Nitrous oxide emissions in proportion to nitrification in moist temperate forests

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Abstract Chronic elevated nitrogen deposition has increased nitrogen availability in many forest ecosystems globally, and this phenomenon has been suggested to increase soil nitrification. Although it is believed that increased nitrogen availability would also increase nitrous oxide (N_2O) emissions from forest ecosystems, its impact on N_2O flux is poorly known. In this study, 3-years monitoring of N_2O emissions was performed in a forested watershed receiving elevated nitrogen deposition and located in the suburbs of Tokyo, Japan. In addition, a comparative field survey was carried out in nine temperate forest sites with varying nitrogen availabilities. In the intensively studied forest site showing typical nitrogen saturation, the average annual N_2O emissions from the whole watershed were estimated to be 0.88 kg N ha^{-1} year^{-1}, comparable to the highest observed levels for temperate forests except for some very high emission sites in Europe. Although no correlation was found for humid spots with WFPS >60%, a clear positive correlation was noted between N_2O flux and net nitrification rate in situ for plots with water-filled pore space (WFPS) < 60%. The N_2O flux varied across nine forest sites almost in proportional to the stream water NO_3^- concentration in the watershed that ranged from 0.14 to 1.64 mg N/L. We conclude that N_2O emissions are related to nitrification in moist temperate forest, which may be associated with the magnitude of nitrogen saturation.

Keywords Nitrous oxide · Nitrogen availability · Nitrogen saturation · Nitrification · Temperate forest

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Introduction

Nitrous oxide (N_2O), the third most influential greenhouse gas in global warming, is estimated to account for approximately 6% of the radiative forcing of the global climate (IPCC 2007). Atmospheric N_2O concentrations have increased nearly linearly by approximately 0.8 ppb year^{-1} over the past few decades, from approximately 270 ppb during the pre-industrial era to 327.1 ± 0.1 ppb in 2014 (WMO 2015). The current increase of atmospheric N_2O has the potential to impact global climate in the next century (Shindell et al. 2009).

Temperate forests have been identified as a major source of N_2O emissions (e.g., Brumme and Beese

1992; Skiba et al. 1999). However, current estimates of N_2O emissions from temperate forests vary markedly. Some forests show high emissions whereas others have negligible emissions. In several temperate forests in Europe receiving elevated levels of atmospheric nitrogen deposition, high annual N_2O flux has been reported; e.g., 0.67–0.92 kgN_2O-N ha^{-1} year^{-1} (Schmidt et al. 1988), 1.15–2.29 kgN_2O-N ha^{-1} year^{-1} (Pitcairn et al. 2002), and 2.6 kgN_2O-N ha^{-1} year^{-1} (Eickenscheidt et al. 2011). The highest N_2O flux was observed in an N-saturated forest site in Germany ('Sölling'), which has a particularly low pH of 3.5 (Eickenscheidt and Brumme 2013). To the contrary, low N_2O emissions have been generally reported in temperate forests in North America; e.g., $-$ 0.03 to 0.06 kgN_2O-N ha^{-1} year^{-1} (Castro et al. 1992) and 0.01–0.02 kgN_2O-N ha^{-1} year^{-1} (Bowden et al. 1990). Due to the adoption of such variable fluxes in different forests to evaluate global emissions, current estimates of N_2O emissions from temperate forests vary widely from 0.1 to 2.0 Tg N_2O-N year^{-1} (Brumme et al. 2005; IPCC 2001; Kroeze et al. 1999), implying significant uncertainty. It is important to understand the key factor associated with this gap in N_2O emissions from forests.

It is generally thought that N_2O emissions from soils are a result of either nitrification or denitrification (Bremner 1997). A factor affecting the major process responsible for N_2O production is water-filled pore space (WFPS) (Bateman and Baggs 2005); nitrification is dominant for N_2O production with WFPS < 60%, whereas denitrification becomes increasingly important when WFPS exceeds 60%. These microbial processes are also controlled by soil chemical and physical properties, including the availability of mineral N and organic matter, pH, and soil type, as well as climate-related soil properties, such as temperature and water content (e.g., Dalal et al. 2003; Skiba and Smith 2000; Bouwman et al. 2002). In addition, soil C/N ratio is suggested as an important indicator for net nitrification and N_2O emissions in forest ecosystems (e.g., Klemedtsson et al. 2005; Pilegaard et al. 2006; Gundersen et al. 2012).

Over several decades, human activity has dramatically increased reactive nitrogen emissions to the global atmosphere by as much as three to fivefold (Hirsch et al. 2006). This has increased atmospheric nitrogen deposition and greatly promoted nitrogen input into temperate forests worldwide (Galloway et al. 2004). Long-term nitrogen deposition has

resulted in the phenomenon called "nitrogen saturation" in temperate forest systems. In this situation, the availability of inorganic nitrogen exceeds the total demand from plants and microorganisms (Aber et al. 1989). A conceptual model of Aber et al. (1989) derived from previous papers postulates temporal patterns of a series of changes in forest ecosystems with nitrogen load, where the forest situation is progressively changed from an initial nitrogen limitation to an eventual nitrogen saturation. The nitrogen saturation is illustrated to activate soil nitrification to increase not only NO_3^- leaching but also N_2O emissions from forest ecosystems. Thus, nitrogen saturation could be a principal factor contributing to N_2O emissions from temperate forests. However, in current nitrogen saturation models that simulate nitrogen cycling affected by nitrogen deposition, N_2O emissions have not been included due to the lack of appropriate basal data (Aber et al. 1989; Lovett and Goodale 2011).

