japonica* (CJ) and *Quercus acutissima* (QA) stands. Bars with different lower-case and capital letters indicate significant differences among sample series.

Examining the vertical distributions of NO_3^- concentrations in soil water samples (Fig. 3-2a and c), NO_3^- concentrations of soil water were higher than those of the O horizon and did not decrease consistently in the soil profile, whereas NO_3^- concentrations were highest in the O horizon and decreased gradually toward deeper layers at Plot QA. The difference of gross NO_3^- consumption rate was the most likely

controlling factor for explaining differences in the distributions of NO_3^- concentration between Plots CJ and QA. That is, although there were the same levels of NO_3^- production at Plots CJ and QA, NO_3^- immobilization activity was significantly higher at Plot CJ.

Although there were no direct measurements of plant N uptake in this study, its impacts can be estimated from earlier studies on N utilization of typical tree species in Japan. Annual N uptake of Plot CJ and broadleaf deciduous tree stands ranged from 28 to 53 kg N ha^{-1} $year^{-1}$ (Harada et al. 1972, Tsutsumi 1962) and 42 to 108 kg N ha^{-1} $year^{-1}$ (Katagiri and Tsutsumi 1978), respectively. Soil mass of organic and A horizons (0–10 cm depths) at Plots CJ and QA were estimated using bulk densities of approximately 0.3 g cm^{-3} for the organic horizon and 0.5 g cm^{-3} for the mineral horizon (Ochiai 2013). Averaged organic horizon thickness, which was the part of organic matter with little mineral soil material (see sample collection description) was 3 cm at both plots. Under these conditions and assuming the same uptake intensities at the organic and mineral horizons, the N uptake rate of broadleaf deciduous tree stands were estimated at 0.42 to 0.81 mg N (kg dry soil) $^{-1}$ day^{-1} for the organic horizon and 0.08 to 0.15 mg N (kg dry soil) $^{-1}$ day^{-1} for the A horizon (0–10 cm depths). These rates of N uptake by trees are an order of magnitude smaller than microbial NO_3^- consumption rate at each horizon (10–79 mg N (kg dry soil) $^{-1}$ day^{-1} for the organic horizon and 3–60 mg N (kg dry soil) $^{-1}$ day^{-1} for the A horizon; Fig. 4-5). For *Cryptomeria japonica*, the uptake rate was 0.35 to 0.90 mg N (kg dry soil) $^{-1}$ day^{-1} for the organic horizon and 0.12 to 0.20 mg N (kg dry soil) $^{-1}$ day^{-1} for the A horizon,

which is still an order of magnitude smaller than the microbial NO_3^- consumption. In general, trees utilize not only NO_3^- but also NH_4^+; thus, the NO_3^- uptake might be even smaller than estimated above.

The mass balance estimations suggest that the microbial NO_3^- consumption was more responsible than plant uptake as a factor for reducing NO_3^- concentration in the soil of Plot QA, and was more effective in differentiating the observed NO_3^- retention mechanisms between the two plots. However, some contribution by plant uptake could not be ignored.

4.2.2 Replacement of atmospheric NO_3^- by soil microbial NO_3^-

The distributions of $\delta^{18}O$ values of NO_3^- in Chapter 3 (Fig. 3-4) indicate that NO_3^- ions derived from atmospheric deposition were replaced by NO_3^- produced effectively by soil microbes in the O and A horizons of both plots, as shown by the gross rates of NO_3^- production and consumption at those plots (Fig. 4-5). Moreover, the median value of $\delta^{18}O$ of NO_3^- in the O horizon at Plot QA was similar to that of soil waters (Fig. 3-4), whereas that in the O horizon at Plot CJ was similar to those of atmospheric deposition (rain, throughfall, and stem flow waters). This is also explained by the immobilization potential of the O horizon at Plot QA being significantly higher than that at Plot CJ. Therefore, NO_3^- was replaced more effectively at Plot QA.

Although NO_3^- concentrations in the O and A horizons were high in both plots

compared with the nationwide average (Urakawa et al. 2013), the major portion of such NO_3^- was not a direct accumulation of excess atmospheric NO_3^- but NO_3^- generated by actively nitrifying microbes in soil.

4.2.3. Controlling factors of gross NO_3^- production and consumption rates

What were the controlling factors of gross NO_3^- production and consumption that accelerated NO_3^- replacement? A statistically significant correlation was found between gravimetric soil moisture content and gross NO_3^- production and consumption at Plot CJ (Fig. 4-6a), although no correlation was detected at Plot QA. Many studies have reported that microbial nitrification is sensitive to soil moisture conditions and is activated under relatively wet conditions (Hirobe et al. 1998, Tokuchi et al. 2000, Tateno *et al*. 2005). Thus, one can reasonably conclude that gross NO_3^- production was affected by soil moisture conditions at Plot CJ (Fig. 4-6a). A similar tendency was detected even at Plot QA, although the correlation was not statistically significant.

