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To cite this article: Ayumi Mizutani, Wakana A Azuma, Mikoto Kaneko & Shuhei Matsuyama (2022) Soil nutrient and moisture environments and shoot growth of *Quercus crispula* canopy trees in two areas that experienced contrasting effects of deer overabundance over an 11-year period, Journal of Forest Research, 27:6, 469-475, DOI: [10.1080/13416979.2022.2061395](https://doi.org/10.1080/13416979.2022.2061395)

To link to this article: <https://doi.org/10.1080/13416979.2022.2061395>



Published online: 12 Apr 2022.



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SHORT COMMUNICATION



Soil nutrient and moisture environments and shoot growth of *Quercus crispula* canopy trees in two areas that experienced contrasting effects of deer overabundance over an 11-year period

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ABSTRACT

To elucidate the effects of modification of the understory aboveground plant biomass and soil nutrient and moisture environments on canopy tree growth in deer overabundance sites, we compared the canopy shoot growth of oak trees (*Quercus crispula*), which are not a target for bark stripping or bark feeding by deer, understory aboveground plant biomass, and components of soil nutrient and moisture between inside (protected) and outside (exposed) a large fenced area. We found that canopy shoot growth in the exposed area was reduced compared with that in the protected area. Net nitrogen (N) mineralization rates did not differ between areas, although understory aboveground plant biomass, total carbon (C) and N, and total soil inorganic N content were significantly lower in the exposed area than in the protected area. Indices of canopy shoot growth correlated significantly with soil water content, but due to the limited scope of our study this result does not confirm an effect of soil moisture on canopy shoot growth. Further study is required to clarify the effects of deer overabundance on canopy tree growth.

ARTICLE HISTORY

Received 24 June 2021

Accepted 30 March 2022

KEYWORDS

Browsing; canopy shoot; enclosure; inorganic nitrogen; mineralization

Introduction

Overabundance of deer and associated changes in forest ecosystems have been reported all over the world (Stewart and Burrows 1989; Kirby 2001; Rooney 2001; Homolka and Heroldová 2003; Takatsuki 2009). Browsing by overabundant deer can alter species composition (Stewart and Burrows 1989; Kirby 2001; Rooney 2001; Homolka and Heroldová 2003) and decrease understory aboveground plant biomass (McInnes et al. 1992; Miyaki and Kaji 2009a; White 2012). Trampling by deer increases soil compaction and changes soil nutrient and moisture environments (Wardle et al. 2002; Bardgett and Wardle 2003; Schrama et al. 2013). Deer browsing also influences soil moisture and nutrient environments through decreased understory aboveground plant biomass and changes in the quality and quantity of plant litter (Wardle et al. 2002; Kumbasli et al. 2010). However, the effects of deer overabundance on soil nutrient and moisture environments and related effects on forest ecosystems are not well understood.

Changes in understory vegetation and soil nutrient and moisture environments associated with deer overabundance can promote or restrict the growth of residual vegetation such as tolerant and unpalatable plants and trees not subject to bark stripping or feeding damage by deer. However, few studies have examined the effect of deer overabundance on residual vegetation. Reductions in understory aboveground plant biomass and plant litter and increases in soil compaction associated with deer overabundance can reduce the mineralization of soil organic matter (Kumbasli et al. 2010; Kardol et al. 2014), which in turn can restrict the growth of residual vegetation. In contrast, deer overabundance increases the abundance of soil

nutrients available for residual vegetation (Tahtinen et al. 2013) and improves light conditions in the ground layer (Sabo et al. 2017), which may promote residual vegetation growth (Miyaki and Kaji 2009a; White 2012).

The growth of an individual tree is influenced by light (Kolb et al. 1990; Pacala et al. 1994; Wainhouse et al. 1998), soil nutrients (Kolb et al. 1990; Canham et al. 1996), and soil moisture (Kolb et al. 1990; Wainhouse et al. 1998; Michelot et al. 2012). Because the upper part of the tree crown is not subject to light restriction, the light environment of canopy shoots does not differ among individuals, unlike understory plants, whose soil nutrient, moisture, and light environments vary among individuals (Pacala et al. 1994; Sefcik et al. 2007). Therefore, shoot growth in the upper part of the tree canopy depends mainly on soil nutrient and moisture availability. While a few studies have examined canopy tree growth under conditions of deer overbrowsing (Suda et al. 2001; Miyaki and Kaji 2009b), the relationship between canopy tree growth and soil nutrient and moisture content remains unclear.

Japanese northern oak (*Quercus crispula*) is a common deciduous canopy tree in the temperate forests of southern Sakhalin, the Kuril Islands, Japan, and Korea (Ohashi et al. 2016). *Quercus crispula* seedlings are palatable to deer (Hashimoto and Fujiki 2014), but they do not strip the bark of mature trees (Miyaki and Kaji 2009b). Therefore, *Q. crispula* is an appropriate tree species for examining the effects of deer browsing on canopy tree growth that result from changes in understory aboveground plant biomass and soil nutrient and moisture environments.

