Multi-objective environment chamber system for studying plant responses to climate change

X. ZHOU[*,+], Z.M. GE[*,**], S. KELLOMÄKI[*], K.Y. WANG[*,**], H. PELTOLA[*], P.J. MARTIKAINEN[***], M. LEMETTINEN[****], A. HASSINEN[****], and R. IKONEN[****]

School of Forest Sciences, University of Eastern Finland, Yliopistokatu 7, P.O. Box 111, FI-80101 Joensuu, Finland[*]
Key Laboratory of Urbanization and Ecological Restoration, East China Normal University, 200062 Shanghai, P.R. China[**]
Department of Environmental Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland[***]
Mekrijärvi Research Station, University of Eastern Finland, FI-82900 Ilomantsi, Finland[****]

Abstract

This paper describes the technical information and performance of a new multi-objective chamber system enabling the control of environmental variables (*e.g.*, temperature, CO_2, air humidity, wind speed, and UV-B radiation) for understanding plant responses to climate change. Over a whole growing season, four different climate scenarios were evenly programmed into the system's 16 chambers as ambient environment (AMB), elevated temperature (ET), elevated CO_2 concentration (EC) and elevated temperature and CO_2 concentration (ETC). Simultaneously, the chamber effects were assessed regarding the physiological responses and growth of a boreal perennial grass (reed canary grass, *Phalaris arundinacea* L.). During the growing season, the chamber system provided a wide variety of climatic conditions for air temperature (T_a), relative humidity (RH) and CO_2 concentration (C_a) in the AMB chambers following outside conditions. The target temperature (+3.5°C) was achieved to a good degree in the ET and ETC chambers, being on average 3.3°C and 3.7°C higher than ambient conditions, respectively. The target concentration of CO_2 (700 ppm) was also well achieved in the EC and ETC chambers, being on average 704 ppm and 703 ppm, respectively. The stable airflow condition inside all of the chambers provided a homogeneous distribution of gases and temperature. The decreases in RH and increases in vapour pressure deficit (VPD) in the elevated temperature chambers were also maintained at a low level. Chamber effects were observed, with some physiological and growth parameters of plants being significantly lower in the AMB chambers, compared to outside conditions. The plant growth was negatively affected by the reduced radiation inside the chambers.

Additional key words: autocontrolled environment chamber; boreal grass; chamber effect; climate change.

Introduction

Global atmospheric carbon dioxide (CO_2) concentrations are expected to be double or more by the end of 21st century, while the temperature will rise by between 1.6–6.4°C in the next 50–100 years (IPCC 2007). Several toxic air pollutants and UV-B radiation induced by climate change have prompted widespread concern about the long-term effects on plants (Jordan 2002, Fiscus *et al*. 2005). The environmental effects on the development and physiological processes of plants would be induced by one or several combined stress factors. The response of agroforestry systems to climate change may be crucial in determining which ecosystems act as global carbon sinks

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[+]Corresponding author; tel.: +358-13-251-4401, fax: +358-13-251-4444, e-mail: xiao.zhou@uef.fi
Abbreviations: *a* – quantum yield; AMB – ambient environment in chamber; C_a – CO_2 concentration; EC – elevated CO_2 concentration in chamber; ET – elevated temperature in chamber; ETC – elevated temperature and CO_2 in chamber; g_s – light-saturated stomatal conductance; L_c – light compensation point; L_s – light saturation; P_{max} – maximum rate of photosynthesis; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; R_D – dark respiration rate; RCG – reed canary grass; RH – relative humidity; T_a – air temperature; VPD – vapour pressure deficit.
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in the future. Consequently, accurate measurements of plant physiological responses and soil dynamics under the predicted climate are invaluable for understanding current and future plant growth and carbon dynamics. Such measurements rely on systems that provide environmental control and are capable of simulating expected climate change.

Numerous plant exposure systems and approaches have been employed in recent decades, including branch bags (*e.g*. Barton *et al*. 1993, Kellomäki and Wang 1997, Saugier *et al.* 1997, Robertnz, 1999), open-top chambers (Leadley and Drake 1993, Norris *et al.* 1996), glass domes with adjustable windows (Urban *et al.* 2001), free air CO_2 enrichment (FACE) systems (Hendrey *et al.* 1999, Herrick and Thomas 2001) and whole-plant closed-top chambers (Kellomäki *et al.* 2000, Medhurst *et al.* 2006). Each approach has contributed greatly to the understanding of the complicated responses of plants under a given environmental condition. However, each of these systems has a particular set of advantages and disadvantages. Therefore, the choice of system design is highly critical in addressing the research questions regarding soil-plant-atmosphere continuum responses to climate change.

A closed autocontrolled environment chamber system has been built at the Mekrijärvi Research Station (belonging to the University of Eastern Finland). This new chamber system was designed to fulfill three important experimental conditions that should be taken into account in ecological studies. Firstly, the facility has adequate space for season- and year-round studies on boreal and temperate plants. Secondly, the automated facility enables the accurate and constant control of environment. Thirdly, the ability of the chamber system allows the ecophysiological and physical responses of soil-plant-atmosphere continuum to changed environment to be examined individually or in combination. These are the advantages over branch bags and open-top chambers, which have difficulties in controlling various environmental variables.

The main objective of this paper is to describe the technical details of this new system and its ability to provide accurate environmental control for studies on the long-term growth and physiological responses of plants to climate change. For the chamber performance evaluation we asked the following questions. Can the chambers be controlled accurately to follow environment fluctuations outside the chamber? Can the temperature elevation and CO_2 enrichment be accurately controlled in order to attain the target values in the "climate change" chambers? Additionally, we also briefly demonstrate the chamber effects on the photosynthesis and growth of a boreal perennial grass (reed canary grass, *P. arundinacea* L., hereafter RCG), which is widely cultivated as a bioenergy crop in North Europe (Zhou *et al.* 2011).

