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Effects of Elevated CO_2 and Nitrogen Availability on Nodulation of *Alnus hirsuta* Turcz.

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Summary

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The effects of elevated CO_2 and soil nitrogen (N) availability on nodulation and N_2 fixation of alder (*Alnus hirsuta* Turcz.) were investigated. Our objective was to determine if elevated CO_2 concentrations enhance nodule mass and alter the inhibitory effect of soil N on nodulation. Potted seedlings of alder were grown at either ambient or elevated CO_2 concentrations (36 Pa and 72 Pa CO_2), with different levels of N supplied as liquid fertilizer (52.5, 5.25 and 0 mgN pot^{-1} $week^{-1}$ for High-N, Low-N and N-free, respectively) in a natural daylight phytotron.

Elevated CO_2 increased both whole-plant mass and nodule mass per plant, but the ratio of nodule mass to whole-plant mass (NMR) was not affected. At High-N, NMR declined under both CO_2 treatments, indicating an inhibitory effect of soil N availability on nodulation regardless of CO_2 concentration. The total amount of N_2 fixation in plants without added N was enhanced under elevated CO_2 conditions, with an increase of nodule mass. At elevated CO_2, both whole-plant N concentration and area-based leaf N decreased.

Leaf litter C:N ratio increased (+14%) under elevated CO_2, suggesting that leaf litter decomposition rates are decreased by elevated CO_2. However, area-based leaf litter N did not decrease under elevated CO_2 due to decreased retranslocation of leaf N per area during leaf senescence. Furthermore, leaf area per plant increased under elevated CO_2 in each of the N-treatments. These results suggest that total leaf litter N input to soil should increase under elevated concentrations of atmospheric CO_2, and that alder may increase soil N availability under elevated CO_2.

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Introduction

Many plant species grown under elevated CO_2 ($[CO_2]^e$) exhibit an enhanced CO_2 assimilation rate and growth when other environmental resources do not limit productivity (NORBY & al. 1999). Forest ecosystems are usually nitrogen (N) -limited (VITOUSEK & HOWARTH 1991), and plants growing under low N conditions usually respond much less to $[CO_2]^e$ (HARTWIG & al. 1996). As trees with symbiotic N_2-fixing capability are largely independent of soil N, they could be more responsive to $[CO_2]^e$ than non-N_2-fixers. In fact, increased growth, N_2 fixation, and nitrogenase activity of nodules or the nodule mass have been documented (NORBY 1987, ARNONE & GORDON 1990, SCHORTEMEYER & al. 1999, THOMAS & al. 2000). However, added fertilisers usually reduce nodule formation or N_2 fixation (EKBLAD & HUSS-DANELL 1995, KOIKE & al. 1997, VOGEL & al. 1997). Because the supply of photosynthate to nodules may increase by enhanced photosynthesis under $[CO_2]^e$ (NORBY 1987), these inhibitions may be reduced as concentrations of atmospheric CO_2 rise (THOMAS & al. 2000).

Nitrogen derived from N_2 fixation cycles into the soil mainly through leaf litter. Many tree species were reported to have decreased leaf N and whole-plant N under $[CO_2]^e$ (COTRUFO & al. 1998), which may result in decreased leaf litter N (O'NEILL & NORBY 1996). Since the decline in leaf N under $[CO_2]^e$ conditions was less in N_2-fixers than in non-N_2-fixers (COTRUFO & al. 1998), the decrease in the leaf litter N of N_2-fixers may also be small. An increase in N biomass through N_2 fixation by litter N under $[CO_2]^e$ conditions may promote the productivity of associated non-N_2-fixers (NORBY 1987, HARTWIG & al. 1996).

Actinorhizal N_2-fixing species, including *Alnus hirsuta* Turcz., can contribute significant amounts of fixed N to temperate forest ecosystems (DAWSON 1983, KOIKE & al. 1997). We examined the effects of $[CO_2]^e$ and soil N availability on nodulation and growth of *A. hirsuta* to determine if $[CO_2]^e$ enhances nodule mass, alters nodulation, or increases total N_2 fixation and leaf litter N input of *A. hirsuta* seedlings.

