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Original article

Growth of a poplar short rotation coppice under elevated atmospheric CO_2 concentrations (EUROFACE) depends on fertilization and species

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Abstract – Growth and woody biomass production of three *Populus* species (*P. nigra* L. clone Jean Pourtet, *P. alba* L. clone 2AS-11 and *P.* × *euramericana* clone I-214), were followed during the first growing season after coppice of a short rotation coppice culture exposed to elevated atmospheric CO_2 concentrations by means of Free-Air Carbon dioxide Enrichment (FACE), and to a nitrogen (N) fertilization treatment. FACE significantly increased the number of shoots per stool, but did not significantly increase height nor total basal area per stool. In September, FACE significantly increased the Leaf Area Index (LAI) with 5.5 to 16.4%, depending on species. FACE significantly stimulated the woody biomass production by up to 25%, but the stimulation of *P. alba* and *P.* × *euramericana* was restricted to the fertilized treatment. Significant differences between species were observed. We concluded that coppice diminished the FACE effect, that the positive FACE effect was restricted under lower soil fertility, and that species differed in their response to FACE.

free-air carbon dioxide enrichment / N-fertilization / short rotation coppice culture / *Populus* / growth

Résumé – La croissance de taillis de peuplier à courte rotation dans une atmosphère à concentration en CO_2 élevée dépend de la fertilisation et de l'espèce. La croissance et la production ligneuse de biomasse de trois espèces de peuplier (*Pinus nigra* L. clone J. Pourtet, *P. alba* L. clone 2AS-11 et *P.* × *euramericana* clone I-214) ont été suivies pendant la première saison de croissance après la coupe du taillis à courte rotation exposé à une concentration élevée en CO_2, au moyen d'un système FACE et à une fertilisation azotée. Le FACE accroît significativement le nombre de pousses par pied. En septembre, le FACE a augmenté l'indice foliaire (LAI) de 5,5 à 16,4 % selon les espèces. FACE stimule significativement la production de biomasse ligneuse (25 %) mais pour *P. alba* et *P.* × *euramericana* cette stimulation ne concerne que le traitement fertilisé. Des différences significatives ont été observées entre les espèces. Nous concluons que le taillis amoindrit l'effet du FACE, que l'effet positif du FACE était limité pour un sol peu fertile et que les espèces diffèrent dans leur réponse au FACE.

enrichissement de l'air en CO_2 / fertilisation azotée / taillis à courte rotation / *Populus* / croissance

1. INTRODUCTION

To investigate the CO_2 response of forests, many experiments have been conducted on young individual plants under artificial circumstances [28]. But young trees are different in their reactions to elevated CO_2, and there is thus a need for more studies on mature trees [20]. To expose mature trees and entire ecosystems to elevated CO_2 under natural conditions [34], a Free-Air Carbon dioxide Enrichment (FACE) system [16] has been developed. Until now, there has almost never been done a survey on the effects of CO_2 on a short rotation coppice culture [42]. In the context of the Kyoto protocol, all participating countries have the commitment to reduce their CO_2 emissions. A short rotation coppice culture with fast growing species represents a considerable capacity to sequester C quickly, because of the fast and large biomass production.

Growth and biomass production are two of the most relevant parameters studied in elevated CO_2 experiments, because it represents the capacity to sequester C. Tree growth is nearly always stimulated in elevated CO_2 [33]. The mean biomass increment due to elevated CO_2 was estimated 63% for deciduous and 38% for coniferous trees [6]. But as Norby et al. [28] reported in their review, there has been a wide range of responses of tree growth reported from field experiments. The growth of trees is directly related to the radiation intercepted by the foliage, which is primarily determined by the leaf area index (LAI). Elevated CO_2 could increase LAI if the light compensation point for photosynthesis is lower so that leaves are retained deeper in the canopy.

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Otherwise, the LAI could be reduced when elevated CO_2 exacerbates nutrient constraints [28], or when increased shading followed by enhanced leaf fall diminishes LAI [12]. Studies on LAI in elevated CO_2 concentrations yielded a variety of results: increased [25, 29, 38], as well as decreased [14, 23] or unchanged [15, 18] LAI's were reported in different studies on the effect of elevated CO_2 on trees.

It is known that a non-deficient supply of nutrients, especially of nitrogen (N), is essential for a non-limited growth response. Interactions between CO_2 and N varied between experiments [28], but it is often accepted that only with non-deficient N availability, elevated CO_2 can stimulate growth and biomass production [25, 30, 32, 41]. Previous findings from the POPFACE study (the present EUROFACE study) showed that FACE enhanced the optical LAI of the high density plantation in the first year and in spring of the second year after planting as a result of a stimulation of individual leaf area and tree dimensions [12]. However, after canopy closure in the second growing season, LAI was no longer affected by FACE, which was confirmed in the third growing season [13]. Because the LAI would only increase under elevated CO_2 if the uptake of N would concurrently increase [17] or if N would be present in super-optimal amounts, it was hypothesized that N was a limiting factor [13]. This was confirmed in a study of the biomass production [2], where a faster depletion of soil N in the FACE treatment explained part of the decrease of the elevated CO_2 stimulation during the 2nd and 3rd years after planting.