This study sought to elucidate the effects of deer overabundance on the canopy shoot growth of trees not subject to bark stripping or feeding damage by deer, as a result of modification of the understory aboveground plant biomass and soil nutrient and moisture environments. We compared *Q. crispula* canopy shoot growth, understory aboveground plant biomass, and soil nutrient and moisture environments between areas inside (protected) and outside (exposed) a deer fence. We hypothesized that canopy shoot growth would be reduced in the exposed area relative to the protected area. Where the growth of canopy shoots differed between the two areas, it was hypothesized that the understory aboveground plant biomass would be smaller in the exposed area than in the protected area, and that the soil nutrient and moisture contents would be lower in the exposed area than in the protected area.

Materials and methods

Site description and study species

This study was conducted in a natural forest in Ashiu Research Forest (ARF; 35°18'N, 135°43'E; elevation, 750 m a.s.l.) in northern Kyoto Prefecture, Japan. The mean annual temperature and annual precipitation of the study site are 10.1°C and 2,290 mm, respectively (Ise and Maeda 2018). The geological components of the area are sandstone, mudstone, and shale of the Tanba Belt of the Mesozoic. Most of the soil in the study area is brown forest soil (Sakai et al. 2012). The ARF consists of cool-temperate mixed forest (Hirayama and Sakimoto 2003) and intermittent Japan Sea coast and Pacific coast vegetation (Yasuda and Nagamasu 1995), including *Cryptomeria japonica*, *Fagus crenata*, *Pterocarya rhoifolia*, *Acer sieboldianum*, and *Aesculus turbinata*; *Q. crispula* is also a common tree in this forest (Hirayama and Sakimoto 2003).

Excessive deer browsing was recognized as a serious problem at the study site in the late 1990s (Tanaka et al. 2008; Sakai et al. 2012), with drastic understory vegetation changes observed until the early 2000s (Kato and Okuyama 2004; Sakai et al. 2012). In 2006, a 16-ha area within the Utsuro catchment was fenced to protect forest vegetation from deer browsing (Sakai et al. 2012). Thus, the protected area has not always been completely free from the effects of deer overabundance; however, in this study, we assumed that differences between the protected and exposed areas reflect the effects of deer overabundance, because the duration of exposure to deer overabundance differed between the two areas. The minimum deer density outside the fence was estimated through yearly block counts on 2 days in December from 2006 to 2010 of 2.30–4.21 (2006), 0.00–5.30 (2007), 1.15–5.75 (2008), 0.00–5.75 (2009), and 4.60–13.80 individuals/km² (2010) (A. Takayanagi, unpublished data cited by Sakai et al. 2012). The tree species composition in the protected and exposed areas was similar prior to fence installation (Sakaguchi et al. 2012), and the species diversity of the forest floor had decreased by 25% by the time the fence was installed (Kato and Okuyama 2004).

Quercus crispula (Fagaceae) is a deciduous canopy tree species with a maximum height of 30 m that flowers from mid-May to mid-June. Acorns mature from August to October, and production fluctuates annually, in patterns

that vary among populations (Nakajima et al. 2012; Odani 2013). *Quercus crispula* is a common tree in the broadleaf and conifer forests of the ARF (Hirayama and Sakimoto 2003). Most natural and/or secondary forests in Japan grow on hill and mountain slopes, with soil nutrient and water conditions differing among locations on the slope (Tateno and Takeda 2003); *Q. crispula* is found mostly on upper slopes (Hirayama and Sakimoto 2003).

Canopy shoot growth

We selected 6 canopy *Q. crispula* trees within the protected area and 15 within the exposed area (Table S1). All trees in the protected area were growing on the upper slopes, whereas trees in the exposed area were growing on lower (seven trees) and upper slopes (eight trees). The average distance between two sample trees was 1,351 m (range: 8–2,811 m). A study conducted before the installation of the deer fence found that soil nutrient and moisture environments were better on the lower slope than on the upper slope (Tateno and Takeda 2003). However, in this study we found little difference in soil nutrient or moisture environments between the lower and upper slopes (Table S2).

In mid-July 2017, to minimize differences in light conditions between and within samples, we collected 1-year-old shoots (1S) attached to current-year shoots (CSs) from the top of the crown by climbing the trees using a rope and jumbar. We measured the 1S stem length, basal diameter, and dry mass of 20 samples from each tree; for CSs, we obtained the same measurements, as well as leaf dry mass. For CSs bearing young acorns, acorn mass was also measured. Dry mass was measured after drying at 70°C for 48 h. The 1S length, basal diameter, and dry mass were used to standardize CS parameters, because CS shoot growth is partly dependent on the size of 1Ss. As indices of canopy shoot growth, we calculated the total CS length per 1S length, total CS cross-sectional area per 1S cross-sectional area, total CS stem mass per 1S stem mass, and total leaf mass per 1S stem mass. The total CS reproductive tissue mass per 1S stem mass was not analyzed, because the young acorn mass was too small to evaluate as an index of reproduction (total CS reproductive tissue mass per 1S: mean \pm standard error (SE) = 0.17 \pm 0.02 g; maximum = 2.53 g). Because acorn production can reduce shoot growth as a reproductive cost (Obeso 2002), we preliminarily analyzed the effect of the reproductive cost and the interaction between the reproductive cost and the deer fence on the shoot growth, with CSs bearing young acorns as reproductive shoots. However, we did not describe the results in this paper, because notable effect was not detected.