Materials and methods

Site description: The Mekrijärvi Research Station (62°47'N, 30°58' E, 145 m a.s.l) is located in Eastern Finland, representing the typical climatic and geographical characteristics of the boreal zone. The climate at the site is generally characterized by cold winters with a persistent snow cover and a short growing season. During the period 1961–2000, the monthly mean temperature was –10.4°C in wintertime and 15.8°C in summertime. The average annual precipitation in the area was about 700 mm, of which about 40% falls as snow (Kellomäki *et al*. 2000).

Description of the chamber system

System structure: The chamber system consists of two main structural sections: (*1*) 16 growth chambers and (*2*) a control and facility center, whose total floor area is 890 m^2 (Fig. 1). The area of each chamber is 16 m^2 with a mean height of 4.0 m, and the volume is 64 m^3. The hull construction of the chambers is stainless steel with tempered glass. The floor of the chambers is 0.7 m above the ground and is made of cement. The four transparent walls and the roof with adjustable windows are made of double-layer glass (16 mm thickness) (*SGG COOL-LITE* and *SGG PLANILUX, Saint-Gobain UK Ltd*, Coventry, UK) (Fig. 1). The glass cuts off part of the infrared radiation and has relatively high transparency for visible light (mean 75% for 380–700 nm), determined using the *Pilkington Spectrum Program v.6* on some sunny days. The chamber has a sloping roof to allow precipitation to run off. Each chamber is equipped with an air condition vent, a gas supply vent, a set of sensors, an air intake and an outlet vent with filters, and two normal lights and a UV-B lamp (Fig. 1). The lateral heating radiator is installed on the side wall in each chamber.

The control and facility center which includes a power room, an air condition unit, a pump unit, a gas cylinder room, a computer control room, a laboratory (for pre-treatment of samples and measurements) and an outdoor weather station is built in the same place with a modular design (Fig. 1). Out of the figure, a pump unit is installed for drawing water from a nearby lake for irrigation.

Chamber automation and data acquisition: Chamber automation system is installed in the building with *RS-485* serial bus for climatic measuring and controlling and reserve unit. The core of automation system uses the *UIO32* PLC (Programmable Logic Controller) module with 12 bit A/D conversion (*Computec Oy*, Joensuu, Finland) and the *CitectSCADA* automation program (*Computec Oy*, Joensuu, Finland). The PLC module is designed for multiple input and output arrangements, extended temperature ranges, immunity to electrical noise, and resistance to vibration and impact. The module works using the computer platform for real-time automation of the electromechanical processes, such as controlling the air conditioning, gas valve, humidifier and UV-B lamp in the chambers. Regarding the automatic measurement of soil conditions (*e.g*. soil temperature and moisture), the *ISM111* module (16 bit A/D conversion) and *ISM112* module (16 bit A/D conversion) with an automation program (*FIX32 v. 7.0*, *Intellution*, USA) are installed and have been previously described by Kellomäki *et al*. (2000).

The *UIO32* PLC module has the dual function of data acquisition/storage and control, and can be employed for both the direct and multichanneled recording and processing of analogue and digital sensor signals or measured values, as well as for regulating the actuators. The measured data from the weather station by the *UIO32 PLC* module are used to compare with those inside the chambers for environmental control. The module with *CitectSCADA* program provides a user-friendly interface based on the *Windows* operating system to set the required parameter values for measurement and control. Additionally, the configuration software can be flexibly combined with a normal text file and *Excel* file to log/store data and display graphs of data processes. All the sensor measurements are logged at 60-s intervals. The *RS-485* serial bus is used for simultaneous connection with all the facilities in the chambers with a 16-bit A/D conversion for the input and 14-bit D/A conversion for the output.

Temperature and humidity control: Temperature control in each chamber is achieved by using the air condition unit, which consists of one cooling compressor and two freezing compressors and radiators (*42JW* and *42HC*, *Carrier*, USA) with a large tank of refrigeration fluid (40% glycol, 45 m^3) (Fig. 1). The *UIO32* PLC module with temperature sensors controls the condenser fluid pump and the operation of the glycol cooler. Temperature control can be based on either a constant or a dynamic air temperature (*e.g*., ambient air temperature), *i.e*., the *UIO32 PLC* module measures the air temperature outside (weather station) and maintains a similar air temperature in the chambers. Moreover, the module can result in the ambient air temperature in a certain degree of difference in the chambers. The chamber air can be cooled in summer by up to 20°C cooler than the outdoor air, even making it frosty at night. The elevated temperature treatment in winter is achieved by a pellet heating plant (700 kW) in the research station. The chamber temperature can be adjusted up to 25°C warmer than the outside air in winter, and 20°C warmer in summer.

The relative humidity can be adjusted in the chambers. When the chamber relative humidity is lower than a set target value, the *UIO32 PLC* module will run an ultrasonic humidifier (*Airwin RB/P, 48V DC, Boga GmbH*, Germany) for changing the humidity in each chamber. The distilled and deionized water was made by one distillatory (*Airtec RO Plus,* 2.8 kW, *Airtec*, Finland) and one UV sterilizer (*Airtec UV-C 405AL*, 30 W, *Airtec*, Finland). The humidifier vent is located below the air condition fan (Fig. 1).

Gas supply and control: The CO_2 control system for each chamber consists of a set of cylinders with pure CO_2 (*AGA Oy*, Finland), a CO_2 transmitter (*GMP 343, Vaisala*, Finland) and a CO_2 proportional valve (*ASCO 363, 24V DC, ASCO Joucomatic*, France) with an adjustable switching frequency of 50–60 Hz. Normally, 12 CO_2 cylinders are connected with pipes to a tank, which contains about 336 kg liquefied CO_2 at a pressure of 5.9 MPa. A pressure regulator (*AGA*) on the CO_2 pipes from the tanks is maintained at a constant pressure of 0.05 MPa, equal to the chambers.