In this paper, we report the first data from the EUROFACE [35] experiment (former POPFACE), where three *Populus* species during the first year after coppice were exposed to N fertilization and elevated CO_2, using the FACE technique. The objective of this study was to investigate the effects of CO_2, N, and species on the growth performance (number of shoots, basal area, height, and woody biomass production) and LAI (measured with a fish-eye-type plant canopy analyzer) of the three *Populus* species. This is the first FACE study ever performed on a short rotation coppice culture [42], and as short rotation, high-density plantations are relevant for the production of renewable energy for the future, it is highly important to understand the CO_2 impact on the woody biomass production of a coppice culture. Because findings from the period before coppice showed that FACE significantly increased the standing root biomass [24], we hypothesize that (1) the FACE effect on different growth parameters will be larger after coppice compared to the first season after planting, because more roots in FACE favor regrowth after coppice. Secondly, the suggested N limitation in the 2nd and 3rd growing season postulates the hypotheses that (2) low N concentrations will limit FACE effects. Finally, given that in the first rotation cycle species appeared to differ in the extent of their response to FACE [2, 3], we speculate that (3) the three *Populus* species will be different in their response to FACE and N.

2. MATERIALS AND METHODS

2.1. Plant material and plantation layout

In late spring 1999, a 9-ha poplar plantation was established using hardwood cuttings at a planting density of 5 000 trees per ha (spacing 2 m × 1 m), and 10 000 trees per ha (spacing 1 m × 1 m) within the six experimental plots. The experimental plots were planted with three *Populus* species, *P.* × *euramericana* (Dode) Guinier (clone I-214), *P. nigra* L. (clone Jean Pourtet), and a local selection of *P. alba* L. (clone 2AS-11), whereas the non-experimental part of the plantation was planted with *P.* × *euramericana* (Dode) Guinier (clone I-214). Each 314-m^2 plot contained 315 plants, and was divided into two parts by a physical resin-glass barrier (1 m deep in the soil) to provide N fertilization in one part of the plot. In the fertilized treatments (half of each plot), a total amount of 212 kg N per hectare was supplied. Hydraulic pumps, installed outside each plot, were used to distribute the fertilizer (Navarson 20-6-6), dissolved in 200 liter-tanks, through the irrigation system. Fertilization was provided once per week for a period of 16 weeks throughout the growing season starting from July 8, 2002 onwards. Each half plot was further divided into three triangular sectors for the different species, thus yielding six sectors per plot. Plantation management included continuous drip irrigation, mechanical herb removal, and a limited application of insecticides. The plantation was designed and managed as a short rotation forest with typical high plant densities [27]. In the winter of 2001, all trees were cut to the base of the stem (5–8 cm above the ground), resulting in stools with many new shoots sprouting of them in the spring of 2002. For further plantation details see Scarascia-Mugnozza et al. [35].

2.2. Site description

The FACE study was located in central Italy, near Viterbo (Tuscania; 42° 22' N, 11° 48' E, altitude 150 m) on 9 ha of former agricultural land. Following detailed soil analysis, six experimental areas, generally called plots (30 m × 30 m, between-plot distance of 120 m) were selected. Three of them were control plots and were left under natural conditions, whereas a FACE design was established in the other three plots. The carbon enrichment was achieved through octagonal PVC rings (22 m diameter) mounted on a tower crane. Pure CO_2 was released through laser drilled holes in the ring to reach a target concentration of 550 $\mu mol \cdot mol^{-1}$ inside the FACE treatment. During the first year after coppice, the CO_2 concentration was 554 ± 1.6 $\mu mol \cdot mol^{-1}$ within the FACE plots (F. Miggliettta, CNR-IATA, Florence, Italy, unpublished results). The elevated CO_2 concentrations, measured at 1-min intervals, were within 20% deviation from the pre-set target concentration for 89.4% of the time. Daytime CO_2 enrichment was provided from bud burst to leaf fall. A meteorological station at the center of each ring collected data used to control the directional release of gas along the ring. The released quantity of gas was determined by wind speed, and by an algorithm based on a 3-D gas dispersion model developed for the facility. A detailed description of the set-up and performance of this FACE facility is given by Miglietta et al. [26].

2.3. Measurements

All measurements were made in the permanent growth plots (PGP's), i.e., six adjacent trees within each sector, surrounded by at least one row of trees from the same species. Growth was followed during the first season after coppice from June until November 2002 at fixed time intervals (every 3 weeks).

2.4. Growth

In each PGP, the number of living shoots per stool was counted, and the diameter of the living shoots at 20 cm above the soil was measured with a digital caliper (Mitutoyo, type CD-15DC, Telford, UK). The height of a stool was determined with an extendable pole as the height of the tallest shoot of the stool. The basal area (BA) of each shoot, and the sum of basal areas (Σ BA) for each stool were calculated. The average per PGP was used in further analyses.

