

Gross nitrification rates in four Japanese forest soils: heterotrophic versus autotrophic and the regulation factors for the nitrification

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Abstract Measurements of gross NH_4^+ and NO_3^- production in forest soils were conducted using the ^{15}N pool dilution method. Mineral topsoils (0–10 cm depth) were collected from four forests from northern to southern Japan with a natural climate gradient to elucidate the mechanisms regulating gross nitrification rates in forest soils. Additionally, we attempted to evaluate the relative importance of heterotrophic nitrification in gross total nitrification using acetylene as a specific inhibitor of autotrophic nitrification. Distinct differences were found among sites in the gross rates of NH_4^+ production (3.1–11.4 mg N kg^{-1} day^{-1}) and gross total nitrification (0.0–6.1 mg N kg^{-1} day^{-1}). The rates of

gross heterotrophic nitrification were low in this study, indicating that heterotrophic nitrification is of minor importance in most forest mineral topsoils in Japan. Significant relations were found between gross autotrophic nitrification and gross NH_4^+ production, soil N, and soil C concentrations, but none was found between gross autotrophic nitrification and soil pH. We determined the critical value of the gross NH_4^+ production rates for gross autotrophic nitrification under which no gross autotrophic nitrification occurred, as well as the critical soil C/N ratio above which gross autotrophic nitrification ceased. Results show that tight coupling of production and consumption of NH_4^+ prevents autotrophic nitrifiers from utilizing NH_4^+ as long as NH_4^+ availability is low.

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Introduction

Nitrification, microbial aerobic oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) via nitrite (NO_2^-), is an important process in the nitrogen (N) cycle of forests, because both the substrate (NH_4^+) and the product (NO_3^-) are important available N for plants and soil microbes (Chapin et al. 2002). Moreover, NO_3^- can leach out from a forest ecosystem because of its high mobility in soil. In addition, NO_3^- is the substrate for denitrification, which is the process of gaseous N loss from an ecosystem (Chapin et al. 2002). These facts have stimulated studies of nitrification to manage N availability for plants and thereby maintain their productivity in N-limited forest ecosystems (Vitousek and Matson 1985). Nitrification, as well as the mineralization that produces NH_4^+ from organic matter, is highly

sensitive to environmental factors such as soil temperature and soil moisture (Prosser 1989). Therefore, it is expected that nitrification and mineralization rates vary among forests according to their different environmental conditions.

Generally, two measures have been frequently used to express the transformation rates of soil inorganic N such as NH_4^+ and NO_3^- : *net mineralization/nitrification rates* as accumulation rates during a certain incubation period (e.g., 1 month) in the laboratory or the field, and *gross mineralization/nitrification rates* as $\text{NH}_4^+/\text{NO}_3^-$ production rates measured by ^{15}N tracer in the laboratory during a short incubation period (e.g., 1 day; Davidson et al. 1991). The net rate can express only the net increase or decrease in the concentration of the target N compound (e.g., net nitrification rate = change in concentration of NO_3^- , i.e., NO_3^- concentration in the incubated sample minus NO_3^- concentration in the initial sample, divided by the incubation period). However, N can turn over quickly because of the high biological demand for N in forest soils (Davidson et al. 1992) by which consumption and production of the target N compound can occur during soil incubation. Consequently, the gross rate, which is useful to distinguish between consumption and production of the target N compound, would be a more appropriate measure to study N turnover in forest soil. Actually, high gross NO_3^- production rates have been observed even in forest soil with low to negligible net nitrification rates (Davidson et al. 1992; Stark and Hart 1997).

Nitrification is generally regarded as a combined process of aerobic ammonia oxidation to NO_2^- by autotrophic ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea and aerobic NO_2^- oxidation to NO_3^- by nitrite-

oxidizing bacteria (Prosser 2007). The rate of ammonia oxidation is considered to regulate the overall nitrification rate (Prosser 2007). Several environmental factors have been proposed as affecting the nitrification rate: NH_4^+ availability, O_2 availability, soil pH, salinity, temperature, and presence of inhibitory compounds (Prosser 2007), as well as the balance of C and N availability (Hart et al. 1994). However, actual regulation of nitrification by those parameters remains unclear (Booth et al. 2005).

Ammonia monooxygenase, an important enzyme of AOB, requires ammonia gas as substrate (Suzuki et al. 1974). Consequently, AOB may have difficulty in conducting ammonia oxidation in soil with low pH because of the high pK_a of ammonia ($\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$; $pK_a = 9.25$). Actually, the pH of Japanese forest soil tends to be low (4.0–6.0 after Kawada 1989; Table 1) because of its humid climate with moderate temperatures. Nevertheless, considerable NO_3^- pool and net nitrification rates in the forest soils are frequently observed, even at low pH such as 4.5–5.5, which is unsuitable for AOB (De Boer et al. 1992). For this reason, heterotrophic nitrification has been highlighted as an important process in NO_3^- production in low-pH forest soils aside from autotrophic nitrification (Killham 1990). Some strains of bacteria and fungi can produce NO_3^- from both inorganic and organic N (Killham 1990). Moreover, autotrophic and heterotrophic can be distinguished using the standard soil incubation protocol with and without acetylene (Hart et al. 1997; Pedersen et al. 1999; Perakis et al. 2005). Nevertheless, the importance of heterotrophic nitrification in acid forest soil remains unclear. The relative contribution of heterotrophic nitrification to total nitrification ranges widely from low

