

# Climate Change Reverses the Competitive Balance of Ash and Beech Seedlings under Simulated Forest Conditions

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**Abstract:** This study identifies the important role of climate change and photosynthetic photon flux density (PPFD) in the regenerative competence of ash and beech seedlings in 12 inter- and intra-specific competition designs in simulated mixed ash-beech forest gaps under conditions of non-limiting soil volume, water and nutrient supply. The growth conditions simulated natural forest conditions as closely as possible. Simulations were performed by growing interacting seedling canopies for one season in temperature-regulated closed-top chambers (CTCs). Eight CTCs were used in a factorial design with replicate treatments of  $[\text{CO}_2] \times \text{temperature} \times \text{PPFD} \times \text{competition}$  design.  $[\text{CO}_2]$  tracked ambient levels or was  $360 \mu\text{mol mol}^{-1}$  higher. Temperature tracked ambient levels or was  $2.8^\circ\text{C}$  higher. PPFD on two plant tables inside each CTC was 16% and 5% of open-field levels, respectively, representative of typical light flux levels in a natural forest gap. In several of the competition designs, climate change made the ash seedlings grow taller than the beech seedlings and, at the same time, attain a larger leaf area and a larger total biomass. Advantages of this type for ash were found particularly at lower PPFD. There was a positive synergistic interaction of elevated temperature  $\times [\text{CO}_2]$  for both species, but more so for ash. There are many uncertainties when a study of chambered seedlings is to be projected to real changes in natural forests. Nevertheless, this study supports a possible future shift towards ash in north European, unmanaged, mixed ash-beech forests in response to the predicted climate change.

**Key words:** Ash, beech, climate change, competition, forest gap, temperature-regulated closed-top chambers (CTCs), unmanaged forests.

## Introduction

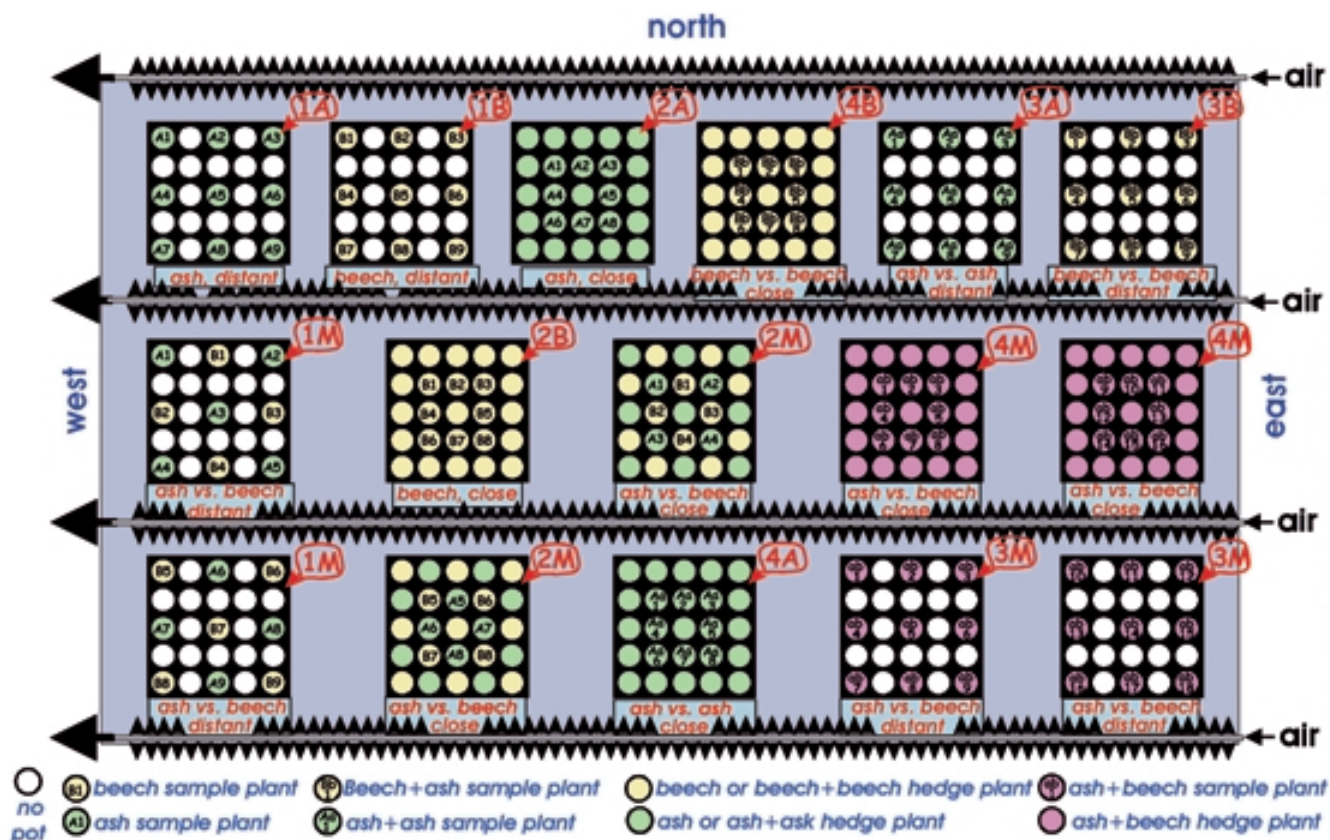
When a storm, lightning, or old age bring down a tall, old tree in an otherwise dense, unmanaged mixed species forest, a gap is created. The improved light flux stimulates the ongoing competition between established seedlings and saplings on the forest floor. More seeds will germinate and competition

becomes fierce. The outcome of this competition between individual plants and between the different species growing in the gap is a matter of genotype, plant size, soil conditions, light, and climate.

In several European countries, managed forest is rapidly being converted to unmanaged forest. With the present climate, beech dominates the unmanaged, mixed ash-beech forests of Northern Europe. However, changes in the global climate may alter the balance between tree species. According to IPCC's Special Report on Emissions Scenarios, the globally averaged surface temperature will increase by  $1.4$  to  $5.8^\circ\text{C}$  over the period from 1990 to 2100, with future atmospheric  $\text{CO}_2$  concentrations reaching  $540$  to  $970 \mu\text{mol mol}^{-1}$  (IPCC, 2001). The increased dependence on natural regeneration and competition, implied by the general conversion to unmanaged forestry, makes climate change studies important if forest managers are to know which species will dominate these forests of the future (Catovsky and Bazzaz, 2002).