Table I. Analysis of variance (ANOVA) of average number of shoots per stool (interactions were not significant) and average height (the interactions CO_2 × N, species × N, and species × CO_2 × N were not significant) of three *Populus* species in FACE and control under fertilized and unfertilized treatments at different dates during the season. Significance (*P*-values of the ANOVA *F*-test) of the effects of CO_2 treatment, species, N and their interactions are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. N: nitrogen.

Date	Number of shoots			Height (cm)			
	CO_2	Species	N	CO_2	Species	N	Species × CO_2
June 18	**	****	*	*	< 0.1		< 0.1
July 9	*	****					*
July 27	*	****	*				< 0.1
Aug. 19	*	****			***		
Sept. 13	**	****			****		
Oct. 1				< 0.1	*		
Nov. 4	**	****					

2.5. Leaf area index

Optical leaf area index ($LAI_{optical}$) was measured at monthly intervals from September till November at sunset, or occasionally, during the daytime on overcast days, with a fish-eye-type plant canopy analyzer (LAI-2000 PCA, Li-Cor, Inc., Lincoln, NE). Measurements were made at 14 points per PGP in different directions to account for spatial variation and plantation design. We used a 45° view cap and all view angles were included in the calculation of the $LAI_{optical}$. The reference measurements were taken at 15-s intervals with an additional PCA installed in a clearing at the experimental site.

As it is often reported that the use of the PCA is restricted by a general tendency towards underestimating LAI [7–9], it is recommended to collect direct reference measurements. In September, at the time of the selective harvest, all leaves from each harvested shoot were removed, weighed, and, using the specific leaf area (SLA), the area of these leaves was calculated. A regression analysis between basal area and total leaf area of each harvested shoot, was used to calculate leaf area for each shoot in a PGP. Summarized for each stool, the leaf area was averaged over a PGP, and the allometric leaf area index ($LAI_{allometric}$) was calculated. A regression analysis between the $LAI_{allometric}$ and the $LAI_{optical}$ compared both methods. It has to be mentioned though that the $LAI_{optical}$ implicitly contains the area of stems and branches of the shoots, and thus in fact it is a plant area index (PAI) [10].

2.6. Living and dead woody biomass

In November 2002, at the end of the growing season, three shoots growing at least one row outside the PGP (in order not to disturb the PGP's) were randomly harvested in each sector. The diameters of the shoots were measured at 20 cm above the soil with a digital caliper, and after removing the leaves, the shoots were oven-dried for three days at 70 °C, and weighed. We developed an allometric relationship between shoot diameter and shoot dry weight for each species, with the data pooled among treatments ($n = 36$) (Software Origin, Version 5.0, Microcal Software, Inc., Northampton, MA). With these relationships, dry weight was calculated from the diameter measurements of all living shoots, and scaled-up to total living above-ground woody biomass production in tons per hectare.

In each PGP, all the dead shoots were collected in November 2002, oven-dried at 70 °C for three days, and weighed. The relative proportion of dead biomass (or necromass = dead biomass/(dead + living biomass) × 100) was calculated for each combination of treatments and species.

2.7. Data analysis

To statistically analyse the main effects of elevated CO_2, N, species, and their interactions on variables of growth and production, we used an analysis of variance (ANOVA). The data were analysed with a three-way ANOVA mixed procedure [21], with a randomized-complete-block design with CO_2, N, species, and their interactions as fixed factors, block as a random factor, and plot as the unit of replication. We used the SAS statistical software package (SAS system 8.2, SAS Inc., Cary, NC). When interactions were significant, an a posteriori comparison of means was performed (using the option least squares means). When not significant, interactions were removed from the model. Effects of fixed factors on variable means where considered significant when the *P*-value of the ANOVA F-test was < 0.05. An arc-sinus transformation was used to analyse the data of proportion of dead biomass. The Shapiro-Wilk statistic (proc univariate in SAS) tested the assumption of normality for each dataset.

3. RESULTS

3.1. Number of living shoots

The number of living shoots showed a small, insignificant increase from June until August, whereafter the number decreased (in some cases significantly). Mortality was strongest in *P. alba* (Fig. 1). During the entire growing season, the number of shoots was significantly increased by FACE (Tab. I). In response to FACE, *P. alba* experienced a 12 to 55% stimulation of number of shoots compared with control values, whereas the maximum observed stimulations for *P. nigra* and *P.* × *euramericana* were 27 and 17%, respectively. The number of shoots differed among species (Tab. I): *P. nigra* had, with an average number of shoots of 23.8, 97% and 138% more shoots than *P. alba* and *P.* × *euramericana*, respectively. In general, the number of shoots did not differ between N treatments. Comparisons with 'least squares means' indicated that fertilization significantly reduced the number of shoots in control in June and at the end of July. On average, the maximum number of shoots was found (for all species) in the unfertilized FACE treatment (not significant). None of the interactions were significant.

