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Growth, Annual Ring Structure and Nutrient Status of Japanese Red Pine and Japanese Cedar Seedlings After Three Years of Excessive N Load

By

T. NAKAJI¹⁾, T. YONEKURA²⁾, M. KUROHA³⁾, S. TAKENAGA³⁾ & T. IZUTA³⁾

Key words: Japanese red pine, Japanese cedar, nitrogen, stress sensitivity.

Summary

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An increase in nitrogen (N) deposition from the atmosphere has been one of the major environmental stresses affecting the net primary production and nutritional status of forest ecosystems. In this study, we experimentally investigated the responses of two representative Japanese coniferous tree species, Japanese cedar (*Cryptomeria japonica*) and Japanese red pine (*Pinus densiflora*), to rising N load during three growing seasons from April 1999 to March 2002. One-year-old seedlings were planted in potted brown forest soil, and treated with five levels of N supply (0, 25, 50, 100 and 300 mg N l⁻¹ fresh soil volume) in April 1999. During the three-year experimental period, N supply to the soil caused soil acidification and leaching of Mn in the soil solution. As for Japanese cedar, the whole-plant dry mass was significantly increased by the higher two N treatments from the end of the second growing season, accompanied with accelerated tree ring growth. The needle Mg concentration of Japanese cedar was also increased by the N treatment. On the other hand, in Japanese red pine seedlings, the two higher N treatments reduced the whole-plant dry mass and needle Mg concentration from the end of the second growing season. The ratio of whole-plant dry mass of N-supplied pine seedlings to the non-treated seedlings negatively correlated with concentration ratios of N/P, N/Mg and Mn/Mg in the needles. No clear relationship was found in the case of Japanese cedar seedlings. We concluded that Japanese red pine has lower tolerance to excessive N input than Japanese cedar, even three years after the experimental N load. The concentration ratios of N/P, N/Mg and Mg/Mn in the needle leaves have potential for use as indicators for evaluating the negative effects of rising N load on the growth of sensitive Japanese coniferous tree species such as Japanese red pine.

¹⁾ Center for Global Environmental Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan, e-mail: nakaji.tatsuro@nies.go.jp

²⁾ Center for Environmental Science in Saitama, 914 Kamitanadare, Saitama 347-0115, Japan.

³⁾ Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan.

Introduction

An increase of N deposition from the atmosphere is affecting forest ecosystems, and becoming one of the environmental stresses. In general, N acts as a fertilizer for tree growth when the amount of N input to forest ecosystems is optimal. In some forests in the Northern hemisphere, however, current increased atmospheric N deposition induces over-nutrition with N, described as 'nitrogen saturation'. Nitrogen saturation usually reduces tree health accompanied with many N-induced processes, such as the accelerated soil acidification, imbalances of foliar nutrients and increased sensitivity to other environmental stresses such as frost, disease and drought (e.g. NIHLGÅRD 1985, ABER & al. 1989, SCHULZE 1989). In European forest ecosystems, the thresholds of N deposition for the appearance of N-saturation and critical N load for the protection of tree health have been proposed as being approximately $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ and 10 to $15 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (WRIGHT & al. 1995, WHO 2000), respectively. Furthermore, in European tree species, criteria based on the tree's responses to increasing N load such as foliar element concentrations of N, P and Mg and their balances (*i.e.* N/P ratio and N/Mg ratio) have been proposed as useful indicators for evaluating forest tree health (DE VRIES & al. 2000).

In Japan, wet N deposition ($\text{NH}_4^+ + \text{NO}_3^-$) to ground surface has been observed at the range from 4 to $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ during the past few decades (HARA 1992), and N deposition ranging from 5 to $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ has been observed in many rural Japanese forest ecosystems (IWATSUBO & al. 1997, OHTÉ & al. 2001). Some field studies have revealed that N saturation has already appeared in suburban forests around Tokyo Metropolis, accompanied by relatively high N deposition over $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (MITCHELL & al. 1997, BABA & OKAZAKI 1998). However, although the effects of 'N fertilizer' on the productivity and nutrient status of Japanese forest tree species have been investigated since 1960s (e.g. TSUTSUMI 1962, KAWADA 1968), information on the influences of N-overload is limited at the present time. Furthermore, although some experimental studies have revealed the short-term responses of Japanese forest tree species to increasing N load (NAKAJI & al. 2001), their long-term responses have not been clarified yet.

