SILVA FENNICA
Silva Fennica 45(2) research articles
www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Morphological Mechanism of Growth Response in Treeline Species Minjiang Fir to Elevated CO_2 and Temperature

Ying Hou, Jintao Qu, Zukui Luo, Chao Zhang and Kaiyun Wang

Hou, Y., Qu, J., Luo, Z., Zhang, C. & Wang, K. 2011. Morphological mechanism of growth response in treeline species Minjiang fir to elevated CO_2 and temperature. Silva Fennica 45(2): 181–195.

To test whether and how morphological traits are linked with growth responses of plants to temperature and CO_2 is important for understanding the mechanism underlying how plant growth will respond to global warming. In this study, using closed-top chambers to mimic future elevated CO_2 and temperature, the growth response, morphological traits of Minjiang fir (*Abies faxoniana* Rehd.et Wils.) and the relationship of the two were investigated after two years of exposure to the single and combined elevation of CO_2 and temperature. The results showed that biomass of Minjiang fir was 21%, 31%, and 35% greater than the control in elevated CO_2, elevated temperature and the combination of elevated CO_2 and temperature treatments, respectively. Elevated CO_2 and temperature significantly affected the morphology of Minjiang fir, and a few morphological traits were highly correlated with growth responses. Larger branch angles at the upper layer, crown volume, and relative crown length contributed to positive growth responses to elevated CO_2, while decreased specific leaf area (SLA) constricted any further growth response. Leaf morphological traits were more closely correlated with the response ratio than crown did in the elevated temperature, while in the combination of elevated CO_2 and temperature, crown was more correlated with the response ratio than the leaf morphological traits. Thus, our results indicate that morphological traits may contribute differently to growth responses under different experimental conditions.

Keywords *Abies faxoniana*, climate change, crown architecture, leaf morphology, response ratio

Addresses *Hou* and *Qu*, Department of Life Sciences, Shangqiu Normal University, Shangqiu, China; *Luo*, School of Environment and Life Sciences, Kaili University, Kaili, China; *Zhang* and *Wang*, Shanghai Key Laboratory of Urbanization and Ecological Restoration, East China Normal University, Shanghai, China, and University of Eastern Finland, School of Forest Sciences, Joensuu, Finland **E-mail** (*Wang*) kywang@re.ecnu.edu.cn

Received 16 December 2010 **Accepted** 25 March 2011
Available at http://www.metla.fi/silvafennica/full/sf45/sf452181.pdf

1 Introduction

Plant growth is a complex phenomenon that integrates many physiological and morphological processes. Growth responses of plants to elevated CO_2 and temperature are closely related with their physiological and morphological adaptations. During the last several decades, many papers have been published describing the growth response of plants to elevated CO_2 and temperature (Gunderson and Wullschleger 1994, Idso and Idso 1994, Pritchard et al. 1999, Ainsworth and Long 2005). However, there have been highly variable results which depend on the species, growing conditions, and the duration of CO_2 and temperature increase. There is abundant information on the physiological mechanisms of growth responses (Bazzaz and McConnaughay 1992, Idso and Kimball 1992, Wang et al. 2008), but it remains unknown how and to what extent the growth response is correlated with morphology. Although examining physiology is valuable in the study of plant responses to elevated CO_2 and temperature, the physiological parameters are a relatively insensitive indicator of what is actually happening to plant growth in terms of structure and function (Stulen and Herton 1993, Sattler and Rutishuser 1997). For example, differences in root depth and branching may influence patterns of water and nutrient uptake independent of total biomass (Tremmel and Bazzaz 1993). White (1984) pointed out that in the vast literature of plant responses to the environment, morphology has been ignored.

Morphology is the study of the size, shape, and structure of organisms or one of its parts (Sattler 1996, Sattler and Rutishauser 1997). Various environmental conditions make different phenotypic expression of the same genotype. Therefore, plant morphology has been recognized to be the consequence of interactions between genotype, environment and developmental history. Elevated CO_2 alters plant structure through its effects on both primary and secondary meristem of shoots and roots. Similarly, elevated temperature may contribute to developmental and morphological changes by influencing meristematic cells within the bud (Pritchard et al. 1999). These changes in morphology, in turn, can influence the growth responses of plants to CO_2 and temperature enrichment through light interception, canopy atmosphere exchange processes, and nutrient and water absorption (Yokozawa et al. 1996, Gielen et al. 2002). Therefore, investigating the effects of elevated CO_2 and temperature on plant morphology will lead us to gain a better understanding of how plant growth will respond to future changes in climate. Such a study can also provide basic information for forest management and carbon cycle models because some process-based growth models include not only physiological indices, but also morphological indices, such as tree crown size, leaf area, and branching (Landsberg and Waring 1997, Cropper 2000).

Recently, some experiments were carried out to explore the relationship between morphology and growth at elevated CO_2 and temperature. For example, a study by Gielen et al. (2002) showed that success of white poplar competing with black poplar and euramerican poplar at elevated CO_2 was due to longer, more horizontally orientated branches. Research on the relationship between crown structure and biomass revealed that crown architecture affected tree growth through the control of leaf area and its display for effective light capture and photosynthesis (Wang and Jarvis 1990). The spatial distribution of leaf area and biomass within a crown might affect carbon assimilation through its effect upon light interception and net CO_2 exchange rates for the whole tree (Reekie and Bazzaz 1989). However, the magnitude and direction of morphological effects on growth response to elevated CO_2 and temperature, or which component of morphological traits are best related with growth response, remains unanswered.

Our primary objective in this study was to investigate the morphological mechanism underlying the growth response of Minjiang fir (*Abies faxoniana* Rehd.et Wils.) to elevated CO_2 and temperature. We first tested the effects of elevated CO_2, elevated temperature and the combination of the two on the growth and morphology of Minjiang fir. Since structure determines the function, we predicted that the growth response would be affected by its morphology. Thus, the relationship between growth response and morphological traits was tested and discussed. We will address the following questions: 1) whether elevated CO_2 and temperature affects the growth and morphology of Minjiang fir; 2) which and how morphological traits are correlated with growth response to elevated CO_2 and temperature.

2 Material and Methods

2.1 Experimental Site and Facility

The experiment was conducted in a closed-top chamber system situated in Maoxian Ecological Research Station (31°41´07˝N, 103°53´58˝E, 1800-m altitude) which belongs to the Chengdu Institute of Biology, Chinese Academy of Science. Mean annual precipitation was 919.5 mm and the mean air temperature was 8.6 °C. The average CO_2 concentration over one growth season was around 360 $\mu mol \cdot mol^{-1}$.