3.2. Shoot height

The stimulating effect of FACE on stem height was limited (between 2.9 and 8.9%) and mostly insignificant (Tab. I). For *P. alba* the average height was significantly reduced by FACE

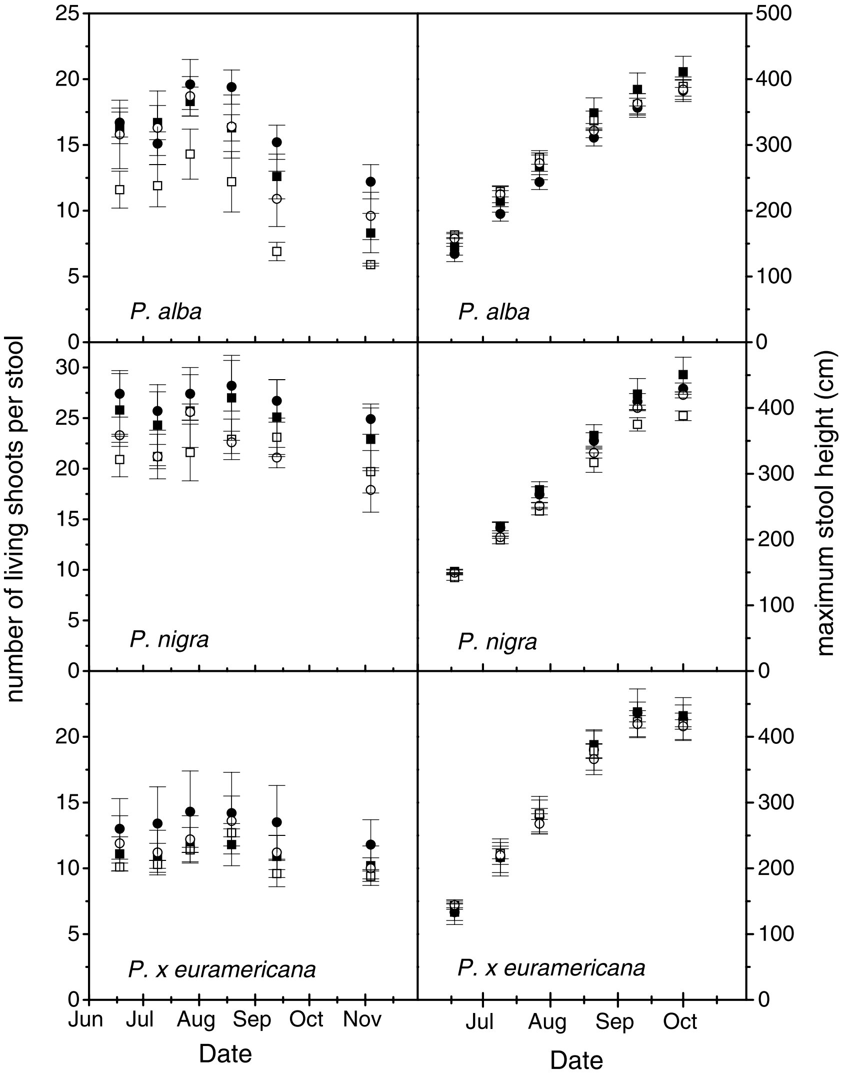

Figure 1. Evolution of the average number of living shoots per stool (left panels) and of height (cm) of the tallest shoot per stool (right panels) during the first growing season after coppice in a short rotation culture of three *Populus* species in FACE (closed symbols) and control (open symbols) under fertilized (square) and unfertilized (circle) treatments. Values are means ± SE ($n = 3$) for *P. alba*, *P. nigra* and *P.* × *euramericana*. The ANOVA results are shown in Table I.

in June and in the beginning of July ($P = 0.0072$ and $P = 0.0489$), whereas for *P. nigra*, the height was significantly increased by FACE at the end of July and in October (results from least squares means; not shown). This explains the significant species × CO_2 interaction. The other interactions were not significant. The average height of the shoots differed among species. Species *P. alba*, which set bud later in the season, grew slower, and at the end of the growing season reached an average height of 392 cm, whereas *P. nigra* and *P.* × *euramericana* reached about the same height (422 and 423 cm, respectively). The decrease of the height of *P.* × *euramericana* in October, falls within the error bars. Within FACE and control, there were no significant differences between unfertilized and fertilized treatments. However, at the end of the first growing season, the shoots of *P. nigra* in control were significantly smaller in the fertilized then in the unfertilized treatment (Fig. 1).

3.3. Sum of basal areas

In general, FACE did not affect the Σ BA (Tab. II), except for *P. nigra*, which was significantly stimulated by FACE from the end of July until the end of the growing season (results from option least squares means, Fig. 2). In September and November, FACE significantly stimulated the Σ BA (Tab. II). Differences between species were significant (Tab. II). The Σ BA of *P. nigra* was in June 17 and 48% larger as compared to *P. alba* and *P.* × *euramericana*, respectively (Fig. 2, ANOVA results are given in Tab. II). *P. nigra* kept this leap during the season. On average, *P. alba* always had the smallest Σ BA. Nitrogen didn't affect the Σ BA. None of the interactions were significant, except the interaction species × CO_2 in August. Remarkably, Σ BA was lowest in the control × fertilized treatment, which was most obvious for *P. nigra*.

