

Original Article

Soil nitrogen transformation dynamics in a suburban forest near Tokyo Metropolitan Area under high nitrogen deposition: A case study using stable isotope tracer techniques

Jun SHI*¹, Nobuhito OHTA*¹, Naoko TOKUCHI*², Kazuo ISOBE*³, Rieko URAKAWA*¹, Naohiro IMAMURA*⁴, Tomoki ODA*¹ and Masakazu SUZUKI*¹

1. Introduction

The nitrogen (N) cycle is one of the most essential material cycles maintaining the whole life activities of forest ecosystems. The N cycle is also one of the most complex biogeochemical cycles in forests, with contributions from multiple functional organisms including plants, microbes, and animals. Both plants and microbes utilize water-soluble N in soils, and often compete to acquire bioavailable N, such as inorganic N and some low-molecular-weight organic N compounds (KAYE and HART, 1997).

Since the industrial revolution, reactive N has been emitted from anthropogenic sources, such as the combustion of fossil fuels and the production of synthetic N fertilizers, causing increased atmospheric N depositions. Currently, large regions of the world have average N deposition rates exceeding $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$, more than 10 times the natural rate (GU *et al.*, 2013). Excess NO_3^- in forest ecosystems can be lost through the hydrological processes (leaching), consequently contaminating aquatic systems such as groundwater aquifers, lakes, and streams (ABER *et al.*, 1997; GRUBER and GALLOWAY, 2008) potentially contributing to eutrophication (BURNS *et al.*, 2009). Excess NO_3^- can also be converted into gaseous forms of N (e.g., NO, N_2O ; BODIRSKY *et al.*, 2012) by denitrifying microbes, thereby becoming reincorporated into the global N cycle (XURI and PRENTICE, 2008). N_2O is one of the most important greenhouse gases contributing to global warming (TIAN *et al.*, 2011).

Since the late 1980s, excess N input has been reported to cause increases in stream NO_3^- concentrations in the forested catchments of the northeast United States and northern Europe, in what is called “N saturation”. A hypothesized scenario of the expected time course of N saturation, including plant decline and high N exports through hydrological systems was proposed, and since then, many studies and discussions have focused on these issues (e.g. SKEFFINGTON and WILSON, 1988; ABER *et al.*, 1989; STODDARD, 1994; ABER *et al.*, 1998). MITCHELL *et al.* (1997) collected the N input-output data from the 24 Japanese forested catchments distributed in nation-wide (from 43°N to 35°N in latitude) in order to evaluate the status of N saturation. They illustrated that the

*1 Department of Forest Science, Graduate School of Agricultural and Life Sciences, University of Tokyo

*2 Field Science Education and Research Center, Kyoto University

*3 Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo

*4 University of Tokyo Forest, Graduate School of Agricultural and Life Sciences, University of Tokyo

forests receiving relatively high inorganic N input (7.6-10.5 kg N ha⁻¹ year⁻¹) were located intensively in the Kanto Plain and its surrounding mountains, while the average of all sites was 6.3 kg N ha⁻¹ year⁻¹, and that those high N input sites discharged higher inorganic N (13.5-28.0 kg N ha⁻¹ year⁻¹) compared to the other sites. They interpreted those as one of the symptoms of N saturation occurred in this region in Japan.

To gain a precise understanding of N saturation in forested ecosystems, elucidating the mechanisms of N dynamics in soil and the responses to excess N input is essential. In particular, the retention mechanisms of N in soils are the most important factors in understanding N saturation in ecosystems.

In most forest ecosystems, organic matter represents the largest stores of N, and soil organic N can be mineralized and partly nitrified. Produced inorganic N species (NH₄⁺ and NO₃⁻) can be immobilized by microbes and then eventually return to organic forms. Among these multiple transformation processes, the most critical functions controlling retention capability are microbial immobilization.

In many previous studies aimed to quantitatively describe 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 ¹⁵N tracers proposed by DAVIDSON *et al.* (1992) allows for the measurement of gross rates of mineralization, nitrification, and immobilization. This method has been applied mainly to the 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. A ¹⁵N tracer experiment by CURTIS *et al.* (2011) provided evidence confirming the importance of C availability in microbial immobilization.

Nitrification, however, is still a key process responsible for the transformation of ammonia (NH₃) into ammonium (NH₄⁺), nitrite (NO₂⁻), and finally nitrate (NO₃⁻), as mediated by microbes. Because of its high solvility, NO₃⁻ is a form of N that can easily be used by plants and microbes, and can also be leached with water flow; therefore, the NO₃⁻ pool size in soil is generally controlled both by biological and hydrological processes in forest ecosystems (OHTE, 2012).

