Original research article

Changes in the carbon and nutrient status of *Cryptomeria japonica* needles and fine roots following 7 years of nitrogen addition

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Abstract: Anthropogenically increased nitrogen (N) deposition may affect the nutrient dynamics of forested ecosystems. To investigate the potential effects of excessive N deposition on Japanese forests, we treated the soil in a 20-year-old Japanese cedar (*Cryptomeria japonica*) stand with 10 l m^{-2} of 10 mM HNO_3 solution, 10 mM NH_4NO_3 solution, or tap water (as a control), monthly for 7 years. A total of 168 and 336 kg N ha^{-1} $year^{-1}$ was added in the HNO_3 and NH_4NO_3 plots, respectively. Tree growth, the amount of nutrients and the carbon concentration of both current shoots and fine roots (<2 mm in diameter) in the surface soil (0–5 cm) were measured. The foliar N concentration increased in both N-fertilized plots during the first 3 years, particularly in the NH_4NO_3 plots. Similarly, the fine-root N concentration was greater in the N-fertilized plots than in the control plots. However, growth in both height and diameter at breast height of Japanese cedar trees were not significantly affected by N fertilization. The foliar K and P concentrations tended to decrease in treatment plots over time when compared with the control plots. Our study suggests that 7 years of excessive N fertilization had no positive or negative effect on the growth of young Japanese cedar trees, although the nutrient status of current shoots and fine roots was altered.

Keywords: $\delta^{13}C$, chemical properties, height and diameter increment, Japanese cedar, nitrogen deposition, nitrogen fertilization, seasonal fluctuation

Introduction

Nitrogen (N) acts a major limiting factor for tree growth in the temperate zone (Reich et al. 1997). However, N input that exceeds the capacity of plants and soil microbes to uptake and assimilate N may have a negative effect on forested ecosystems (Aber et al. 1998). Nitrogen deposition caused by human activity has increased, and the effects of N deposition on forested ecosystems have been examined, especially in North America and Europe (Wright and Rasmussen 1998, Magill et al. 2004).

In Japan, some forested watersheds close to metropolitan areas receive abnormally high amounts of N deposition from anthropogenic sources (Yoshinaga et al. 2012). A further increase in N deposition caused by human activity is expected, especially in Asia (Liu et al. 2013), creating a need for studies related to the effects of increased N deposition on tree growth in Japanese forested ecosystems. We performed a long-term N fertilization experiment in a Japanese cedar (*Cryptomeria japonica*) stand over 7 years (Nagakura et al. 2006). That study showed that N fertilization resulted in increased concentrations of NO_3^-, Ca^{2+}, Mg^{2+} and Al in the soil solution. Therefore, the nutrient status of Japanese cedar trees is expected to change with N fertilization. The foliar nutrient concentration is often used to evaluate the nutrient status of trees, because it reflects the availability of nutrients in the soil and the effects of other environmental factors (Van den Driessche 1974).

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Nagakura et al. (2006) indicated that soil in N-fertilized plots was drier than that in control plots in early spring and summer; this may occur because an increased supply of N often results in an increased use of water by trees (Mitchell and Hinckley 1993, Nilsen 1995). In fact, an experiment using 1-year-old Japanese cedar seedlings suggested that N addition has the potential to increase water consumption of Japanese cedar (Nagakura et al. 2008). Drier soil might reflect an increase in water consumption of N-fertilized Japanese cedar trees when compared with control trees. In addition, plants growing under dry conditions generally have a high level of water use efficiency. Therefore, we measured the foliar $\delta^{13}C$ content of Japanese cedar, a measure that is often used as a short- or long-term indicator of water use efficiency (Ehleringer et al. 1993).

The objective of this study is to evaluate the changes in the nutrient status of Japanese cedar trees to 7 years of excessive N fertilization. The response of Japanese cedar, a major tree species that is planted widely in Japan, to increased N deposition is closely related to the nutrient dynamics of these forested ecosystems.

