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Responses of leaf photosynthesis, pigments and chlorophyll fluorescence within canopy position in a boreal grass (*Phalaris arundinacea* L.) to elevated temperature and CO_2 under varying water regimes

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Abstract

The effects of elevated growth temperature (ambient + 3.5°C) and CO_2 (700 μmol mol^{-1}) on leaf photosynthesis, pigments and chlorophyll fluorescence of a boreal perennial grass (*Phalaris arundinacea* L.) under different water regimes (well watered to water shortage) were investigated. Layer-specific measurements were conducted on the top (younger leaf) and low (older leaf) canopy positions of the plants after anthesis. During the early development stages, elevated temperature enhanced the maximum rate of photosynthesis (P_{max}) of the top layer leaves and the aboveground biomass, which resulted in earlier senescence and lower photosynthesis and biomass at the later periods. At the stage of plant maturity, the content of chlorophyll (Chl), leaf nitrogen (N_L), and light response of effective photochemical efficiency (Φ_{PSII}) and electron transport rate (ETR) was significantly lower under elevated temperature than ambient temperature in leaves at both layers. CO_2 enrichment enhanced the photosynthesis but led to a decline of N_L and Chl content, as well as lower fluorescence parameters of Φ_{PSII} and ETR in leaves at both layers. In addition, the down-regulation by CO_2 elevation was significant at the low canopy position. Regardless of climate treatment, the water shortage had a strongly negative effect on the photosynthesis, biomass growth, and fluorescence parameters, particularly in the leaves from the low canopy position. Elevated temperature exacerbated the impact of water shortage, while CO_2 enrichment slightly alleviated the drought-induced adverse effects on P_{max}. We suggest that the light response of Φ_{PSII} and ETR, being more sensitive to leaf-age classes, reflect the photosynthetic responses to climatic treatments and drought stress better than the fluorescence parameters under dark adaptation.

Additional key words: chlorophyll fluorescence; CO_2; layer position; photosynthesis; pigment; temperature; water deficit.

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Abbreviations: Cars – carotenoid; Chl – chlorophyll; Chl *a*(*b*) – chlorophyll *a*(*b*); CON – ambient environment; EC – elevated CO_2 concentration; ET – elevated temperature; ETC – combination of temperature elevation and CO_2 enrichment; ETR – electron transport rate; F_m – maximal chlorophyll fluorescence of dark-adapted state; F_m' – maximal chlorophyll fluorescence of light-adapted state; F_0 – minimum chlorophyll fluorescence of dark-adapted state; F_0' – minimum chlorophyll fluorescence of light-adapted state; F_s – steady state fluorescence; F_v/F_m – maximal photochemical efficiency of PSII; g_{sat} – light-saturated stomatal conductance; HW – high water level; LW – low water level; N_L – leaf nitrogen; NW – normal water level; NPQ – nonphotochemical quenching; P_{max} – maximum rate of photosynthesis; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux densities; PSII – photosystem II; q_P – photochemical quenching; R_D – dark respiration rates; RCG – Reed canary grass; Rubisco – ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose bisphosphate; α – apparent quantum yield; Φ_{PSII} – the effective photochemical efficiency.

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Introduction

Climate change is expected to influence plant photosynthesis, development and biomass growth in many regions of the world, including boreal conditions, as a result of significant elevation of temperature and atmospheric CO_2 concentration (Kellomäki *et al*. 2005). The warming climate in the boreal zone will also decrease the water availability in the soil profile and consequently affect the carbon assimilation and plant growth (Kellomäki *et al*. 2005).

Numerous studies of photosynthetic acclimation to changing environmental conditions have only focused on the top layer (young and sunlit) leaves in the canopy. However, the leaves in the low layer that is shaded within canopy, show different photosynthetic responses compared with young leaves, representing the typical ageing- and shading-acclimated manner (Osborne *et al*. 1998, Del Pozo *et al*. 2007, Pérez *et al*. 2007). The leaf-age distribution within the canopy with varying leaf nitrogen and Chl content determines the photosynthetic capacity and sink strength of the whole plant (Pérez *et al*. 2007). Moreover, one should bear in mind that the seasonality of annual or perennial herbaceous plants is highly sensitive to climate change. The change in photosynthetic capacity in regard to the elevated temperature may represent an acclimation of photosynthesis to temperature combined with the changes in phenological events (Berry and Björkman 1980). Yamasaki *et al*. (2002) found that a rise in growth temperature stimulated plant development and increased the photosynthesis during the peak growing period. However, the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) has been found to be inactive under high temperatures (Crafts-Brandner and Salvucci 2000). Regarding elevation in growth CO_2, a decrease in Rubisco content was observed, coupled with lower content of Chl and leaf nitrogen when the nitrogen supply was insufficient (Martínez-Carrasco *et al*. 2005, Del Pozo *et al*. 2007).

