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ORIGINAL ARTICLE

Zhi-Bin Luo · Rosemarie Langenfeld-Heyser · Carlo Calfapietra · Andrea Polle

Influence of free air CO_2 enrichment (EUROFACE) and nitrogen fertilisation on the anatomy of juvenile wood of three poplar species after coppicing

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Abstract *Populus* × *euramericana*, *P. alba*, and *P. nigra* clones were exposed to ambient or elevated (about 550 ppm) CO_2 concentrations under field conditions (FACE) in central Italy. After three growing seasons, the plantation was coppiced. FACE was continued and in addition, one-half of each experimental plot was fertilised with nitrogen. Growth and anatomical wood properties were analysed in secondary sprouts. In the three poplar clones, most of the growth and anatomical traits showed no uniform response pattern to elevated [CO_2] or N-fertilisation. In cross-sections of young poplar stems, tension wood amounted to 2–10% of the total area and was not affected by elevated CO_2. In *P. nigra*, N-fertilisation caused an about twofold increase in tension wood, but not in the other clones. The formation of tension wood was not related to diameter or height growth of the shoots. In *P.* × *euramericana* N-fertilisation resulted in significant reductions in fibre lengths. In all three genotypes, N-fertilisation caused significant decreases in cell wall thickness. In *P.* × *euramericana* and *P. alba* elevated [CO_2] also caused decreases in wall thickness, but less pronounced than nitrogen. In *P. nigra* and *P.* × *euramericana* elevated [CO_2] induced increases in vessel diameters. These results show that elevated [CO_2] and N-fertilisation affect wood structural development in a clone specific manner. However, the combination of these environmental factors resulted in overall losses in cell wall area of 5–12% in all three clones suggesting that in future climate scenarios negative effects on wood quality are to be anticipated if increases in atmospheric CO_2 concentration were accompanied by increased N availability.

Keywords Climate change · Elevated CO_2 · N-fertilisation · Wood anatomy · *Populus*

Z.-B. Luo · R. Langenfeld-Heyser · A. Polle (✉)
Institut für Forstbotanik, Georg-August Universität,
Büsgenweg 2,
37077 Göttingen, Germany
e-mail: apolle@gwdg.de
Tel.: +49-551-393480
Fax: +49-551-392705

C. Calfapietra
Department of Forest Environment and Resources (DISAFRI),
Universita degli Studi della Tuscia,
Via S. Camillo de Lellis,
01100 Viterbo, Italy

Introduction

Forest ecosystems cover 43% of the terrestrial biosphere (Melillo et al. 1993). During the past century human activities, such as combustion of fossil fuels, deforestation, wide application of nitrogen-containing fertilisers, etc., have resulted in a dramatic increase in the atmospheric CO_2 concentration and enhanced nitrogen deposition (Huang et al. 1999). Increased [CO_2] is expected to increase biomass accumulation and net primary productivity of forest ecosystems (Melillo et al. 1993; Wullschleger et al. 1995; Gielen and Ceulemans 2001; Calfapietra et al. 2003a). Increasing nitrogen deposition also has been observed to stimulate wood production (Brix 1981; McGuire et al. 1992, 1993).

Despite compelling evidence that these environmental factors can result in enhanced above-ground biomass and increased annual ring width (Hättenschwiler et al. 1996; Telewski et al. 1999; Yazaki et al. 2001; Ceulemans et al. 2002; Mäkinen et al. 2002), there is no clear picture how CO_2 and nitrogen affect the anatomical properties of wood. For example, Atkinson and Taylor (1996) found that elevated [CO_2] significantly increased both vessel number and mean vessel size of *Quercus* seedlings, but had no influence on the vessel number and size of *Prunus* seedlings. In *Pinus radiata* CO_2 enrichment caused no differences in tracheid length, lumen diameter or wall thickness (Donaldson et al. 1987). In contrast to these results, Conroy et al. (1990) reported that tracheid wall thickness increased by 44% in *Pinus radiata*, whereas Yazaki et al. (2001) found reduced wall thickness in *Larix sibirica* under elevated [CO_2]. Ceulemans et al. (2002) found that the wood of *Pinus sylvestris* grown at elevated

[CO_2] contained significantly increased numbers of tracheids, tracheid diameters, and annual ring widths, whereas wood density remained unchanged and wood strength decreased.

Although high nitrogen increased the growth rate and resulted in higher stem wood production, impacts of high nitrogen supply on wood quality were questioned (Shupe et al. 1996; Pape 1999). Several studies have shown that fertilisation enhanced tracheid lumen diameter, decreased cell wall thickness and the basic density of wood (Brolin et al. 1995; Lindström 1996). The results of Yang et al. (1988) and Dutilleul et al. (1998) suggest that increased nutrient availability might decrease fibre length; however, contradictory results were also reported (Schmidtling 1973; Zobel and Van Buijtenen 1989).

Obviously, these contrasting data demand further investigations before general conclusions about the influence of enriched [CO_2] and nitrogen on wood structure and quality can be drawn (Saxe et al. 1998; Pritchard et al. 1999; Ward and Strain 1999; Ceulemans et al. 2002). With respect to these uncertainties it is important to analyse the wood of trees exposed to elevated [CO_2] and additional nitrogen fertilisation under field conditions and to compare the CO_2 responses in different species to find out whether the responsiveness to CO_2 depends strongly on the genetic background or not.