Materials and Methods

Study site

The study was conducted in a 20-year-old Japanese cedar (*Cryptomeria japonica*) plantation at the Chiyoda Experimental Station of the Forestry and Forest Products Research Institute (FFPRI), in eastern Japan (36°10′N, 140°13′E). The mean annual temperature and precipitation at a weather station operated by the Chiyoda Experimental Station from 1987–1998 were 13.8°C and 1567 mm, respectively (excluding missing data for 1989, 1992, 1994 and 1995). The mostly flat site has an elevation of 41 m a.s.l.. The Andisol soil type (Sakai et al. 2010) had a pH (0–5cm) of 5.8 and a soil C/N ratio of 17. Two years old seedlings from cuttings were planted in 1978 at a density of 3000 trees ha^{-1} (1.8 m × 1.8 m spacing); 50% of the trees were thinned in 1988.

At the beginning of the experiment in March 1997, the trees were 20 years old, at that time the stand density was 1500 trees ha^{-1}, and the mean stem diameter at breast height (DBH) and height were 20.8 cm and 14.0 m, respectively.

Nitrogen application

In March 1997, two 1.8 m × 9 m plots including three trees, spaced 3.6 m apart, were established for use in each of the three treatments: HNO_3, NH_4NO_3 and control (six plots in total; see Fig. 1 for the layout of plots compared to rows of planted trees). These plots were fertilized monthly with either 10 l m^{-2} of 10 mM HNO_3, 10 mM NH_4NO_3, or tap water (as a control), from March 1997 to March 2004. The amount of N fertilizer was 168 kg N ha^{-1} $year^{-1}$ in the HNO_3 plots, and 336 kg N ha^{-1} $year^{-1}$ in the NH_4NO_3 plots. These respective values are approximately 12- and 24-fold greater than the annual N input by precipitation in this area; the mean annual N load for 1995–1996 was 13.9 kg N ha^{-1} (Itoh and Kato 2004).

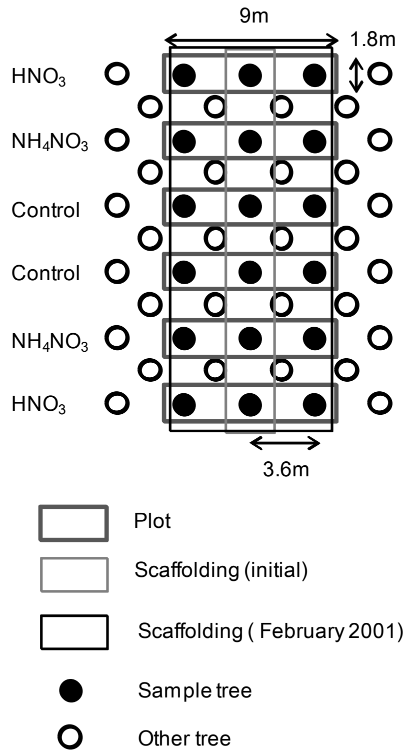

Fig. 1. Plot design at the study site.

Measuring stem increments and element status

Scaffolding was constructed in February 1997 so as to surround the central tree of each plot for access to the canopy and for accurate measurement of height growth (Fig. 1). Scaffolding was initially 12 m in height, but it was sequentially expanded upward or transversely; scaffolding reached 15 m in height in February 1998, was expanded transversely so as to surround all sample trees in February 2001, and to 20 m in height in December 2001. The height and stem diameter at breast height (DBH) were measured annually, in winter or early spring. The height was measured to the nearest cm.

Current shoots were sampled from the upper crown of each tree three times annually, in March, July, and November, from 1997 to 2003. At this site, shoot elongation of Japanese cedar began in late April, and stopped between late August and September (Shigenaga 2009). For Japanese cedar, old suppressed shoots mainly die during September and October (Tange et al. 1989). March, July and November are the months just before new shoot growth, the peak season of shoot growth, and just after mass death of old suppressed shoots, respectively. Shibamoto and Tsutsumi (1979) found that the best season for collecting samples for foliar analysis was November and December because the shoot growth has stopped and the nutrient concentration in foliage of Japanese cedar was relatively stable.

