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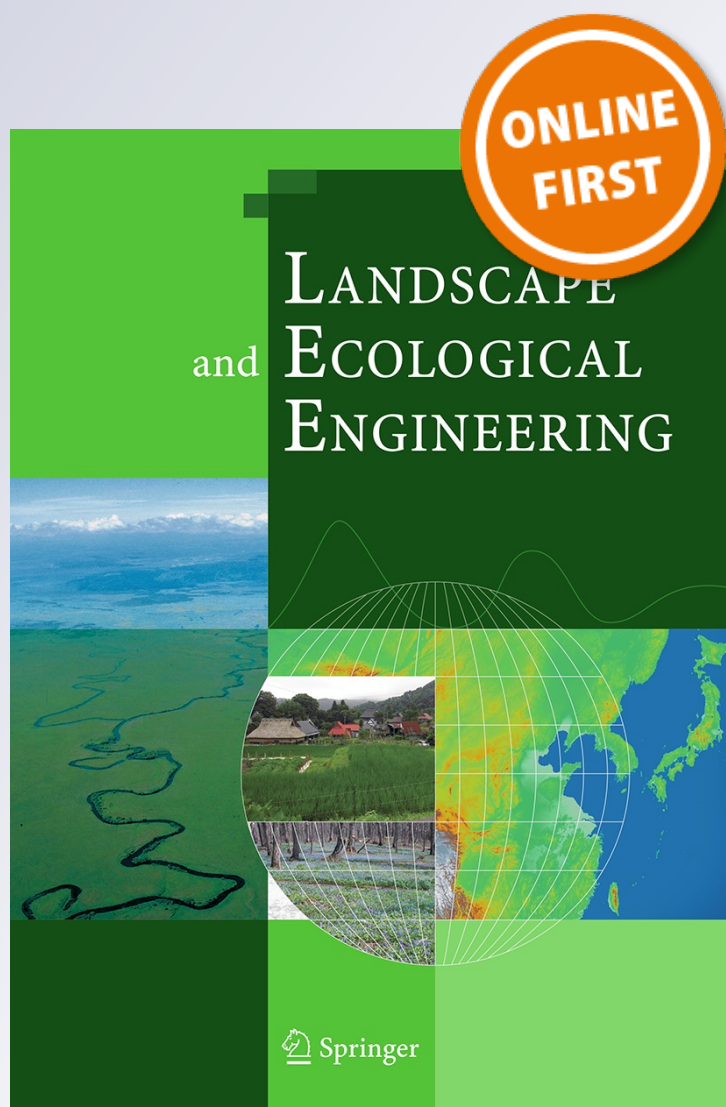
Hitomi Furusawa, Teruaki Hino, Hiroshi Takahashi & Shinji Kaneko

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Nitrogen leaching from surface soil in a temperate mixed forest subject to intensive deer grazing

Hitomi Furusawa[1] · Teruaki Hino[2] · Hiroshi Takahashi[3] · Shinji Kaneko[1]

Abstract   We aimed to determine the grazing impact on soil nitrogen (N) mineralization and N leaching in a temperate mixed forest on Mt. Ohdaigahara, central Japan, where Sika deer (*Cervus nippon* Temminck; hereafter deer) grazing has decreased biomass of *Sasa nipponica* Makino et Shibata (hereafter sasa) that dominates the forest understory. Soil N mineralization and N leaching of the top 0.3-m layer were measured in situ from 2006 to 2007 by an ion-exchange resin core method inside and outside a deer exclosure. Deer grazing decreased the carbon to nitrogen (C:N) ratio of sasa litter because of the declining dead culm C:N ratio and the increasing ratio of leaves with a lower C:N ratio than culms in the aboveground biomass. Grazing increased N mineralization and nitrification rates as well as annual N (ammonium-N and nitrate-N) and nitrate-N leaching. In contrast, deer grazing did not increase the total soil C and N content. The positive effect of grazing on the N mineralization rate may have resulted from a qualitative change in sasa litter due to grazing. The higher annual N leaching resulting from deer grazing may have been related to the facilitated N mineralization rate and decreased N uptake in plants, e.g., sasa with less aboveground biomass due to grazing.