From each 1S, we selected a single complete leaf at random from a CS that was a leader shoot. An optical scanner was used to obtain an image of each sample, from which leaf area was calculated using ImageJ software (Schneider et al. 2012). The sample was then dried at 70°C for 48 h, weighed, and ground for total carbon (C) and nitrogen (N) content measurements. Leaf total C and N contents (leaf TC and TN) were measured using a Sumigraph NCH-22A auto analyzer (Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Single leaf area, mass, specific leaf area, leaf TC, leaf TN, and the leaf C/N ratio (CN) were used as indices of leaf characteristics.

Understory aboveground plant biomass

To compare the understory aboveground plant biomass quantitatively between the protected and exposed areas, in mid-July 2017 we set four 30 cm × 30 cm quadrats 2 m apart from the trunk of each sample tree, in an orthogonal direction along the contour line of the sample tree. The aboveground plant biomass in each quadrat was collected and weighed after drying at 70°C for 48 h.

Soil nutrient and moisture environments

The CN and net N mineralization rate of soil collected in mid- to late July 2017 were evaluated. For each tree, a composite soil sample (ca. 360 mL) was prepared from individual soil cores (ca. 90 mL) taken from the four quadrats from which understory aboveground plant biomass was collected. The soil samples were sieved to < 2 mm and large fragments (e.g. plant roots) were removed. Soil samples were stored at 4°C until analysis.

The soil total C and N (soil TC and TN) contents of 3-mg subsamples were measured using a NC analyzer (Sumigraph NCH-22A; Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Each subsample was dried at 40°C for 60 h, sieved, and ground to powder prior to analysis. The CN was calculated as the total C content divided by the total N content.

Soil water content, and ammonium (NH_4^+ -N) and nitrate (NO_3^- -N) concentrations, were measured before and after incubation to calculate the net N mineralization rates of the soil samples. Soil water content was calculated by drying a 3-g subsample at 105°C for 48 h. To measure NH_4^+ -N and NO_3^- -N concentrations, a 5-g subsample was extracted in 50 mL 2 N KCl and filtered. The NH_4^+ -N and NO_3^- -N concentrations of the filtrates were analyzed using an autoanalyzer (Autoanalyzer III; BL TEC K. K., Osaka, Japan) and recorded as NH_4^+ -N and NO_3^- -N concentrations g – 1 dry soil. Another 5-g subsample was weighed and incubated for 4 weeks at 25°C, with water content maintained based on weight throughout this period. The incubated subsample was also extracted and analyzed as described above. The net N mineralization rate was calculated by comparing the NH_4^+ -N and NO_3^- -N concentrations g – 1 dry soil before and after incubation. The net nitrification rate was calculated as the difference in NO_3^- -N contents (g⁻¹ dry soil) before and after incubation. The net ammonification rate was calculated as the difference in NH_4^+ -N contents (g⁻¹ dry soil) before and after incubation.

Statistical analyses

All statistical analyses were performed using R v3.5.1 software (R Core Team 2018). The effects of the deer enclosure fence on tree height and diameter at breast height (DBH) were examined using linear models with tree height or DBH as the response variable and fence treatment (protected = 1, exposed = 0) as the explanatory variable.

The effect of the fence on indices of shoot growth (total CS length per 1S length, total CS cross-sectional area per 1S cross-sectional area, total CS stem mass per 1S stem mass,

and total leaf mass per 1S stem mass) were analyzed using a linear mixed model (LMM). The full models for the CSs (y_{CS}) and 1S (y_{1S}) data were set as follows:

$$\ln(y_{CS}) = a_0 + a_1 \cdot F1 + b + \text{offset}(\ln(y_{1S})) \quad (1)$$

where a_0 and a_1 are the fixed-effect coefficients, b is a random variable, and $\ln(y_{1S})$ was defined as an offset term because we were not interested in the coefficient of $\ln(y_{1S})$. The response variables were total length, stem cross-sectional area, stem mass, and leaf mass for CSs on a single 1S, and the explanatory variables were fence treatment (protected = 1, exposed = 0). We log-transformed y_{CS} and y_{1S} in the model under the assumption that they had an allometric relationship. Differences in response variables among trees were used as a random variable. The LMMs were computed using the *lme4* R package (Bates et al. 2015).

Understory aboveground plant biomass was compared between the protected and exposed areas using a LMM, where the response, explanatory, and random variables were understory aboveground plant biomass, fence treatment (protected = 1, exposed = 0), and differences in response variables among trees, respectively. The LMM was computed using the *lme4* package in R (Bates et al. 2015).

The effects of the fence on the soil TC and TN contents, soil water content, NH_4^+ -N and NO_3^- -N concentrations g⁻¹ dry soil, nitrification rate, and net N mineralization rate were examined using an LMM, where fence treatment was used as the explanatory variable and differences in response variables among trees were used as random variables.