To discuss the N sensitivity and new criteria for the health of Japanese coniferous tree species under high N load, in this study, we investigated the effects of varied N load on the growth and nutrient status of two representative Japanese coniferous tree species, Japanese cedar (*Cryptomeria japonica*) and Japanese red pine (*Pinus densiflora*), over three growing seasons.

Material and Methods

One-year-old seedlings of Japanese cedar (*Cryptomeria japonica* D. Don.) and Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) were transplanted into plastic pots containing 7.2 liters of brown forest soil, and grown under field conditions at the experimental field in Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan) for three growing seasons from 25 April 1999 to 17 March 2002. The annual average of air temperature in 1999, 2000 and 2001 were 15.8°C , 15.6°C and 15.2°C , respectively. From 25 May 1999 to 10 June 1999, N was added as $25 \text{ mM NH}_4\text{NO}_3$ solution to the potted soil surface at 0 (N0), 25 (N25), 50 (N50), 100 (N100) and 300

(N300) mg N l⁻¹ fresh soil volume. These N amounts added to the soil were equivalent to 0, 28, 57, 113 and 340 kg N ha⁻¹ on the basis of soil surface area, respectively. The stem base diameter and whole-plant dry mass obtained at the beginning of the experiment were 3.0±0.4 mm and 1.09±0.12 g in Japanese cedar seedlings, and 3.2±0.4 mm and 1.38±0.17 g in Japanese red pine seedlings, respectively. The seedlings were irrigated using deionized water as necessary during the experimental period. To avoid N input from natural precipitation, all the seedlings were covered with a transparent polyvinylchloride roof on rainy and snowy days. The mean transmittance of polyvinylchloride roof was 89 % at wavelength from 400 to 700 nm.

Soil solutions of the potted soil were collected 6 times (July and October in 1999, 2000 and 2001) using a soil moisture sampler (Eijkelkamp Co., The Netherlands). The pH and concentrations of inorganic N (NH₄⁺ and NO₃⁻) were determined using a pH meter (M-12, Horiba Co., Japan) and ion chromatography (IC200, Yokogawa Co., Japan), respectively. The concentrations of P, K, Ca, Mg and Mn in the soil solution were analysed by using ICP (JY48P, Seiko Instruments Inc., Japan).

Harvesting of seedlings was performed 3 times during the experiment: on 25 October 1999, 25 October 2000 and 16 March 2002. The harvested seedlings were washed with deionized water, and separated into needles, stem and roots. A trunk sample was collected for tree ring analysis at the final harvest. All the plant organs except the trunk sample were dried at 60 °C for 1 week and then weighed. The stem gravity was calculated as total stem dry mass divided by total stem volume. Dried needles were pooled for each seedlings and ground to a fine powder using a vibrating sample mill (TI-100, Heiko Co., Japan). Total N concentration in the needle sample was determined by the combustion method using a CHN analyser (EA1108, Fisons Ins., Italy). Concentrations of P, K, Ca, Mg, Mn and Al were determined with ICP (ICAP-750, Nippon-Jarrel-Ash, Japan) after digestion of the needle powder by HNO₃ and H₂O₂. For the tree ring analysis, a small segment (approx. 1 cm long) was cut from the trunk at 3 cm above the ground and fixed in a solution of formaldehyde-acetic acid-ethanol-water (1:1:9:9, v/v). Fixed trunk samples were dehydrated through a graded ethanol series and embedded in celloidin. Transverse sections with 20 µm thick were cut using a sliding microtome (LS-113, Yamato-Kohki, Japan) and stained with a 1% aqueous solution of safranin. The width of the annual ring formed during the experimental period was observed using a light microscope (TE-300, Nikon, Japan). The number of resin canals was counted in the trunks of the pine seedlings.

The Kruskal-Wallis test was used to test the effects of N treatment on the chemical property of soil solution. Analysis of variance (ANOVA) was used to test the effects of N treatment on the growth and nutrient parameters of Japanese cedar and Japanese red pine seedlings. Significant differences ($p < 0.05$) in dry mass, tree ring width and element concentrations in the needles among the five N treatments were determined by Tukey's HSD test.