Studies have been conducted over the last three decades to understand the fundamental implications of nitrogen saturation in forest ecosystems. The most widely adopted approach is the short- or long-term observation of forests after N addition (e.g., Venterea et al. 2004; Zhang et al. 2012). A recent compilation of 313 observations from N addition experiments indicated that N_2O emissions increased by an average of 216% relative to the control (Liu and Greaver 2009) although no significant linear correlation was noted between N addition and N_2O emissions. In other studies of forest ecosystems, the risk of N_2O flux increase under elevated nitrogen deposition has been underscored (e.g., Eickenscheidt et al. 2011). However, N addition did not always stimulate N_2O flux: simulated nitrogen deposition decreased (e.g., Skiba et al. 1999) or did not change (e.g., Borken and Beese 2005) soil N_2O emissions. As a reason why N addition did not promote N_2O emissions from N-poor temperate forest soils, Cheng et al. (2016) have suggested that soil N_2O flux is under strong control by the competitive interactions for soil available N between plants and nitrifiers. Although simulated N addition approaches have been commonly employed, applicable quantitative data on the impact of nitrogen saturation on N_2O emissions from forest ecosystems have been limited.

A comparative field survey to study the in situ situation of forest ecosystems across a gradient of

nitrogen availability could be an alternative effective approach. Perakis and Sinkhorn (2011) indicated that nitrate leaching has a positive relationship with net nitrification and soil N in forests having a wide soil N gradient in the United States. One group of Japanese forest researchers measured N_2O emissions in 26 forests for 2 years and found a positive correlation between N_2O flux and nitrification rate (Morishita et al. 2011). However, there have been few systematic field studies of the effects of nitrogen saturation on N_2O emissions in natural temperate forest ecosystems.

Theoretically, nitrogen availability should be an important factor regulating N_2O emissions because temperate forest ecosystems are nitrogen-limited in general (Vitousek and Howarth 1991; LeBauer and Treseder 2008). Nitrification, a key process for N_2O production, is the weakest competitor for NH_4^+ (Jones and Richards 1977) and it has been reported that forest soils may have no detectable nitrification activity (Vitousek et al. 1982; De Boer et al. 1988; Killham 1990). On the other hand, N_2O emission is expected to only start in terrestrial ecosystems when nitrifiers can acquire NH_4^+ that will be increased with elevated nitrogen availability.

In this study, N_2O emissions were monitored for 3 years in a small watershed of a deciduous forest located in the suburbs of Tokyo, in which nitrogen saturation has been indicated from the elevated NO_3^- concentrations in deep soils (Yoh et al. 2001). In addition, a comparative field survey was carried out in nine temperate forest sites in central Japan having different stream water NO_3^- concentrations and different nitrogen deposition rates. We tested the hypothesis that N_2O emissions would increase with nitrogen availability associated with the progress of nitrogen saturation, as indicated by a positive correlation between N_2O flux and nitrate leaching.

Materials and methods

Research site

Intensively studied forest site

Monthly field research was conducted in an N-saturated forest site (Tama) located in the suburbs of Tokyo, Japan (35° 38′ N, 139° 22′ E). Nitrogen saturation status was indicated by elevated nitrate concentrations in deep soil and stream water (Yoh et al. 2001). Mean annual temperature and annual precipitation were 14.4 °C and 1600 mm, respectively. Open bulk nitrogen deposition was 19.7 kgN ha^{-1} $year^{-1}$ and forest throughfall was 19.1 kgN ha^{-1} $year^{-1}$ in oak forest (Kimura et al. 2009). Tama is a terrace underlain by interglacial marine deposits that emerged from the sea 300,000 to 500,000 years ago (Baba et al. 1995). Aeolian volcanic ashes from Mt. Hakone and Mt. Fuji deposited on Tama, resulting in a thick andosol layer. Vegetation in the studied area consists of oak (*Quercus serrata*) with understory bamboo grass. Forest floor type is mull.

A small watershed was used for flux measurement as described below to consider heterogeneity of microbial processes due to topographical position. The watershed contains a gentle slope and a steep slope with elevations ranging from 145 to 175 m (Fig. 1). Near the turning point between the steep slope and the gentle slope, a small discharge sometimes occurs in summer owing to elevated groundwater levels (Fig. 1).

Gradient sites

In order to investigate the effect of nitrogen availability on N_2O emissions, we selected eight temperate forest sites in rural or mountainous regions having a range of nitrogen statuses as gradient sites (Table 1, Fig. 2). Stream NO_3^- concentrations varied by nearly 12-fold, which means that the selected forest sites have a large range of nitrogen statuses. Deciduous broad-leaved trees were predominant in those sites, similar to Tama, as described above.

All of the field studies at the gradient sites were conducted once in summer as a snapshot study, when high emissions were expected due to elevated temperatures and humid conditions. Detailed field properties are described in Table 1. Kusaki site was reported to be N-saturated (Ohrui and Mitchell 1997).

Field survey and sampling

Measurement of N_2O flux

N_2O flux was measured by means of a closed chamber method using a rectangular plastic chamber (25 cm × 15 cm and 20 cm in height). Twelve permanent chamber bases were embedded in soil to

(a)