To examine the effects of the soil nutrient and moisture environments on canopy shoot growth, we regressed indices of canopy shoot growth against the net N mineralization rate, soil water content, total inorganic N (sum of NH_4^+ -N and NO_3^- -N concentrations g⁻¹ dry soil), CN ratio, and soil TC using LMMs. In these models, CS (y_{CS}) values were used as response variables, whereas 1S (y_{1S}) values were included as offset variables, as follows:

$$\ln(y_{CS}) = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_5 + b + \text{offset}(\ln(y_{1S})) \quad (2)$$

and net N mineralization rate, soil water content, total inorganic N (NH_4^+ -N and NO_3^- -N concentrations g⁻¹ dry soil), soil CN, and soil TC were used as explanatory variables (x_1, x_2, \dots, x_5). Differences in the response variables among trees were used as random variables.

For each analysis, the full model and the model that reduced the explanatory variable were compared using the likelihood ratio test.

Results

Canopy shoot growth

Tree height and DBH in the protected area were not significantly different from those in the exposed area (height: $F = 0.582$, $P = 0.455$; DBH: $F = 0.650$, $P = 0.430$). The means ± SE of tree height in the protected and exposed areas were 18.9 ± 1.7 and 17.8 ± 0.6 m, respectively, whereas those of DBH in the protected and exposed areas were 50.5 ± 4.0 and 45.3 ± 3.7 cm, respectively.

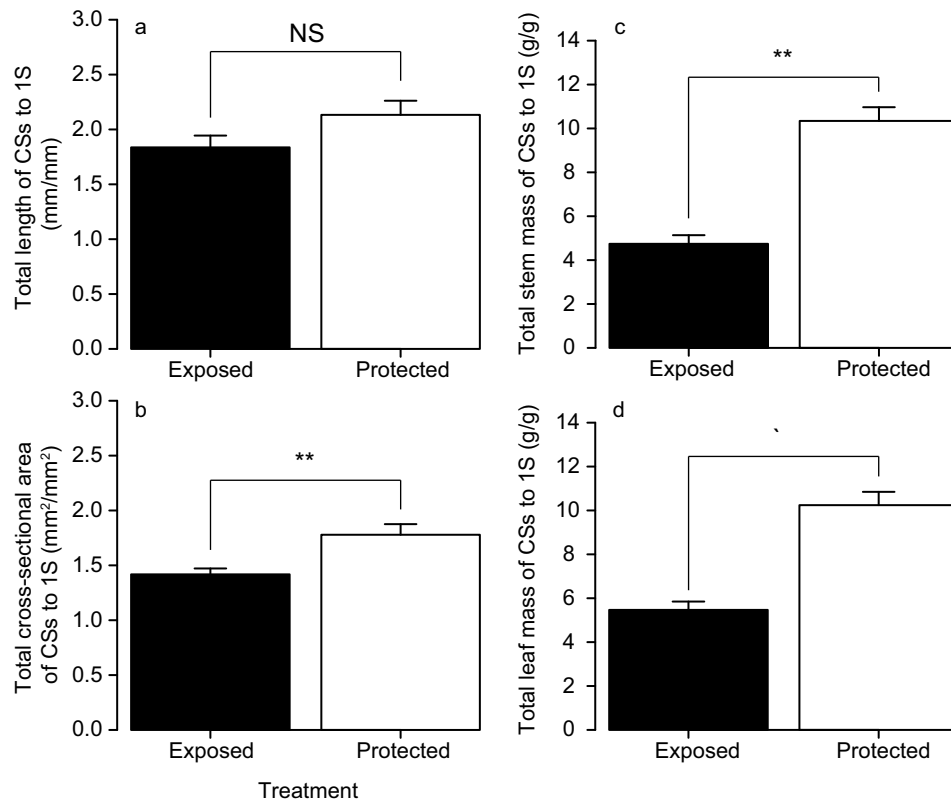


Figure 1. Means and standard errors for total current-year shoot (CS) length per one-year-old (1S) length (a), cross-sectional area per 1S cross-sectional area (b), stem mass (c) and leaf mass (d) per 1S mass in the exposed and protected areas (exposed: solid; protected: open). Asterisks indicate that the difference between variables of each group is significant (NS; $P > 0.05$, *; $P < 0.05$, **; $P < 0.01$).

Table 1. Single leaf area, specific leaf area, total carbon (TC) and nitrogen contents (TN), and carbon-nitrogen ratio (CN) for *Quercus crispula* in the protected and exposed areas. Means and standard errors, and statistics (χ^2 and P) are shown.

Variables	Exposed (15)	Protected (6)	χ^2	P
Single leaf area (cm ²)	312.26 ± 7.90	333.59 ± 19.96	0.129	0.720
Specific leaf area (cm ² /g)	138.60 ± 5.17	136.54 ± 6.01	0.011	0.918
Leaf TC (g/kg)	45.80 ± 0.65	46.62 ± 0.83	0.528	0.467
Leaf TN (g/kg)	1.97 ± 0.05	1.74 ± 0.04	4.749	0.029
Leaf CN	23.94 ± 0.77	26.95 ± 0.57	2.830	0.093

The total cross-sectional area of the CSs, total CS stem mass and leaf masses per 1S in the exposed area were significantly smaller than those in the protected area (total cross-sectional area: $\chi^2 = 6.913$, $P = 0.009$; total CS stem: $\chi^2 = 7.485$, $P = 0.006$; total CS leaf mass: $\chi^2 = 6.545$, $P = 0.011$; Figure 1). The total length of the CSs to 1S in the exposed area was smaller than that in the protected area, but the difference was not statistically significant ($\chi^2 = 1.985$, $P = 0.159$; Figure 1).