Fine roots (< 2 mm in diameter) were collected from surface soil (0–5 cm) at each treatment site (*n* = 1–2) irregularly from April 1997 to September 2002. The current shoot and fine-root samples were washed separately with deionized water. The dried samples of each were finely ground for analysis. Total N and C concentrations were measured using an NC analyzer (Sumigraph NC-22F, Sumika Chemical Analysis Service, Osaka, Japan). The Ca, Mg, K and P concentrations were measured after wet digestion by HNO_3 and $HClO_4$ (2:1) using inductivity coupled plasma optical spectrometry (ICP-OES; Optima 4300DV, PerkinElmer, Norwalk, CT, USA). The $\delta^{13}C$ of the current shoot sampled in November and March was measured using an isotope ratio mass spectrometer (Delta V, Thermo Fisher Scientific, San Jose, CA, USA) connected to an elemental analyzer (FlashEA 1112, Thermo Fisher Scientific). Foliar $\delta^{13}C$ collected in March is expected to reflect growth condition of previous year.

Statistical analysis was performed using JMP version 8.0 (SAS Institute, Cary. NC, USA). One-way analysis of variance (ANOVA) was used to assess differences among treatments and years. Significant results ($P < 0.05$) were analyzed further using Tukey's honestly significant difference test.

Results

The annual precipitation observed at the Tsuchiura Weather Station 10 km south of the study site (Japan Meteorological Agency, http://www.jma.go.jp/jma/menu/report.html) ranged from 928 to 1441 mm during the experiment, from 1997 to 2003 (Fig. 2). The annual precipitation in 1997, 1999, 2001, and 2002 was lower than the average annual precipitation from 1981 to 2010 (1190 mm). Summer precipitation (from June to September) was particularly low in 1997 and 2001.

Annual increments of tree height were not significantly different among the treatments ($P > 0.05$) from 1996 to 2003 (Fig. 3). Growth in height in 2003 was very low (Fig. 3), in part because small arthropods (perhaps spider mites) appeared on Japanese cedar needles in May 2003, not only at the experimental site

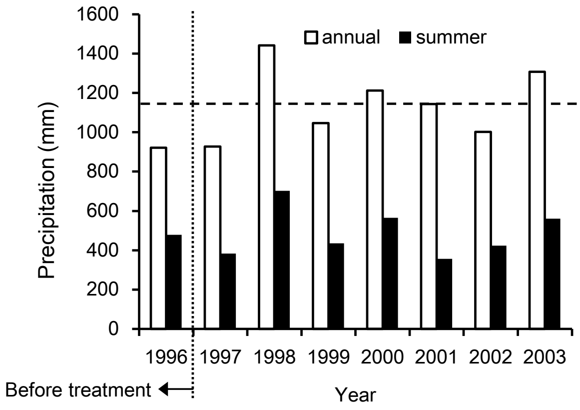

Fig. 2. Precipitation observed at the Tsuchiura Weather Station 10 km south of the study site during 1996–2003. Black columns indicate the annual precipitation, and white columns indicate the precipitation in summer (from June to September). A dashed line indicates the average annual precipitation from 1981–2010 (1190 mm).

but also in neighboring Japanese cedar stands; crowns of infected trees turned a reddish color. The annual increments in DBH were also not significantly different among the treatments ($P > 0.05$; Fig. 3).

The concentrations of foliar nutrients and carbon (C) showed similar seasonal changes, regardless of the treatments. The foliar N, potassium (K), phosphorus (P) and C concentrations decreased from July to March, although there were exceptions (July 2000 for N, July 2002 for P, July 1999 for K, and July 2002 for C; Fig. 4). The foliar magnesium (Mg) concentrations peaked in November (Fig. 4). The foliar calcium (Ca) concentrations were low in July except 1997 and 2002, and peaked in March, except in 1998 (Fig. 4).

Nitrogen fertilization resulted in higher foliar N concentrations, particularly in the NH_4NO_3 plots. The foliar N concentrations were significantly higher in the NH_4NO_3 plots than in the control plots in March 1998, 1999, 2000 and 2003 (Fig. 5) and July 1999 ($P < 0.05$).