As a causal factor, how N input from atmospheric deposition affects or disturbs the N cycle in forest ecosystems is also critical. Isotopic signatures of NO₃⁻ can provide useful information to trace how atmospheric NO₃⁻ is incorporated into the internal cycling of N in the forest soils (KENDALL and MCDONNELL, 1998; ELLIOTT *et al.*, 2007; OHTE, 2013). In terrestrial systems, the method has been recognized for its ability to distinguish between atmospheric (OHTE *et al.*, 2004; SEBESTYEN *et al.*, 2008; TOBARI *et al.*, 2010) and soil microbial sources of NO₃⁻. Atmospheric NO₃⁻ has distinctly higher δ¹⁸O values (60 to 80 ‰) than NO₃⁻ originating from microbial processes (-15 to 15‰; KENDALL *et al.*, 2007).

Atmospherically deposited NO₃⁻ 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 NO₃⁻ is consequently replaced by the NO₃⁻ produced by microbes in soils. Changes in the

$\delta^{18}\text{O}$ value of NO_3^- in soils reflects this replacement of NO_3^- (OHTE, 2013), which suggests that changes in $\delta^{18}\text{O}$ can be used as an indicator to evaluate the N transformation activities in soils.

Prior to the study presented in this paper, we monitored the NO_3^- concentration and isotope compositions in rain and soil waters along the canopy-soil continuum of the same study site (SHI *et al.*, 2014). Quick decrease in the $\delta^{18}\text{O}$ of NO_3^- in the soil waters of the organic horizons was observed and suggested that the atmospheric NO_3^- supplied to the forest floor was effectively replaced by the NO_3^- produced by the microbes in soils.

In this paper, in order to clarify the current N status and to elucidate the impact of excess N input on the N cycles in soils, and specifically to describe the detail mechanisms of N transformation as the incorporating processes of the atmospheric N, we investigated the transformation dynamics of N in the forest floor of a typical suburban forest stands near the Tokyo metropolitan area in Japan receiving heavy atmospheric N deposition. For the present purpose, we utilized the pool dilution method using ^{15}N tracer (DAVIDSON *et al.*, 1992) to measure the gross rates of N transformation.

2. Material and methods

2.1. Study site

Field observations were conducted in the University of Tokyo, Tanashi Forest, which is located in the city of Nishi-Tokyo, 20 km west of the Tokyo metropolitan area (35°44' N, 139°32' E). 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°C which is based on the data published by THE UNIVERSITY OF TOKYO FORESTS (2014). Lack data (March 2012) was filled with the AMeDAS (Automated Meteorological Data Acquisition System) data at Fuchu located approximately 10 km southwest from the study site published by JAPAN METEOROLOGICAL AGENCY (2014). Averaged annual precipitation for 2009-2012 was 1718 mm (IMAMURA, 2014).

According to IMAMURA (2014), averaged annual inorganic N input was 10.4 kg N ha⁻¹ year⁻¹ (2009-2012). As we mentioned by citing the investigation by MITCHELL *et al.* (1997) in the introductory section, the N deposition of the study site was typical in the Kanto Plain, and the significantly higher than the nation wide average.

We selected two different tree stands within the forest: one dominated by *Cryptomeria japonica* and another dominated by *Quercus acutissima*. *C. japonica* is an evergreen coniferous tree and one of the most popular plantation species for timber production in Japan, with plantations accounting 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 used in this study site 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*.

Q. acutissima is an oak native to East Asia. The secondary deciduous forest dominated by *Q. acutissima* is quite common on a large part of Honshu Island of Japan, and had been utilized as coppices often situated in agricultural or suburban regions. It is a medium-sized, broadleaf

deciduous tree that grows 25-30 m in height, with a trunk that can reach up to 1.5 m in diameter. The bark is dark gray and deeply furrowed, while leaves are 8-20 cm in length and 3 - 6 cm in width. The *Q. acutissima* stand in this study site 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, while December-March is the dormant season, during which leaves are absent. Hereafter, we call the *C. japonica* plantation as “Plot A” and the *Q. acutissima*-dominated stand as “Plot B.” Also, we use the *Q. acutissima*'s growing season (April-November) as “the growing season” of this study site.