Leaf TN was significantly higher in the exposed area than in the protected area ($\chi^2 = 4.749$, $P = 0.029$; Table 1). Single-leaf area, specific leaf area, leaf TC and TN did not differ statistically between the protected and exposed areas (Table 1).

Understory aboveground plant biomass

Understory aboveground plant biomass was significantly lower in the exposed area (28.0 ± 23.6 g) than in the protected area (91.2 ± 81.1 g) ($F = 13.292$, $P < 0.001$).

Table 2. Comparisons of the components of soil nutrient and moisture environments in protected and exposed areas. Means and standard errors of soil water content, nitrate (NO₃⁻) and ammonium (NH₄⁺) content, total inorganic nitrogen content, net nitrification rate, net ammonification rate, net nitrogen (N) mineralization rate, total carbon and nitrogen contents (TC and TN), and carbon-nitrogen ratio (CN) for soil around *Quercus crispula* in the protected and exposed areas. Means and standard errors, and statistics (F and P) are shown.

Variables	Exposed (15)	Protected (6)	F	P
Soil water content (%)	47.63 ± 2.51	52.82 ± 3.66	1.272	0.273
NO ₃ ⁻ content (mg/kg)	1.52 ± 0.09	1.52 ± 0.05	0.005	0.945
NH ₄ ⁺ content (mg/kg)	11.47 ± 1.36	20.27 ± 2.07	12.063	0.003
Total inorganic nitrogen content (mg/kg)	13.00 ± 1.32	21.78 ± 2.03	12.794	0.002
Net nitrification rate (mg/kg/day)	1.16 ± 0.48	0.19 ± 0.13	1.576	0.225
Net ammonification rate (mg/kg/day)	1.49 ± 0.32	1.66 ± 0.32	0.093	0.764
Net N mineralization rate (mg/kg/day)	2.65 ± 0.51	1.85 ± 0.31	0.909	0.352
Soil TC (g/kg)	12.25 ± 6.86	20.76 ± 6.87	5.948	0.025
Soil TN (g/kg)	0.74 ± 0.29	1.05 ± 0.29	4.234	0.054
Soil CN	15.49 ± 0.89	19.58 ± 0.69	7.600	0.013

Soil nutrient and moisture environments

The NH₄⁺-N content, total inorganic N content, and soil TC and TN were significantly lower in the exposed area than in the protected area (NH₄⁺-N content: $F = 12.063$, $P = 0.003$; total inorganic N content: $F = 12.794$, $P = 0.002$; soil TC: $F = 5.948$, $P = 0.025$; soil CN: $F = 7.600$, $P = 0.013$; Table 2). Soil TN was marginally lower in the exposed area than in the protected area (Table 2). Soil water content, NO₃⁻-N content, net nitrification rate, and net N mineralization rate did not differ statistically between the protected and exposed areas (Table 2).

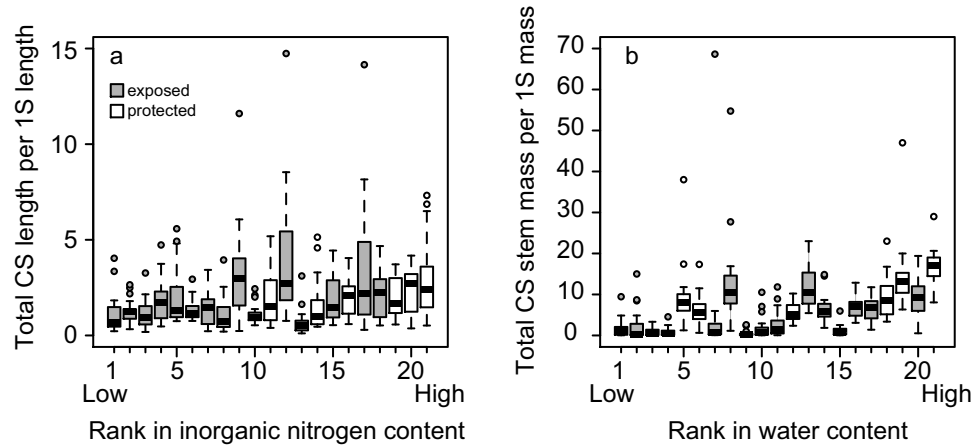


Figure 2. Ranks in the components of the conditions of the soil and the CS growth components per 1S components: (a) inorganic nitrogen content and stem length and (b) soil water content and stem mass for the trees in the exposed and protected areas (exposed: gray; protected: open). Bold line in the box, and upper and bottom of the box denote median and quartiles. Bars show maximum and minimum. Plots show outliers.

Table 3. Statistics of likelihood ratio test for model comparisons on total current-year shoot (CS) length, cross-sectional area, stem mass, or leaf mass. Explanatory variables for the model were soil water content, total inorganic nitrogen (N) content, net N mineralization rate, soil carbon:N (CN ratio), and soil total carbon content (soil TC). Models included one-year shoot size measurement (1S) as an offset term. Chi-square values are shown. *NS* and asterisks denote *P* values for the likelihood ratio test (*NS*: $P \geq 0.05$; *: $P < 0.05$; **: $P < 0.01$).