It is well documented that trees and forests will respond to climate change (e.g., Mohren et al., 1997; Mickler and Fox, 1998; Bruhn et al., 2001; Iverson and Prasad, 2001; Larsen and Saxe, 2001). Most studies have focused on responses to elevated atmospheric  $[\text{CO}_2]$  (e.g., Saxe et al., 1998; Catovsky and Bazzaz, 2002), while fewer studies have attempted to isolate the effects of elevated temperature (e.g., Kozłowski and Pallardy, 1997; Saxe et al., 2001). Nevertheless, the response to climate change cannot be predicted from separate responses to elevated  $[\text{CO}_2]$  and elevated temperature because, with a few exceptions (e.g., Kellomäki and Wang, 2001), these interact in their effects, which also depend on the species being studied, plant age, season, and microclimatic conditions (Tjoelker et al., 1998; Leverenz et al., 1999a; Bruhn et al., 2000; Nagy et al., 2000; Hättenschwiler, 2001; Kuokkanen et al., 2001; Usami et al., 2001; Norby and Luo, 2004). The varying results of these and other studies have generated much uncertainty (Karnosky, 2002). To yield reliable results, future studies need to be highly controlled, complex, and as close to nature as possible.

The present study is a factorial experiment of ash and beech competition  $\times$  elevated temperature  $\times$  elevated  $[\text{CO}_2]$   $\times$  PPFD under conditions of non-limiting soil volume, water and nutrient supply. Competition was represented by 12 inter- and intra-specific designs. Despite the fact that this study is limited to one season and uses chambers and potted seedlings to



**Fig. 1** One of 18 plant tables. Frames, pots, plants, and competition were arranged identically on all 18 tables, each of which measured  $2.05 \times 1.20$  m. Cooling air was released along and between rows of

plants growing in nine or 25 pots per frame, with one or two seeds per pot of ash, beech or both species. The competition designs are summarised in Fig. 3.

simulate natural forest gaps, the complexity of the setup yields results that provide new insight into possible interactions between climate change and competitive regenerative growth of two important European deciduous species.

## Materials and Methods

### Soil and pots

All the seedlings were sown and grown in a standard soil blended in a cement mixer in 60-l batches. Each batch was composed of 38 l fine sphagnum (pH 2.6–4.4, Pindstrup Mosebrug Ltd., Denmark), 12 l leca pearls (2–4 mm, Danish Leca Ltd., Denmark), 6 l beech forest soil, and 4 l ash forest soil. The forest topsoil was dug out in 1.5 m perimeters around mature trees in the same beech and ash plantations where the seeds had been harvested. The topsoil added clay and natural mycorrhizal fungi to the standard soil mixture.

The soil mixture was filled into 18000 280-ml tube pots (Pöbelman GmbH, Germany), and 200 1-l pots, and kept well watered until the seeds were sown.

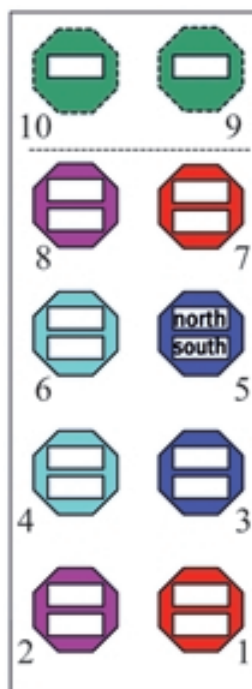
### Plant material

The ash seeds (*Fraxinus excelsior*) were harvested in Tisvilde Hegn ( $12^{\circ}05'E$ ,  $56^{\circ}02'N$ ) and the beech seeds (*Fagus sylvatica*) in Alsted forest ( $11^{\circ}38'E$ ,  $55^{\circ}24'N$ ), both in Denmark. The seeds were pre-treated to facilitate germination.

The seeds were sown in early April 2000 with either one or two seeds per 280-ml tube pot. In order to investigate the possible effects of pot size, additional seeds were sown in 1-l pots, allowing two seeds of either species to develop in each. Both species began germinating around 22 April, and by 4 May most seeds had germinated. A large number of tube pots with uniform seedlings were selected and placed in frames ( $276 \times 276$  mm) with 9 or 25 pots per frame. The frames were placed on ebb/flood container tables (Fig. 1) both in chambers and outdoors at the KVL Arboretum ( $12^{\circ}50'E$ ,  $55^{\circ}88'N$ ) on 16 May. Ten 1-l pots were placed on each table in each chamber and outdoors. All the chambered plants grew under controlled climate conditions for 123 days from 17 May to 17 September 2000.

### Fertigation

The plants were watered daily by raising the water level around the pots for 20 min, beginning at 2 a.m. The water used was rainwater caught from the roof of a nearby greenhouse. pH



treatment	chamber type	CO <sub>2</sub> conc.	Air temp.	soil temp	RH%	PPFD north table	PPFD south table
1: + °C	CTC	416	18.3	16.9	72.3	64	175
2: + °C & +CO <sub>2</sub>	CTC	748	18.4	17.4	71.7	64	184
3: reference	CTC	416	15.5	14.4	73.2	58	178
4: +CO <sub>2</sub>	CTC	785	15.5	14.1	75.2	58	187
5: reference	CTC	420	15.6	13.7	76.7	58	204
6: +CO <sub>2</sub>	CTC	792	16.1	14.5	72.6	61	180
7: + °C	CTC	400	18.3	18.2	77.1	58	180
8: + °C & +CO <sub>2</sub>	CTC	766	18.6	18.8	77.4	61	181
9-10: outdoors	no chamber	423	15.5	15.3	79.7	721	

**Fig. 2** There were 10 experimental plots. Eight were closed-top chambers (CTCs) each containing two plant tables, a north table, and a south table, and two were outside plots with one table on each. 24-h mean values averaged over the total exposure period of 123 days from 17 May to 17 September 2000 are given for atmospheric [CO<sub>2</sub>], air and soil temperature (°C), and relative humidity. Photosynthetic photon

flux densities (PPFD,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) are mean values for daytime hours immediately above the north and south table canopies, and outdoors. Outdoor PPFD was measured at 1.75 m above the ground, and represented approximately  $\frac{2}{3}$  of PPFD measured at 8 m above the ground, considered to be an open-field reading.

and EC (electrical conductivity) were regulated by an AMI 5000 (DGT-Volmatic Ltd., Denmark). pH was adjusted to 6.5, while EC initially remained at 0.7  $\mu\text{S}$  (normal for rainwater). After a week, EC was increased to 1.0  $\mu\text{S}$  by adding fertiliser. On 20 June it was increased to 1.2  $\mu\text{S}$ , on 15 July to 1.5  $\mu\text{S}$ , and finally on 5 August to 3.2  $\mu\text{S}$  in all treatments. Increasing the EC was necessary to keep the rapidly growing chambered plants healthy and green. There were no symptoms of overdosing even in the slowest-growing plants. The outdoor plants were always less green than the plants grown inside chambers, probably due both to dilution of the nutrient treatment common to all plants on ebb/flood tables by rain and to the high light flux and wind outdoors.