The objective of the present study was to investigate the impact of elevated [CO_2] and N-fertilisation on wood structure and quality of field-grown trees. For this purpose the study was conducted at the EUROFACE field site, where poplars have been grown under ambient and elevated [CO_2] of about 550 ppm since 1999. In 2001 the trees had reached heights of 8.5–9.3 m (Calfapietra et al. 2003b) and the plantation was cut. Secondary sprouts developed from the stools in 2002. We used this experimental approach to address the following questions: do elevated [CO_2] and nitrogen as single factors or in combination: (1) affect tension wood formation or is tension wood formation mainly related to growth characteristics (radial growth, height), (2) affect the structure of normal wood, and (3) affect the anatomical properties of wood elements (fibre lumen, vessel lumen, length, etc.)? Since the EUROFACE field site contains three different poplar clones (*P. alba*, *Populus* × *euramericana* and *P. nigra*) with different growth characteristics, the results of this study can furthermore contribute to elucidate whether the responsiveness of certain traits to [CO_2] and nitrogen enrichment is uniform or is genotype-dependent.

Materials and methods

Site description

The study site is located in central Italy (42°22′N, 11°48′E, altitude 150 m) on 9 ha of former agricultural land. In spring 1999, following detailed soil analysis, six 30 m ×30 m experimental areas ("plots") were selected and FACE facilities were installed in three of the plots whereas the other three plots, representing the control treatment, were left under natural conditions. The minimum distance between plots is 120 m to avoid cross-contamination between FACE and control treatments. The CO_2 enrichment was realised through octagonal polyethylene rings (22 m diameter) mounted on telescopic poles. Pure CO_2 (Messer Griesheim) was released through laser-drilled holes in the polyethylene rings to achieve the target [CO_2] (550 μmol mol^{-1}) inside the FACE plots. A meteorological station located at the centre of each FACE plot was used to control the release of CO_2, whose amount was determined by wind direction and speed and by an algorithm developed for the FACE facility according to a 3-D gas dispersion model. The FACE system was controlled and monitored by a computer to reach the target [CO_2]. In the FACE plots daytime [CO_2] was 554 ±1.6 μmol mol^{-1} during the growing season of 2002 (from bud burst to leaf fall; F. Miglietta, CNR-IATA, Florence, Italy, unpublished data). A detailed description of FACE facilities was given by Miglietta et al. (2001).

Plant material and plantation layout

In spring 1999, on 9 ha of land six experimental plots with homogenous soil and microclimatic conditions were selected and on the surrounding land *P.* × *euramericana* (Dode) Guinier (clone I-214) was planted at a planting density of 5,000 trees per ha (2 m ×1 m). Each experimental plot was divided into halves by a physical resin-glass barrier (1 m deep in the soil) to provide N-fertilisation in each half plot. Each half plot was further divided into three slices (subplots), planted with one of three poplar clones, *P. alba* L. (2AS-11), *P.* × *euramericana* (Dode) Guinier (I-214), and *P. nigra* L. (Jean Pourtet), at a planting density of 10,000 trees per ha (1 m ×1 m). A detailed description of the clone properties was given by Calfapietra et al. (2001). In 2001, the trees reached heights of 8.5–9.3 m (Calfapietra et al. 2003b) and all trees were cut to the base of the stem at 5–8 cm above the ground. As a result, secondary sprouts developed from the stools in the spring of 2002. During the growing season of 2002, Navarson (Amiad, Imago, Italy), a fertiliser with a 10:3:3 NPK content (nitrogen with a 4:1 NH_4^+: NO_3^- ratio) and micro-nutrients, dissolved in 200 l tanks, was applied once per week, starting July 8, for a period of 16 weeks, through hydraulic pumps installed outside plots and a drip-irrigation system. A total amount of 212 kg N ha^{-1} was supplied throughout the growing season.

Sampling

In the first week of September 2002, the first harvest was achieved by the following sampling strategy: two stools were marked randomly per subplot; on each marked stool, the shoot with the second thickest diameter, measured at

the height of 20 cm above the stool, was harvested. The shoot with the third thickest diameter was marked for the following harvest in March 2003. A total of 72 shoots were harvested from the six experimental plots. Each selected shoot was cut at the stool level. Its height and biomass were determined. An 8 cm long stem segment was removed from the height of 1.92 m to 2.00 m of each shoot and preserved for anatomical studies in FAE (37% formalin/glacial acetic acid/70% ethyl alcohol =5 parts/5 parts/90 parts) in wide-mouthed jars with lids. The sampling was repeated in March 2003.

Analysis of wood anatomy

Stem cross-sections (30 μm) were obtained with a sliding microtome (Reichert-Jung, Heidelberg, Germany) and mounted in 50% glycerol for microscopy. For an overview sections were viewed by fluorescence microscopy (Axioskop, Zeiss, Oberkochen, Germany) using the filter combination G365/FT395/LP420 to document autofluorescence. Sections were stained for 10 min with toluidine blue (pH 7.0, w/v=0.05%). Well-stained sections and a micrometer scale were photographed under a light microscope (Axioskop, Zeiss, Oberkochen, Germany) with a digital camera (Nikon CoolPix 990, Nikon, Tokyo, Japan) with 40× and 400× magnifications. In addition, photographs of stem cross-sections from cambium to pith were taken under a binocular (Stemi SV11, Zeiss, Oberkochen, Germany) with 6× magnification. A 2.5 cm part of the same stem segment was removed and dried at room temperature. Then its cross-sectional plane was polished, stained with zinc chloriodide (Purvis et al. 1966) which stained cellulose indicative for tension wood and photographed under a binocular with 6× magnification.