Variables	Soil water content	Total inorganic nitrogen content	Net N mineralization rate	CN ratio	Soil TC
Total CS length	0.186 <i>NS</i>	6.848**	0.792 <i>NS</i>	0.043 <i>NS</i>	0.465 <i>NS</i>
Total CS cross-sectional area	0.012 <i>NS</i>	5.006 *	0.001 <i>NS</i>	0.006 <i>NS</i>	0.172 <i>NS</i>
Total CS stem mass	9.915 *	0.333 <i>NS</i>	1.364 <i>NS</i>	0.037 <i>NS</i>	1.447 <i>NS</i>
Total CS leaf mass	9.512 *	0.008 <i>NS</i>	1.255 <i>NS</i>	0.043 <i>NS</i>	3.093*

Canopy shoot growth and soil N and C content

Total inorganic N was a significant explanatory variable in the model for total CS length (Figure 2a, Table 3) and total CS cross-sectional area (Table 3). For CS total stem mass and total leaf mass per 1S, soil water content was a significant explanatory variable in the model (Figure 2b, Table 3). Soil TC was also a significant explanatory variable for total CS leaf mass per 1S (Table 3).

Discussion

Our results for the total stem length, total cross-sectional area, and total stem and leaf mass of CSs per 1S (Figure 1) indicated that canopy shoot growth was reduced in the exposed area compared to the protected area. Understory shoot growth was influenced by the soil nutrient and moisture environments, and by the CS light environment (Kolb et al. 1990; Canham et al. 1996). However, greater effects on shoot growth of soil moisture and nutrients have been observed in high-light environments than in low-light environments (Kolb et al. 1990; Canham et al. 1996). As all of the CSs examined in this study were collected from the tops of canopy trees, we assume that there was no significant difference in the light environments among the CS samples from the trees within the two areas. The lack of differences in leaf

parameters according to light environment, such as single-leaf area and specific-leaf area (Poorter et al. 2009) between the protected and exposed areas (Table 1), implies that the soil nutrient environment are important for canopy shoot growth.

We detected significantly lower total inorganic N contents in the exposed area than in the protected area (Table 2), indicating that soil inorganic N may be more limited in the former area. This finding was supported by lower understory aboveground plant biomass, soil TC and TN in the exposed area (Table 2), implying less soil organic matter as a substrate of soil inorganic N. However, it remains unclear whether the lower canopy shoot growth observed in the exposed area was attributable to limited N availability. It should be considered that the results of this study are based on one-time sampling in a single pair of experimental plots, since inorganic N pools are one of the most spatio-temporally dynamic soil properties (Reich et al. 1997). It should be also considered that we detected no significant difference between the protected and exposed areas in the net N mineralization rate, which is a good index of the soil N availability for plants (Reich et al. 1997) (Table 2). A recent study of subtropical forests also reported differences in the spatial patterns of inorganic N content and the net N mineralization rate (Wang et al. 2007).

In addition to the lack of difference in the net N mineralization rate between the protected and exposed areas, leaf TN was significantly higher in the latter area (Table 1), implying that the lower canopy shoot growth in the exposed area was not caused by limited N availability. Leaf N content increases in response to soil fertilization (Forkner and Hunter 2000). However, the increase of leaf N content could also occur when one of other crucial factors limited the tree growth even under similar soil N availability (Tateno et al. 2002). Additionally, Funk et al. (2007) reported that the shoot growth increased but leaf N content less increased in *Populus deltoides* on irrigated condition. Therefore, our results might indicate that the lower canopy shoot growth in the exposed area was not caused by limited N availability, but caused by one of other uninvestigated factors.

The significant relationship between soil water content and the CS growth indices (Figure 2; Table 3) implies that local soil moisture environments affect canopy shoot growth. However, the lack of a significant difference in soil water content between

the exposed and protected areas (Table 2) does not support an effect of the soil moisture environment on the observed differences in canopy shoot growth in the two areas. As with our soil nutrient results, the spatial and temporal dynamics of the soil moisture environment must be considered, such that our results may have been influenced by the limited sampling protocol.

Thus, although we detected lower canopy shoot growth in the exposed area, seasonal monitoring of soil nutrient and moisture environments, and/or replication of the experimental plots, will be required to understand the relationship between canopy shoot growth and the soil nutrient and moisture environments in the study area.

Acknowledgments

We thank Takeshi Hirai for his help with the sample collection, and Dr. Masae Ishihara and Dr. Atsushi Takayanagi for preparation of our field survey. We also thank the staff of the Ashiu Forest Research Station for the permission to conduct our field study and their support.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

