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ORIGINAL PAPER

Effects of elevated CO_2 and nitrogen on wood structure related to water transport in seedlings of two deciduous broad-leaved tree species

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Abstract Wood structure might be altered through the physiological responses to atmospheric carbon dioxide concentration ([CO_2]) and nitrogen (N) deposition. We investigated growth, water relations and wood structure of 1-year-old seedlings of two deciduous broad-leaved tree species, *Quercus mongolica* (oak, a ring-porous species) and *Alnus hirsuta* (alder, a diffuse-porous species and N_2–fixer), grown under a factorial combination of two levels of [CO_2] (36 and 72 Pa) and nitrogen supply (N; low and high) for 141 days in phytotron chambers. In oak, there was no significant effect of [CO_2] on wood structure, although elevated [CO_2] tended to decrease stomatal conductance (g_s) and increased water use efficiency regardless of the N treatment. However, high N supply increased root biomass and induced wider earlywood and larger vessels in the secondary xylem in stems, leading to increased hydraulic conductance. In alder, there was significant interactive effect of [CO_2] and N on vessel density, and high N supply increased the mean vessel area. Our results suggest that wood structures related to water transport were not markedly altered, although elevated [CO_2] induced changes in physiological parameters such as g_s and biomass allocation, and that N fertilization had more pronounced effects on non-N_2-fixing oak than on N_2-fixing alder.

Keywords Elevated carbon dioxide · Oak (*Quercus mongolica*) · Alder (*Alnus hirsuta*)

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Introduction

Because of increasing atmospheric carbon dioxide concentrations ([CO_2]) caused by combustion of fossil fuels and enhanced nitrogen (N) deposition by human activities, it is predicted that forest dynamics will change, especially in the regions of middle and high latitudes in northern hemisphere (e.g., Bucher et al. 1998; Oren et al. 2001). It is urgent to evaluate not only forest dynamics but also wood properties, because forested trees are expected to be sinking organs for CO_2. However, there is little information on changes in wood properties induced by elevated [CO_2] and N deposition (Kostiainen et al. 2004).

Wood structure might be altered through the physiological responses to elevated [CO_2]. In general, it has been reported that elevated [CO_2] reduced stomatal conductance (g_s) and transpiration (T_r) and enhanced water use efficiency (WUE) in trees grown in phytotron chambers and under field conditions (e.g., Ceulemans and Mousseau 1994; Saxe et al. 1998; Urban 2003; Ainsworth and Rogers 2007). Elevated [CO_2] also enhanced total biomass and root:shoot ratio in many tree species (Saxe et al. 1998). These physiological and morphological changes will induce change not only in the internal water balance, but also in the anatomical features of conduit cells such as tracheids and vessel elements in secondary xylem.

A few studies have addressed changes in wood structure caused by elevated [CO_2] (Telewski et al. 1999; Yazaki et al. 2005). Atkinson and Taylor (1996) showed that elevated [CO_2] increased vessel diameters in the ring-porous wood of *Quercus robur* seedlings. On the other hand, no obvious effect was found in diffuse-porous woods including that of *Populus tremuloides* (Kaakinen et al. 2004) and *Betula pendula* (Kostiainen et al. 2006), whereas the vessel lumen area increased significantly in seedlings of *Quercus ilex* under high [CO_2] (Gartner et al. 2003).

Increasing N deposition would also affect the physiological characteristics of forest trees and stimulate wood production (e.g., Ericsson et al. 1996; Wallace et al. 2007). Some investigations showed that combination of high [CO_2] and high N fertilization stimulated biomass allocation such as increased aboveground biomass (e.g., Tognetti and Johnson 1999) and enhanced root biomass (e.g., Oh and Choung 2005). Furthermore, it has been reported that N_2-fixing species respond differently from non-N_2-fixing species under elevated [CO_2], because they have N_2-fixing microorganisms *Frankia* in their root system (e.g., Tobita et al. 2005). For N-limited boreal forest, N_2-fixing species are important because of their N_2 fixation ability, and elevated [CO_2] can increase their rate of N_2 fixation (Hibbs et al. 1995; Vogel et al. 1997). However, increasing N deposition would have strong influences on the activity of N_2-fixing species, and a combination of elevated [CO_2] and N deposition will influence physiological and morphological traits of N_2-fixing species.

The effects of [CO_2] on the wood of forest trees and with nitrogen acting in combination have been investigated (e.g., Hättenschwiler et al. 1996; Yazaki et al. 2001; Kostiainen et al. 2004). Elevated [CO_2] and N-fertilization altered the dimensions of xylem vessels and wood fibers in a genotype-specific manner in three species of *Populus* (Luo et al. 2005). However, little information is available regarding the effects of the changing environment on wood formation (Kirschbaum 2004; Yazaki et al. 2004, 2005). Therefore, we studied combined effects of [CO_2] and nitrogen on wood formation and water relations in tree species in northern Japan.