(b)
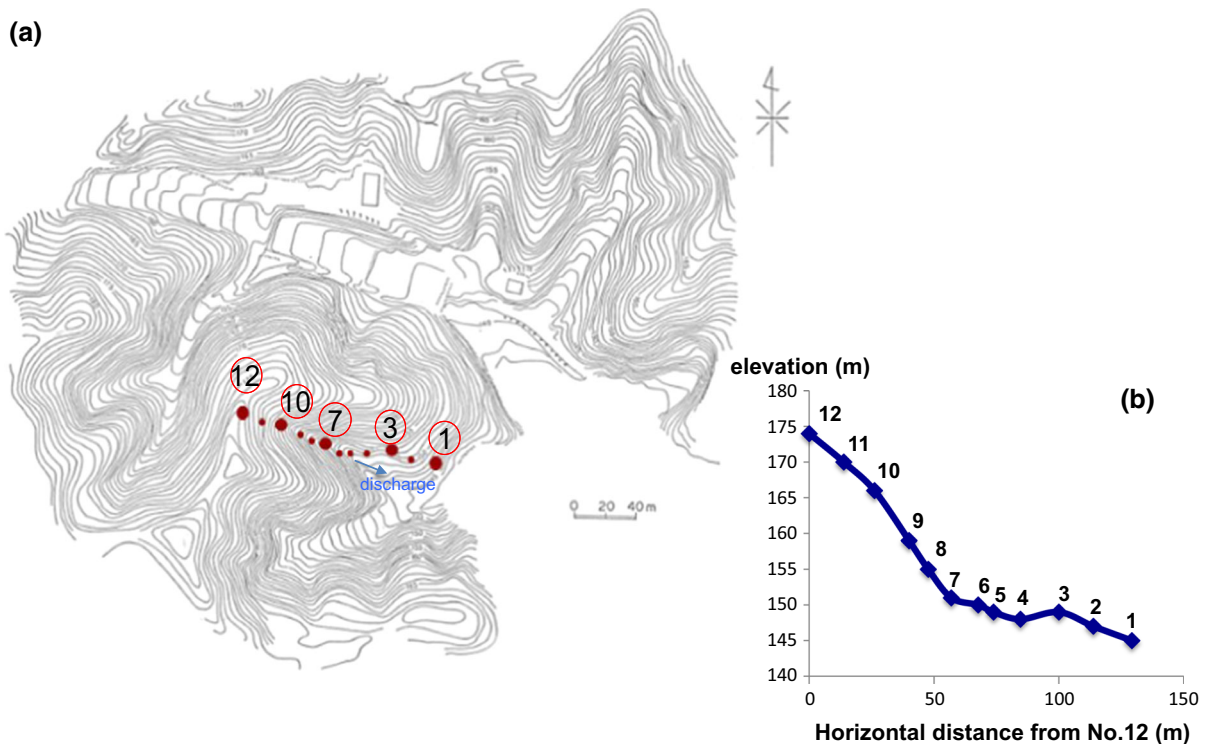
Fig. 1 Topographic map (**a**) and cross-section drawing (**b**) of twelve fixed plots set along a slope for N_2O flux measurement in Tama

approximately 5 cm depth to reduce the effects of soil disturbance resulting from the chamber embedment in March 2012. Fifteen milliliters of gas sample was collected 0, 15, and 30 min after closing the plastic chamber and transferred into a 12-mL pre-evacuated vial.

It has been suggested that slope position is important for the determination of spatial variability of N_2O emissions (McSwiney et al. 2001; Osaka et al. 2006), although knowledge of spatial topographic factors is limited (Nishina et al. 2009; Christiansen et al. 2012). Therefore, twelve plots were set along a slope covering the whole watershed (Fig. 1) to determine the influence of slope position and understand the N_2O flux in the entire watershed. Measurement was conducted monthly from May 2012 to May 2015.

We selected eight gradient sites with various watershed sizes, slopes and directions that were all covered with broad-leaved forests (mostly oak trees), but were expected to have different nitrogen status. Six same chambers as above were distributed along the slope of the watershed to cover spatial variation.

The chambers were embedded in soil to approximately 5 cm depth 0.5–1 h before measurement.

Preparation of samples for related parameters

Surface soil (5 cm) temperature was measured near the chamber while gas was sampled. Three soil samples were collected from surface mineral soil (0–10 cm) at respective sites near the chambers for chemical analyses. One soil sample was prepared from 3–5 sampling points. Stream water was also collected from respective watersheds to measure NO_3^- concentration. All the watersheds had no inflows from anthropogenic activities such as human habitation and agricultural practices. All samples were kept cool during the transport. In the laboratory, soils were sieved immediately by passing through 2 mm sieves for late analyses.

In situ soil incubation experiment

Net nitrification rate was determined in situ for surface soil (0–10 cm) in Tama by a covered cylinder method

Table 1 General locations and nitrogen statuses of sites

Site	Location N	E	Soil type	Elevation m	Soil NO_3^- * mg N/kg	Stream water NO_3^- mg N/L	Soil temperature °C	Soil C/N*	Sampling time
Ichinose	35° 50′ 18.9″	138° 49′ 53.4″	Brown forest soil	1356–1369	5.1 ± 0.5	0.14	15.4	16.6 ± 1.4	Jun 2013
Daibosatsu	35° 44′ 18.0″	138° 50′ 16.4″	Brown forest soil	1690–1715	9.0 ± 1.8	0.22	13.5	13.8 ± 0.7	Jun 2013
Izu	34° 45′ 20.3″	138° 58′ 48.0″	Brown forest soil	63–83	2.0 ± 0.4	0.25	24.6	15.6 ± 0.4	Aug 2012
Iwaki	36° 57′ 06.7″	140° 49′ 31.4″	Brown forest soil	31–49	2.3 ± 1.0	0.32	21.7	15.4 ± 0.9	Jul 2012
Yabitsu-toge	35° 25′ 34.9″	139° 13′ 03.8″	Brown forest soil	830	3.3 ± 0.3	0.34	18.6	–	Jun 2015
Nokogiriyama	35° 46′ 43.7″	139° 6′ 12.3″	Brown forest soil	980–1008	4.2 ± 0.8	0.47	19.9	14.4 ± 0.6	Jul 2013
Karibasaka-toge	35° 58′ 09.3″	139° 11′ 01.3″	Brown forest soil	693	8.5 ± 2.0	1.19	17.8	14.6 ± 1.2	Jul 2014
Kusaki	36° 33′ 25.3″	139° 24′ 38.0″	Brown forest soil	850	7.3 ± 0.6	1.40	17.2	16.7 ± 0.5	Jul 2014
Tama	35° 38′ 14.0″	139° 22′ 40.3″	Brown forest soil	145–175	23.7 ± 2.1	1.64	24.9	12.2 ± 0.7	Aug 2012

*Means ± one standard error; n = 3

(Hatch et al. 1998) in July and October 2012, June 2014, and February 2015. Measurements were conducted for plots 1, 3, 7, 10, and 12 (Fig. 1a). In situ soil incubation experiments were conducted near the chamber (within 1 m) to compare with the N_2O fluxes.

Six PVC cylinders (inserted depth: 10 cm, inner diameter: 5.5 cm) per plot were embedded in the soil perpendicularly. Three of the six cylinders were immediately excavated and transported to the laboratory. The soil samples were kept cold during transport. In the laboratory, 0–10 cm of surface mineral soil from each plot was mixed. Half of the soil was used for water content measurement and the other half was used for water extraction for chemical analysis of soil mineral N (t_0 samples). The remaining three cylinders were incubated in situ. After one month, the cylinders were excavated and the soil samples from the cylinders (t_1 samples) were subjected to the same procedure as that for the t_0 samples.