Material and Methods

One-year-old seedlings of *Alnus hirsuta* Turcz. obtained from a commercial nursery (Oji Forestry & Landscaping, Sapporo, Japan), were transplanted into five liter pots filled with 1:1 (v/v) Kanuma pumice and clay loam and grown in a natural daylight phytotron. The CO_2 partial pressure was regulated with a CO_2 controller (DAIWA Air, Sapporo, Japan) at either 36 Pa (ambient) or 72 Pa (elevated) CO_2 from mid-May. Each treatment was replicated twice. Seedlings were supplied with nitrogen (N) at 52.5 mgN pot^{-1} $week^{-1}$ (High-N), 5.25 mgN pot^{-1} $week^{-1}$ (Low-N) or no N ('N-free') in 0.5x Hoagland solution. Other nutrient concentrations were the same. Air temperature was maintained at 26/16 °C (day/night) from May to September. Pots were set in trays with water in order to avoid desiccation. After 100 days (late-August), six seedlings of each treatment were harvested, and dry mass and N content of each organ were determined by combustion using an NC analyzer (NC-800; Sumica Chem., Osaka, Japan). Twenty-five seedlings were harvested before treatment to provide a baseline for the initial mass and N content of plants.

Leaf gas exchange measurements were made on six mature leaves of each treatment on day 59 (mid-July). Light-saturated net photosynthetic rates per leaf area (P_{max}) were determined using an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) at each CO_2 condition (36 Pa or 72 Pa CO_2).

Total N_2 fixation (TNF) was calculated only in the N-free treatments. Because symbiotic N_2 fixation was considered to be the only source of N at N-free, TNF was considered to be equal to the total plant N (TPN) increment during two harvests. The average specific nitrogenase activity (SNA) was calculated as: SNA = TPN * NW^{-1} * $[day]^{-1}$, where NW is the average nodule mass per plant during two harvests and [day] is the period of the treatment when it was harvested (TISSUE & al. 1997).

Leaf litter from each individual was collected daily as leaves abscised after gradually decreasing the ambient temperature from 20/10 °C to 14/10 °C (day/night) from September to November. Area-based N resorption efficiency (RE) before senescence was calculated as: RE = 100 * (1 - $[N]_{fallen}$ * $[N]_{green}^{-1}$), where $[N]_{fallen}$ was average area based leaf litter N within each treatment and $[N]_{green}$ was average area-based leaf N of each plant harvested in August (VAN HEERWAARDEN & al. 2003).

Analysis of variance (ANOVA) was used to evaluate the effects of CO_2 and N-treatments on biomass, leaf area, P_{max} and N content at the probability level p<0.05 using StatView Version 5.0 (Abacus Concepts, Inc., Berkeley, CA). The probability level p<0.1 was considered to indicate a trend. Analysis of co-variance (ANCOVA) was used to compare the relationship between whole-plant mass and nodule mass per plant. Statistically significant differences of SNA of the seedlings in the N-free treatment between CO_2 treatments were tested using Student's t-test (p<0.05).

Results and Discussion

Elevated CO_2 ($[CO_2]^e$) had a significant positive effect on whole-plant mass, leaf mass and leaf area per plant as well as on the P_{max} of mature leaves despite of N treatments (Table 1). Trees growing under $[CO_2]^e$ conditions tended to have a higher nodule mass per plant than control plants at ambient CO_2 concentrations (Fig. 1). Elevated CO_2, however, had no effect on the relationship between whole-plant mass and nodule mass (Fig. 2, ANCOVA, p>0.1) as reported for *Alnus rubra* (HIBBS & al. 1995), suggesting that $[CO_2]^e$ increased the nodule mass of *A. hirsuta* as a function of the increase in whole-plant mass rather than by an increase in the ratio of nodule mass to whole-plant mass (NMR).

Table 1. Whole-plant mass (g $plant^{-1}$), leaf mass (g $plant^{-1}$) and area (m^2 $plant^{-1}$) per plant, and light-saturated photosynthesis (P_{max}) (μmol m^{-2} s^{-1}) of *Alnus hirsuta*. * = p<0.05, ** = p<0.01, *** = p<0.001, n.s. = not significant at p<0.1.