Table 1 Study site characteristics

Site	Uryu	Kusaki	Kamigamo	Takakuma
Mean annual temperature (°C)	3.1	9.0	14.6	14.5
Mean annual precipitation (mm)	1390	1460	1582	3410
N deposition ($\text{kg N ha}^{-1} \text{ year}^{-1}$)	7.1 ^a	17.8 ^b	6.6 ^c	–
Stream water NO_3^- (mg N L^{-1}) ^d	0.085	0.57	0.061	0.098
Soil pH (H_2O) ^e	4.7	5.0	4.1	5.6
Soil C (g C kg^{-1})	103.1 ± 15.0 ^b	163.7 ± 18.2 ^a	34.9 ± 3.3 ^c	21.7 ± 1.5 ^c
Soil N (g N kg^{-1})	6.7 ± 0.6 ^b	9.9 ± 1.2 ^a	1.5 ± 0.1 ^c	1.4 ± 0.1 ^c
Soil C/N ratio	15.3 ± 0.8 ^b	16.7 ± 0.2 ^b	23.5 ± 1.3 ^a	15.6 ± 0.2 ^b

Data presented as mean ± standard error (SE) for soil C, soil N, and soil C/N ratio

Values with different letters in each row differed significantly ($P < 0.05$)

^a Wet deposition modified from Ogawa et al. (2006)

^b Bulk deposition modified from Ichikawa et al. (2003)

^c Bulk deposition (unpublished results)

^d After Shibata et al. (2001)

^e Averaged values after Shibata et al. (2011)

(Pennington and Ellis 1993) to 100% (Perakis et al. 2005), independent of environmental conditions and geological settings. There is a great need for more information related to the relative contribution of heterotrophic and autotrophic nitrification to NO_3^- production for better understanding of nitrification in forest ecosystems.

For this study, we measured gross mineralization (NH_4^+ production) and gross nitrification (NO_3^- production) rates of forest soils collected from four different sites from northern to southern parts of Japan to elucidate the mechanisms regulating gross NO_3^- production in forest soils. Additionally, we attempted to evaluate the relative importance of heterotrophic nitrification against autotrophic nitrification. Our testing hypothesis was that heterotrophic nitrification is more important in ecosystems with lower N availability, higher C availability, and lower soil pH. We conducted ^{15}N tracer experiments using acetylene to measure gross N transformation rates in forest soils, with emphasis on nitrification.

Materials and methods

Study sites

We investigated four sites of natural forest in northern Hokkaido, northern Kanto, central Kinki, and southern Kyushu regions by considering the natural climate gradient in the Japanese Archipelago [Table 1; see details in Shibata et al. (2011)]. In northern Hokkaido (hereinafter designated Uryu), the study site is located in Uryu Experimental Forest of Hokkaido University; it has a cold, snowy climate and a clean atmosphere. The forest type is mixed forest; the soil is moderately moist, brown forest soil (B_D ; Forest Soil Division 1976) or Cambisols (IUSS Working Group WRB 2006) on Tertiary andesite. In northern Kanto (hereinafter designated Kusaki), the study site is located in Field Museum Kusaki of Tokyo University of Agriculture and Technology with humid and hot summers and dry and cool winters, with high N deposition (Table 1). The forest type is deciduous hardwood forest, and the predominant soil is a drier subtype of moderately moist, brown forest soil or Andosols (IUSS Working Group WRB 2006). In central Kinki (hereinafter designated Kamigamo), the study site is located in Kamigamo Experimental Forest of Kyoto University with warm-temperate climate with hot and humid summers. The forest type is coniferous forest; forest resources such as charcoal and firewood have been used around this region for more than several hundred years, but they have not been used since the late 1960s. The soil type is dry brown forest soil (B_B ; Forest Soil Division 1976) or Cambisols (IUSS Working Group WRB 2006) on sandstone and

slate. In southern Kyushu (hereinafter designated Takakuma), the study site is located in the Takakuma Experimental Forest of Kagoshima University, with hot and humid summers and a warm temperate climate. The forest type is evergreen hardwood forest; the soil is immature soil (Im; Forest Soil Division 1976) or volcanic Regosols (IUSS Working Group WRB 2006) derived mainly from Mt. Sakurajima.

Sampling was conducted from the end of August to early September in 2009. We collected surface mineral soil (0–10 cm) from the A horizon at five randomly selected spots in representative plots ($20 \times 20 \text{ m}^2$) on gentle or flat areas on a slope or ridge at each site. At each site, the five collected soils were sieved (4 mm) individually to remove coarse roots and gravel. The collected soil samples were then stored at 4°C and used for ^{15}N experiments within 15 days after sampling.

^{15}N experiments

Of the sieved soil, 7 g was put into a centrifuge tube (50 mL; Corning Int'l. K.K., Tokyo, Japan). After weighing, soils were preincubated at 26°C for 1 day to remove the effects of low temperatures on nitrifier activity. One milliliter of 5 mM $\text{Na}^{15}\text{NO}_3$ (99.8 ^{15}N atom%) or $^{15}\text{NH}_4\text{Cl}$ (99.7 ^{15}N atom%) was injected into the tube, where ^{15}N atom% = $100 \times ^{15}\text{N}/(^{14}\text{N} + ^{15}\text{N})$. The soils with ^{15}N were incubated at 26°C . After 2 h incubation, half of the tubes were extracted with 35 mL 2 M KCl to obtain the initial concentration and ^{15}N levels of NH_4^+ and NO_3^- . Other samples were extracted after 26 h incubation. The 2 M KCl extract was prepared by 1 h shaking of the soil with KCl solution, filtration using a glass-fiber filter (GF/F; Whatman Int. Ltd., Maidstone, UK; muffled at 450°C for 4 h), and storage at 4°C until later measurements.