We studied oak and alder seedlings under strictly controlled conditions in a phytotron, because young seedlings are most responsive to [CO_2] (Saxe et al. 1998; Medlyn et al. 2001) and may be indicators of wood quality in mature trees (Yazaki et al. 2001). Oak, a ring-porous tree lacking the ability for N_2 fixation, was expected to be more sensitive to nutrient limitations and show more severe changes in wood structure than alder, a diffuse-porous tree and N_2 fixer. We tested this prediction in oak and alder seedlings raised under factorial combinations of elevated [CO_2] and nitrogen.

Material and methods

Plant materials

One-year-old seedlings of oak [*Quercus mongolica* Fisch. ex Ledeb. var. *crispula* (Blume) Ohashi] and alder (*Alnus hirsuta* Turcz.) obtained from Oji Forestry and Landscaping, Sapporo, Japan, were cultured in 5-l pots filled with 1:1 (v/v) Kanuma pumice and clay loam, and grown in a natural daylight phytotron in spring 2003. The initial height of seedlings was 14–18 cm in both species.

Both species are representative deciduous broad-leaved trees in boreal forests of northeast Asia; the sensitivity to environmental stressors is similar between seedlings and adult trees (Koike 1988). *Alnus hirsuta* is an actinorhizal N_2-fixer that may contribute significant amounts of N to N-limited temperate forest ecosystems (Dawson 1983; Tobita et al. 2005). The patterns of water transport in the stem are different in oak and alder, which produce ring-porous wood and diffuse-porous wood, respectively (Chaney and Kozlowski 1977; Utsumi et al. 1998, 1999).

Treatments

Two natural daylight phytotron chambers (Koito Industries, Yokohama, Japan) equipped with [CO_2] controllers (DAIWA Air Co. Ltd, Sapporo, Japan) were used as described previously (e.g., Koike 1995; Tobita et al. 2005). Batches of six seedlings (48 seedlings per species) were grown for up to 141 days at 36 Pa (ambient) or 72 Pa (elevated) CO_2 from mid-May. Seedlings were supplied with N at 52.5 mg N pot^{-1} week^{-1} (high-N) or 5.25 mg N pot^{-1} week^{-1} (low-N) in 0.5 × Hoagland solution (Asher and Edwards 1983; 3 mM KNO_3, 2 mM $Ca(NO_3)_2/4\ H_2O$, 0.5 mM $NH_4H_2PO_4$, 1 mM $MgSO_4/7\ H_2O$, 25 μM EDTA-Fe, 4.5 μM $MnCl_2/4\ H_2O$, 23 μM H_3BO_3, 0.4 μM $ZnSO_4/7\ H_2O$, 0.15 μM $CuSO_4/5\ H_2O$, 0.007 μM $(NH_4)_6Mo_7O_{24}/4\ H_2O$). In the low-N treatment, KCl, $CaCl_2$ and KH_2PO_4 were added to provide the same concentrations of K^+ and Ca^{2+} as in high-N tests. Air temperature was maintained at 26/16°C (day/night). Pots were kept in trays with water to avoid desiccation.

Gas-exchange

Leaf gas exchange measurements were made on six mature leaves of each treatment on days 112–114 (*Q. mongolica*) and on days 93–100 (*A. hirsuta*). The age of the leaves was about 60 days (*Q. mongolica*) and 21 days (*A. hirsuta*). In alder, the sixth and the eighth leaves, counted from the tip of leader shoots, were used, whereas in oak, secondary flushed leaves produced during the experimental period were examined.

Light-saturated net photosynthetic rates per leaf area (A_{sat}), stomatal conductance (g_s), and transpiration rate (T_r) of mature leaves were determined using an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) at both 36 and 72 Pa CO_2. Saturating photon flux density (PFD) at the upper leaf surface was 1,200 μmol m^{-2} s^{-1}, as determined based on light response curves of photosynthetic rates (data not shown). Leaf temperature was maintained at 25°C and the relative humidity within the measuring chamber was kept at 70%. Instantaneous WUE (A_{sat} per T_r) and leaf mass per area (LMA) of leaves used for gas-exchange measurements were determined.

Growth and biomass allocation

After 141 days, 6 seedlings of each treatment were harvested, and dry masses of organs (leaf, shoot, stem and root) as well as total leaf area (LA) were determined. The LA ratio (LAR; LA per total biomass) and top-to-root ratio (T/R ratio; aboveground biomass per root biomass) of each seedling were calculated.

Wood anatomy

We took wood samples from stem bases after harvesting and fixed them in 4% FAA (formaldehyde, acetic acid and 70% ethanol) immediately. Thick transverse sections (15 μm thickness) were cut with a sliding microtome, stained with safranin (1%, w/v) for 10 min and mounted permanently.