Unlike most infrared CO_2 analyzers, the CO_2 transmitter is a single-wavelength nondispersive infrared gas sensor that is not sensitive to other gases, such as water vapour. On the other hand, the sensor is diffusion aspirated, *i.e*., CO_2 enters through a permeable membrane. The transmitter measures the CO_2 concentration in the chamber at 60-s intervals. In order to maintain the chamber CO_2 concentration at the set target level, pure CO_2 is injected into the circulating chamber air through an electric proportional valve. Given a relatively constant rate of ventilation, the target concentration of CO_2 in each chamber is achieved by coordination between the *UIO32 PLC* module, transmitter and CO_2 proportional valve. When receiving a signal from the transmitter, the module compares the measured value with the target value, which determines whether the proportional valve needs adjusting. The required accuracy of CO_2 control can be attained by repeated adjustment of the related values in the regulation system of the module. The valve works on the basis of automatic pulse width modulation. The lag in the response time of the regulator to the CO_2 transmitter and valve can also be taken into account. To follow the ambient conditions, the *UIO32 PLC* module can obtain the ambient atmospheric CO_2 (weather station) and maintain the same concentration in the chambers.

Measurements of environmental variables and air ventilating control: Currently, the available measurements inside each chamber include air temperature, relative humidity, photosynthetic photon flux density, CO_2 concentration, UV-B radiation, soil temperature and moisture. The same measurements are conducted by the weather station outside the chambers. Details of all the instruments used in the system are summarized in Table 1.

For fresh air ventilation, there are inlet and outlet vents (150 mm in diameter) in each chamber (in the wall) with controlled air fan and air filters (absorbent activated charcoal for gas purification) (*Lämpökarelia Oy*,

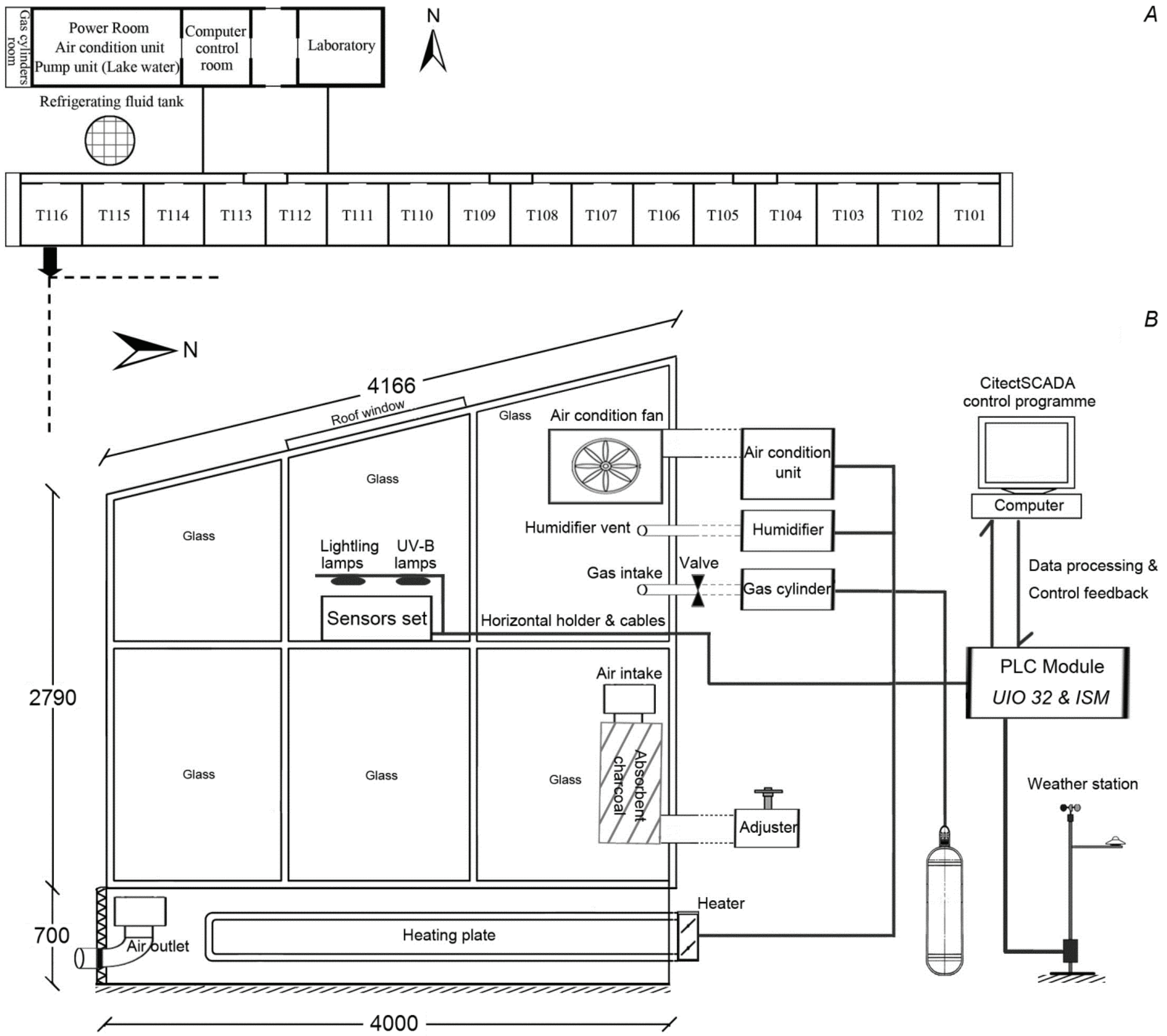

Fig. 1. Overview of the design of the chamber system construction (*A*) and a schematic profile of one chamber of the system (*B*) indicates the pipes/cables of the chamber, weather station and gas cylinders connecting to the control modules and computers. The dimensions are in mm.

Finland). The air flowing through the inlet (0.4–0.6 $m^3 s^{-1}$ during the growing season) is measured and controlled using an air velocity instrument (*470-I, C&G Industrial Supply*, USA). The fresh air turnover time in the chamber is about 2 min.