Soils were also incubated with 1% (v/v) of acetylene to measure heterotrophic nitrification (Stein et al. 2005). After preincubation (at 26°C for 1 day), 10 g sieved soil was put in a 200-mL glass vial (#0501-24; Maruemu Corp. Co. Ltd., Osaka, Japan). After addition of 1 mL 5 mM $^{15}\text{NH}_4\text{Cl}$ (99.7 ^{15}N atom%), the vial was crimp-sealed using an aluminum seal (#1307-04; Maruemu Corp. Co. Ltd.) with a butyl stopper (#1306-04; Maruemu Corp. Co. Ltd.). Then 20 mL Ar-acetylene (90:10) gas was injected via syringe into the vial; the vials were incubated for 2 or 26 h at 26°C . After incubation, the soils were extracted using 50 mL 2 M KCl, as described above.

Chemical analysis

Concentrations of NH_4^+ in the soil extract were measured using the indophenol method (Keeney and Nelson 1982); the NO_3^- concentration was measured using gas

chromatography–mass spectrometry (GC–MS) with conversion of NO_3^- to N_2O by the denitrifier (Isobe et al. 2011). The ^{15}N atom% in NH_4^+ and NO_3^- were also measured using the “denitrifier method” with GC–MS after the conversion of each N compound to N_2O (Isobe et al. 2011). Then, the ^{15}N atom% in N_2O was determined using the equation presented in Stevens et al. (1997) and Isobe et al. (2011). Soil C and N concentrations were measured using a CN Analyzer (Sumigraph NC Analyzer NC-90A; Sumika Chemical Analysis Service Ltd., Osaka, Japan).

Calculation of gross rates

Concentration and ^{15}N atom% of NH_4^+ and NO_3^- in the 2- and 26-h incubated samples were used to calculate the NH_4^+ and NO_3^- production rates as well as NH_4^+ and NO_3^- consumption rates according to Davidson et al. (1992). The concentrations of $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ were calculated, respectively, with NH_4^+ and NO_3^- concentrations multiplied by each ^{15}N atom%. We use the term “gross total nitrification” for the NO_3^- production that occurred both autotrophically and heterotrophically throughout this study. Gross autotrophic nitrification was determined by subtracting the gross heterotrophic nitrification from the gross total nitrification rate. The gross NH_4^+ immobilization rate was determined by subtracting the gross total nitrification rate from the gross NH_4^+ consumption rate. It was assumed that the gross NO_3^- consumption rate was equivalent to the gross NO_3^- immobilization rate in the present study, because gross rates measured in the absence of plant uptake and leaching and under aerobic condition were expected to minimize the denitrification rate.

The net production rates were calculated from the difference in NH_4^+ or NO_3^- pool size during the incubation period (1 day), where the pool size of NH_4^+ was NH_4^+ concentration with $^{15}\text{NO}_3^-$ addition, and the pool size of NO_3^- was NO_3^- concentration with $^{15}\text{NH}_4^+$ addition.

Mean residence time (MRT) was calculated as

$$\text{MRT (days)} = \text{NH}_4^+ \text{ or } \text{NO}_3^- \text{ pool size} / \text{gross NH}_4^+ \text{ or } \text{NO}_3^- \text{ production rate,}$$

where the pool size of NH_4^+ was the NH_4^+ concentration of 2-h incubated sample with $^{15}\text{NO}_3^-$ addition, and the pool size of NO_3^- was the NO_3^- concentration of 2-h incubated sample with $^{15}\text{NH}_4^+$ addition.

Statistical analysis

Statistical analysis was conducted using R software (R Development Core Team 2008) with significance level of 0.05. Comparisons of soil properties and gross rates among sites were conducted using analysis of variance (ANOVA)

with post hoc Tukey–Kramer tests. Simple regression analysis was used to assess relations among gross rates or between gross rates and soil properties. Welch two-sample *t* tests were used to compare the MRT of NH_4^+ and that of NO_3^- .

Results

Soil C, N concentration, and C/N ratio

Soil C, N concentration, and C/N ratio differed significantly among sites (Table 1). Uryu and Kusaki had high C and N concentrations, whereas Kamigamo and Takakuma showed low C and N concentrations (Table 1). Kamigamo had the highest C/N ratio of all the sites (Table 1).

Gross production, immobilization, and MRT of NH_4^+ and NO_3^-

Gross NH_4^+ production was 3.1–11.4 mg N kg^{-1} day^{-1} , whereas NH_4^+ immobilization was 10.6–24.7 mg N kg^{-1} day^{-1} (Table 2). Both gross production and immobilization rates of NH_4^+ were highest at Kusaki, whereas Kamigamo and Takakuma showed low rates (Table 2). MRTs of NH_4^+ were 0.2–1.0 day (Table 2). Kusaki had long MRT of NH_4^+ despite its high production rate because of its large NH_4^+ pool size, whereas the MRT at Kamigamo was short because of its small pool size of NH_4^+ (Table 2).