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Introduction

Herbivores occasionally enhance (McNaughton 1979; Frank and McNaughton 1993) and other times decelerate (Pastor et al. 1993; Ritchie et al. 1998) the rate of soil nitrogen (N) dynamics in ecosystems. In some cases, grazing increases plant net primary production and litter input into the soil (McNaughton et al. 1988; Holland et al. 1992; Frank and McNaughton 1993) and may enhance the decomposition of plant litter and soil N mineralization by reducing carbon:nitrogen (C:N) ratios in plant shoots and roots (Frank and Groffman 1998). In addition, herbivores excrete urine and feces, both of which increase the available N and soil N mineralization rates (McKendrick et al. 1980; Pastor et al. 1993). In contrast, some studies have suggested that herbivores decelerate soil N dynamics. Herbivores selectively feed on plant species with nutrient-rich tissues and thus increase the dominance of nutrient-poor plants as well as those defended by secondary compounds. Litter from these latter species decomposes slowly and reduces nutrient turnover and availability. In this case, herbivores enhance the positive feedback that reduces aboveground productivity and the soil N mineralization rate (Pastor et al. 1993). The direction of the grazing effect on soil N dynamics depends on various factors controlling plant species composition, plant productivity, and soil microbial processes, e.g., trophic level, climate (McNaughton et al. 1988; Côté et al. 2004), and grazing intensity (Shariff et al. 1994).

Sika deer (*Cervus nippon* Temminck; hereafter deer) populations have increased in Japan (Natural

Environmental Research Center 1997), and deer grazing has induced various changes in the forest ecosystems. The effects of deer grazing first appear as a decrease in biomass and/or a change of species composition of understory plants in forests (Takatsuki 1977). These changes in understory plants would induce changes in N uptake in plants and the quantity and quality of input of organic materials from plants to the soil, which affect soil N dynamics. Moreover, loss of N from the forest ecosystem due to deer grazing is of growing concern in terms of ecosystem sustainability. One pathway of N loss is leaching of mineral N from the surface soil layer into the subsoil (hereafter, N leaching), which may occur more often in Japanese forests than elsewhere because precipitation and the resulting runoff are higher in Japan than in other comparable regions (Kondo 1994). Previous studies indicate that the decline in understory plants due to deer grazing may increase N loss from forest ecosystems in Japan. Iwatsuki et al. (2014) observed mineral N movement in soil inside and outside a deer exclosure in a deciduous forest and suggested that the impoverished understory due to deer grazing may induce N leaching. Fukushima et al. (2014) showed that nitrate concentrations in soils and streams were significantly lower in a fenced watershed than those in an unfenced watershed 2 years after the fence was constructed, probably because of N uptake due to an increase in understory plants in the fenced watershed. However, the effects of changes in understory plants by deer grazing on N leaching associated with soil N dynamics in forest ecosystems are not well known.

We have observed N dynamics inside and outside deer exclosures since 1997 in a temperate mixed forest on Mt. Ohdaigahara, central Japan, where understory plants have grazed by deer (Furusawa et al. 2005, 2011). Deer grazing decreased the biomass of *Sasa nipponica* Makino et Shibata (hereafter sasa), a dwarf bamboo that dominates the understory (Ito and Hino 2005; Furusawa et al. 2011). N input from aboveground sasa litter inside the exclosures was nearly twice the sum of N input from aboveground sasa litter and deer excrement outside the exclosures (Furusawa et al. 2011), suggesting that deer grazing has decreased sasa aboveground productivity and could lower its N uptake (Furusawa et al. 2011). The observed N input from sasa litter in the deer-exclusion plots almost amounted to N input from woody litter; thus, sasa would have a substantial effect on forest N dynamics.

We expected that the decrease in the aboveground sasa productivity affects soil N mineralization and N leaching on Mt. Ohdaigahara and considered two contrasting N leaching hypotheses. The decrease in N input to soil due to reduced sasa productivity may decelerate the soil N mineralization rate, resulting in less N leaching. In contrast, if grazing lowers the sasa litter C:N ratio, both the change in litter quality and input of deer excrement could stimulate the soil N mineralization rate and increase N leaching. A decrease of N uptake in sasa would also increase N leaching. Thus, our aims in this study were to determine the grazing impact on soil N mineralization and N leaching rates in the temperate mixed forest at Ohdaigahara and examine these alternative hypotheses.