The sections were photographed using a light microscope (Axioskop 2 plus, Zeiss, Oberkochen, Germany) with a 10× objective equipped with a digital camera (Nikon digital sight, Nikon, Tokyo, Japan). Five micrographs (the resolution of a micrograph: 1,280 × 960 pixel) were obtained from the outermost (=the most recently formed) annual ring of secondary xylem in each section. We measured vessel lumen area using the Image J software (National Institute of Health, Bethesda, MD) and calculated the total vessel area, proportion of vessel area per total micrograph area (about 2.8 mm^2), mean vessel area for one vessel and number of vessels per square millimeter (mm^2) on the micrograph. To distinguish vessels from other cell types in the automated analysis, we determined the minimum vessel lumen area and used it as a threshold value; cells with smaller lumen areas were not included in our analysis (oak, 1,256 μm^2 lumen area; alder, 314 μm^2 lumen area). This decision was made according to the frequency distribution of porous areas in all annual rings of one sample (data not shown).

In oak, vessel elements were selected from earlywood formed in the same year. In alder, vessel elements were selected from the central part of the annual ring, because average vessel sizes in diffuse-porous woods decrease from earlywood to latewood. Frequencies of vessel sizes were determined in all samples. To evaluate the hydraulic conductance of the wood sampled, a "hydraulic mean" vessel diameter, calculated using the assumption that hydraulic conductance was proportional to diameter raised to fourth power as predicted by the Hagen–Poiseuille law, was computed from data of vessel lumen area (Sperry and Ikeda 1997).

Statistical analysis

Two-way analysis of variance (ANOVA) for split-plot designs was used to evaluate the effects of [CO_2] and N-treatments on physiological properties (A_{sat}, g_s, T_r, WUE), growth properties (total biomass, LA, LAR, T/R ratio, stem basal diameter, LMA) and wood properties (total vessel area, mean vessel area, vessel proportion, vessel density, vessel size frequency, "hydraulic mean" vessel diameter) at the probability level $P < 0.05$, using JMP (SAS Institute 2003). The probability level $P < 0.15$ was considered to indicate a trend.

Results

Effects of elevated [CO_2] and N supply on photosynthesis and water relations

In oak, stomatal conductance (g_s) and transpiration (T_r) were slightly, but not significantly, decreased under elevated [CO_2] as compared to ambient [CO_2] (Table 1). Elevated [CO_2] enhanced light-saturated net photosynthetic rates per LA, in particular in high-N plants (Table 1). There was a significant [CO_2]-dependent promotion of the instantaneous WUE (Table 1). g_s and T_r in high-N plants were significantly higher than in low-N plants, but WUE was not significantly affected by N supply (Table 1).

Alder showed similar statistically insignificant tendencies for g_s in response to elevated [CO_2] (Table 2). Elevated [CO_2] enhanced A_{sat}, whereas there were no significant [CO_2] effects on T_r and WUE. No significant N effects were found on g_s, T_r and WUE in alder (Table 2). No significant interactive effects of [CO_2] and N on g_s, T_r and WUE were detected in oak (Table 1) and alder (Table 2).

Effects of elevated [CO_2] and N supply on biomass allocation

In oak, there were no significant effects of [CO_2] on total LA and T/R ratio, whereas LAR tended to decline under

Table 1 Physiological parameters related to photosynthesis, water availability and biomass allocation and their dependence on [CO_2] and nitrogen supply in *Quercus mongolica*

	High N		Low N		Two-way ANOVA		
	36 Pa	72 Pa	36 Pa	72 Pa	CO_2	N	CO_2 × N
A_{sat} (μmol m^{-2} s^{-1})	12.42 (±1.33)	19.84 (±2.45)	5.60 (±0.63)	6.4 (±1.21)	0.003	<0.0001	0.056
Stomatal conductance (mol m^{-2} s^{-1})	0.20 (±0.05)	0.16 (±0.03)	0.10 (±0.02)	0.04 (±0.01)	0.084	0.003	NS
Transpiration (mmol m^{-2} s^{-1})	2.02 (±0.39)	1.73 (±0.33)	1.13 (±0.18)	0.49 (±0.13)	0.142	0.002	NS
Water use efficiency (μmol CO_2 [mmol H_2O]$^{-1}$)	6.79 (±0.71)	13.05 (±1.78)	5.24 (±0.46)	14.12 (±0.80)	0.021	NS	NS
Leaf area (m^2 $plant^{-1}$)	0.15 (±0.01)	0.14 (±0.01)	0.10 (±0.01)	0.10 (±0.01)	NS	<0.0001	NS
Leaf area ratio (m^2 kg^{-1})	2.41 (±0.08)	1.79 (±0.10)	2.66 (±0.12)	2.45 (±0.17)	0.148	0.001	0.073
Top:root dry mass ratio (g g^{-1})	0.76 (±0.07)	0.59 (±0.06)	0.53 (±0.02)	0.52 (±0.04)	NS	0.007	0.139
Stem basal diameter (mm)	11.77 (±0.24)	12.61 (±0.50)	8.30 (±0.39)	7.62 (±0.16)	NS	<0.0001	0.031
Total dry biomass (g)	61.68 (±4.00)	80.23 (±3.01)	36.90 (±1.94)	37.27 (±2.96)	0.031	<0.0001	0.010
LMA (g m^{-2})	71.95 (±2.62)	77.87 (±2.70)	63.55 (±2.65)	79.79 (±2.24)	0.019	NS	0.068