In contrast to the foliar N concentration, N fertilization resulted in lower foliar K and P concentrations, particularly in July (Fig. 4). When compared to the control plots, foliar K and P concentrations were significantly lower in the NH_4NO_3 plots in July 1998 and 2002; the foliar P concentrations were significantly lower in the NH_4NO_3 plots also in March 2001 ($P < 0.05$).

Compared with the foliar Mg concentrations in the control plots, those in the NH_4NO_3 plots were significantly higher in March 2000 but lower in July 2002. The foliar Ca and C concentrations were not significantly affected by N fertilization.

The foliar $\delta^{13}C$ varied by year (Fig. 6). The foliar

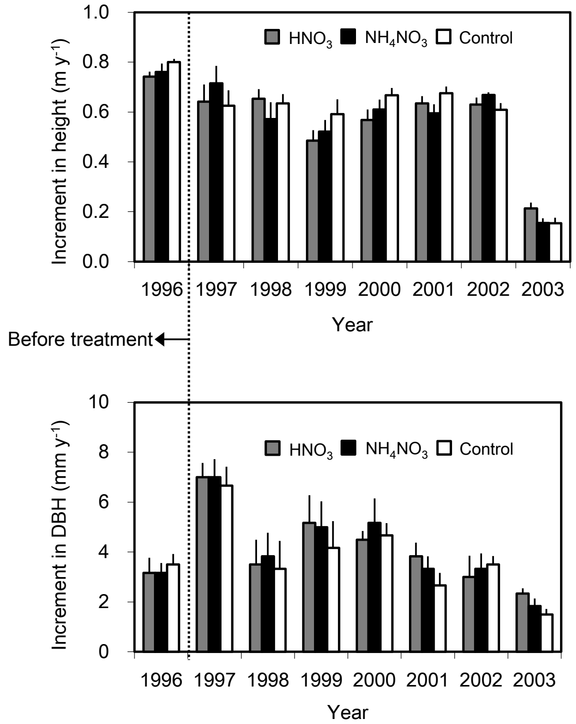

Fig. 3. Changes in the annual increments in (a) height, and (b) diameter at breast height (DBH). Gray, black and white columns indicate data on trees at HNO_3, NH_4NO_3, and control plots, respectively. Data shown are means with SE values (n = 6).

$\delta^{13}C$ in the control plots tended to be lower than those in the both the N-fertilized plots, although the difference was not significant. The foliar $\delta^{13}C$ in the control plots was highest in 1997 and low in 1998, 2000, and 2003 (Fig. 6a).

The N concentration of fine roots (< 2 mm in diameter) in surface soil (0–5 cm) as well as that of the current year shoots was higher in the N-fertilized plots than in the control plots (Fig. 7). The N concentration of fine roots ranged from 8.3 to 13.3 mg g^{-1} in the control plots, and from 9.3 to 18.4 mg g^{-1} in the N-fertilized plots. The C concentrations of fine roots were not different among treatments (Fig. 7).

Discussion

The foliar N concentration increased, and the foliar K and P concentrations decreased over time during 7 years of N fertilization relative to those of the control plots (Fig. 4). Although N fertilization resulted in increased concentrations of Ca^{2+} and Mg^{2+} in the soil solution (Nagakura et al. 2006), the foliar Ca and Mg concentrations did not change significantly within 7 years. Generally, the foliar N concentration has a positive relationship with photosynthetic capacity. For example, Ito (1976) reported that a foliar N concentration of more than 15.0 mg g^{-1} for Japanese cedar is required for superior growth. Moreover, Shigenaga et al. (2008) assessed the nutrient status of Japanese cedar plantations in Japan by measuring the foliar N concentration in Japanese cedar at various sites across Japan between August and October and reported a mean value of 14.0 mg g^{-1}. The foliar N concentration in November in our study was not high even in N-fertilized plots when compared with the value required for superior growth of Japanese cedar and with the average foliar N concentration of Japanese cedar across Japan as reported above.