On the other hand, water stress is a well-known limiting factor for carbon uptake and growth of plants. The water deficiency resulted in stomatal closure, decreased intercellular CO_2 content, Rubisco inactivity, accumulation of free radicals and disruption of light-harvesting complexes (Parry *et al*. 2002, Bota *et al*. 2004, Hu *et al*. 2010). Moreover, the synthesis of Chl was inhibited leading to decreases in the light-harvesting protein associated with photosystem II (PSII) (Hu *et al*. 2010). Consequently, we asked the questions with respect to whether the response of plants changes in response to combined elevation in temperature and CO_2 and combined treatments of climate and irrigation (Hamerlynck *et al*. 2000, Erice *et al*. 2006). Additionally we looked at this in the context of the variation in impacts according to leaf canopy position (Del Pozo *et al*. 2007, Pérez *et al*. 2007). Based on the literature, a united measurement on gas exchange and Chl fluorescence can provide useful information about the physiological performance of plants under environmental stress (Sayed 2003).

Reed canary grass (henceforth given as RCG, *Phalaris arundinacea* L.), a typical boreal perennial grass, is widely cultivated in Europe for bioenergy crop production with a further objective to balance the active carbon source of exploited peatlands (in Finland and Sweden). However, limited knowledge of boreal grass physiology and biochemistry would likely hinder improved carbon sequestration and biomass production under the conditions of climate change. Therefore, characterization of the physiological responses of RCG plants to the future increases in temperature and atmospheric CO_2 and changes in other predicted environmental factors (*e.g*. soil water availability) is critically needed.

In the above context, an experiment was conducted on intact blocks of field peatland with RCG plants to test the effects of temperature, atmospheric CO_2 and water regimes, over a whole vegetation period in an auto-controlled environment chamber system. The objective of this work was to investigate the seasonal variability of photosynthesis and growth responses to climatic treatments (temperature and CO_2) and water regimes, as well as the synergetic effects. The emphasis was on the possible variations of biochemical acclimation between the leaves of different canopy positions when the plant was fully mature. With this purpose, leaf photosynthesis and aboveground biomass, Chl fluorescence, content of pigments and N_L within two canopy positions were measured and estimated.

Materials and methods

Plant material and environment chambers: The sampling of RCG plants was carried out in the Linnansuo peatland (62°30'N, 30°30 E), eastern Finland, cultivated by a peat/bioenergy production company (*Vapo Oy*). The 50 cm topsoil had, on average, pH of 5.3, while the organic C and N contents were 32% and 0.9%, respectively. In January–February 2009, the frozen peat soil (no sprout emerged) with RCG plants were transplanted into high-density polyethylene containers (size of 0.8 m × 0.6 m × 0.4 m, large size selected to reduce stress on the root system). The area of the peat bulk sample was almost equal to the size of container bottom, and the gap between peat and container was tamped with small peat pieces. The peat soil was further fertilized with a per hectare surface application of 60 kg N, 14 kg P, and 45 kg K.

In April 2009, 48 containers with peat soil substrates were moved into a greenhouse with 16 auto-controlled

environmental chambers (three containers in each chamber) at the Mekrijärvi Research Station (62°47'N, 30°58'E, belonging to the University of Eastern Finland), 30 km from the Linnansuo peatland. The chambers were independent research units, each up to 16 m^2 in size. The internal chamber height is 3.5 m and the volume is 64 m^3. The four transparent walls and the top are made of glass (16 mm thickness). Each chamber was equipped with an air condition vent, a gas supply vent, a set of sensors (air temperature, relative humidity, CO_2 concentration, soil temperature and moisture), one air intake and one outlet vent with filters, two normal light lamps and a heating plate. The climate controlling and measuring in the chambers uses a 16-bit *UIO32* PLC module (16 bit A/D conversion) integrated in the *CitectSCADA* automation program (*Computec Oy*, Finland). The technical details and performance of the chamber system have been described in details by Zhou *et al*. (submitted).