Net nitrification rate was calculated as follows:

$$\text{Net nitrification rate } (\text{mg N/m}^2/\text{day})$$
$$= \left(\left[NO_3^- \right]_{\text{afterincubation}} - \left[NO_3^- \right]_{\text{beforeincubation}} \right) \times \rho$$
$$\times h/t$$

$[NO_3^-]$: NO_3^- concentration (mg N/g soil); ρ: soil density (g soil/m^3); h: depth of soil sampled (0.1 m; assumed); t: incubation time (30 days).

Analyses

Analysis of N_2O concentration and calculation

N_2O concentrations in the vials were determined with a gas chromatograph (GC) equipped with an electron capture detector (ECD). The ECD-GC (Shimadzu GC-14B, Kyoto, Japan) was equipped with a 3 m Porapak Q column (80–100 mesh). Oven, injection, and detection temperatures were 60 °C, 80 °C, and 340 °C, respectively. Pure nitrogen (> 99.99995%) was used as carrier gas at the flow rate of 20 mL min^{-1}.

N_2O flux was determined by calculating the linear regression of the change in N_2O concentration in the chamber with time and expressed as µg N m^{-2} h^{-1}. Linear regression analyses of the measurement series with an R^2 value higher than 0.8 were used for flux calculation.
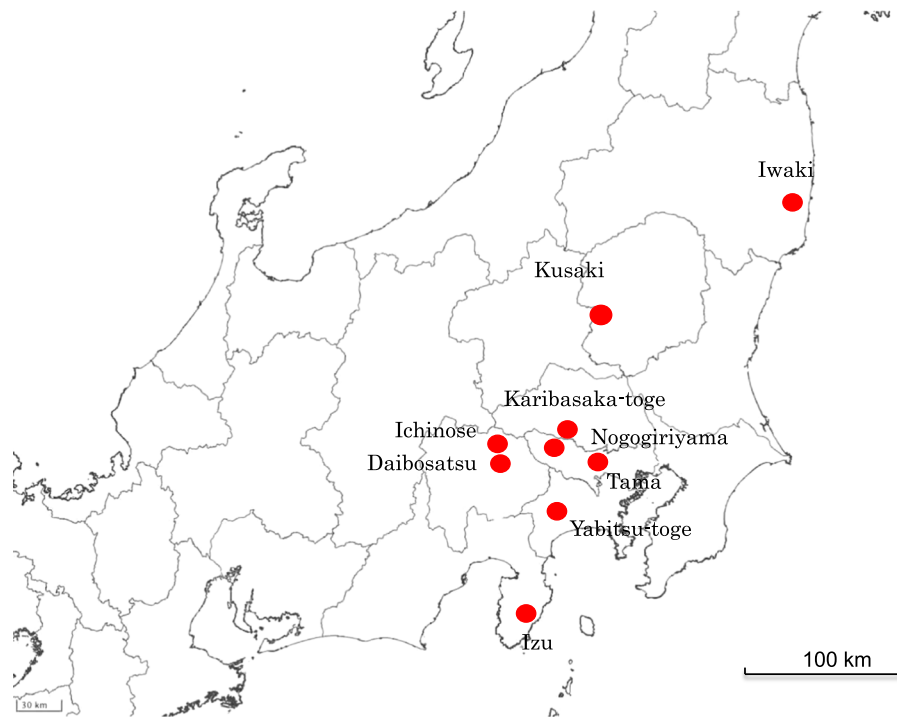
Fig. 2 Locations of intensively studied forest site (Tama) and eight gradient sites

N$_2$O flux at each plot was calculated using the following equation:

$$\text{N}_2\text{O flux } (\mu\text{gN m}^{-2}\text{h}^{-1}) = \rho \times V/A \times dc/dt \times \alpha$$

ρ: gas density of N$_2$O at STP (1.978×10^9 µg m^{-3}); V: volume of chamber (m^3); A: cross-sectional area of chamber (m^2); dc/dt: rate of change in gas concentration (c) inside the chamber per unit time (t) (m^3 m^{-3} h^{-1}); α: conversion factor for transforming N$_2$O into N (28/44).

Because it was expected that N$_2$O flux would be different among plots due to different soil conditions, N$_2$O flux was quantified for each of the twelve plots. Furthermore, monthly N$_2$O flux from the entire watershed studied was estimated by summing them up.

$$\text{Monthly N}_2\text{O flux } \left(\text{g N ha}^{-1}\text{month}^{-1}\right)$$
$$= \sum_{1-12}\left[\text{N}_2\text{O flux} \times A \times 24 \times 30 \times 10^4/10^6\right]/S$$

A: area represented by each plot (m^2).

We assume that all of the plots have the same area; i.e., A = S $\times$ 1/12.

S: total watershed area (ha).

Annual N$_2$O flux was calculated by summing up monthly N$_2$O fluxes.

Nitrate concentration

Soil water extract was collected after shaking 5 g of subsamples of sieved soil with 50 mL of pure water for 60 min and centrifuging for 10 min. The supernatant was filtered using glass filters (GF/F; Whatman Int. Ltd.) and stored at − 20 °C until analysis. Nitrate was analyzed by ion chromatography (Dionex DX-120). Nitrite was negligible throughout.

C/N ratio

The determination of total nitrogen and total carbon in mineral soils of 0–10 cm depth was carried out by dry combustion using a CN coder (Yanaco MT-700). Soil samples dried at 60 °C were comminuted and approximately 100 mg of the thoroughly mixed sample was used for measurement.

Physical properties

Bulk density of soil samples was determined by oven drying at 105 °C. Soil temperature at 5 cm depth was measured with a digital thermometer (YOKOGAWA MODEL 2455). pH of a soil–water mixture (1:2.5 (v/v)) was determined using a glass electrode. Water-filled pore space (WFPS) was calculated from the following formula:

$$WFPS(\%) = \text{Soil water content}(\%) \times \rho_V/(1 - \rho_v/2.65)$$

ρ_v: soil bulk density (g cm^{-3}); 2.65: particle density of soil (assumed).

