

論文 (Original article)

Stable nitrogen and carbon isotope ratios and related leaf properties of four tree species at high and low nitrogen-deposition sites in the Kanto district of Japan

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Abstract

We compared the leaf properties of four tree species (Japanese cedar, hinoki cypress, and two deciduous hardwood species) at sites in Tsukuba (a high nitrogen-deposition area) and Katsura (a low nitrogen-deposition area) in the Kanto district of Japan. Nitrogen concentration in fresh leaves of Japanese cedar did not differ between the two sites, whereas those for the other three species were higher at Tsukuba than at Katsura. Leaf mass per area of hinoki cypress and the two deciduous hardwood species was lower at Tsukuba than at Katsura, but the effects on leaf nitrogen content per area varied among tree species. The nitrogen resorption efficiency of Japanese cedar was lower at Tsukuba than at Katsura, but there was no clear difference in the other three species. The nitrogen isotope ratio in all species was higher at Tsukuba than at Katsura, but the carbon isotope ratio did not differ between the two sites. These results suggested that the effects of nitrogen deposition on leaf properties varied among tree species and that Japanese cedar at Tsukuba was in a more nitrogen-saturated condition than were the other three species.

Key words : Japanese cedar, hinoki cypress, nitrogen saturation, stable isotopes, leaf traits

1. Introduction

Recently, nitrogen deposition by precipitation or dry deposition in forest ecosystems has been increasing because of human activity. When the supply of ammonium and nitrate exceeds plant and microbial demand, the presence of excess nitrogen may result in higher soil nitrification rates, soil acidification, greater nitrogen loss in stream waters, and a decline in forest productivity. These conditions are considered to constitute nitrogen saturation, which has been examined in many studies in Europe and North America (Aber et al. 1989, Gundersen et al. 2006). In Japan, several studies have reported high rates of nitrogen loss in the stream waters in forests along the periphery of the Kanto Plain; this is symptomatic of nitrogen saturation (Mitchell et al. 1997, Ohrui et al. 1997, Itoh et al. 2004, Yoshinaga et al. 2012).

The effects of nitrogen deposition on trees differ among tree species (Gundersen et al. 2006), and the effects need to be clarified for each of the major tree species in a region. Nitrogen use by trees can be evaluated by examining leaf properties. Fresh-leaf nitrogen concentration is used as an index of photosynthetic ability (Evans 1989). Leaf-litter nitrogen concentration is used as an index of nitrogen use efficiency (Vitousek 1982) or nitrogen resorption proficiency (Killingbeck 1996). The natural abundance of nitrogen isotope in leaves ($\delta^{15}\text{N}$) reflects soil nitrogen availability (Craine et al. 2009), nitrogen deposition (Pardo et al. 2006, Fang et al. 2013), and

the form of nitrogen utilized by plants (Takebayashi et al. 2010). Therefore, leaf nitrogen isotope ratio would be a possible indicator of nitrogen saturation of trees.

Decline of Japanese cedar trees is sometimes observed on the Kanto plain (Sakata et al. 1996, Sase et al. 1998). This decline is likely related to changes in water use associated with dry deposition or decreasing humidity (Sase et al. 1998). Nitrogen deposition can also be a factor affecting water use by trees. In one experiment, nitrogen addition increased transpiration by Japanese cedar seedlings (Nagakura et al. 2008). Information on tree water use is required to evaluate the effects of nitrogen deposition in the Kanto district. The carbon isotope ratio ($\delta^{13}\text{C}$) is used as an index of water use efficiency, because the leaf carbon isotope ratio is a function of both the supply of CO_2 to sites of carbon fixation and photosynthetic capacity (i.e. chloroplast demand for CO_2) (Farquhar et al. 1982). A high $\delta^{13}\text{C}$ in leaves indicates a high water-use efficiency (i.e. a high ratio of assimilation to conductance).

Here, we compared the leaf properties of tree species in two areas of Ibaraki Prefecture in central Japan. The Tsukuba study site receives high levels of nitrogen deposition, whereas the Katsura study site receives moderate levels of nitrogen deposition. We compared the leaf properties of four tree species to determine species-specific response of water and nitrogen utilization to atmospheric nitrogen deposition.