Gross heterotrophic nitrification rates were low, ranging from 0.0 to 0.3 mg N kg^{-1} day^{-1} ; they did not differ among sites (Table 2). Gross autotrophic nitrification rates were -0.2 to 5.8 mg N kg^{-1} day^{-1} . The rate was highest at Kusaki (Table 2). At Kamigamo, the higher rate of gross heterotrophic nitrification than that of gross total nitrification engendered the negative autotrophic nitrification rate. The relative contribution of gross heterotrophic nitrification to gross total nitrification was also low at all sites except Kamigamo, ranging from 8% to 23%. The relative contribution for Kamigamo was not calculated because autotrophic nitrification was not detected (Table 2). Immobilization rates of NO_3^- were 0.4–0.9 mg N kg^{-1} day^{-1} , although no NO_3^- immobilization was detected at Kusaki or Takakuma (Table 2). The MRT of NO_3^- was 0.7–7.5 days, although the negligible pool size of NO_3^- at Kamigamo prevented us from calculating MRT (Table 2). At Kusaki, the MRT of NO_3^- was significantly longer than the MRT of NH_4^+ ($P < 0.05$), although no significant difference was found between MRT of NH_4^+ and NO_3^- at Uryu and Takakuma.

Positive correlation was found between gross NH_4^+ production rates and gross autotrophic nitrification rates (Table 3; Fig. 1a), especially when gross NH_4^+ production

Table 2 Production and consumption rates and MRTs of inorganic N

Sites	NH ₄ ⁺			NO ₃ ⁻				
	Production (mg N kg ⁻¹ day ⁻¹)	Immobilization (mg N kg ⁻¹ day ⁻¹)	MRT (days)	Heterotrophic nitrification (mg N kg ⁻¹ day ⁻¹)	Autotrophic nitrification (mg N kg ⁻¹ day ⁻¹)	Immobilization (mg N kg ⁻¹ day ⁻¹)	% of heterotrophic nitrification	MRT (days)
Uryu	7.3 ^b (0.5)	18.4 ^b (0.6)	0.9 ^{ab} (0.2)	0.11 (0.03)	1.4 ^b (0.5)	0.94 (0.3)	7.7 (1.5)	0.7 (0.1)
Kusaki	11.4 ^{ab} (1.3)	24.7 ^a (1.4)	1.0 ^a (0.2)	0.27 (0.12)	5.8 ^{ab} (1.3)	ND ^B	7.6 (3.5)	2.3 (0.3)
Kamigamo	3.1 ^c (0.5)	12.6 ^c (0.6)	0.2 ^b (0.08)	0.23 (0.19)	-0.2 ^b (0.2)	0.41 (0.3)	ND ^B	ND ^A
Takakuma	3.3 ^c (0.4)	10.6 ^c (0.3)	0.6 ^{ab} (0.06)	0.04 (0.01)	0.4 ^b (0.2)	ND ^B	23 (10)	7.5 (4.6)

Data shown as mean (standard error, SE)

n = 5 except for Hokkaido (*n* = 4)

NH₄⁺ immobilization was calculated by subtracting NO₃⁻ production from NH₄⁺ production

% of heterotrophic nitrification was calculated as gross heterotrophic nitrification divided by gross total nitrification

Different letters denote significant differences among sites (*P* < 0.05) based on one-way analysis of variance (ANOVA) with post hoc Tukey–Kramer

^A ND means not determined because of unmeasurable size of NO₃⁻ pool

^B ND means not determined because these calculated values were negative

rates were greater than 6 mg N g⁻¹ day⁻¹ (*R*² = 0.90, *P* < 0.0001, *n* = 9; Fig. 1a). Gross NH₄⁺ production rates also correlated significantly with NH₄⁺ immobilization rates (Table 3; Fig. 1b), soil C (Table 3), and soil N concentrations (Table 3; Fig. 1c). A significant relation was found between soil C or N concentration and gross total and autotrophic nitrification rates (Table 3). No significant relation was found between gross total and autotrophic nitrification rates and pH (Table 3). The gross autotrophic nitrification rate was negligible with high soil C/N ratio (>20) or low soil N (<2 g N kg⁻¹), but soils with lower C/N ratio and higher soil N concentration had significant gross autotrophic nitrification rates (Fig. 2).

Discussion

Site characteristics of N transformation rates

Our measurement of gross N transformation rates revealed the different characteristics of N dynamics among different forest sites (Fig. 3). Pool sizes and net N transformation rates have been used to characterize N dynamics in forest soils. Davidson et al. (1992) pointed out, however, that neither the relative size of the NO₃⁻ pool nor net changes in NO₃⁻ pool sizes over time is a good index of the importance of NO₃⁻ in the internal N cycle of an ecosystem. Actually, some studies describe rapid turnover of NO₃⁻ and high significant gross nitrification rate despite small NO₃⁻ pool and low net nitrification in forest soils (Davidson et al. 1992; Tokuchi et al. 2000). Hart et al. (1994) reported that the best measure of the relative dynamics of NH₄⁺ and NO₃⁻ pools in the internal N cycle is the MRT of that pool, because this index integrates information of both pool size and the process rate into a single measure. The MRT of NH₄⁺ and NO₃⁻ in mineral topsoils are generally observed in the range of 0–5 days (Davidson et al. 1992; Hart et al. 1994; Stark and Hart 1997; Corre et al. 2003, 2007; Hirobe et al. 2003; Venterea et al. 2004), and our MRT data were within this range, except for at Takakuma (Table 2). Although the short MRT of NH₄⁺ at all sites (ca. 1 day) indicates that the produced NH₄⁺ was used rapidly at all sites, the MRT of NH₄⁺ differed significantly among sites, which implies different N cycles among study sites (Table 2).