Results

Soil moisture

WFPS values varied according to slope position (Fig. 3). Plot 1 had low values of 30–45%, whereas plot 7 showed significantly high values of 55–68%. Although plot 1 located at the end of a gentle slope had the highest soil moisture content, its low soil density resulted in the lowest calculated values of WFPS. Most of the WFPS values were < 60%, but WFPS values in plots 7 and 10 in July 2012 were > 60%, suggesting that anaerobic processes could be operative. Generally, WFPS tended to be high in summer, the season that had more rainfall.
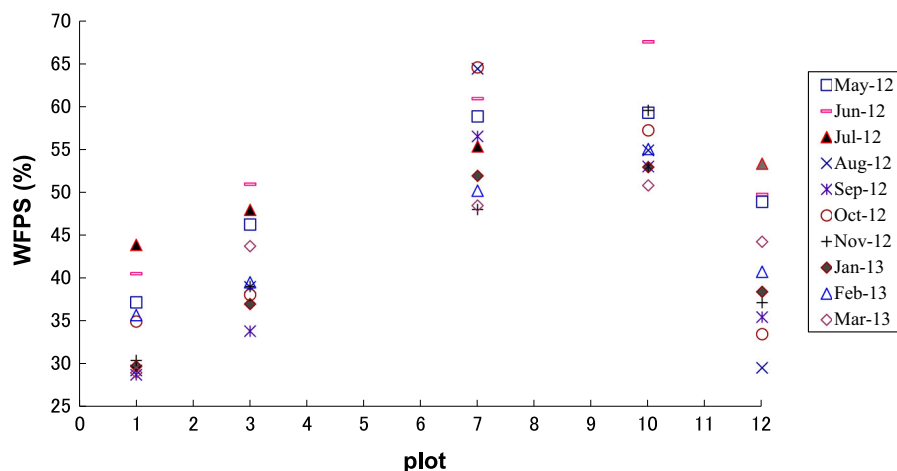
N$_2$O flux

Considerable spatial variation in annual N$_2$O flux was found in the twelve plots set along a slope in Tama (Fig. 4), ranging from 0.4 kg N ha^{-1} year^{-1} (plot 1) to 1.9 kg N ha^{-1} year^{-1} (plot 8). The highest fluxes were observed at the inflection point between the gentle slope and the steep slope (plots 6–8), where high WFPS values were observed (Fig. 3). The fluxes were relatively low at both ends of the slope where low WFPS values were observed (Fig. 3).

The N$_2$O fluxes in the entire watershed measured monthly in Tama are shown in Fig. 5. The N$_2$O fluxes showed typical seasonal variations, which were closely related to soil temperature at 5 cm depth (data not shown): high N$_2$O emissions in summer and minimum emissions, almost close to zero, in winter (Fig. 5).

In this intensively studied N-saturated forest, the average annual N$_2$O emissions from the entire watershed were estimated to be 0.88 kg N ha^{-1} year^{-1}.

N$_2$O fluxes at the gradient sites and the intensively studied forest site (Tama), which were all measured in summer, are compared in Fig. 6. N$_2$O fluxes varied by approximately 34-fold across the nine forest sites, ranging from 0.13 g N ha^{-1} day^{-1} to 4.38 g N ha^{-1} day^{-1}. Markedly high N$_2$O flux was observed in Tama (Fig. 6).

Soil NO$_3^-$ concentration in Tama was also several times higher than those in the gradient sites. Stream water NO$_3^-$ concentration varied by approximately 12-fold across the nine forest sites, ranging from 0.14

Fig. 3 Spatial and temporal variations of water-filled pore spaces during the study period in Tama