Each chamber (3 m in height; 9.35 m^2 in ground area; 24.5 m^3 in volume) was composed of a prism as its main body that was made up of a hendecahedron and a corresponding partial sphere as its top. Chambers were connected with a heating-cooling system, a CO_2 flow system and a wind exchange system, which were controlled by a computer. The computer-controlled heating and cooling system (CAJ-4511YHR, L'Unité-Hermetique, Barentin, France), together with a set of magneto-electric valves (controlling the pure CO_2 supply), enabled the temperature and CO_2 concentration to be adjusted automatically inside the chambers to conform to the ambient conditions, or to achieve a specified enrichment in CO_2, a rise in temperature, or a combined elevation of CO_2 and temperature.

2.2 Experimental Design

This experiment was conducted at two levels of CO_2 concentration and temperature, thus four treatments were formed: (1) outside ambient temperature and CO_2 concentration (CON); (2) elevated temperature at ambient CO_2 (ET, i.e. CON+ (2.0 ± 0.5 °C); (3) elevated concentration of CO_2 at ambient temperature (EC, i.e. CON + (350 ± 25) $\mu mol \cdot mol^{-1}$); (4) combined elevation of CO_2 and temperature (ECT). CO_2 concentration in EC and ECT, temperature in ET and ECT were elevated continuously (24 $h \cdot day^{-1}$) throughout the growing season. In order to provide a comparison on the CON treatment and to detect whether the chamber itself affects the morphology of plants, an "O" treatment was conducted with six trees and without a chamber. A chamber was used for each treatment, so four chambers was used in total. There were six containers in each chamber. Containers rotated among chambers once a week (simultaneously with a change in CO_2 concentration and temperature) during the growing season, which ensured that all containers spent approximately equal time within each chamber. This rotating averaged out any chamber differences (Gavazzi et al. 2000).

In this system, the hourly means of the 15 second readings taken from April to October in 2005 and 2007 indicated that the target temperature and CO_2 concentrations were achieved (Fig. 1). The mean ambient CO_2 concentration was 363.8 (±34.2) $\mu mol \cdot mol^{-1}$. The CO_2 concentration in EC and ECT was 347.1 (±22.1) $\mu mol \cdot mol^{-1}$ and 352.8 (±27.6) $\mu mol \cdot mol^{-1}$, respectively, higher than in the CON. The CO_2 concentrations were within 650~800 $\mu mol \cdot mol^{-1}$ for 91.32% (EC chamber) and 91.67% (ECT chamber) of the exposure time. The temperature in ET and ECT was 2.0 (±0.12) °C and 2.0 (±0.18) °C, respectively, higher than that in the CON. The air temperature was within 1.0~3.0 °C for 95.06% (ET) and 88.73% (ECT) of the exposure time. The actual rise in CO_2 concentration and temperature in this experiment suggests that the control on temperature was less precise than on CO_2 concentration due to many difficulties, such as a lack of technical resources.

2.3 Plant Material

Minjiang fir (*Abies faxoniana* Rehd.et Wils.) is very common in Western China and sometimes occurs as a pioneer species on clear-cutting sites in subalpine coniferous forests (the so-called dark coniferous forests). It is also one of the main constituents of the timberline climax communities in the Qinghai-Tibetan Plateau. In order to simulate conditions in the natural ecotone, we randomly collected native soil with seeds and roots of herbs from the subalpine ecotone of east Qinghai-Tibetan Plateau in the winter of 2005. The volume of soil clod was approximately

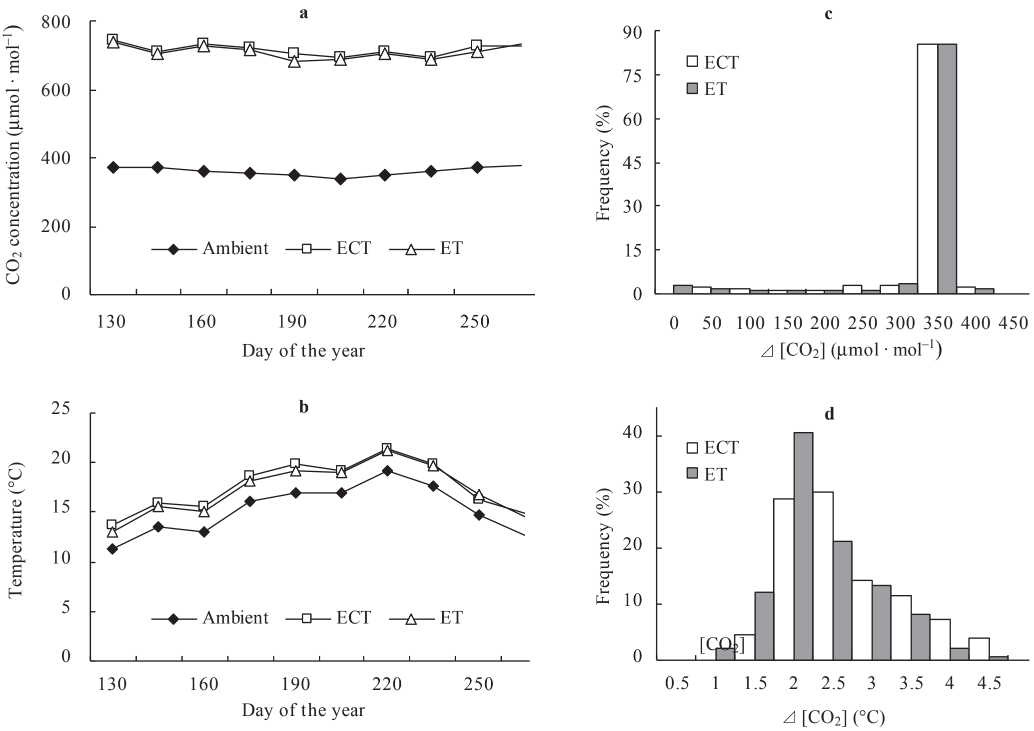

Fig. 1. Time course (a, b) and frequency distribution (c, d) of changes in CO_2 concentration and air temperature among treatments. Plots are based on hourly means of 15 seconds. EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature.

0.168 m^3 (70 cm length, 60 cm width, and 40 cm in height). An intact clod was put into one woody container. Healthy and uniform size (height, basic diameter, crown length and width; Table 1) Minjiang fir seedlings (9 years old) were transplanted into the containers, with one seedling in each container. The volume of container was also 0.168 m^3. This container size was selected to eliminate root binding in Minjiang fir growth as known from our previous survey. To avoid the influence of the container wall on the seedling root, the seedling was planted in the middle of the container. Containers were then moved into chambers for CO_2 and temperature treatment in the spring of 2006 (before bud burst). During the two-year treatment, no fertilizer was added to the containers. The native soil moisture content was maintained through a drip irrigation system. Since the experiment was carried out in the subalpine zone, the temperature in late autumn and winter was very low and the plants stopped growing. Therefore, the climate treatments began in April and ended in October when plants began to go through natural senescence. The rest of the time, the seedlings were growing in the ambient atmosphere (from November to March the following year). At the end of experiment, only the upper two whorls born during the treatments were totally affected by the treatments, whereas elsewhere in the canopy, only part of the shoots were born during the treatments because at the beginning of treatments, the seedlings were 9 years old.