Values in parentheses are standard errors of the means ($n = 6$); results of two-way ANOVA (actual P values) are also shown

NS not significant, A_{sat} light-saturated net photosynthetic rates per leaf area, *LMA* leaf mass per area

elevated [CO_2]. There were significant effects of N on LA, LAR and the T/R ratio (Table 1), and also significant interactive effects of [CO_2] and N on stem basal diameter and total biomass (Table 1). High N treatment enhanced total biomass in both [CO_2] treatments after 141 days, whereas high-N plants showed significantly increased total biomass under elevated [CO_2]. LA, T/R ratio, basal stem diameter and total biomass increased with increased N supply. There was a significant CO_2-induced increase of LMA in low-N plants (Table 1); a similar trend occurred in high-N plants.

In alder, high-N plants showed high LA at elevated [CO_2] (Table 2). No significant [CO_2] effects on LAR and T/R ratio were found in any N treatment. Stem basal diameter tended to be larger under elevated [CO_2], in particular in high-N plants (Table 2). Total biomass accumulation under elevated [CO_2] was stimulated (Table 2). Stem diameter and total biomass under elevated [CO_2] and low-N tended to be larger than that at ambient [CO_2] in alder but not in oak. Elevated [CO_2] tended to increase LMA in both N treatments. Higher N supply stimulated total biomass, but no significant interactive effects of [CO_2] and N on biomass became evident.

Effects of elevated [CO_2] and N supply on wood anatomy

Transverse sections of secondary xylem of oak seedlings were analyzed (Fig. 1). Oak produces ring-porous wood, but young seedlings showed semi-ring-porous traits. Under

Table 2 Physiological parameters related to photosynthesis, water availability and biomass allocation and their dependence on [CO_2] and nitrogen supply in *Alnus hirsuta*

	High N		Low N		Two-way ANOVA		
	36 Pa	72 Pa	36 Pa	72 Pa	CO_2	N	CO_2 × N
A_{sat} (μmol m^{-2} s^{-1})	15.35 (±0.63)	16.31 (±0.61)	14.11 (±0.47)	16.02 (±0.72)	0.0002	NS	NS
Stomatal conductance (mol m^{-2} s^{-1})	0.69 (±0.16)	0.39 (±0.09)	0.71 (±0.07)	0.53 (±0.09)	0.146	NS	NS
Transpiration (mmol m^{-2} s^{-1})	4.30 (±0.72)	3.28 (±0.57)	4.59 (±0.16)	3.96 (±0.34)	NS	NS	NS
Water use efficiency (μmol CO_2 [mmol H_2O]$^{-1}$)	3.98 (±0.81)	6.34 (±1.59)	3.08 (±0.14)	4.23 (±0.50)	NS	NS	NS
Leaf area (m^2 $plant^{-1}$)	0.39 (±0.04)	0.55 (±0.04)	0.40 (±0.05)	0.40 (±0.04)	0.048	0.153	0.110
Leaf area ratio (m^2 kg^{-1})	5.83 (±0.37)	5.10 (±0.32)	5.79 (±0.36)	5.22 (±0.39)	NS	NS	NS
Top:root dry mass ratio (g g^{-1})	1.83 (±0.19)	1.70 (±0.15)	2.06 (±0.16)	1.75 (±0.12)	NS	NS	NS
Stem basal diameter (mm)	14.82 (±0.77)	18.07 (±0.12)	14.97 (±0.50)	15.94 (±0.61)	0.059	0.109	0.089
Total dry biomass (g)	69.01(±9.46)	106.80 (±4.53)	67.89 (±5.82)	77.12 (±6.10)	0.040	0.045	0.076
LMA (g m^{-2})	40.28 (±3.57)	48.73 (±2.16)	34.06 (±2.64)	47.40 (±3.23)	0.114	NS	NS

Values are means with standard errors ($n = 6$); for details on statistical analysis, see Table 1 high-N treatment (Fig. 1a, b), earlywood regions were wider than under low-N seedlings (Fig. 1c, d) and larger vessels appeared.

In oak, total vessel area and the mean vessel area were not significantly influenced by [CO_2] in any N treatment, while N increased both the total vessel area and the mean vessel area (Fig. 2). No interactive effects of [CO_2] and N were detected.