Foliar N concentrations were often higher in the NH_4NO_3 plots than in the HNO_3 plots (Figs. 4 and 5). This would be associated with concentrations of NO_3^- in the soil solution that were higher in the NH_4NO_3 plots than in the HNO_3 plots (Nagakura et al. 2006). In addition, the N load for the NH_4NO_3 plots was two times greater than that for the HNO_3 plots. NH_4^+ in the soil solution may be converted into NO_3^- by nitrification, and the pH obviously decreased and the Al concentration increased in the soil solution in the NH_4NO_3 plots (Nagakura et al. 2006). Fertilization using NH_4NO_3 would have the possibility of declining tree vitality, although it would be effective in improving the nitrogen status of foliage and fine roots.

Shigenaga et al. (2011) found the average foliar K, Ca and Mg concentrations of Japanese cedar across Japan were 6.45 mg g^{-1}, 11.6 mg $g^{-1}$and 1.97 mg g^{-1}, respectively. At our study site, the foliar K and Ca concentrations in November were lower, and the foliar Mg concentration was higher than the average values across Japan (Fig. 4). The foliar Ca concentration was lower than those of Xue and Luo (2002) who reported data from a 13-year-old Japanese cedar stand. However, the foliar K and Ca concentrations were not lower than the values of Japanese cedar that exhibited inferior growth rates (< 4.0 mg g^{-1} for K, < 6.0 mg g^{-1} for Ca; Ito 1976). The foliar N:K, and Ca:K ratios were also within the upper range reported by Ito (1976; N:K = 2.1–3.3, Ca:K = 0.9–2.5). However, the foliar Ca:Mg ratio was below the lower range reported by Ito (1976; Ca:Mg = 3.5–6.5).

The total amount of N fertilizer used during the present experiment was 1190 kg N ha^{-1} for the HNO_3 plots and 2380 kg N $ha^{-1}$for the NH_4NO_3 plots. Excessive N fertilization had no obvious effect on the growth in height and DBH of Japanese cedar in the present experiment (Fig. 3). The foliar N concentration that was not high even in N-fertilized plots when compared with the value required for superior growth of Japanese cedar might be responsible for lack of positive effects of N fertilization on height and DBH growth.

In the present experiment, the foliar N, P and K concentrations decreased, whereas the foliar Ca concentrations increased with needle maturation (Fig.