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2. Materials and methods

2.1 Study sites

The study was conducted in the Tsukuba and Katsura areas of Ibaraki Prefecture. The Tsukuba site was located in the Tsukuba Forest Experimental Watershed of the Forestry and Forest Products Research Institute on the periphery of the Kanto Plain, about 60 km northeast of Metropolitan Tokyo (N36°10', E140°10', 320 to 390 m in altitude). The mean annual temperature from 1980 to 2010 is 13.8 °C at the nearest weather station of Japan Meteorological Agency (Tsukuba station: N36°3', E140°8', 25 m in altitude) and the annual precipitation from 1979 to 1990 at the study site was 1430 mm (Kabeya et al. 2014). The Katsura site was located in the Katsura Headwater Catchment of the Forestry and Forest Products Research Institute, about 120 km northeast of Tokyo (N 36°32', E 140°18', 210 to 270 m in altitude). The mean annual temperature from 1980 to 2010 is 12.7 °C and the annual precipitation is 1340 mm at the nearest weather station of Japan Meteorological Agency (Hitachiomiya station: N36°36', E140°20', 95 m in altitude). The soil parent material is a volcanic ash over biotite gneiss at Tsukuba and over Mesozoic shale and sandstone at Katsura. The soils in the two areas are classified as brown forest soils (Forest Soil Division 1976) and are classified as Fulvudand or Dystrudept, depending on the influence of the volcanic ash material at the site in US Soil Taxonomy (Soil Survey Staff 2010).

The two sites receive different nitrogen depositions. Annual nitrogen input by throughfall in Japanese cedar plantations is 20.0 kg N ha⁻¹ year⁻¹ at Tsukuba and 8.8 kg N ha⁻¹ year⁻¹ at Katsura (Yoshinaga et al. 2012). Nitrogen loss in stream nitrates from watersheds including Japanese cedar plantation was 11.0 kg N ha⁻¹ year⁻¹ in the Tsukuba area and 1.9 kg N ha⁻¹ year⁻¹ in the Katsura area (Kobayashi et al. 2011). Rates of nitrogen deposition and nitrogen loss in the Tsukuba area are relatively high among Japanese forest ecosystems in Japan (Mitchell et al. 1997); the high rate of nitrogen loss indicates that forests in the area are in a nitrogen-saturated condition.

2.2 Sample collection and analysis

At each site, we selected a Japanese cedar (*Cryptomeria japonica*) plantation, a hinoki cypress (*Chamaecyparis obtusa*) plantation, and a deciduous broad-leaved forest. In the deciduous broad-leaved forest, *Quercus serrata* and *Clethra barbinervis* were selected from the canopy and sub-canopy layers of the forest, respectively. Mean diameters at breast height (DBH) are given in Table 1. The ages of the trees in the Japanese cedar and hinoki cypress plantations ranged from 34 to 55 years, but those of the hardwood species were not known (Table 1). Fresh leaves of each species were collected from five trees in August 2009. A slingshot was used to collect leaves

Table 1. Mean DBH of trees, and forest age.

	Katsura		Tsukuba	
	DBH (cm)	Age (yr)	DBH (cm)	Age (yr)
Hinoki cypress	21.8	34	18.9	40
Japanese cedar	26.3	43	23.8	55
<i>Quercus serrata</i>	21.1	-	17.6	-
<i>Clethra barbinervis</i>	6.4	-	4.9	-

from the Japanese cedar and hinoki cypress trees, whereas high-branch scissors were used for *Q. serrata* and *C. barbinervis*. Leaf litter of Japanese cedar and hinoki cypress was collected by using litter traps (3-8 traps). Litterfall was collected at 1- to 2- month intervals from July 2009 to June 2010. Collected samples in each plot were combined into one sample and divided into leaves and other parts. Leaf samples were analyzed for nitrogen content. Freshly fallen leaf litter of *Q. serrata* and *C. barbinervis* was collected from the forest floor in November 2009 (n=5).

Leaf area of the collected hinoki cypress, *Q. serrata*, and *C. barbinervis* samples was determined by using LIA32 software (<http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32>). Leaf area of Japanese cedar was not determined, as it was difficult to measure because of the needle shape of the leaf. The leaf samples were dried at 70 °C for 72 h and weighed. Leaf mass per area (LMA) was calculated as leaf mass divided by leaf area. The nitrogen concentration of fresh leaves and leaf litter was measured with an NC analyzer (NC 22F; Sumika Analytical Center). The carbon and nitrogen isotope ratios were measured with an on-line C and N analyzer (NC 2500; CE Instruments) coupled with an isotope ratio mass spectrometer (MAT252; Thermo Electron). Results are expressed as δ values with ‰ deviations from standard reference materials, where

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$ and appropriate standards were Pee Dee Belemnite (PDB) and atmospheric nitrogen for carbon and nitrogen, respectively.