The fertilisers used were “Pioner Macro Green” (19-2-15 NPK+Mg) and “Pioner Micro Solution” (B, Cu, chelated Fe, Mn, Mo, Zn), both made up as prescribed and mixed 1:1 (Brøste Ltd., Denmark).

#### Pest control

All the plants were sprayed with the microbiological insecticide DiPel (*Bacillus thuringiensis*, Cillus Inc., Denmark) at the beginning of the experiment to control caterpillars. To control aphids, we initially applied *Adalia bipunctata* (Biological Systems, Belgium) and later *Chrysoperla carnea* (Borggård Bioplant, Denmark) every two weeks. Problems with pests were negligible throughout the experiment.

#### Chambers and treatments

The plants were grown on 18 ebb/flood container tables (Fig. 1), with two in each of eight 15-m<sup>2</sup> hexadecagonal temperature-regulated closed-top chambers (CTCs) with domed roofs (Saxe et al., 1998; Leverenz et al., 1999b) and two on outdoor “reference” plots (Fig. 2). Outdoor growth is included in the graphic presentation of results (Figs. 4–6) but not in the statistical analyses of plant responses to climate change and competition design. The outdoor plots were left out of the statistical analyses because the chambers tracking outdoor conditions provided better references, with light conditions being representative of forest gaps. The plants in the outdoor plot received unnaturally high photosynthetic photon flux density (PPFD), far more intense than in forest gaps. Using the chambers tracking outdoor conditions as the reference for our study of climate change effects ensured that any chamber effects were comparable in controls and treatments.

The surrounding buildings and trees reduced the diffuse light reaching the CTCs by about 30% relative to open-field PPFD. Light-absorbing black/green plastic nets (Mononet 70AR, Rolloos Sørensen Ltd., Holland) mounted on the outside of the CTC glass panels removed another 70% of the incoming light, and the glass and aluminium structure of the CTCs removed 25% of the remaining light. The resulting light flux on south tables inside the CTCs was 16% of open-field PPFD, comparable to a forest edge or the open part of a forest gap. The northern plant table in each chamber was shaded by a second layer



Competition design		Intensity			
		1 seed / pot		2 seeds / pot	
		9 pots/frame	25 pots/frame	9 pots /frame	25 pots /frame
Type	Ash vs. ash	1 A (1)	2 A (4)	3 A (7)	4 A (10)
	Beech vs. beech	1 B (2)	2 B (5)	3 B (8)	4 B (11)
	Ash vs. beech	1 M (3)	2 M (6)	3 M (9)	4 M (12)

**Fig. 3** The competition design included 12 individual competition combinations (competitions 1–12), i.e., four intensities (one or two seeds per pot combined with pots set at two different pot distances) and three types (ash competing versus ash, beech vs. beech, and ash vs. beech). A, ash; B, beech; M, mixed species within frame or pot. See Fig. 1.

of Mononet 70AR, which reduced the light on these tables to 5% of open-field PPFD, comparable to the darker areas of a forest gap.

The PPFD was measured continuously in each chamber by means of four sensors, two above each plant table. An outdoor sensor was placed between the two outdoor plots at canopy level. The PPFD sensors were calibrated before and after the experiment. The sensors used were spherical sensors made from Hamamatsu G1126-02 GaAs photodiodes (Hamamatsu Photonics, Germany) mounted inside celluloid table tennis balls (Stiga, Sweden). This sensor responded similarly to the Li-Cor PAR sensor (Aaslyng and Rosenqvist, 1999), and was much cheaper. The light flux values of all 33 spherical PPFD sensors were recorded every 5 min. The values for each chamber and outdoors given in Fig. 2 are daytime mean values (sunrise to sundown) averaged over the total exposure period.

The atmospheric  $[\text{CO}_2]$  was enriched day and night in four of the chambers to an average of  $773 \mu\text{mol mol}^{-1} \text{CO}_2$  (Hydrogas, Denmark), measured as 24-h mean values averaged over the total exposure period. The  $[\text{CO}_2]$  in the other four chambers was  $413 \mu\text{mol mol}^{-1}$ , comparable to the mean outdoor concentration of  $423 \mu\text{mol mol}^{-1} \text{CO}_2$ . The exposure resulted in a relatively stable increase of  $360 \mu\text{mol mol}^{-1} \text{CO}_2$  day and night (Fig. 2). Due to the dark respiration of the ever-increasing canopy volume, the  $[\text{CO}_2]$  increased by  $80\text{--}120 \mu\text{mol mol}^{-1}$  during the night compared with daytime, with the highest absolute amplitudes occurring in chambers with the most plant material.

Cooling air was released along and between the rows of plant frames and below the tables to avoid shading the plants (Fig. 1). The air exchange rate in each  $60\text{-m}^3$  chamber was two per minute. Each  $\text{CO}_2$  chamber needed to be supplied with only 40 kg of liquid  $\text{CO}_2$  per week because the cooling system recycled 95% of the air.

In four of the chambers, two with elevated and two with outdoor  $[\text{CO}_2]$ , the air temperature tracked the outdoor air temperature, while the air temperature in the other four chambers, two with elevated and two with outdoor  $[\text{CO}_2]$ , was elevated by  $2.8^\circ\text{C}$  day and night relative to outdoors (Fig. 2). Air temperature and relative humidity were measured every 5 min in each chamber and outdoors using inter-calibrated, ventilated sensors (RTF-5C, DGT-Volmatic Ltd., Denmark), placed well above the canopies to the northeast but calibrated to represent the air above the top of the canopy.

Soil temperature probes (PF-5, DGT-Volmatic Ltd.) in one pot per chamber were evenly distributed between the north and south tables, and values were recorded every 5 min. Soil temperature followed the pattern of air temperature, though, on average, the soil temperature was  $1.0\text{--}1.5^\circ\text{C}$  cooler due to evaporative cooling.