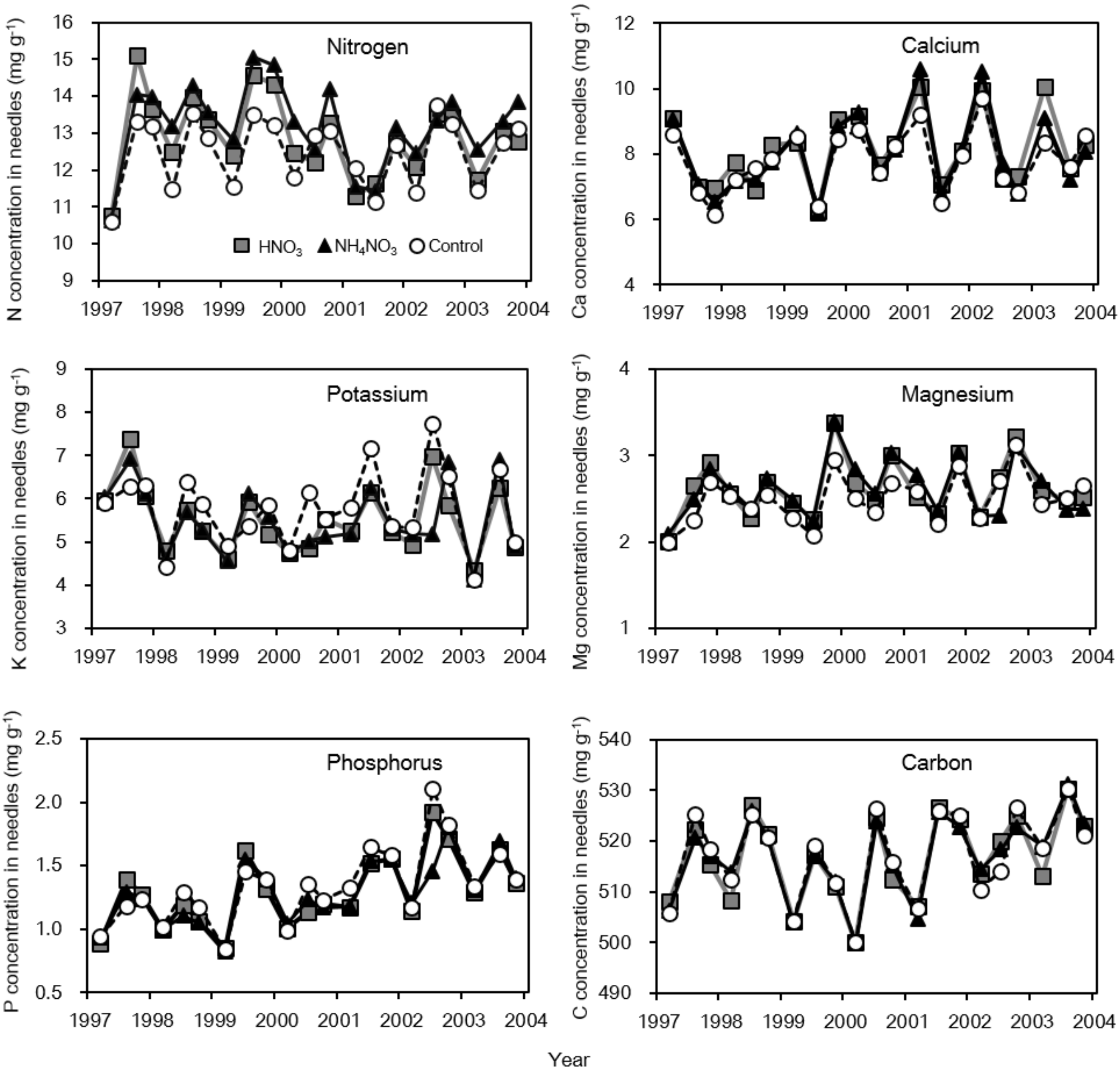

Fig. 4. Changes in nutrients (N, P, K, Ca, Mg) and carbon (C) concentration of current year shoots during 1997–2003. Data shown are means (n = 6).

4). These seasonal changes in each foliar nutrient have been previously well reported (Marschner, 1995), and a similar result has been reported in a 13-year-old Japanese cedar stand (Xue and Luo 2002).

The foliar C concentrations showed seasonal change (Fig. 4) that was similar to that of foliar N, P and K. The decrease in the foliar concentrations of these elements from July to November is thought to be a result of a dilution effect during needle maturation, and the decrease from November to March would be a result of translocation prior to new shoot growth.

The N concentrations of fine roots tended to be higher in the N-fertilized plots than in the control plots (Fig. 7). This may correspond to the increased concentration of NO_3^- in the soil solution (Nagakura et al. 2006). Noguchi et al. (2013) conducted a 3-year N fertilization experiment in a Japanese cedar stand adjacent to our study stand. In that study, the soil had been treated with an NH_4NO_3 solution at the same rate as our study (336 kg N ha^{-1} y^{-1}). They found that the N concentration of fine roots (< 1 mm in diameter) in surface soil (0–10 m) was higher in the N-fertilized plots than in the control plots, which is consistent with our results. Other reports have also noted that N fertilization resulted in an increase in the N concentration of fine roots (Stober et al. 2000, Genenger et al. 2003, Magill et al. 2004). The nutrient status of fine roots would indicate soil nutrient conditions very well.