- Bardgett RD, Wardle DA. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*. 84 (9):2258–2268. doi:10.1890/02-0274.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 67(1):1–48. doi:10.18637/jss.v067.i01.
- Canham CD, Berkowitz AR, Kelly VR, Lovett GM, Ollinger SV, Schnurr J. 1996. Biomass allocation and multiple resource limitation in tree seedlings. *Canadian J For Res*. 26(9):1521–1530. doi:10.1139/x26-171.
- Forkner RE, Hunter MD. 2000. What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*. 81(6):1588–1600. doi:10.1890/0012-9658(2000)081[1588:WGUMCD]2.0.CO;2.
- Funk JJ, Jones CG, Lerdau MT. 2007. Leaf- and shoot-level plasticity in response to different nutrient and water availabilities. *Tree Physiol*. 27(12):1731–1739. doi:10.1093/treephys/27.12.1731.
- Hashimoto Y, Fujiki D. 2014. List of food plants and unpalatable plants of sika deer (*Cervus nippon*) in Japan. *Hum Nat*. 25: 133–160. Japanese.
- Hirayama K, Sakimoto M. 2003. Spatial distribution of canopy and subcanopy species along a sloping topography in a cool-temperate conifer-hardwood forest in the snowy region of Japan. *Ecol Res*. 18 (4):443–454. doi:10.1046/j.1440-1703.2003.00568.x.
- Homolka M, Heroldová M. 2003. Impact of large herbivores on mountain forest stands in the Beskydy Mountains. *For Ecol Manag*. 181(1–2):119–129. doi:10.1016/S0378-1127(03)00121-X.
- Ise T, Maeda M. 2018. Kyoto University Field Science and Education and Research Center, Ashiu Research Forest. *Jp J Ecol*. 68: 75–80. in Japanese.
- Kardol P, Dickie IA, St John MG, Husheer SW, Bonner KI, Bellingham PJ, Wardle DA. 2014. Soil-mediated effects of invasive ungulates on native tree seedlings. *J Ecol*. 102(3):622–631. doi:10.1111/1365-2745.12234.
- Kato M, Okuyama Y. 2004. Changes in the biodiversity of a deciduous forest ecosystem caused by an increase in the Sika deer population at Ashiu, Japan. *Contr Biol Lab Kyoto Univ*. 29:437–448.
- Kirby KJ. 2001. The impact of deer on the ground flora of British broadleaved woodland. *Forestry*. 74(3):219–229. doi:10.1093/forestry/74.3.219.
- Kolb TE, Steiner KC, McCormick LH, Bowersox TW. 1990. Growth response of northern red-oak and yellow-poplar seedlings to light, soil nutrients and moisture in relation to ecological strategy. *For Ecol Manag*. 38(1–2):65–78. doi:10.1016/0378-1127(90)90086-Q.
- Kumbasli M, Makineci E, Cakir M. 2010. Long term effects of red deer (*Cervus elaphus*) grazing on soil in a breeding area. *J Environ Biol*. 31 (1–2):185–188.
- McInnes PF, Naiman RJ, Pastor J, Cohen Y. 1992. Effects of moose browsing on vegetation and litter of the boreal forest, Isle Royale, Michigan, USA. *Ecology*. 73:2059–2075. doi:10.2307/1941455.
- Michelot A, Bréda N, Damesin C, Dufrêne E. 2012. Differing growth responses to climatic variations and soil water deficits of *Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris* in a temperate forest. *For Ecol Manag*. 265:161–171. doi:10.1016/j.foreco.2011.10.024.
- Miyaki M, Kaji K. 2009a. Shift to litterfall as year-round forage for Sika deer after a population crash. In: McCullough DR, Takatsuki S, Kaji K, editors. *Sika deer: biology and management of native and introduced populations*. Tokyo: Springer; p. 171–180.
- Miyaki M, Kaji K. 2009b. The dynamics of forest stands affected by sika deer on Nakanoshima Island - change of size structure similar to the thinning effect. In: McCullough DR, Takatsuki S, Kaji K, editors. *Sika deer: biology and management of native and introduced populations*. Tokyo: Springer; p. 181–191.
- Nakajima A, Koike S, Masaki T, Shimada T, Kozakai C, Nemoto Y, Yamazaki K, Kaji K. 2012. Spatial and elevational variation in fruiting phenology of a deciduous oak (*Quercus crispula*) and its effect on foraging behavior of Asiatic black bear (*Ursus thibetanus*). *Ecol Res*. 27(3):529–538. doi:10.1007/s11284-011-0919-z.
- Obeso JR. 2002. The cost of reproduction. *New Phytologist*. 155 (3):321–348. doi:10.1046/j.1469-8137.2002.00477.x.
- Odani J. 2013. Estimating the acorn crops from the number of fallen male inflorescences of three Fagaceae species (*Fagus crenata*, *Quercus crispula*, and *Q. serrata*). *J Jpn For Soc*. 95(1):45–50. Japanese. doi:10.4005/jjfs.95.45.
- Ohashi H, Kadota Y, Murata Y, Yonokura K, Kihara H. editors. 2016. *Wild flowers of Japan: Rosaceae-Meliaceae*. Tokyo: Heibonsha. Japanese.
- Pacala SW, Canham CD, Silander JAJ, Kobe RK. 1994. Sapling growth as a function of resources in a north temperate forest. *Can J For Res*. 24(11):2172–2183. doi:10.1139/x94-280.
- Poorter, H, Niinemets, U, Poorter, L, Wright, IJ, Villar, R 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol*. 182:565–588. doi:10.1111/j.1469-8137.2009.02830.x
- R Core Team. 2018. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.
- Reich PB, Grigal DF, Aber JD, Gower ST. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology*. 78(2):335–347. doi:10.1890/0012-9658(1997)078[0335:NMAPIH]2.0.CO;2.
- Rooney TP. 2001. Deer impacts on forest ecosystems: a North American perspective. *Forestry*. 74(3):201–208. doi:10.1093/forestry/74.3.201.
- Sabo AE, Frerker KL, Waller DM, Kruger EL. 2017. Deer-mediated changes in environment compound the direct impacts of herbivory on understory plant communities. *Journal of Ecology*. 105 (5):1386–1398. doi:10.1111/1365-2745.12748.
- Sakaguchi S, Fujiki D, Inoue M, Yamasaki R, Fukushima K, Takayanagi A. 2012. The effects of deer browsing on forest regeneration processes and diversity of tree communities in a mountainous region with heavy snowfall of Central Japan. *For Res Kyoto*. 78:57–69.
- Sakai M, Natuhara Y, Imanishi A, Imai K, Kato M. 2012. Indirect effects of excessive deer browsing through understory vegetation on stream insect assemblages. *Popul Ecol*. 54(1):65–74. doi:10.1007/s10144-011-0278-1.
- Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 9(7):671–675. doi:10.1038/nmeth.2089.
- Schrama M, Heijning P, Bakker JP, van Wijnen HJ, Berg MP, Olff H. 2013. Herbivore trampling as an alternative pathway for explaining differences in nitrogen mineralization in moist grasslands. *Oecologia*. 172(1):231–243. doi:10.1007/s00442-012-2484-8.