Microphotographs of normal wood were analysed by an image analysis software (analySIS 3.2, Soft Imaging System, Münster, Germany) for the following parameters: diameter of vessel and fibre lumina, thickness of double fibre wall (the wall between two adjacent fibre cells), areas of ray parenchyma cells, vessel lumina, and fibre lumina as well as the number of vessels per unit area. The unit area was set as a square-shaped area of 598,333 μm^2. The percentage of cell wall area (CWA) was calculated as follows:

$$\mathrm{CWA}(\%) = \left[\text{total area} - \begin{pmatrix}\text{vessel lumen area}\\ +\text{fibre lumen area}\\ +\text{ray parenchymaarea}\end{pmatrix}\right] \times 100/\text{total area}$$

The xylem width was measured as the distance from the cambium to the pith. For tension wood determination, sections stained purple by zinc chloriodide in whole-stem cross-sections were measured. The percentage of tension wood area (TWA, portion of purple sections) was calculated as follows:

$$\mathrm{TWA}(\%) = \text{tension wood area} \times 100/(\text{total area} - \text{pith area})$$

To determine the lengths of vessel elements and fibres, the same stem segments as those used for cross-sections were chosen. About 1 mm width of wood next to the cambium was discarded to avoid young xylem cells and the residual wood was cut longitudinally into pieces for chemical maceration in 65% nitric acid (Merck, Darmstadt, Germany) and traces of sodium chlorate (Merck, Darmstadt, Germany) after Kitin et al. (1999). After 2 h of maceration at room temperature, the materials were transferred into reaction cups and centrifuged (3,200*g*, 2 min, Eppendorf Centrifuge). The pellets were washed with distilled water, centrifuged again, and then preserved in 70% ethanol for further analysis. The prepared cell mixture was stained with toluidine blue (pH 7.0, w/v=0.05%) and mounted in 50% glycerol for microscopy. The short lengths of vessel elements (the short distance between the perforations) were measured after the definition of Chalk and Chattaway (1934).

Statistical analysis

To determine the main effects of species (clone), CO_2 treatment (CO_2) and N-fertilisation (nitrogen) on all variables, an ANOVA, i.e. a randomised-complete-block design, with species, CO_2 treatment, N-fertilisation and their interactions as fixed factors and block as a random factor, was applied. All statistical tests were performed in Statgraphics (STN, St Louis, Mo., USA) using the mixed procedure and plot as a replicate. When interactions were significant, a posteriori comparison of means was done. To reduce the chance on type I errors, all *P*-values of these multi-comparisons were corrected by Tukey method. Data were tested for normality with the Shapiro–Wilk's test. Differences between parameter means were considered significant when the *P*-value of the ANOVA *F*-test was less than 0.05.

Results

Growth and tension wood as affected by FACE and N-fertilisation

The heights and diameters of the second and third thickest shoot were measured in September, when the trees were still in the active phase of growth, and in March, when the trees were dormant. There were significant differences between the clones with *P.* × *euramericana* displaying tallest and thickest shoots and *P. alba* the shortest (Table 1). Exposure to FACE conditions caused increases in height and diameter of the selected shoots (Table 1, *P*-values for CO_2 main effect). Nitrogen fertilisation had no

Table 1 Relative tension wood area (*TWA*), shoot diameter (*D*) and shoot height (*SH*). *A*: ambient [CO_2], *E*: elevated [CO_2], *L*: no N-fertilisation, *H*: N-fertilisation. The fractional TWA was determined per whole cross-sectional area. The whole cross-sectional area (100%) was defined as the total xylem cross-sectional area minus pith area. The shoot diameter was determined at 0.2 m above stool level. *0902*: harvest in September 2002; *0303*: harvest in March 2003. Data indicate means (±SD, *n*=6). The values followed by different letters in the same column indicate significant differences at $P \leq 0.05$ for all treatments and species. *P*-values of the ANOVA *F*-test of the main effects of clone, CO_2, nitrogen and their interactions are also indicated

Clones	CO_2	Nitrogen	TWA 0902 (%)	TWA 0303 (%)	D 0902 (mm)	D 0303 (mm)	SH 0902 (m)	SH 0303 (m)
P. alba	A	L	9.7±4.4 c	2.1±2.0 a	23.3±5.1 abc	20.9±4.3 abc	3.7±0.5 abcd	3.6±0.5 abcd
	E	L	4.3±3.1 a	1.5±1.7 a	23.3±4.4 abc	18.9±1.8 abc	3.5±0.4 a	3.5±0.4 abc
	A	H	8.9±4.1 bc	3.3±5.4 ab	21.5±2.3 ab	17.7±3.9 ab	3.7±0.3 ab	3.3±0.4 ab
	E	H	7.0±3.2 abc	3.6±4.7 ab	21.4±2.5 ab	17.1±3.1 ab	3.6±0.3 a	3.5±0.4 abc
P. × euramericana	A	L	5.4±4.3 ab	3.6±3.4 ab	26.8±3.5 cde	21.0±7.7 abc	4.0±0.4 bcdef	3.3±1.0 a
	E	L	3.8±3.4 a	2.4±3.8 a	28.6±3.7 def	22.9±5.2 c	4.1±0.2 def	3.5±0.5 abc
	A	H	3.6±2.2 a	4.9±2.2 ab	30.5±3.6 ef	23.7±5.3 c	4.4±0.3 ef	3.9±0.6 bcd
	E	H	4.4±1.9 a	2.6±1.5 a	32.6±5.0 f	28.9±3.1 d	4.4±0.3 f	4.2±0.3 d
P. nigra	A	L	4.2±2.1 a	3.3±2.5 ab	20.9±5.9 ab	17.4±3.3 ab	3.7±0.6 abc	3.4±0.6 abc
	E	L	5.6±3.9 ab	3.5±2.6 ab	25.5±4.5 bcd	22.1±5.7 bc	4.0±0.2 bcde	3.9±0.5 cd
	A	H	6.5±2.4 abc	7.1±7.3 b	20.4±5.1 a	16.5±3.6 a	3.5±0.5 a	3.3±0.4 a
	E	H	8.9±2.9 bc	7.2±4.9 b	28.1±4.9 cdef	19.3±2.9 abc	4.1±0.2 cdef	3.8±0.5 abcd
P-values (main effects)	Clones		0.0051	0.0625	0.0000	0.0000	0.0000	0.3212
	CO_2		0.3560	0.5216	0.0111	0.0581	0.1616	0.0254
	Nitrogen		0.1675	0.0279	0.3323	0.9768	0.1987	0.3103
P-values (interactions)	Clone × CO_2		0.0169	0.6636	0.0468	0.0939	0.0248	0.2697
	Clone × nitrogen		0.1989	0.4012	0.0802	0.0160	0.1227	0.0099
	CO_2 × nitrogen		0.1455	0.9199	0.5714	0.6473	0.5943	0.5971
	Clone × CO_2 × nitrogen		0.8053	0.8893	0.7750	0.5801	0.8249	0.8024