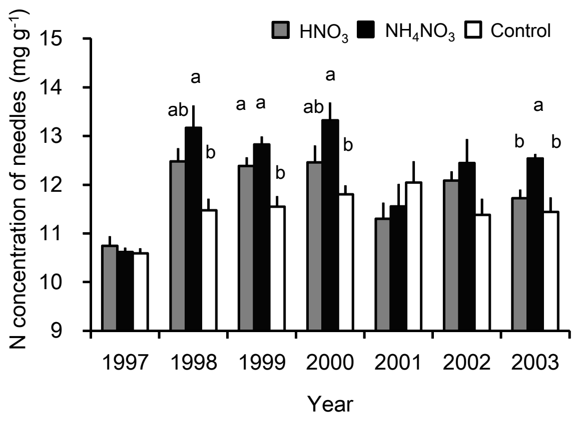

Fig. 5. Changes in nitrogen (N) concentration of current shoots sampled in March during 1997–2003. Gray, black and white columns indicate data on trees at HNO_3, NH_4NO_3, and control plots, respectively. Data shown are mean values with SE values (n = 6). Different letters indicate a significant difference in each year (Tukey's honestly significant difference test; $P < 0.05$).

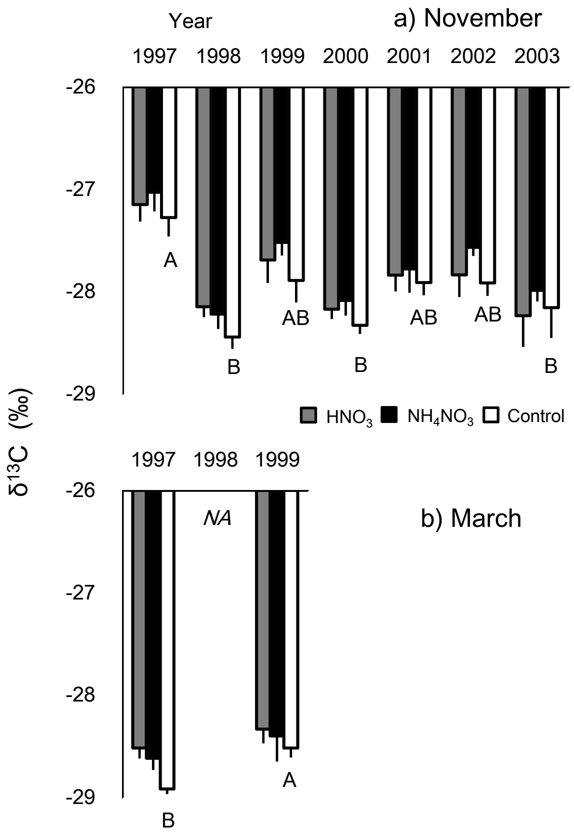

Fig. 6. a) Changes in the $\delta^{13}C$ of current needles collected in November during 1997–2003. b) Difference in the $\delta^{13}C$ of current needles collected in March 1997 and 1999. Gray, black and white columns indicate data on trees in HNO_3, NH_4NO_3, and control plots, respectively. Data shown are mean with SE values (n = 6). Different capital letters indicate a significant difference among years in control plots (Tukey's honestly significant difference test; $P < 0.05$).

The foliar N concentration was higher in the N-fertilized plots than in the control plots (Fig. 4), and the effects were significant in March (Fig. 5). Old branch and needle death of Japanese cedar occurred mainly from September to October (Tange et al. 1989). In autumn, the effects of a lack of nutrients with high mobility, such as N, might be partly compensated for by the translocation of those nutrients to current needles from old branches and needles. The foliar N concentration was high in July and/or November and decreased largely in March (Fig. 4). Xue and Luo (2002) reported that the foliar N concentration remained constant from June to February in a Japanese cedar stand and decreased to its lowest level in March. Because radial growth in Japanese cedar begins in March, the N of current shoots may be used particularly in the control plots during new radial growth. Therefore, the nitrogen status of Japanese cedar might be well reflected in March rather than November. The foliar N concentrations in the present experiment were also significantly lower in the control plots than in the NH_4NO_3 plots in March (Fig. 5).