The correlation between soil moisture and gross NO_3^- consumption at Plot CJ (Fig. 4-6b) also suggests soil moisture control of immobilization at that plot. However, it has been recognized that carbon availability for microbes is one of the major controls on immobilization (e.g., Vitousek et al. 1982, Aber et al. 1998). This is because NO_3^- immobilization is mainly governed by heterotrophic microorganisms requiring

biologically available C compounds as energy sources, whereas nitrification is mainly modulated by autotrophic microorganisms. Several studies have shown that a greater N retention capability requires greater C availability in soil systems (Gundersen et al. 1998; Curtis et al. 2011).

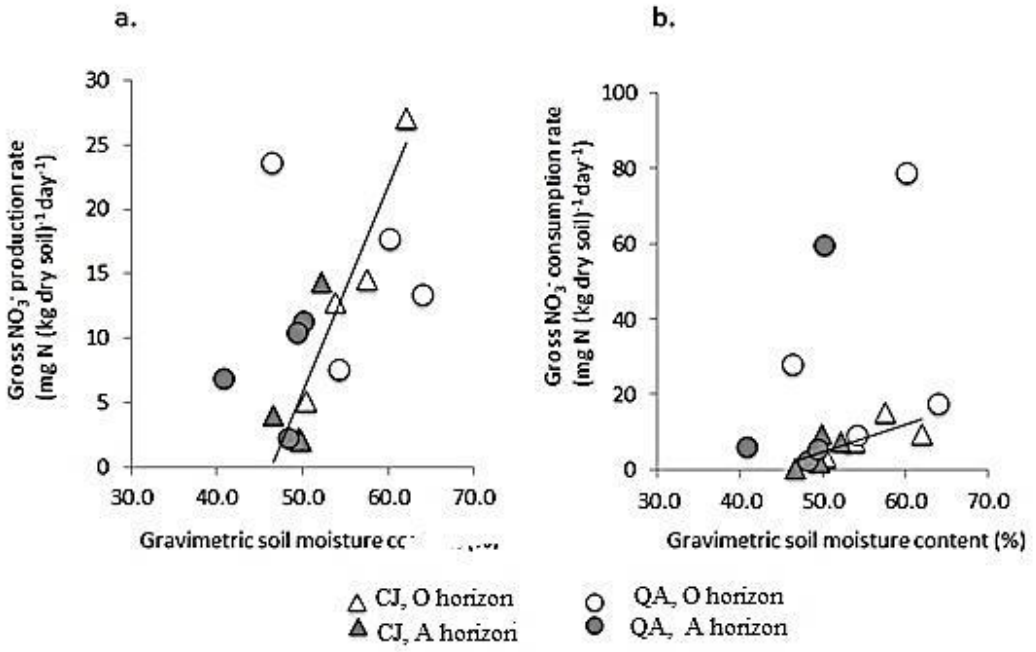

Fig. 4-6 (a) Relationship between gravimetric soil moisture content and gross nitrate (NO_3^-) production rate in *Cryptomeria japonica* (Plot CJ) and *Quercus acutissima* (Plot QA) stands. Positive correlation was found for Plot CJ, including both O and A horizon samples (R^2= 0.85207, $P < 0.05$). (b) Relationship between gravimetric soil moisture content and gross nitrate (NO_3^-) consumption rate for the same sample series. Positive correlation was found for Plot CJ, including both O and A horizon samples (R^2= 0.51803, $P < 0.05$).

The gross immobilization rate at the study site was higher at the forest floor of Plot QA than of Plot CJ (Fig. 4-5). However, there were no significant differences found in total C concentration and the C/N ratio (Table 4-1). Based on the earlier

understanding that carbon availability is a primary control of microbial NO_3^- immobilization, it can be hypothesized that presence of labile and bioavailable organic carbon substances was more significant at Plot QA than Plot CJ.

To find evidence supporting this hypothesis, one must seek more consistent information on presence and quantity of microbially available and labile organic carbon compounds for immobilizing microorganisms, in addition to the total C concentration. Still, comparative settings between the two plots of this study should be suitable for addressing the above issue.

4.2.4High N deposition and high gross N transformation rates in the Tanashi Forest: a comparative perspective

The results of gross rate measurements at the study site showed that the N transformation cycles included a very active NO_3^- production–immobilization cycle in addition to that cycle of NH_4^+. To address the relationship between high N deposition and high gross N transformation capability, a comparison may provide greater insight into the mechanisms of this linkage.