In alder, there was significant interactive effect of [CO_2] and N on vessel density, and there were also trends of interaction between [CO_2] and N on total vessel area and vessel proportion (Fig. 3). Total vessel area and vessel proportion tended to be larger under elevated [CO_2] at high N. There was a significant positive effect of N on mean vessel area (Fig. 3).

We analyzed vessel size frequency to clarify effects of [CO_2] and N (Fig. 4). No effect of elevated [CO_2] was shown in vessel size frequency in both species, except class A (<1,000 μm^2) in alder. In oak, the size frequency was higher in high-N than in low-N plants in class D (3,000–4,000 μm^2), E (4,000–5,000 μm^2) and F (>5,000 μm^2). In alder, N supply increased vessel size frequency in class C (2,000–3,000 μm^2).

"Hydraulic mean" vessel diameters in oak showed significant effects of N supply but not of [CO_2] (Fig. 5). In alder, high-N plants had a tendency to increase the "hydraulic mean" vessel diameter, but none of the differences observed were significant (Fig. 5).

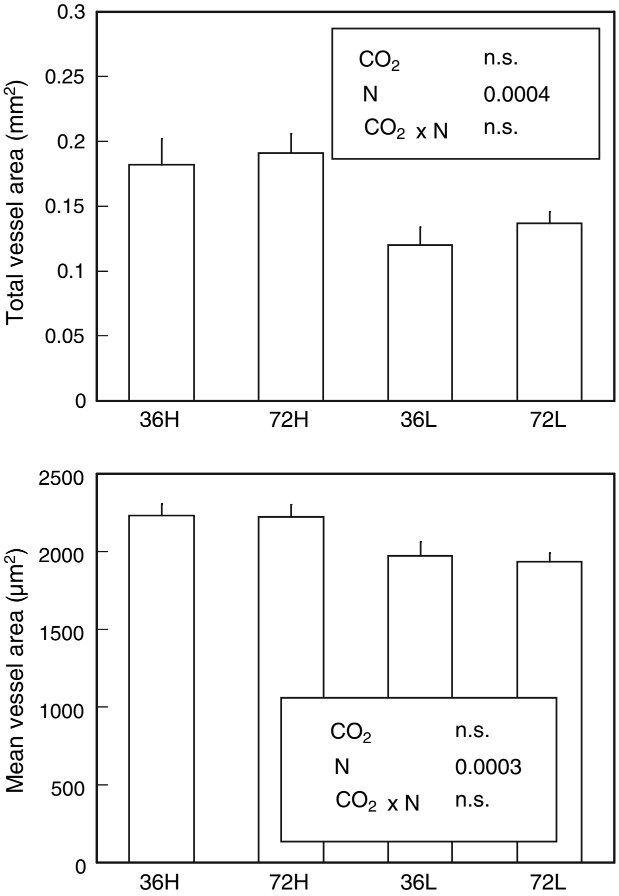

Fig. 2 Total vessel area and mean vessel area of *Quercus mongolica* seedlings. Values shown are means + SE ($n = 6$). H, high-N; L, low-N; 36, ambient-[CO_2]; 72, elevated-[CO_2]. Results of two-way ANOVA are also shown (*n.s.* not significant). Actual *P* values are shown when $P < 0.15$

Discussion

Reductions in g_s and T_r, and enhanced WUE induced by elevated [CO_2] will ameliorate the internal water balance in plants (e.g., Tyree and Alexander 1993). Furthermore, elevated [CO_2] enhances whole-plant biomass, in particular root biomass and LA (Ceulemans and Mousseau 1994; Saxe et al. 1998). In this study, we examined whether

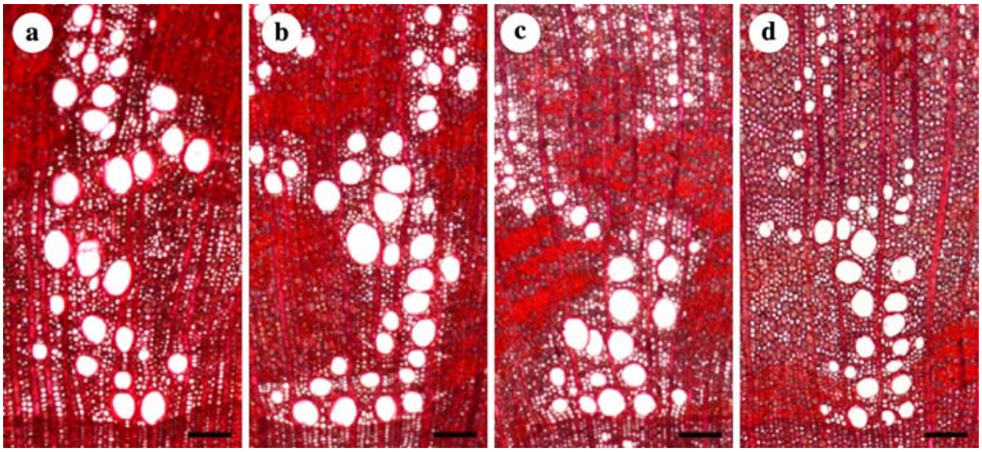

Fig. 1 Vessel elements in earlywood of *Quercus mongolica* seedlings. **a** 36 Pa [CO_2]-high N. **b** 72 Pa [CO_2]-high N. **c** 36 Pa [CO_2]-low N. **d** 72 Pa [CO_2]-low N. *Scale bars* 100 µm