2.4 Measurement

Minjiang firs were destructively harvested after two growing seasons in October, 2007. Plants with soil were dug out, and the roots were carefully washed. For each of the trees, we measured height, diameter, crown length, crown width along

Table 1. Height, diameter, crown length, crown width and dry mass of Mingjiang fir before treatment (mean ± standard error, n = 6).

Measured variables	CON	EC	ET	ECT
Height (cm)	51.89 ± 1.21	51.20 ± 1.63 (0.97) *	53.76 ± 1.40 (0.60)	52.83 ± 1.29 (0.93)
Diameter (mm)	9.17 ± 0.36	8.91 ± 0.60 (0.96)	10.17 ± 0.28 (0.30)	10.40 ± 0.50 (0.17)
Crown length	38.14 ± 2.01	37.84 ± 1.34 (0.91)	42.09 ± 2.69 (0.40)	39.94 ± 1.85 (0.87)
Crown width	22.03 ± 1.58	21.42 ± 1.02 (0.98)	24.21 ± 1.83 (0.62)	23.60 ± 1.53 (0.81)
Dry mass	35.89 ± 1.55	33.33 ± 2.92 (0.84)	36.90 ± 3.31 (0.98)	37.40 ± 2.73 (0.96)

* Data in crackets indicate P-value between CON and EC, ET, ECT. CON: the control; EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature.

the widest branch (the widest part of the crown, without stretching the branch), total leaf area (TLA: using leaf area meter (Li-3100, Li-Cor), the leaf area of sub-sampled leaves were measured, and these leaves were then dried at 65 °C. The quotient of leaf area divided by dry weight was SLA. All leaves were weighed and then converted to leaf area with sub-sampled SLA measurements). On average, the tree crown was separated into 3 layers along bole from the base of the crown to the tip (i.e. upper layer, middle layer and lower layer). At each layer, whorl length, branch length, and branch angle (bole as axis) were measured. All plant parts were oven-dried at 65 °C until reaching constant mass and then weighed. Simplified indices of morphology were calculated: root to shoot ratio (RSR) is the partitioning of dry mass between root and shoot; relative crown length (RCL) is the ratio of crown length to tree height; crown length to width ratio (CLWR) is the ratio of crown length to widest crown width, reflecting crown shape; crown radius (CR) is the product of branch length and the sine of the corresponding branch angle, i.e. the distance of the branch tip from the tree trunk; branch length to stem length ratio (BLS) is the ratio of total branch length per cm bole length. crown volume (*Vc*) represents the composite of different crown traits and reflects spatial occupancy. Tree crown is assumed to be the regular cone shape because it is still a seedling, so *Vc* is calculated as:

$$Vc = \pi\, L * D^2 / 12 \tag{1}$$

where *L* and *D* represent crown length and crown width.

Additionally, simplified indices related to leaf morphological traits were calculated as follows: specific leaf area (SLA, leaf area per g leaf dry mass); leaf area ratio (LAR, leaf area to total dry mass ratio); leaf area density (LAD, leaf area per dm^3 crown volume); leaf mass ratio (LMR, proportion of leaf mass to total dry mass).

To estimate the effect size of the treatment, a response ratio was calculated as the magnitude of an experimental mean relative to the control mean according to Hedges et al. (1999):

$$r = Xe / Xa \tag{2}$$

where *Xe* is the biomass of an experimental treatment; *Xa* is the biomass of a control treatment; $r > 1$, represents the biomass increased in the experimental treatment compared to the control (positive response); $r = 1$, no response; and $r < 1$, biomass decreased (negative response).

2.5 Statistical Analysis

In the experiment, a chamber was an experimental unit, and a container was a subunit. However, the rotation of containers among chambers ensured that the container could be used as a unit of replication of the variable, and thus n = 6. The effect of elevated CO_2, elevated temperature and the combination of elevated CO_2 and temperature on biomass and morphology traits was tested using independent samples t-test.

To test which of the morphological traits of Minjiang fir may have contributed to their growth response, stepwise regression analysis was used. Biomass was used as the dependent variable. However, when assessing a large number of independent variables in a regression analysis, it is possible for the independent variables to be mutually correlated. Furthermore, a previous study showed that the morphological features of plants are always highly correlated with each other (Szymura 2005). To avoid the effect of collinearity while minimizing the loss of information resulting from a reduced number of possible predictors, principal component analysis (PCA) was used to summarize the information in original independent variables into a fewer number of orthogonal (non-correlated) variables. In PCA, the varimax the rotation was used. This rotation maximized variations of the eigenvalues of variables for each factor. These new synthetic morphological variables (the principal components) were used as independent variables in the regression analysis as described above. Then, we hypothesized that morphological traits, which were not only significantly effected by treatments but also had high loadings in the principal components, might contribute to the growth response. The correlation between these morphological traits and the growth response was analyzed by Pearson's correlation analysis.

All statistical analyses were all performed with SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Statistical difference were considered significant at $P \leq 0.05$.

3 Results

3.1 Growth Response and Biomass Allocation

The growth of Minjiang fir showed significantly positive responses to elevated CO_2 and temperature (Fig. 2), with a biomass of 21%, 31%, and 35% greater than the control in elevated CO_2, elevated temperature and the combination of elevated CO_2 and temperature, respectively. Accordingly, relative to the control the response ratio was 1.21, 1.31, and 1.35 in elevated CO_2, elevated temperature and the combined elevation, respectively.

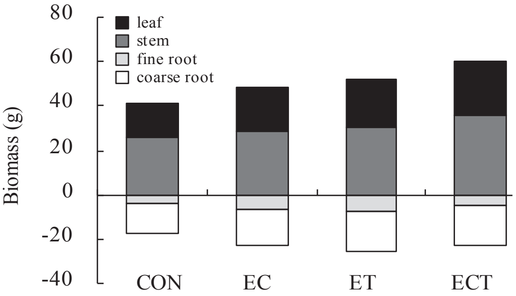

Fig. 2. Total biomass and biomass allocation in Minjiang fir after 2 years of growth in ECT, ET, EC, and CON. Mean values per tree of 6 replicates are shown. The values along Y axes above X axes are the above-groud biomass and below X axes are the below-ground biomass. CON: the control; EC: elevated CO2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature.