The soil in the Tanashi Forest is Andisol (i.e., volcanic ash soil) with a relatively thick A horizon (50-55 cm in depth from the surface), underlain by >60 cm in depth of B-horizon soil. Apparent aggregates were observed in the topsoil horizon (<5 cm in depth). Both Plots A and B had similar mineral soil horizon profiles. The organic horizon was 6-8 cm in thickness at Plot A and 4-7 cm in thickness at Plot B. Within the organic horizon, the Oi horizon was thicker at Plot A than at Plot B. Both at Plots A and B, there were the Oa horizon between the accumulations of organic matters and the surface of mineral soil horizon consisting of the mixture of well-decomposed organic materials and mineral soils, and its thickness was approximately 3 cm.

2.2. Sample collections and field observations

Soil samples for chemical analysis and experiments on N transformation were collected in Plots A and B during the growing season: June and August in 2012, and April and September in 2013. Organic soil of the lowest part of the organic horizon was sampled (hereafter, O horizon) and mineral soil from 0 to 10 cm in depth (hereafter, A horizon). Precisely annotating, the samples of the “O horizon” here included not only organic matters, but also little amount of the mineral soil materials from the Oa horizon.

Sampling was conducted basically during 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 measurements of N transformations. The soil samples were stored at 4°C until analysis and used for chemical analysis and net N transformation measurements within 7 days, and gross N transformation measurements using ¹⁵N tracers were performed within 14 days after sampling.

Figure 1 shows changes in the monthly mean air temperature observed at the meteorological station of the Tanashi Forest located 100 m southwest of Plot A (THE UNIVERSITY OF TOKYO FORESTS, 2014). Seasonal patterns of air temperatures both in 2012 and 2013 were almost identical. Thus, we present the results of our experiments throughout the growing season from April to September, although the sampling was conducted in June and August of 2012, and in April and September of 2013.

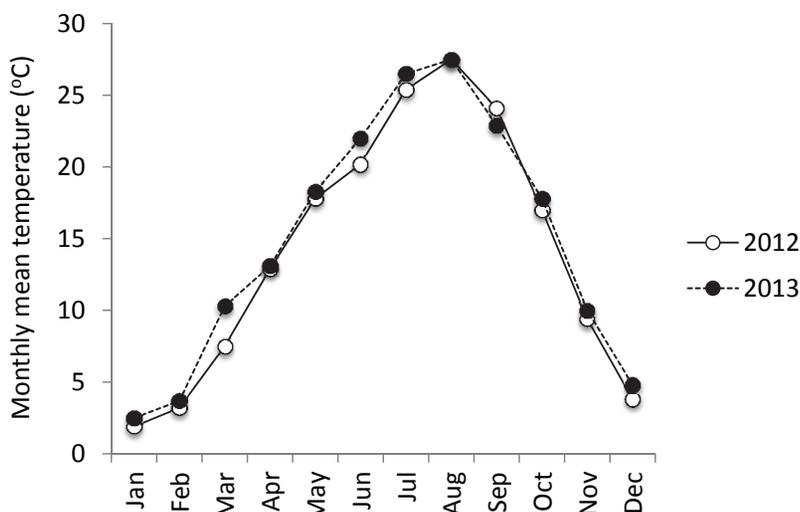


Fig. 1. Monthly mean temperature in 2012 and 2013 (data source: the data published by THE UNIVERSITY OF TOKYO FORESTS (2014). Missing data (March 2012, Jun 2013) was filled with the AMeDAS (Automated Meteorological Data Acquisition System) data at Fuchu located approximately 10 km southwest from the study site published by JAPAN METEOROLOGICAL AGENCY (2014).

2.3. Soil chemical analysis and measurements of gross N transformation rates

Organic matter and soil samples were sieved using 4- and 2-mm mesh, respectively. Soil water content was measured by drying soil at 105 °C in a ventilated oven. Soil samples for total N and C concentrations (hereafter, called “total N and C,” respectively) were ground and measured using an NC analyzer (SUMIGRAPH NC-22A, Sumika Chemical Analysis Service, Ltd., Osaka, Japan) at the Field Science Education and Research Center, Kyoto University.

The gross NH_4^+ production (mineralization) rates and nitrification rates were determined using the following measurements and calculations. Gross NH_4^+ production rates were determined using the $^{15}\text{NO}_3^-$ 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 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 hours 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 °C for 26 hours, and was then extracted using 50 mL of 2 M KCl to obtain the 24-hour incubation measurement. The moisture content of each incubation sample was kept as the condition of the sampling time.