significant influence as a main factor, perhaps because of the relatively short duration of the treatment (ca. 2 months before harvest in September). Only *P. × euramericana* shoots responded positively with respect to diameter and height growth to N-addition (Table 1, significant *P*-value for the interaction of clone × nitrogen).

To find out whether clone-specific growth characteristics or growth stimulation by FACE or N-fertilisation affected wood properties, we determined the occurrence of tension wood in stem cross-sections of *P. alba*, *P. nigra* and *P. × euramericana*. Tension wood formation was significantly dependent on the clone and in most cases lower in *P. × euramericana* than in the two other species (Table 1). The main factor CO_2 had no and nitrogen only in March significant effects on the proportion of tension wood formed (Table 1). To test whether correlations existed between tension wood and growth parameters, we performed linear correlation analysis between shoot height or diameter and the portion of tension wood area. No significant relationships were found (R^2=0.0006 for tension wood area vs diameter; R^2=0.0001 for tension wood area vs height). The shoot diameters were measured 0.2 m above ground, whereas the stem sections analysed for tension wood were collected about 2 m above the ground. To investigate the possibility that the tension wood area was related to the radial growth observed 2 m above ground, correlation analysis was also conducted for tension wood area and the xylem width of the wood

Table 2 Statistical results of the percentages of cell wall area (*PCWA*), the percentages of vessel lumina area (*PVA*), the percentages of fibre lumina area (*PFA*), the percentages of ray parenchyma area (*PRA*), the vessel frequency (*VF*) and the thickness of double fibre wall (*TDFW*) of three *Populus* species grown under either ambient (*A*) or elevated (*E*) [CO_2] in the presence (*H*) or absence (*L*) of N-fertilisation. *P*-values of the ANOVA *F*-test of the main effects of clone, CO_2, nitrogen and their interactions are indicated

P-values		PCWA	PVA	PFA	PRA	VF	TDFW
Main effects	Clone	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	CO_2	0.0000	0.1554	0.0000	0.0001	0.2851	0.0000
	Nitrogen	0.0000	0.0074	0.0000	0.0078	0.0931	0.0000
Interactions	Clone × CO_2	0.0000	0.1960	0.0000	0.0000	0.0020	0.0240
	Clone × nitrogen	0.0000	0.4350	0.0000	0.5420	0.1890	0.0000
	CO_2 × nitrogen	0.0000	0.8500	0.0020	0.8230	0.0400	0.0000
	Clone × CO_2 × nitrogen	0.7250	0.9380	0.5410	0.0020	0.1430	0.0000