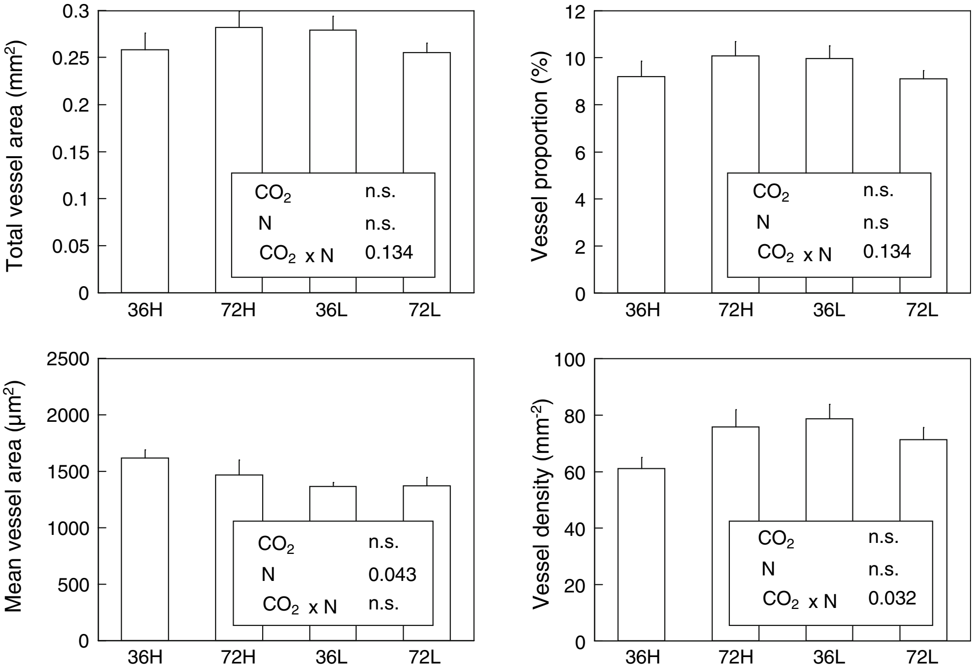

Fig. 3 Total vessel area, mean vessel area, vessel proportion and vessel density (per mm^2) of *Alnus hirsuta* seedlings. Values shown are means + SE ($n = 5$–6). H, high-N; L, low-N; 36, ambient-[CO$_2$]; 72, elevated-[CO$_2$]. Results of two-way ANOVA are also shown (*n.s.* not significant). Actual *P* values are shown when $P < 0.15$

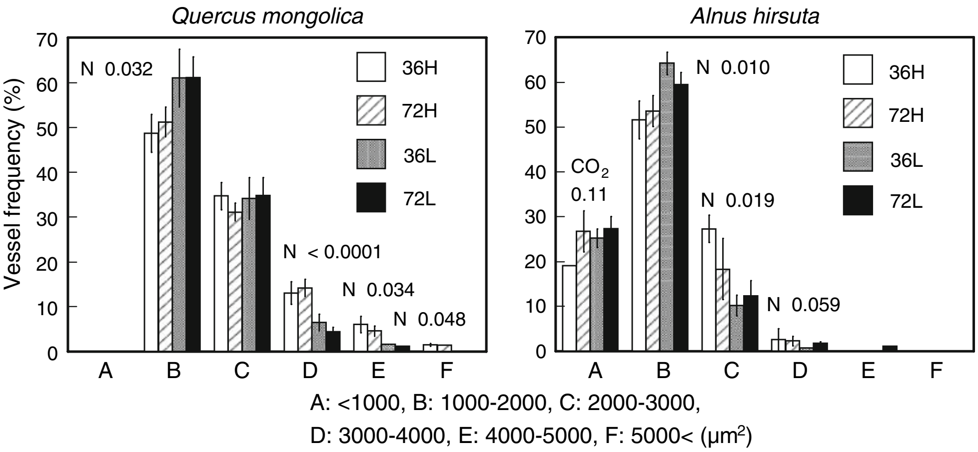

Fig. 4 Vessel frequency in *Quercus mongolica* and *Alnus hirsuta* seedlings. Values shown are means ± SE ($n = 5$–6). H, high-N; L, low-N; 36, ambient-[CO$_2$]; 72, elevated-[CO$_2$]. Results of two-way ANOVA are also shown. Actual *P* values are shown when $P < 0.15$. No significant interactive effects of [CO$_2$] and N were found in any of the classes (at $P < 0.15$)