Methods

Site description

This study was conducted in a temperate mixed forest with a 5°–9° slope on Mt. Ohdaigahara on the Kii Peninsula, central Japan (lat. 34°10′44″N, long. 136°5′22″E; altitude 1540 m) (Fig. 1). Deer density is 20–30 individuals km^{-2} and has remained stable for the past 20 years (Fukushima et al. 1984; Maeji et al. 1999; Environmental Agency of Japan 2001). The mean annual temperature in the forest was 6.4 °C in 2001 (Furusawa et al. 2006). Annual precipitation on the mountain is very high, usually >4000 mm, with a maximum of 8214.3 mm observed in 1920 (Doei et al. 1989). The dominant tree species are *Fagus crenata* (48.2 % of basal area), *Abies homolepis* Sieb. et Zucc. (23.9 %), and *Quercus crispula* Blume (12.3 %; Ito and Hino 2004). N input from woody litter is 3.7 g/m^{-2} $year^{-1}$ at the experimental site (Furusawa et al. 2011). Almost all woody litter fall is derived from *Fagus crenata*; this species is unaffected by deer browsing. Hence, experimental deer exclusion was not expected to
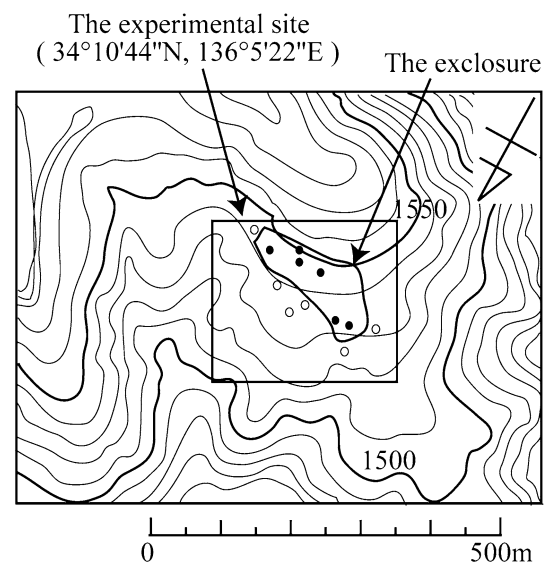
Fig. 1 Location of the experimental site. *Filled circles* indicate positions of deer-exclusion plots; *open circles* indicate positions of deer-grazed plots

affect woody litter in the forest. Although sasa covers >90 % of the forest floor, it is <0.1 m tall as a result of deer grazing. Plants except sasa are rare on the forest floor. The ratio of grazed to total sasa aboveground yield is 0.46 at the site (Hino, unpublished data). Furusawa et al. (2001) provided a soil profile description, and Ito and Hino (2004) and Furusawa et al. (2005) described the study site in more detail.

We used deer-exclusion plots constructed in 1997 and modified in 2005. In 1997, ten plots were constructed for a research project to estimate the effects of deer grazing on plants, soil, and insects (e.g., Ito and Hino 2004; Furusawa et al. 2005, 2011; Ueda et al. 2008, 2009). Each deer-exclusion plot was enclosed by a 2-m-high fence to keep deer out (plot size, 5 × 10 m). The research project finished in spring 2005, and a new 1.22-ha deer exclosure with a 2-m-high fence, which enclosed all previous plots, was constructed by the Environmental Agency of Japan in autumn 2005. Removal of the old fence from the previous deer-exclusion plots followed construction of the new fence. Thus, deer exclusion has continued at the previous deer-exclusion plots. In this study, we used six of the ten deer-exclusion plots. We also deployed six plots established outside the new exclosure in 2006 that permitted deer grazing and have never been manipulated.