NH_4^+ in the extracts was recovered as $(\text{NH}_4)_2\text{SO}_4$ 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 above procedures for ^{15}N isotopic analysis was converted into N_2O using same procedure of the microbial denitrifier method for the measurement of $\delta^{18}\text{O}$ from

NO_3^- proposed by as described above. Isotopic measurement of N_2O was conducted using a gas chromatography-mass spectrometer (GCMS-QP2010 Ultra, Shimadzu, Kyoto, Japan). Then, the ^{15}N atom % in the N_2O was determined using the equations presented by ISOBE *et al.* (2011). The NH_4^+ and NO_3^- production rates, as well as the NH_4^+ and NO_3^- consumption rates, were determined from changes in the fractional abundance above the natural abundance in the soils and the 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 and BARTHOLOMEW (1954):

$$m = \frac{M_0 - M_t}{t} \times \frac{\log(H_0 M_t / H_t M_0)}{\log(H_0 / M_t)} \quad (1)$$

$$c = \frac{M_0 - M_t}{t} \times \frac{\log(H_0 / H_t)}{\log(M_0 / M_t)} \quad (2)$$

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

2.4. Statistical analysis

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

3. Results

3.1. Total C and N concentrations and NH_4^+ and NO_3^- concentrations in soils

Although statistical differences were found among the sampling times in several items (total N concentration, C/N ratio, and gravimetric water content), clear seasonal variations were not identified (Table 1). Significant difference between Plots A and B was detected only for the total N concentration of the O horizon ($P < 0.05$). The NH_4^+ and NO_3^- concentrations of extracted samples from each sampling time are shown in Figure 2. While the NH_4^+ and NO_3^- concentrations were higher in the A horizon than the O horizon at a few sampling times, those concentrations were higher in the O horizon than the A horizon at most sampling times. Although statistically significant

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

	Total C (mg C (g dry soil) ⁻¹)		Total N (mg N (g dry soil) ⁻¹)		C/N		Gravimetric water content (%)	
	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon	O horizon	A horizon
Plot A								
<i>Cryptomeria japonica</i>								
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)
Plot B								
<i>Quercus acutissima</i>								
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)

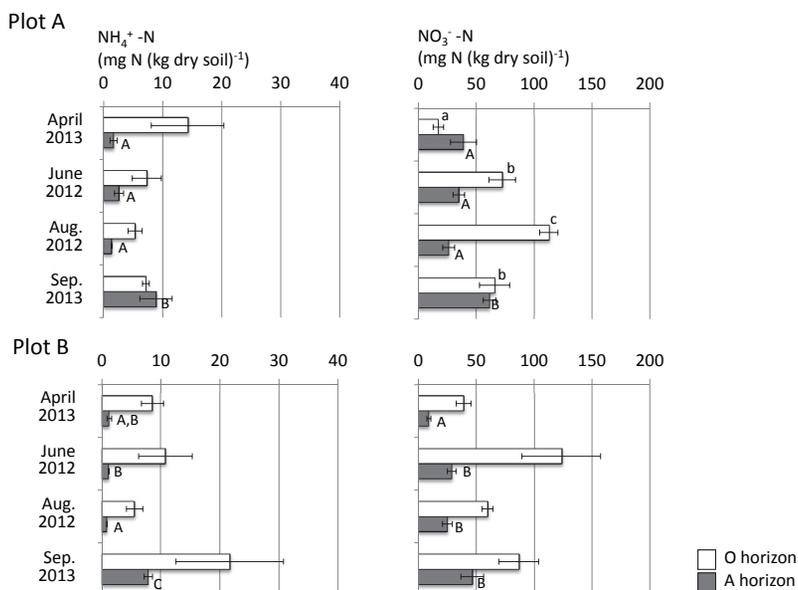


Fig. 2. Ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations of each sampling time in the O and A horizons in the *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands. Bars with different upper and lower-case letters indicate significant differences between each sample series.

differences were found among the sampling times in all the A horizon samples and in the NO_3^- concentrations of the O horizon at Plot A, consistent seasonal patterns were not identified.

3.2. 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 Figures 3 and 4, respectively. In most cases, a gross transformation rate of NO_3^- is an order of magnitude lower than that of NH_4^+ . The gross NO_3^- production rates at both plots (3 - 26 $\text{mg N (kg dry soil)}^{-1} \text{ day}^{-1}$) were similarly higher than those previously reported from Japanese forests (KUROIWA *et al.*, 2011; TOKUCHI *et al.*, 2000; TOKUCHI *et al.*, 2014).

Although an extremely high gross NO_3^- consumption rate was observed in the O and A horizons of Plot B site in June, consistent seasonal patterns were not identified. The gross NO_3^- consumption rates in Plot B from June to September were slightly higher than those in Plot A, especially in the O horizon.