Experimental treatments: The 16 chamber units were divided into 4 experiment groups under four climate treatments: (*1*) ambient temperature and CO_2 concentration (CON), (*2*) elevated temperature and ambient CO_2 concentration (ET), (*3*) elevated CO_2 concentration and ambient temperature (EC), and (*4*) elevated CO_2 concentration and temperature (ETC). During the growing season (April–September), the differences in temperature between the outside air atmosphere and the CON chambers were small. The air temperature scenario was set at +3.5°C higher in the elevated temperature chambers (ET and ETC) than in the CON chambers, with the temperatures in the ET chambers and ETC chambers being, on average, +3.3°C and +3.7°C, respectively. The target CO_2 concentration was around 370 μmol mol^{-1} in the ambient CO_2 chambers (CON and ET) and 700 μmol mol^{-1} in the elevated CO_2 chambers (EC and ETC).

In each chamber, the containers were treated with three types of irrigation, which were simulated as high water table level (HW, 100% volumetric soil water content), normal water table level (NW, ~50% volumetric soil water content), and low water table level (LW, ~30% volumetric soil water content). The moderate drought of 30% was identified considering that the wilting point of drained agriculture peatlands in Finland occurs at about 20–30% volumetric soil water content. The continuous monitoring of the soil moisture was carried out with the program-controlled soil water/heat sensors (Kellomäki *et al*. 2000).

Measurement layout: Generally, a mature RCG shoot has 6–8 leaves. The first (younger) and the fourth (elder) fully expanded leaves under the flag leaf were identified as top layer (L1) and low layer (L2) leaves within the shoot. The development stage of seed ripening after anthesis (period V, *see* below) is the stable growth characteristic (Sahramaa and Jauhiainen 2003), when the leaf photosynthesis, Chl fluorescence, pigments and N_L content were measured in the leaves located in the two layers (L1 and L2). Measurements were restricted to the hours from 08:00 h to 11:00 h on sunny and generally cloud-free days. Four RCG plants of similar morphology (height and diameter) in each container in the 16 chambers were measured for replicates.

A seasonal measurement of photosynthesis of L1 leaves and aboveground biomass of RCG plants was conducted. The measurements were started after 45 days (30 May) of exposure acclimation in the chambers. There were six measurement periods from the end of May to the middle of September: they were labeled using Roman numerals I–VI (*i.e.*, I: 30th May–15th June, II: 16th June–30th June, III: 1st July–15th July, IV: 16th July–31st July, V: 1st August–15th August, VI: 16th August–15th September), roughly following the development stages of RCG plants (from "before flag leaf emerged" to "seed ripened and stem turning yellow") identified by Sahramaa and Jauhiainen (2003).

P_N and dry matter: The response of RCG leaf P_N to photosynthetic photon flux densities (PPFD) (0, 20, 50, 100, 150, 250, 350, 500, 700, 1,000; 1,200; and 1,500 μmol m^{-2} s^{-1}) was measured in L1 and L2 layer leaves using an infrared gas analyzer built into a leaf chamber in a portable *Li-6400* infrared gas-exchange system (*Li-6400*, *Li-cor Inc.*, Lincoln, NE, USA), under growth conditions of CO_2 (370 μmol mol^{-1} or 700 μmol mol^{-1}). The CO_2 source for the measurements was a computer-controlled CO_2 mixing system supplied with *Li-6400*. The light source was provided by a red-blue LED light source (*Li-6400-02B*, *Li-cor Inc*., Lincoln, NE, USA). Leaves were equilibrated at saturating PPFD before initiation of the light-response curve. Sufficient time was allowed for the new PPFD to stabilize before logging the measurements (typically requiring 20 min or less). The rate of assimilation was based on leaf area. The leaf area was measured using a leaf area meter (*Li-3100*, *Li-cor* Inc., Lincoln, NE, USA). Dark respiration rates (R_D) of the shoots were measured after at least a 30-min dark period, achieved by covering the leaf chamber with a black polyethylene film. The light-saturated stomatal conductance (g_{sat}) was recorded at the PPFD of 1,500 μmol m^{-2} s^{-1}. During all measurements, the temperature inside the leaf chamber was kept at 20±2°C, and vapour pressure deficit was kept at about 1.0 kPa. The relative humidity of the air in the leaf chamber was set at 60%.