The 24-h average relative humidity (RH) was primarily defined by temperature, transpiration, and condensation in the cooling system, with average values of 72–77% and a range of 50–90%.

#### Competition design

To investigate how climate change and light flux affect competitive regenerative growth of beech and ash, a large number of seedlings were placed in 12 different competition designs (Figs. 1–3).

The competition *types* and *intensities* (Fig. 3) may be functionally categorized as (a) competition for *light* (1A, 1B, 1M, 2A, 2B, 2M) and (b) simultaneous competition for *light* and *root space* (3A, 3B, 3M, 4A, 4B, 4M). These may be functionally sub-categorised into *close* and *distant* positioning of pots (Fig. 1).

In the light competition design, the seedlings competed with their neighbours for light as they grew larger, but had a fixed root space of 280 ml. In the root + light competition design, as they grew larger the seedlings competed with their neighbours in surrounding pots as well as their neighbour in the same pot. This resulted in more intense competition for light than in the light competition design. Furthermore, the two seedlings in each pot competed for root resources, i.e., physical space, water and nutrients, and perhaps “chemical space” (allelopathy).

In the close competition design with 25 pots per frame, only the eight plants in the inner “circle” of each frame were sample plants, while the 16 outer edge plants and the single centre plant in each frame were not analysed (Fig. 1). This design gave a fixed distance to all neighbouring plants, i.e., 46 mm or 65 mm on average, provided that each plant was at the centre of its pot. In the distant competition design with nine pots per frame and 16 empty spaces, all the plants were sample plants, as the plants in the neighbouring frames acted as “hedge plants”. The empty spaces in the frames ensured a fixed distance between plants, i.e., 92 mm or 130 mm on average, provided that each plant was at the centre of its pot.

The frames were initially placed 8–18 cm from each other, to avoid shading from plants in neighbouring frames. During the exposure experiment, all the canopies developed from being less than 1-layered canopies to being multi-layered. As plants grew outside the perimeters of the frames, the distances between individual frames were successively adjusted to minimise interference by neighbouring frames in the competition design specific to each frame. This means that distant competition designs were given an increasing distance to neighbouring frames, while close competition designs were given decreasing distances to neighbouring frames.

### Measurements

On 17 May, 10 ash and 10 beech plants representative of the initial size of seedlings in all pots were weighed and measured.

Plant height was measured regularly from the beginning of the experiment (17 May, 29 May, 20 June, 5 July, 31 July, 11 September). Plant height was defined as the vertical distance from the top of the pot to the maximum effective “shading” height of the top leaf. This parameter increased with stem length but decreased with the bending of seedlings and leaves (particularly in ash), as they grew taller or “leaned over”. Plant height was measured in this way since it was felt to be more relevant to competition than stem length.

The canopy density was measured towards the end of the experiment by measuring PPFD transmission below and immediately above the canopies belonging to each of the 288 frames.

On 17 September, 123 days after the exposure was initiated, growth had nearly ceased. The 3672 edge plants were discarded and the 3672 sample plants were harvested and analysed along with the control plants in 1-l pots. In pots with two seedlings of the same species, the plants were marked during harvest as the smaller or the larger specimen. Each plant of both species was separated into three fractions: leaves, stems, and roots.

The number of leaves on each plant was counted, total leaf area measured with a Li-Cor Li-3000 Portable Area Meter mounted on the Li-3050 A belt conveyor (Li-Cor Inc., USA), and fresh weight measured using a Mettler Toledo PG 503-S scale (Mettler Toledo GmbH, Switzerland). Total stem length was measured with a ruler, fresh weight determined, and the maximum stem diameter near the stem basis measured with calipers (Helixpoint 150, Helios-Messtechnik, Germany). Data from the scale and the calipers were digitally transferred (Winwedge Ltd., USA) to a computer. All the sample plant roots were rinsed thoroughly and the fresh weight determined. The three fractions (leaf, stem, and root) of each plant were dried separately at 70°C for 72 h and the dry mass determined.

### Statistical methods

The species differences in average growth were calculated and analysed using linear models in the JMP software (SAS Institute, USA), including all biometric values. The experimental design was a split-split-split-plot design. By stepwise regression, the seven factors of significance in the linear response model were:  $[\text{CO}_2]$ , temperature, light,  $[\text{CO}_2] \times \text{temperature}$ ,

$[\text{CO}_2] \times \text{light}$ , temperature  $\times \text{light}$ , and  $[\text{CO}_2] \times \text{temperature} \times \text{light}$ .

## Results

### Overall growth responses

The PPFD at the outdoor reference plots was much higher than below a forest canopy and not conducive to the growth of the tree seedlings under investigation. Beech was far more tolerant to the light flux outside the chambers than ash, and grew significantly better ( $p < 0.0001$ ) under these conditions, as measured by all growth variables. Inside the chambers, plant growth depended on the species response to the modified climate:  $[\text{CO}_2]$ , temperature and PPFD, and on competition design.

At the end of the first season, the seedling canopies absorbed as much as 99.5% of the incoming PPFD, and the densest canopies were those with the highest initial competition intensity and the highest PPFD, temperature, and  $[\text{CO}_2]$  during the experiment. Ash canopies typically developed less densely than beech canopies, while mixed canopies developed intermediate densities.