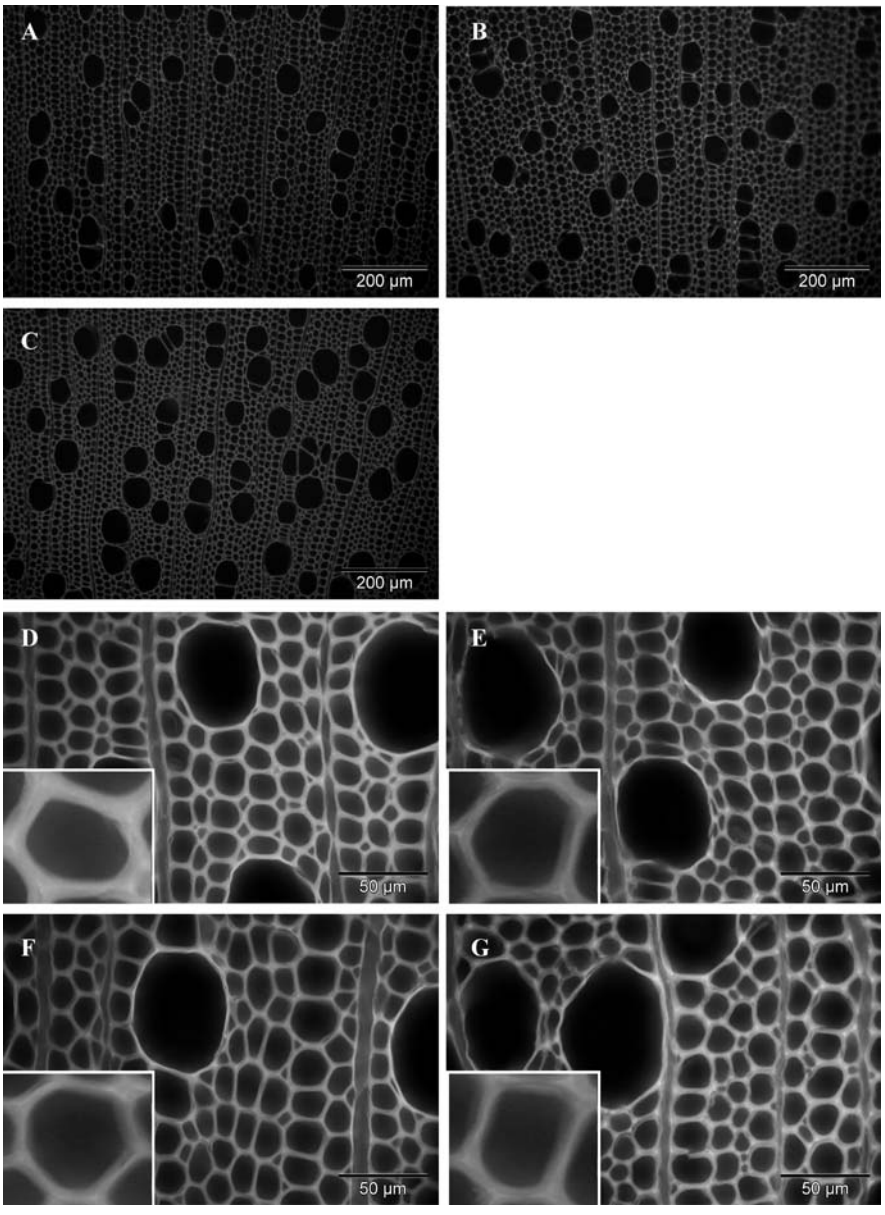

Fig. 1 Typical cross-sections of *P. alba* (**A**), *P.* × *euramericana* (**B**) and *P. nigra* (**C**) grown under ambient [CO_2] in the absence of additional N-fertilisation. For more details, cross-sections of *P. nigra* **D**–**G** were photographed with higher magnification and insets in **D**–**G** show details of typical thickness of double fibre wall under different conditions (**D**: under ambient [CO_2] in absence of N-fertilisation; **E**: under ambient [CO_2] in presence of N-fertilisation; **F**: under elevated [CO_2] in absence of N-fertilisation; **G**: under elevated [CO_2] in presence of N-fertilisation). The sections were viewed by fluorescence microscopy. Magnifications are indicated by scale bars

slice used for anatomical studies (September 2002 samples only). Again no significant correlation was observed (R^2=0.0179).

Influence of FACE and N-fertilisation on structural wood composition

The three poplar clones showed significant structural differences in their normal wood composition (Table 2). The secondary xylem of *P. nigra* displayed larger vessel lumina and higher vessel numbers than those of *P. alba* and *P.* × *euramericana* (Fig. 1a–c). This accumulated to significantly larger vessel lumen areas in *P. nigra* than in the other two genotypes (Fig. 2). In all three poplar species, FACE significantly decreased the percentages of cell wall area compared with those grown at the ambient CO_2 concentration (Fig. 2, Table 2). The decreased portion of cell wall area was mainly caused by increased fibre lumen areas in the three poplar clones (Fig. 2). Furthermore, elevated [CO_2] also resulted in increased percentages of ray parenchyma areas compared with the ambient [CO_2] (Fig. 2, Table 2). N-fertilisation also significantly reduced the percentages of cell wall area in comparison with shoots from unfertilised plots (Fig. 2, Table 2). These decreases were accompanied by decreases in vessel lumina but increases in fibre lumina areas (Fig. 2, Table 2). The observation that N-fertilisation had these consistent effects on wood properties was surprising since the nitrogen