The shape of the average light-response curve was modeled by fitting the data to a nonrectangular hyperbola equation (Lambers *et al*. 1985) by means of a nonlinear least squares curve-fitting program (SPSS): $P_N = \{\alpha\ PPFD + P_{max} - [(\alpha\ PPFD + P_{max})^2 - 4\ \theta\ \alpha\ PPFD\ P_{max})^{0.5}]/ 2\theta - R_D\}$, where α is the apparent quantum yield (mol mol^{-1}, the initial slope of the light-response curve), P_{max} is the maximum rate of photosynthesis, θ is a dimensionless parameter. The P_{max} was identified as the indicator of photosynthesis capacity in this study.

Since the plants measured for physiological parameters needed to be destructively sampled for further pigment and N_L analysis, the adjacent RCG clusters in the same container were harvested for aboveground biomass determination. The RCG materials (leaf and stem) were harvested in each container in the 16 chambers during growing periods I–VI, using a steel ring with an area of 154 cm^2 (14 cm diameter) down to the soil surface for identification of the harvest area. The shoot number in each sample plot was recorded. The harvested plants were dried in a forced-air oven at 70°C for 48 h to determine dry mass. The biomass of the RCG plants was calculated as an average at shoot level. As the seed production of RCG is slightly unreliable because of seed shattering and occasionally poor panicle production, we did not take seed biomass into account.

Fluorescence and analysis of measurements: The leaves undergoing photosynthetic measurements were also used to determine Chl fluorescence, using an integrated leaf chamber fluorometer (*Li-6400-40*, *Li-cor Inc*., Lincoln, NE, USA) under growth conditions of CO_2. Two groups of measurements were made, including measurements on dark-adapted leaves to estimate the optimal photochemical efficiency (F_v/F_m) of PSII and PPFD-response curves (50, 100, 150, 250, 350, 500, 700, 1,000; and 1,500 mmol m^{-2} s^{-1}). The experimental protocol originally described by Genty *et al*. (1989) and revised by Maxwell and Johnson (2000) was basically followed. Fluorescence was excited with a modulated red radiation of *ca*. 2 μmol m^{-2} s^{-1} by setting a pulse-width of 3 μs and a frequency of 20 kHz. A saturating radiation pulse (0.8 s) of *ca*. 8,000 μmol m^{-2} s^{-1} was provided. Firstly, 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*. 8,000 μmol m^{-2} 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 (λ = 740 nm) light after switching off the actinic PPFD.

Based on the measurements, the following parameters were calculated: (*1*) the maximum (dark-adapted) PSII photochemical efficiency [$F_v/F_m = (F_m - F_0)/F_m$]; (*2*) the effective (light-adapted) photochemical efficiency [$\Phi_{PSII} = (F_m' - F_s)/F_m'$]; (*3*) the photochemical quenching [$q_P = (F_m' - F_s)/(F_m' - F_0')$]; (*4*) the nonphotochemical quenching [$NPQ = (F_m - F_m')/F_m'$]; and (*5*) the apparent linear electron transport rate through PSII [$ETR = \Phi_{PSII} \times 0.5 \times 0.84 \times PPFD$].

Pigment content and leaf nitrogen: After the fluorescence measurement, the measured leaves were sampled for the pigment and N_L analysis (additional parallel leaves were also collected for sample supplies). The dark-adapted dry leaves (70°C for 48 h) were extracted with acetone (80%) in a pestle with quartz. 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). The dried leaves were then ground and sieved for analyzing N_L using an *Elemental Vario Micro Cube CHNS* analyzer (*Elementar Analysensysteme GmbH*, Germany). The content of pigments and leaf nitrogen was expressed on a leaf-area basis.

Statistical analyses were carried out using the *SPSS* (Chicago, IL) software package (*version 16.0*). Mean values of the parameters of leaf photosynthesis, Chl fluorescence, pigments, N_L and biomass were tested for the various treatment effects (temperature, CO_2, and water regimes) using *Tukey*'s HSD test (p<0.05).