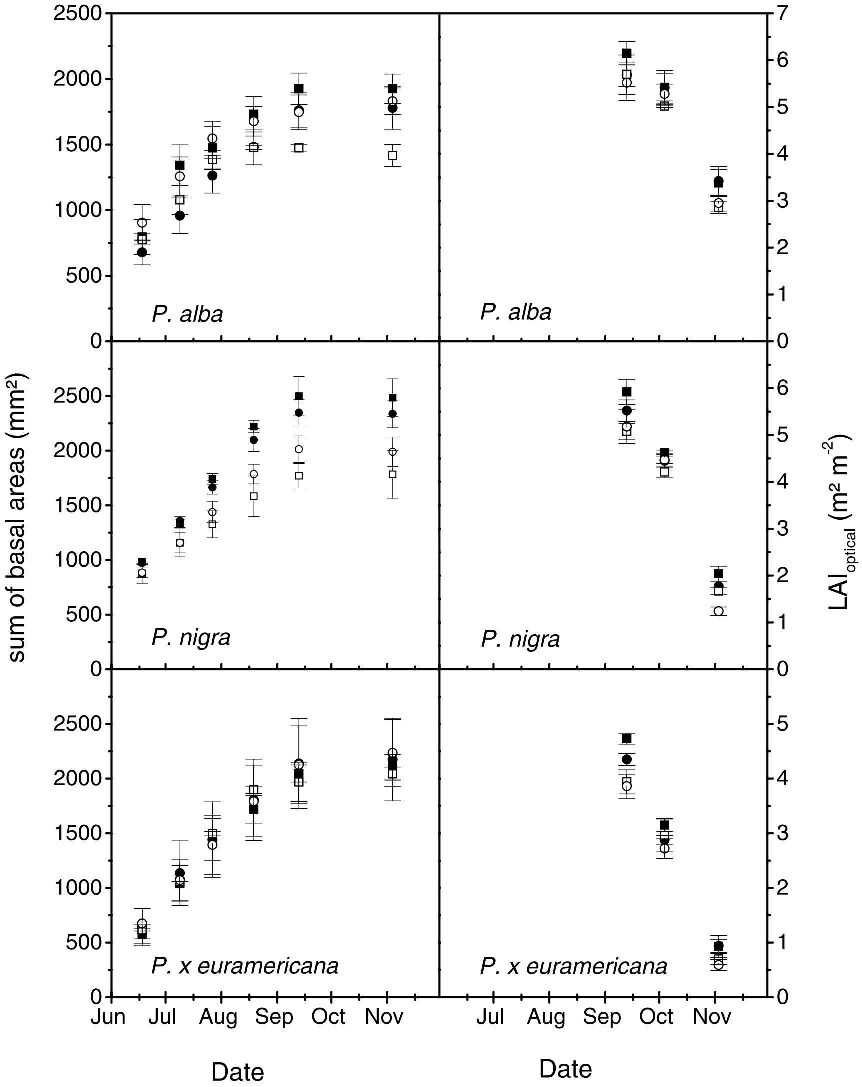

Figure 2. Evolution of sum of basal areas (mm^2, left panels) and optical leaf area index ($LAI_{optical}$ $m^2 \cdot m^{-2}$, right panels) during the first growing season after coppice in a short rotation culture of three *Populus* species in FACE (closed symbols) and control (open symbols) under fertilized (square) and unfertilized (circle) treatments. Values are means ± SE ($n = 3$) for *P. alba*, *P. nigra* and *P.* × *euramericana*. The ANOVA results are shown in Tables II and III.

Table II. Analysis of variance (ANOVA) of the sum of basal areas of three *Populus* species in FACE and control under fertilized and unfertilized treatments at different dates during the season. Significance (*P*-values of the ANOVA *F*-test) of the effects of CO_2 treatment, species, N and their interactions are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. N: nitrogen.

Date	CO_2	Species	N	Species × CO_2	CO_2 × N	Species × N	Species × CO_2 × N
June 18		****					
July 9		< 0.1					
July 27							
Aug. 19		*		*			
Sept. 13	*	**					
Nov. 4	*	**			< 0.1		

Table III. Analysis of variance (ANOVA) of $\text{LAI}_{\text{optical}}$ of three *Populus* species in FACE and control under fertilized and unfertilized treatments at different dates during the season. Significance (*P*-values of the ANOVA *F*-test) of the effects of CO_2 treatment, species, N and their interactions are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. N: nitrogen.