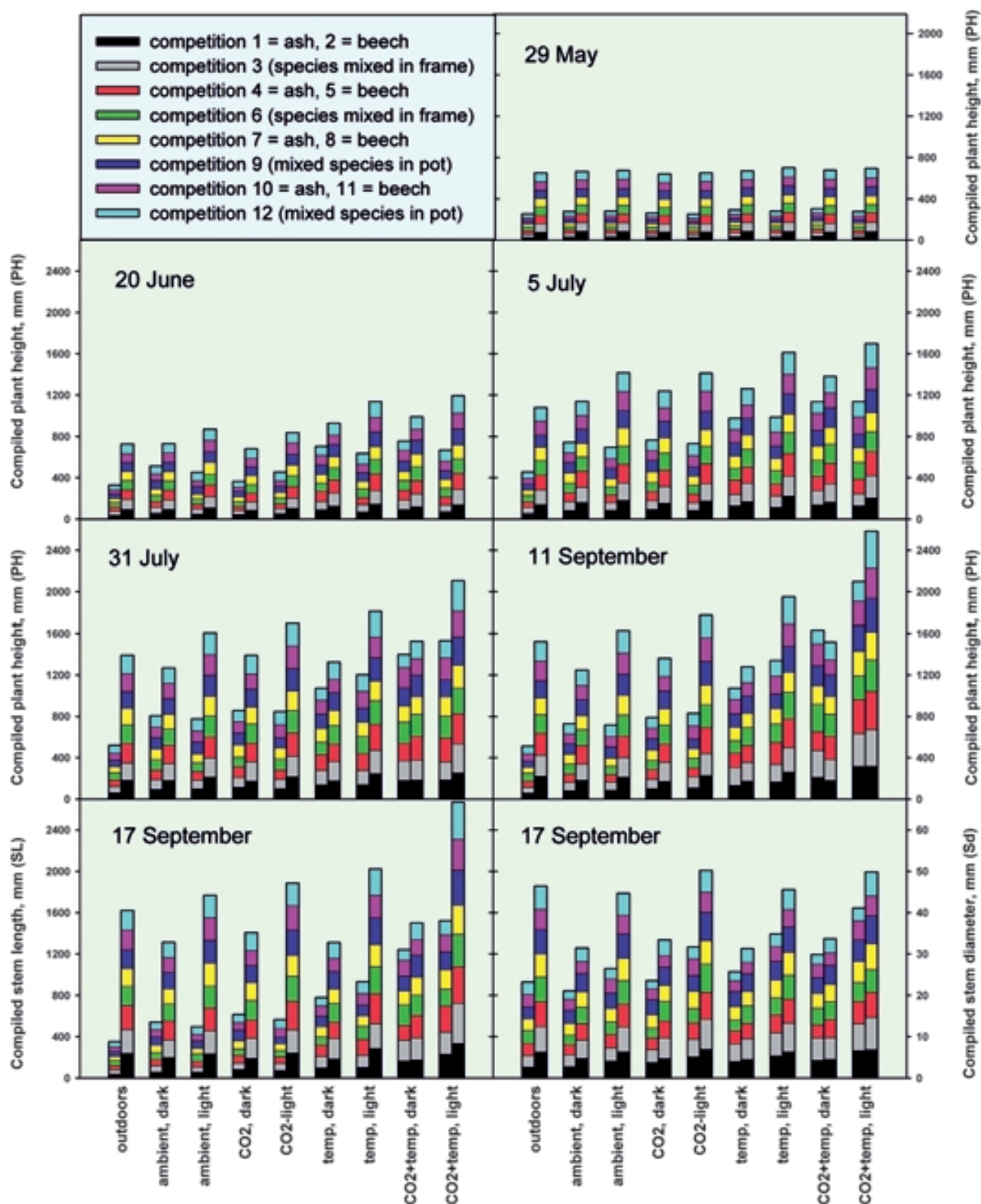
Seedlings of both species grew better on the south tables (16% of open-field PPFD) than on the north tables (5% of open-field PPFD), measured by all growth variables ( $p < 0.0001$ ).

While the growth of beech seedlings responded more positively to the higher PPFD inside chambers than ash seedlings, elevated temperature, and simultaneously elevated  $[\text{CO}_2]$  and temperature stimulated ash growth and canopy density more than beech growth and canopy density, whether in monoculture or in mixed species canopies (Figs. 4, 5). These results were statistically significant for most biometric indices.

Beech grew taller (plant height) than ash in the year 2000 climate and when temperature and  $[\text{CO}_2]$  were increased separately. However, when  $[\text{CO}_2]$  and temperature were increased *simultaneously*, ash grew taller than beech at the lower PPFD of the north table ( $p < 0.05$ ), giving it the potential to shade out beech in later competition (Fig. 4). At the higher PPFD of the south table, beech still grew taller than ash, though less so than in the present climate, i.e., CTC 3 and 5 (Fig. 2).

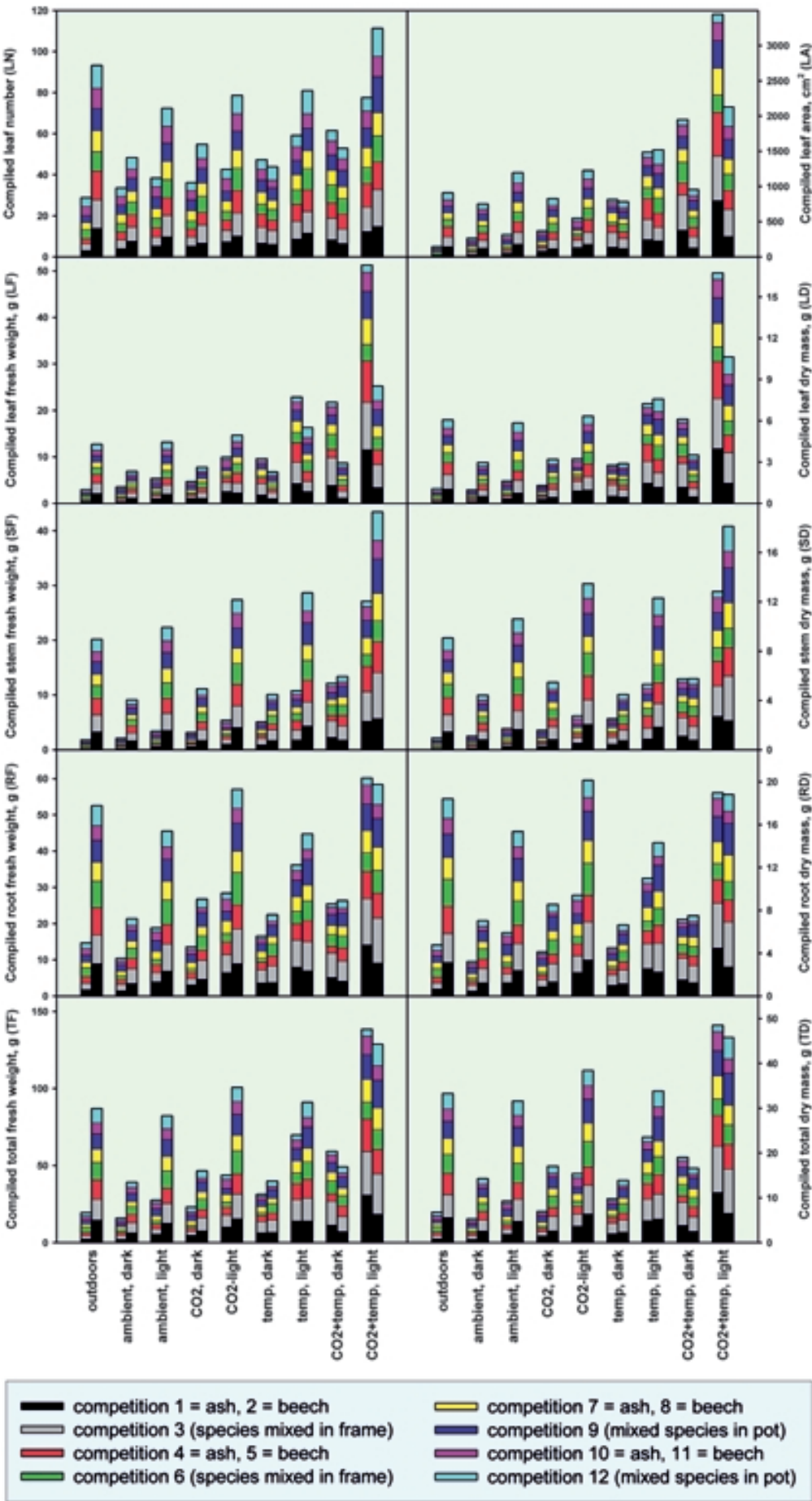
The leaf area ( $p < 0.001$ ), leaf fresh weight ( $p < 0.001$ ), and dry mass ( $p < 0.01$ ), and total plant fresh weight ( $p < 0.05$ ) and dry mass ( $p < 0.05$ ) of ash were all greater than for beech at both light levels with simultaneously elevated temperature and  $[\text{CO}_2]$  (Fig. 5). Ash improved its competitive capacity over beech mainly by allocating resources to leaves, as reflected by the improved total leaf area. However, the number of leaves was higher in ash only at the lower PPFD ( $p < 0.05$ ).

