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Seasonal responses of photosynthesis and growth of a  
bioenergy crop (*Phalaris arundinacea* L.) to climate  
change under varying water regimes

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Academic dissertation

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## ABSTRACT

The aim of this study was to investigate how the elevated temperature and CO<sub>2</sub> and varying water regimes affected the physiological characteristics (leaf photosynthesis, chlorophyll fluorescence and pigment) and growth of a bioenergy crop, reed canary grass (*Phalaris arundinacea* L.). For this purpose, the plants (with peat monoliths) was grown in an auto-controlled environment chamber system (Paper I) over two growing seasons (2009–2010) under elevated temperature (ambient + 3.5°C) and CO<sub>2</sub> (700 μmol mol<sup>-1</sup>). The plants were also treated as three levels of soil moisture, ranging from high (100% volumetric content), to normal (~50%) and low (~30%).

The elevated temperature stimulated the leaf photosynthesis and carbon storage in the biomass during the early growth periods compared to the ambient temperature, while it might result in earlier senescence and lower photosynthesis and biomass during the later periods (Paper II & V). The maximum rate of photosynthesis ( $P_{\max}$ ), the maximum rate of ribulose-1,5-bisphosphate carboxylase-oxygenase activity ( $V_{\max}$ ) and the potential rate of electron transport ( $J_{\max}$ ) at gradient measurement temperature (5–30°C) showed also significant seasonal variations regardless of climate treatment (Paper IV). At the early stages of growing season, the elevated temperature decreased  $V_{\max}$  and  $J_{\max}$  compared to the ambient temperature at the lower measurement temperatures (5–15°C), opposite to the higher measurement temperatures (20–30°C). Later in the growing period,  $V_{\max}$  and  $J_{\max}$  were under the elevated temperature consistently lower across the measurement temperatures. The CO<sub>2</sub> enrichment significantly increased the photosynthesis and slightly decreased  $V_{\max}$  and  $J_{\max}$  compared to the ambient CO<sub>2</sub> across the measurement temperatures. The CO<sub>2</sub> enrichment led also to a slight down-regulation in the leaf nitrogen, chlorophyll content and fluorescence characters (Paper III).

Low soil moisture decreased clearly the photosynthesis performance and chlorophyll fluorescence, which eventually also decreased the carbon storage in plant biomass, particularly under the elevated temperature (Paper II, III & V). Furthermore,  $V_{\max}$  and  $J_{\max}$  decreased significantly under low soil moisture (Paper IV). This provided further evidence that not only diffusive conductance but also photosynthetic capacity would be reduced in the plants subjected to long-term drought. Nevertheless, the temperature- and drought-induced stresses were partially mitigated by the elevation of CO<sub>2</sub>.

To conclude, the seasonal estimation of the physiological and growth parameters for reed canary grass makes it possible to simulate its photosynthesis and carbon storage in biomass over the whole growing season under varying environmental conditions. The growth of plants on organic soils could also be expected to be favored under warming climate if the water availability is high.

**Keywords:** Climate change, Water regimes, *Phalaris arundinacea* L., Seasonal photosynthetic acclimation, Leaf characteristics, Carbon storage

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Finally, I would like to dedicate this PhD thesis especially to my husband, Dr. Zhen-Ming Ge, as without his love and great support I could have not confidence and happiness in my life. I also would like to dedicate this thesis to my parents and relatives due to their love and support beyond borders. Last, but not the least, I would like to extend my gratitude to the Chinese Friends in Joensuu for their warm support and joyous times shared.

Xiao Zhou

Joensuu May 11<sup>th</sup> 2011

## LIST OF ORIGINAL ARTICLES

This thesis is a summary of the following papers, which are referred to in the text by the Roman numerals I–V. Articles I, III and V are reproduced with the kind permission from the publishers, while the studies II and IV are the author version

- I Zhou, X., Ge, Z.M., Kellomäki, S., Wang, K.Y., Martikainen, P., Lemettinen, M., Hassinen, A., Ikonen, R. & Peltola, H. 2011. Multi-objective environment chamber system for studying plant responses to climate change. *Photosynthetica*. (In press). <http://www.springer.com/life+sciences/plant+sciences/journal/11099>.
- II Zhou, X., Ge, Z.M., Kellomäki, S., Wang, K.Y., Peltola, H., Biasi, C., Shurpali, N. & Martikainen, P. 2011. Effects of elevated CO<sub>2</sub> concentration and temperature on photosynthesis of a bioenergy crop (*Phalaris arundinacea* L.) subjected to different water levels in boreal conditions. Manuscript.
- III Ge, Z.M., Zhou, X., Kellomäki, S., Wang, K.Y., Peltola, H. & Martikainen, P. 2011. Responses of leaf photosynthesis, pigments and chlorophyll fluorescence within canopy position in a boreal grass (*Phalaris arundinacea* L.) to elevated temperature and CO<sub>2</sub> under varying water regimes. *Photosynthetica* 49: 172-184.
- IV Zhou, X., Ge, Z.M., Kellomäki, S., Peltola, H., Wang, K.Y. & Martikainen, P. 2011. Seasonal acclimation of photosynthesis, carboxylation efficiency and electron transport capacity of a boreal grass (*Phalaris arundinacea* L.) to elevated temperature and CO<sub>2</sub> under different water availability. Manuscript.
- V Zhou, X., Ge, Z.M., Kellomäki, S., Wang, K.Y., Peltola, H. & Martikainen, P. 2011. Effects of elevated CO<sub>2</sub> and temperature on leaf characteristics, photosynthesis and carbon storage in above-ground biomass of a boreal bioenergy crop (*Phalaris arundinacea* L.) under varying water regimes. *Global Change Biology Bioenergy* 3: 223-234.

Xiao Zhou had the main responsibility for all the work done in Papers I–V. The co-authors of the separate Papers I–V participated in the work mainly by commenting on the manuscripts and supporting the data analyses. Paper II was written jointly by Zhen-Ming Ge and Xiao Zhou.

## TABEL OF CONTENTS

|  |           |
|--|-----------|
| <b>ABSTRACT</b>  | <b>3</b>  |
| <b>ACKNOWLEDGMENTS</b>   | <b>4</b>  |
| <b>LIST OF ORIGINAL ARTICLES</b>   | <b>5</b>  |
| <b>LIST OF SYMBOLS</b>   | <b>7</b>  |
| <b>1 INTRODUCTION</b>  | <b>9</b>  |
| 1.1 General background   | 9         |
| 1.2 Physiological responses  | 10        |
| 1.3 Growth responses   | 11        |
| 1.4 Aims of the study  | 12        |
| <b>2 MATERIALS AND METHODS</b>   | <b>12</b> |
| 2.1 General outlines   | 12        |
| 2.2 Temperature and CO <sub>2</sub> treatments and water regime design (I)                         | 15        |
| 2.3 Gas exchange measurements (II, III, IV, V)   | 16        |
| 2.3.1 <i>Outlines of measurements</i>  | 16        |
| 2.3.2 <i>Measurements for seasonal variation of leaf level photosynthesis</i>                      | 16        |
| 2.3.3 <i>Measurements for chlorophyll fluorescence and pigment content within layer position</i>   | 16        |
| 2.3.4 <i>Measurements for estimating photosynthetic parameters</i>                                 | 17        |
| 2.3.5 <i>Method for analyzing gas exchange</i>   | 18        |
| 2.4 Growth and carbon storage measurements (V)   | 19        |
| 2.5 Statistical data analyses  | 20        |
| <b>3 RESULTS</b>   | <b>20</b> |
| 3.1 Stability and accuracy of auto-controlled environment chamber system (I)                       | 20        |
| 3.2 Physiological responses of RCG to temperature, CO <sub>2</sub> and water regimes (II, III, IV) | 21        |
| 3.2.1 <i>Gas exchange</i>  | 21        |
| 3.2.2 <i>Pigments and chlorophyll fluorescence within canopy positions</i>                         | 21        |
| 3.2.3 <i>Temperature dependency of photosynthetic parameters</i>                                   | 22        |
| 3.3 Growth responses of RCG to temperature, CO <sub>2</sub> and water regimes (V)                  | 23        |
| 3.3.1 <i>Leaf characteristics</i>  | 23        |
| 3.3.2 <i>Carbon storage in above-ground biomass</i>  | 23        |
| <b>4 DISCUSSION AND CONCLUSIONS</b>  | <b>24</b> |
| 4.1 Effects of temperature and CO <sub>2</sub>   | 24        |
| 4.2 Effects of water regime  | 27        |
| 4.3 Interactive effects of temperature, CO <sub>2</sub> and water regimes                          | 28        |
| 4.4 Conclusion   | 28        |
| <b>REFERENCES</b>  | <b>29</b> |