Tokuchi et al. (2014) presented N status and gross N transformation rates of soils in a coniferous forest with relatively low N deposition in the Kiryu Experimental Watershed (KEW). Annual total inorganic N deposition was approximately 10 kg N ha^{-1} yr^{-1} (Osaka et al. 2010), compared with more than 40 kg N ha^{-1} yr^{-1} at Plot CJ in the present study (Imamura 2014).

Although total C and N concentrations and C/N ratio of soils in the Tanashi Forest were not extremely different from those of the KEW, pool sizes of NH_4^+ and NO_3^- were substantially different. NH_4^+ pool size in the O horizon of KEW was several times larger than those in the Tanashi Forest. In contrast, sizes of the NO_3^- pool in the O horizon of the latter forest (20–125 mg N (kg dry soil)$^{-1}$) were several tens of times larger than those at KEW (1–1.7 mg N (kg dry soil)$^{-1}$). Also, the gross NO_3^- production rate of the O horizon in the Tanashi Forest (5–24 mg N (kg dry soil)$^{-1}$ day^{-1}; Fig. 4) was an order of magnitude higher than those in the KEW (0–2.1 mg N (kg dry soil)$^{-1}$ day^{-1}) (Tokuchi et al. 2014).

These differences clearly reflect whether only the NH_4^+ production-immobilization cycle is active, or both it and that of NO_3^- is active. In other words, in the Tanashi Forest, produced NH_4^+ was not only immobilized by heterotrophic microorganisms but also by autotrophic nitrifying microbes that can compete in nitrification.

Higher reactive N deposition can be considered a possible external factor inducing the difference between the KEW and Tanashi Forest. High N deposition might bloat the NH_4^+ production-immobilization cycle and consequently activate that of NO_3^-. There are other differences of environmental factors that potentially affect N dynamics between the KEW and Tanashi Forest. The KEW organic horizon is thinner (Tokuchi 1993) than that of Tanashi. The mineral soil of KEW is sandy, whereas that of Tanashi is silty. It is also important to understand how these factors affect the difference of N transformation dynamics in addition to the difference of atmospheric N input. The comparison between the KEW and Tanashi forests is still a useful means

for further understanding of the mechanisms behind the N saturation in Japan that was addressed by Mitchell et al. (1997), because those two sites have significant differences of N deposition and both have detailed information on soil N dynamics.

4.3 Conclusion

To clarify N dynamics of forest soils under high reactive N deposition, I conducted field observations of soil N status and N transformation rates in the Tanashi Forest in a suburban area of Tokyo.

N dynamics of the Tanashi Forest revealed by this investigation consisted of extremely large N transformation activities relative to those of KEW, which is in a region of low N deposition. Their comparison suggested that the high reactive N deposition was one of the important factors altering the N cycling structure from the NH_4^+ production-immobilization-dominated system to the NH_4^+ system plus NO_3^- system.

The high NO_3^- accumulation in soil is the most visible symptom of N saturation (Aber et al. 1989). The difference of NO_3^- immobilization capability between Plots CJ and QA of the study site (lower at Plot CJ) can be useful information to elucidate the mechanism of generation of high NO_3^- accumulation in the soil profile. Further insight into the above difference may show the conditions critical to the occurrence of high NO_3^- accumulation in soils and N saturation.

Additionally, I concluded that the combination of isotopic tracer and measurement

techniques of natural isotope ratios of NO_3^- used in this study are promising for attaining a mechanistic understanding of the impact of excess N input on the N dynamics in forest soils.

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Chapter 5

Summary and Conclusion

In Chapter 3, nitrogen (N) deposition and transformation dynamics along the canopy-soil continuum was investigated by nitrate isotopic composition. The investigation of N status and transformation rates in Chapter 4 revealed N dynamics of the internal nitrogen cycle in forest soils, determined using isotope tracer techniques during the growing season. In this chapter, I summarize new findings from the investigations and analysis of the present study and state requirements for future directions.

5.1 Input and incorporating processes of atmospheric NO_3^- in Tanashi Forest

The values of $\delta^{18}O$ and $\delta^{15}N$ may help clarify the sources of NO_3^-. According to the relationship between $\delta^{18}O$ and $\delta^{15}N$ values (Figs. 5-1 and 5-2), there are two centralized distribution areas at the two study sites. The largest $\delta^{18}O$ values of rain and throughfall were from 60‰ to 80‰, whereas the values of soil water were mainly below 20‰.