Aboveground biomass of sasa and total C and N contents in dead sasa material

Aboveground biomass of sasa was measured in the six deer-exclusion and the six deer-grazed plots in August 2006. Aboveground sasa was clipped with shears at ground level in 0.32-m × 0.34-m quadrats from each plot. We divided the clipped sasa from each plot into fresh leaves, fresh culms, dead leaves, and dead culms and measured the dry weights of the fresh samples. Although we tried to collect fresh litter of the aboveground parts of sasa to estimate its total C and N contents, we were unsuccessful because it was not possible to place litter traps owing to the high density and extremely low height of the sasa. In addition, the sasa litter in the O layer had already begun to decompose; thus, it was not possible to consider it as fresh litter. Therefore, we regarded standing dead leaves and culms as fresh litter. The total C and N contents in the divided materials were measured with a Sumigraph NC900 NC analyzer (Sumika Chemical Analysis Co., Japan).

In situ N mineralization and N leaching

Sample collection and incubation

Ion exchange resin (IER) core methods (DiStefano and Gholz 1986; Wu et al. 1996; Hirai et al. 2007) were used
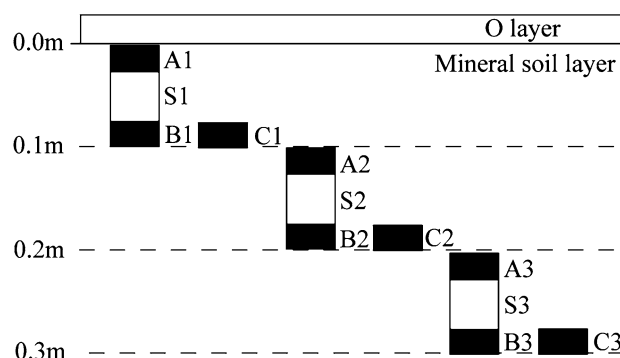
Fig. 2 Ion exchange resin (IER) core field setup and soil cores for measuring nitrogen (N) mineralization and N leaching. *A1–A3* IER core-A; *B1–B3* IER core-B; *C1–C3* IER core-C; *S1–S3*, soil cores from each layer. IER core-B and the soil cores were used to measure net N mineralization. IER cores-A and core-C were used to measure N leaching from upper layers

to estimate soil N mineralization and N leaching rates in the deer-exclusion and deer-grazed plots from 2006 to 2007. The IER core (50 mm in diameter and 25-mm long) consisted of polyvinyl chloride with polyester mesh attached to the bottom. Moist processed cation (Amberlite IR-120: H+) and anion (IRA-410: OH−) resin (20 g each) were mixed and placed in the IER core (Hirai et al. 2007).

We took intact soil core samples in each plot using a polyvinyl chloride cylinder (50-mm diameter, 50-mm long) in July and August 2006 from the middle of three mineral soil layers (0.0–0.1 m, 0.1–0.2 m, and 0.2–0.3 m), because N uptake in plants and N mineralization occurs mostly in the root mat layers at a depth ∼0.2 m (Terai et al. 2009). Two soil cores were taken from each layer, resulting in 72 soil core samples. One core was used to describe the initial soil properties and be transported in a cooler and kept cool until laboratory analysis; the other was used for a field-incubation experiment using the following procedure: IER cores (core-A and core-B) were fixed to the top and bottom of the soil core and fastened using plastic tape (Hirai et al. 2007). The soil core with the fixed IER cores was immediately returned to the original soil layer in each plot (n = 36; Fig. 2). IER core-C was sealed with nylon stocking material and directly embedded at 0.1-, 0.2-, and 0.3-m depths in each plot (n = 36; Fig. 2). These cores were retrieved and transported to the laboratory in August 2007, after about 1 year of incubation.

Chemical analysis and calculations

The 20-g soil core samples were initially extracted, and after incubation with 100 ml 2 M KCl, 4-g resin subsamples in the IER cores were extracted with 1 M KCl

using the procedure described by Haibara et al. (1990). Concentrations of ammonium and nitrate in the extracts were colorimetrically determined using an automatic N analyzer (TN-30NN, FI-N50; Mitsubishi Chemical Analytech Co., Ltd., Tokyo, Japan). The N mineralization rate of each soil layer, defined as the total net accumulation of mineral N in the soil core and the IER core fixed to the bottom of the soil cores (IER core-B), was determined as follows:

$$Nm = Sa + Ra - Si \tag{1}$$

where Nm (g N m^{-2} 0.05 m^{-1} year^{-1}) is the total ratio of net mineralized N (ammonium-N + nitrate-N), Sa is mineral N content in the incubated soil core, Si is initial mineral N content in the soil core, and Ra is mineral N content in IER core-B. Annual N leaching (ammonium-N and nitrate-N) from the O layer was defined as net accumulation of mineral N in the IER core-A1 (Fig. 2). Annual N leaching at 0.1 and 0.2 m was defined as the average net accumulation of mineral N in IER cores-C1 and -A2 and IER cores-C2 and -A3, respectively (Fig. 2). Annual N leaching at 0.3 m was defined as the net accumulation of mineral N in the IER core-C3.