Date	CO_2	Species	N	Species × CO_2	CO_2 × N	Species × N	Species × CO_2 × N
Sept. 13	****	****	**		*		
Oct. 04	*	****					
Nov. 03	***	****	< 0.1			*	

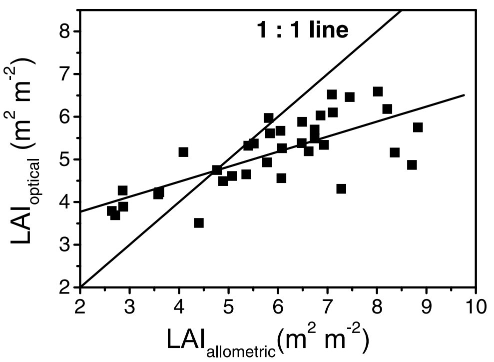

Figure 3. Relationship between LAI estimated by allometric relations ($\text{LAI}_{\text{allometric}}$) and by PCA ($\text{LAI}_{\text{optical}}$). The coefficients for the lineair regression $\text{LAI}_{\text{optical}} = a \times \text{LAI}_{\text{allometric}} + b$ are: 0.3534 (a) and 3.0672 (b). The 1:1 relationship is illustrated.

3.4. Leaf Area Index

A significant correlation ($R^2 = 0.53$, $P < 0.0001$) between the allometric ($\text{LAI}_{\text{allometric}}$) and optical LAI ($\text{LAI}_{\text{optical}}$) was observed (Fig. 3). Nevertheless, a bias from the 1:1 relationship was present, with $\text{LAI}_{\text{optical}}$ values overestimating the $\text{LAI}_{\text{allometric}}$ for low LAI values, and underestimating the $\text{LAI}_{\text{allometric}}$ for high LAI values (Fig. 3).

The FACE treatment significantly increased the $\text{LAI}_{\text{optical}}$ (Fig. 2, the ANOVA results are given in Tab. III). In September, $\text{LAI}_{\text{optical}}$ of *P.* × *euramericana* was stimulated by 16.4% by FACE ($P < 0.0001$), and was significantly smaller than the $\text{LAI}_{\text{optical}}$'s of *P. alba* and *P. nigra*. During the following months, FACE caused a smaller, but still significant increase of the $\text{LAI}_{\text{optical}}$. Species differed significantly (Tab. III): absolute values of maximum $\text{LAI}_{\text{optical}}$ in the control treatment were 3.9, 5.13, and 5.61 for *P.* × *euramericana*, *P. nigra,* and *P. alba*, respectively. In September, the fertilized treatment significantly increased $\text{LAI}_{\text{optical}}$ of *P. alba* and *P.* × *euramericana*, but only in FACE (significant CO_2 × N interaction, Tab. III). In November, the fertilized treatment stimulated $\text{LAI}_{\text{optical}}$ in control.

3.5. Living and dead biomass

Allometric relationships between shoot diameter and dry weight (stem plus branches) were established for each species with the data pooled for all treatments (R^2 values between 0.93 and 0.98; Tab. IV). There were no significant differences between treatments. FACE significantly stimulated the living woody biomass production of *P. nigra* with 25% ($P = 0.0054$), whereas *P. alba* and *P.* × *euramericana* experienced a stimulation of only 10.4% and 9.9%, respectively (Fig. 4, the ANOVA results are given in Tab. V). For *P. alba* and *P.* × *euramericana*, the stimulation of woody biomass production was only significant in the fertilized treatment ($P < 0.1$ for the interaction CO_2 × N). Differences between species were significant (Tab. V); although the three species reached the same woody biomass production in the control treatment, values of woody biomass production in the FACE treatment were 13.7, 12.7 and 11.07 ton·ha^{-1}·y^{-1} for *P. nigra*, *P. alba* and *P.* × *euramericana*, respectively (Fig. 4). Within control, fertilization significantly reduced the woody biomass production averaged over the three species ($P < 0.1$ for the interaction CO_2 × N, Tab. V). Although not significant, fertilization slightly increased the woody biomass production of *P. nigra* and *P. alba* in FACE. FACE significantly reduced the relative proportion of dead biomass with 54.8, 27, and 36.8% for *P. nigra*, *P. alba* and *P.* × *euramericana*, respectively (Fig. 4, the ANOVA results are given in Tab. V). The relative percentage of dead biomass differed significantly between species: *P. alba* had significantly more (2.65%) dead biomass than *P.* × *euramericana* (0.15%) and *P. nigra* (0.30%) (Fig. 4). This was confirmed by the larger decline of living shoots per stool of *P. alba* (Fig. 1). Fertilization affected the proportion of dead biomass: fertilization significantly increased the percentage of dead biomass of *P. alba* in FACE (significant interaction N × species, Tab. V).

Table IV. Allometric relationships used to determine the living woody biomass production (x: shoot diameter (mm) measured at 20 cm above soil, y: shoot dry weight (g)). Symbols: a and b are parameters of a non-linear least squares fit for the equation $y = ax^b$, with R^2 as indicated. *n*: number of replicates.

	n	a	b	R^2
P. alba	36	0.2346	2.2871	0.933
P. nigra	36	0.5541	1.9252	0.973
P. × *euramericana*	36	1.0413	1.7012	0.979

4. DISCUSSION

FACE stimulated the above-ground woody biomass production in our experiment up to 25%. An observed woody biomass production between 10.2 and 14.0 ton·ha^{-1}·y^{-1} corresponds with the 'working maximum' of 10–12 ton·ha^{-1}·y^{-1} proposed

Table V. Analysis of variance (ANOVA) of living and dead biomass (% of total biomass) of three *Populus* species in FACE and control under fertilized and unfertilized treatments at different dates during the season. Significance (*P*-values of the ANOVA *F*-test) of the effects of CO_2 treatment, species, N and their interactions are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. N: nitrogen.