Fig. 4 Spatial variations of N$_2$O fluxes in Tama. Points and error bars are the averages and the standard deviations of annual N$_2$O fluxes for 3 years
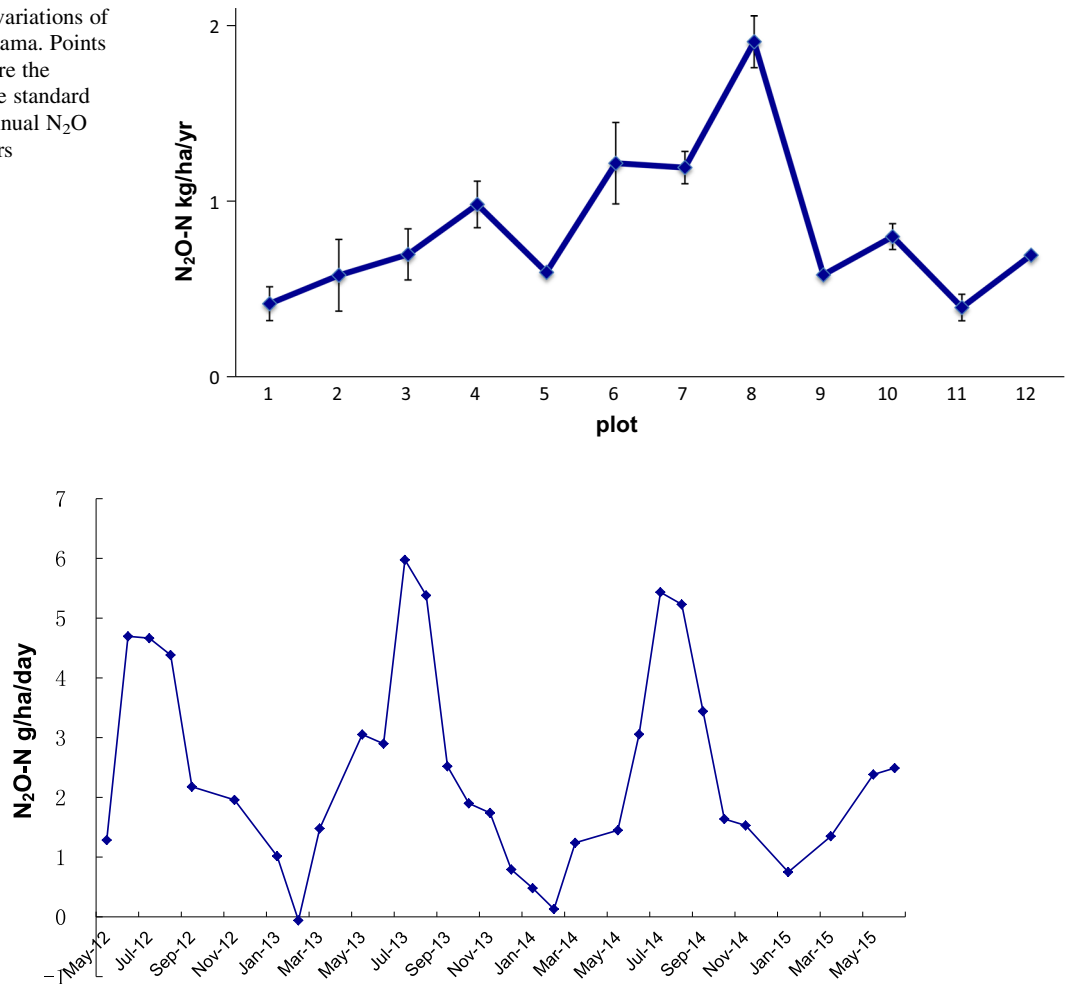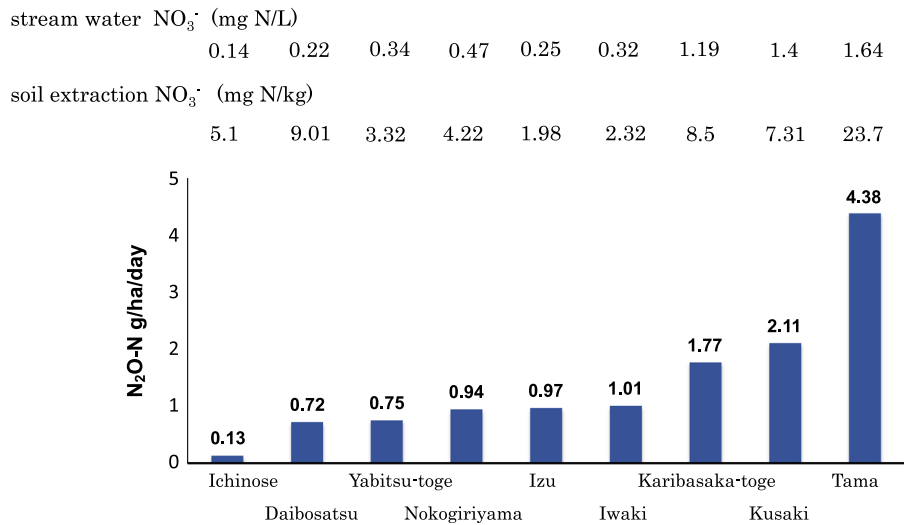

Fig. 5 Seasonal variations of N$_2$O fluxes in Tama. The N$_2$O flux was measured for the entire watershed (See text for details)

to 1.64 mg N/L. Stream water NO$_3^-$ concentration varied almost in parallel with N$_2$O emissions; a high concentration was associated with high N$_2$O emissions and vice versa. Soil C/N ratio (Table 1) showed no significant correlation with stream water NO$_3^-$ concentration or N$_2$O emissions among the eight forest sites studied here, although previous studies have suggested that soil C/N ratio is a contributing factor to nitrate leaching (Gundersen et al. 1998) and N$_2$O emissions (Klemedtsson et al. 2005; Pilegaard et al. 2006; Gundersen et al. 2012). The lack of correlation may be due to the relatively low soil C/N ratios in the present study; clear correlations were just observed for large-spans of soil C/N ratios in the previous studies.

Nitrification

The net nitrification rate in Tama showed a wide variation ranging from -3 to 69.8 mg N/m^2/day. For the same plots, the net nitrification rate varied generally with soil temperature, being high in summer and low in winter (Table 2). Spatially, there was a consistent pattern in the net nitrification rate; it was generally higher at lower positions (plots 1 and 3) than upper positions (plots 10 and 12) (Table 2). Enhanced aeration due to relatively low soil density as well as low WFPS value could result in the high net nitrification rates in the gentle-slope plots near the bottom. For plot 7, which is located at the inflection point between the gentle slope and the steep slope, the net nitrification rates were invariably low and there were

stream water NO_3^- (mg N/L)

| 0.14 | 0.22 | 0.34 | 0.47 | 0.25 | 0.32 | 1.19 | 1.4 | 1.64 |

soil extraction NO_3^- (mg N/kg)

| 5.1 | 9.01 | 3.32 | 4.22 | 1.98 | 2.32 | 8.5 | 7.31 | 23.7 |

Fig. 6 N_2O fluxes in forest sites having different nitrate concentrations in stream water and soil. Data were collected at the sampling times shown in Table 1

Table 2 Net nitrification rates and related variables in the incubation experiment in Tama

Incubation period	Plot	Soil temperature °C	WFPS%	Net nitrification rate mg N/m^2/day
Jul-2012	1	22.4	44	55.3
	3	22.8	48	38.6
	7	22.8	61	− 3
	10	23.5	70	33.7
	12	22.8	53	41.4
Oct-2012	1	16.8	30	69.8
	3	17.4	39	29.7
	7	16.9	48	3.9
	10	16.9	58	7.4
	12	17.1	37	21
Jun-2014	1	19.5	34	20.9
	3	19.7	39	15.5
	7	19.5	66	− 0.7
	10	19.9	65	10
	12	19.7	38	3
Feb-2015	1	4.5	40	9.2
	3	4.7	41	6.9
	7	4.7	60	0.9
	10	4.6	72	6.1
	12	4.8	41	2.3

WFPS, soil temperature, and N_2O fluxes were surveyed at the same time

two negative values, both of which were measured in summer (July 2012 and June 2014). These exceptionally low fluxes would be due to the excessive moisture (higher than 60%) in soil arising from abundant rainfall in summer and groundwater seepage at this location (Fig. 3). The high WFPS condition should