Elevated CO_2 significantly affected the pattern of biomass allocation among organs of Minjiang fir seedlings (Fig. 2). Minjiang fir seedlings allocated more biomass to root in elevated CO_2, with an increase of 15% in root to shoot ratio (RSR). Furthermore, more biomass was allocated to organs related to capturing resources. Leaf mass ratio (LMR) and fine root to total biomass ratio of Minjiang fir was 13% and 39% respectively, greater than in the control in elevated the CO_2. These results indicate that biomass was transferred from the stem and coarse root to the leaf and fine root.

The pattern of biomass allocation of Minjiang fir seedlings was also significantly influenced by elevated temperature (Fig. 2). RSR was 18% greater in the elevated temperature than in the control, which indicates that more biomass was allocated to belowground. LMR and fine root to total biomass ratio of Minjiang fir was 7% ($P = 0.08$) and 47%($P \leq 0.05$) respectively, greater than the control in the elevated temperature. This indicates that the magnitude of biomass enhance-

Table 2. The effects of elevated CO_2 and temperature on plant size and leaf morphology of Minjiang fir (mean ± standard error, n = 6).

Measured variables	CON	EC	ET	ECT
Vc(dm^3)	8.7 ± 0.30a*	10.4 ± 0.42b	10.6 ± 0.59b	11.6 ± 0.58b
Height (cm)	62.3 ± 2.20a	71.6 ± 2.89b	72.4 ± 2.00b	67.4 ± 2.11a
Diameter (mm)	12.5 ± 0.50a	13.5 ± 0.79a	14.1 ± 0.67a	15.2 ± 0.88b
Total leaf area (dm^2)	11.0 ± 0.90a	13.1 ± 0.58b	17.8 ± 0.96b	17.1 ± 0.99b
SLA ($dm^2 \cdot g^{-1}$)	0.71 ± 0.04a	0.60 ± 0.02b	0.83 ± 0.03b	0.82 ± 0.02b
LAR ($dm^2 \cdot g^{-1}$)	0.19 ± 0.01a	0.18 ± 0.01a	0.23 ± 0.01b	0.21 ± 0.01b
LAD ($dm^2 \cdot dm^{-3}$)	0.30 ± 0.01a	0.29 ± 0.01a	0.36 ± 0.02b	0.33 ± 0.01b

* Letters in the same row indicate statistically significant differences ($P \leq 0.05$) between CON and EC, ET, ECT. CON: the control; EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature. SLA: specific leaf area; LAR: leaf area ratio; LAD: leaf area density.

ment was larger in leaf and fine root than in the stem and coarse root.

Biomass allocation to organs of Minjiang fir seedlings was not or only slightly affected by the combination of elevated CO_2 and temperature (Fig. 2). No significant effect of the combination of elevated CO_2 and temperature on RSR and LMR was detected. The combination of elevated CO_2 and temperature only induced a non-significant increase in fine root to total biomass ratio.

3.2 Morphology of Minjiang Fir

Effects of elevated CO_2 and elevated temperature on tree size traits were significant except for diameter (Table 2). Height and *Vc* of Minjiang fir seedlings were significantly greater in the elevated CO_2 than in the control by 15% and 19%, respectively. Similarly, height and *Vc* of Minjiang fir seedling increased by 16% and 22% in the elevated temperature compared to the control, respectively. However, the combination of elevated CO_2 and temperature had no significant influence on the height of Minjiang fir, but increased the diameter and *Vc* by 21% and 33%, respectively.

Elevated CO_2 and elevated temperature both stimulated crown growth of Minjiang fir seedling (Fig. 3). Crown length, RCL, and CLWR were 19%, 15%, and 7%, respectively, greater than the control in elevated CO_2, and 29%, 20%, and 25%, respectively, greater in elevated temperature. There were no significant effects of elevated CO_2 and elevated temperature on crown width. The combination of elevated CO_2 and temperature increased crown length, width, and RCL by 24%, 31%, and 24%, respectively, compared to the control, whereas CLWR was not significantly affected by the combination of elevated CO_2 and temperature.

Elevated CO_2 differently affected upper, mid, and lower crown layers of Minjiang fir seedlings (Table 3). In the elevated CO_2 treatment, the branch angle became steeper (17% smaller than in the control) at the upper crown layer, but more horizontal (17% greater) at the mid crown layer, and was not influenced at the lower crown layer. Elevated CO_2 decreased the crown radius by 20% at the upper layer, while increased it by 16% and 59% at mid and lower layer, respectively. Minjiang fir seedlings allocated more branches to the mid layer in the elevated CO_2, with an increase of 22% for the branch length to stem length ratio (BLS). Accordingly, BLS decreased by 26% and 26% at the upper and lower layers, respectively. Elevated CO_2 stimulated whorl length, with an increase of 46% at the upper layer. However, whorl length at the mid and lower layer was not affected or negatively affected by elevated CO_2.

Elevated temperature had no significant effect on upper and lower branch angles of Minjiang fir seedlings, but induced more horizontal branches at the mid layer, with an increase in angle by 10%. The crown radius at the upper crown layer was not influenced by elevated temperature, while it was 22% and 58% greater than the control at the mid and lower crown layer, respectively. The effects

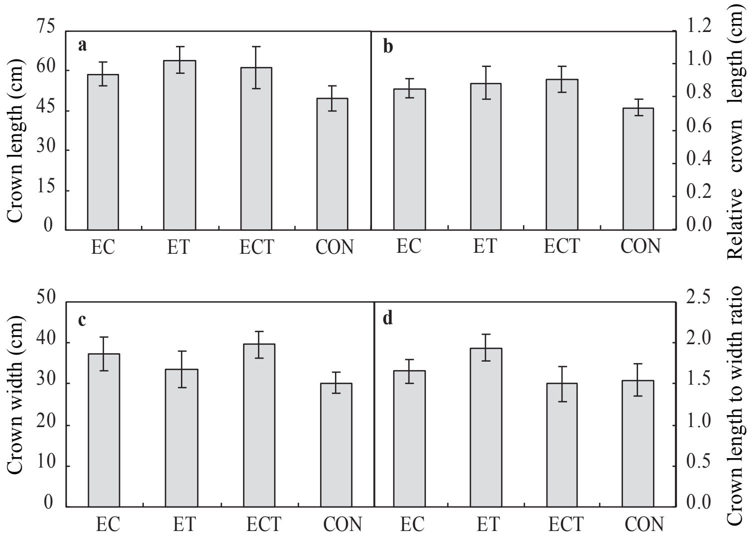

Fig. 3. Tree crown shape of Minjiang fir after 2 years of grown at ECT, ET, EC, and CON. Mean values of Minjiang fir as well as SE of 6 replicates are shown. a, Crown length; b, Relative crown length; c, crown width; d, Crown length to crown width ratio. CON: the control; EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature.