	High-N		Low-N		N-free		Source of variance		
	ambient	elevated	ambient	elevated	ambient	elevated	CO_2	N	CO_2xN
whole-plant mass	34.5	43.5	34.3	40.6	39.2	51.6	**	n.s.	n.s.
leaf mass	13.1	16.3	12.6	14.8	14.3	17.7	*	n.s.	n.s.
leaf area	0.37	0.42	0.34	0.40	0.43	0.50	*	*	n.s.
P_{max}	13.8	16.7	12.8	14.3	11.4	14.2	***	**	n.s.

Increased N availability had a negative effect on both nodule mass (Fig. 1) and NMR (Fig. 2) regardless of CO_2 concentration, indicating that the inhibitory effect of high soil N availability on nodulation of *A. hirsuta* was retained under $[CO_2]^e$. Some *Alnus* species had a similar inhibitory effect on nodulation under

$[CO_2]^e$ (NORBY 1987, KOIKE & al. 1997, TEMPERTON & al. 2003), though some legume species showed no limitation (THOMAS & al. 2000).

The whole-plant N content after 100 days tended to be higher under $[CO_2]^e$ (Table 2). The enhanced whole-plant N uptake under $[CO_2]^e$ with N-free soil amendment means that N_2 fixation increased under $[CO_2]^e$. On the other hand, the average specific nitrogenase activity (SNA) tended to decrease under $[CO_2]^e$ (2.5 mmolN g nodule^{-1} day^{-1} under ambient conditions and 2.2 mmolN g nodule^{-1} day^{-1} under $[CO_2]^e$; t-test, p=0.06). Some studies of *Alnus* species have shown that $[CO_2]^e$ increased the total amount of N_2 fixation per plant by having greater nodule nitrogenase activity (TEMPERTON & al. 2003), by greater nodule mass (HIBBS & al. 1995) or by both (NORBY 1987, ARNONE & GORDON 1990, VOGEL & al. 1997). This current work suggests that the increase in N_2 fixation under $[CO_2]^e$ occurred mainly because of an increase in nodule mass proportionate with whole-plant mass, and not by increased SNA.

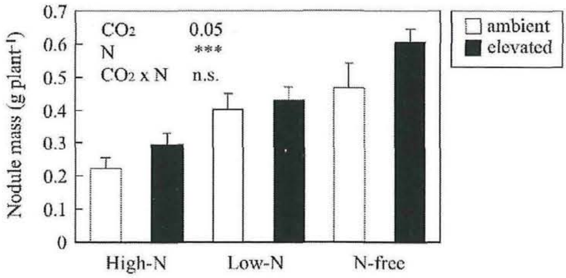

Fig. 1. Nodule mass per plant of *Alnus hirsuta* after 100 days. Values shown are means + SE (n = 6). *** = p<0.001, n.s. = not significant at p<0.1.

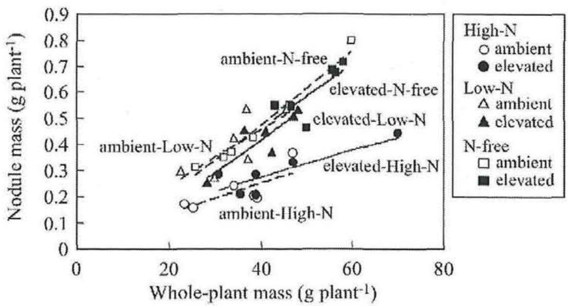

Fig. 2. Relationships between whole-plant mass and nodule mass per plant. Lines represent statistically significant (p<0.05) power functional regression at each treatment [(nodule mass) = a * (whole-plant mass)^b]. Dotted lines; ambient CO_2, solid lines; elevated CO_2.

Since the nitrogenase activity of nodules was not measured, we could not evaluate N_2 fixation at High-N and Low-N. In an earlier study, it was reported that $[CO_2]^e$ had no effect on the proportion of nitrogen derived from symbiotic fixation (VOGEL & al. 1997, SCHORTEMEYER & al. 1999). As whole-plant N accretion was larger under $[CO_2]^e$ in each of the N-treatments (Table 2), it is possible that N_2 fixation at High-N and Low-N are greater under $[CO_2]^e$ conditions than under ambient concentrations.