We analyzed the differences in these parameters between the Tsukuba site (high nitrogen deposition) and the Katsura site (low nitrogen deposition) by using a *t*-test. Because the litterfall in Japanese cedar and hinoki cypress was combined into one sample in each collection, we calculated the weighted mean for the leaf-litter nitrogen concentration. Nitrogen resorption efficiency was calculated as

$$\text{Nitrogen resorption efficiency (\%)} = (N_{\text{fresh}} - N_{\text{litter}}) / N_{\text{fresh}} \times 100$$

where N_{fresh} and N_{litter} are the nitrogen concentrations in fresh leaves and leaf litter, respectively.

3. Results and Discussion

3.1 Leaf nitrogen concentration and nitrogen resorption

The nitrogen concentration in the fresh leaves of Japanese cedar did not differ between the two sites ($P = 0.17$, Table 2), whereas those in the other three species were higher at Tsukuba (high nitrogen deposition) than at Katsura (low nitrogen deposition) ($P < 0.05$). LMA was not determined for Japanese cedar but LMA for other three species was lower at Tsukuba than at Katsura ($P < 0.01$). The pattern of leaf nitrogen content per area differed among the three species, namely the leaf nitrogen content per area at Tsukuba was higher in hinoki cypress ($P < 0.01$) and lower in *Q. serrata* ($P < 0.02$), whereas there was no clear difference in the case of *C. barbinervis* ($P = 0.09$). The leaf-litter nitrogen concentration of the three species was higher at Tsukuba than at Katsura (Table 3). The nitrogen resorption efficiency of Japanese cedar was lower than those of the other three species. Moreover, the nitrogen resorption efficiency of Japanese cedar was lower at Tsukuba (5.5%) than at Katsura (14.7%).

A previous study reported that the mean (range) nitrogen concentration in fresh leaves of hinoki cypress in Kinki and Shikoku districts was 10.3 (7.4 to 13.7) mg g⁻¹ (Inagaki et al. 2011a). We found that the nitrogen concentration in fresh leaves of hinoki cypress was relatively high at Tsukuba (12.7 mg g⁻¹) and moderate at Katsura (9.4 mg g⁻¹). A previous study reported that the mean nitrogen concentration in leaf litter of hinoki cypress forests in Kanto, Kinki and Shikoku districts was 8.1 (5.7 to 10.9) mg g⁻¹ (Inagaki et al. 2010). The nitrogen concentration in the leaf litter of hinoki cypress was moderate at Tsukuba (9.0 mg g⁻¹) and relatively low at Katsura (5.7 mg g⁻¹). A previous study reported that the mean nitrogen resorption

efficiency of hinoki cypress in Kinki and Shikoku districts was 32% (5% to 46%) (Inagaki et al. 2010). Nitrogen resorption efficiency of hinoki cypress in this study was moderate at Tsukuba (29%) and relatively high at Katsura (39%). Thus the rate of nitrogen cycling in hinoki cypress forests was low at Katsura and moderate at Tsukuba. If the hinoki cypress trees were nitrogen saturated, then one would expect high nitrogen concentrations in the leaf litter and low nitrogen resorption efficiency. However, the leaf-litter nitrogen concentrations and nitrogen resorption efficiency in the hinoki cypress at Tsukuba were moderate and not indicative of nitrogen saturation. The fact that leaf-litter production in the Tsukuba hinoki cypress forest was very high compared with that in other hinoki cypress forests in Japan (Inagaki et al. 2012) likely indicated that there was a high rate of leaf production in response to high levels of nitrogen deposition. Stem production of hinoki cypress plantation at Tsukuba was larger than that of Japanese cedar plantations at Tsukuba and Katsura (Inagaki et al. 2011b, 2012). These findings suggest that nitrogen deposition promotes forest growth in hinoki cypress and that the degree of nitrogen saturation of the hinoki forest was not extreme.

In the case of Japanese cedar, a previous study reported that the mean nitrogen concentration in fresh leaves was 14.0 mg g⁻¹ (Shigenaga et al. 2008). The nitrogen concentrations in fresh leaves in our study were relatively low (7.5 mg g⁻¹ at Katsura and 8.7 mg g⁻¹ at Tsukuba). A previous study reported that the mean of nitrogen concentration in leaf litter was 8.1 (5.3 to 12.7) mg g⁻¹ (Inagaki et al. 2012). The leaf-litter nitrogen concentration in Japanese cedar in our study was low at Katsura (6.4 mg g⁻¹) and moderate at Tsukuba (8.2 mg g⁻¹) compared with reported values. The nitrogen resorption efficiency of

Table 2. Nitrogen concentrations, nitrogen content per area, and leaf mass per area (LMA) in four tree species at Katsura and Tsukuba.