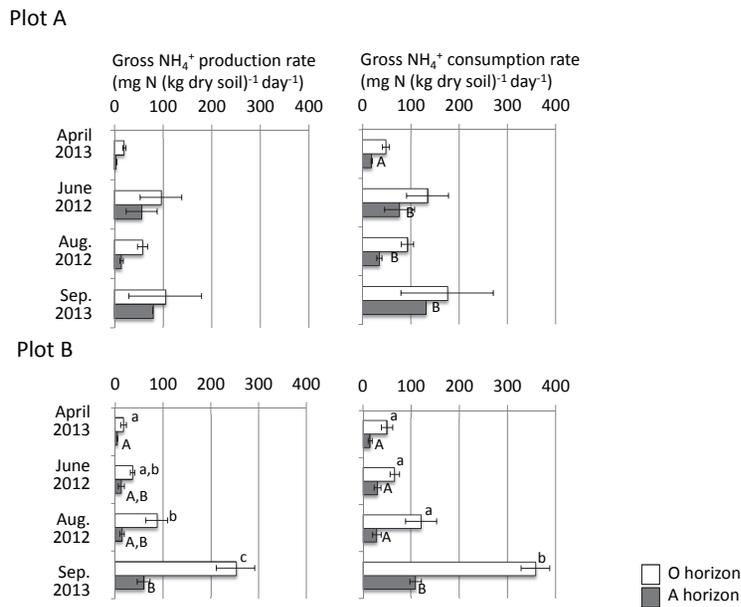


Fig. 3. Gross ammonium (NH_4^+) production and consumption rates of each sampling time in the O and A horizons in the *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands. Bars with different upper and lower-case letters indicate significant differences between each sample series.

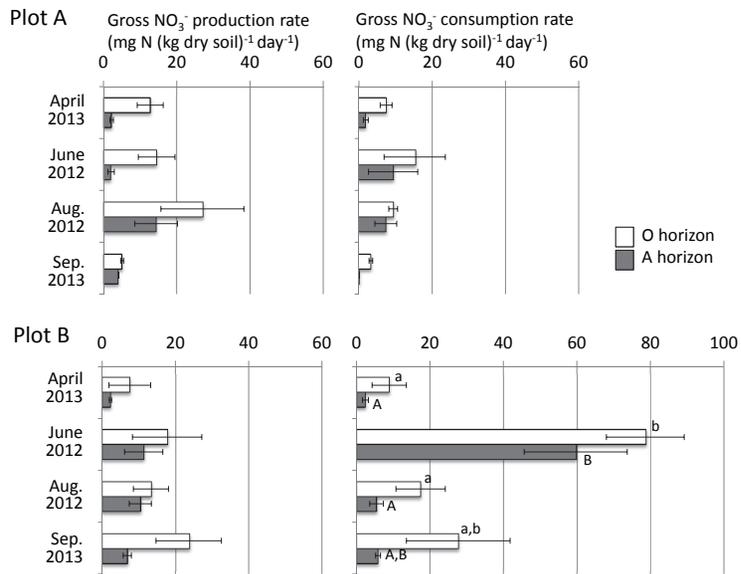


Fig. 4. Gross nitrate (NO_3^-) production and consumption rates during each sampling time in the O and A horizons at the *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands. Bars with different upper and lower-case letters indicate significant differences between each sample series.

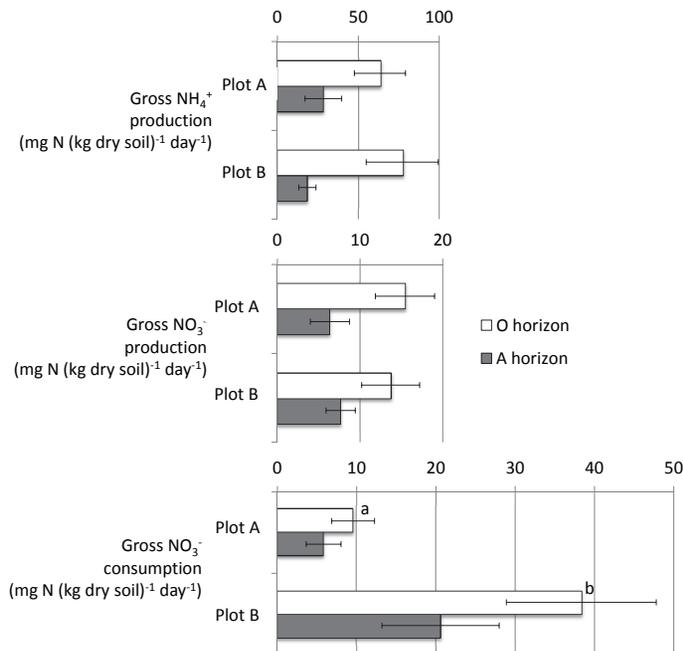


Fig. 5. Average values of gross ammonium (NH_4^+) production rate, nitrate (NO_3^-) production, and consumption rates for all four sampling times in the *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands. Bars with different upper and lower-case letters indicate significant differences between each sample series.