Results

Seasonal leaf photosynthesis and biomass growth: The seasonal variations of P_{max} in L1 leaves and the aboveground biomass are shown in Fig. 1. During the early growth stage (periods I–III), P_{max} and the biomass were, on average, 12 and 24% higher (p<0.05), respectively, under elevated temperature treatments (ET and ETC) than under ambient ones (CON and EC) regardless of water regime. While after period III, P_{max} and the rate of increment in biomass began to decline to a lower level under elevated temperature treatments than under ambient ones. At the final harvest the biomass was, on average, 8% lower in the elevated temperature chambers than in the ambient temperature chambers, regardless of water regime. In the elevated CO_2 chambers (EC and ETC), the P_{max} and the biomass were, on average over the growing period, 33 and 14% higher (p<0.05), respectively, than in the ambient CO_2 chambers (CON and ET) across the water table level. Regardless of growth period and climate treatment, the P_{max} and the biomass in HW and NW were significantly (p<0.05) higher than in LW (Fig. 1). The P_{max} in LW showed the lowest values in ET in both layer leaves.

Leaf photosynthesis, pigments and leaf nitrogen: In the period after anthesis (period V), the P_{max} was 14% lower for ET compared to CON and 24% lower for ETC compared to EC (both p<0.05). Elevation of CO_2 increased the P_{max} and decreased the R_D and g_{sat} significantly (p<0.05) regardless of water regime and leaf location (Table 1). Irrespective of climate treatment and

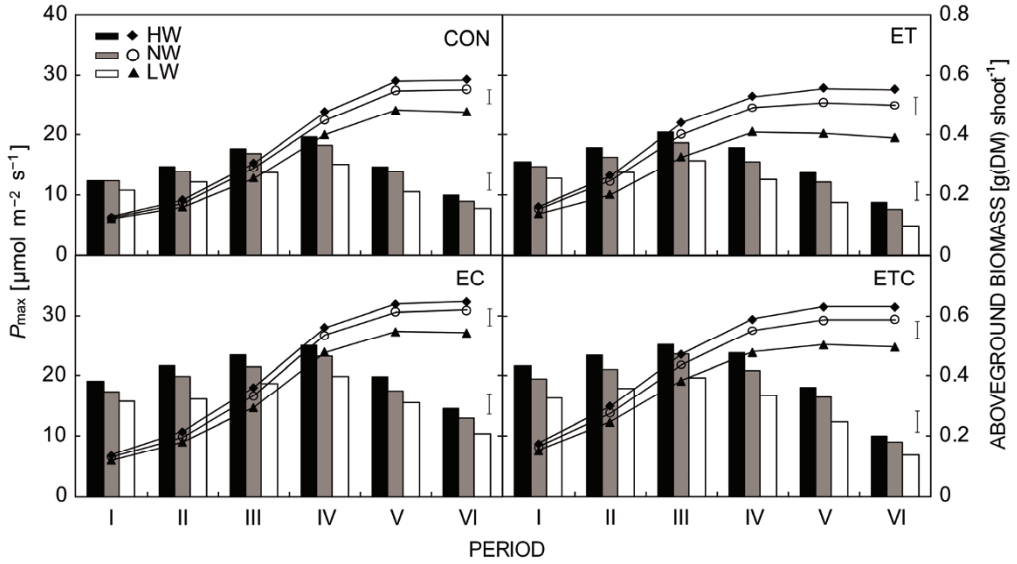

Fig. 1. Maximum photosynthetic rates (P_{max}, *histograms*) and aboveground biomass (*lines*) of top layer leaves in control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC) chambers combined with high water table level (HW), normal water table level (NW) and low water table level (LW), during 6 development stages. The values of P_{max} were fitted by the nonrectangular hyperbola equation with the data of PPFD-response curves of photosynthesis. Mean and average SE (*bars*) are presented.

water regime, the values of P_{max}, α, R_D and g_{sat} of L1 leaves was significantly (p<0.05) higher than L2 leaves. LW produced the lowest values of photosynthesis parameters in ET in leaves at both layers (Table 1).

The content of Chl *a* and N_L and ratio of Chl *a*/*b* were higher in L1 leaves than in L2 leaves, which was opposite to the ratio of Cars/Chl (*a*+*b*), regardless of climate treatment and water regime (Table 2). Irrespective of water regime and leaf location, elevated temperature significantly (p<0.05), on average, decreased the Chl *a* content by 33%, the Chl *a*/*b* by 16%, the N_L content by 27%, while significantly (p<0.05) increased the Cars/Chl (*a*+*b*) by 26%, compared with ambient temperature. Regardless of water regime, a slightly lower content of Chl *a* and N_L was observed in EC and ETC compared with the ambient CO_2 treatments in L1 leaves, while the discrepancy was significant (p<0.05) in L2 leaves (Table 2). Under each climate treatment, LW produced lower Chl *a*, N_L and Chl *a*/*b* and higher Cars/Chl (*a*+*b*), and in the case of elevated temperature treatments it led to a significant (p<0.05) difference, compared with NW and HW (Table 2).