Soil properties

The total soil C and N contents, pH, and bulk density of the initial soil core samples were analyzed from each plot. Total dry soil C and N contents were measured using the NC analyzer described above. Bulk density was determined by dividing the dried soil mass by volume; pH was determined in a soil-H$_2$O solution mixture of 1:2.5.

Statistical analysis

The nonparametric Wilcoxon test was used to compare the effects of deer treatments on aboveground biomass and leaf ratio to the total aboveground biomass of sasa, total C and N contents, and the C:N ratio of dead sasa materials. Two-way analysis of variance (ANOVA) was used to examine the effects of deer and soil depth on soil N mineralization rate, net nitrification rate, and annual N (ammonium-N and nitrate-N) leaching, as well as ammonium-N leaching and nitrate-N leaching. In addition, the Tukey–Kramer method was used to find the significant differences in the soil N mineralization rate among each depth in each plot. Two-way ANOVA was also used to examine the effects of deer and soil depth on soil properties. All statistical tests were performed using JMP6.0 (SAS Institute, Cary, NC, USA) or Statview 5.0 (Hulinks Co., Tokyo, Japan) software.

Results

Aboveground biomass of sasa and total C and N contents in sasa materials

The total aboveground biomass of sasa was 172.6 ± 79.2 g m^{-2} in the deer-grazed plots and 447.3 ± 164.5 g m^{-2} in the deer-exclusion plots (Table 1). Deer grazing significantly decreased the total aboveground biomass of sasa ($p < 0.05$). Deer treatment did not affect the amount of leaves, whereas the amount of culms was significantly higher in the deer-exclusion plots ($p < 0.01$). Thus, deer grazing significantly increased the ratio of leaves to the total aboveground biomass ($p < 0.01$).

The amount of N required for the aboveground parts of sasa (sasa required N) was calculated by multiplying the plant's biomass by its N concentration (sasa biomass N) in the deer-exclusion plots. Because deer have grazed and consumed N in the portion of aboveground part of sasa, sasa required N was calculated by sasa biomass N and adding the deer's N intake from sasa (1.0 g m^{-2}; shown by Furusawa et al. 2011) in the deer-grazed plots. The sasa required N was 4.51 g m^{-2} in the deer-grazed and 5.60 g m^{-2} in the deer-exclusion plots. The average of N content of dead sasa leaves and culm were 16.9 and 8.6 g kg^{-1} in the deer grazed plot, and were 16.5 and 5.4 g kg^{-1} in the deer-exclusion plots, respectively (Table 1). The N input from litter of the aboveground parts was estimated by multiplying the biomass determined in

Table 1 Aboveground biomass of *Sasa nipponica* and total carbon (C) and nitrogen (N) contents in dead *S. nipponica* materials (mean ± standard deviation) in the deer-grazed and deer-exclusion plots in 2006