Fig. 7 Relationships between net nitrification rate and N$_2$O flux. Data were collected from field incubation experiments in Tama and are shown in Table 2
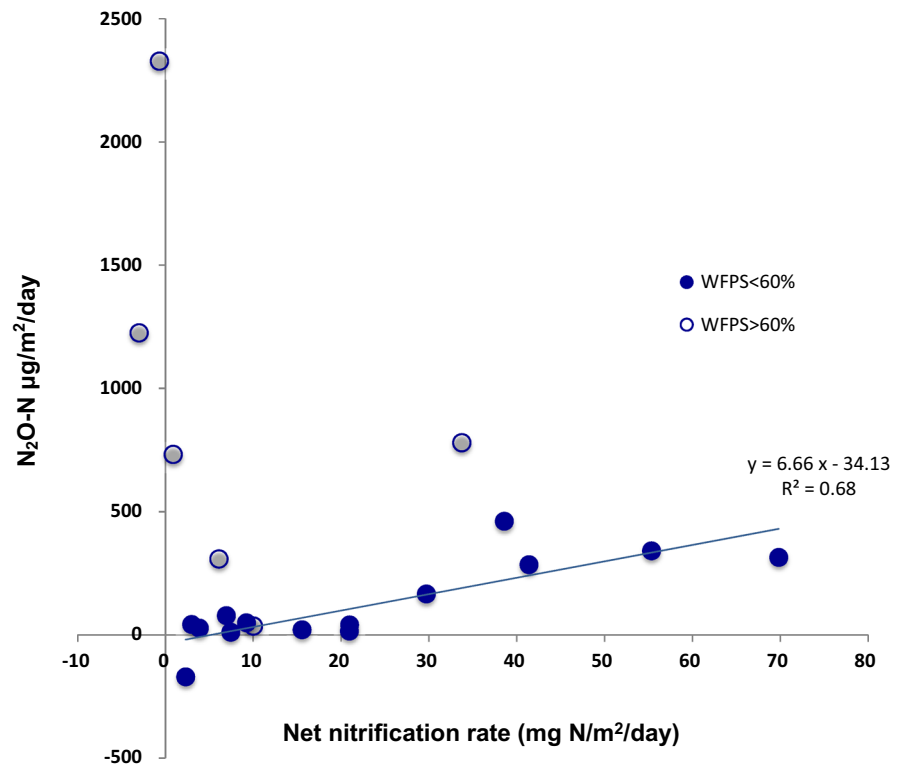
create an anaerobic condition in the soil to suppress nitrification and NO$_3^-$ production, and at the same time promote denitrification resulting in consumption of available NO$_3^-$.

The relationship between N$_2$O flux and net nitrification rate in Tama is shown in Fig. 7. A clear positive correlation was found between N$_2$O flux and net nitrification rate for WFPS < 60% (Fig. 7), indicating that nitrification is the major process responsible for N$_2$O production under well-aerated conditions. The slope of the regression line, which corresponds to the ratio of N$_2$O production during nitrification, is equivalent to 0.66%. For WFPS > 60% (data from plots around the bottom of a steep slope (plots 7 and 10) in the rainy season (July 2012 and June 2014)), in contrast, no correlation was found between N$_2$O flux and net nitrification rate. The scattered data for WFPS > 60% in Fig. 7 might be due to nitrate consumption and elevated N$_2$O emissions affected by denitrification, which occurred under exceedingly humid conditions at a particular period (summer) and at particular locations with groundwater seepage.

Discussion

The average annual N$_2$O emissions from the N-saturated forest (Tama) were estimated to be 0.88 kg N ha^{-1} year^{-1}; instantaneous flux rate ranged from − 0.03 to 90 µg N m^{-2} h^{-1}. The fluxes observed in the present study are higher than those reported for other forest ecosystems in Japan (e.g., 0.106 kg N ha^{-1} year^{-1} (Morishita et al. 2011), 0.66 ± 0.03 µg N m^{-2} h^{-1} (Inatomi et al. 2010), and 0.17–12.5 µg N m^{-2} h^{-1} at 26 sites (Morishita et al. 2007)) and in North America (− 0.03 to 0.06 kg N ha^{-1} year^{-1} (Castro et al. 1992) and 0.01–0.02 kg N ha^{-1} year^{-1} (Bowden et al. 1990)). In Europe, similar high N$_2$O fluxes, e.g., 0.67–0.92 kg N ha^{-1} year^{-1} (Schmidt et al. 1988) and 0.55–0.62 kg N ha^{-1} year^{-1} (Kesik et al. 2005), were reported. Still higher N$_2$O fluxes, e.g., 1.15–2.29 kg N ha^{-1} year^{-1} (Pitcairn et al. 2002) and 2.6 kg N ha^{-1} year^{-1} (Eickenscheidt et al. 2011), were reported for N-saturated forests having particularly low soil pH values. Thus, except for the high N$_2$O emissions in some European forests, the

Fig. 8 Relationship between N_2O flux and stream water NO_3^- concentration in all the sites. The regression line and the correlation coefficient are for all points excluding Tama
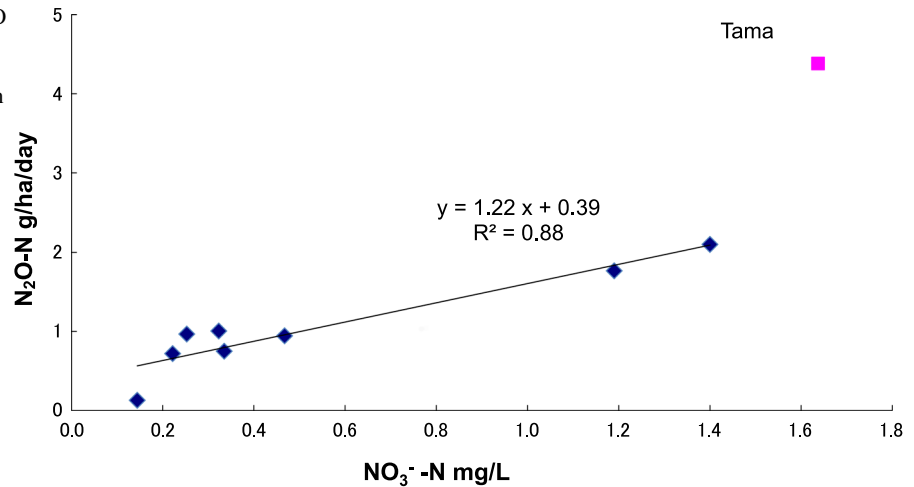
value estimated for Tama in this study is comparable to the highest level ever reported for temperate forests.