Layout of the reed canary grass experiments

Chamber performance test: The environmental dataset measured in the chambers for the growing season (15th April–15th September) of 2009 were used in the evaluation of chamber control performance on air temperature (T_a), relative humidity (RH), vapour pressure deficit (VPD) and CO_2 concentration (C_a). 16 chambers were divided evenly according to their climatic treatments, representing ambient environment (AMB), elevated temperature (ET), elevated CO_2 concentration (EC), and elevated temperature and CO_2 concentration (ETC), with four replications. Although the UV-B facilities are available, they were not employed in the current study. For the ET and ETC chambers, the *UIO32 PLC* module was set to control the chamber temperature at +3.5°C higher than the AMB chambers. For the EC and ETC chambers, a constant CO_2 concentration was set at 700 ppm. This was done based on the official FINADAPT climate change scenario (Carter *et al.* 2005).

Experimental design: All 16 chambers of the system were utilized for understanding the physiological responses and growth of RCG under changed environments during the growing season. In early spring 2009, frozen peat with cultivated RCG were collected, and placed in high density polyethylene containers (0.8 m long × 0.6 m wide × 0.4 m deep). The area of peat bulk sample was close to the size of the container bottom, and the gap between peat and container was tamped with peat pieces. The peatland, regularly cultivated by *Vapo Bioenergy Ltd*., is located in Linnansuo (62°30'N, 30°30'E), about 30 km from the Mekrijärvi Research Station.

In mid-April 2009, 64 containers with peat-RCG were evenly moved into the chambers. Simultaneously, the system was operated to control the environment. For comparison, four containers with peat-RCG were placed outside, providing information on the chamber effects. Before starting the chamber cultivation, a harvest was done by removing the previous aerial biomass. The peat was fertilized with 5.4 g(N) m^{-2}, 1.2 g(P) m^{-2} and 4.2 g(K) m^{-2} (*Vapo Bioenergy Ltd*., Finland) after harvesting, and the soil was irrigated to a soil moisture of ~50% (volumetric content) during the cultivation period representing a similar level as in the Linnansuo field.

Test on chamber effects: To study the chamber effects in detail, measurements of net photosynthesis (P_N) *vs*. photosynthetic photon flux density (PPFD) were made on sixteen intact, top-layer fully expanded leaves of RCG in four AMB chambers as well as for plants cultivated outside, using a leaf chamber attached to a portable *Li-6400* infrared gas-exchange system (*Li-6400, Li-Cor*, Lincoln, USA). The measurements were carried out in three phases (around 15th June, 15th July, and 15th August) with an air temperature of around 20°C on the sunny days (8:00–11:00 h). The air flow in the leaf chamber was controlled at 400 ml min^{-1}, leaf temperature was 20 ± 1°C, VPD was 1.0 ± 0.1 kPa, RH was set above 60%, and the CO_2 concentration was kept at 370 ± 1 μmol(CO_2) mol^{-1}.

The parameter values of the maximum rate of photosynthesis (P_{max}), quantum yield (a), light-compensation point (L_c) and light-saturated point (L_s) were obtained using a nonrectangular hyperbola (Marshall and Biscoe 1980) by means of a nonlinear least squares curve-fitting program (*SPSS V. 16.0*, Chicago, IL, USA). Dark respiration rate (R_D) was measured at 20°C after dark adaptation for at least 30 min. The light-saturated stomatal conductance (g_s) for water was recorded at a PPFD of 1,500 μmol(photon) m^{-2} s^{-1}.

Furthermore, with a sample area of 0.0154 m^2 (diameter of 140 mm) in the containers in the AMB chambers and outside, RCG plants were harvested for the growth parameters of stem height, stem diameter, leaf length, leaf area and biomass of leaf and stem, which were calculated at single-shoot level based on plant density. The phenological phases of RCG grown in the AMB chambers and outside conditions were recorded based on Sahramaa and Jauhiainen (2003).

Results

Effects of the chamber system on the microenvironment: The performance of the chambers was analyzed by comparing the main environmental variables outside and inside the AMB chambers. The distribution of the environmental fluctuations indicated that T_a inside the AMB chambers followed the diurnal and seasonal dynamics of the outside conditions (Fig. 2), with a small (mean of 0.6°C) deviation of T_a (ΔT_a) through the whole growing period (Fig. 3). RH outside ranged from 25% to 100% and RH inside the AMB chambers was effectively controlled higher than the original target (50%). The largest deviation of RH (ΔRH) was in the range of –20% to 30% (Fig. 3). Higher RH inside the chambers partly resulted from the plant transpiration and dew fall from the air conditioner. C_a outside was around 350–450 ppm during the growing season. C_a inside the AMB chambers was controlled to a similar level as the ambient, with a minor deviation of –25 to 25 ppm through the study period (Fig. 3).

The wind velocity outside the chamber system was in the range of 0.7–5.6 m s^{-1} during the growing season. Inside all of the 16 chambers, the airflow was mainly affected by the relatively constant air circulation from the air conditioning fans (location *see* in Fig. 1). The wind velocity was highest near the air conditioning fans. The airflow was relatively stable in the middle of the chamber (lower than 0.5 m s^{-1}), but slightly higher (0.6–0.8 m s^{-1}) in the area near to the glass walls due to the backflow. The mean wind velocity of most of the chamber areas was slightly lower than the outside conditions.

Temperature elevation and CO_2 enrichment: The target was to elevate T_a in the ET and ETC chambers by +3.5°C above the AMB chambers, and maintain C_a in the EC and ETC chambers at 700 ppm throughout the study period. The climatic measurements showed that the target was in general well obtained (Figs. 4,5). T_a in the ET and ETC chambers was, on average, 3.3 ± 0.9°C and 3.7 ± 1.4°C higher than the AMB chambers, respectively, for more than 90% of the time during the growing season (Fig. 4). Furthermore, the mean C_a was 703.9 ± 34.8 ppm and 703.2 ± 35.9 ppm in the ET and ETC chambers, respectively, for more than 95% of the time during the growing season (Fig. 5). The two occurrences of drops in concentration were due to the replenishment of the gas tank. Although there was a particular method for controlling air humidity, elevated temperature treatment chambers affected, to some extent, RH and VPD, compared to the AMB chambers. The results indicated that elevated temperature led to a 5–15% reduction in RH for about 60% and 55% of the time in the ET and ETC chambers, respectively, and a 0.1–0.5 kPa increase in VPD for about 70% and 65% of the time in the ET and ETC chambers, respectively (Fig. 6).