	CO_2	Species	N	Species × CO_2	CO_2 × N	Species × N	Species × CO_2 × N
Living biomass (ton·ha^{-1}·y^{-1})	**	< 0.1			< 0.1		
Dead biomass (%)	*	****	< 0.1			**	

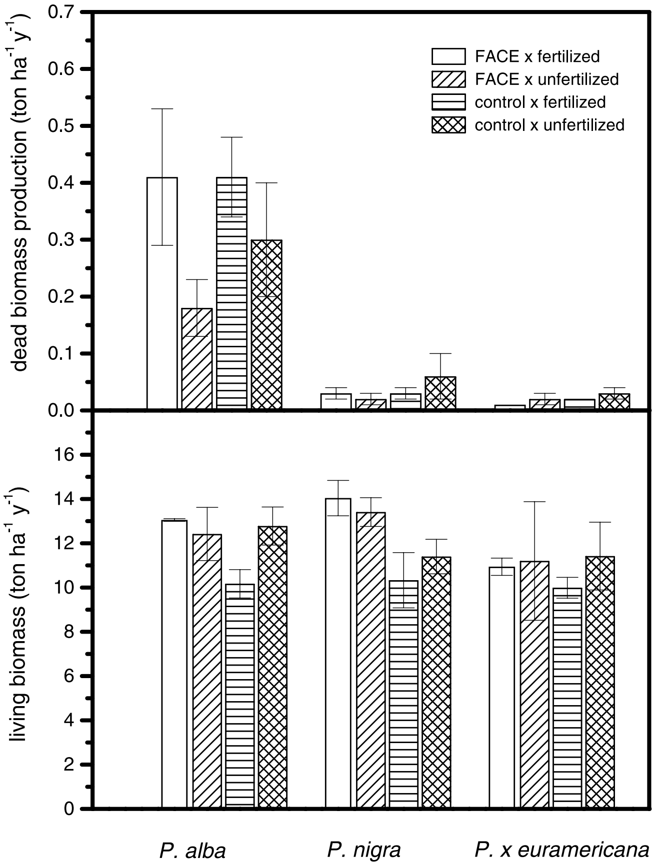

Figure 4. Dead and living woody biomass production (ton·ha^{-1}·y^{-1}) for three *Populus* species in the first year after coppice of a short rotation coppice culture in FACE and control under fertilized and unfertilized treatments. Values are means ± SE for *P. alba*, *P. nigra* and *P.* × *euramericana*. The ANOVA results are shown in Table V.

by Cannell and Smith [4] for close-spacing hardwood plantations. FACE did not affect mean canopy height, as was also found in the first year after planting of POPFACE [3]. Nevertheless, growth stimulation of height is one of the frequently reported effects of elevated CO_2 [38–40]. The maximum LAI that was observed in the control treatment of our study is similar to values reported for poplars during the first year of intensive, high-density plantations [5]. An estimation of leaf area index with the PCA in deciduous forests [8] showed that overlapping of leaves, the presence of gaps, and light at the horizon level seem to be important variables that influence LAI estimation by PCA. Figure 3 indicates that the $LAI_{optical}$ overestimated the $LAI_{allometric}$ at low LAI values. We did not validate the contribution of the stem and branch area to the $LAI_{optical}$, which were thus included in the $LAI_{optical}$ measurements. In low LAI canopies, especially in a coppice culture rich of branches, woody area may play an important role in determining the leaf area index as 'seen' by the PCA. This could explain the overestimation for low LAI. For high LAI values, the $LAI_{optical}$ underestimated the $LAI_{allometric}$. Here the relative contribution of the branches and stems to the $LAI_{optical}$ will be less, but the assumptions of the instrument will play a role. The PCA assumes that the foliage is randomly distributed. Foliage is never random, but is clumped along stems and branches.