Chl fluorescence and ETR: F_m were dependent on climate treatments concurrent with a significant (p<0.05) difference between leaf locations, with the effects being larger on the F_m than on the F_0 (Table 3). Elevated temperature slightly decreased the F_0 and F_v/F_m, and significantly (p<0.05) reduced the F_m, on average, by 22% compared with the ambient temperature treatments, irrespective of water regime and leaf location. Elevated CO_2 treatments led to a slight decrease in the value of F_0, F_m, and F_v/F_m. LW produced the lowest values of chlorophyll fluorescence parameters in ET in both layer leaves (Table 3).

The responses of Φ_{PSII} to PPFD showed a linear (in L1) and exponential (in L2) decline to a low level at low PPFD, and then maintained quite low constant values above a "threshold" PPFD (Fig. 2). The elevated temperature led to a significantly (p<0.05) lower value of initial Φ_{PSII} and an earlier decline (around 500–600 μmol m^{-2} s^{-1} in L1 leaves and 250–350 μmol m^{-2} s^{-1} in L2 leaves), when comparing ET with CON and ETC with EC, regardless of water regime and leaf location (Fig. 2). EC produced a slightly lower value of Φ_{PSII} across PPFD, compared with CON, regardless of water regime and leaf location. Comparing LW with NW and HW regardless of climate treatment, the Φ_{PSII} in L1 leaves was significantly (p<0.05) lower when PPFD was more than 400 μmol m^{-2} s^{-1} (Fig. 2), while the difference was marginal in L2 leaves due to the similar small values.

The threshold value of ETR peaked at around 500 μmol m^{-2} s^{-1} and 350 μmol m^{-2} s^{-1} in L1 and L2 leaves, respectively, regardless of climate treatment and water regime. The values of ETR were significantly (p<0.05) higher in L1 leaves than those of L2 when PPFD was more than 100 μmol m^{-2} s^{-1}, regardless of climate treatment and water regime (Fig. 3). On the contrary to CON, the values of ETR were lower in ET and EC, and lowest in ETC regardless of water regime (Fig. 3). In LW, the ETR were significantly (p<0.05) lower when PPFD was more than 250 μmol m^{-2} s^{-1}, regardless of climate treatments and leaf location (Fig. 3). The elevated CO_2 treatments slightly lessened the deviation of Φ_{PSII} and ETR in L1 leaves between LW and NW (and HW).

Table 1. PPFD-saturated photosynthetic rates (P_{max}), apparent quantum yield (α), dark respiration rates (R_D) and light-saturated stomatal conductance (g_{sat}) of top (L1) and low (L2) layer leaves in RCG after anthesis. The values of P_{max}, α and R_D were fitted by the nonrectangular hyperbola equation with the data of PPFD-response curves of photosynthesis in Fig. 2. The values (Mean±SE) were acquired on the basis of four replicates under the treatment of control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC), combined with high water table level (HW), normal water table level (NW) and low water table level (LW). Different *uppercase letters* denote significant differences among means within each column ($p<0.05$, *Tukey*'s HSD test).