## LIST OF SYMBOLS

| Symbol            | Unit   | Description  |
|-------------------|--|--|
| CON               | /  | Ambient temperature and CO <sub>2</sub> concentration              |
| ET                | /  | Elevated temperature and ambient CO <sub>2</sub> concentration     |
| EC                | /  | Elevated CO <sub>2</sub> concentration and ambient temperature     |
| ETC               | /  | Combined temperature and CO <sub>2</sub> concentration elevation   |
| HW                | /  | High soil moisture   |
| NW                | /  | Normal soil moisture   |
| LW                | /  | Low soil moisture  |
| (period) I–VI     | /  | Six development periods for photosynthesis and growth measurements |
| GP-I, GP-II       | /  | Two periods for photosynthetic parameters measurements             |
| <i>RuBP</i>       | /  | Ribulose biphosphate   |
| <i>Rubisco</i>    | /  | Ribulose 1,5-biphosphate carboxylase/oxygenase                     |
| <i>NADPH/</i>     | /  | Nicotinamide adenine dinucleotide phosphate                        |
| <i>NADP</i>       |  |  |
| <i>PGA</i>        | /  | Phosphoglyceric acid   |
| $P_n$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Net photosynthetic rate  |
| $P_{\text{max}}$  | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Maximum photosynthetic rate  |
| $P_c$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Rubisco-limited rate of photosynthesis                             |
| $P_i$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | RuBP-regeneration-limited rate of photosynthesis                   |
| $C_i$             | $\mu\text{mol mol}^{-1}$                       | Intercellular CO <sub>2</sub> concentration                        |
| $C_c$             | $\mu\text{mol mol}^{-1}$                       | Chloroplast CO <sub>2</sub> concentration                          |
| <i>PPFD</i>       | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Photosynthetic photon flux densities                               |
| $\Gamma^*$        | $\mu\text{mol mol}^{-1}$                       | CO <sub>2</sub> compensation point (absence of dark respiration)   |
| $V_{\text{cmax}}$ | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Maximum rate of carboxylation by Rubisco                           |
| $J_{\text{max}}$  | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Maximum rate of electron transport                                 |
| <i>TPU</i>        | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Triose phosphate use   |
| $R_d$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Mitochondrial respiration in light                                 |
| $K_c$             | $\mu\text{mol mol}^{-1}$                       | Rubisco Michaelis constants for CO <sub>2</sub>                    |
| $K_o$             | $\mu\text{mol mol}^{-1}$                       | Rubisco Michaelis constants for O <sub>2</sub>                     |
| $O$               | $\text{mmol mol}^{-1}$                         | O <sub>2</sub> concentration                                       |
| $V_o$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Maximum rate of oxygenation by Rubisco                             |
| $J$               | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Rate of electron transport   |
| $\alpha$          | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Initial slope of the light response curve                          |
| $L_c$             | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Light compensation point   |
| $I$               | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Absorbed irradiance  |
| $\theta$          | /  | Curvature of the light response curve                              |
| $T_{\text{opt}}$  | K  | Optimal temperature  |
| $\Delta H_a$      | $\text{kJ mol}^{-1}$                           | Activation energy  |
| $\Delta H_d$      | $\text{kJ mol}^{-1}$                           | Deactivation energy  |
| $\Delta S$        | $\text{kJ K}^{-1} \text{mol}^{-1}$             | Entropy of the desaturation equilibrium                            |
| $R$               | $\text{J mol}^{-1} \text{K}^{-1}$              | Molar gas constant   |
| <i>VPD</i>        | kpa  | Vapor pressure deficit   |
| $E_t$             | $\text{mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$ | Light-saturated transpiration                                      |
| <i>WUE</i>        | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Light-saturated water use efficiency                               |

|                      |  |   |
|----------------------|--|---|
|                      | $\text{mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$ |   |
| $g_s$                | $\text{mol m}^{-2} \text{s}^{-1}$              | Stomatal conductance                                      |
| $g_m$                | $\text{mol m}^{-2} \text{s}^{-1}$              | Mesophyll conductance                                     |
| $g_{\text{sat}}$     | $\text{mol m}^{-2} \text{s}^{-1}$              | Light-saturated stomatal conductance                      |
| $F_0$                | /  | Minimum chlorophyll fluorescence of dark-adapted state    |
| $F_m$                | /  | Maximal chlorophyll fluorescence of dark-adapted state    |
| $F_v/F_m$            | /  | Maximal photochemical efficiency of PSII                  |
| $\Phi_{\text{PSII}}$ | /  | Effective photochemical efficiency of light-adapted state |
| $F_s$                | /  | Steady state fluorescence                                 |
| $F_0'$               | /  | Minimum chlorophyll fluorescence of light-adapted state   |
| $F_m'$               | /  | Maximal chlorophyll fluorescence of light-adapted state   |
| $q_P$                | /  | Photochemical quenching                                   |
| $NPQ$                | /  | Non-photochemical quenching                               |
| $ETR$                | $\mu\text{mol m}^{-2} \text{s}^{-1}$           | Electron transport rate                                   |
| $C_l$                | $\text{g C shoot}^{-1}$                        | Carbon storage in leaf                                    |
| $C_s$                | $\text{g C shoot}^{-1}$                        | Carbon storage in stem                                    |
| $LA$                 | $\text{cm}^2 \text{shoot}^{-1}$                | Leaf area   |
| $SLW$                | $\text{g cm}^{-2}$                             | Specific leaf weight                                      |
| $N_L$                | $\text{g cm}^{-2}$                             | Nitrogen content based on leaf area                       |
| Chl $a(b)$           | $\text{mg g}^{-1}$                             | Chlorophyll $a(b)$  |
| Cars                 | $\text{mg g}^{-1}$                             | Carotenoid  |



# 1 INTRODUCTION

## 1.1 General background

The amount of carbon dioxide in the atmosphere has increased by more than 28% since the beginning of the industrial revolution, largely as a result of land-use change and anthropogenic emissions from the burning of fossil fuels. Since then CO<sub>2</sub> has been accumulating in the global atmosphere at an accelerating rate (IPCC 2007). By the end of this century, CO<sub>2</sub> is projected to surpass 550–700 μmol mol<sup>-1</sup> (Prentice et al. 2001). A predicted consequence of the rise in atmospheric CO<sub>2</sub> is an increase in temperature between 1.6–6.4 °C in the next 50–100 years (IPCC 2007). Furthermore, drought episodes are expected to become more frequent during summertime in many regions of the world, including the boreal zone (Jylhä et al. 2009, 2010). These stresses are often expected to occur simultaneously and affect the physiological acclimation of plants to the changing environmental conditions (Martínez-Carrasco et al. 2005, Erice et al. 2006, Kellomäki et al. 2008).

Boreal peatlands contain one-third of the world's soil organic carbon, equivalent to more than half the amount of carbon in the atmosphere (Dorrepaal et al. 2009). Historically, drainage of peatlands for resource utilization is a common practice in Scandinavia, Canada and Russia. However, when drained for forestry, agriculture or peat extraction, the thickness of their aerobic soil layer increases. As a result, drained extracted peatlands are turned into atmospheric carbon sources (Maljanen et al. 2002, Minkkinen et al. 2002), the development of which could be expected to accelerate under climate warming (Dorrepaal et al. 2009). Therefore, organic soils have been included among the areas with high risk of significant soil carbon losses, and even recommended to be kept out of biomass production for bioenergy (OECD 2007).

In Europe, the area under the cultivation of bioenergy crop, reed canary grass (*Phalaris arundinacea* L., here after RCG) is rapidly increasing (e.g., in Finland and Sweden). RCG is preferred in Finland as a bioenergy crop cultivation and applied as an after-use option on cut-over peat mining site because it produces substantial biomass (6–8 t ha<sup>-1</sup>) under northern, long day conditions (Pahkala et al. 2008). Furthermore, the cultivation of RCG is also environmentally sound, because it has a high storage capacity for fertilizer nutrients in the root system (Kätterer and Andréén 1999), and additionally only small amounts of nitrogen are removed from the soil in harvested biomass (Partala et al. 2001). However, one of the most important issues is in regard to its potential for raw material for energy production, how to optimize the biomass production of RCG and the carbon balance of sites with organic soil (Shurpali et al. 2009).

In Finland, the mean annual temperature is expected to increase by 2–7°C with a concurrent elevation of CO<sub>2</sub> by the end of the 21<sup>st</sup> century (Jylhä et al. 2009, 2010). The changing climate will also affect water availability in the soil profile and the consequent carbon uptake and plant growth. In fact, the drought episodes are expected to become more frequent in the future and limit plant growth even in the central boreal zone (Kellomäki et al. 2008). However, limited knowledge of the physiology and biochemistry of RCG would hinder how to manage it in an optimal way for the carbon sequestration and biomass production under the changing climate. On the other hand, RCG is well known as a semi-aquatic plant. On the other hand, whether RCG cultivated in the cut-over and drained

peatlands with lower water table level would have a positive or negative effect on the RCG growth is still unknown. Consequently, the question has arisen of how to operate the field cultivation and management (water table level regulation) for sustainable RCG production and maintenance of positive carbon balance under the changing climate.

## 1.2 Physiological responses

Both temperature and atmospheric CO<sub>2</sub> are key variables affecting plant growth, development and functions. Higher temperature and the atmospheric CO<sub>2</sub> will directly influence plant physiology, through their effects on photosynthesis, transpiration and respiration (Drake et al. 1997). However, soil water availability has contrasting influences on these primary processes. Among the plant physiology, the photosynthesis characteristics are fundamental issues to be considered, because changing climate may significantly affect the carbon uptake and growth of plants.

The uptake rate of CO<sub>2</sub> is one of the key parameters needed to understand how plants respond to changes in the environmental conditions over long periods. In recent years, several studies have been done to understand how the photosynthetic responses in C<sub>3</sub> plants growing under the elevated CO<sub>2</sub> and temperature may change, regarding to various environmental factors (Bernacchi et al. 2001, 2002, Long et al. 2004, Alonso et al. 2008, 2009). In this respect, both experimental and model-based studies are needed in order to understand the effects of climate change on plants (Medlyn et al. 2002a,b). The models can also be used to test the effects of short-term physiological responses to the changes in environmental conditions, and to identify how sensitive plants are to climate change in the long run (Medlyn 2002a,b). In this regard, the widely used biochemical photosynthetic model developed by Farquhar et al. (1980) and Farquhar and von Caemmerer (1982), offers a means to study the photosynthetic response of C<sub>3</sub> plants and possible acclimation of photosynthesis to the elevated temperature and atmospheric CO<sub>2</sub>. In this model, the biochemical reactions of photosynthesis are considered to be in one of three distinct steady states. In one state, the capacity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) consumes ribulose bisphosphate (RuBP). This state is called Rubisco-limited photosynthesis and normally occurs when the CO<sub>2</sub> supply is low. Because of this, the initial slope of the net photosynthesis rates versus intercellular CO<sub>2</sub> concentration response is determined by Rubisco capacity ( $V_{\text{cmax}}$ ) at light saturation (Farquhar and von Caemmerer 1982). In the other state, photosynthetic rates are predicted assuming that the rate of regeneration of RuBP is limiting. RuBP regeneration capacity at light saturation generally reflects limitations in electron transport capacity ( $J_{\text{max}}$ ), a feature that allows for the estimation of  $J_{\text{max}}$  using net photosynthesis rates versus photosynthetic photon flux densities response at saturated CO<sub>2</sub> (von Caemmerer and Quick 2000). A third state occurs when the chloroplast reactions have a higher capacity than the capacity of the leaf to use the products of the chloroplasts, triose phosphate. This third state is called triose phosphate use (TPU) limitation, but it rarely affects photosynthesis under natural conditions (von Caemmerer and Quick 2000, Sharkey et al. 2007).