Table 3. The effects of elevated CO_2 and temperature on the crown architecture of Minjiang fir after a 2-year cultivation (mean ± standard error, n = 6).

Measured variables	Treatments			
	CON	EC	ET	ECT
Branch angle (degree)				
Upper	71.6 ± 1.7a*	59.5 ± 1.5b	76 ± 2.5a	72.4 ± 3.3a
Mid	67.8 ± 1.8a	79.2 ± 1.4b	74.8 ± 2.0b	62.0 ± 2.2a
Lower	61.5 ± 2.2a	61.5 ± 2.7a	64.9 ± 2.6a	51.5 ± 1.2b
Crown radius (cm)				
Upper	17.6 ± 0.68a	14.1 ± 0.49b	17.9 ± 0.83a	12.8 ± 0.63b
Mid	17.3 ± 0.75a	20.1 ± 0.71b	21.1 ± 0.97b	15.1 ± 0.89a
Lower	18.8 ± 0.70a	30.0 ± 1.00b	30.0 ± 1.00b	25.2 ± 0.71b
Branch length per cm bole (cm/cm)				
Upper	6.3 ± 0.39a	4.7 ± 0.23b	4.8 ± 0.39b	3.6 ± 0.41b
Mid	9.1 ± 0.43a	11.0 ± 0.79a	10.8 ± 0.64b	8.0 ± 0.49a
Lower	12.7 ± 0.60a	9.3 ± 0.34b	7.1 ± 0.37b	12.3 ± 0.51a
Whorl length (cm)				
Upper	7.5 ± 0.35a	10.9 ± 0.33b	10.5 ± 0.25b	10.8 ± 0.26b
Mid	9.9 ± 0.63a	8.5 ± 0.56a	10.0 ± 0.64a	10.4 ± 0.38a
Lower	5.1 ± 0.27a	3.8 ± 0.12b	4.9 ± 0.15a	3.4 ± 0.16b

* Letters in the same row indicate statistically significant differences ($P \leq 0.05$) between CON and EC, ET, ECT. CON: the control; EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature.

of elevated temperature on BLS were similar to elevated CO_2. BLS of Minjiang fir seedlings at the mid layer was 20% greater than the control, whereas it was 24% and 44% less than the control at the upper and lower layers, respectively. Whorl length was 41% greater at the upper layer, but was not influenced by elevated temperature at the mid and lower layers (Table 3).

The combination of elevated CO_2 and temperature had no significant effects on branch angle at the upper and mid crown layers, but it decreased branch angle at the lower layer by 16% compared to the control. Crown radius and BLS significantly decreased by 28% and 43% at the upper crown layer, but was not significantly affected at the mid crown layer. At the lower crown layer, crown radius was 34% greater, and BLS was not influenced by the combination of elevated CO_2 and temperature. The combination stimulated stem growth at the upper layer, with an increase of 44% for whorl length. However, whorl length at the mid and lower layers was not affected, or negatively affected, by the combination of elevated CO_2 and temperature (Table 3).

Elevated CO_2 stimulated leaf growth of Minjiang fir seedling, increasing total leaf area by 19% (Table 2). The leaf mass increased even more because SLA decreased significantly by 15% in the elevated CO_2. Leaf area ratio (LAR) and leaf area density (LAD) decreased by 9% and 11%, respectively, compared to the control in the elevated CO_2. All the leaf morphological traits of Minjiang fir seedlings were significantly greater in the elevated temperature than the control. Total leaf area, specific leaf area (SLA), LAR, and LAD increased by 62%, 17%, 23%, and 22%, respectively, compared to the control. The combination of elevated CO_2 and temperature had the similar effects on leaf morphological traits as elevated temperature. Total leaf area, SLA, LAR, and LAD increased by 55%, 15%, 11%, and 12%, respectively, compared to the control.

3.3 Relationship between Growth and Morphology

The principal component analysis of the original 25 morphological variables in Minjiang fir seedlings indicated that 95.8% of the variation in the morphological variables could be summarized by the first four principal components. The first principal component, which explained 50.7% variation of the original variables, was primarily related to crown shape and architecture. The original variables which contributed the most to this factor were crown volume, crown length, crown width, and relative crown length, crown radius

Table 4. Factor patterns for principal components 1 and 3 of the original independent variables used in the regression analysis.

Variables	PC1*	PC3
HEIGHT	.781	.229
DIAMETER	.732	.477
Crown volume	**.839**	.406
CL	**.884**	.328
CW	**.835**	.174
RCL	**.879**	.438
CLWR	.505	.494
BAU	.023	.895
BAM	.419	–.224
BAL	.100	.154
CRU	–.290	.437
CRM	.354	.094
CRL	**.840**	.056
BLSU	–.313	.067
BLSM	.440	–.009
BLSL	–.107	.004
WLU	**.962**	.116
WLM	.264	.676
WLL	–.421	.377
TLA	.589	**.800**
SLA	.184	**.960**
LAR	.322	**.935**
LAD	.276	**.958**
LMR	.560	.105
RSR	.434	.071
Partial R^2	68.7%	22.2%
P-value	0.000	0.000

Abbreviations:
CL: crown length; CW: crown width; RCL: relative crown length; CLWR: crown length to width ratio; BAU: branch angle at upper layer; BAM: branch angle at mid layer; BAL: branch angle at lower layer; CRU: crown radius at upper layer; CRM: crown radius at mid layer; CRL: crown radius at lower layer; BLSU: branch length per cm bole at upper layer; BLSM: branch length per cm bole at mid layer; BLSL: branch length per cm bole at lower layer; WLU: whorl length at upper layer; WLM: whorl length at mid layer; WLL: whorl length at lower layer; TLA: total leaf area; SLA: specific leaf area; LAR: leaf area ratio; LAD: leaf area density; LMR: leaf mass ratio; RSR: root to shoot ratio.

* Principal 1 and 3 accounted for 50.6% and 17.2% of the variation in the original variables, respectively. Variables with high loadings on each of the principal components are indicated boldfaced.

Table 5. Pearson correlation coefficients between the response ratio and morphological traits at the elevated CO_2, elevated temperature and the combination of elevated CO_2 and temperature (n=6).