	Katsura(a)		Tsukuba(b)		(b)/(a)	<i>P</i> (<i>t</i> -test)
	Mean	(SD)	Mean	(SD)		
Leaf N concentration (mg g ⁻¹)						
Hinoki cypress	9.4	(0.7)	12.7	(1.5)	1.36	0.01
Japanese cedar	7.5	(1.4)	8.7	(1.1)	1.16	0.17
<i>Quercus serrata</i>	19.8	(0.9)	22.6	(1.9)	1.14	0.03
<i>Clethra barbinervis</i>	16.2	(1.8)	21.7	(1.2)	1.34	0.01
Leaf mass per area (g m ⁻²)						
Hinoki cypress	250.9	(11.3)	210.1	(17.7)	0.84	0.01
Japanese cedar	-		-			
<i>Quercus serrata</i>	70.7	(5.6)	53.1	(4.1)	0.75	0.01
<i>Clethra barbinervis</i>	72.0	(9.4)	43.9	(5.2)	0.61	0.01
Leaf N content per area (g m ⁻²)						
Hinoki cypress	2.35	(0.14)	2.65	(0.12)	1.13	0.01
Japanese cedar	-		-			
<i>Quercus serrata</i>	1.40	(0.08)	1.20	(0.11)	0.86	0.02
<i>Clethra barbinervis</i>	1.17	(0.22)	0.95	(0.10)	0.81	0.09

Table 3. Leaf-litter nitrogen concentration and nitrogen resorption efficiency in four tree species at Katsura and Tsukuba.

	Katsura(a)		Tsukuba(b)		(b)/(a)	<i>P</i> (<i>t</i> -test)
	Mean	(SD)	Mean	(SD)		
Litter N concentration (mg g ⁻¹)						
Hinoki cypress	5.7		9.0		1.58	
Japanese cedar	6.4		8.2		1.28	
<i>Quercus serrata</i>	7.3	(0.2)	8.5	(0.4)	1.17	0.01
<i>Clethra barbinervis</i>	9.2	(1.3)	13.5	(1.6)	1.47	0.01
N resorption efficiency (NRE) (%)						
Hinoki cypress	39.2		29.3		0.75	
Japanese cedar	14.7		5.5		0.37	
<i>Quercus serrata</i>	63.5	(1.4)	62.2	(3.3)	0.99	0.44
<i>Clethra barbinervis</i>	42.9	(9.7)	37.2	(9.9)	0.87	0.39

Table 4. Stable nitrogen ($\delta^{15}\text{N}$) and carbon isotope ratios ($\delta^{13}\text{C}$) in four tree species at Katsura and Tsukuba.

	Katsura(a)		Tsukuba(b)		(b) — (a)	<i>P</i> (<i>t</i> -test)
	Mean	(SD)	Mean	(SD)		
$\delta^{15}\text{N}(\text{‰})$						
Hinoki cypress	-6.4	(0.6)	-3.3	(0.4)	3.1	0.01
Japanese cedar	-4.9	(0.8)	-2.4	(0.2)	2.5	0.01
<i>Quercus serrata</i>	-5.5	(0.5)	-3.7	(1.0)	1.8	0.01
<i>Clethra barbinervis</i>	-6.6	(1.2)	-4.9	(0.6)	1.8	0.03
$\delta^{13}\text{C}(\text{‰})$						
Hinoki cypress	-27.0	(0.8)	-27.1	(1.0)	-0.1	0.90
Japanese cedar	-30.1	(0.8)	-29.6	(0.9)	0.5	0.37
<i>Quercus serrata</i>	-29.2	(1.0)	-30.2	(0.6)	-0.9	0.16
<i>Clethra barbinervis</i>	-30.3	(2.1)	-31.2	(0.6)	-0.9	0.41