We use the ratio of gross autotrophic nitrification to gross NH₄⁺ production (denoted the gross autotrophic nitrification fraction) and the ratio of gross NH₄⁺ immobilization to gross NH₄⁺ production (denoted the gross NH₄⁺ immobilization fraction) to gain insight into the fate of NH₄⁺ (Table 4) that determines the MRT of NH₄⁺. The gross autotrophic nitrification fraction can represent the relative NH₄⁺ availability for autotrophic nitrifiers, whereas

Table 3 Relations among gross rates, and between gross rates and environmental factors

			R^2	P value	n
Gross total nitrification	vs.	NO_3^- pool size	0.93	<0.0001	14
		Gross NH_4^+ production	0.85	<0.0001	19
		Total soil C	0.81	<0.0001	19
		Total soil N	0.80	<0.0001	19
		Net NH_4^+ production	0.64	<0.0001	19
		NH_4^+ pool size	0.63	<0.0001	19
		C/N ratio	0.08	0.26	19
		pH	0.04	0.80	4
Gross autotrophic nitrification	vs.	NO_3^- pool size	0.94	<0.0001	14
		Gross NH_4^+ production	0.83	<0.0001	19
		Total soil C	0.79	<0.0001	19
		Total soil N	0.79	<0.0001	19
		Net NH_4^+ production	0.62	<0.0001	19
		NH_4^+ pool size	0.62	<0.0001	19
		C/N ratio	0.10	0.20	19
		pH	0.05	0.78	4
Gross NH_4^+ production	vs.	Total soil C	0.87	<0.0001	19
		Total soil N	0.86	<0.0001	19
		Gross NH_4^+ consumption	0.85	<0.0001	19
		Gross NH_4^+ immobilization	0.77	<0.0001	19
		NH_4^+ pool size	0.53	<0.001	19
		C/N ratio	0.09	0.22	19

Simple regression analysis was used to investigate the relationships

the gross NH_4^+ immobilization fraction can indicate the relative NH_4^+ demand by heterotrophic microbes. The ratio of net nitrification to net mineralization—usually called percent nitrification—is frequently used to express the relative activity of autotrophic nitrifiers in forest soils (Hirobe et al. 1998; Aber et al. 2003; Fang et al. 2011). However, the gross autotrophic nitrification fraction presented here would be more appropriate in principle regarding the actual proportion of the produced NH_4^+ that is being converted to NO_3^- by autotrophic nitrifiers. Similarly, the gross NH_4^+ immobilization fraction can be a good indicator of competition between heterotrophs and autotrophic nitrifiers. At Kusaki, the highest gross autotrophic nitrification fraction and the lowest gross NH_4^+ immobilization fraction (Table 4) show that the soils at this site have higher availability of NH_4^+ for autotrophic nitrifier than other soils (Table 4). In contrast, Kamigamo soils are characterized by the lowest gross nitrification fraction and considerably high gross NH_4^+ immobilization fraction (Table 4). Takakuma soils had intermediate values for each

fraction (Table 4). Uryu soils also had intermediate values for the gross nitrification fraction, although the gross immobilization fraction was significantly lower than that at Takakuma and as low as that at Kusaki (Table 4). These reflect the N-rich environment at Kusaki and the N-limited environment at Kamigamo.

Comparison of gross and net N transformation data at the same sites

Next we compare the data of gross N transformation obtained in this study and data from a net N transformation study conducted by Shibata et al. (2011). Our results support their results of in situ incubation experiments in many respects and add more detailed observations. They reported distinct differences between net production of NH_4^+ and NO_3^- among soils that had been incubated at each native site during the growing season. They presented the following three observations: (1) net nitrification was significantly higher in Kusaki soils than in Uryu or Kamigamo soil; (2) on the other hand, net NH_4^+ production was significantly higher at Uryu; and (3) Kamigamo soil tended to show a very small rate for both net NH_4^+ and NO_3^- production. Similarly to their observation (1), our gross N transformation study showed that the gross total nitrification was significantly higher at Kusaki than at other sites (Table 2; Fig. 3). Moreover, the ratio of gross autotrophic nitrification fraction was significantly higher at Kusaki than at other sites (Table 4), suggesting higher availability of NH_4^+ for nitrifiers at Kusaki than at other sites. Based on these results, the high net nitrification at Kusaki is probably attributable to the high rates of gross total nitrification, not to the low NO_3^- immobilization. Contrary to their observation (2), gross NH_4^+ production at Uryu was moderate in the present study (Table 2). At Uryu, the gross autotrophic nitrification fraction was not significantly different from that at either Kamigamo or Takakuma (Table 3). Therefore, the low gross NH_4^+ immobilization fraction at Uryu, possibly because of the low microbial biomass at low temperature (Table 1), might be responsible for NH_4^+ accumulation in the soil observed by Shibata et al. (2011). In Kamigamo, the gross total nitrification rate was quite low; moreover, the net nitrification rate and gross NH_4^+ production rate were the lowest among the four sites (Table 2). The lowest MRT of NH_4^+ for Kamigamo soils represents the rapid turnover with a small pool (Table 2). Consequently, the observation (3) was attributed to the low production and fast turnover of inorganic N in Kamigamo soil.