Variables	Growth response ratio					
	EC		ET		ECT	
	Pearson correlation	P	Pearson correlation	P	Pearson correlation	P
Crown volume	0.946	**0.004**	0.661	0.153	0.910	0.012
Crown length	0.757	0.081	0.736	0.096	0.832	0.040
Crown width					0.919	**0.009**
RCL	0.934	**0.006**	0.565	0.243	0.927	**0.008**
CRL	0.852	0.031	0.824	0.044	0.973	**0.001**
WLU	0.786	0.064	0.854	0.030	0.940	**0.005**
TLA	0.864	0.027	0.967	**0.002**	0.906	0.013
SLA	0.906	0.013	0.937	**0.006**	0.899	0.015
LAR			0.997	**0.000**	0.794	0.059
LAD			0.954	**0.003**	0.911	0.011
BAU	0.950	**0.004**				

CON: the control; EC: elevated CO_2; ET: elevated temperature; ECT: the combined elevation of CO_2 and temperature. Crown volume: crown volume; RCL: relative crown length; CRL: crown radius at lower layer; WLU: whorl length at upper layer; TLA: total leaf area; SLA: specific leaf area; LAR: leaf area ratio; LAD: leaf area density; BAU: branch angle at upper layer.

at the lower layer, and whorl length at the upper layer. The second component, which accounted for 20.1% of original variables, was connected with crown architecture and RSR. The original variables with large loading in the second component were branch angle at the mid and lower layer, crown radius at the upper and mid crown layers, BLS at the mid crown layer, and RSR. The third component which explained 16.6% of original variables was a measure of leaf morphology. The original variables that contributed the most to this factor were TLA, SLA, LAR, and LAD. The fourth component accounting for 8.4% of original variables was related only to BLS at the lower layer. These results indicate that morphological traits were resolved as crown shape, crown architecture, leaf morphology, and biomass allocation between aboveground and belowground.

Stepwise regression of biomass of Minjiang fir seedlings on the four new orthogonal variables revealed that 90.9% of the variation of biomass could be accounted for by the first and the third principal components, with 68.7% by the former component and 22.2% by the latter component (Table 4). This suggests that the growth of Minjiang fir seedlings is mainly related to crown shape, crown architecture and leaf morphology.

Finally, the correlations between the growth response ratio and morphological traits in the treatments were respectively analyzed by Pearson's correlation analysis (Table 5). In the elevated CO_2, the growth response ratio was significantly correlated with crown size and shape (Pearson's $R^2=0.95$ and 0.93, $P=0.004$ and 0.006, respectively). In the elevated temperature, the growth response ratio was better correlated with leaf morphology than with crown characters. The growth response ratio was more strongly correlated with crown architecture than with crown shape in the combination of elevated CO_2 and temperature. The correlation between crown architecture and growth response ratio was stronger in the combination of elevated CO_2 and temperature than in the elevated temperature (Table 5).

4 Discussion

4.1 Growth Response

Minjiang fir seedlings exhibited positive growth response to elevated CO_2, elevated temperature and the combination of elevated CO_2 and temperature. Increased biomass of Minjiang fir seedlings in elevated CO_2 was consistent with previous studies where trees grew in mixture (Gavazzi et al. 2000). However, the magnitude of enhancement (21%) in biomass of Minjiang fir in elevated CO_2 was smaller than the average value (29%), which was from a set of 102 measurements of total tree biomass (Curtis and Wang 1998).

Temperature increase can have either positive or negative effects on a given process depending on the current relationship to the temperature optimum curve. For example, photosynthesis processes have an optimum temperature, with reaction rates increasing from 0 °C to the optimum, and then declining rapidly with further increase in temperature. At high altitude, the ambient temperature in the early and later periods of the growing season is generally lower than the optimum temperature for photosynthesis. Therefore, increase in temperature over the ambient temperature provided more propitious temperature conditions for photosynthesis and thus stimulated growth (Kellomäki and Wang 2001). In this experiment, the growth of Minjiang fir was stimulated by elevated temperature, which was consistent with the above findings.

The response ratio in the combination of elevated CO_2 and temperature was larger than in the elevated CO_2 or temperature alone (1.35 versus 1.21 or 1.31, respectively). There are several reasons that might explain this difference. The first is the interaction of elevated CO_2 and temperature; however, due to the limitation of repetition, this interaction needs additional investigation. The second is nutrients: in our experiment, nutrient availability was poor and no fertilizer was added during experiment. When temperature was elevated, mineralization and availability of nutrients likely increased (Peltola et al. 2002), which may have indirectly accelerated the positive response of growth to elevated CO_2. This also supports a previous conclusion that the response of plants to elevated CO_2 and temperature is related to other factors (Matala et al. 2006).

4.2 Morphological Change

Tree height was greater in the elevated CO_2 and in the elevated temperature than in the control, which is consistent with reports on Scots pine (Jach and Ceulemans 1999) and Norway spruce (Skre and Nes 1996). However, Kilpelainen et al. (2005) reported that there were no significant treatment effects on height growth during their 6-year study. We found no significant difference for diameter which is consistent with findings by Groninger et al. (1996) and Gavazzi et al. (2000) that elevated CO_2 affected tree height growth more than diameter growth.

Elevated CO_2, elevated temperature and the combination of two positively affected tree crown length and crown width, resulting in overall larger crown size. Furthermore, The combination of elevated CO_2 and temperature increased *Vc* more than in the elevated CO_2 or elevated temperature alone, which is consistent with the change in biomass (Fig. 2). These results supported the findings by Lee et al. (2001). Elevated temperature stimulated lengthways growth of Minjiang fir due to larger crown length to width ratio, which is consistent with reports by Hollister et al. (2005). They found that shoot length was considerably larger in seedlings due to earlier bud break under elevated temperatures. On the contrary, Apple (1998) reported that trees from elevated temperature chambers tended to be shorter. These discrepancies could be due to the average temperature of the study site: Our study was carried out in a relatively cold site, whereas the study of Apple (1998) was carried out in a relatively warm site.

Elevated CO_2, elevated temperature and the combination of two also significantly affected the crown architecture of Minjiang fir. However, these changes in crown architecture were different in three treatments. In the elevated CO_2 and the combination of elevated CO_2 and temperature, increased whorl length in the upper layer led to reduced branch length to stem length ratio (BLS), which resulted in sparse branches. The sparse branches decreased the possibility of self-shading (Deleuze et al. 1996). This was advantageous for Minjiang fir growth because within-crown self-shading had strong effects on light interception prior to canopy closure (Oker-Blom and Kellomäki 1983). Simultaneously, longer branches and larger angles at the mid layer in elevated CO_2 have been also attributed to light interception (Goulet and Nikinmaa 2000). Longer branches and sharper angles at lower layer were detected in the combination, which is consisted with previous results that branch length was negatively correlated with termination angle (Gielen et al. 2002). These changes induced branches at lower layer to grow upwards to the light source. In elevated temperature, an increased branch angle, crown radius, and BLS at the mid layer caused a wider mid crown with denser branches in the elevated temperature than the control, which might have implications for light interception (Chmura et al. 2007).