Japanese cedar at the two sites (5.5 to 14.7%) was lower than that in the other three species. The mean nitrogen concentrations in previous studies were 14.0 mg g⁻¹ in fresh leaves (Shigenaga et al. 2008) and 8.1 mg g⁻¹ (Inagaki et al. 2012) in leaf litter. The nitrogen resorption efficiency calculated from these values was 42%. The nitrogen use efficiency of Japanese cedar in our study was likely lower among Japanese cedar forests. The nitrogen resorption efficiency of Japanese cedar was lower at Tsukuba than at Katsura. In contrast to our expectation, the nitrogen concentration in fresh leaves of Japanese cedar at Tsukuba was not high. The low nitrogen resorption efficiency at Tsukuba was caused mainly by the low fresh-leaf nitrogen concentration. These findings suggest that the increase in nitrogen uptake by Japanese cedar in response to nitrogen deposition is very small. A previous study has suggested that stem growth of tall trees in Japanese cedar forests in the Tsukuba site is inhibited (Inagaki et al. 2012). These results suggested that trees at Tsukuba did not show increased growth and nitrogen uptake in response to nitrogen deposition, indicating that conditions in the cedar forest were nitrogen saturated.

The nitrogen concentrations in the deciduous hardwood species in our study were similar to those in previous studies of

Q. serrata (Migita et al. 2007) and *C. barbinervis* (Nagakura et al. 2009). Nitrogen concentrations in the fresh leaves were higher at Tsukuba than at Katsura, but nitrogen content per leaf area was not higher owing to a decrease in LMA at Tsukuba (Table 3). The nitrogen use efficiency of the two species did not differ between the two sites (*P* > 0.05) (Table 3). These results suggest that, in these hardwood species, nitrogen deposition strongly decreases leaf thickness and may not increase nitrogen uptake as much as in hinoki cypress. The decrease in leaf thickness may reduce leaf production (on a mass basis) if the leaf area of a forest remains constant. To evaluate nitrogen uptake in response to nitrogen deposition, leaf production should be clarified in a future study.

3.2 Nitrogen isotope

Fresh leaf $\delta^{15}\text{N}$ of four tree species ranged from -6.6‰ to -2.4‰ (Table 4). This is within the range in previous studies of Japanese cedar and hinoki cypress (-7.1‰ to -2.1‰; Koba et al. 2003, Tateno et al. 2009, Takebayashi et al. 2010). Leaf $\delta^{15}\text{N}$ was higher at Tsukuba than at Katsura for all tree species (*P* < 0.05). This result is consistent with the report of Pardo et al. (2006), which showed a positive correlation between nitrogen

Table 5. $\delta^{15}\text{N}$ (‰) in soil at depths of 0 to 10 cm in a hinoki cypress plantation. Values are means with SE ($n=3$). Data are from the work of Hayashi (2014).

	Total N	NH_4^+	NO_3^-
Katsura	2.4(0.9)	4.4(0.5)	-2.0(0.3)
Tsukuba	1.4(0.2)	1.9(0.6)	-4.1(1.2)

deposition and leaf $\delta^{15}\text{N}$ in a large data set from the United States and Europe. On the other hand, Fang et al. (2013) reported very low leaf $\delta^{15}\text{N}$ values in heavily nitrogen-saturated forests in China. In the Kanto and Chubu districts of Japan, Takebayashi et al. (2010) found a decreasing trend in leaf $\delta^{15}\text{N}$ content in hinoki cypress with increasing nitrogen deposition. Takebayashi et al. (2010) also found that leaf $\delta^{15}\text{N}$ in a nitrogen-saturated forest was close to soil nitrate $\delta^{15}\text{N}$; they suggested preferential uptake of soil nitrate as a reason for the low leaf $\delta^{15}\text{N}$.

Soil $\delta^{15}\text{N}$ has been investigated in the hinoki cypress forests at Tsukuba and Katsura in another study by Hayashi (2014) (Table 5). The leaf $\delta^{15}\text{N}$ of hinoki cypress at Tsukuba (-3.3‰) in our study was between the soil ammonium $\delta^{15}\text{N}$ (1.9‰) and nitrate $\delta^{15}\text{N}$ (-4.1‰) in the study by Hayashi (2014). Isotope discrimination during plant uptake is generally very small when the soil inorganic nitrogen pool in forest ecosystems is small (Nadelhoffer and Fry 1994). If we assume that there is no isotope discrimination during plant uptake and that plants absorb nitrogen from the two above-mentioned sources, the contribution of each nitrogen source can be calculated as mixing ratio of the two $\delta^{15}\text{N}$ sources (Takebayashi et al. 2010). The contribution of nitrate to plant nitrogen uptake in hinoki cypress at Tsukuba is 87% and that of ammonium is 13%. This result and the findings of Takebayashi et al. (2010) suggest that hinoki cypress has a strong ability to utilize soil nitrate when nitrogen deposition rates are high.