Treatment	Water regime	P_{max} [μmol m^{-2} s^{-1}]		α [mol mol^{-1}]		R_D [μmol m^{-2} s^{-1}]		g_{sat} [mol m^{-2} s^{-1}]	
		L1	L2	L1	L2	L1	L2	L1	L2
CON	HW	14.44 ± 2.14^{bc}	6.29 ± 0.14^{ab}	0.05 ± 0.001^{a}	0.03 ± 0.002^{a}	0.99 ± 0.10^{a}	0.44 ± 0.10^{bc}	0.18 ± 0.02^{a}	0.11 ± 0.03^{a}
	NW	13.72 ± 1.15^{bc}	6.04 ± 0.17^{b}	0.05 ± 0.001^{a}	0.02 ± 0.001^{ab}	0.86 ± 0.04^{ab}	0.39 ± 0.12^{c}	0.16 ± 0.02^{a}	0.10 ± 0.01^{a}
	LW	10.45 ± 1.27^{c}	5.83 ± 0.13^{b}	0.05 ± 0.003^{a}	0.02 ± 0.001^{ab}	0.82 ± 0.04^{ab}	0.38 ± 0.08^{c}	0.14 ± 0.02^{ab}	0.08 ± 0.02^{ab}
ET	HW	13.50 ± 1.63^{bc}	5.33 ± 0.18^{b}	0.04 ± 0.001^{ab}	0.02 ± 0.002^{ab}	1.01 ± 0.09^{a}	0.87 ± 0.04^{a}	0.14 ± 0.01^{ab}	0.10 ± 0.01^{a}
	NW	11.95 ± 1.69^{c}	4.91 ± 0.22^{bc}	0.04 ± 0.002^{ab}	0.02 ± 0.002^{ab}	0.91 ± 0.07^{a}	0.61 ± 0.13^{b}	0.12 ± 0.02^{b}	0.08 ± 0.01^{ab}
	LW	8.62 ± 0.20^{d}	4.39 ± 0.05^{bc}	0.04 ± 0.003^{ab}	0.01 ± 0.001^{b}	0.95 ± 0.22^{a}	0.55 ± 0.13^{bc}	0.09 ± 0.01^{b}	0.05 ± 0.02^{b}
EC	HW	19.63 ± 2.25^{a}	9.41 ± 0.33^{a}	0.06 ± 0.002^{a}	0.04 ± 0.004^{a}	0.92 ± 0.14^{a}	0.39 ± 0.12^{c}	0.10 ± 0.01^{b}	0.05 ± 0.01^{b}
	NW	17.54 ± 2.26^{ab}	8.41 ± 0.22^{a}	0.06 ± 0.002^{a}	0.03 ± 0.002^{a}	0.84 ± 0.08^{ab}	0.26 ± 0.14^{cd}	0.09 ± 0.01^{b}	0.04 ± 0.01^{b}
	LW	15.24 ± 2.38^{b}	7.15 ± 0.19^{ab}	0.05 ± 0.004^{a}	0.02 ± 0.002^{ab}	0.76 ± 0.08^{b}	0.12 ± 0.03^{d}	0.05 ± 0.01^{c}	0.03 ± 0.04^{bc}
ETC	HW	15.87 ± 1.25^{b}	6.56 ± 0.32^{ab}	0.05 ± 0.003^{a}	0.03 ± 0.004^{a}	0.93 ± 0.06^{a}	0.70 ± 0.22^{b}	0.11 ± 0.01^{b}	0.08 ± 0.01^{ab}
	NW	14.23 ± 1.23^{bc}	6.16 ± 0.16^{ab}	0.05 ± 0.002^{a}	0.02 ± 0.002^{ab}	0.85 ± 0.08^{ab}	0.45 ± 0.11^{bc}	0.10 ± 0.01^{b}	0.04 ± 0.01^{b}
	LW	10.17 ± 1.15^{c}	5.61 ± 0.18^{b}	0.05 ± 0.002^{a}	0.02 ± 0.002^{ab}	0.71 ± 0.09^{b}	0.38 ± 0.11^{c}	0.07 ± 0.01^{bc}	0.03 ± 0.01^{bc}

Table 2. Pigment characters [Chl *a*, Chl *a*/*b* and Cars/Chl (*a*+*b*)] and leaf nitrogen (N_L) of top (L1) and low (L2) layer leaves in RCG after anthesis. The values (Mean±SE) were acquired on the basis of four replicates under the treatment of control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC), combined with high water table level (HW), normal water table level (NW) and low water table level (LW). Different *uppercase letters* denote significant differences among means within each column ($p<0.05$, *Tukey*'s HSD test).