Minjiang fir allocated more biomass belowground in elevated CO_2 treatment and elevated temperature treatment, resulting in increased root to shoot ratio (RSR). This might imply an increased ability for Minjiang fir to exploit belowground resources. The larger RSR in elevated CO_2 was also reported by Gavazzi et al. (2000), but contrasts with findings by Groninger et al. (1996) and Curtis and Wang (1998). Although leaf biomass both increased in elevated CO_2 treatment and elevated temperature treatment, specific leaf area (SLA) decreased in elevated CO_2, while increased in elevated temperature. Reduced SLA is the common response to elevated CO_2 and may have been due to an increase in a combination of non-structural carbohydrates and/or more cell layers (Ferris et al. 1996). Increased SLA in elevated temperature implied the magnitude of enhancement in total leaf area was greater than biomass. This supports the finding that high temperature accelerates leaf extension (Ferris et al. 1996). The combination of elevated CO_2 and temperature did not alter biomass allocation of Minjiang fir among organs. Since total leaf area increased significantly, SLA and LAR were greater in the combination than in the control. Larger SLA and LAR increased the probability of intercepting radiation, and consequently stimulated the growth of Minjiang fir (Cornelissen et al. 1996).

4.3 Relationship of Morphology and Growth Response

The principal component analysis indicated that morphological traits of Minjiang fir could be simplified to four factors: crown shape, crown architecture, leaf morphology, and biomass allocation between aboveground and belowground. This was consistent with the typical classical morphology definition, which reduces the diversity of plant form to mutually exclusive morphological categories (Sattler 1996). The stepwise regression revealed that growth performance was highly correlated with the first and third principal component, i.e. crown and leaf morphology. This implies that it is possible to interpret the growth of Minjiang fir in terms of morphology. The importance of phenotypic plasticity and crown architecture in the understanding of community and stand structure, functioning and production has been stressed by several authors (Sachs 2004, Pearcy et al. 2005, Barthelemy and Caraglio 2007). However, morphological traits that contribute the most to the growth response should be those not only significantly affected by elevated CO_2 and temperature but also with high loading in the first and third principal components. The morphological traits that were significantly affected in different treatments varied, suggesting that morphological traits correlated with growth response were specific in different treatments.

The t-test and regression analysis suggested that growth response of Minjiang fir to elevated CO_2 was correlated with a few of the morphological traits that were related to crown shape, such as crown volume (*Vc*), relative crown length (RCL) and branch angle at the upper layer (BAU) (Table 4). *Vc* was positively correlated with growth response, consistent with other studies showing that plants increased competitive predominance by growing larger individuals (Sultan 2000). A larger branch angle at the upper layer was highly correlated with the growth response ratio (Pearson's $R^2 = 0.95$, Table 5). This agrees with findings of Gielen et al. (2002) that success of white poplar when competing with black poplar and euro-american poplar in elevated CO_2 was due to longer, more horizontally orientated branches.

The growth response of Minjiang fir in the elevated temperature was significantly correlated with crown architecture and leaf morphological traits. Furthermore, leaf traits were better correlated with the response ratio than crown (Table 5). This is consistent with previous findings that leaf development and morphology are important as it determines the amount of intercepted radiation which, together with photosynthetic efficiency, determines productivity (Ferris et al. 1996). The greater TLA, SLA, LAR, and LAD (Table 2) suggested that leaf biomass increased and leaf was thinner in the elevated temperature than in the control. On the one hand, a larger and thinner leaf likely allowed a higher interception of light and thus stimulated growth. On the other hand, it would also imply a higher vulnerability to water stress and thus restrict positive growth response (Cardillo and Bernal 2006). Therefore, the trade-off between leaf-mediated changes in light interception and water loss when plants are grown at different temperatures should be highlighted.

The growth response of Minjiang fir to the combination of elevated CO_2 and temperature was correlated with crown morphology (Table 5, $P<0.01$). Furthermore, crown architecture (relative crown length, crown radius at lower layer, whorl length at the upper layer) was more strongly correlated with the response ratio than crown shape (crown length, crown width, and crown volume). This is consistent with the findings by Chmura (2007) that crown shape itself seems to be less important for light interception than its distribution within the crown. In addition, the relationship between crown architecture and the growth response ratio was stronger in the combination than in elevated temperature (Table 5). This suggests that the same morphological traits might contribute to growth response differently in various environments.

In conclusion, our results show that growth and morphology of Minjiang fir are greatly affected by elevated CO_2 and temperature, which confirms our first hypothesis. Growth of Minjiang fir was stimulated by elevated CO_2 and temperature, and morphological traits were specifically altered by different treatments. We also demonstrate that the growth response of Minjiang fir is correlated with morphology. However, the different relationship (extent and direction) between growth response and morphological traits suggests that the same morphological traits may contribute differently to the growth response in various conditions. The results imply that simple crown and leaf morphological variables can be good predictors of future seedling growth, and thus provide useful information to forest managers.

References

ACIA. 2005. Presentation of the Arctic Climate Impact Assessment overview report and international workshop on climate changes in the Arctic and international polar year 2007/2008 in the Arctic. Cambridge University Press, Cambridge, UK. p. 28.

Ainsworth, E.A. & Long, S.P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist 165(2): 351–371.

Apple, M.E., Lucash, M.S., Olszyk, D.M. & Tingey, D.T. 1998. Morphogenesis of Douglas fir buds is altered at elevated temperature but not at elevated CO_2. Environmental and Experimental Botany 40(2): 159–172.

Barthelemy, D. & Caraglio, Y. 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Annals of Botany 99(3): 375– 407.

Bazzaz, F.A. & McConnaughay, K.D.M. 1992. Plant–plant interactions in elevated CO_2 environments. Australian journal of Botany 40(5): 547–563.

Cardillo, E. & Bernal, C.J. 2006. Morphological response and growth of cork oak (Quercus suber L.) seedlings at different shade levels. Forest Ecology and Management 222(1–3): 296–301.

Chmura, D.J., Rahman, M.S. & Tjoelker, M.G. 2007. Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine. Forest ecology and management 143(2–3): 219–230.

Cornelissen, J.H.C., Diez, P.C. & Hunt, R. 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. Journal of Ecology 84(5): 755–765.

Cropper, W.P., Jr. 2000. SPM2: A simulation model for slash pine (Pinus elliottii) forests. Forest Ecology and Management 126(2): 201–212.

Curtis, P.S. & Wang, X.A. 1998. Meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113(1–3): 299–313.