Regulating factors of gross autotrophic nitrification

The measured gross total nitrification rates in this study (0.02–6.08 mg N kg^{-1} day^{-1}) were within the ranges

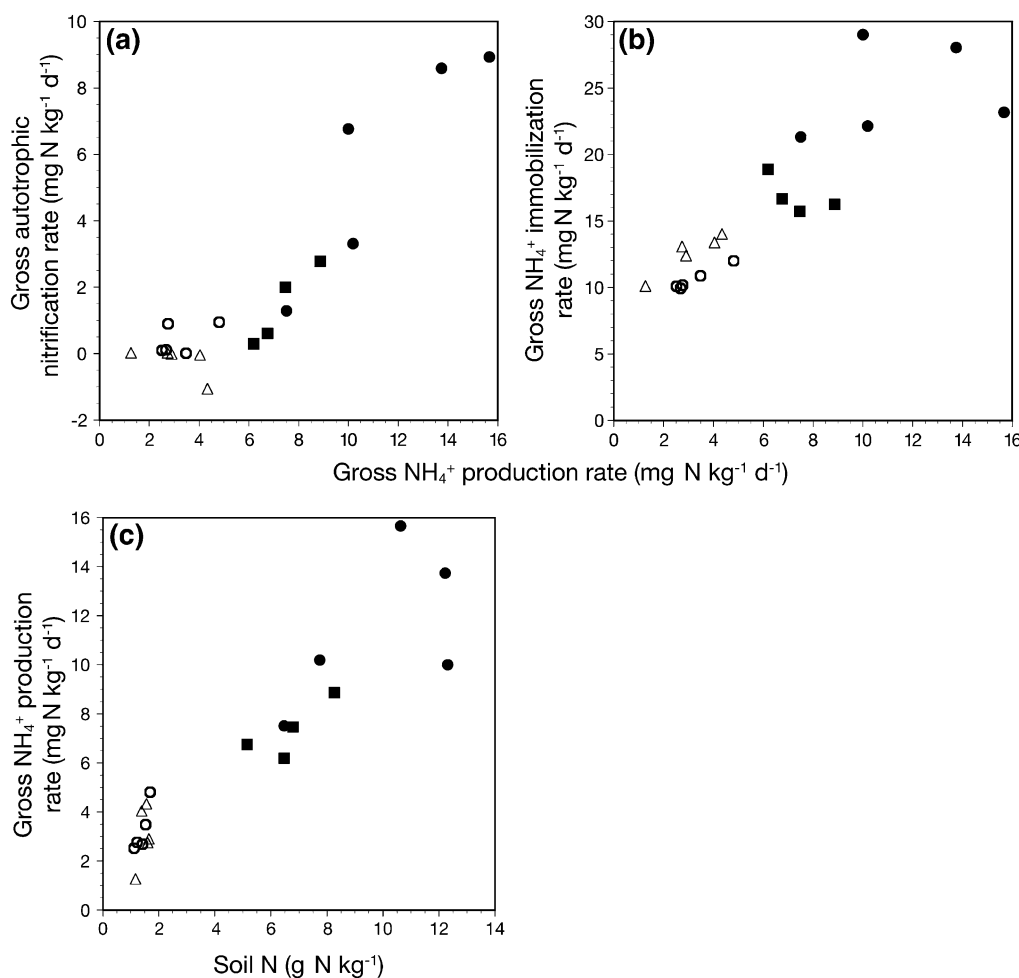


Fig. 1 Relations between gross NH_4^+ production and soil properties at: closed squares Uryu, closed circles Kusaki, open triangles Kamigamo, and open circles Takakuma. Relations between **a** gross

NH_4^+ production and gross autotrophic nitrification rate, **b** gross NH_4^+ production and gross NH_4^+ immobilization, and **c** soil N and gross NH_4^+ production

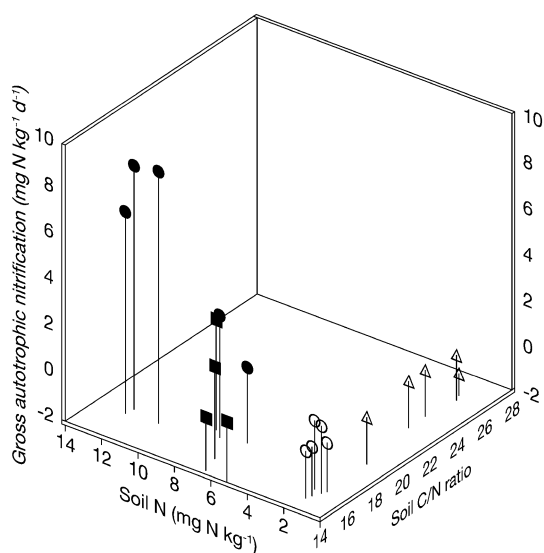


Fig. 2 Relations among soil N, soil C/N ratio, and gross autotrophic nitrification rate. Symbols as in Fig. 1

reported for other forest sites. Corre et al. (2007) measured gross nitrification rates of $0.1\text{--}0.26\text{ mg N kg}^{-1}\text{ day}^{-1}$ in 86–132-year-old spruce forests, whereas Boyle-Yarwood et al. (2008) reported rates of $0.14\text{--}3.69\text{ mg N kg}^{-1}\text{ day}^{-1}$ in Douglas fir and red alder forests. In Japan, Tokuchi et al. (2000) investigated the gross NO_3^- production rates in upper ($0.01\text{--}0.04\text{ mg N kg}^{-1}\text{ day}^{-1}$) and lower mountain slopes ($0.04\text{--}11.07\text{ mg N kg}^{-1}\text{ day}^{-1}$) in a 45-year-old Japanese cedar forest. Booth et al. (2005) assembled data from 100 studies conducted in forests, shrublands, grasslands, and agricultural systems and examined the relation between gross N transformation rates and soil characteristics using regression analyses. They concluded that gross nitrification is a log-linear function of NH_4^+ production, C, N, and extractable NH_4^+ concentrations in soil. Moreover, they found that gross nitrification rates show no relation either to soil pH or to the C/N ratio.