Under the elevated CO<sub>2</sub>, a decrease in Rubisco specific activity could be found (Pérez et al. 2005). The changes in temperature dependence of the  $V_{\text{cmax}}$  and the  $J_{\text{max}}$  under elevated CO<sub>2</sub> concentrations have also been addressed in the previous studies (Alonso et al. 2008, 2009), indicating that the elevated CO<sub>2</sub> may produce an upward shift in the temperature optimum of photosynthesis. In addition, elevated temperature will shift the specificity of

Rubisco for O<sub>2</sub> relative to CO<sub>2</sub>, which will increase the proportion of photorespiration in photosynthesis (Jordan and Ogren 1984). However, increases in CO<sub>2</sub> will shift the balance towards carboxylation and reduce photorespiratory loss (Stitt and Krapp 1999). On the other hand, the net photosynthesis is also related to the respiration. Thus, higher temperatures would increase the ratio of respiration to photosynthesis (Fitter and Hay 1987), while increased CO<sub>2</sub> may decrease it (Ziska and Bunce 1994). Nonetheless, the balance of these processes and the extent and direction of acclimation (both regulation and changes in capacity) to elevation of temperature and CO<sub>2</sub> alone or in interaction still remain uncertain.

Furthermore, the rising temperature may increase a drought frequency and intensity (IPCC 2007), which induce changes in various physiological processes (Boyer 1982). Stomatal closure is an early response to drought and an efficient way to reduce water loss under water-limiting conditions. Some earlier studies have suggested that elevated CO<sub>2</sub> may alleviate the drought situation (Morison 1993, Sage 1996), by enhancing the water use efficiency (*WUE*). However, the inhibitory effects of drought on photosynthesis can be associated with low CO<sub>2</sub> availability as caused by limitations of diffusion through the stomata and the mesophyll for photosynthesis (Flexas et al. 2004a).

### 1.3 Growth responses

There are many processes in plant growth at a wide range of scales that are affected by temperature and CO<sub>2</sub> alone or in interactions (Morison and Lawlor 1999). Temperature is one of the most important factors affecting plant ontogeny and morphogenesis (Morison and Lawlor 1999). Normally, this relationship is often conveniently summarized in terms of thermal time. The effect of increase in temperature is acting cumulatively over long periods of time, and it may significantly affect the rates of initial development and growth in leaf area and biomass (Pinter et al. 1996). The impact can be large over the range of temperature elevation. For example, in winter wheat (*Triticum aestivum* L., cv. Mercia), 50% of anthesis happened 21 days earlier under a +4°C elevation of temperature treatment than under the ambient temperature (Mitchell et al. 1993). This effect of temperature increment will substantially affect the annual growth of a crop, like RCG. The stimulation of total plant leaf area under the elevated CO<sub>2</sub> has been widely reported, too. However, most of the previous work have not distinguished whether the stimulation was due to the increase of leaf numbers or increased leaf size (Mitchell et al. 1993, Hakala and Mela 1996, Ghannoum et al. 1997). It is obvious that even small effects on leaf size and total leaf area can have major impacts on total plant photosynthesis (Lawlor, 1995). However, only small effects on the development rate, for example, in winter wheat have been reported under the CO<sub>2</sub> elevation (e.g. Marc and Gifford 1984, Mitchell et al. 1993), and the effect was certainly much smaller than that of temperature.

As RCG is an aquatic plant, water table management is the main concern regarding the cultivation of RCG, especially in peatland where the water table has fallen following peat exploitation. In some previous studies, plants grown under water shortage have had smaller leaves and lower plant biomass than those without water shortage (e.g. Marcelis et al. 1998, Sinclair and Muchow 2001, Qaderi et al. 2006). This is because drought stress affects also the leaf expansion to a larger extent than the photosynthesis (e.g. Tardieu et al. 1999), and makes leaves thicker than under sufficient water supply. These modifications are suggested to be due to a self-adaptive mechanism under low water availability (e.g. Wright et al. 1994, Craufurd et al. 1999, Qaderi et al. 2006).

## 1.4 Aims of the study

The main aim of this study was to study the effects of elevated temperature and CO<sub>2</sub> on the physiological characteristics and growth of a bioenergy crop, reed canary grass (*Phalaris arundinacea* L.), under varying water regimes. For this purpose, the reed canary grass (with peat monoliths) was grown in an auto-controlled environment chamber system over two growing seasons (2009–2010) based on a factorial design with four replicates for each climate treatment: Ambient temperature and CO<sub>2</sub> concentration (CON); Elevated temperature and ambient CO<sub>2</sub> concentration (ET); Elevated CO<sub>2</sub> concentration and ambient temperature (EC) and Elevated temperature and CO<sub>2</sub> concentration (ETC). Each chamber contained three containers represented three different levels of soil water, ranging from high (HW, 100% volumetric soil water content), to normal (NW, ~50% volumetric soil water content, roughly as field measurement) and low (LW, ~30% volumetric soil water content). The specific objectives of the study were as follows:

- i. To study the stability and accuracy of a new auto-controlled environment chamber system in order to study reed canary grass response to climate change under boreal conditions (**Paper I**);
- ii. To study the effects of elevated temperature and CO<sub>2</sub> on photosynthesis, chlorophyll fluorescence and pigments content of reed canary grass under varying water regimes (**Paper II, III**);
- iii. To study the seasonal acclimation of carboxylation efficiency and electron transport capacity in reed canary grass to elevated temperature and CO<sub>2</sub> under varying water regimes (**Paper IV**);
- iv. To study the effects of elevated temperature and CO<sub>2</sub> alone and in interaction on leaf characteristics and carbon storage in above-ground biomass of reed canary grass under varying water regimes (**Paper V**).

## 2 MATERIALS AND METHODS

### 2.1 General outlines

Understanding of the environmental factors, which limit plant physiological processes are important for estimating the effects of climate change on plants. In Figure 1, it is shown the framework for this thesis, considering the seasonal photosynthesis and growth of short life-cycle plants (RCG) under given environment. For the interface between stomata and atmosphere, the seasonal variations of acclimation of the leaf photosynthesis and respiration are needed to be understood. In this context, it is crucial to study the key parameters defining the carboxylation efficiency and electron transport capacity. At the leaf-shoot level, the determination of leaf area-based carbon uptake, water use efficiency and chlorophyll fluorescence can also provide useful information about the physiological performance of the whole plant under various environmental stresses. Regarding biomass production, the amount of carbon stored in response to varying environmental conditions

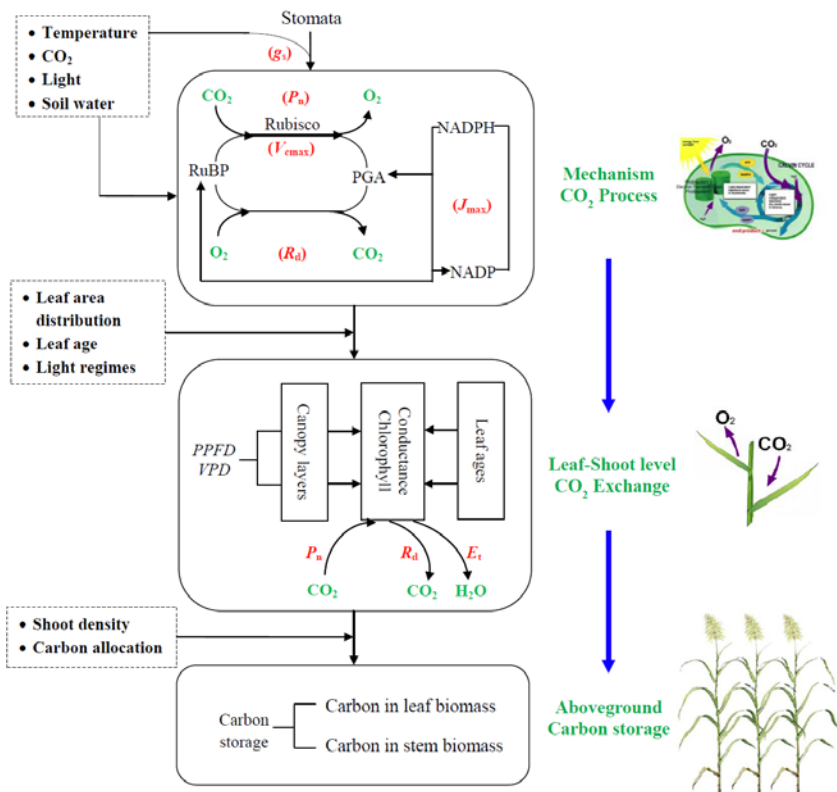
will also be studied in this work.

In Paper I, the stability and accuracy of a new auto-controlled chamber system were studied and demonstrated, because accurate environmental control is crucial for further studies (Papers II-V) on seasonal response of photosynthesis and growth of RCG to climate change under boreal conditions.