Leaf $\delta^{15}\text{N}$ of hinoki cypress at Katsura (-6.4‰) in this study was lower than the soil ammonium $\delta^{15}\text{N}$ (4.4‰) or nitrate $\delta^{15}\text{N}$ (-2.0‰) in Hayashi (2014). Therefore, leaf $\delta^{15}\text{N}$ cannot be explained by these two nitrogen sources only. Nitrogen deposition is a possible factor in low leaf $\delta^{15}\text{N}$, as suggested by Fang et al. (2013), but its contribution would not be substantial at Katsura because nitrogen deposition in the area is low. Another possible nitrogen source at Katsura is the soil organic horizon. Accumulation of the soil organic horizon in the hinoki cypress forest at Katsura is very small (Inagaki Y, personal observation). This suggests that there is very rapid decomposition and nitrogen release from this horizon. The organic horizon should have a $\delta^{15}\text{N}$ close to that of fresh leaves; efficient uptake of nitrogen from this horizon may maintain the low $\delta^{15}\text{N}$ values in the leaves.

Therefore, from these findings, leaf $\delta^{15}\text{N}$ in the two sites cannot be explained only by the $\delta^{15}\text{N}$ of ammonium and nitrate,

but the analysis of soil nitrogen sources revealed the presence of an unknown nitrogen source. Our results suggest that leaf $\delta^{15}\text{N}$ is not a simple index of nitrogen saturation but may provide valuable information about nitrogen sources. The role of nitrogen from the organic horizon should be clarified in future research.

3.3 Carbon isotope

Leaf $\delta^{13}\text{C}$ was higher in hinoki cypress than in the other three species. There was no clear difference in leaf $\delta^{13}\text{C}$ between the two areas ($P > 0.05$) (Table 4). The lack of increase in leaf $\delta^{13}\text{C}$ at Tsukuba suggests that reduced transpiration through stomatal closure did not occur. In contrast, Sakata et al. (1996) found an increase in leaf $\delta^{13}\text{C}$ in Japanese cedar trees on the Kanto plain that were in decline. They suggested that stomatal closure caused by water stress was the primary reason for the decrease in $\delta^{13}\text{C}$ in Japanese cedar trees. However, their results differed from those of our study. There are two interpretations of this: first, low leaf $\delta^{13}\text{C}$ at Tsukuba is indicative of lower water stress at a site, and second, low leaf $\delta^{13}\text{C}$ indicates a lack of response to water stress. It is difficult to distinguish which is the case from our data. However, because a previous study showed that stem growth of tall trees was inhibited at the Tsukuba study area (Inagaki et al. 2012), lack of stomatal control could be one of the factors negatively affecting tree growth at the site. Sase et al. (1998) showed that decline in Japanese cedar is associated with a decrease in leaf wax content and an increase in transpiration due to dry deposition. Loss of stomatal control is harmful, because humidity levels in Kanto district have recently been decreasing (Sase et al. 1998). These results suggest that factors other than nitrogen deposition can negatively affect stomatal control. Future research is required to reveal the combined effects of climate change, nitrogen deposition, and other substances derived from human activities.

4. Conclusion

Analysis of leaf properties revealed species-specific responses to nitrogen deposition. For hinoki cypress, nitrogen deposition increases nitrogen uptake indicated by large nitrogen content in litterfall, and no symptoms of decline were observed. Soil $\delta^{15}\text{N}$ analysis suggested that hinoki cypress in the nitrogen-saturated area had a strong ability to absorb soil nitrate. In the case of Japanese cedar, nitrogen deposition did not increase nitrogen uptake and stem growth was reduced among the taller trees. The lack of change in leaf $\delta^{13}\text{C}$ at the nitrogen-saturated site indicated a lack of stomatal control in response to nitrogen addition or other factors. In the case of deciduous trees, nitrogen deposition did not increase leaf nitrogen content per area. These results suggest that the effects of nitrogen deposition on leaf properties vary among tree species and that the Japanese cedars at Tsukuba were in more nitrogen-saturated condition than were the other three species.