Ash has a competitive advantage due to its growth form, i.e., its ability to arch long leaves upwards as a kind of extension of the stem. Beech has a competitive advantage due to its mosaic-like positioning of leaves, which results in very dense canopies, allowing less growth beneath beech canopies than beneath ash canopies. Six weeks into the exposure period, beech began to branch, efficiently building its mosaic canopies. At this stage, ash has two options for survival: it can either arch its leaves



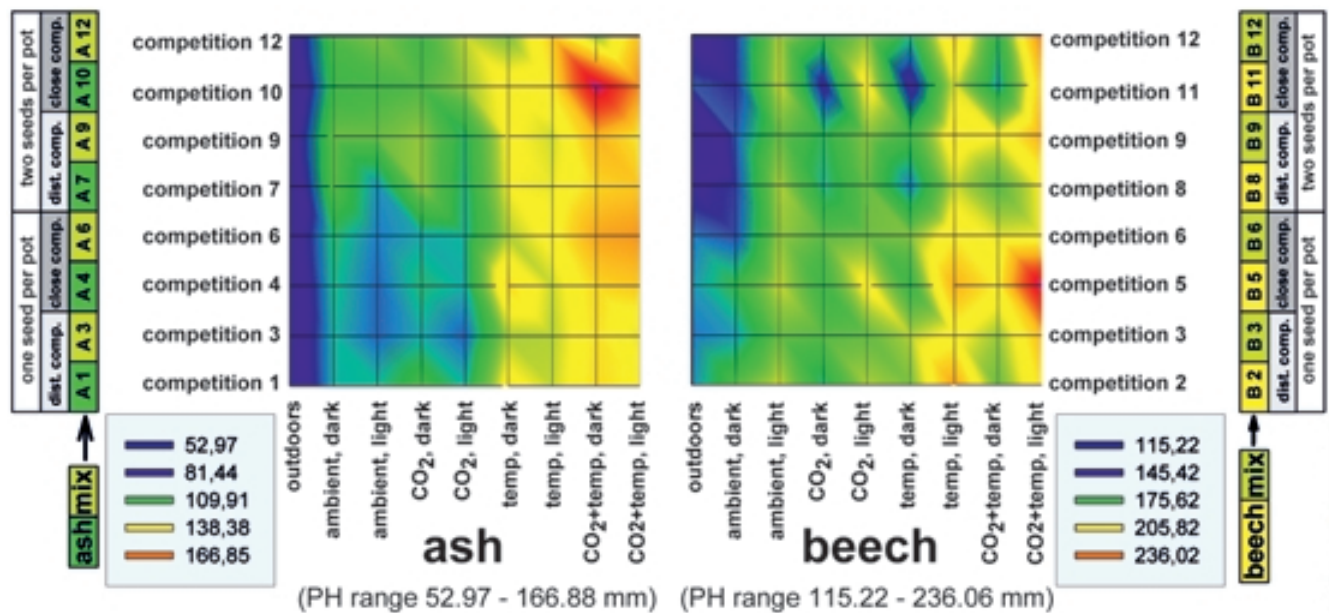
**Fig. 4** Responses to climate change and 12 types and intensities of competition measured by three different biometric indices on the indicated dates. The first five graphs show the compiled plant height (PH) on five dates throughout the experiment. PH is the vertical distance from the top of the pot to the maximum effective "shading" height of a top leaf. The other two indices include the compiled length of harvested stems (SL) and their compiled diameter (Sd). The legends in the top left corner indicate the individual competition designs de-

scribed in Fig. 3. Each species participates in eight of the twelve designs. For each climate treatment and biometric index there are two compounded bars; the left is for ash and the right is for beech. The eight sections of each bar represent the eight competition designs of one species. "Ambient" refers to the outdoor temperature and [CO<sub>2</sub>] levels. "Dark" and "light" refer to 5% and 16% of open-field PPFD, respectively. "Temp" and "CO<sub>2</sub>" refer to the elevated temperature and [CO<sub>2</sub>] in Fig. 2. The dates indicate the day of measurement or harvest.



**Fig. 5** Response of 10 different biometric indices to climate change measured at the end of the growth season. The indices include the combined number and area of leaves (LN, LA), the combined leaf fresh weight and dry mass (LF, LD), stem fresh weight and dry mass (SF, SD), root fresh weight and dry mass (RF, RD), and the combined total plant fresh weight and dry mass (TF, TD). Legends for competition types and abbreviations for climate treatments are given in Fig. 4, the actual climate treatments in Fig. 2, and competition numbers in Fig. 3.





**Fig. 6** The growth of both species was analysed for each growth index using contour plots of competition design versus climate treatments. The largest growth was assigned the colour red, the smallest growth, dark blue. This figure is an example of these contour plots, in this case competitive plant height on 5 July (PH57). The main results (red hot spots) for contour plots of all biometric indices are given in Fig. 7.