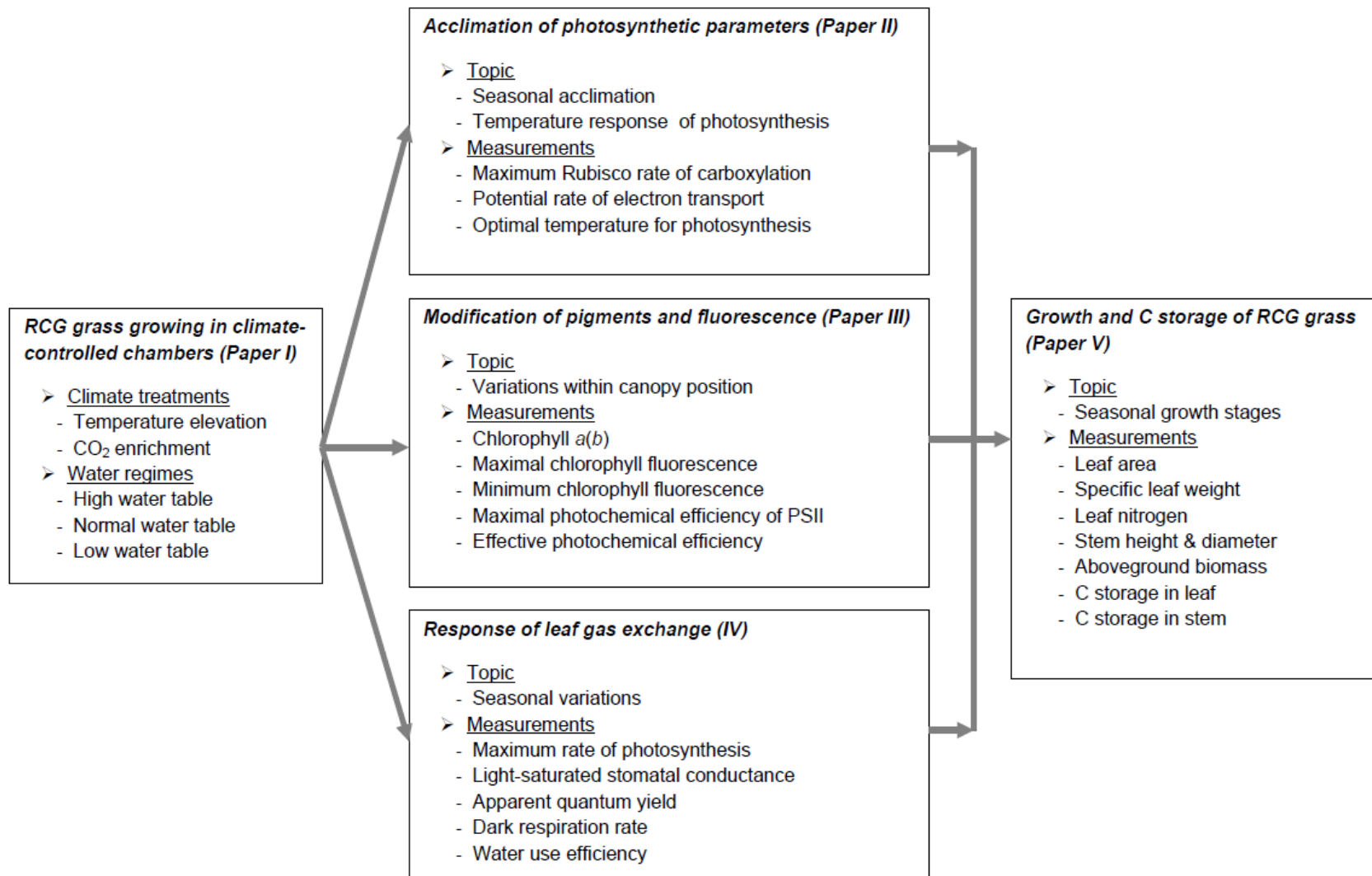
In Papers II and III, in general, it was studied the seasonal variations of photosynthesis and the canopy positions (leaf age classes) related chlorophyll fluorescence of RCG under elevated temperature and CO<sub>2</sub> with varying soil water regimes. More specifically, the light saturated stomatal conductance, water use efficiency, the pigment and nitrogen content were considered.

In Paper IV, the seasonal acclimation of carboxylation efficiency ( $V_{\text{cmax}}$ ) and electron transport capacity ( $J_{\text{max}}$ ) under elevated temperature and CO<sub>2</sub>, subjected to different soil moistures were studied utilizing the Farquhar's model (Farquhar et al. 1980, Farquhar and von Caemmerer 1982). The modifications on the temperature dependency of the parameters at two different periods during the growing season were investigated. In addition, the estimated parameter values were tested against a set of measurements not used for the estimation of the parameter values of the model.

In Paper V, the effects of elevated CO<sub>2</sub> and temperature alone and in interaction, on the leaf characteristics and the carbon storage in above-ground biomass (leaves and stem) of RCG were estimated under varying water regimes. The outlines of measurement work is also shown in Figure 2.



**Figure 1.** Frame of carbon uptake processes and storage in reed canary grass under a given environment.



**Figure 2.** Outline of the measurement work in Papers I–V.

## 2.2 Temperature and CO<sub>2</sub> treatments and water regime design (I)

In March 2009, 48 microcosms consisting of organic soil monoliths (80 cm × 60 cm × 35 cm, sufficient volume for root growth) with intact RCG plants were cored from the Linnansuo peatland (62°30'N, 30°30'E, Eastern Finland, belonging to Vapo Bio-energy Ltd.). The RCG plants were cultivated in environment-controlled chambers at the Mekrijärvi Research Station (62°47'N, 30°58'E, belonging to the University of Eastern Finland, Figure 3) for two growing season from 2009 to 2010. The plants were fertilized with 5.4 g N m<sup>-2</sup>, 1.2 g P m<sup>-2</sup> and 4.2 g K m<sup>-2</sup> each year applying the management practices used by Vapo Bio-energy Ltd.

The greenhouse consists of 16 chambers working independently from each other, which facilitated a factorial design with four replicates for each climate treatment: Ambient temperature and CO<sub>2</sub> concentration (CON); Elevated temperature and ambient CO<sub>2</sub> concentration (ET); Elevated CO<sub>2</sub> concentration and ambient temperature (EC) and Elevated temperature and CO<sub>2</sub> concentration (ETC).

During the growing period (15th April to 15th September), the CON chambers were set to follow the outside free air CO<sub>2</sub> concentration (around 370-390 μmol mol<sup>-1</sup>) and temperature. In the chambers with elevated temperature (ET and ETC), the target temperature was set at +3.5°C above that in the CON chambers. The target CO<sub>2</sub> concentration in the chambers with the elevated CO<sub>2</sub> (EC and ETC) was 700 μmol mol<sup>-1</sup>.

Each chamber contained three containers with different levels of soil water, ranging from high (HW, 100% volumetric soil water content), to normal (NW, ~50% volumetric soil water content, roughly as field measurement) and low (LW, ~30% volumetric soil water content). LW represented the wilting point (20–30% volumetric soil water content) typical on drained peatlands used for agriculture in Finland. Soil moisture was monitored with manual soil moisture sensors (*Theta Probe ML 1*, *Delta-T Devices*, Cambridge, U.K.) to keep the soil moisture at the target level through irrigation.



**Figure 3.** The climate chamber system used for RCG cultivation.

## 2.3 Gas exchange measurements (II, III, IV, V)

### 2.3.1 Outlines of measurements

There are three different sets of gas exchange measurements done in this study. Firstly, the gas exchange measurements for seasonal variation of leaf level photosynthesis were conducted during the 2009 growing season. The measurements were started after 45 days (30 May) of exposure to the treatments. Altogether, six different measurement periods were used from the end of May to the middle of September as labeled using Roman numerals I–VI (i.e., I: 30<sup>th</sup> May–15<sup>th</sup> June, II: 16<sup>th</sup> June–30<sup>th</sup> June, III: 1<sup>st</sup> July–15<sup>th</sup> July, IV: 16<sup>th</sup> July–30<sup>th</sup> July, V: 1<sup>st</sup> August–15<sup>th</sup> August, VI: 16<sup>th</sup> August–15<sup>th</sup> September). The periods are roughly following the RCG development stages (from “before flag leaf emerged” to “seed ripened and stem turning yellow”) identified earlier by Sahramaa and Jauhiainen (2003). Secondly, the gas exchange measurements for the chlorophyll (Chl) fluorescence of leaves representing upper and lower canopy (young leaves in upper canopy and old leaves in lower canopy) were done in measurement period V, 2009. Thirdly, the gas exchange measurements for mechanism of CO<sub>2</sub> processes of RCG were done in 15<sup>th</sup>–25<sup>th</sup> June (GP-I, growth period I, before florescence) and 5<sup>th</sup>–15<sup>th</sup> August (GP-II, growth period II, after completed florescence) of 2010.

All the leaf gas exchange measurements were conducted with a 2 cm×3 cm standard leaf cuvette in a portable steady-state photosynthesis system (Li-6400, *Li-cor* Inc., Nebraska, USA), on the intact, second fully expanded top layer leaves. The leaves undergoing photosynthesis measurements were also used to determine the Chl fluorescence, using an integrated 2 cm<sup>2</sup> leaf cuvette fluorometer (Li-6400-40, *Li-cor* Inc., Nebraska, USA). The CO<sub>2</sub> source of the measurements was a computer-controlled CO<sub>2</sub> mixing system supplied with the Li-6400. All the gas exchange measurements were conducted between early and mid-morning (8:00–11:00 h) to avoid afternoon stomatal closure. The leaf area was measured using a leaf area meter (Li-3100, *Li-cor* Inc., Nebraska, USA) for four plants in each climate chamber and water regime ( i.e. having four replicates).

### 2.3.2 Measurements for seasonal variation of leaf level photosynthesis

The response of net photosynthetic rates ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in relation to photosynthetic photon flux densities ( $PPFD$ ) were measured from 1500 to 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (including 11 points) at  $20\pm 1^\circ\text{C}$ . The stomatal conductance ( $g_{\text{sat}}$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) and transpiration rate ( $E_t$ ) were recorded under saturating  $PPFD$  ( $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The CO<sub>2</sub> concentration was kept at  $370\pm 1 \mu\text{mol mol}^{-1}$  in ET and CON and  $700\pm 2 \mu\text{mol mol}^{-1}$  in ETC and EC. Leaves were equilibrated at saturating  $PPFD$  before initiation of the light response. Sufficient time was allowed for photosynthesis to stabilize under the new  $PPFD$  before logging the measurements (typically requiring up to 10 min). In each chamber, four leaves (one leaf per shoot) in each container were measured for replicates in each measurement period.