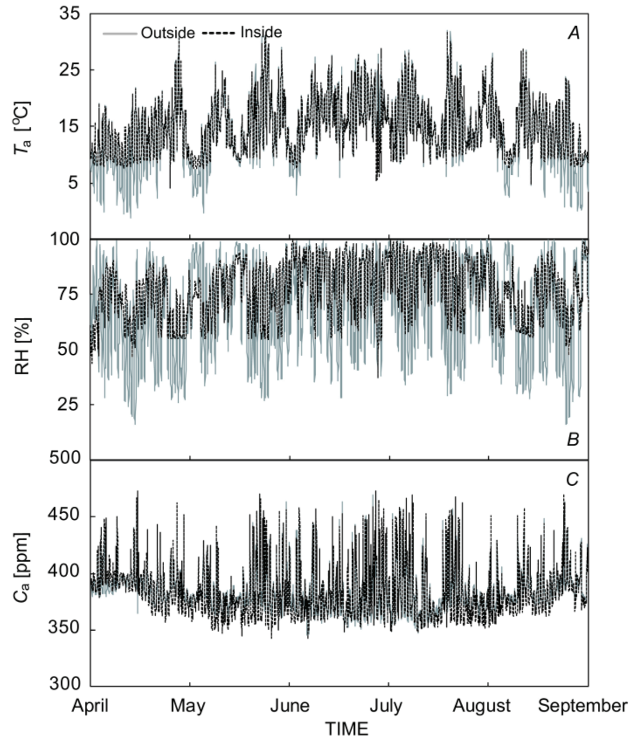

Fig. 2. Mean hourly patterns of air temperature (T_a), relative humidity (RH), and CO_2 concentration (C_a) inside the AMB chambers and outside conditions. The data are based on hourly means of 60-s readings taken outside and from four replicates in the AMB chambers. The readings cover the period of 15th April–15th September, 2009.

Effects of the chamber system on reed canary grass photosynthetic responses and growth: The parameters of RCG photosynthetic responses and growth used to evaluate the chamber effects during the growing season are presented in Table 2. At the late growing period (around 15th August), the parameters of P_{max}, L_c, L_s and g_s were significantly lower in the AMB chambers, compared to outside (Fig. 7). When measured in around 15th July and 15th August, the growth parameters of stem height, stem diameter, and stem biomass, and leaf area were significantly lower in the chambers than those outside (Table 2). The parameters of leaf length and leaf biomass were not modified significantly in the AMB chamber. There was an earlier development of RCG plants grown in the chambers, such as earlier leafing, flowering, seed setting as well as premature senescence (by 6–9 days).

Discussion

Performance of the chamber system: Compared to the current exposure and closed systems, our chamber system described here has several architectural and functional advantages: (*1*) it provides 16 individual research units with a large space (16 m^2 area) for large-volume planting containers (Fig. 1), and (*2*) it demonstrates a good control performance of growth environment of closed plants, over the "current climate" and "changed climate" scenarios such as expected temperature elevation and gas pollutants.

As the experimental control unit, the AMB chambers were effectively controlled following the same environment fluctuations, at hourly and daily levels, as observed outside the chambers. The chamber temperature was slightly higher during the beginning (15th–30th April) and the end (1st–15th September) of the study period. In retrospect the deviation can be caused by the presence of people inside the chambers working on adjusting the air conditioning, sensor configuration, and calibration and

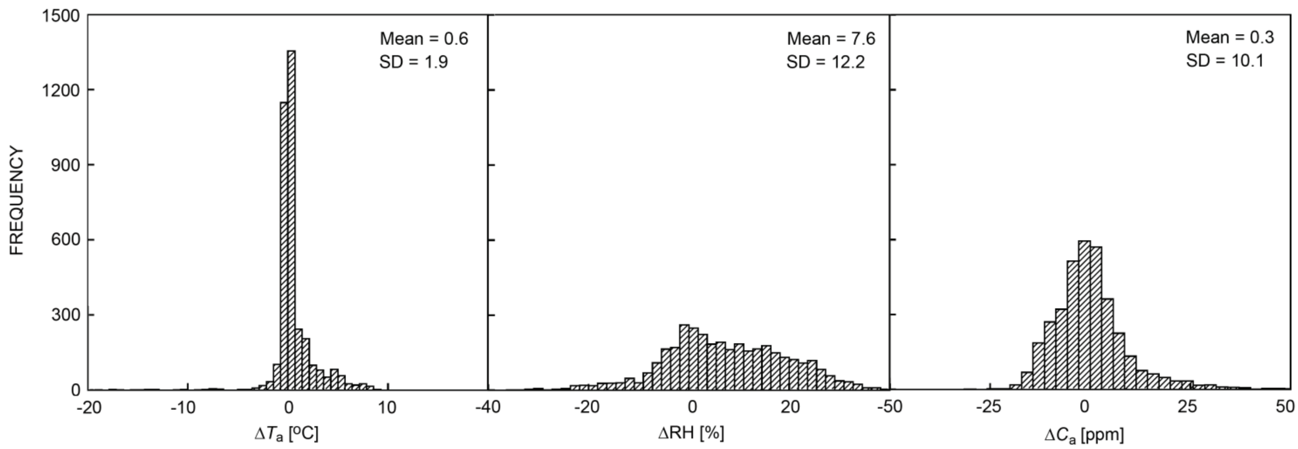

Fig. 3. Frequency distribution of ΔT_a, ΔRH, and ΔC_a in the AMB chambers. The values were calculated as the difference of air temperature (T_a), relative humidity (RH), and CO_2 concentration (C_a) between the measurements in the AMB chambers and outside conditions. The data were based on hourly means of 60-s readings taken from four replicates of the AMB chambers. The readings cover the period of 15th April–15th September, 2009.