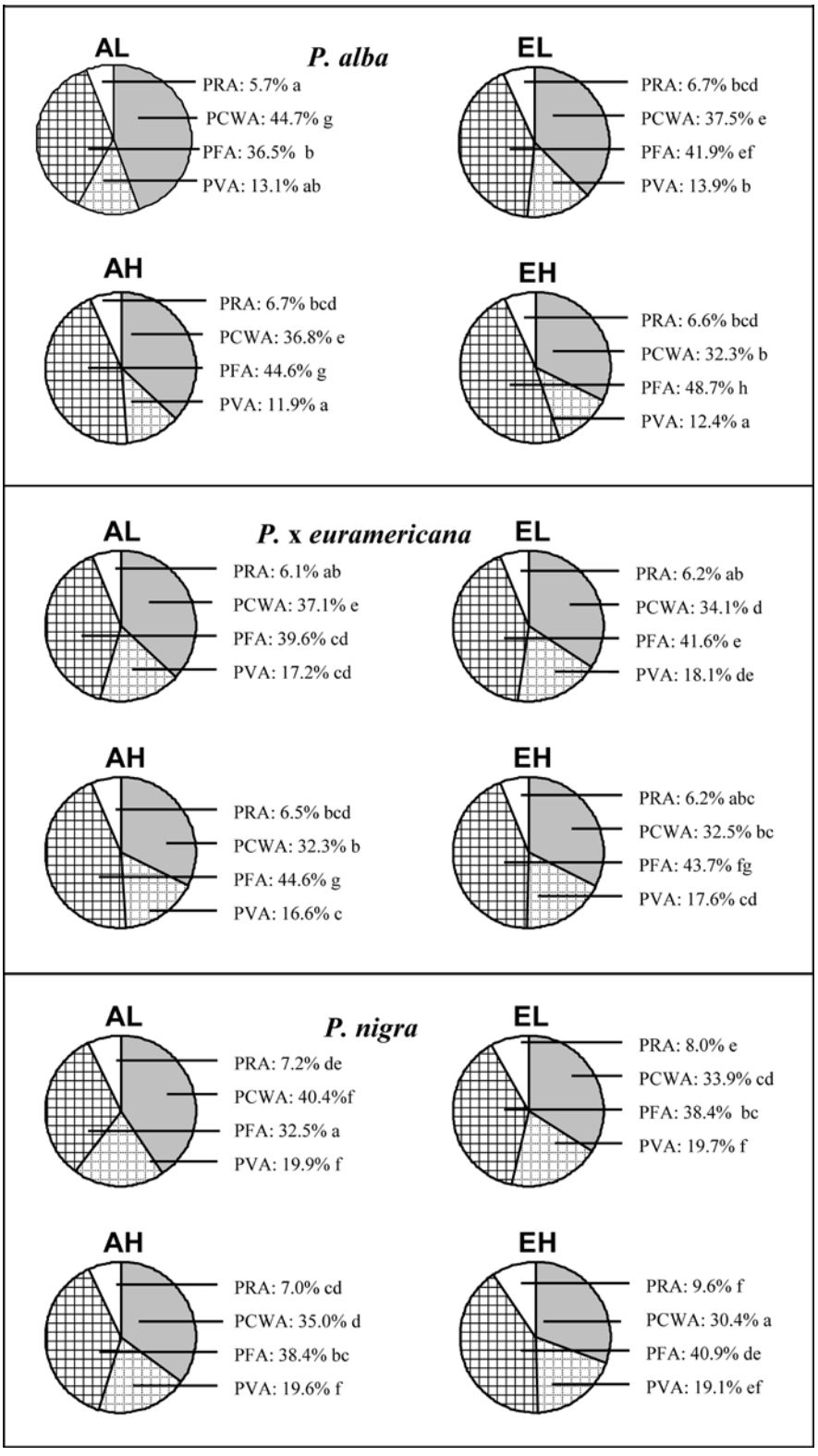

Fig. 2 Relative abundance of cell lumina and wall areas in cross-sections of secondary xylem of *P. alba*, *P.* × *euramericana* and *P. nigra* grown under either ambient (*A*) or elevated (*E*) [CO_2] in the presence (*H*) or absence (*L*) of N-fertilisation. The pies correspond to fractional areas of cell walls (*PCWA*), vessel lumina (*PVA*), fibre lumina (*PFA*), and ray parenchyma (*PRA*). Data indicate means (n=18) and *different letters* indicate significant differences at $P \leq 0.05$

influence on radial or height growth was genotype-specific (Table 1).

Effects of FACE and N-fertilisation on anatomical characteristics of wood

There were significant differences in vessel anatomical traits among the three poplar genotypes (Figs. 1a–c, 3, Tables 2, 3). Under most conditions *P.* × *euramericana*

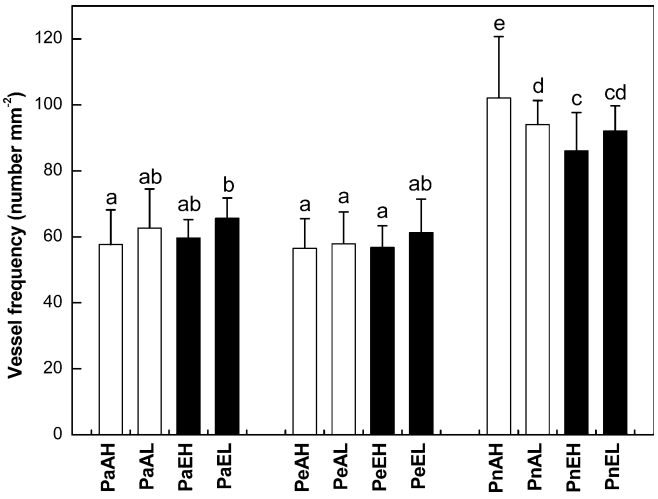

Fig. 3 Vessel frequency (number mm^{-2}) in cross-sections of *P. alba* (*Pa*), *P.* × *euramericana* (*Pe*) and *P. nigra* (*Pn*) grown under either ambient (*A*) or elevated (*E*) [CO_2] in the presence (*H*) or absence (*L*) of N-fertilisation. Bars indicate means (±SD, *n*=18). Different letters indicate significant differences at $P \leq 0.05$

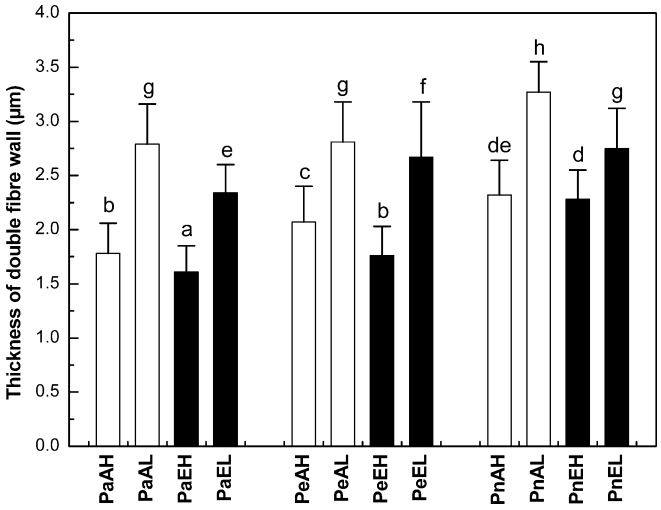

Fig. 4 Thickness of double fibre walls in cross-sections of *P. alba* (*Pa*), *P.* × *euramericana* (*Pe*) and *P. nigra* (*Pn*) grown under either ambient (*A*) or elevated (*E*) [CO_2] in the presence (*H*) or absence (*L*) of N-fertilisation. The bars indicate means (±SD, *n*=216). Different letters indicate significant differences at $P \leq 0.05$

contained the longest vessel elements with diameters intermediate between those of *P. alba* (smaller) and *P. nigra* (larger) (Table 3). The main factors (CO_2) and nitrogen also had significant influence on vessel properties (Table 3). With the exception of *P. alba*, FACE caused decreases in vessel element lengths and increases in vessel element diameters (Table 3). Under most conditions, nitrogen fertilisation resulted in the production of shorter vessel elements (Table 3). A notable exception was *P. alba* under ambient CO_2, where nitrogen fertilisation resulted in about 12% longer vessels.