### 2.3.3 Measurements for chlorophyll fluorescence and pigment content within layer position

During period V, the Chl fluorescence was measured from leaves of RCG grown under the ambient or elevated CO<sub>2</sub>, using an integrated leaf chamber fluorometer (Li-6400-40, *Li-cor* Inc., Nebraska, USA). Two groups of measurements were made, including measurements on the dark adapted leaves to estimate the maximum photochemical efficiency ( $F_v/F_m$ ) of



PSII and PPFD-response curves (50, 100, 150, 250, 350, 500, 700, 1000, 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The experimental protocol originally described by Genty et al. (1989) and revised by Maxwell and Johnson (2000) was followed. Fluorescence was excited with a modulated red radiation of ca. 2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by setting a pulse-width of 3  $\mu\text{s}$  and a frequency of 20 kHz. A saturating radiation pulse (0.8 s) of ca. 8000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was provided. The minimum chlorophyll fluorescence of the open PSII centre ( $F_0$ ) and the maximal chlorophyll fluorescence of the closed PSII centre ( $F_m$ ) were measured after 30 min of dark-adaptation. Subsequently the leaves were continuously irradiated. The fluorescence at the steady state ( $F_s$ ) was thereafter recorded and a second saturating pulse at ca. 8000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was imposed to determine the maximal fluorescence of light-adapted state ( $F_m'$ ). The minimum fluorescence of light-adapted state ( $F_0'$ ) was determined in the presence of far-red ( $\lambda = 740$  nm) light after switching off the actinic PPFD. In each chamber, four leaves (one leaf per shoot) in each container were measured for replicates in each measurement period.

After the fluorescence measurements, the measured leaves were sampled for the pigment analysis (additional parallel leaves were also collected for sample supplies). The dark-adapted dry leaves were extracted immediately after dried (70°C) for 48 h, with acetone (80%) in a pestle with quartz. As presented by Mathura et al. (2006), the dried samples could be used for pigment analysis, as the dried leaves that were stored for seven days resulted in the least amount of chlorophyll degradation followed by 28 days ice storage, seven day ice storage and lastly 28 days dried storage. The absorbance was determined with a recording spectrophotometer (*U3200, Hitachi, Ibaraki, Japan*). The concentrations of Chl *a*, Chl *b*, and total carotenoids (*Cars*) were calculated per dry mass using the equations and absorption coefficients according to Lichtenthaler (1987).

### 2.3.4 Measurements for estimating photosynthetic parameters

For the photosynthetic parameters, two sets of  $P_n$  measurements were done, including the responses of photosynthesis to the  $\text{CO}_2$  concentration in the intercellular spaces ( $P_n-C_i$ ) and photosynthetic photon flux density ( $P_n-PPFD$ ). Firstly, The  $P_n-C_i$  curves were produced under saturating *PPFD* (1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). After inducing steady-state photosynthesis, the photosynthetic response to varying intercellular  $\text{CO}_2$  concentration ( $C_i$ ) was measured. The  $\text{CO}_2$  concentration in the cuvette ( $C_a$ ) was lowered stepwise from 370 to 20  $\mu\text{mol mol}^{-1}$  (including 6–7 points) and then returned to 370  $\mu\text{mol mol}^{-1}$  to re-establish the initial steady state value of photosynthesis. Thereafter  $C_a$  was increased steadily from 370 to 1400  $\mu\text{mol mol}^{-1}$  (including 5–6 points). Gas exchange measurements were determined as soon as the inlet air  $\text{CO}_2$  concentration was stable, but not necessarily at a steady state (Long and Bernacchi 2003). Secondly, the  $P_n-PPFD$  curves were produced under 1400  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentration by the stepwise reduction of the value of *PPFD* from 1500 to 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (including 10–11 points). Sufficient time was allowed for photosynthesis to stabilize under the new *PPFD* and the concentration of  $\text{CO}_2$  before logging the measurements (typically requiring up to 10 min). All these measurements were done at 5°C intervals from 5 to 30 °C for leaf temperature. In each chamber, four leaves (one leaf per shoot) in each container were measured for replicates in each measurement period.

### 2.3.5 Method for analyzing gas exchange

#### $P_{max}$

The maximum rate of photosynthesis ( $P_{max}$ ) at saturating photon flux density is calculated in this work with the average light response curve for the RCG plants grown under the ambient or elevated  $CO_2$  by fitting a non-rectangular hyperbola to the data by means of a nonlinear least squares curve-fitting program (Marshall and Biscoe 1980):

$$P_n = \frac{\alpha PPF D + P_{max} - \sqrt{(\alpha PPF D + P_{max})^2 - 4\theta\alpha PPF D P_{max}}}{2\theta} - R_d \quad (1)$$

where  $\alpha$  is the apparent quantum yield ( $\mu\text{mol mol}^{-1}$ , the initial slope of the light response curve),  $P_{max}$  is the maximum rate of photosynthesis at saturating  $PPFD$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $\theta$  is a dimensionless parameter with  $0 < \theta < 1$  (Thornley and Johnson 1990). The light compensation point ( $L_c$ ) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was calculated as the  $PPFD$  at which the net photosynthetic rate equaled zero for each curve from the non-rectangular hyperbola model. Instantaneous water use efficiency ( $WUE$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$ ) was calculated for each plant at each measurement time by dividing the rate of  $P_n$  by  $E_t$  at saturating  $PPFD$ .

#### Fluorescence parameters

Following parameters were estimated:

- (i) the maximum (dark-adapted) PSII photochemical efficiency [ $F_v/F_m = (F_m - F_0)/F_m$ ];
- (ii) the effective (light-adapted) photochemical efficiency [ $\Phi_{PSII} = \Delta F/F_m' = (F_m' - F_s)/F_m'$ ];
- (iii) the photochemical quenching [ $q_P = (F_m' - F_s)/(F_m' - F_0)$ ];
- (iv) the non-photochemical quenching [ $NPQ = (F_m - F_m')/F_m'$ ]; and
- (v) the apparent linear electron transport rate through PSII [ $ETR = \Phi_{PSII} \times 0.5 \times 0.84 \times PPF D$ ].

#### $V_{cmax}$ and $J_{max}$

The seasonal acclimation of carboxylation efficiency and electron transport capacity were studied utilizing the Farquhar's model (Farquhar et al. 1980, Farquhar and von Caemmerer 1982), according to which the rate of net photosynthesis ( $P_n$ ) is the minimum of two factors, the Rubisco-limited rate of photosynthesis ( $P_c$ ) and the RuBP-regeneration-limited rate of photosynthesis ( $P_j$ ), considering the regulation by mesophyll conductance:

$$P_n = \min(P_c, P_j) \quad (2)$$

$$P_c = C_i - P_n / g_m \quad (3)$$

where  $g_m$  is the mesophyll conductance (the conductance for  $CO_2$  diffusion from the intercellular space to the chloroplast stroma),  $C_i$  is the intercellular  $CO_2$  concentration and  $C_c$  is the  $CO_2$  concentration in the chloroplast, the values of which were calculated based on Sharkey et al. (2007) from  $P_n$ - $C_i$  curve. According to Alonso et al. (2009), a constant  $g_m$  for

the entire range of  $C_i$  is assumed. This is because the use of a single  $g_m$  value has negligible effects on the parameter estimation excluding the case when  $g_m$  is largely reduced.

The Rubisco-limited photosynthesis is given by:

$$P_c = (1 - \Gamma^* / C_c) \frac{V_{cmax} C_c}{C_c + K_c (1 + O / K_o)} - R_d \quad (4)$$

where  $V_{cmax}$  is the maximum rate of carboxylation,  $\Gamma^*$  is the  $CO_2$  compensation point in the absence of dark respiration,  $K_c$  and  $K_o$  are the Michaelis constants of Rubisco for  $CO_2$  and  $O_2$ , respectively, and  $O$  is the oxygen concentration.  $\Gamma^*$  is a function of the  $CO_2/O_2$  specificity ( $K_o V_c / K_c V_o$ ) and the maximum rate of oxygenation by Rubisco ( $V_o$ ) is taken as  $0.21 V_c$  (Farquhar et al. 1980).  $R_d$  is the mitochondrial respiration in light. Consequently:

$$P_c = V_{cmax} \frac{K_o C_c - 0.105 K_c O}{K_c O + K_c K_o + K_o C_c} - R_d \quad (5)$$

Similar to the function (4), the RuBP-limited photosynthesis rate is:

$$P_j = J \frac{K_o C_c - 0.105 K_c O}{4.5 K_o C_c + 1.1025 K_c O} - R_d \quad (6)$$

where  $J$  is the rate of electron transport.  $J$  is related to the absorbed irradiance,  $I$ , by:

$$J = \frac{\alpha I + J_{max} - \sqrt{(\alpha I + J_{max})^2 - 4\theta\alpha J_{max}}}{2\theta} \quad (7)$$

where  $\theta$  is the curvature of the light response curve (taken to be 0.88) and  $J_{max}$  is the maximum rate of electron transport, and  $\alpha$  is the initial slope of the light response curve.

Based on Eqs. 5–6, the parameter values of  $V_{cmax}$  and  $J_{max}$  were estimated from the  $P_n-C_c$  and  $P_n-PPFD$  curves using a non-linear regression with SPSS (Chicago, IL) software package (Version 16.0). The temperature dependencies of  $K_c$  and  $K_o$  used in this study were the same given by Bernacchi et al. (2001; 2002).

The optimal temperature ( $T_{opt}$ ) for the photosynthesis was calculated as (Medlyn et al. 2002a):

$$T_{opt} = \frac{\Delta H_d}{\Delta S - R \ln[\Delta H_a / (\Delta H_d - \Delta H_a)]} \quad (8)$$

where  $\Delta H_a$  is the activation energy for  $CO_2$  and light-saturated assimilation,  $\Delta H_d$  is the energy of deactivation,  $\Delta S$  is the entropy of the desaturation equilibrium of  $CO_2$  and light-saturated assimilation, and  $R$  is the molar gas constant.

## 2.4 Growth and carbon storage measurements (V)

The RCG plants were harvested down to the soil surface immediately after photosynthetic measurements were finished using a steel ring with an area of  $154 \text{ cm}^2$  (14 cm diameter) for

identifying the harvest area. The shoot number in each sample plot was recorded. The plant material was separated into leaves and stems. The leaf area (*LA*) of the fully expanded leaves was determined by using a leaf area meter (Li-3100, *Li-cor* Inc., Nebraska, USA). Harvested plant parts were dried in a forced-air oven at 70°C for at least 72 h to determine dry mass (DM, g). Dried plant samples were analyzed for their carbon (% of DM) and nitrogen content (% of DM), the latter one using an Elemental VarioMicroCube CHNS instrument (*Elementar Analysensysteme GmbH*, Germany).