Results and Discussion

As shown in Table 1, the addition of NH₄NO₃ to brown forest soil reduced soil solution pH in both tree species, but in no case to a value less than pH 4.0 which might induce growth inhibition of Japanese cedar and Japanese red pine seedlings (MIWA & al. 1998, LEE & al. 1998). Although the both concentrations of NO₃⁻ and NH₄⁺ in the soil solution significantly increased as a result of N treatment of the soil, NH₄⁺ concentration was lower than NO₃⁻ concentration. This suggests that the N addition by the form of NH₄NO₃ stimulated the dominant uptake of NH₄⁺ rather than NO₃⁻ by these coniferous seedlings (SANADA & TSUTSUMI 1978) and the nitrification by the nitrobacteria. The concentrations of P and cations (K, Ca, Mg and Mn) tended to be increased by N treatment, and significant increases were observed on the Mn concentration in the soils of both tree species and concentrations of P, Ca and Mg in the soil in

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which the pine seedlings were growing.

Table 1. Chemical properties of soil solution. Each value is the average value of all measurements. The significance of treatment effect is shown as an asterisk (Kruskal-Wallis test; * $p < 0.05$, ** $p < 0.01$, NS not significant).

	Japanese cedar						Japanese red pine					
	N 0	N25	N50	N100	N300		N 0	N25	N50	N100	N300	
pH	5.1	5.1	4.9	4.6	4.2	*	5.1	5.0	4.9	4.8	4.1	**
NH ₄ ⁺ (μM)	0.3	0.4	0.5	2.3	34.2	*	0.5	0.7	2.5	15.8	65.4	*
NO ₃ ⁻ (mM)	2.6	1.8	5.1	6.1	15.9	*	5.1	5.8	6.4	7.2	18.8	**
P (μM)	1.1	1.4	1.0	1.4	2.7	NS	0.8	0.8	1.0	1.1	2.5	**
K (mM)	0.12	0.09	0.13	0.10	0.16	NS	0.06	0.11	0.12	0.07	0.11	NS
Ca (mM)	1.49	2.24	2.25	2.87	4.74	NS	0.99	0.74	1.48	1.98	5.77	**
Mg (mM)	0.35	0.48	0.48	0.56	0.83	NS	0.25	0.19	0.37	0.44	1.07	*
Mn (μM)	7.7	31.1	24.6	42.3	150.9	*	6.9	6.2	13.0	24.2	154.9	**

In Japanese cedar seedlings, the N treatment significantly increased the whole-plant dry mass from the second growing season (Fig. 1A). A decline of the Japanese cedar has been observed mainly in the Kanto district of Japan, since early 1960s (YAMBE 1978), and as one of the possible reasons, it has been emphasized that high N deposition may reduce its drought tolerance due to reduction of the root mass relative to above-ground part mass (KOHNO 2001). However, in this study, since no significant effect of N treatment was found in the biomass of fine roots or root/shoot ratio of cedar seedlings (data not shown), the foregoing hypothesis could not be confirmed. As for Japanese red pine, the highest N treatment and two higher N treatments significantly reduced the whole-plant dry mass from the first and second growing seasons (Fig. 1B), respectively. This N-induced reduction in the whole-plant dry mass was accompanied with reduction in the dry mass of all plant organs (needles, trunk, branches, coarse roots and fine roots) without any significant change in root/shoot ratio (data not shown). In our previous study, a short-term (6 months) experiment revealed that the Japanese red pine has lower tolerance to excess N load than Japanese cedar with respect to dry matter production, because the excess N loads above 100 mg N l⁻¹ soil fresh volume induced growth inhibition in Japanese red pine seedlings (NAKAJI & al. 2001). The results of this study coincide with the previous views, even over long-term observation. As shown in Table 2, the stem diameter at the end of the third growing season increased in Japanese cedar seedlings but reduced in Japanese red pine seedlings as a result of the N treatment. The responses of annual ring widths to increasing N load also showed similar trends to the whole-plant dry mass (Table 2 and Fig. 1). No significant effect of N treatment was found in the stem gravity of either tree species or the resin canal of Japanese red pine seedlings, suggesting that increasing N load does not affect the lumber quality of these coniferous tree species.