4. Discussion

4.1. Effects of NO_3^- production and consumption balance

To compare the gross rates of NH_4^+ and NO_3^- transformation between Plots A and B, average values for all four sampling times at each site are presented in Figure 5. For both NH_4^+ and NO_3^- in the O and A horizons, no statistically significant differences were found between Plots A and B. Gross production rates of both NH_4^+ and NO_3^- were higher in the O horizon than the A horizon at both Plots A and B, although the gross NO_3^- consumption rate at Plot B was significantly higher than that of Plot A (Figure 5).

According to the observations of the vertical distributions in NO_3^- concentrations in soil water samples of the same site presented in SHI *et al.* (2014), the NO_3^- concentrations of soil water were higher than those of the O horizon and did not decrease consistently in the soil profile at Plot A (denoted as the CJ stands in SHI *et al.*, 2014), while the NO_3^- concentrations were highest in the O horizon and decreased gradually toward deeper layers at Plot B (denoted as the QA stands in SHI *et al.*, 2014). The difference in the gross NO_3^- consumption rate was the most likely controlling factor explaining differences in the distributions of NO_3^- concentration between Plots A and B. That is, while the same levels of NO_3^- production occurred at Plots A and B, the NO_3^- immobilization activity was significantly higher at Plot B.

Although direct measurements have not been conducted for the N uptake of plants in this study, this impact can be estimated from previous studies on the N utilization of typical tree species in Japan. Annual N uptake of the *Cryptomeria japonica* stands and broad leaf deciduous tree stands ranged from 28 to 53 kg N ha⁻¹ year⁻¹ (HARADA *et al.*, 1972; TSUTSUMI, 1962) and from 42 to 108 kg N ha⁻¹ year⁻¹ (KATAGIRI and TSUTSUMI, 1978), respectively. Soil mass of organic and A horizons (0-10 cm in depth) at Plots A and B can be 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 matters with little mineral soil material (see Sample collection description) was 3 cm at both Plots A and B. Under these conditions, assuming the same uptake intensities at the organic and mineral horizons, N uptake rate of broad leaf deciduous tree stands are estimated using the data from KATAGIRI and TSUTSUMI (1978) as 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 in depth). 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, see Figure 5). For *Cryptomeria japonica*, uptake rate are also estimated using the data from HARADA *et al.* (1972) and TSUTSUMI (1962) as 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. Moreover in general, trees utilize not only NO_3^- but also NH_4^+ ; thus, the NO_3^- uptake might be even smaller than above estimations.

These mass balance estimations suggested that the microbial NO_3^- consumption was more responsible than plant uptake as a decreasing factor of the NO_3^- concentration in the soil of Plot B,

and played more effective role to differentiate the observed NO_3^- retention mechanisms between Plots A and B, even though some contribution by plant uptake could not be ignored.

4.2. 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 the gravimetric soil moisture content and the gross NO_3^- production and consumption at Plot A (Figure 6a), although no correlation was detected at Plot B. Many previous 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 condition at Plot A (Figure 6a). The similar tendency can be detected even for Plot B, although the correlation was not statistically significant.

The correlation between soil moisture and gross NO_3^- consumption was also found at Plot A (Figure 6b), implies the possibility of the soil moisture control also on immobilization at Plot A. However, it has previously been recognized that the carbon availability for the microbes is one of the major controlling factors of immobilization (e.g. VITOUSEK *et al.*, 1982; ABER *et al.*, 1998), because the NO_3^- immobilization is mainly governed by heterotrophic microorganisms requiring biologically available C compounds as energy sources, while nitrification is mainly governed by autotrophic microorganisms. Several previous studies have shown that higher N retention capability requires higher C availability in soil systems (GUNDERSEN *et al.*, 1998; CURTIS *et al.*, 2011).