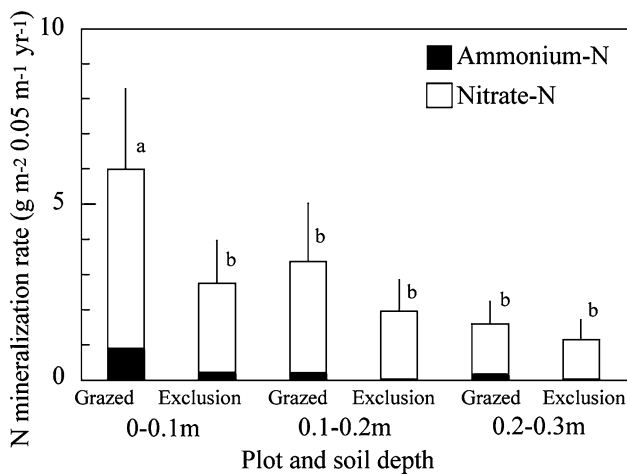|	Grazed	Exclusion	*P* value
Aboveground biomass			
Leaves (g m^{-2})	91.7 ± 38.6	121.3 ± 42.2	NS
Culms (g m^{-2})	80.9 ± 41.4	326.0 ± 131.2	<0.01
Total (g m^{-2})	172.6 ± 79.2	447.3 ± 164.5	<0.05
Ratio of leaves to total	0.54 ± 0.04	0.28 ± 0.05	<0.01
Dead leaves			
Total C content (g kg^{-1})	420.6 ± 12.5	431.8 ± 22.9	NS
Total N content (g kg^{-1})	16.9 ± 1.9	16.5 ± 2.1	NS
C:N ratio	25.2 ± 2.6	26.6 ± 3.8	NS
Dead culms			
Total C content (g kg^{-1})	469.2 ± 1.8	479.9 ± 4.6	<0.01
Total N content (g kg^{-1})	8.6 ± 0.9	5.4 ± 0.4	<0.01
C:N ratio	55.4 ± 6.9	88.9 ± 7.7	<0.01

NS not significant

2006 by the total N concentration in dead leaves and culms. In this respect, we assumed that the biomass in 2006 represented the litter input for 2007, because culms and leaves of this species sprout in spring and finish growing by autumn, when they overwinter and then die by the autumn of the next year. N input from litter of the aboveground parts was 2.22 g m^{-2} in the deer-grazed and 3.73 g m^{-2} in the deer-exclusion plots.

The C:N ratio of dead leaves was lower than that of dead culms in both plots (Table 1). Deer grazing had no significant effect on the total C and N contents of dead sasa leaves. In contrast, it significantly decreased the total C content and increased the total N content in dead sasa culms, thus, lowering their C:N ratio ($p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). C:N ratios of dead sasa leaves and culms were weight-averaged to calculate a weighted mean C:N ratio of sasa litter, which was significantly lower in the deer-grazed plots (34.3 vs. 55.7; $p < 0.01$).

N mineralization and leaching and soil properties

Soil N mineralization and nitrification rates were significantly higher in the deer-grazed plots (Fig. 3; Table 2) and decreased significantly with soil depth (Fig. 3; Table 2). Using the Tukey–Kramer method, soil N mineralization rate at a depth of 0–0.1 m in the deer-grazed plots was determined to be significantly higher than at other depths and within and without the exclosure.
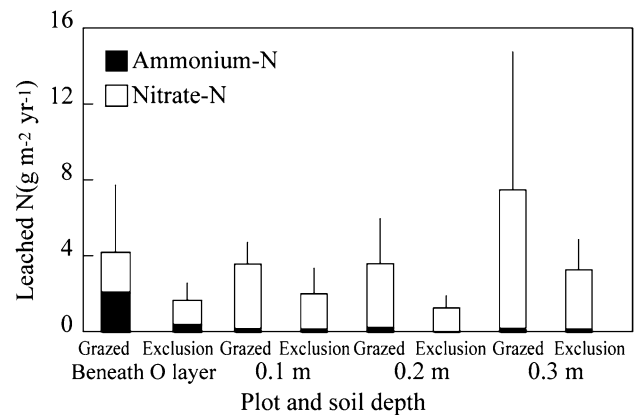
Annual N (ammonium-N and nitrate-N) leaching and nitrate-N leaching were significantly higher in the deer-grazed plots (Fig. 4; Table 2). Deer had no significant effect on annual ammonium-N leaching. The effect of soil depth on nitrate-N leaching but not on N or ammonium-N

Table 2 Two-way analysis of variance results of soil nitrogen (N) mineralization rate and N leaching

	F value	P value
Soil N mineralization rate		
Deer treatment	14.8	<0.01
Soil depth	15.1	<0.01
Deer treatment × soil depth	3.2	0.06
Nitrification rate		
Deer treatment	11.9	<0.01
Soil depth	13.7	<0.01
Deer treatment × soil depth	2.8	0.08
N leaching		
Deer treatment	8.7	<0.01
Soil depth	2.2	0.10
Deer treatment × soil depth	0.4	0.76
Ammonium-N leaching		
Deer treatment	2.1	0.15
Soil depth	2.7	0.06
Deer treatment × soil depth	1.5	0.24
Nitrate-N leaching		
Deer treatment	6.8	<0.05
Soil depth	3.5	<0.05
Deer treatment × soil depth	0.8	0.52