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References

- Aber, J. D., Nadelhoffer, K. J., Steudler, P. and Melillo, J. M. (1989) Nitrogen saturation in northern forest ecosystems. *Bioscience* 39, 378-386.
- Craine, J. M., Elmore, A. J., Aidar, M. P. M., Bustamante, M., Dawson, T. E., Hobbie, E. A., Kahmen, A., Mack, M. C., McLauchlan, K. K., Michelsen, A., Nardoto, G. B., Pardo, L. H., Penuelas, J., Reich, P. B., Schuur, E. A. G., Stock, W. D., Templer, P. H., Virginia, R. A., Welker, J. M. and Wright, I. J. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol.* 183, 980-992.
- Evans, J. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78, 9-19.
- Fang, Y. T., Koba, K., Yoh, M., Makabe, A. and Liu, X. Y. (2013) Patterns of foliar $\delta^{15}N$ and their control in Eastern Asian forests. *Ecol. Res.* 28, 735-748.
- Farquhar, G. D., O'Leary, M. H. and Berry, J. A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 13, 281-292.
- Forest Soil Division (1976) Classification of forest soils in Japan (1975). *Bull. Gov. For. Exp. Sta.* 280, 1-28 (in Japanese with English summary).
- Gundersen, P., Schmidt, I. K. and Raulund-Rasmussen, K. (2006) Leaching of nitrate from temperate forests - effects of air pollution and forest management. *Environ. Rev.* 14, 1-57.
- Hayashi, T. (2014) Dynamics of high and low molecular weight extractive organic nitrogen (EON) in forest soils elucidated by $\delta^{15}N$. Master Thesis. Tokyo University of Agriculture and Technology, pp. 71p (in Japanese).
- Inagaki, Y., Inagaki, M., Hashimoto, T., Kobayashi, M., Itoh, Y., Shinomiya, Y., Fujii, K., Kaneko, S. and Yoshinaga, S. (2012) Aboveground production and nitrogen utilization in nitrogen-saturated coniferous plantation forests on the periphery of the Kanto Plain. *Bull. For. For. Prod. Res. Inst.* 11, 161-173.
- Inagaki, Y., Nakanishi, A. and Fukata, H. (2011a) Soil properties and nitrogen utilization of hinoki cypress as affected by strong thinning under different climatic conditions in the Shikoku and Kinki districts in Japan. *J. For. Res.* 16, 405-413.
- Inagaki, Y., Noguchi, K., Kaneko, S., Hashimoto, T. and Miura, S. (2011b) Biomass allocation to leaves, stems and reproductive organs in Japanese cedar plantations with different stand densities. *Jpn. J. For. Environ.* 53, 23-29 (in Japanese with English summary).
- Inagaki, Y., Okuda, S., Sakai, A., Nakanishi, A., Shibata, S. and Fukata, H. (2010) Leaf-litter nitrogen concentration in hinoki cypress forests in relation to the time of leaf fall under different climatic conditions in Japan. *Ecol. Res.* 25, 429-438.
- Itoh, Y., Miura, S., Kato, M. and Yoshinaga, S. (2004) Regional distribution of nitrate concentrations in the stream water of forested watersheds in the Kanto and Chubu districts. *J. Jpn. For. Soc.* 86, 275-278 (in Japanese with English summary).
- Kabeya, N., Shimizum, A., Zhang, J. and Nobuhiro, T. (2014) Effect of Hydrograph Separation on Suspended Sediment Concentration Predictions in a Forested Headwater with Thick Soil and Weathered Gneiss Layers. *Water* 6, 1671-1684.
- Killingbeck, K. T. (1996) Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. *Ecology* 77, 1716-1727.
- Koba, K., Hirobe, M., Koyama, L., Kohzu, A., Tokuchi, N., Nadelhoffer, K., Wada, E. and Takeda, H. (2003) Natural ^{15}N abundance of plants and soil N in a temperate coniferous forest. *Ecosystems* 6, 457-469.
- Kobayashi, M., Yoshinaga, S., Itoh, Y., Tsuboyama, Y., Tamai, K., Kabeya, N. and Shimizu, T. (2011) Nitrogen leaching from two forested watershed in Ibaraki, Japan. Abstract of Japan Geoscience Union, AHW026-12: (in Japanese).
- Migita, C., Chiba, Y. and Tange, T. (2007) Seasonal and spatial variations in leaf nitrogen content and resorption in a *Quercus serrata* canopy. *Tree Physiol.* 27, 63-70.
- Mitchell, M. J., Iwatsubo, G., Ohnui, K. and Nakagawa, Y. (1997) Nitrogen saturation in Japanese forests: an evaluation. *For. Ecol. Manag.* 97, 39-51.
- Nadelhoffer, K. J. and Fry, B. (1994) Nitrogen isotope studies in forest ecosystems. In: Lajtha, K. and Michener, R.H. (eds) *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Oxford, 22-44.
- Nagakura, J., Kaneko, S., Akama, A. and Shigenaga, H. (2009) Nutrient concentrations in leaves of 39 hardwood species at the summer and the fall. *Kanto For. Res.* 60, 195-198 (in Japanese with English summary).
- Nagakura, J., Kaneko, S., Takahashi, M. and Tange, T. (2008)