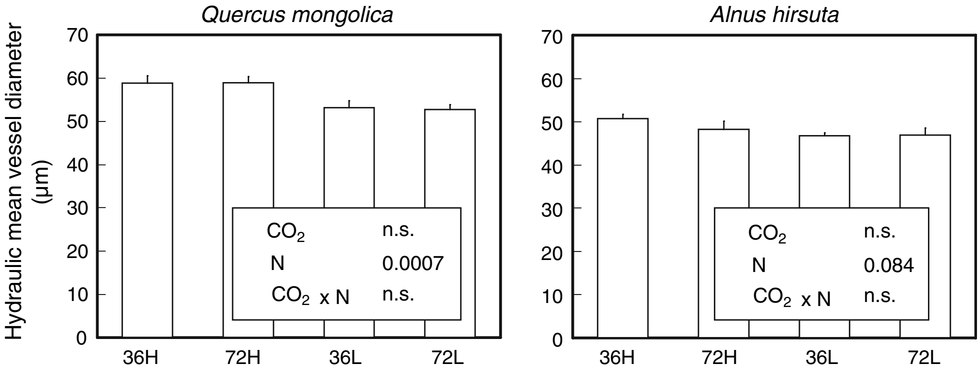

Fig. 5 Hydraulic mean vessel diameter in *Quercus mongolica* and *Alnus hirsuta* seedlings. Values shown are means + SE ($n = 5$–6). H, high-N; L, low-N; 36, ambient-[CO$_2$]; 72, elevated-[CO$_2$]. Results of two-way ANOVA are also shown (*n.s.* not significant). Actual *P* values are shown when $P < 0.15$

CO$_2$-dependent changes in water balance and water allocation patterns correlated with modified wood structure in the stem of two hardwood species under doubled [CO$_2$], 72 Pa, which is predicted to be reached by the end of this century as an effect of fossil fuel burning and land cover change (Houghton et al. 2001).

The physiological properties except A_{sat} (g_s, T_r and WUE) of oak seedling were affected by elevated [CO$_2$]

similarly in both N treatments, and the seedlings grown under elevated [CO_2] tended to reduce T_r and enhance WUE (Table 1). These results agree with those for *Quercus alba* (e.g., Norby et al. 1995), *Q. robur* (e.g., Heath and Kerstiens 1997) and *Q. mongolica* (Oh and Choung 2005). Though Norby et al. (1995) pointed out that increased LA under high [CO_2] results in a decrease of g_s, which helps to balance water supply and demand in *Q. alba*, LA of oak seedlings was unaffected by elevated [CO_2] in this study (Table 1). The [CO_2]-induced physiological effects we observed in *Q. mongolica* were not matched by any significant changes of wood structure related to water transport (Fig. 2). In contrast, Atkinson and Taylor (1996) found that, in *Q. robur* seedlings, total stem vessel area, mean vessel area and vessel number increased significantly under elevated [CO_2]. Because we measured vessel lumen area in earlywood formed at about 2 weeks after CO_2 exposure, it is possible that physiological changes had not affected wood structure yet.

The effects of elevated [CO_2] on the morphological properties except LA of oak seedlings were different between N treatments (Table 1). With high N supply, elevated [CO_2] enhanced total biomass, in particular root biomass indicated by lower T/R ratio compared to ambient [CO_2] (Table 1), which is in accord with a previous study (Oh and Choung 2005). Similarly, seedlings of *Q. virginiana* treated with high [CO_2] combined with high N levels enhanced total biomass, in particular height and stem diameter (Tognetti and Johnson 1999). On the other hand, an imbalance of other nutrient elements to N could be a reason for the observed T/R ratios (Ericsson et al. 1996).

Increased biomass allocation to roots in high-N seedlings grown under elevated [CO_2] might result in increased water and nutrient uptake. Increased N supply had stronger effects on vessel formation than elevated [CO_2] and induced larger vessels in wider earlywood region in oak (Figs. 1, 2, 4). Consequently, the “hydraulic mean” vessel diameter increased, suggesting increased hydraulic conductance in the stem (Fig. 5). In oak, combined effects of elevated [CO_2] and nitrogen supply might enhance water uptake and transport, but larger vessels also affect wood quality in oak (Savill and Mather 1990).

In alder, there was no interactive effect of [CO_2] and N on the physiological properties, and the seedlings grown under elevated [CO_2] enhanced A_{sat} and tended to reduce g_s (Table 2). In contrast, Hibbs et al. (1995) showed that elevated [CO_2] enhanced g_s and WUE in well-watered *Alnus rubra* seedlings. In our study, responses to elevated [CO_2] in terms of biomass allocation, LA, stem basal diameter and total biomass differed between N treatments (Table 2). The enhanced total biomass and stem basal diameter with unchanged LA observed at low N appears to indicate changes in the internal water balance. At high N, elevated [CO_2] enhanced both LA and total biomass and stem basal diameter.

