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Effects of Soil Moisture and Nitrogen on Growth Responses of *Cryptomeria japonica* and *Chamaecyparis obtusa* Seedlings

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Summary

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An increase in anthropogenic nitrogen (N) deposition and fluctuations in the distribution of precipitation is a concern because of the potential influence on forest ecosystem dynamics. To evaluate the influence of changes in soil moisture and N availability on Japanese cedar (*Cryptomeria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*), three-month-old seedlings were subjected to treatment with combinations of two watering (dry and moist) and three N levels (low: LN, moderate: MN and high: HN) for 12 weeks. In both species, dry treatment increased biomass allocation to roots and decreased radial growth. Nitrogen levels increased needle N concentrations and decreased biomass allocation to roots in both species. In *C. japonica*, the whole plant biomass, root collar diameter and water-use efficiency (WUE) increased in response to N levels, while height growth was restricted with dry treatment. In *C. obtusa*, these parameters did not change significantly with these treatments; however, the whole plant biomass of *C. obtusa* in HN was smaller than that in MN. Soil N levels in HN likely exceeded the requirements of *C. obtusa*. This suggests that *C. obtusa* might be more sensitive to excess N load than *C. japonica*. We conclude that *C. japonica* is more responsive to soil moisture and N than *C. obtusa*, and therefore, deficiencies in soil moisture and N are likely to limit the growth of *C. japonica* more remarkably than that of *C. obtusa*. It is also suggested that growth responses to soil water deficiencies were mostly unaffected by N availability in both *C. japonica* and *C. obtusa*, because interactions between soil moisture and N levels were significant only with regard to the WUE of *C. obtusa*.

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Introduction

Availability of water and nitrogen (N) in soil are major limiting factors for plant growth. Global climate models have been used to predict future fluctuations in the distribution of precipitation as well as temperature (IPCC 2001). Moreover, increases in anthropogenic N deposition are a concern because of the influence on forest ecosystem responses (NIHLGÅRD 1985, ABER & al. 1998). OHRUI & MITHCHELL 1997 suggested that some Japanese watersheds have already been saturated with N. Nitrogen availability often influences the water economy of plants; for example, several studies have reported that the water-use efficiency (WUE), the ratio of the total increase in biomass to total transpiration, increases in response to N fertilization (LIVINGSTON & al. 1999, WELANDER & OTTOSSON 2000, RIPULLONE & al. 2004). On the contrary, other studies have indicated that N supply has no effect on WUE (MITCHELL & HINCLEY 1993, GUEHL & al. 1995) or rather it decreases WUE (WANG & al. 1998). These results suggest that WUE seems to be influenced not only by N availability, but also by several factors such as tree species (GUEHL & al. 1995) and soil moisture conditions (WANG & al. 1998).

In Japan, Japanese cedar (*Cryptomeria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*) are the most common plantation tree species. *C. japonica* is favorably planted in moist and nutrient-rich soil, whereas *C. obtusa* is planted in relatively dry and nutrient-poor soil. Therefore, *C. japonica* and *C. obtusa* are expected to display different responses to water and N availability in the soil. Although several studies have reported the responses of Japanese conifers to changes in soil moisture and/or N conditions (SHIBAMOTO 1952, TSUTSUMI 1962, NAKAJI & al. 2001), the growth and WUE of *C. japonica* and *C. obtusa* have not been compared. The purpose of this study is to evaluate the effects of soil moisture and N availability on the growth and WUE of *C. japonica* and *C. obtusa*, and to clarify whether N availability influences growth responses to soil water deficiency. Furthermore, we discuss the influence of N load on Japanese plantation forests dominated by these species.