Fig. 4 Annual nitrogen (N) (ammonium-N and nitrate-N) leaching at each depth in the deer-grazed and deer-exclusion plots. *Bars* represent standard deviations

Fig. 3 Soil nitrogen (N) mineralization rate at each depth in the deer-grazed and deer-exclusion plots. *Bars* represent standard deviations. *Different letters* indicate significant differences between plots or soil depths by the Tukey–Kramer method ($p < 0.05$)

leaching was significant (Table 2). Deer exclusion had no apparent effect on pH, total C and N soil contents, C:N ratio, or bulk density (Table 3). However, total C and N contents decreased significantly with soil depth ($p < 0.01$ and $p < 0.01$, respectively), whereas pH and bulk density increased ($p < 0.01$ and $p < 0.01$, respectively). We attempted to calculate the annual rate of N taken up by plants based on the method by Wu et al. (1996), whereas

Table 3 Soil characteristics (mean ± standard deviations) at each depth in the deer-grazed and deer-exclusion plots

	0.0–0.1 (m)		0.1–0.2 (m)		0.2–0.3 (m)	
	Grazed	Exclusion	Grazed	Exclusion	Grazed	Exclusion
pH-H_2O	3.7 ± 0.2	3.8 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Total C content (g kg^{-1})	143.0 ± 29.1	150.1 ± 39.1	94.4 ± 35.7	77.5 ± 19.3	52.7 ± 13.1	56.4 ± 11.0
Total N content (g kg^{-1})	10.1 ± 1.9	10.3 ± 2.5	6.8 ± 2.5	5.5 ± 1.4	3.6 ± 0.9	3.8 ± 0.6
C:N ratio	14.1 ± 0.4	14.6 ± 1.1	13.9 ± 0.6	14.0 ± 0.8	14.7 ± 1.0	14.8 ± 0.7
Bulk density (g m^{-3})	373.6 ± 69.9	333.3 ± 69.7	449.1 ± 47.4	420.8 ± 90.8	473.3 ± 77.2	453.8 ± 82.7

C carbon, *N* nitrogen

we assumed that the difference between the input N (addition from the upper layer plus mineralized N in a soil layer) and the output N (leached N from the soil layer) is the annual rate of N taken up by plants. The mean value of the difference was 6.6 and 2.4 Ng m^{-2} $year^{-1}$ at a depth of 0–0.1 m, 3.3 and 2.6 Ng m^{-2} $year^{-1}$ at 0.1–0.2 m, and −2.3 and −0.9 Ng m^{-2} $year^{-1}$ at 0.2–0.3 m in the deer-grazed and deer-exclusion plots, respectively. The mean value of the difference tended to be higher in the deer-grazed plots at a depth of 0–0.1 m and 0.1–0.2 m.

Discussion

Similar to the findings of previous studies (Ito and Hino 2005; Furusawa et al. 2011), aboveground biomass of sasa in the deer-grazed plots was significantly lower than that in the deer-exclusion plots, indicating that heavy grazing continues at the site. We expected that the aboveground N input of sasa litter would be lower in the grazed plots, as shown in previous studies. This expectation was supported by the calculated N input from litter of the aboveground parts of sasa, which had a lower value in the deer-grazed plots.

Deer grazing increased the ratio of leaves to total aboveground biomass of sasa, and the dead leaves had a relatively low C:N ratio compared with that of dead culms. In addition, deer grazing significantly increased the N content and lowered the C:N ratio of dead sasa culms. Thus, the weighted mean C:N ratio in sasa litter was lower in the deer-grazed plots. New leaves and culms which grow after defoliation by grazing have been reported to contain more N (Ruess et al. 1983), resulting in a declining C:N ratio in green leaves and consequently in their litter (Ruess and McNaughton 1987). A declining C:N ratio could facilitate litter decomposition (Kielland et al. 1997; Olofsson and Oksanen 2002). Our results suggest that more decomposable litter was supplied by the aboveground sasa to soil in the deer-grazed plots.