Soil moisture conditions would influence the foliar $\delta^{13}C$ during shoot growth (Warren et al. 2001). The foliar $\delta^{13}C$ corresponded negatively to high levels of summer precipitation. For example, 1998 was the year with the highest summer precipitation during this experiment and the lowest foliar $\delta^{13}C$ collected in November of any year was observed in 1998; (Figs. 2 and 6a). Conversely, the highest foliar $\delta^{13}C$ was observed in 1997, a year receiving only a small amount of precipitation. The foliar $\delta^{13}C$ tended to be lower in the control plots than in the N-fertilized plots that had drier soil (Nagakura et al. 2006). Furthermore, Livingston et al. (1999) reported that N stressed white spruce seedlings had low foliar $\delta^{13}C$, and suggested a positive correlation existed between the foliar N and $\delta^{13}C$ concentrations that was likely associated with increased photosynthetic capacity. It is possible that the relatively low foliar N concentration in the control plots reflects low foliar $\delta^{13}C$. The difference in the foliar N concentration caused by N fertilization was the most significant in March (Fig. 5). In March 1999, the foliar $\delta^{13}C$ was not different among treatments (Fig. 6b), although the foliar N concentration was significantly higher in the N-fertilized plots than in the control plots (Fig. 5). The foliar $\delta^{13}C$ would be influenced by soil moisture conditions rather than by N fertilization.

In our study, 7 years of N fertilization had no positive or negative effect on the growth of young Japanese cedar trees, although the nutrient status of current shoots and fine roots was altered. Magill et al. (2004) reported that 15 years of N addition resulted in increased biomass in a hardwood stand, but decreased

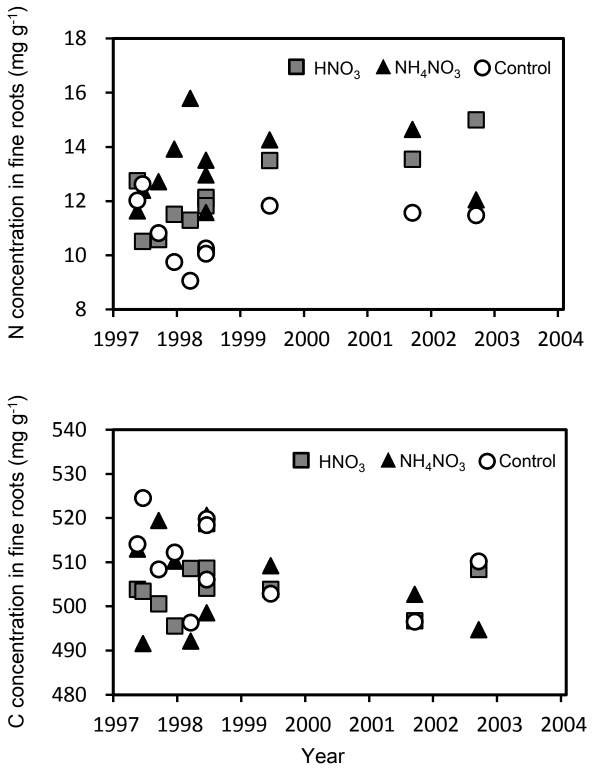

Fig. 7. Changes in the nitrogen (N) and the carbon (C) concentration of fine roots (< 2 mm in diameter). Data shown are measured values or mean values (n = 2).

biomass and high mortality was observed in a pine stand. Many factors may cause variations in the responses of trees to N addition, such as soil nutrient conditions, physiological characteristics of the tree species, and the amount of N added. Further long-term N loading experiments in various types of forests would be necessary to clarify the effects of these various factors over the long term on Japanese cedar.

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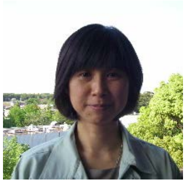

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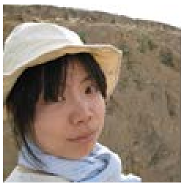

Dr. Ayumi Tanaka-Oda studies plant ecophysiology and forest ecology. Her recent research is focused on the carbon and nitrogen stable isotope ratio of plant species.

Dr. Takeshi Tange studies site condition and growth of planted Japanese cedar in central Japan. His recent research is effect of atmospheric drought on growth of old and tall Japanese cedar trees.