Comp. Design →		1A (1)	1M (3)	2A (4)	2B (5)	4 A (10)	4 M (12)
Climate treatment	Temp. And light				Beech: PH295		Ash: PH295
	CO <sub>2</sub> + temp. and low light					Ash: PH206, PH57	
	CO <sub>2</sub> + temp. and light	Ash: LN,LA,Sd, RF,LF,RD,SD LD,TF,TD	Ash: SF Beech: LN,LA, Sd,SL,RF,SF,LF, RD,SD,LD,TF,TD	Ash: SL PH317 PH119	Beech: PH57 PH317 PH119		Beech: PH206

**Fig. 7** Results of analyses by contour plots, indicating at which combination of competition design and climate change the individual growth indices show absolute peak values for each species. Number codes for dates were given as month number extended by the day number of that month: 295 = 29 May; 206 = 20 June; 57 = 5 July; 317 = 31 July; 119 = 11 September; A, ash; B, beech; LA, leaf area; LD, leaf dry mass; LF, leaf fresh weight; LN, leaf number; M, mixed species within frame or pot; PH, competitive plant height; RD, root dry mass; RF, root fresh weight; SF, stem fresh weight; Sd, stem diameter; SD, stem dry mass; TD, total dry mass; TF, total fresh weight.

above the dense beech canopies to avoid being shaded, or tolerate a higher degree of shading than beech by building a larger area of shade leaves, shading the lower parts of the beech canopy. In the present climate, ash did mostly the latter, but with a simulated future climate, mostly the former.

The plants grown using two seeds of the same species in 1-l pots did not grow significantly larger in terms of root and total dry mass than plants grown under similar conditions in 280-ml tube pots (frames nos. 3A and 3B, Fig. 1). The limited pot volume of 280 ml was therefore considered non-restrictive to root growth during the length of this study.

Dependence on competition design

The growth responses of beech and ash seedlings to climate change (Figs. 4, 5) depended on the competition design (Figs. 1, 3). Though the growth of beech seedlings responded

more positively to light than ash seedlings, there was a tendency for this advantage to diminish with increasing competition density.

Analyses of growth activity using contour plots of competition design versus climate treatments (Fig. 6) pinpointed the overall conditions which induced the largest growth activity for each species and measured by each biometric growth index. The main results of these analyses are given in Fig. 7.

Measured by most growth indices, both species grew best at combined elevated temperature and [CO<sub>2</sub>], and in the more open competition designs (Figs. 4, 5), and both species competed most successfully against ash when two seeds of either species were allowed to germinate in each pot.



	PPFD % of open field conditions	Growth at ambient climate	Growth at elevated [CO <sub>2</sub> ]	Growth at elevated temp.	Growth at combined elevated [CO <sub>2</sub> ] and temp.
Ash	5%	100%	132%	184%	355%
	16%	100%	167%	256%	524%
Beech	5%	100%	119%	98%	115%
	16%	100%	122%	108%	145%

**Fig. 8** Comparison of the synergistic effect of elevated [CO<sub>2</sub>] + temperature on ash and beech total dry mass as an average of all competition conditions.

Comparing the growth of both species in all the competition designs suggested the possibility that, in the predicted future climate, beech may retain only *some* of the advantages it has today in competition with ash. In this experiment, beech developed the largest total leaf area *only* in competition design no. 12, which is the highest density, mixed species ash-beech competition design, and only at high PPFD ( $p < 0.05$ ). The competitive plant height of ash gained the advantage over beech in the predicted future climate, but beech stayed ahead of ash except in the most open competition designs. The latter was the reason why, as an average of all the competition designs, ash grew taller than beech in very low light (north tables) with simultaneously elevated temperature and [CO<sub>2</sub>] (CTC nos. 2 and 8).

While beech root mass in the present climate was twice the ash root mass in first-year seedlings, the predicted future climate gives ash roots a fresh weight and dry mass comparable to beech roots in all competition designs except no. 12.

While the overall beech growth was 2–3 times greater than for ash in the present climate, the predicted future climate gives ash larger overall growth in several of the open (1A, 1B, 1M) competition designs ( $p < 0.05$ ), and comparable growth in intermediate (2A, 2B, 2M, 3A, 3B, 3M) designs. However, beech retains larger growth in several of the densest (4A, 4B, 4M) competition designs ( $p < 0.05$ ), and may still be able to outperform ash under these conditions.

There were no detectable interactions between the two species when grown in the same pot (data not shown), which indicate the absence of allelopathic mechanisms.

## Discussion

### *More-than-additive responses to elevated [CO<sub>2</sub>] and temperature*

The present study confirms that the relative increase in tree growth caused by elevated [CO<sub>2</sub>] may be enhanced by temperature (Idso and Idso, 1994; Idso et al., 1995; Tjoelker et al., 1998; Leverenz et al., 1999a; Bruhn et al., 2000), though there are known exceptions to this rule (Teskey, 1997; Kellomäki and Wang, 2001). The study also confirms that the effects of elevated temperature on CO<sub>2</sub> growth responses in trees are species-dependent (e.g., Norby et al., unpublished: red maple and sugar maple; King et al., 1996: loblolly pine and ponderosa pine; Tjoelker et al., 1998: five boreal species).

Bruhn et al. (2000) reported a more-than-additive interaction between elevated temperature and elevated atmospheric [CO<sub>2</sub>] in beech. This was partly confirmed by the present study (Fig. 8).

Temperature and [CO<sub>2</sub>], elements of climate change, are not the only growth conditions which may change the relative growth rate ranking of woody species. It is well known that the relative growth rate of woody seedlings and saplings may also change rank depending on PPFD levels, growing conditions such as nitrogen availability, and the duration of the study (Finzi and Canham, 2000; Caspersen and Kobe, 2001; Sack and Grubb, 2001).

By way of example, we found that the positive synergistic effects on beech growth were only true at the high PPFD condition (Fig. 8). Hättenschwiler (2001) also described interaction between PPFD and [CO<sub>2</sub>] for beech. Ash responded relatively more than beech to both elevated [CO<sub>2</sub>] and to elevated temperature, as well as to the combined exposures, and, in contrast to beech, all positive growth effects were relatively higher under the low than under the high light flux conditions. For ash, there was a positive synergistic effect ( $p < 0.001$ ) on the total dry mass at both PPFD levels (Fig. 8).