In our study site, the gross immobilization rate was higher in the forest floor of Plot B than in Plot A (Figure 5). However, the significant differences could not be found in the total C concentration

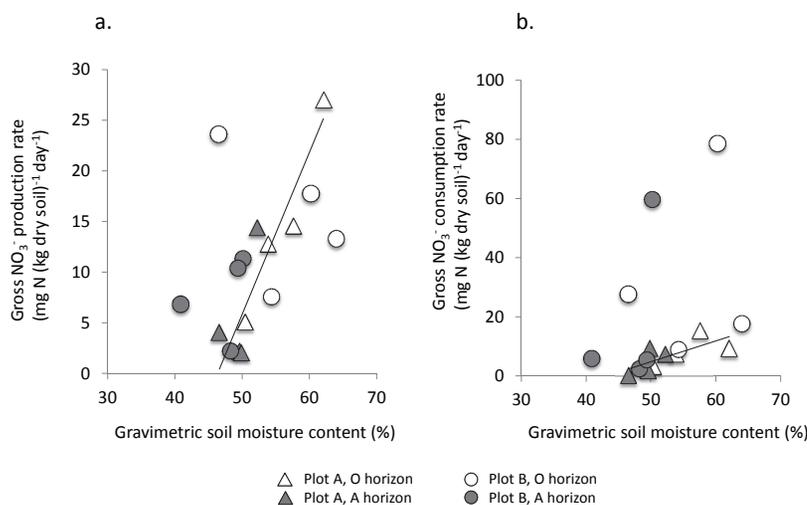


Fig. 6. (a) Relationship between gravimetric soil moisture content and gross nitrate (NO_3^-) production rate in the *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands. Positive correlation was found for Plot A including both the O and A horizon samples ($R^2 = 0.85207$, $P < 0.05$).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

and the C/N ratio (Table 1). Based on the previously proposed understanding that the carbon availability is a primary controlling factor of microbial NO_3^- immobilization, it can be hypothesized that presence of specifically labile and bioavailable organic carbon substances was more significant in Plot B compared to Plot A.

To find evidences supporting this hypothesis, we need to 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 Plots A and B of this study should be suitable for the above discussion.

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

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

TOKUCHI *et al.* (2014) presented the N status and the 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 $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (OSAKA *et al.*, 2010), while it was more than $40 \text{ kg N ha}^{-1} \text{ year}^{-1}$ at Plot A in our 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, the pool sizes of NH_4^+ and NO_3^- were significantly different. The NH_4^+ pool size in the O horizon of KEW was several times higher than those found in the Tanashi Forest, and in contrast, the sizes of the NO_3^- pool in the O horizon of the Tanashi Forest ($20\text{-}125 \text{ mg N (kg dry soil)}^{-1}$) were several ten times higher than those in KEW ($1\text{-}1.7 \text{ mg N (kg dry soil)}^{-1}$). Also the gross NO_3^- production rate of the O horizon in the Tanashi Forest ($5\text{-}24 \text{ mg N (kg dry soil)}^{-1} \text{ day}^{-1}$, Figure 4) was an order of magnitude higher than those in KEW ($0\text{-}2.1 \text{ mg N (kg dry soil)}^{-1} \text{ day}^{-1}$, TOKUCHI *et al.*, 2014).

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

Higher reactive N deposition can be considered as a possible external factor inducing this difference between KEW and the Tanashi Forest. High N deposition might bloat the NH_4^+ production-immobilization cycle and consequently activate the NO_3^- production-immobilization cycles. There are the other differences of environmental factors potentially affecting the N dynamics between KEW and the Tanashi Forest: The organic horizon of KEW is thinner (TOKUCHI, 1993) than that of the Tanashi Forest, and the mineral soil of KEW is sandy, while that of Tanashi is silty. It is also important to understand how these factors affect the difference of the N transformation

dynamics in addition to the difference in the atmospheric N input. The comparison between KEW and the Tanashi forest is still a notable opportunity for further understanding of the mechanisms behind the N saturation in Japan that have been focused by MITCHELL *et al.* (1997), because those sites have significant difference in N deposition, and both have detailed information on the soil N dynamics.

5. Conclusion

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

The N dynamics of the Tanashi Forest revealed by our investigation consisted of extremely large N transformation activities compared to those of KEW, which is located in the region with low N deposition. The 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 the NO_3^- production-immobilization system.

The high NO_3^- accumulation in soil is the most visible symptom of N saturation (ABER *et al.*, 1989). In our cases, while the NO_3^- concentrations at the O and A horizons were significantly high in both Plots A and B compared to the nation wide average level (URAKAWA *et al.*, 2013), the major portion of those NO_3^- was not direct accumulation of excess atmospheric NO_3^- , but the NO_3^- generated by active nitrifying microbes in soils. Difference in the NO_3^- immobilization capability between Plots A and B of our study site (lower in the Plot A) can be useful information to elucidate the mechanism behind the generation of high NO_3^- accumulation in soil profile. Further insight of the above difference may show the critical conditions of the occurrence in high NO_3^- accumulation in soils and N saturation.