Soil N mineralization rates were higher in the deer-grazed plots, although deer grazing had no apparent effect on soil N content. The increase in soil N mineralization rate in the deer-grazed plots was apparently not related to the soil N content but to other factors, such as litter quality. Soil N mineralization rate can be accelerated by the input of litter with a low C:N ratio, which decomposes faster (Kielland et al. 1997; Ritchie et al. 1998; Olofsson and Oksanen 2002). Moreover, N was added as excrement in the deer-grazed plots. The positive effects of enriched litter quality through deer grazing, including deer excrement, may override the negative effects of the decrease in the quantity of N input by sasa litter. However, these positive effects were only seen within surface soil (0–0.1 m depth) and not at depth lower than 0.1 m in the deer-grazed plots, as suggested by results using the Tukey–Kramer method.

The amount of required N for the aboveground parts, calculated from the aboveground biomass, was less in the deer-grazed plots. However it was considered that the amount of required N for the belowground parts did not differ between plots, because Furusawa et al. (2011) showed the insignificant effect of deer grazing on N stock in belowground sasa. These suggest that sasa probably takes up less N in the deer-grazed plots because aboveground sasa productivity is lower there. Although it was not directly measured, it was considered that the amount of N taken up by trees in the overstory did not differ between plots during the experimental period in this study for two reasons. First, upper tree density was not expected to vary in the short term, because the dominant tree species is *Fagus crenata*, which is unaffected by deer grazing. Second, this study was conducted from 2006 to 2007, at a point in time when the new deer exclosure had just been constructed. Hence, we expected that the total amount of N taken up by both sasa and trees would probably be less in the deer-grazed plots. In contrast, the difference between input N (addition from the upper layer plus mineralized N in the soil layer) and output N (leached N from the soil

layer)—which is regarded as an annual rate of N taken up by plants in the soil layer—tended to be higher in the deer-grazed plots at depth of 0–0.1 m and 0.1–0.2 m. Although we assume that a difference between input N and output N is an annual rate of N uptaken by plants, soil N dynamics would be more complicated than our assumption, and the difference would contain not only plant uptake but also diffuse N and ammonium volatilization. We suggest that it was not reasonable to regard the calculated value as N taken up by plants.

In this study, it is shown that deer grazing increased annual N leaching and soil N mineralization rates, suggesting that the surplus of available soil N would increase in the deer-grazed plots and then leach in the forest. Consistent with the observations of Iwatsuki et al. (2014) and Fukushima et al. (2014), our results also support that a decrease in N uptake by understory vegetation may increase N leaching. These results therefore agree with the predictions of our second hypothesis: the change in litter quality caused by deer grazing and input of deer excrement may stimulate soil N mineralization and increase N leaching. This observation suggests that a relative increase in N compared with C is more important to the soil N mineralization rate and N leaching than the decrease in total N input.

In general, upper-tree biomass and production in forests are much larger compared with those of understory plants; thus, trees have a significant impact on soil N dynamics. Conversion of dominant tree species by herbivore grazing would affect soil N dynamics through the change of litter quality (Pastor et al. 1993). In this respect, deer would not affect N concentration, C:N ratio, or amount of litter from upper trees in this study, because the density and species composition of upper trees probably did not vary from 1997 to 2007.

Our results suggest that deer grazing on understory plants could substantially influence soil N dynamics and N leaching, even though it does not affect the upper-story trees. Increased N leaching might cause a decrease soil N stock, which is the substrate for N mineralization, and hence decrease the N mineralization rate in the grazed area in mixed temperate forests on Mt. Ohdaigahara. We suggest that understory plants may need to be protected from deer grazing to decrease N leaching. Although sasa has been dominant for several decades on the forest floor on Mt. Ohdaigahara, in the long term, it must die and germinate following mass flowering, and a succession of plant species might occur at that time on the forest floor. Moreover, it is necessary to consider changes in relation to upper-story trees, because density will decrease over the long term due to the high mortality rate of tree seedling caused by deer grazing on Mt. Ohdaigahara (Akashi and Nakashizuka 1999). Changes in plant and soil N dynamics

should be monitored to describe the long-term effect of deer grazing.

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