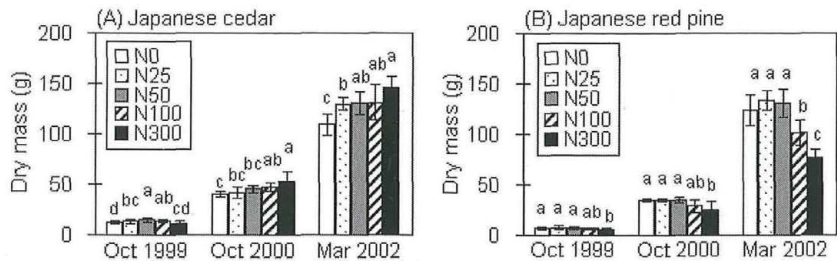


Fig. 1. Effects of N supply on the whole-plant dry mass of (A) Japanese cedar and (B) Japanese red pine seedlings. Each value is the mean (\pm s.d.) of eight seedlings. Different letters above a bar indicate significant differences among the N treatments in each observation period (Tukey's HSD test; $p < 0.05$).

Table 2. The annual ring structure of Japanese cedar and Japanese red pine seedlings grown under varied soil N levels. Each value is the mean of five seedlings. Values followed by different letters are significantly different among the N treatments (Tukey's HSD test; $p < 0.05$).

	Stem diameter (mm)	Stem gravity (g cm ⁻³)	Annual ring width (mm)			Bark width (mm)	Resin canal (cm ⁻²)
			1999	2000	2001		
Japanese cedar							
N0	10.6 b	0.50	1.26 b	0.93 b	1.16 b	1.07	—
N25	10.8 ab	0.50	1.39 ab	0.94 b	1.29 b	1.03	—
N50	10.7 ab	0.52	1.46 ab	1.01 b	1.16 b	1.03	—
N100	11.5 ab	0.51	1.70 a	0.95 b	1.26 b	1.11	—
N300	11.9 a	0.50	1.34 ab	1.36 a	1.45 a	1.11	—
Japanese red pine							
N0	13.4 ab	0.60	1.23	1.53 ab	1.91	1.21	276
N25	14.5 a	0.54	1.30	1.63 a	1.91	1.35	258
N50	14.7 a	0.54	1.40	1.58 a	2.11	1.35	287
N100	13.5 ab	0.53	1.12	1.58 a	1.77	1.35	288
N300	12.0 c	0.57	1.02	0.99 b	1.79	1.23	252

Table 3 indicates the concentrations of nutrients (N, P, K, Ca, Mg and Mn) and Al in the needles of Japanese cedar and Japanese red pine seedlings after the third growing season. In both tree species, no significant treatment effect was found in needle concentrations of P, K, Ca or Al. In the cedar seedlings, although the highest needle N concentration was observed in the seedlings grown in the highest N treatment, no significant difference was found between the N-treated seedlings (N25-N300) and non-treated seedlings (N0). Needle concentrations of Mg and Mn significantly increased by the two higher N treatments and the highest N treatment (Table 3), respectively. The Mn/Mg ratio significantly increased as a result of the highest N treatment, but the value was within the range which did not reduce the photosynthetic activity of cedar needles via excess Mn-induced inhibition of Rubisco activation (NAKAJI & al. 2001). On the

other hand, in the pine seedlings, although needle N concentration significantly increased as a result of the highest N treatment, needle Mg concentration fell due to the two higher N treatments, resulting in an increase in the needle N/Mg ratio at the highest N treatment (Table 3). Reduction of needle Mg concentration and the relatively high concentration of needle Mn also caused a significant increase of Mn/Mg ratio in the highest N treatment (Table 3). In our previous studies, increasing the soil N of Japanese red pine seedlings significantly reduced the needle concentration of P and/or Mg after one or two growing seasons, accompanied with reduced mycorrhizal infection ratio (NAKAJI & al. 2002, 2004). Although the mycorrhizal infection ratio was not investigated, reduced fine root biomass and observed poor mycorrhizal development in this study would be the main cause of reduced accumulation of Mg in pine seedlings under high N load. These results indicate that responses of nutrient status to increasing N load differ between Japanese cedar and Japanese red pine, and long-term exposure to N may induce Mg deficiency in sensitive tree species such as Japanese red pine.

Table 3. The element concentration and element ratio in the needles of Japanese cedar and Japanese red pine seedlings. Each value is the mean of five determinations. Values followed by different letters are significantly different among the N treatments (Tukey's HSD test; $p < 0.05$).