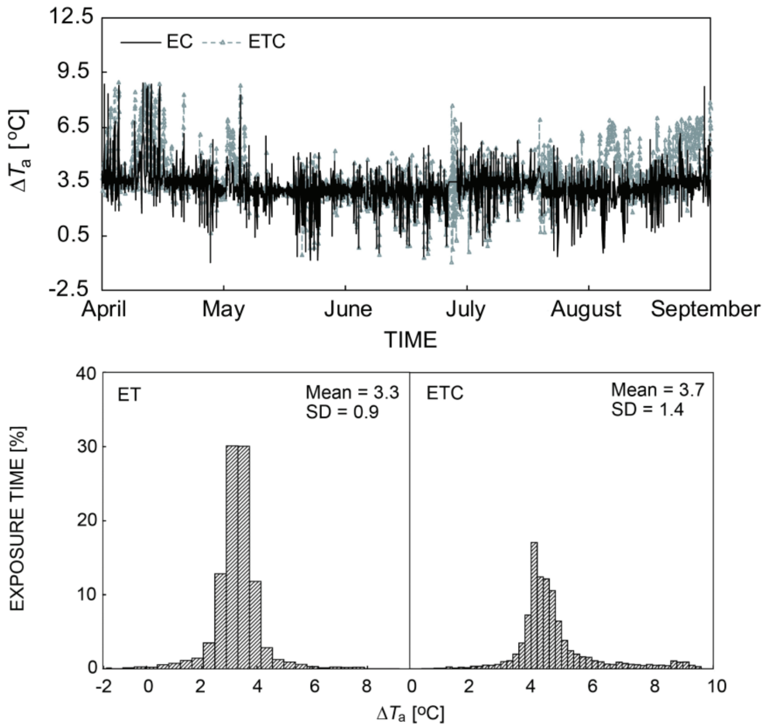

Fig. 4. Mean hourly patterns (*line charts*) and frequency distribution (*bar charts*) of ΔT in the ET and ETC chambers. The values of ΔT_a were calculated as the difference of air temperature (T_a) between the temperature measured in the ET, ETC, and AMB chambers. The data are based on hourly means of 60 s readings taken from four replicates of each treatment chamber. The readings cover the period of 15th April–15th September, 2009.

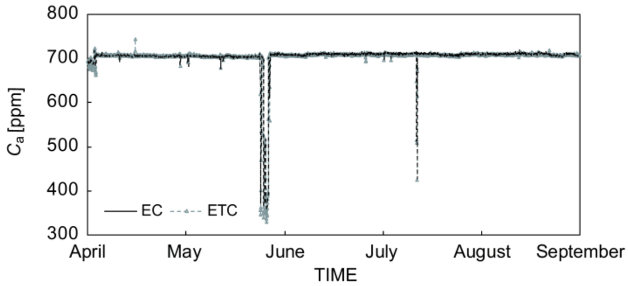

Fig. 5. Mean hourly patterns of CO_2 concentration in the EC and ETC chambers. The data were based on hourly means of 60-s readings taken from four replicates in each treatment chamber. The readings cover the period of 15th April–15th September, 2009. The two large drops in the CO_2 concentration were due to the renewal of the gas tanks.

measurements. The environment control was not disturbed during most of the growing season. Although the current study on grass with a short life-cycle excluded the winter season, lower temperature in wintertime would be easier to achieve in the chambers, compared to summertime. It is because the Mekrijärvi site is characterized by a boreal climate with extremely low winter temperatures (monthly mean temperature is around –10°C). To maintain the lower temperature in the chambers during the winter season, the full cooling capacity would not be necessary.

Furthermore, the targets of temperature elevation (+3.5°C) and CO_2 enrichment (700 ppm) were achieved well in the ET and EC chambers during the test period from April to September. The chamber system also offers controlled temperature and CO_2 elevation simultaneously in the ETC chambers, allowing important insights into the interactive effects of temperature and CO_2 on plants. Previously, a lack of temperature control has been a major limitation of many closed or semiopen chambers used for exposing plants to elevated CO_2 concentrations (Saxe *et al.* 1998, Kellomäki *et al.* 2000, Urban *et al.* 2001). The utilization of the high-power air condition unit overcomes this problem under the control of the PLC automation module.

An important methodological issue for environmental experiments is the physical effect of warming on the atmospheric humidity and the difference in air VPD between substomatal cavities and the local atmosphere (Amthor *et al.* 2010). The changes in VPD will affect the stomatal function and transpiration and carbon uptake patterns of the plant. Amthor *et al.* (2010) pointed out that some studies did not assume or set up any atmospheric water content control (relative air humidity), and this might lead to large increases in air VPD in the warmed ecosystem. In many growth chambers, the air relative humidity inside the chamber is significantly lower than the outside conditions during the daytime (Urban *et al.* 2001). As simulated by Amthor *et al.* (2010), an experimental increase in air temperature from 25°C (with RH of 50%) to 29°C would cause a drop in RH by 40%, resulting in an increase of 64% in VPD. The closed chamber generally induces the hot house effect (Urban *et al.* 2001). The previous whole-tree closed