The carbon storage in leaves ( $C_l$ ) and stems ( $C_s$ ) were calculated based on the dry mass. The  $C_l$  (g C shoot<sup>-1</sup>),  $C_s$  (g C shoot<sup>-1</sup>) and *LA* (cm<sup>2</sup> shoot<sup>-1</sup>) were calculated as an average at the shoot level. The specific leaf weight (*SLW*, g cm<sup>-2</sup>) was determined as dried leaf mass divided by leaf area (Gardner et al. 1988). The leaf area-based nitrogen concentration ( $N_L$ , g cm<sup>-2</sup>) was expressed as the nitrogen content multiplied by the *SLW*. As the seed production of RCG is slightly unreliable because of seed shattering and occasionally poor panicle production, the seed biomass was not taken into account (see e.g. Lewandowski 2003). The carbon contents of leaf and stem were, on average, 44 ± 0.19% and 46 ± 0.81%, respectively, regardless of growth period and climatic treatment and water regime. Therefore, constant mean values were also used as carbon conversion factors for biomass. The total biomass carbon content was calculated by the sum of leaf and stem carbon content.

## 2.5 Statistical data analyses

Statistical data analyses were carried out using the SPSS software package (Version 16.0, Chicago, IL). Mean values of photosynthetic parameters and above-ground biomass of RCG plants with standard errors (SE) were calculated separately for each treatment and measurement period. They were also tested for the effects of elevated CO<sub>2</sub>, elevated temperature and water regimes alone and in interaction using ANOVA. The differences between each climate treatments and water regimes were further analyzed based on Tukey's test ( $p < 0.05$ ).

## 3 RESULTS

### 3.1 Stability and accuracy of auto-controlled environment chamber system (I)

The new auto-controlled environment chamber system provided a wide variety of climatic conditions for air temperature, relative humidity and CO<sub>2</sub> concentration in CON compared to outside conditions (Paper I, Fig. 3). The stable airflow condition inside the chamber provided a homogeneous distribution of gases and temperature. The target increase in temperature (+3.5°C) was achieved well in ET and ETC, being on average 3.3°C and 3.7°C higher than in CON and EC, respectively (Paper I, Fig. 4). The decrease in relative humidity and increase in vapor pressure deficit in the temperature treatment chambers were also small compared to ambient temperature chambers. The target concentration of CO<sub>2</sub> (700 μmol mol<sup>-1</sup>) was also well achieved in the EC and ETC chambers, on average 703.9 and 703.2 μmol mol<sup>-1</sup>, respectively (Paper I, Fig. 5). The chamber effects were observed regarding the physiological responses and growth of RCG plants, with some parameters

being significantly lower in CON at the end of the growing season, compared to the outside conditions. The growth indicators were also negatively affected by the reduced radiation inside the chambers.

### 3.2 Physiological responses of RCG to temperature, CO<sub>2</sub> and water regimes (II, III, IV)

#### 3.2.1 Gas exchange

During the early stage of growing season (periods I–III), the  $P_{\max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was, on average, 12% higher under elevated temperature (ET and ETC) than under ambient temperature (CON and EC) chambers regardless of water regime (Paper II, Fig. 1). After period III, the  $P_{\max}$  began to decline to a lower level under the elevated temperature compared to the ambient temperature. In the elevated CO<sub>2</sub> chambers (EC and ETC), the  $P_{\max}$  was, on average, 33% higher, during the measurement period, than in the chambers with the ambient CO<sub>2</sub> (CON and ET) across the water regimes (Paper II, Fig. 1). Regardless of stage of growing season and climate treatment, the  $P_{\max}$  in HW and NW were significantly higher than in LW (Paper II, Fig. 1) (Table 1).

The  $g_{\text{sat}}$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ) was clearly reduced by the elevation of CO<sub>2</sub> (Paper IV, Fig. 5). However, it was not significantly affected by elevation of temperature during the measurement period. The *WUE* was significantly stimulated by the elevated CO<sub>2</sub> (Table 1) in contrast to the elevated temperature regardless of the water regime and stage of growing season (Paper IV, Fig. 6). Irrespective of stage of growing season and climate treatment, the  $g_{\text{sat}}$  of RCG in HW and NW were significantly higher (on average 23.5% higher) than in LW (Paper IV, Table 2). The *WUE* ( $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$ ) was also affected by the water regimes (Table 1); i.e. it was, on average, 18.7% lower in HW compared to that in LW, regardless of climate treatment.

#### 3.2.2 Pigments and chlorophyll fluorescence within canopy positions

In the period after anthesis (period V), the Chl *a* ( $\text{mg g}^{-1}$ ) content and the ratio of Chl *a/b* were higher in the upper canopy leaves (young leaves) than in the lower canopy leaves (old leaves), which was opposite to the ratio of Cars/Chl *a+b*. This was found regardless of climate treatment and water regime (Paper III, Table 2). Irrespective of water regime and leaf location, the elevated temperature significantly decreased the Chl *a* and Chl *a/b*, opposite to the Cars/Chl *a+b*, compared to the ambient temperature (Table 1). Regardless of water regime, a slightly lower content of Chl *a* was observed in EC and ETC compared to the ambient CO<sub>2</sub> chambers in the young leaves, while the discrepancy was significant in the old leaves (Paper III, Table 2). Regardless of climate treatment, LW produced lower values for Chl *a* and Chl *a/b* and higher ones for Cars/Chl *a+b*, compared to NW and HW (Table 1).

The elevated temperature led to a significantly lower  $\Phi_{\text{PSII}}$  at lower *PPFD* and its earlier decline (around *PPFD* of 500–600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the young leaves and 250–350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the old leaves) in ET compared to CON and in ETC compared to EC, regardless of water regime and leaf location (Paper III, Fig. 2). In EC chambers, the  $\Phi_{\text{PSII}}$  across *PPFD* were slightly lower compared to those for CON, regardless of water regime and leaf location. When *PPFD* was higher than 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , the  $\Phi_{\text{PSII}}$  in the young leaves were

significantly lower in the RCG plants grown in LW compared to NW and HW, regardless of climate treatment (Paper III, Fig. 2). However, the difference was marginal in the old leaves.

In the young leaves, the  $q_p$  were higher than those in the old leaves, regardless of climate treatment and water regime (Paper III, Fig. 4). On the other hand, the ET, EC and ETC did output lower values of  $q_p$ , compared to CON, regardless of water regime. The  $NPQ$  in the young leaves was slightly higher under elevated  $CO_2$  (i.e. in EC compared to CON, and in ETC compared to ET), while this effect was not found in the old leaves. Low soil moisture led to lower values for the  $q_p$  and higher values for the  $NPQ$ , compared to well-watered soil conditions (Paper III, Fig. 4).

### 3.2.3 Temperature dependency of photosynthetic parameters

Based on  $P_n-C_c$  and  $P_n-PPFD$  curves, the curvilinear response estimations of  $V_{cmax}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $J_{max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in relation to the measurement temperature gradient are shown in Fig. 2 in Paper IV. The temperature dependent of  $V_{cmax}$  and  $J_{max}$  were significantly higher during GP-I (before florescence) compared to GP-II (after completed florescence) (Paper IV, Fig. 2) (Table 1), regardless of climate treatment and water regime. At GP-I, the  $V_{cmax}$  and  $J_{max}$  in plants growing under the elevated temperature were 10.7% and 5.6% lower at 5–15°C compared to those in plants grown under the ambient temperature, regardless of water regime. When the measurements were done at 20–30°C, the situation was the opposite with the higher  $V_{cmax}$  and  $J_{max}$  by 5.6 and 15.8%, respectively. At GP-II, the  $V_{cmax}$  and  $J_{max}$  in the plants grown under the elevated temperature were consistently lower across the measurement temperatures compared to under the ambient temperature regardless of water regime. On average, the elevation of  $CO_2$  slightly modified the temperature response of  $V_{cmax}$  and  $J_{max}$ , i.e., it was 9.3 and 5.0% lower compared to under the ambient  $CO_2$  across the measurement temperatures, regardless of water regime (Paper IV, Fig. 2).

The effect of water regimes on  $V_{cmax}$  and  $J_{max}$  was significant regardless of climate treatment (Paper IV, Table 1) (Table 1). The  $V_{cmax}$  and  $J_{max}$  were, on average, 36.4 and 30.6% lower, respectively, in LW compared to those in HW and NW both at GP-I and GP-II, regardless of climate treatment. Furthermore, the temperature response curves of  $V_{cmax}$  and  $J_{max}$  in LW were flatter compared to those in HW and NW (Paper IV, Fig. 2). No significant interactions were found between climatic factors (elevation of temperature and  $CO_2$ ) and water regimes regarding  $V_{cmax}$  and  $J_{max}$ , respectively (Paper IV, Table 1) (Table 1).

There was found a significant linear correlation between the  $V_{cmax}$  and  $J_{max}$  regardless of climate treatment and water regime over both GP-I and GP-II (Paper IV, Table 2). The  $V_{cmax}$  and  $J_{max}$  at 25°C declined along with the decrease in leaf nitrogen content at both GP-I and GP-II (Paper IV, Fig. 3), regardless of climate treatment and water regime.

The modeled  $P_{max}$  based on the use of the estimated photosynthetic parameters was also compared against a set of measurements of  $P_{max}$  not used in the parameter estimation. This was done in order to study the performance of the photosynthetic model with the parameter values acclimated to the climatic treatment. As a result, it was found that the modeled values of  $P_{max}$  generally coincided well with the measured values of  $P_{max}$  (Paper IV, Fig. 4).

### 3.3 Growth responses of RCG to temperature, CO<sub>2</sub> and water regimes (V)

#### 3.3.1 Leaf characteristics

The elevation in temperature had no effect on the  $LA$  (cm<sup>2</sup> shoot<sup>-1</sup>) (Table 1) unlike the elevation of CO<sub>2</sub>. Regardless of the measurement periods and water regimes, the values of  $LA$  under the elevated CO<sub>2</sub> were, on average, 14.7% higher than in the ambient CO<sub>2</sub> (Table 1). Nevertheless, the values of  $LA$  in LW were, on average, 12.0 and 7.7% lower compared to those in HW and NW, respectively. Regarding  $SLW$ , the values were significantly higher under the elevated temperature than under the ambient temperature (Paper V, Table 3). On the other hand, the  $SLW$  (g cm<sup>-2</sup>) in LW was, on average, 12.7 and 8.7% higher than in HW and NW, respectively (Paper V, Table 3).