Additionally, we concluded that combination of isotopic tracer techniques used in this study and measuring techniques of natural isotope ratios of NO_3^- applied in SHI *et al.* (2014) can hold promise as a method used to gain a mechanistic understanding of the impact of the excess N input on the N dynamics in forest soils.

6. Acknowledgments

This work was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology for Science Research (24658133) and a grant for cooperative research with the Center for Ecological Research, Kyoto University (FYs. 2011, 2012). The authors would like to thank Dr. Takashi NAKAMURA of the University of Yamanashi and Dr. Ichiro TAYASU of Kyoto University for their support in isotopic analysis.

Summary

To describe the nitrogen (N) dynamics of forest soils under heavy N deposition, gross N transformation rates of the organic (O horizon) and mineral soils (A horizon) of the forest floor were measured in *Cryptomeria japonica* (Plot A) and *Quercus acutissima* (Plot B) stands in a suburban forest of the Tokyo metropolitan area, which receives higher atmospheric N deposition than the national average level. At both Plots A and B, the extractable NO₃⁻ concentration ranged from 10 to 120 mg N (kg dry soil)⁻¹ at the O and A horizons, which was significantly higher than the national average. While the gross nitrification rates at both plots (3 - 26 mg N (kg dry soil)⁻¹ day⁻¹) were also higher than those previously reported from Japanese forests, the gross NO₃⁻ immobilization rate at Plot B (3 - 78 mg N (kg dry soil)⁻¹ day⁻¹) was significantly higher than that at Plot A (3 - 17 mg N (kg dry soil)⁻¹ day⁻¹). These data explained the previously reported fact that the NO₃⁻ concentration of soil waters at the A horizon of Plot B decreased, while that decrease was not observed at Plot A. From a comparison with the case of a low N deposition site such as Kiryu Experimental Watershed in the central part of Japan, it was suggested that the high reactive N deposition was one of the important factors altering the N cycling structure from the NH₄⁺ production-immobilization-dominated system to the NH₄⁺ system plus the NO₃⁻ production-immobilization system.

Keywords: forest soil, nitrogen transformation, ¹⁵N pool dilution method, suburban forest

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(Received July 14, 2014)

(Accepted Jan. 21, 2015)

東京近郊の森林における高窒素沈着下の土壌窒素の形態変化 について：安定同位体トレーサーを利用した事例研究

石碯 *1・大手信人 *1・徳地直子 *2・磯部一夫 *2・浦川梨恵子 *1・

今村直広 *4・小田智基 *1・鈴木雅一 *1

*1 東京大学大学院農学生命科学研究科森林科学専攻

*2 京都大学フィールド科学教育研究センター

*3 東京大学大学院農学生命科学研究科応用生命化学専攻

*4 東京大学大学院農学生命科学研究科附属演習林

要 旨

大気沈着による窒素の負荷が過剰な森林における土壌中の窒素ダイナミクスの実態を明らかにするために、東京の都市近郊に位置する東京大学田無演習林内のスギ林分 (Plot A) とクヌギ林分 (Plot B) において、植物の成長期における有機物層、鉍質土層表層部の無機態窒素 (NO_3^- , NH_4^+) 現存量、総窒素無機化・硝化速度を測定した。両方の林分において、無機態の窒素濃度 ($10 - 120 \text{ mg N (kg dry soil)}^{-1}$) は有機物層、鉍質土層表層部で、日本の森林における平均的な値より顕著に高かった。総硝化速度も ($3 - 26 \text{ mg N (kg dry soil)}^{-1} \text{ day}^{-1}$)、両林分において我が国でこれまでに報告されている事例に比べて高かったが、総 NO_3^- 不動化速度は Plot B ($3 - 78 \text{ mg N (kg dry soil)}^{-1} \text{ day}^{-1}$) において、Plot A ($3 - 17 \text{ mg N (kg dry soil)}^{-1} \text{ day}^{-1}$) よりも顕著に高かった。これらのことは、先行の調査結果で Plot B の A 層における土壌水中の NO_3^- の濃度がその上層に比べて低下し、Plot A ではその傾向が見られない現象を説明するものであった。日本の中央部に位置する桐生水文試験地のような窒素沈着量が少ないサイトとの比較から、反応性の窒素沈着が多いことが、 NH_4^+ の生成 - 不動化が支配的なシステムから、それに NO_3^- の生成 - 不動化システムが付加されたシステムへの移行を生じさせる重要な要因であることが示唆された。

キーワード：森林土壌、窒素形態変化、 ^{15}N 同位体希釈法、都市近郊林