In alder, interactive effects of [CO_2] and N causes changes in wood properties, such as total vessel area, vessel proportion and vessel density. Low-N plants tended to decrease total vessel area, because vessel density tended to decrease without change in mean vessel area under elevated [CO_2] (Fig. 3). The observed imbalance between increased stem diameter (water supply) and unchanged LA (water demand) is in line with the decreased vessel density.

On the other hand, high-N plants of alder tended to increase total vessel area due to increased vessel density at elevated [CO_2], and increased N supply induced larger mean vessel area (Fig. 3). These results imply an increased hydraulic conductance under high-N (Fig. 5). In alder, we measured vessel lumen area in the central parts of annual rings formed in the same year. Therefore, changes in biomass allocation caused by interaction between elevated [CO_2] and N supply, i.e., increased stem diameter and unchanged LA at low N and increased stem diameter and LA at high N, cause an inner water balance, resulting in the altered wood structure.

Because earlywood vessels in ring-porous woods are the main determinants of water transport capacity (Chaney and Kozlowski 1977; Utsumi et al. 1999), they may show responses to elevated [CO_2] in the long term. In our study, however, oak seedlings grown under elevated [CO_2] showed no short-term effects. CO_2 exposure might induce changes in wood structure following alterations in the internal water balance in oak. However, because vessels formed in diffuse-porous wood remain functional for several years (Chaney and Kozlowski 1977; Utsumi et al. 1998), structural changes of wood tissue may not occur immediately after the onset of CO_2 exposure. In alder (*A. hirsuta*), there were interactive effects of [CO_2] and N on wood structure related to water transport (Fig. 3). No significant changes in mean vessel size and total vessel area were found in seedlings of *Prunus avicum* × *pseudocerasus* (Atkinson and Taylor 1996) and *Betula pendula* (Kostiainen et al. 2006) grown under elevated [CO_2]. On the other hand, Gartner et al. (2003) showed that *Q. ilex* grown under elevated [CO_2] developed a higher proportion of large-diameter vessels and a greater mean vessel area than plants grown under ambient [CO_2]. Enrichment of [CO_2] led to wider vessels in *Populus nigra* and *Populus* × *euramericana* but not in *Populus alba* (Luo et al. 2005). These discrepancies might be due to different durations of CO_2 exposure or specific sensitivities to [CO_2] stimulation in different species. Our study suggests that diffuse-porous woods might regulate vessel size and/or numbers in response to changes in the internal water balance induced by elevated [CO_2] and N supply.

Both oak and alder seedlings showed an increase in mean vessel area in response to increased N supply (Figs. 2, 3). Cooke et al. (2003) indicated that N-dependent changes in gene expression will alter the partitioning of C and N and subsequently influence wood quality and the quantity of materials provided for the biosynthesis of cell wall components. Therefore, changes in wood structure such as wider earlywood and an increased number of large vessels in oak, as well as an increase in mean vessel area in alder, may be due to altered gene expression patterns under enhanced N supply. *Quercus mongolica* is a gap-dependent species and probably responds to nutrient conditions. On the other hand, alder is an N_2-fixing species. At low N supplies, *A. hirsuta* forms large amount of root nodules; photosynthates may be allocated to nodulated roots to support the activity of the *Frankia* symbionts in the nodules (Tjepkema 1985). At high-N, plants may preferentially take up N provided as fertilizer, reducing the demand for photosynthates. It is likely that these different allocation patterns affect cambial activity, leading to altered wood structure.

This study conducted short-term CO_2 exposure in controlled environments using phytotron chambers with 1-year-old seedlings in pots. Since the exposure techniques used for CO_2 enrichments are likely to alter the environment surrounding the seedlings (e.g., Arp 1991; Ainsworth and Rogers 2007), it will be impossible to scale the seedling responses of our study to whole trees and forest stands under natural conditions as previously suggested (Körner 1995; Nowak et al. 2004). However, the controlled environments should be useful to clear the interactive effects of elevated [CO_2] and N supply on the physiological properties, such as stomatal conductance and biomass allocation, and wood properties, because the other environmental factors will be removed during experiments. In addition, it is important to determine the characteristic response of each species to elevated [CO_2] and N deposition, because the responses to global climate changes depend on species (Nowak et al. 2004). Therefore, our results might contribute an indicator on wood anatomical features of trees in the near future.

Conclusion

Nitrogen deposition affects wood structure more strongly than elevated [CO_2]. Combinatory effects of [CO_2] and N enhance total biomass, but the increase in the proportion of larger vessels decreases wood quality in oak and alder, irrespective of the ability to fix N_2 of the latter. Changes in wood properties would affect CO_2 sink ability of forest trees.

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