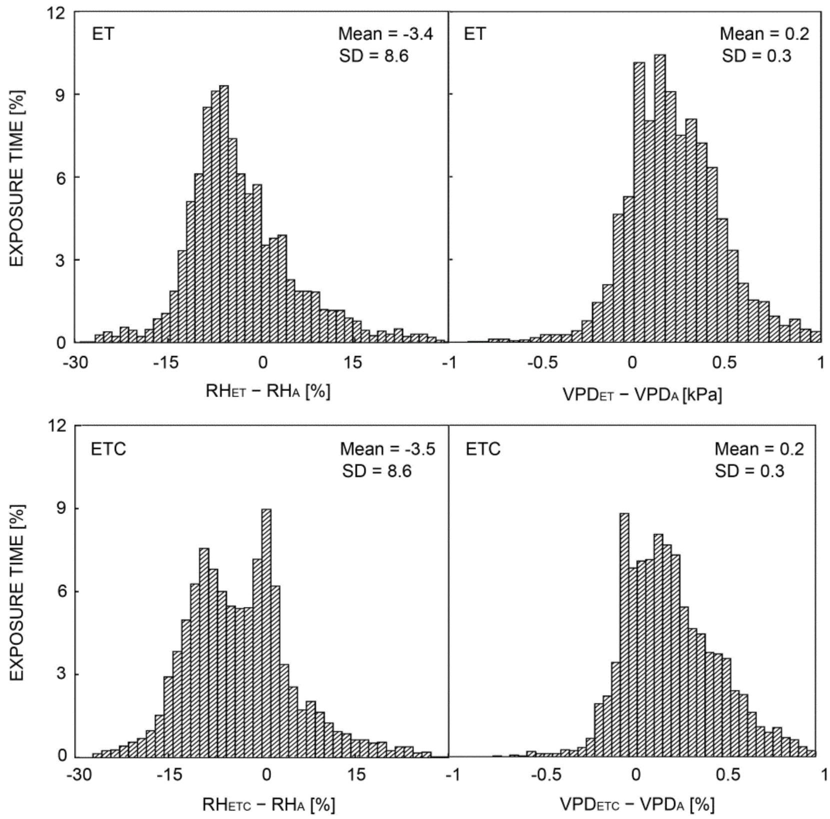

Fig. 6. Changes in relative humidity (RH) and vapour pressure deficit (VPD) in the ET and ETC chambers, compared to the AMB chambers. The data were based on hourly means of 60-s readings taken from four replicates in each treatment chamber. The readings cover the period of 15th April–15th September, 2009.

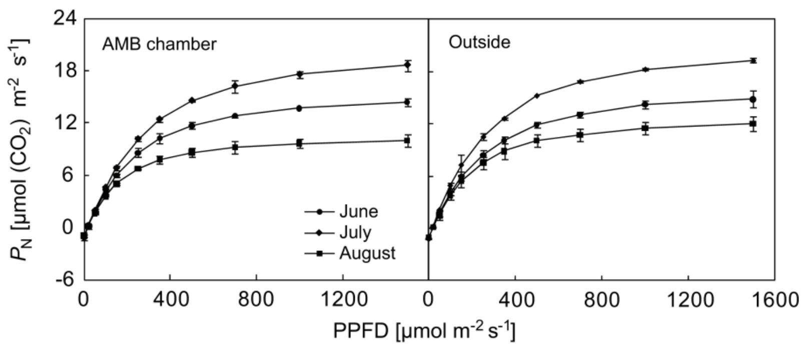

Fig. 7. Net photosynthetic rate (P_N) *vs*. photosynthetic photon flux density (PPFD) of reed canary grass leaves grown in the AMB chambers and outside. Shown are the means ± SE for 16 measured leaves. The curves are the best fits to the nonrectangular hyperbolic equation.

chambers in the Mekrijärvi Research Station (Kellomäki *et al.* 2000) were also found to have greatly RH and higher VPD inside the elevated temperature chambers than outside conditions. Consequently, Bronson *et al.* (2009) suggested that a well-designed chamber-based warming experiment can accommodate a parallel control of humidity. This will become more important as field experiments explore the full possible range of changes in temperature and humidity during the coming decades. In our new chamber system, an adjustable humidifier was installed to mitigate the dry conditions inside by means of setting a RH threshold (50% in this case), which maintains a similar VPD variation as free air outside the chambers. Consequently, the decreases in RH and increases in VPD in the temperature-treatment chambers were maintained at a low level (Fig. 6).

A broad range of physiological responses to wind has been found between species (Clark *et al*. 2000). Strong winds potentially damage plants, however, too low wind velocities lead to insufficient atmospheric mixing of gases and leaf cooling in certain circumstances (Clark *et al*. 2000). In the chambers, the fans are installed at a height of > 3 m (Fig. 1). The wind velocity close to the air conditioning fans is relatively high, providing a relatively stable airflow of 0.3–0.8 m s^{-1} in most of the space below. This wind velocity is enough to guarantee a homogeneous distribution of gases and temperature and to maintain a low leaf boundary resistance within the chambers (Kellomäki and Wang 1999, Kellomäki *et al.* 2000).

Chamber effects were observed when comparing the physiological and growth parameters of RCG grown inside and outside the chambers. The photosynthetic rate and stomatal conductance of plants were lower in the

Table 1. List of the instruments for environmental variables for 16 chambers and 1 weather station of the system. PPFD – photosynthetic photon flux density; ● – facilities are available.

Measurement	Location	Measurement range	Accuracy	Model, company and state	Chamber (inside)	Weather station (outside)
Air temperature	2 m above the ground	–50–120ºC	± 0.3ºC	*Pt-1000, Computec,* Joensuu, Finland	●	●
Relative humidity	2 m above the ground	0–100%	± 3%	*TRH-302A, Nokeval Rixen*, Nokia, Finland	●	●
Solar radiation	2 m above the ground	0–4,000 W m^{-2}	± 1%	*CM11, Kipp and Zonen*, Delft, the Netherlands		●
Net radiation	2 m above the ground	–2,000–2,000 W m^{-2}	± 1%	*NR-LITE, Kipp and Zonen*, Delft, the Netherlands		●
PPFD	3 m above the ground	0–10,000 µmol s^{-1} m^{-2}	< ± 1%	*LI-190, LiCor*, Lincoln, U.S.A.	●	●
CO_2 concentration	2 m above the ground	0–1,000 ppm	± 6–9 ppm	*GMP 343, Vaisala*, Helsinki, Finland	●	●
Air pressure	2 m above the ground	500–1,100 hPa	± 25 hPa	*PTB220BAA1A1, Vaisala*, Helsinki, Finland		●
Wind speed	16 m above the ground	0.4–75 m s^{-1}	± 2%	*WAA15A, Vaisala*, Helsinki, Finland		●
Precipitation	1.5 m above the ground	0.5–2,000 mm h^{-1}	± 5–10%	*VRG101, Vaisala*, Helsinki, Finland		●
UV-B radiation	2 m above the floor/ground	0–0.5 W m^{-2}	10%	*E-1.c, Adolf Thies GmbH & Co. KG*, Göttingen, Germany	●	●
Soil temperature	Soil depth of 50 and 150 mm	–40–60 ºC	± 0.05 ºC	*Pt-100, Elektroniikka Muurlan OY*, Helsinki, Finland	●	●
Soil water content	Soil depth of 50 and 150 mm	0–100%	± 5%	*Theta Probe ML 1, Delta-T Devices*, Cambridge, U.K.	●	●