### *Competition under the predicted future climate*

In this study, a simultaneous increase in temperature and [CO<sub>2</sub>] either gave ash the advantage over beech or it equalled beech in height growth, leaf area, and canopy density in all but the fiercest competition situations. Ash will increase its competitive capacity relative to beech, particularly at PPFD levels comparable to those of the darker sub-canopy regions in mixed ash-beech forests. Beech may still have the advantage in the centre of a forest gap where the PPFD is highest, if the ash-beech competition were to start from seeds. However, in mature mixed ash-beech forests, there is sufficient light below the closed canopy for both species to germinate and begin competition as seedlings and saplings before a gap is formed by storm or lightning. Under these low PPFD conditions, the results of this study indicate that in the predicted future climate this competition may be won by ash and, when a gap forms, ash would already be ahead of beech.

Our data suggest that, in the predicted future climate in Danish unmanaged mixed forests, ash could be more predominant in competition with beech than it is today. However, it is important to express a number of reservations when interpreting data from seedling studies in controlled climates in terms of what may happen to mature forests under natural conditions

with climate change. As a minimum, these reservations include the following:

1. All chambers are bound to have a microclimate somewhat different from that in a forest. Results from chamber experiments on, e.g., leaf area are not always in agreement with results from free air carbon dioxide enrichment (FACE) experiments (Taylor et al., 2001). Nevertheless, the air and soil temperature levels and patterns in our chambers were very similar to those in a nearby, unmanaged forest (data not shown). The fertiliser administered did not lead to an excess of available nutrients in the soils compared with the forest. During the daytime in one week in July, PPFD within the forest canopy varied from 6–132  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , depending on the day and on the position within a gap. The chamber light flux on north and south tables averaged 52–93  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 141–292  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively, on the same days. The darker north tables thus represented the most realistic light flux conditions throughout the natural gap. Even wind speeds were comparable. We conclude that there were only minor microclimatic differences between our chambers and true forest conditions.
2. The chambers protected seedlings from insects and mammal predators. Browsing, for example, may have an effect on competition, since ash is more favoured by browsers than beech (Gill, 2000; Johnson, 2005). Allowing faunal interference would introduce a great deal of experimental noise which would blur the main objective of this study, namely to investigate ash-beech competition in a mixed forest gap.
3. The use of potted plants ensured well-defined above-ground distances between plants, without having to take out or replant thousands of plants to obtain a symmetrical setup. And the use of potted plants made it possible to separate the root systems of individual plants at the final harvest and to complete all measurements within a realistic time span. Comparison with seeds grown in 1-l pots indicated that the confined root space in 280-ml pots did not restrict growth for the duration of this experiment. However, growing plants in pots does represent an artificial situation in that the root systems are enclosed and do not experience natural belowground competition, while the shoots are allowed to compete for light directly with their neighbours. We did, however, simulate belowground competition in pots with two seeds, though this is still far from natural conditions with many competing neighbouring seeds.
4. Our experiment supplied non-limiting water and nutrient conditions and constant light conditions, rather than variable light flecks. This sets our experimental conditions apart from the natural conditions in a forest (Stitt and Krapp, 1999; Leakey et al., 2002). Rust and Savill (2000) studied competitive relationships in root systems of ash and beech *in vivo* and *in vitro* in the present climate. The distribution of ash roots was determined by competition for light under a closed overstorey of beech, while the distribution of beech roots changed if there was competition with ash roots. Interspecific competition for water strongly reduced the growth and survival of beech saplings. Competition for water was concluded to be the reason for beech sapling dieback at some sites. This evidence supports rather than contradicts the hypothesis that ash may improve more than beech with the expected climate change, since this also includes drier summers in Denmark.
5. Since tree seedlings and saplings may acclimate to elevated  $[\text{CO}_2]$  (Medlyn et al., 1999; Liozon et al., 2000; Polle et al., 2001; Catovsky and Bazzaz, 2002), any difference between species in this respect would affect the outcome of long-term competition. It was not possible to measure acclimation in this relatively short-term study, limited to one growing season. Leaving acclimation aside, elevated temperature and  $[\text{CO}_2]$  improved the relative size of ash vs. beech by 200–400% during the first growing season, giving ash a significant impetus for competition in subsequent growing seasons. However, severe reservations must be expressed when projecting short-term experiments to long-term results, such as ultimate changes in the forest tree composition in natural forests.
6. The genetic variation within a stand will affect its response to climate change and, in the long term, climate change may affect the genetic variation within the stand (Gunter et al., 2000; Catovsky and Bazzaz, 2002). As an example, Tognetti et al. (1998) demonstrated that acclimation of long-term shade-grown beech seedlings to changing light conditions depended on their geographical origin. As the present study included only one provenance of each species, our conclusions are valid for these provenances only, not for the two species in general.
7.  $[\text{CO}_2]$  is known to accelerate senescence in sun leaves and prolong leaf function in shade leaves of oak (Cavender-Bares et al., 2000). If such an interaction between  $[\text{CO}_2]$  and light is different for ash and beech, this could affect the outcome of competition in mature forests with full exposure of the leaves in the top of the canopy. This mechanism was not tested in the experiment.

The reservations expressed illustrate that even results from complex, highly controlled climate change seedling studies such as the present one cannot easily be generalised to apply to natural growing conditions and to the later developmental stages of mature forests. Furthermore, we did not investigate other important species, e.g., oak, and interaction with such species further complicate the picture of what may happen in mixed, unmanaged forests under future climate change.

## Conclusions

In the coming 50–100 years, atmospheric  $[\text{CO}_2]$  and global temperatures are expected to increase. In Denmark, managed forestry will move towards unmanaged forestry. Our data support the assertion that climate change reverses the competitive balance of ash and beech seedlings under simulated forest conditions. The strength of our chamber-based study is the high degree of complexity, with interaction between a precisely regulated, elevated temperature, elevated  $[\text{CO}_2]$ , two PPFD levels, and 12 competition designs.

The results suggest that unmanaged mixed ash-beech forests could possibly shift in species composition from beech- to ash-dominated. However, the outcome of competition in the real world depends on more factors and more species than were investigated here, and a chamber-based seedling study can never predict with any certainty the outcome under natural conditions. To society at large, a change in forest tree species composition is of little consequence (Kaae and Madsen, 2003). Nevertheless, it is important for forest managers and industry to be able to anticipate these changes. We therefore en-

courage future field-based climate change studies of competition between tree species under elevated temperature  $\times$  elevated  $[\text{CO}_2]$   $\times$  PPFD. This could be facilitated by a FACE system with temperature control.

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