Deleuze, C., Herve, J.C., Colin, F. & Ribeyrolles, L. 1996. Modeling crown shape of Picea abies spacing effects. Canadian Journal of Forest Research 26(11): 1957–1965.

Ferris, R., Nijs, I. & Impens, I. 1996. Contrasting CO_2

and temperature effects on leaf growth of perennial ryegrass in spring and summer. Journal of Experimental Botany 47(8): 1033–1043.

Gavazzi, G., Seiler, J., Aust, W. & Zedaker, S. 2000. The influence of elevated carbon dioxide and water availability on herbaceous weed development and growth of transplanted loblolly pine (Pinus taeda). Environmental and Experimental Botany 44(3): 185–194.

Gielen, B., Calfapietra, C. & Claus, A. 2002. Crown architecture of Populus spp. was differentially modified by free-air CO_2 enrichment (POPFACE). New Phytologist 153(1): 91–99.

Goulet, J., Messier, C. & Nikinmaa, E. 2000. Effect of branch position and light availability on shoot growth of understory sugar maple and yellow birch saplings. Canadian Journal of Botany 78(1): 1077–1085.

Groninger, J.W., Seiler, J.R., Zedaker, S.M. &Berrang, P.C. 1996. Effects of CO_2 concentration and water availability on growth and gas exchange in greenhouse-grown miniature stands of Loblolly Pine and Red Maple. Functional Ecology 10(6): 708–716.

Gunderson, C.A. & Wullschleger, S.D. 1994. Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. Photosynthesis Research. 39(3): 369–388.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80(4): 1150–1156.

Hollister, R., Webber, P. & Weedie, C. 2005. The response of Alaskan arctic tundra to experimental warming: differences between short- and long-term responses. Global Change Biology 11(4): 525–536.

Idso, K.E. & Idso, S.B. 1994. Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years' research. Agricultural and Forest Meteorology 69(3/4): 153–203.

Idso, S.B. & Kimball, B.A. 1992. Effects of atmospheric CO_2 enrichment on photosynthesis, respiration and growth of sour orange trees. Plant Physiology 99(1): 341–344.

Jach, M.E. & Ceulemans, R. 1999. Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (Pinus sylvestris) seedlings after two years of exposure in the field. Tree Physiology 19(4–5): 289–300.

Kellomäki, S. & Wang, K.Y. 2001. Growth and resource use of Birch seedlings under elevated carbon dioxide and temperature. Annals of Botany 87: 669–682.

Kilpeläinen, A., Peltola, H., Ryyppo, A., and Kellomäki, S. 2005. Scots pine responses to elevated temperature and carbon dioxide concentration: growth and wood properties. Tree Physiology, 25(1): 75–83.

Lee, J., Usami, T. & Oikawa, T. 2001. High performance of CO_2-temperature gradient chamber newly built for studying global warming effect on a plant population. Ecological Research 16(2): 347–358.

Landsberg, J.J. &Waring, R.H. 1997. A generalised model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest Ecology and Management 95(3): 209–228.

Matala, J., Ojansuu, R., Peltola, H., Raitio, H. & Kellomaki, S. 2006. Modeling the response of tree growth to temperature and CO_2 elevation as related to the fertility and current temperature sum of a site. Ecological Model 199(1): 39–52.

Oker-Blom, P. & Kellomäki, S. 1983. Effect of grouping of foliage on the within-stand and within-crown light regime: comparison of random and grouping canopy models. Agricultural and Forest Meteorology 28(2): 143–155.

Pearcy, R.W., Muraoka, H. & Valladares, F. 2005. Crown architecture in sun and shade environments: assessing function and tradeoffs with a 3-D simulation model. New Phytologist 166(3): 791–800.

Peltola, H., Kilpeläinen, A. & Kellomaki, S. 2002. Diameter growth of Scots pines (Pinus sylvestris L.) grown at elevated temperature and CO_2 under boreal conditions. Tree Physiology 22(14): 963–972.

Poorter, H. & Remkes, C. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83(4): 553–559.

Printchard, S., Rogers, H., Prior, S. & Peterson, C. 1999. Elevated CO_2 and plant structure: a review. Global Change Biology 5(7): 807–837.

Reekie, E.G. & Bazzaz, F.A. 1989. Competition and patterns of resource use among seedlings of five tropical trees grown at ambient and elevated CO_2. Oecologia 79(2): 212–222.

Sachs, T. 2004. Self–organization of tree form: a model for complex social systems. Journal of Theoretical Biology 230(2): 197–202.

Sattler, R. 1996. Classical morphology and continuum morphology: opposition and continuum. Annals of Botany 78(5): 577–581.

— & Rutishauser, R. 1997. The fundamental relevance of morphology and morphogenesis to plant research. Annual Botany 80(5): 571–582.

Skre, O. & Nes, K. 1996. Combined effects of elevated winter temperature and CO_2 on Norway spruce seedlings. Silva Fennica 30(2–3): 135–143.

Stirling, C.M., Heddell-Cowie, M., Jones, M.L., Ashenden, T.W. & Sparks, T.H. 1998. Effects of elevated CO_2 and temperature on growth and allometry of five native fast-growing annual species. New Phytologist 140(2): 343–354.

Stulan, I. & Herto, D. 1993. Root growth and functioning under atmospheric CO_2 enrichment. Vegetation 104/105(1): 99–115.

Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history. Trends Plant Science 5(12): 537–542.

Szymura, T.H. 2005. Silver fir sapling bank in seminatural stand: Individuals architecture and vitality. Forest Ecology and Management 212(3): 101–108.

Tremmel, D.C. & Bazzaz, F.A. 1993. How neighbor canopy architecture affects target plant performance. Ecology 74(7): 2114–2124.

Wang, X.W., Zhao, M., Mao, Z.J., Zhu, S.Y., Zhang, D.-L. & Zhao, X.Z. 2008. Combination of elevated CO_2 concentration and elevated temperature and elevated temperature only promote photosynthesis of Quercus mongolica seedlings. Russian Journal of Plant Physiology 55(1): 54–58.

Wang, Y.P. & Jarvis, P.G. 1990. Influence of crown structural properties on PAR absorption, photosynthesis and transpiration in Sitka spruce: application of a model (Maestro). Tree Physiology 7(1_2_3_4): 297–316.

White, J. 1984. Plant metamerism. In: Dirzo, R. & Sarukhan, J.(eds.). Perspectives on plant population ecology. Sinauer, Sunderland, MA. p. 15–47.

Yokozawa, M., Kubota, Y. &Hara, T. 1996. Crown architecture and Species Coexistence in Plant Communities. Annals of Botany 78(4): 437–447.

Total of 46 references