The vessel frequency in wood of *P. nigra* was significantly higher than in that of *P.* × *euramericana* or *P. alba* (Figs. 1a–c, 3). The main factors [CO_2] and nitrogen had no significant influence on the vessel frequencies (Table 2). Only in *P. nigra* grown under ambient [CO_2] nitrogen fertilisation caused increased vessel numbers, an effect which disappeared when this clone was grown under FACE conditions (Fig. 3).

The anatomical characteristics of fibres were also significantly affected by the three main factors, clone, [CO_2], and nitrogen (Tables 2, 3). *P. alba* wood generally contained fibres with larger average lumen diameters than that of wood of *P.* × *euramericana* or *P. nigra* (Table 3). Growth under FACE had no effect on fibre lumen diameter

Table 3 Anatomical characteristics of vessels and fibres in the secondary xylem. *A*: ambient [CO_2], *E*: elevated [CO_2], *L*: no N-fertilisation, *H*: N-fertilisation; *ADV* average diameter of vessel lumen, *VEL* vessel element length, *ADF* average diameter of fibre lumen; *FL* fibre length. Data indicate means (±SD, $n \geq 36$). The values followed by different letters in the same column indicate significant differences at $P \leq 0.05$ for all treatments and species. *P*-values of the ANOVA *F*-test of the main effects of clone, CO_2, nitrogen and their interactions are indicated

Clones	CO_2	Nitrogen	ADV (µm)	VEL (µm)	ADF (µm)	FL (µm)
P. alba	A	L	67.1±5.9 cd	227.2±52.4 a	20.6±2.7 de	617.8±87.2 e
	E	L	64.1±4.5 b	276.0±45.7de	20.3±2.3 d	669.9±68.4 g
	A	H	65.9±4.6 bcd	268.4±40.5 cde	21.8±2.7 g	677.4±85.9 g
	E	H	65.0±4.1 bc	228.3±43.9 a	21.1±2.6 ef	598.4±71.1 d
P. × *euramericana*	A	L	61.1±6.2 a	328.5±56.9 g	19.6±2.9 c	641.9±84.0 f
	E	L	68.1±7.2 de	321.1±61.5 g	19.6±2.3 c	702.8±86.5 h
	A	H	67.1±6.5 cd	303.7±65.5 f	19.2±2.5 c	551.7±105.9 a
	E	H	77.7±5.4 I	255.2±62.1 bc	21.4±2.6 fg	569.2±132.6 b
P. nigra	A	L	71.9±6.8 g	264.4±58.9 cd	16.2±2.3 a	578.2±63.9 bc
	E	L	75.0±5.6 h	254.7±39.7 bc	17.5±1.5 b	583.1±72.0 c
	A	H	70.1±5.8 ef	282.1±43.9 e	15.7±1.8 a	609.9±79.3 e
	E	H	73.8±5.2 gh	246.8±43.4 b	17.6±1.9 b	597.9±77.5 d
P-values of main effects	Clone		0.0000	0.0000	0.0000	0.0000
	CO_2		0.0000	0.0000	0.0000	0.0012
	Nitrogen		0.0002	0.0001	0.0000	0.0000
P-values of interactions	Clone × CO_2		0.0000	0.0010	0.0000	0.0000
	Clone × nitrogen		0.0000	0.0000	0.0000	0.0000
	CO_2 × nitrogen		0.0490	0.0000	0.0000	0.0000
	Clone × CO_2 × nitrogen		0.5350	0.0010	0.0000	0.0000

of *P. alba* but caused significant increases in *P. nigra* (Table 3). Nitrogen fertilisation caused increased fibre lumina in *P. alba* and *P.* × *euramericana* under FACE (Table 3).

The influence of FACE or nitrogen fertilisation on fibre lengths was also strongly clone-specific. For instance, in *P.* × *euramericana*, N-fertilisation caused strong reductions in fibre lengths, whereas *P. nigra* showed clear increases (Table 3). In *P. alba* the response of fibre length to N-fertilisation was modulated by FACE (Table 3).

An important feature affecting wood density is the thickness of the fibre wall. For practical purposes, we measured the thickness of the walls between two adjacent fibre cells, denominated as "double fibre wall thickness". This parameter was also significantly affected by clone, CO_2, and nitrogen (Fig. 1d–g, Table 2). The double fibre walls of *P.* × *euramericana* and *P. nigra* were significantly thicker by 9.4% and 24.9%, respectively, than those of *P. alba* (Fig. 4). In all three poplar genotypes, FACE significantly decreased the thickness of double fibre wall compared with the ambient [CO_2] (Figs. 1d–g, 4, Table 2). N-fertilisation consistently caused strong decreases of the thickness of double fibre cell walls (Figs. 1d–g, 4, Table 2).

Discussion

Is tension wood formation affected by FACE or N-fertilisation or related to growth characteristics?

An important difference of our study compared with previous studies on tree responses to elevated [CO_2] was that we analysed newly formed poplar shoots after coppicing. The number of shoots formed on stools differed between the different poplar clones (data not shown). Coppicing increased source-to-sink relationships (Hovenden 2003) probably explaining that in contrast to the previous single-stem system (Calfapietra et al. 2001, 2003a) or to other CO_2-response and N-response studies (Conroy et al. 1990; Prior et al. 1997; Jach and Ceulemans 1999; Peltola et al. 2002; Günthardt-Goerg et al. 1996; Yazaki et al. 2001) FACE and N-fertilisation did not always have positive effects on individual shoot heights and diameters (Table 1). This does not imply a lack of nitrogen or FACE effects on biomass because we analysed only selected shoots for the present study.

A major question addressed in this investigation was whether FACE or N-fertilisation stimulated tension wood formation or whether tension wood production was mainly related to growth characteristics (radial growth, height) or clone-specific traits. The observation that the clones showed height and radial diameter differences as well as differences in tension wood but no correlation between these parameters (Table 1 and R^2-values under Results), indicates that tension wood formation was clone-specific but not related to their typical growth characteristics. This was corroborated by the finding that the taller and, thus, probably more wind-exposed *P.* × *euramericana* shoots (2.4–5.4%) produced less tension wood than *P. nigra* (3.3–8.9%).