Material and Methods

One-month-old seedlings of *C. japonica* and *C. obtusa* were obtained from the Nursery of the Forestry and Forest Products Research Institute (FFPRI; Tsukuba, Japan) on 2 July, 2001. Each seedling was transplanted in a 1.5-liter Wagner pot (100 cm^2 surface area and 18.5 cm deep) containing about 1000 g of air-dried Akadama soil derived from the loamy B horizon of Andisol. Potted seedlings were acclimated during the following 2 months then subjected to treatment with combinations of two soil moisture (dry and moist) × three N levels for approximately 12 weeks from 3 September, 2001. Nitrogen levels were controlled by the different nutrient solutions used for watering: LN (low nitrogen, 7.0 mgN L^{-1}), MN (moderate nitrogen, 28.0 mgN L^{-1}) and HN (high nitrogen, 112.0 mgN L^{-1}). These N amounts were equivalent to 4.2, 16.8, 67.2 kgN ha^{-1}, respectively. The solution in the soil was replaced with new nutrient solution at each watering. The nitrogen source was NH_4NO_3. Other nutrient concentrations in all solutions were kept the same (P: 10.8, K: 23.5, Ca: 28.1, Mg: 9.7, Fe: 3.0, Cu: 0.03, Zn: 0.03, Mn: 0.03, B: 0.35, and Mo: 0.04 mg L^{-1}). For the moist treatment, seedlings were watered once every two or three days. The driest soil water content (θ; m^3 m^{-3}) observed in the moist treatment was 0.36 (-0.005 Mpa). For the dry treatment, seedlings were watered when the soil dried to θ = 0.30 (-0.06 Mpa). Conversion of θ values to soil matric potentials (-MPa) were predetermined by a soil water relation curve. Watering intervals in the dry treatment were once every 5 to 7 days for *C. japonica* and once every 7 to 8 days for *C. obtusa*. Soil moisture conditions during the experiment were estimated by weighing the pots. The pH (H_2O) of the soil suspension (dry soil:H_2O = 1:2.5) was measured with a glass electrode pH meter before and after the experiments. Ten seedlings of each species underwent each treatment. Seedlings were grown in a phytotron with day/night temperatures and relative humidities of 25/20°C and 70/80%, respectively; they were given natural sunlight with supplemental illumination to maintain a 14-h photoperiod. From the 7^{th} to the 12^{th} week after commencing treatment (hereafter referred to as the TMP: transpiration measurement period), seedling transpiration was measured as the weight loss during watering intervals. The details of culture and transpiration measurements have been published elsewhere (NAGAKURA & al. 2004).

To estimate the dry mass of the seedlings at the beginning of the TMP, half the seedlings in each treatment were harvested (n=5). The rest were harvested at the end of the experiments (n=5). The growth parameters (height, root collar diameter, and branch lengths) of the harvested seedlings were measured. The aboveground parts and roots were separated and washed with deionized water, and all parts were weighed after drying at 75°C for 48 h. Dried needles were finely ground and N concentrations were measured using a NC analyzer (NC-900, SUMIGRAPH, Japan).

WUE is defined as the biomass increment per unit of transpiration, and is calculated as: WUE = $(W_2 - W_1)(T)^{-1}$, where W_1 is the whole plant dry weight on day 1 (t_1), W_2 is the whole plant dry weight on the last day (t_2) of the TMP, and T is the total transpiration from t_1 to t_2. W_2 is the actual weight at t_2, but W_1 is estimated from the allometric equation calculated by the dry weight at t_1 (NAGAKURA & al. 2004).

Growth parameters of the seedlings were analyzed using two-way ANOVA to test the effects of water, nitrogen and their interaction for each species.

Results

The whole plant biomass, height and root collar diameter were smallest in the dry × LN treatment for both species. Largest values were observed in the moist × HN treatment for *C. japonica*, and in the moist × MN treatment for *C. obtusa* (Table 1). The soil pH (H_2O) decreased from 5.5 (before treatment) to 5.3 (moist × LN), 5.4 (dry × LN), 5.2 (moist × MN), 5.3 (dry × MN), 4.2 (moist × HN) and 4.6 (dry × HN) regardless of the species.

In both species, dry treatment increased biomass allocation to the roots and decreased root collar diameter (Table 1). The root-to-shoot ratio (R/S) in the dry treatment was larger than that in the moist treatment, particularly under low nitrogen conditions. The whole plant biomass tended to be small in the dry treatment, although the effect was not significant for either species. Heights of *C. japonica* were about 30% smaller in the dry treatment than in the moist treatment, whereas those of *C. obtusa* were not inhibited by dry treatment. Soil moisture treatment did not affect the WUE and needle N concentration of either species (Table 2).

Nitrogen levels increased needle N concentrations for both species (Table 1). Needle N concentrations of *C. japonica* and *C. obtusa* in HN were 1.9 and 1.25 times greater than those in LN, respectively. In *C. japonica*, the whole plant biomass, root collar diameter and WUE increased with increasing N, but no significant effect was observed in *C. obtusa*. The R/S of both species tended to decrease as N levels increased, and this tendency was more obvious in the dry treatment than moist treatment (Tables 1 and 2). Nitrogen levels did not have a significant effect on height growth in either species (Table 2). The interactive effect of soil moisture and N levels was significant only with regard to the WUE of *C. obtusa* (Table 2).

Table 1. Whole plant biomass (Biomass), height, root collar diameter (RCD), root-to-shoot ratio (R/S), water-use efficiency (WUE), and needle N concentration of seedlings grown in different treatments. Values represent the mean ± SE (n=5).