When rain falls through the forest vegetation to the floor, NO_3^- derived from the atmosphere is absorbed by plants and/or microbes. Owing to the distribution of microbes in the canopy and on tree trunks or stems, nitrification may occur.

Atmospheric NO_3^- is gradually mixed with microbial NO_3^- during contact with those three surfaces. Consequently, the NO_3^- of stemflow is a mixture of those two sources. Here, $\delta^{18}O$ values were less than 40‰–80‰ for *Cryptomeria japonica* (Fig. 5-1) and 30–80‰ for *Quercus acutissima* (Fig. 5-2).

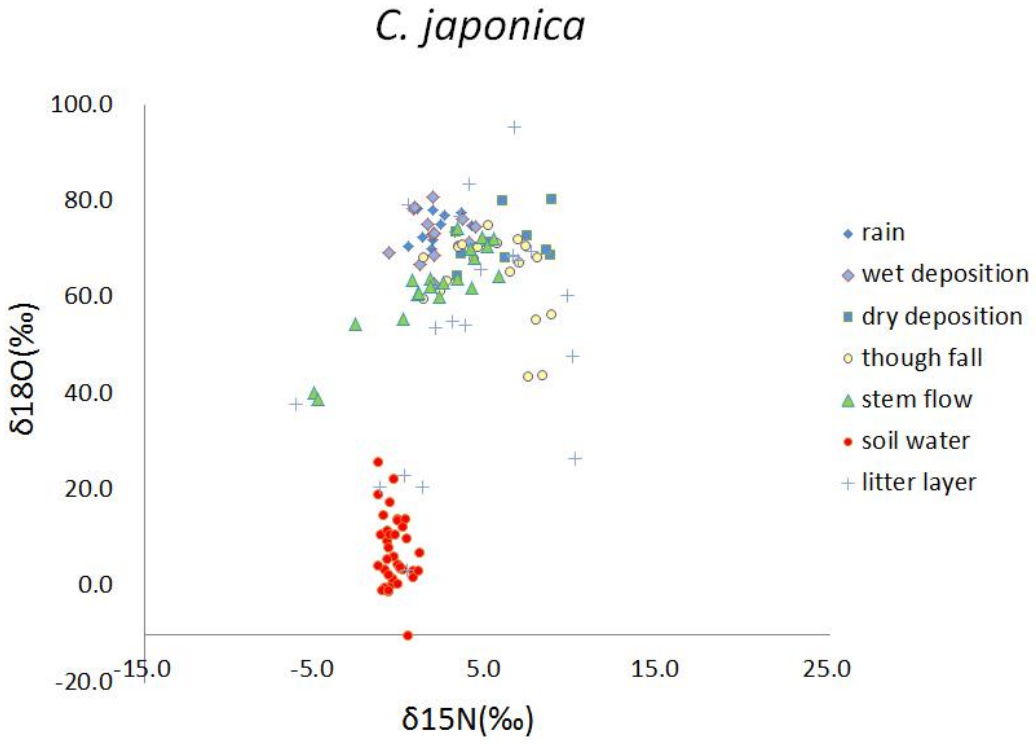

Fig. 5-1 Relationship between $\delta^{18}O$ and $\delta^{15}N$ values of NO_3^- at CJ site