- Sefcik LY, Zak DR, Ellsworth DS. 2007. Seedling survival in a northern temperate forest understory is increased by elevated atmospheric carbon dioxide and atmospheric nitrogen deposition. *Glob Change Biol.* 13(1):132–146. doi:[10.1111/j.1365-2486.2006.01293.x](https://doi.org/10.1111/j.1365-2486.2006.01293.x).
- Stewart GH, Burrows LE. 1989. The impact of white-tailed deer *Odocoileus virginianus* on regeneration in the coastal forests of Stewart Island, New Zealand. *Biol Conser.* 49(4):275–293. doi:[10.1016/0006-3207\(89\)90048-7](https://doi.org/10.1016/0006-3207(89)90048-7).
- Suda K, Araki R, Maruyama N. 2001. The effects of sika deer on the structure and composition of the forests on the Tsushima Islands. *Biosph Conser.* 4(1):13–22.
- Tahtinen B, Murray BD, Webster CR, Tarasoff CS, Burton AJ. 2013. Does ungulates foraging behavior in forest canopy gaps produce a spatial subsidy with cascading effects on vegetation? *Forest Science.* 60(5):819–829. doi:[10.5849/forsci.13-080](https://doi.org/10.5849/forsci.13-080).
- Takatsuki S. 2009. Effects of sika deer on vegetation in Japan: a review. *Biol Conser.* 142(9):1922–1929. doi:[10.1016/j.biocon.2009.02.011](https://doi.org/10.1016/j.biocon.2009.02.011).
- Tanaka Y, Takatsuki S, Takayanagi A. 2008. Decline of *Sasa palmata* community by grazing of sika deer (*Cervus nippon*) at Ashiu Research Forest Station [Kyoto, Japan]. *For Res Kyoto Univ.* 77:13–23.
- Tateno R, Kawaguchi H. 2002. Differences in nitrogen use efficiency between leaves from canopy and subcanopy trees. *Ecol Res.* 17(6):695–704. doi:[10.1046/j.1440-1703.2002.00526.x](https://doi.org/10.1046/j.1440-1703.2002.00526.x).
- Tateno R, Takeda H. 2003. Forest structure and tree species distribution in relation to topography-mediated heterogeneity of soil nitrogen and light at the forest floor. *Ecol Res.* 18(5):559–571. doi:[10.1046/j.1440-1703.2003.00578.x](https://doi.org/10.1046/j.1440-1703.2003.00578.x).
- Wainhouse D, Ashburner R, Ward E, Rose J. 1998. The effect of variation in light and nitrogen on growth and defense in young Sitka Spruce. *Funct Ecol.* 12(4):561–572. doi:[10.1046/j.1365-2435.1998.00232.x](https://doi.org/10.1046/j.1365-2435.1998.00232.x).
- Wang L, Mou PP, Huang J, Wang J. 2007. Spatial heterogeneity of soil nitrogen in a subtropical forest in China. *Plant Soil.* 295(1–2):137–150. doi:[10.1007/s11104-007-9271-z](https://doi.org/10.1007/s11104-007-9271-z).
- Wardle DA, Bonner KI, Barker GM. 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Funct Ecol.* 16(5):585–595. doi:[10.1046/j.1365-2435.2002.00659.x](https://doi.org/10.1046/j.1365-2435.2002.00659.x).
- White MA. 2012. Long-term effects of deer browsing: composition, structure and productivity in a northeastern Minnesota old-growth forest. *For Ecol Manage.* 269:222–228. doi:[10.1016/j.foreco.2011.12.043](https://doi.org/10.1016/j.foreco.2011.12.043).
- Yasuda S, Nagamasu H. 1995. Flora of Ashiu, Japan. *Contr Biol Lab Kyoto Univ.* 28:367–486.