	Low nitrogen (LN)		Moderate nitrogen (MN)		High nitrogen (HN)	
	moist	dry	moist	dry	moist	dry
C. japonica						
Biomass (g)	6.1±0.3	5.4±0.3	6.5±0.7	6.0±0.6	8.0±0.8	7.1±1.0
Height (mm)	278±21	196± 6	309±20	220±15	315±19	217± 9
RCD (mm)	3.85±0.12	3.21±0.06	4.31±0.19	3.55±0.12	4.69±0.17	3.95±0.25
R/S	0.25±0.01	0.39±0.04	0.28±0.02	0.33±0.02	0.24±0.02	0.29±0.02
WUE ($g\ g^{-1}\ day^{-1}$)	4.1±0.6	3.6±0.3	5.0±0.4	5.3±0.2	5.2±0.23	5.3±0.5
Needle N ($mg\ g^{-1}$)	11.7±0.5	11.0±0.8	19.5±1.3	18.5±1.2	20.9±1.3	22.2±1.1
C. obtusa						
Biomass (g)	3.2±0.5	2.5±0.3	3.7±0.7	2.9±0.4	3.0±0.7	2.8±0.5
Height (mm)	294±24	296±17	339±12	275±26	306±32	303±14
RCD (mm)	3.01±0.17	2.36±0.14	3.11±0.27	2.53±0.15	2.85±0.27	2.66±0.21
R/S	0.26±0.01	0.37±0.03	0.25±0.01	0.33±0.02	0.24±0.01	0.28±0.01
WUE ($g\ g^{-1}\ day^{-1}$)	5.3±0.5	4.5±0.5	4.2±0.7	6.5±0.1	4.7±0.7	5.1±0.5
Needle N ($mg\ g^{-1}$)	18.4±1.3	17.5±0.7	20.1±0.5	20.7±0.6	22.9±1.4	22.4±0.9

Table 2. The effects of watering treatment, nitrogen levels, and water × nitrogen in the analysis of variance for seedling growth parameters (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$, ns = not significant).

	Water	Nitrogen	interaction
C. japonica			
Whole plant biomass	ns	**	ns
Height	***	ns	ns
Root collar diameter	***	***	ns
Root-to-shoot ratio (R/S)	***	*	ns
Water-use efficiency (WUE)	ns	***	ns
Needle N	ns	***	ns
C. obtusa			
Whole plant biomass	ns	ns	ns
Height	ns	ns	ns
Root collar diameter	**	ns	ns
Root-to-shoot ratio (R/S)	***	***	ns
Water-use efficiency (WUE)	ns	ns	**
Needle N	ns	***	ns

Discussion

Biomass production was stimulated by N levels and height growth was restricted by drought in *C. japonica*. However, the biomass production and height growth of *C. obtusa* were not influenced by soil moisture or N levels. In both species, biomass allocation and radial growth were more sensitive to water deficiency than biomass production. The larger whole plant biomass of *C. japonica* in MN and HN was likely caused by high needle N concentrations, which, in general, have a positive relationship with productivity (LAMBERS & al. 1998). Needle N concentrations of *C. obtusa* also increased with N levels but the increasing rate was smaller compared with *C. japonica*. This might have resulted in the modest response of biomass production in *C. obtusa*.

As found in *C. japonica*, an increase in WUE in response to elevated N availability has also been reported for *Pinus pinaster* (GUEHL & al. 1995), *Quercus robur* (WELANDER & OTTOSSON 2000), *Picea abies* and *Fagus sylvatica* (SONNLEITNER & al. 2001). Application of nitrogen fertilization rather than water consumption increased productivity in these species. However, for *C. obtusa*, the WUE of the dry treatment was lower in HN than MN while the WUE of the moist treatment was highest in LN. In addition, the whole plant biomass of *C. obtusa* in HN was smaller than that in MN. Soil N levels in HN likely exceeded the requirements of *C. obtusa*, and the high N load might therefore have adversely affected the growth of *C. obtusa*. This suggests that increasing anthropogenic N deposition is likely to decrease the production of Japanese plantation forests dominated by *C. obtusa* rather than *C. japonica*.

In conclusion, *C. japonica* is highly responsive to changes in soil moisture and N availability compared with *C. obtusa*. Deficiencies in water and N were shown to limit the growth of *C. japonica* more remarkably than that of *C. obtusa*. It is also suggested that growth responses to soil moisture deficiency are mostly unaffected by N availability in *C. japonica* and *C. obtusa* because interactions between soil moisture and N levels were significant only with regard to the WUE of *C. obtusa*.

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