	Concentration (mg g ⁻¹ DW)							Ratio (g g ⁻¹)		
	N	P	K	Ca	Mg	Mn	Al	N/P	N/Mg	Mn/Mg
Japanese cedar										
N0	6.41 ab	0.45	3.1	9.6	1.13 b	0.079 b	0.16	14.1	5.7	0.070 b
N25	5.85 b	0.46	2.9	9.7	1.40 ab	0.069 b	0.14	12.6	4.2	0.049 b
N50	5.43 b	0.44	2.9	10.4	1.38 ab	0.084 b	0.16	12.4	3.9	0.061 b
N100	5.59 b	0.45	2.8	11.1	1.46 a	0.107 b	0.20	12.4	3.8	0.073 b
N300	7.58 a	0.47	2.5	10.4	1.40 a	0.297 a	0.23	16.3	5.4	0.213 a
Japanese red pine										
N0	13.31 b	0.67	2.1	3.7	1.30 a	0.94	0.26	19.8	10.2 b	0.723 b
N25	14.87 ab	0.72	2.1	4.0	1.16 ab	0.99	0.26	20.7	12.8 b	0.853 b
N50	13.41 ab	0.74	2.5	3.5	1.19 ab	0.98	0.27	18.2	11.3 b	0.824 b
N100	14.60 ab	0.68	2.4	3.9	0.99 b	0.90	0.27	21.5	14.7 ab	0.909 ab
N300	17.47 a	0.71	2.1	4.7	1.01 b	1.12	0.23	24.7	17.3 a	1.109 a

Fig. 2 shows the relationships between relative whole-plant dry mass of N treated seedlings to non-treated seedlings and foliar nutrient ratios (N/P, N/Mg and Mn/Mg) in the needles of Japanese cedar and Japanese red pine seedlings after three growing seasons. In the cedar seedlings, no clear relationship was found between relative dry mass and the proposed needle nutrient indices. On the other hand, although the relation was not linear, the relative whole-plant dry mass of red pine seedlings negatively correlated with the N/P, N/Mg and Mn/Mg ratios. We therefore believe the concentration ratios of N/P and N/Mg in tree leaves to be useful indices for evaluating tree health. The criteria for N/P ratio and N/Mg ratio for the optimal levels in mature European pine trees has been defined as 17 g g⁻¹ and 28.3 g g⁻¹ with other element concen-

trations and ratios (DE VRIES & al. 2000), respectively. The foliar Mn/Mg ratio, proposed by NAKAJI & al. 2001 as an indicator of the negative effects of excessive N load on the Rubisco activation state, is expected to evaluate the degree of foliar Mg deficit with excessive Mn accumulation under high N-induced soil acidification. The results of this study suggest that the ratios of N/P, N/Mg and Mn/Mg have potential for use as indices for evaluating the negative effects of increasing N load on the growth of Japanese red pine. The optimal levels of these ratios with no significant growth inhibition of Japanese red pine seedlings are lower than 21.5 (N/P), 14.7 (N/Mg) and 0.9 (Mn/Mg), respectively. Because of high N tolerance, the critical points of nutrient ratios for Japanese cedar was not defined in this experimental study. The N sensitivity of other many plant species has not yet been clarified in Japan. We conclude that criteria based on the plant responses to increasing N load should be developed to evaluate and conserve Japanese forests in a healthy state.

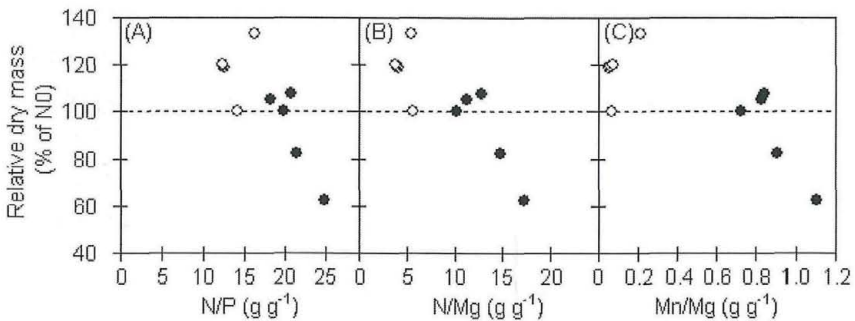


Fig. 2. The relationships between the relative whole-plant dry mass of N-treated coniferous seedlings and foliar (A) N/P ratio, (B) N/Mg ratio and (C) Mn/Mg ratio. The open circle and closed circle symbols indicate data for Japanese cedar and Japanese red pine, respectively.

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