The  $N_L$  (g cm<sup>-2</sup>) declined continually from the beginning of the early measurement periods, regardless of the climate treatment and water regime (Paper V, Fig. 3). Under the elevated CO<sub>2</sub>, it was, on average, 6.0% lower than under the ambient CO<sub>2</sub>. Under the elevation of temperature the  $N_L$  was, on average, 7.9% higher compared to under the ambient temperature. No clear differences were found among water regimes (Table 1).

#### 3.3.2 Carbon storage in above-ground biomass

Regardless of the climate treatment and water regime, the  $C_1$  (g C shoot<sup>-1</sup>) showed a clear increase from period I and a peak during period IV under the ambient temperature. Similarly, the  $C_1$  peaked during period III under the elevated temperature (Paper V, Fig. 4). The elevated CO<sub>2</sub> significantly increased the  $C_1$  and  $C_s$  (g C shoot<sup>-1</sup>) consistently during most of the measurement periods (III–VI) across the water regime (Paper V, Table 3) (Table 1). Additionally, the  $C_s$  increased rapidly during the periods I–V under the ambient temperature, while under the elevated temperature, the increase leveled off during period IV (Paper V, Fig. 4). No decrease was identified in the  $C_s$  at the end of the whole measurement period regardless of climatic treatment and water regime. The  $C_1$  and  $C_s$  were slightly and significantly lower in LW than in HW and NW, respectively, regardless of the measurement period and climatic treatment (Paper V, Fig. 4).

At the final harvest (period VI), the lowest total carbon storage in the above-ground biomass was found for the RCG plants grown under the elevated temperature (ET) chambers, while the total carbon storage was highest in elevated CO<sub>2</sub> across all water regimes (Paper V, Fig. 4). In EC and ETC, the carbon storage was also, on average, 11.7 and 6.5% higher than in the CON chambers, respectively. The carbon storage in the above-ground biomass in LW was, on average, 21.2 and 15.9% lower compared to HW and NW, regardless of climatic treatment. Low soil moisture and elevated temperature resulted, thus, in the lowest carbon storage in biomass (Paper V, Fig. 4).

**Table 1.** Summary table for photosynthetic and growth parameters of reed canary grass (Papers II, III, IV and V). Statistical results from ANOVA analysis of effects of elevated temperature (T), CO<sub>2</sub> enrichment (CO<sub>2</sub>) and water regimes (W) based on the measurements during the years 2009 and 2010. \*: significant effect ( $p < 0.05$ ), ns: no significant effect. The significant effect in terms of increase or decrease of parameter values due to elevated temperature compared to ambient temperature, or elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub>, and high soil moisture compared to low soil moisture were shown as ↑ and ↓ in parentheses, respectively ( $p < 0.05$ ).

| Parameters  | T     | CO <sub>2</sub> | W     | T×CO <sub>2</sub> | T×W | CO <sub>2</sub> ×W | T×CO <sub>2</sub> ×W |
|---|-------|-----------------|-------|-------------------|-----|--------------------|----------------------|
| <b>Gas exchange</b>   |       |                 |       |                   |     |                    |                      |
| $P_{\max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )   | ns    | * (↑)           | * (↑) | ns                | ns  | ns                 | *                    |
| $g_{\text{sat}}$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )  | ns    | * (↓)           | * (↑) | ns                | ns  | *                  | *                    |
| $WUE$ ( $\mu\text{mol m}^{-2} \text{s}^{-1} / \text{mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$ ) | ns    | * (↑)           | * (↓) | ns                | ns  | ns                 | ns                   |
| <b>Pigments</b>   |       |                 |       |                   |     |                    |                      |
| Chl a ( $\text{mg g}^{-1}$ )  | * (↓) | ns              | * (↑) | ns                | ns  | ns                 | ns                   |
| Chl a/b   | * (↓) | ns              | * (↑) | ns                | ns  | ns                 | ns                   |
| Cars/Chl a+b  | * (↑) | ns              | * (↓) | ns                | ns  | ns                 | ns                   |
| <b>Biochemical parameters</b>   |       |                 |       |                   |     |                    |                      |
| $V_{\text{cmax}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )                                    | ns    | ns              | * (↑) | ns                | ns  | ns                 | ns                   |
| $J_{\text{max}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )                                     | ns    | ns              | * (↑) | ns                | ns  | ns                 | ns                   |
| <b>Leaf characteristics</b>   |       |                 |       |                   |     |                    |                      |
| LA ( $\text{cm}^2 \text{shoot}^{-1}$ )  | ns    | * (↑)           | * (↑) | ns                | ns  | ns                 | ns                   |
| SLW ( $\text{g cm}^{-2}$ )  | * (↑) | * (↑)           | * (↓) | ns                | ns  | ns                 | ns                   |
| $N_L$ ( $\text{g cm}^{-2}$ )  | * (↑) | * (↓)           | ns    | ns                | ns  | ns                 | ns                   |
| <b>Carbon storage in biomass</b>  |       |                 |       |                   |     |                    |                      |
| $C_1$ ( $\text{g C shoot}^{-1}$ )   | * (↓) | * (↑)           | * (↑) | ns                | ns  | ns                 | ns                   |
| $C_s$ ( $\text{g C shoot}^{-1}$ )   | * (↓) | * (↑)           | * (↑) | ns                | ns  | ns                 | ns                   |

## 4 DISCUSSION AND CONCLUSIONS

### 4.1 Effects of temperature and CO<sub>2</sub>

Previously, it was not totally clear whether the change in photosynthetic capacity of plants with a short-life cycle under the elevated temperature is a part of an acclimation process to temperature or a part of a phenological phenomenon driven by temperature (Medlyn et al. 2002a,b). In this study, an important issue was to investigate the effects of climate treatments on the seasonal variations in the acclimation of photosynthesis and growth of reed canary grass.

The temperature effects on the development and growth of plants, such as RCG, are often described in terms of effective temperature time (Sahramaa and Jauhiainen 2003). In this work, during the early measurement periods, the elevated temperature enhanced the photosynthesis and the biomass accumulation relative to ambient temperature (Papers II &



V). However, both ones rapidly declined in the leaves and both in the upper and lower canopy towards the end of the growing season. The stimulation of carbon uptake was due to the high nitrogen content and Chl of the leaves during the early stages of growth (Papers III & V). During the later growing periods, the inhibition of photosynthesis and biomass growth might be attributed to the acceleration of senescence with lower content of the Chl *a* and leaf nitrogen. This finding was consistent with earlier results in wheat (Pérez et al. 2007).

The temperature-induced leaf senescence was layer-specific, as reflected in the increased ratio of Cars/Chl *a+b* due to the progressive loss of Chl coinciding with the partial retention of carotenoids. Under higher temperature conditions, the leaves from the lower canopy (old leaves) turned yellow and senesced earlier than those from the upper canopy (young leaves), as indicated by the higher Cars/Chl *a+b*. In old leaves, the  $\Phi_{\text{PSII}}$  declined rapidly under low *PPFD*, and the sensitivity of  $\Phi_{\text{PSII}}$  and *ETR* to high level of *PPFD* was much less than in leaves from the upper layer (young leaves). The down-regulation of PSII photochemistry observed in the senescent leaves was in line with the previous studies on wheat (Lu et al. 2003). This can be seen also as a decrease in the efficiency of excitation energy capture by open PSII center, occurring concurrently with decreases in the  $q_p$  (Paper III).

A significant seasonal variation of the photosynthetic parameters ( $V_{\text{cmax}}$  and  $J_{\text{max}}$ ) was found regardless of climate treatment (Paper IV). During the early stages of the growing period, the elevated temperature increased the  $V_{\text{cmax}}$  and  $J_{\text{max}}$ , under higher measurement temperatures. This was due to the earlier development and high nitrogen content during the early stage (Papers III & V). However, later in the growing period, the  $V_{\text{cmax}}$  and  $J_{\text{max}}$  under the elevated temperature were consistently lower across the measured temperatures may due to the early senescence.

The  $V_{\text{cmax}}$  in ET were lower at lower measurement temperatures than those in CON, which was the opposite at higher measurement temperatures. Photosynthetic performance is largely determined by affinity for  $\text{CO}_2$  (Rubisco kinetics) at low measurement temperatures and by the state of Rubisco activation under higher measurement temperatures (Yamori et al. 2006, Urban et al. 2007). Generally, there is a trade-off relationship between the  $V_{\text{cmax}}$  and affinity for  $\text{CO}_2$  (von Caemmerer and Quick 2000). Under lower measurement temperatures, the affinity for  $\text{CO}_2$  was generally larger in plants grown in ET, and therefore the  $V_{\text{cmax}}$  decreased. When measured at higher temperatures, the optimal temperature of Rubisco activation was higher in the plants grown in ET (Paper IV). Consequently, when the measurement temperature is lower than the optimum temperature, Rubisco activation may be less effective. However, during the late growing period, it was found that at the higher measurement temperatures, the Rubisco was slightly less active in ET, as has also been found in some previous studies (e.g. Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004). It has also been previously suggested that higher measurement temperatures loosen the catalytic site of Rubisco, thus facilitating the release of inhibitors (Schrader et al. 2006), with a resulting decrease in the values of photosynthetic parameters. In this work, the response of  $J_{\text{max}}$  to the elevated temperature was similar to that of  $V_{\text{cmax}}$ . Both the photosynthetically active radiation absorbed by leaves and the activity of the RuBP regeneration system have been reported that may largely limit the rate of RuBP regeneration and determine the temperature dependence of  $J_{\text{max}}$  (Hikosaka 2005, Hikosaka et al. 2006).