- Nitrogen promotes water consumption in seedlings of *Cryptomeria japonica* but not in *Chamaecyparis obtusa*. For. Ecol. Manag. 255, 2533-2541.
- Ohrui, K. and Mitchell, M. J. (1997) Nitrogen saturation in Japanese forested watersheds. Ecol. Appl. 7, 391-401.
- Pardo, L. H., Templer, P. H., Goodale, C. L., Duke, S., Groffman, P. M., Adams, M. B., Boeckx, P., Boggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjonaas, J., Lovett, G., Mack, M., Magill, A., Mbila, M., Mitchell, M. J., McGee, G., McNulty, S., Nadelhoffer, K., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schleppi, P., Spoelstra, J. and Wessel, W. (2006) Regional assessment of N saturation using foliar and root $\delta^{15}\text{N}$. Biogeochemistry 80, 143-171.
- Sakata, M. (1996) Evaluation of possible causes for the decline of Japanese cedar (*Cryptomeria japonica*) based on elemental composition and $\delta^{13}\text{C}$ of needles. Environ. Sci. Technol. 30, 2376-2381.
- Sase, H., Takamatsu, T., Yoshida, T. and Inubushi, K. (1998) Changes in properties of epicuticular wax and the related water loss in Japanese cedar (*Cryptomeria japonica*) affected by anthropogenic environmental factors. Can. J. For. Res. 28, 546-556.
- Shigenaga, H., Takahashi, M., Nagakura, J. and Akama, A. (2008) Spatial variations in needle nitrogen content in sugi (*Cryptomeria japonica* D. Don) plantation across Japan. J. Jpn. For. Soc. 90, 182-189.
- Soil Survey Staff (2010) Keys to Soil Taxonomy, 11th ed., Natural Resources Conservation Service USDA, Washington DC.
- Takebayashi, Y., Koba, K., Sasaki, Y., Fang, Y. and Yoh, M. (2010) The natural abundance of ^{15}N in plant and soil-available N indicates a shift of main plant N resources to NO_3^- from NH_4^+ along the N leaching gradient. Rapid Commun. Mass Spectrom. 24, 1001-1008.
- Tateno, R., Fukushima, K., Fujimaki, R., Shimamura, T., Ohgi, M., Arai, H., Ohte, N., Tokuchi, N. and Yoshioka, T. (2009) Biomass allocation and nitrogen limitation in a *Cryptomeria japonica* plantation chronosequence. J. For. Res. 14, 276-285.
- Vitousek, P. (1982) Nutrient cycling and nutrient use efficiency. Am. Nat. 119, 553-572.
- Yoshinaga, S., Itoh, Y., Aizawa, S. and Tsurita, T. (2012) Variation in nitrate concentrations in streamwater of forested watersheds in the northeastern Kanto Plain as a function of distance from the Tokyo metropolitan area. J. Jpn. For. Soc. 94, 84-91 (in Japanese with English summary).

関東地方の窒素負荷量の異なる 2 地域における 4 樹種の 窒素炭素安定同位体比と葉の性質

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要旨

4 樹種（スギ、ヒノキ、落葉広葉樹 2 種）の葉の特性を関東地域の筑波試験地（窒素負荷の多い地域）と桂試験地（窒素負荷の少ない地域）で比較した。スギでは生葉窒素濃度に差が認められなかったが、他の 3 樹種では生葉窒素濃度は筑波試験地で桂試験地よりも高かった。ヒノキと落葉広葉樹では葉面積当たりの重量は、筑波試験地で桂試験地よりも小さかった。葉面積当たりの窒素量の試験地間の傾向は樹種によって異なっていた。落葉前の窒素引き戻し率は、スギではつくば試験地で桂試験地よりも低い、他の 3 樹種では試験地間で差は認められなかった。4 樹種の葉の窒素同位体比は筑波試験地で桂試験地よりも高かったが、葉の炭素安定同位体比は試験地間で差は認められなかった。これらの結果より、窒素負荷が葉の性質に及ぼす影響は樹種によって異なっており、筑波試験地のスギは他の 3 樹種よりも窒素飽和の進んだ状態にあることが示唆された。

キーワード：スギ、ヒノキ、窒素飽和、安定同位体比、葉の特性

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