Although the results from the first year after planting of POPFACE indicated a strong stimulation of the LAI in FACE (in the first growing season the LAI of *P. nigra* was stimulated 252% by FACE [12]), the stimulation that we observed after coppice was much smaller. In fact, the first-year's LAI after coppice was comparable with the second-year's LAI before coppice [12], and this LAI was not affected by FACE after canopy closure [12]. The authors postulated that increased shading and competition in FACE enhanced leaf fall and turnover, and therefore, decreased the FACE effect. Because the shoots grew so rapidly after coppice, canopy closure was already reached at the end of the first growing season in our study. After all, coppice shoots benefit from the existing root system and the rapid development of a high LAI, by which they grow faster than seedlings or cuttings. This might explain the decrease of the effects of FACE on growth after coppice, since competition begins to play soon after the coppicing. Most studies indicate that elevated CO_2 stimulates the root production [34], and fine roots have been shown to be especially responsive to CO_2 [28]. Lukac et al. [24] demonstrated that for the first 3 years after planting the trees in POPFACE, FACE significantly stimulated the standing root biomass from 47% (for *P. alba*) up to 71% and 76% (for *P.* × *euramericana* and *P. nigra* respectively). However, shoots of stools growing in FACE were not favored through this larger root system and better nutrient acquisition. We thus have to reject our first hypothesis (i.e. that the installed root system favors FACE effects on growth after coppice).

As FACE significantly stimulated both the woody biomass production and the $LAI_{optical}$, we can speculate that also the net primary production (NPP, i.e. the sum of the woody biomass and leaf biomass production) will have been stimulated by FACE in the first growing season after coppice. However, additional data are needed to verify this because elevated CO_2 may alter leaf turnover.

The magnitude of the growth responses to elevated CO_2 has been linked to soil fertility in studies with different species [19, 25, 40]. Experiments with elevated CO_2 and N fertilization, showed that with low N fertilization, the effects of CO_2 enrichment on leaf area and biomass accumulation were lost [25]. Nevertheless, it was found that elevated CO_2 could increase forest productivity even in N-limiting conditions owing to increased N acquisition and use efficiency [37]. In our study, FACE increased the LAI even in the unfertilized treatments, except in October (data not shown), when the LAI stimulation was lost in the unfertilized treatment. Our results of woody biomass production of *P. alba* and *P.* × *euramericana* confirmed our second hypothesis that FACE effects were limited to fertilized treatments (significant interaction $CO_2 \times N$, $P < 0.1$). Remarkably, *P. nigra* experienced a FACE stimulation in both the unfertilized as the fertilized treatment (Fig. 4). Responses to elevated CO_2 under low N availability result in a large accumulation of carbohydrates that cannot be used for growth. These carbohydrates could be involved in signaling N deficiency through down-regulation of photosynthesis [31].

In the control treatment, fertilization significantly limited the woody biomass production (significant interaction $CO_2 \times N$, $P = 0.0345$, results from the option least squares means, not shown), which may indicate an unbalanced nutrient supply. The N supply provided 212 $kg \cdot ha^{-1}$, about the highest application for N fertilizer recommended [22]. This might have been more than needed, and therefore resulted in an unbalance of N versus other mineral elements. Indeed a long-term study indicated that plants grown at high N showed the lowest P contents [25, 40]. This limiting effect was also detected in the different growth parameters. As Calfapietra et al. [2] suggested that in the 3rd growing season after planting, the [N] became depleted in FACE, adding N in FACE might have balanced the system, whereas adding N in control might have brought the [N] up to supra-optimal amounts.

We observed a significant effect of species on almost all variables at nearly all times. It is known that elevated CO_2 enhances growth and biomass production to a different degree depending on species [6]. *P. nigra* was often the only species that experienced a significant CO_2 stimulation. The good performance of *P. nigra* confirmed the results of the first growing season after planting when *P. nigra* had the highest volume index and seemed to profit more than the other two species from the CO_2 enrichment [3]. Surprisingly, the woody biomass production of *P. nigra* was stimulated in both the fertilized as unfertilized treatments. How can this greater sensitivity of *P. nigra* to FACE be explained? Lukac et al. [24] observed that, after three years of growth, *P. nigra* had the largest stimulation of roots by FACE. After coppice, this could have favored the positive response to FACE only in *P. nigra*. Nevertheless, these higher reserves of carbohydrates of *P. nigra* could also have been limiting growth. After all, Bernacchi et al [1] found that in June 2002 *P. nigra* experienced the largest down-regulation of photosynthesis. The authors stated that a larger translocation of carbohydrates to the regrowing shoots had caused the down-regulation of the photosynthetic potential [1]. Further research will have to clarify this contradiction. *P. alba* had the largest necromass among the three species. *P. alba* is characterized by longer and more horizontally orientated branches as compared to both other species [11]. This makes that in the *P. alba* stands, branches do interact more frequently and therefore competition, leading to the death of more shoots, could have been more important. The relative proportion of dead shoots was below the limit of 10%, proposed for willows by Telenius and Verwijst [36].

In conclusion, FACE significantly affected growth and woody biomass production after coppice, but the effects were not as unequivocal as in the first year after planting. Coppice accelerated growth so strongly, that canopy closure was already reached during the first year. Possibly, growth is so strongly stimulated by coppice, that FACE effects were blurred. This study confirmed our hypotheses that stimulation of the woody biomass production by CO_2 is limited by N availability, and that species differ in their response to FACE en N. Although fertilization sometimes executed a stimulating effect in FACE, it limited woody biomass production in control. This could indicate an unbalance of N versus other elements. These findings will guide further study to clarify remaining uncertainties with regard to $CO_2 \times N$ interactions.

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