The stimulation of photosynthesis with higher biomass accumulation under the  $\text{CO}_2$  elevation is mostly related to the increased availability of  $\text{CO}_2$  for Rubisco and the

inhibition action for the oxygenation of Ribulose-1,5-bisphosphate (RuBP) (Drake et al. 1997). However, in the mature plants, the elevated CO<sub>2</sub> caused in this study a decline in  $N_L$  and Chl content in the leaves both in the lower and upper layers, in agreement with some previous acclimation studies (Del Pozo et al. 2007, Pérez et al. 2007).

Under the long-term elevation of CO<sub>2</sub>, “the downward acclimation” is usually observed in photosynthetic parameters, and several hypotheses have been proposed to explain this phenomenon (Long et al. 2004, Ainsworth and Rogers 2007). On average, a 10% reduction of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  in grasses and crops has been reported (Nowak et al. 2004, Rogers et al. 2006, Ainsworth and Rogers 2007, Zhang et al. 2009). Among several explanations, the re-allocation of nitrogen in the leaf has been assumed to occur under the elevated CO<sub>2</sub> (Wullschlegel et al. 2002a,b). The shift of nitrogen from Rubisco and towards RuBP regeneration would enable plants to reduce the Rubisco content under the elevated CO<sub>2</sub> and to optimize their investment in photosynthetic machinery (Drake et al. 1997). The current findings in this work inferred that Rubisco activity was reduced under the elevated CO<sub>2</sub> more than the capacity for RuBP regeneration (Paper IV). On the other hand, nitrogen deficit may cause a large down-regulation of photosynthetic capacity as reported by Drake et al. (1997) and Long et al. (2004). This was not found in this study due to the sufficient nitrogen supply.

According to Martínez-Carrasco et al. (2005), the elevated CO<sub>2</sub> not only decreases Rubisco activity, but also  $ETR$  and  $q_p$ . The depression of  $\Phi_{\text{PSII}}$  under the CO<sub>2</sub> enrichment increases the probability of excitation energy being dissipated by the increased  $NPQ$  in the antenna of PSII at high  $PPFD$  (Hymus et al. 2001). In previous studies, the modification of  $NPQ$  have occurred in the leaves in the upper canopy (Martínez-Carrasco et al. 2005, Pérez et al. 2007), but not in the lower canopy. This was probably due to the offset effects of ageing and shading (Paper III).

Due to the sufficient CO<sub>2</sub> supply to Rubisco and the inhibition of photorespiration, the CO<sub>2</sub> enrichment led to a significant increase in the rate of photosynthesis throughout the growing season, compared to that under the ambient CO<sub>2</sub> treatment. Finally, the carbon storage in the above-ground biomass of RCG was significantly increased by the elevated CO<sub>2</sub>. In a previous review, Poorter (1993) has reported a significant biomass increase (on average by 41%) for several C<sub>3</sub>-species groups (herbaceous and woody plants) under the CO<sub>2</sub> enrichment in the greenhouse conditions. Similarly, in a short grass prairie, the doubling of the CO<sub>2</sub> concentration has increased the carbon fixation and above-ground biomass (Morgan et al. 2001). The observed stimulation due to the elevation of CO<sub>2</sub> may also be explained by the fact that both the photosynthesis and the leaf area were significantly enhanced by the elevated CO<sub>2</sub> during the growing season (see Paper V).

The elevation of temperature and CO<sub>2</sub> in interaction affected the photosynthesis and growth of RCG plants in this study (Paper V). During the earlier measurement period, the elevated temperature increased the photosynthesis in the ETC chambers compared to in the EC chambers, which was in agreement with findings by Alonso et al. (2008, 2009). Higher temperature at the beginning of growing season will accelerate carboxylation of Rubisco with earlier development (Alonso et al., 2008, 2009). Although, under high measurement temperature, an increase in inhibitors might be due to the loose of Rubisco’s catalytic site (Schrader et al. 2006), the CO<sub>2</sub> enrichment would suppress the inhibitor release (Zhu et al. 1998a,b). During the later stages of growing season, the response of photosynthesis and the temperature response of photosynthetic parameters to the combined elevated temperature and CO<sub>2</sub> were found in this study quite different compared to those caused by the elevated temperature alone. The temperature-induced earlier senescence may offset the effect of

“CO<sub>2</sub>-fertilization”. However, the CO<sub>2</sub> enrichment slightly mitigated the temperature-induced adverse impact in the ETC chambers, relative to the impacts in the chambers with ET, representing a higher photosynthesis and biomass growth than in the ambient conditions.

## 4.2 Effects of water regime

Regarding the water availability, low soil moisture content had strong negative effects on the net carbon fixation rate, which eventually also decreased the carbon storage in the biomass of RCG plants (Papers III & V). Furthermore, the LW had strongly negative effects on the chlorophyll fluorescence, particularly in leaves with low canopy position. In some previous studies regarding herbaceous crops, photosynthesis characteristics and PS II activity have also been directly affected by water deficit. Similar results have been found based on use of eddy covariance data from a RCG cultivation field (Shurpali et al. 2009), i.e., during drought episodes in growing seasons, low soil moisture content and atmospheric stress restricted the photosynthetic activity. This also implies that RCG plants are well adapted in HW and NW.

In this study, the reduced photosynthesis and chlorophyll fluorescence were accompanied by a decline in the  $g_{\text{sat}}$  in LW in relation to stomatal closure, which corresponded well with the drought response (Flexas et al. 2006a,b). Restrained stomatal behavior helps plants to conserve water under drought conditions (Shaw et al. 2005). However, a closure of stomata reduces water loss but also decreases the entering of CO<sub>2</sub> in leaves with a consequent reduction of the concentration of CO<sub>2</sub> in the leaves (Flexas and Medrano 2002). This decreases the CO<sub>2</sub>:O<sub>2</sub> ratio and, therefore, also increases photorespiration, which decreases net photosynthesis.

In the low soil water availability, photosynthesis may also be limited by biochemical impairments such as decreasing photosynthetic enzyme activity and regeneration as reported by Bota et al. (2004) and Flexas et al. (2004a). In this study, the seasonal discrepancy of photosynthesis and biochemical parameters between water regimes was much more significant than between the climate treatments (Paper IV). The large decreases in  $V_{\text{cmax}}$  and  $J_{\text{max}}$  provided further evidence that not only diffusive conductance but also photosynthetic capacity was reduced in leaves of RCG subjected to long-term drought, which has also been found in some previous studies (e.g. Flexas et al. 2004a, 2006a,b, Hu et al. 2010). The negative effect of drought conditions on the  $V_{\text{cmax}}$  (Paper IV) is consistent with the results reported for C<sub>3</sub> herbaceous plants, i.e. in wheat (Zhou et al. 2007) and turfgrass (Hu et al. 2010). The limitation of the biochemical responses to water stress could be ascribed to a decrease in Rubisco activity. The non-activation of other enzymes in the Calvin cycle or a decrease in ATP and RuBP synthesis could explain this result. The limitation of  $J_{\text{max}}$  is among the earliest responses of plants to water stress (Flexas et al. 2004b, Zhou et al. 2007, Hu et al. 2010). The decreased  $J_{\text{max}}$  indicated down-regulation of electron transport, and it has been commonly accompanied by limited RuBP regeneration and activity of soluble enzymes of the stroma (Flexas et al. 2004b). Furthermore, plants under water stress have a higher respiration, which offsets carbon gaining (Hu et al. 2010).

The leaf nitrogen in RCG was much lower in low soil moisture than in higher one in both GP-I and GP-II, leading to a flatter “linear relationship” between photosynthetic parameters and leaf nitrogen (Paper V). Drought stress decreased the *LA* and increased the *SLW* under ambient CO<sub>2</sub> treatments. This might reflect a cooperative adjusting mechanism

on self-protection of enzyme and nitrogen allocation under drought conditions (Hu et al. 2010).

### **4.3 Interactive effects of temperature, CO<sub>2</sub> and water regimes**

In most climate change scenarios, high temperature, CO<sub>2</sub> increase and water deficit occur simultaneously. For the C<sub>3</sub> herbaceous species (Poorter 1993, Gutiérrez et al. 2009), the elevated atmospheric CO<sub>2</sub> concentrations have been found to stimulate plant photosynthesis and to increase dry matter production. As a comparison, the above ambient temperatures have not found to have significant effects on the assimilation and yield, but they have found to accelerate the leaf senescence instead. Moreover, water deficit clearly exacerbates the impacts of high temperature (Xu and Zhou 2005; 2006). In this study, the interactive effects of climatic treatments and water regimes could be observed during different measurement periods (Paper V). For instance, the effects of elevated temperature on photosynthesis, leaf growth and carbon storage of plants were profound at low soil moisture under the ambient CO<sub>2</sub> (Aranjuelo et al. 2005). Nevertheless, the effects of temperature and drought stress on the crops could be mitigated by the CO<sub>2</sub> enrichment, which increases the water use efficiency (e.g. Hamerlynck et al. 2000, Kang et al. 2002, Manderscheid and Weigel 2007, Qaderi et al. 2006, Gutiérrez et al. 2009).

### **4.4 Conclusion**

In conclusion, the climate change (elevation in temperature and CO<sub>2</sub>) will most likely affect the carbon storage in above-ground biomass of the bioenergy crop such as RCG cultivar in boreal conditions in Finland in interaction with different water availabilities. The elevated temperature may accelerate the rate of organ development and expansion and shorten the length of the growing period. As a consequence, the total carbon accumulation may be reduced. The CO<sub>2</sub> enrichment increased the carbon storage in this work, but did not in general affect RCG development as the elevated temperature did. The combination of elevated temperature and CO<sub>2</sub> did not significantly enhance the above-ground biomass and carbon storage in RCG (on average 6.5% higher compared to ambient conditions), regardless of the water regime. This indicates that the local RCG cultivars might not necessarily grow very well under the expected climate change, especially if drought episodes become more frequent, as suggested by the climate change scenarios. Although larger carbon storage under sufficient water conditions could be obtained, it might be expensive to maintain the water table level high enough for RCG cultivations. As documented by the Finnish Agrifood Research Institute (MTT), there are several successfully cultivated RCG cultivars available, representing different climatic zones or breeding lines in Finland. Therefore, different cultivars of RCG need to be tested under varying water regimes in the future. This would be crucial in order to identify those ones that could adapt to climate change, or even take advantage of the expected climate change.

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