Gartner et al. (2003) induced tension wood formation experimentally by inclining the pots by 30° and found no influence of elevated [CO_2] on tension wood formation in *Quercus ilex*. In this experimental system the highest tension wood formation was found at the stem base. In upright stems the frequency of tension wood formation at the stem base was similar to that in the middle (Gartner et al. 2003). In our study with naturally inclined or upright shoots, tension wood formation in the middle of the stem was quite variable (1.5–9.7%). This suggests that under field conditions tension wood is primarily a response to inclination angles and other forces acting by chance affecting shoots individually. Our data show that the extent of this response was determined by the genetic constitution and stimulated by N-fertilisation (Table 1). The latter observation is important because it indicates that an overabundance of nitrogen is likely to have negative effects on wood quality. The mechanisms which lead to the stimulation of tension wood production are not clear, but it is possible that the decreased wall thickness found here (Fig. 4) may render stems more flexible and, thus, more prone to tension wood formation. In conclusion, tension wood formation in poplar shoots was genotype-dependent and stimulated by nitrogen fertilisation but not related to radial and height growth.

Are the structural properties of normal wood affected by FACE or N-fertilisation?

Environmental factors, such as CO_2-enrichment and N-fertilisation, can affect cell division and differentiation finally causing changes in xylem anatomy and wood structural composition. However, as outlined in the introduction, the data found in the literature give no conclusive picture as to whether and how elevated [CO_2] affects xylem anatomy and tissue composition. The present study shows that CO_2-enrichment and N-fertilisation under field conditions mainly increased the fraction of fibre lumen area (2–8%) and decreased the cell wall area (−3 to −8%). Decreased cell wall thickness was also found after fertilisation of conifers (Brolin et al. 1995; Lindström 1996). These observations indicate that wall thickness is regulated in a yet unknown manner by nitrogen availability in a genotype-independent manner. Cooke et al. (2003) have shown recently that gene expression in phloem versus xylem was altered within few days in response to N-fertilisation. It will be interesting to analyse in future studies expression patterns of cambial genes to find out which are regulated by N-fertilisation.

The observation that growth under elevated [CO_2] also caused reductions in fibre wall thickness, which were more pronounced in poplars grown on non-fertilised plots than in those on fertilised plots (Fig. 4), is surprising and unexpected because it has been suggested that elevated [CO_2] will increase the internal resources of assimilated carbon for processes such as cell wall formation (Conroy

et al. 1990). Thus, in contrast to the results obtained here, increases in wall thickness would be expected. Actually, increased wall thickness was found in *Pinus radiata* seedlings grown under elevated [CO_2] (Conroy et al. 1990). Currently, we can only speculate about the reasons for these contrasting responses to CO_2-enrichment. It is possible that sink/source relationships, unknown environmental factors in the field, species-inherent features, etc., were decisive for wall thickness. But regardless of the reasons, the variable responses indicate that increased photosynthesis, which was generally found under elevated [CO_2] (Medlyn et al. 1999; Hovenden 2003) is not a factor directly stimulating increased cell wall synthesis.

The consistent loss in cell wall area in response to FACE and N-fertilisation in all three genotypes is remarkable since other traits showed significant species-specific variations. It should be noted that these structural changes occurred, even though [CO_2] and fertilisation effects on height or radial growth were small and not uniform between the three species (Table 1). A wider interpretation of these data may be premature because the results of this study were restricted to juvenile wood, which has anatomical properties different from mature wood (Zobel and Van Buijtenen 1989; Ceulemans et al. 2002). However, if these responses persisted and were also found in other tree species, we predict an aggravation of technological wood properties for trees in future environmental scenarios with increased availability of atmospheric CO_2 and nitrogen in forest ecosystems. In conclusion, independent from the genotype elevated [CO_2] and N-fertilisation had negative effects on the structural properties of juvenile poplar wood.

Is wood anatomy affected by FACE and N-fertilisation and what are possible implications?

In addition to technological wood properties, anatomical features of the wood cells are of interest. For example, vessel lumina determine the capacity for water transport and, thus, contribute to climatic adaptation of trees. In our study, where water was not limiting, CO_2-enrichment resulted in wider vessel lumina. This suggests that poplars grown in the FACE system were more sensitive to drought since wider vessels make trees more prone to cavitation (Hargrave et al. 1994; Tyree et al. 1994). However, a generalisation of these results is not possible since the experimental plots were irrigated and we observed that the anatomy of the secondary xylem in poplar is responsive to water availability (Polle, unpublished data).

Vessel frequency is another important factor for water conduit in the stem. The vessel frequency decreased in *P. nigra* grown on fertilised plots under FACE, but not in the other clones (Fig. 3). In *P.* × *canescens* grown with CO_2-enrichment in a greenhouse Gross (2002) also found diminished vessel frequencies. In other studies no influence of elevated [CO_2] on the vessel frequencies (*Q. ilex*, Gartner et al. 2003; *Prunus avium*, Atkinson and Taylor 1996) or increased vessel frequencies (*Q. robur*, Atkinson and Taylor 1996) were found. These contrasting observations suggest that vessel frequency is influenced by strong CO_2 × genotype interactions.

In conclusion, elevated [CO_2] and N-fertilisation altered the dimensions of wood cells in a genotype-specific manner, whereas increased N-availability resulted in thinner fibre cell walls independent of the genotype. The combination of both factors led to anatomical alterations in the xylem structure with potentially negative effects on wood quality such as decreased density and decreased mechanical strength in addition to an increased risk of cavitation.

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