Elevated CO_2 had a negative effect on whole-plant N concentration and mass-based and area-based leaf N (Table 2), but no effect on area-based leaf litter N (Fig. 3a). These results suggest that the resorption efficiency (RE) of leaf N was affected by CO_2 concentrations, and indeed RE tended to decrease under $[CO_2]^e$ at low N and N-free (Fig. 3b). Thus, N resorption from leaves to seedlings before abscision, seems to be lower in $[CO_2]^e$ than in ambient CO_2. Because $[CO_2]^e$ increased total leaf area (Table 1) and had no effect on area-based leaf litter N in all N-treatments (Fig. 3a), total per plant leaf litter N input into soil may increase under $[CO_2]^e$. VOGEL & al. 1997 also reported that total leaf litter N of *Alnus glutinosa* increased with $[CO_2]^e$, though autumnal leaf N resorption of *A. glutinosa* was minimally affected by $[CO_2]^e$.

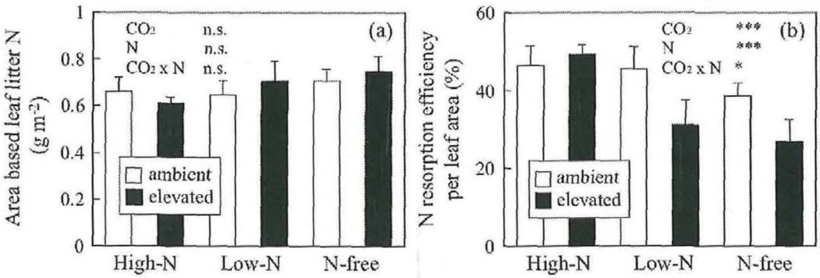

Fig. 3. Area based leaf litter N (a) and N resorption efficiency (b) of *Alnus hirsuta*. Values shown are means + SE (n = 6). * = p<0.05, *** = p<0.001, n.s. = not significant at p<0.1.

Table 2. Whole-plant N increment (g $plant^{-1}$), whole-plant N concentration (mg g^{-1}), leaf N (mass and area based, mg g^{-1}, g m^{-2}), and leaf litter N (mass based, mg g^{-1}) and C:N ratio of *Alnus hirsuta*. * = p<0.05, ** = p<0.01, *** = p<0.001, n.s. = not significant at p<0.1.

	High-N		Low-N		N-free		Source of variance		
	ambient	elevated	ambient	elevated	ambient	elevated	CO_2	N	CO_2xN
plant N increment	0.72	0.81	0.61	0.72	0.80	0.90	0.07	*	n.s.
whole-plant N	21.4	19.2	18.5	18.0	20.9	17.7	***	*	n.s.
mass based leaf N	34.8	31.0	31.1	29.9	36.1	28.7	***	0.07	*
area based leaf N	1.23	1.20	1.13	1.11	1.18	1.02	*	**	0.08
leaf litter N	18.1	15.8	17.7	15.4	19.2	16.3	***	n.s.	n.s.
leaf litter C:N	29.0	33.2	29.0	33.9	26.8	32.3	***	n.s.	n.s.

Elevated CO_2 increased the C:N ratio of leaf litter of *A. hirsuta* (14 ± 0.35 %, mean of three N-treatments ± SE) compared to ambient CO_2 (Table 2). The initial C:N ratio of leaf litter is one of the main factors that affects decomposition rates (O'NEILL & NORBY 1996). Though *Alnus glutinosa* exhibited a modest increase of 8 % in litter C:N ratio (25.9 ± 0.35) under $[CO_2]^e$, VOGEL & al. 1997 proposed that there would be little effect of CO_2 concentration on litter decomposition rates. Further study will be needed to evaluate the effect of $[CO_2]^e$ on leaf litter decomposition rates.

In conclusion, it was suggested that *A. hirsuta* would accumulate a greater biomass N through increased N_2 fixation by increased nodule mass, and may increase soil N availability by increased leaf litter N under $[CO_2]^e$.

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