The N_2O flux in Tama was higher than those in the gradient sites when values obtained in summer observations were compared. Soil NO_3^- concentration and stream water NO_3^- concentration were also the highest compared to the gradient sites. In the eight gradient sites, N_2O fluxes were generally higher in the sites that had higher soil NO_3^- and stream water NO_3^- concentrations (Fig. 6). The N_2O flux varied across nine forest sites almost in proportional to the stream water NO_3^- concentration in the watershed that ranged from 0.14 mg N/L to 1.64 mg N/L (Fig. 8). In addition, a significant linear correlation between N_2O flux and net nitrification rate was observed for well-aerated plots in Tama (Fig. 7). Thus, the results described above indicate that N_2O emissions in the temperate forests studied here are proportional to the magnitude of nitrification. Morishita et al. (2011) also reported a positive correlation between N_2O flux and nitrification rate in a 3-year experiment conducted in temperate forests (in the Kanto region) having the same climate as Tama. Several studies have pointed out that nitrification is the major process involved in N_2O emissions from forest ecosystems (e.g., Castro et al. 1992; Venterea et al. 2004; Fang et al. 2009).

The slope of the regression line between N_2O flux and net nitrification rate (except for humid condition) in Fig. 7, which corresponds to the ratio of N_2O production during nitrification, was 0.66%. This value is consistent with the ratio of N_2O-N emissions to nitrification of 0.05–0.15% reported by Hynes and

Knowles (1984) in a pure culture of *Nitrosomonas europaea* and the ratio of N_2O-N emissions to gross nitrification varying between 0.4%–2.1% in a ^{15}N-labeled incubation of soil in the laboratory reported by Venterea et al. (2004). Similar values of 0.39% (Maag and Vinther 1996) in a laboratory incubation experiment of forest soil and 0.23% (Morishita et al. 2011) in a field incubation experiment in a temperate forest have been also reported. Previous works on agricultural soil in the aerobic condition also showed the ratio of N_2O-N emissions to nitrification varying from 0.02 to 0.2% (mostly < 0.1%) (e.g., Breitenbeck et al. 1980; Tortoso and Hutchinson 1990; Stevens et al. 1997; Mathieu et al. 2006).

The concurrent increase in N_2O emissions and nitrate leaching is in accordance with the nitrogen saturation conceptual model proposed by Aber et al. (1989); both are negligible at 'stage 1' and increase simultaneously after 'stage 2' with the initiation of nitrification. Few such concurrent behaviors have been reported in previous studies of N_2O emissions in temperate forests. The unique findings of clear relationships in the present study may be partly because microbial processes are much less limited by soil moisture due to the humid climate in central Japan, unlike forest research sites in Europe and the United States. Typical seasonal changes in N_2O emissions observed in the present study, which have hardly been reported in previous studies of temperate forests, should also stem from this humid condition, which allows temperature to be the sole controlling factor. Relatively abundant rainfall and mountainous

topographic features would also allow collection of stream waters. In addition, the contribution of denitrification in the watershed would be partial, if any, due to such conditions. It is likely that those conditions enabled us to clarify the relationships between N_2O emissions and relevant parameters.

The setting of twelve plots along a slope covering the whole watershed revealed a unique feature of spatial variability and topographic factor in N_2O emissions. The inflection point of the gentle slope and the steep slope (No. 6 and No. 7 in the intensively studied forest site) emits more than twice the amount of N_2O in other plots. Such relatively high N_2O emissions at the bottom of slope, which have also been pointed out by previous researchers (e.g., Osaka et al. 2006; Nishina et al. 2009), are related to the high WFPS value. The data points for Tama were located far from the regression line in Fig. 8. This could partly be attributed to this topographic effect. It is likely that under slightly elevated WFPS values, denitrification may contribute partly to N_2O production because it has been shown that denitrification becomes increasingly important when WFPS exceeds 60% in agricultural and natural soils (e.g., Dobbie et al. 1999; Abbasi and Adams 2000; Skiba and Ball 2002; Bateman and Baggs 2005). It is also known that N_2O yield during nitrification is elevated at low oxygen concentration (Goreau et al. 1980).

It is generally understood that nitrogen is limited in temperate forest ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008). It is common for forest soils to have no detectable nitrification activity (Vitousek et al. 1982; De Boer et al. 1988; Killham 1990). Aber et al. (1989) have also hypothesized no nitrification before starting chronic nitrogen deposition. The lack of nitrification activity is understandable when considering that nitrifiers are weak competitors for NH_4^+ (Jones and Richards 1977). However, nitrogen availability has been widely elevated due to chronic nitrogen deposition to initiate nitrification in forest ecosystems, a key process promoting N_2O emissions. Nitrification inevitably produces N_2O as a by-product and moreover enables denitrification by providing the substrate (NO_3^-), which also contributes N_2O emission. The correlations of N_2O emissions to soil NO_3^- concentration (Fig. 6), stream water NO_3^- concentration (Figs. 6 and 8), and soil net nitrification rate (Fig. 7) strongly suggest that N_2O emissions occur in parallel with the magnitude of nitrification. The large variation of N_2O emissions in temperate forests, ranging from negligible to high

emissions, was adequately accounted for by these variables associated with nitrification. Thus, it is reasonable to conclude that increased nitrogen availability due to anthropogenic nitrogen depositions will stimulate N_2O emissions from temperate forests through enhanced nitrification. Percentage N_2O emission is approximately 0.66% relative to NO_3^- production. Thus, it would be important to re-evaluate the global flux of N_2O from forests from the perspective of changes in anthropogenic nitrogen emissions.

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