In our study, as in previous studies, strong correlations were found between the rate of gross autotrophic

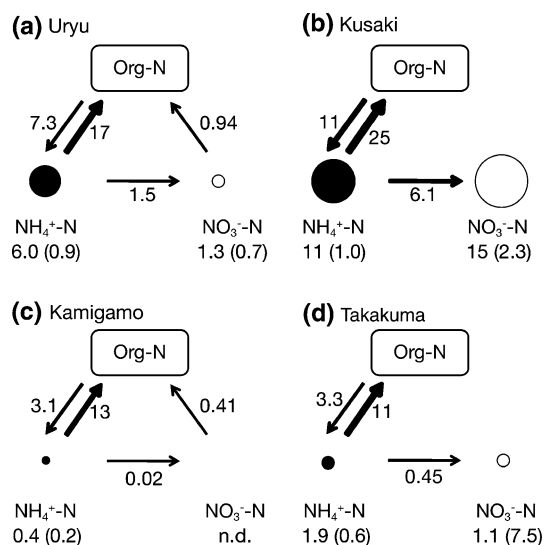


Fig. 3 Characteristics of N dynamics at four study sites. *Arrows* indicate the following: from Org-N (organic N) to $\text{NH}_4^+\text{-N}$, gross NH_4^+ production; from $\text{NH}_4^+\text{-N}$ to Org-N, NH_4^+ immobilization; from $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$, gross autotrophic nitrification; from $\text{NO}_3^-\text{-N}$ to Org-N, NO_3^- immobilization. The figures adjacent to each *arrow* show the rates ($\text{mg N kg}^{-1} \text{ day}^{-1}$). The figure below the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ is the pool size (mg N kg^{-1}) with MRT (days) in *parentheses*. The sizes of the NH_4^+ and NO_3^- pools (*closed and open circles*, respectively) and the arrow thickness reflect their relative sizes

Table 4 Ratios of gross NH_4^+ consumption to production rates

Sites	Autotrophic nitrification fraction	NH_4^+ immobilization fraction
Uryu	0.18 ^{ab} (0.06)	2.4 ^b (0.2)
Kusaki	0.47 ^a (0.09)	2.3 ^b (0.2)
Kamigamo	-0.05 ^b (0.04)	4.7 ^a (0.8)
Takakuma	0.12 ^b (0.05)	3.4 ^{ab} (0.2)

Data shown as mean (SE)

$n = 5$ except for Hokkaido ($n = 4$)

Different letters denote significant differences among sites ($P < 0.05$) based on one-way analysis of variance (ANOVA) with post hoc Tukey–Kramer

Gross autotrophic nitrification fraction was calculated as gross autotrophic nitrification rates divided by gross NH_4^+ production rates

Gross NH_4^+ immobilization fraction was calculated as gross NH_4^+ immobilization rates divided by gross NH_4^+ production rates

nitrification and gross NH_4^+ production (Fig. 1a), and soil C and N concentrations (Table 3). A stronger positive correlation was found between gross NH_4^+ production and gross autotrophic nitrification than that between NH_4^+ pool size and gross autotrophic nitrification (Table 3), suggesting that gross autotrophic nitrification was principally regulated by the gross NH_4^+ production rate rather than by the NH_4^+ pool size. The strong linear correlation between

the gross NH_4^+ production and soil C or N (Table 3; Fig. 1c) further suggests that the quantity of soil C or N controls the gross autotrophic nitrification through regulation of the gross NH_4^+ production rate.

We also found the critical gross NH_4^+ production rate under which autotrophic nitrification did not occur (Fig. 1a). Above the critical value of $6 \text{ mg N kg}^{-1} \text{ day}^{-1}$, the rates of gross autotrophic nitrification increased linearly and significantly, whereas gross autotrophic nitrification did not occur below this value (Fig. 1a). This nonlinear relation between the gross NH_4^+ production rate and gross autotrophic nitrification indicates that the production rate of NH_4^+ does not simply engender NH_4^+ availability for autotrophic nitrification because of the competition for NH_4^+ between nitrifier and heterotrophic bacteria. In soil, two processes of NH_4^+ consumption can occur: immobilization and nitrification. NH_4^+ immobilization and heterotrophic nitrification are mediated by soil C as a substrate, although autotrophic nitrification is not considered to be affected by soil C. It has been suggested that heterotrophic microbes are superior to autotrophic nitrifiers in NH_4^+ acquisition when C availability is high (Verhagen and Laanbroek 1991; Strauss and Lamberti 2000). The heterotrophic immobilization of NH_4^+ is anticipated to prevent the produced NH_4^+ from being available to nitrifiers unless the NH_4^+ production can exceed the N demands of heterotrophs, which is likely to be regulated by soil C/N ratio. Aber et al. (2003) showed threshold C/N ratios of 20–25 by illustrating a pattern between the percent nitrification and the C/N ratio in organic and mineral soils for forests throughout the USA. Similar trends between net nitrification and C/N ratio have been reported across a range of North American, European, and Japanese sites (Tietema and Beier 1995; Dise et al. 1998; Emmett et al. 1998; Gundersen et al. 1998; Goodale and Aber 2001; Yoh 2001; Ollinger et al. 2002; Hirai et al. 2006). We investigated the relation between the C/N ratio and autotrophic nitrification to explore such a threshold of C/N ratio from heterotrophy to autotrophy with respect to the fate of NH_4^+ in soil (Fig. 2). Our data were consistent with perceptions derived from previous studies in that significant autotrophic nitrification occurred only when C/N was relatively low, although the effect of C/N on the autotrophic nitrification was not clear because of low variability of C/N among sites in this study (Table 1; Fig. 2). The correlation between autotrophic nitrification and soil N (Table 3) implied that not C/N ratio but soil N would drive autotrophic nitrification. Regarding mineralization, Toda and Haibara (1999) showed the critical C/N ratio using reaction kinetics analysis in Kusaki soil. It is clear that our limited data should be complemented with those of more studies to derive a more general, detailed relation between the C/N ratio and gross autotrophic rates.