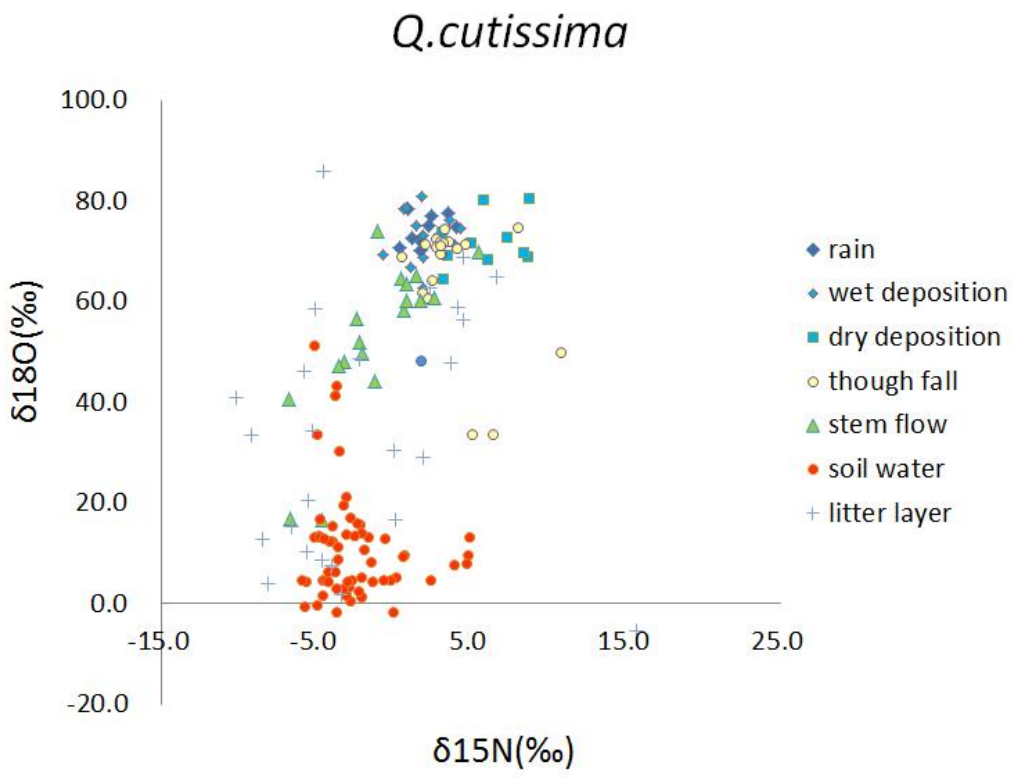

Fig. 5-2 Relationship between $\delta^{18}O$ and $\delta^{15}N$ values of NO_3^- at QA site

In the organic horizon and shallowest part of the mineral horizon, $\delta^{18}O$ values of NO_3^- indicate that NO_3^- molecules were effectively replaced by microbially produced NO_3^-. This observation and interpretation are reasonably confirmed by high gross rates of NO_3^- production at both CJ and QA sites.

5.2 Impact of high nitrogen deposition in suburban forests

Nitrogen input via throughfall has been reported at more than 40 kg N ha^{-1} yr^{-1} at the CJ site in Tanashi Forest (Imamura 2014). That value is significantly greater than the average 10–20 kg N ha^{-1} yr^{-1} in Japan, and is typical in regions surrounding the

Tokyo metropolitan area (Mitchell et al. 1997). Comparison of nitrogen dynamics between the Tanashi Forest and Kiryu Experimental Forest (KEW) suggest differences in the dominant cycling of inorganic N in soils. Whereas low N deposition (<10 kg N kg N ha^{-1} yr^{-1}; Osaka et al. 2010) at KEW involved the NH_4^+ production-immobilization cycle, that cycle for NO_3^- was involved in Tanashi Forest, in addition to the NH_4^+ cycle.

This hypothetically indicates that high atmospheric inorganic N input activates the nitrifying microbes. Consequently, the NO_3^- production-immobilization cycle is generated incrementally as an extra N cycle.

Because other factors possibly affect the difference of N dynamics between the KEW and Tanashi forests, such as pool size of organic N and carbon availability, it is difficult to verify the aforementioned hypothesis. More comprehensive comparisons are needed, not only between those two sites but also between those with varying atmospheric N input, to clarify the impact of high N deposition on structural alteration of nitrogen cycles in soil.

5.3 Future topic suggested by the difference between CJ and QA stands: Nitrogen dynamics in organic horizon as a key process

The A horizon at the surface of forest soil is the interface between the atmospheric and soil systems in the forest. The results of the present study show that nitrogen dynamics at the forest floor (organic horizon and shallow part of the A horizon) is a

key process of the nitrogen cycle dynamic within the entire forest nitrogen system. The results of gross rate measurements of soils also evidenced that the nitrogen dynamic in the A horizon is a key process in the soil internal nitrogen cycle.

An especially notable finding is that the gross immobilization rate was significantly higher on the forest floor of the QA stands than of the CJ stands, whereas the difference between their gross NO_3^- production rates was not significant (Fig. 4-4). It is hypothesized that carbon availability for heterotrophic microbes governing immobilization is a major determinant of the aforementioned difference between the CJ and QA stands.

To find evidence supporting this hypothesis, more consistent information is required on the presence and quantity of microbially available and labile organic carbon compounds for immobilizing microorganisms, in addition to the total carbon concentration. Nevertheless, comparison between the CJ and QA sites of the present study should provide a good opportunity for the above discussion and for further elucidating mechanisms of the progress of N saturation. This is because N status at the CJ stands indicated typical signs of N saturation, whereas that of the QA stands showed that N retention dynamics were ongoing, although the level of gross NO_3^- production was similar between the two stands.

Finally, I conclude that the combination of isotopic tracer techniques and measurement of natural isotope ratios of NO_3^- used in the present study are promising for attaining a mechanistic understanding of the impact of excess N input on nitrogen

dynamics in forest soils, and furnish a subject for further study.

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