Table 2. Comparison of parameters related to photosynthetic responses (mean ± SE for 16 replicates of measured leaves) and growth (mean ± SE for 16 replicates of samples) of reed canary grass between plants grown in four AMB chambers and outside conditions. The statistical significance (*p*) of the difference between AMB chambers and outside conditions was determined by the pair-*t* test, in which * – $p<0.05$, ns – nonsignificant. P_{max} – maximum rate of photosynthesis; R_D – dark respiration rate; *a* – quantum yield; L_c – light compensation point; L_s – light saturation; g_s – light-saturated stomatal conductance.

Variables	15 June			15 July			15 August		
	AMB chamber	Outside	*p*	AMB chamber	Outside	*p*	AMB chamber	Outside	*p*
Physiology (single leaf)									
P_{max} [µmol(CO_2) m^{-2} s^{-1}]	13.9 ± 0.1	14.0 ± 0.2	ns	19.7 ± 0.1	21.1 ± 0.6	ns	9.7 ± 0.2	12.8 ± 0.1	*
R_D [µmol(CO_2) m^{-2} s^{-1}]	0.8 ± 0.1	0.8 ± 0.1	ns	1.0 ± 0.1	1.0 ± 0.1	ns	0.8 ± 0.1	0.8 ± 0.1	ns
a [µmol(CO_2) mol^{-1}(photon)]	0.05 ± 0.002	0.05 ± 0.002	ns	0.06 ± 0.001	0.06 ± 0.004	ns	0.05 ± 0.003	0.05 ± 0.002	ns
L_c [µmol(photon) m^{-2} s^{-1}]	17.3 ± 2.3	17.9 ± 2.8	ns	18.6 ± 2.0	19.0 ± 3.0	ns	18.1 ± 1.7	19.8 ± 2.7	*
L_s [µmol(photon) m^{-2} s^{-1}]	878.6 ± 5.0	877.9 ± 42.3	ns	977.5 ± 32.9	989.9 ± 40.0	ns	612.8 ± 19.0	762.8 ± 16.4	*
g_s [mol(H_2O) m^{-2} s^{-1}]	0.3 ± 0.04	0.4 ± 0.1	ns	0.2 ± 0.03	0.3 ± 0.1	*	0.2 ± 0.01	0.3 ± 0.01	*
Growth (per plant)									
Stem height [m]	0.3 ± 0.1	0.3 ± 0.4	ns	0.5 ± 0.1	0.6 ± 0.1	*	0.8 ± 0.2	1.0 ± 0.1	*
Stem diameter [mm]	1.6 ± 0.5	2.2 ± 0.3	*	2.0 ± 0.1	2.5 ± 0.3	*	2.1 ± 0.3	2.6 ± 0.4	*
Leaf length [mm]	140.4 ± 17.4	145.7 ± 14.6	ns	152.8 ± 9.8	151.8 ± 6.2	ns	159.2 ± 19.0	161.1 ± 10.4	ns
Leaf area [mm^2]	1853 ± 581	2017 ± 813	*	2828 ± 663	3137 ± 239	*	3193 ± 1021	3436 ± 808	*
Stem biomass [g]	0.3 ± 0.2	0.3 ± 0.1	ns	0.6 ± 0.2	0.7 ± 0.2	*	1.1 ± 0.6	1.3 ± 0.4	*
Leaf biomass [g]	0.01 ± 0.01	0.02 ± 0.005	ns	0.02 ± 0.01	0.03 ± 0.003	ns	0.05 ± 0.04	0.05 ± 0.008	ns

chambers, which is in line with the work of Kellomäki *et al.* (2000) and Medhurst *et al.* (2006) on closed trees. Especially, P_{max} and g_s of grass were significantly reduced at the late stages of growing season. The reduced capacity of carbon uptake negatively affects the plant growth, *i.e.*, decline in stem height, stem diameter and biomass. Generally speaking, it may be difficult to determine the effects of the chamber as such on the physiology and growth of the plants because numerous aspects of the physiological processes are involved and they vary according to the spatial and temporal scales on which the observations are made. In our case, the low air humidity inside the chamber, as found in the previous system (Kellomäki *et al.* 2000), is partly resolved with the help of humidity adjusters. The measurements showed that the PPFD in the chambers was 20–30% lower during the growing season, compared to the outside conditions. The reduced light availability may be the main reason for the modifications in certain parameters of photosynthesis and growth, especially for the light-demanding species. On the other hand, it is well known that the developmental stages of herbaceous plants (such as reed canary grass) are triggered by thermal time. As presented by Oijen *et al*. (1999), facilities such as growth chambers that lack a cooling machine typically have 1–3°C higher temperature than ambient. Accordingly, the significant chamber effects at the late stage of growing season could be due to the earlier senescence induced as a result of higher temperature sum for grass development in the chambers.

Conclusions: In summary, the chamber system is suitable for growing a wide range of plant sizes from crops and herbaceous plants to seedlings and young trees. The system, with its calibrated sensor set and control module, is able to follow the free-air environment over prolonged periods as required in the AMB chambers. The system is also able to maintain the set targets of air temperature and CO_2 concentration within each "climate change" chamber on a continuous basis. This provides a relatively reliable and stable means for studying long-term responses of plants to environmental change under boreal and temperate conditions. Moreover, the application of the humidity adjusters can accommodate the parallel control of humidity for a reasonable change in vapour pressure deficit for stomatal behavior and growth of plants under warmer environment. As most of the closed chamber systems, the environment of low light availability mainly contributed to the chamber effects, leading to a reduced light-saturated net photosynthesis and lower biomass. For the light-demanding species, an external irradiation source would be necessary for PPFD supplement in the future applications.

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