Heterotrophic nitrification in mineral soil

It is reported in studies using pure cultures that the highest rates of NO_3^- production by heterotrophic nitrifiers are just one-tenth of those of autotrophic nitrifiers, which suggests that heterotrophic nitrifiers are of minor importance (Silvia et al. 2005). However, some reports describe that heterotrophic nitrification is the dominant pathway of NO_3^- production in forest soils (Hart et al. 1997; Pedersen et al. 1999; Perakis et al. 2005). Hart et al. (1997) reported that more than 60% of total gross nitrification was heterotrophic in red alder forest and coniferous forest soils. Perakis et al. (2005) reported that 64–100% was heterotrophic in pristine Chilean forests. In contrast, other reports describe that heterotrophic nitrification was not statistically significant and that nitrification was regarded as derived from autotrophic nitrifiers (De Boer et al. 1989; Pennington and Ellis 1993; Barraclough and Puri 1995; Islam et al. 2007). In Japan, Inagaki and Miura (2002) demonstrated that net NO_3^- production in soils from under Japanese cedar and hardwood forests were completely stopped by acetylene, which means that NO_3^- production was performed entirely by autotrophic nitrifiers.

Unlike autotrophic nitrifiers, heterotrophic nitrifiers gain energy not from nitrification but from breakdown of organic matter, which indicates no energy advantage for heterotrophic nitrifiers from nitrification. Furthermore, the low soil pH, which is unsuitable for autotrophic nitrifiers, suggests the hypothesis that heterotrophic nitrifiers can be important in an ecosystem with low N availability, high C availability, and low pH. In this study, however, the rates of heterotrophic nitrification and relative contribution of heterotrophic nitrification were low among sites with various pH, total soil C and N contents, and C/N ratios (Tables 1, 2). Our data indicate that heterotrophic nitrification in mineral topsoil would have only a slight contribution at our study sites (Table 2), although the importance of heterotrophic nitrification was reported in some forested sites in Japan (Inagaki and Yamada 2002). However, the environmental factors affecting the low importance of heterotrophic versus autotrophic remain unclear. The significant autotrophic production was observed in a low-pH environment in Uryu with soil pH of 4.7 (Table 1) with high NH_4^+ availability (Table 2), implying that NH_4^+ oxidation by acid-tolerant or even acidophilic autotrophic bacteria and archaea would occur.

Conclusions

The rates of heterotrophic nitrification were negligible across a range of pH, total soil C and N contents, C/N ratios, and the climate gradient in the Japanese Archipelago

(Table 2). Results showed that heterotrophic nitrifiers would be of minor importance in forest mineral topsoils in Japan, although more investigations are required to reach general conclusions regarding the importance of heterotrophic nitrification.

We portray a schematic picture of nitrification regulation in forest soil in Fig. 4. We found a significant positive relation between gross autotrophic nitrification and gross NH_4^+ production (Table 3; Fig. 1a) with a critical NH_4^+ production rate ($6 \text{ mg N kg}^{-1} \text{ day}^{-1}$ in this study) below which autotrophic nitrification did not occur. Additionally, strong correlation was found between the rate of gross NH_4^+ production and soil C or N (Table 3; Fig. 1c), implying that NH_4^+ production is regulated mainly by the quantity of soil C or N. The critical value of soil C/N (15–20 in this study) at which gross autotrophic nitrification ceased was also found (Fig. 2). These results suggest that the balance of C and N determines NH_4^+ availability through the heterotrophic microbial C and N requirements, resulting in the regulation of gross autotrophic nitrification. Our results showed that gross autotrophic nitrification was determined by soil N and the C/N ratio simultaneously through regulation of the gross NH_4^+ production rate and competition among heterotrophic and autotrophic

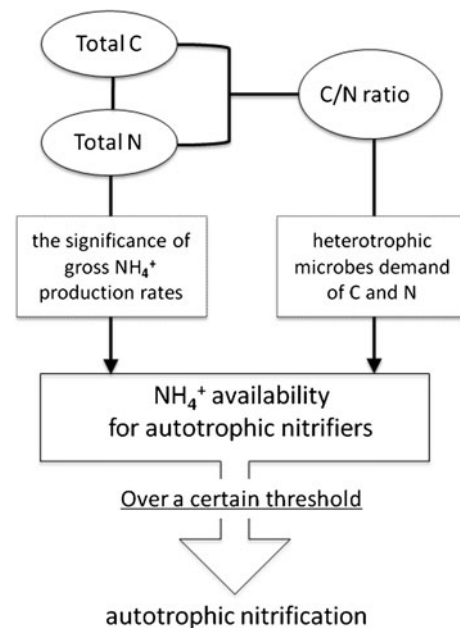


Fig. 4 Schematic showing nitrification regulation in forest soil. Total soil C or N regulates the rate of NH_4^+ production and affects NH_4^+ availability. The C/N ratio alters NH_4^+ availability through heterotrophic microbial demand of C and N, resulting in regulation of gross nitrification. The threshold value of the soil C/N ratio is shown (15–20, in this study), along with the gross NH_4^+ production ($6 \text{ mg N kg}^{-1} \text{ day}^{-1}$, in this study) below which gross nitrification ceased

microbes, which produced the distinctive patterns of N dynamics (Fig. 3).

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