Treatment	Water regime	Chl *a* [g m^{-2}]		Chl *a*/*b*		Cars/Chl (*a*+*b*)		N_L [g m^{-2}]	
		L1	L2	L1	L2	L1	L2	L1	L2
CON	HW	0.59 ± 0.02^{a}	0.41 ± 0.03^{a}	3.45 ± 0.32^{a}	3.07 ± 0.36^{a}	0.19 ± 0.04^{b}	0.31 ± 0.03^{b}	0.93 ± 0.05^{a}	0.80 ± 0.05^{a}
	NW	0.57 ± 0.04^{a}	0.36 ± 0.03^{ab}	3.17 ± 0.25^{ab}	2.87 ± 0.12^{a}	0.20 ± 0.03^{b}	0.32 ± 0.04^{b}	0.90 ± 0.08^{a}	0.71 ± 0.02^{ab}
	LW	0.37 ± 0.02^{bc}	0.23 ± 0.02^{b}	3.12 ± 0.27^{ab}	2.85 ± 0.27^{a}	0.35 ± 0.03^{a}	0.44 ± 0.05^{b}	0.55 ± 0.04^{c}	0.44 ± 0.03^{c}
ET	HW	0.45 ± 0.04^{b}	0.29 ± 0.04^{b}	2.84 ± 0.19^{b}	2.44 ± 0.17^{ab}	0.25 ± 0.05^{a}	0.37 ± 0.03^{b}	0.72 ± 0.02^{b}	0.54 ± 0.06^{bc}
	NW	0.34 ± 0.04^{bc}	0.25 ± 0.02^{b}	2.81 ± 0.23^{b}	2.36 ± 0.14^{ab}	0.28 ± 0.04^{a}	0.51 ± 0.03^{a}	0.57 ± 0.02^{c}	0.47 ± 0.05^{c}
	LW	0.23 ± 0.02^{d}	0.18 ± 0.01^{c}	2.74 ± 0.12^{b}	2.23 ± 0.18^{ab}	0.38 ± 0.05^{a}	0.63 ± 0.03^{a}	0.45 ± 0.03^{cd}	0.29 ± 0.04^{dc}
EC	HW	0.51 ± 0.04^{ab}	0.25 ± 0.04^{b}	3.50 ± 0.16^{a}	3.04 ± 0.33^{a}	0.18 ± 0.05^{b}	0.28 ± 0.05^{bc}	0.83 ± 0.03^{ab}	0.62 ± 0.02^{b}
	NW	0.50 ± 0.06^{ab}	0.24 ± 0.02^{b}	3.07 ± 0.19^{ab}	2.76 ± 0.25^{a}	0.18 ± 0.04^{b}	0.30 ± 0.05^{b}	0.82 ± 0.07^{ab}	0.60 ± 0.04^{b}
	LW	0.30 ± 0.02^{c}	0.11 ± 0.02^{d}	2.98 ± 0.34^{b}	2.58 ± 0.16^{ab}	0.29 ± 0.06^{b}	0.41 ± 0.04^{b}	0.47 ± 0.01^{cd}	0.30 ± 0.04^{dc}
ETC	HW	0.42 ± 0.03^{b}	0.19 ± 0.03^{c}	2.98 ± 0.16^{b}	2.43 ± 0.18^{ab}	0.23 ± 0.01^{ab}	0.36 ± 0.04^{b}	0.68 ± 0.05^{b}	0.48 ± 0.02^{c}
	NW	0.32 ± 0.02^{c}	0.16 ± 0.03^{c}	2.96 ± 0.24^{b}	2.29 ± 0.26^{ab}	0.26 ± 0.01^{a}	0.46 ± 0.04^{a}	0.66 ± 0.03^{b}	0.37 ± 0.03^{d}
	LW	0.21 ± 0.02^{d}	0.05 ± 0.01^{e}	2.62 ± 0.17^{b}	2.27 ± 0.12^{ab}	0.36 ± 0.05^{a}	0.59 ± 0.04^{a}	0.37 ± 0.30^{d}	0.19 ± 0.03^{e}

Table 3. Minimum chlorophyll fluorescence of dark-adapted state (F_0), maximal chlorophyll fluorescence of dark-adapted state (F_m) and maximum PSII photochemical efficiency (F_v/F_m) of top (L1) and under (L2) layer leaves in RCG after anthesis. The values (mean ± SE) were acquired on the basis of four replicates under the treatment of control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC), combined with high water table level (HW), normal water table level (NW) and low water table level (LW). Different upper case letters denote significant differences among means within each column (p<0.05, *Tukey*'s HSD test).

Treatment	Water regime	F_0		F_m		F_v/F_m	
		L1	L2	L1	L2	L1	L2
CON	HW	143 ± 2^{a}	139 ± 2^{a}	674 ± 82^{a}	639 ± 63^{a}	0.79 ± 0.03^{a}	0.78 ± 0.02^{a}
	NW	140 ± 2^{a}	128 ± 2^{a}	653 ± 76^{a}	629 ± 118^{a}	0.78 ± 0.02^{a}	0.79 ± 0.04^{a}
	LW	123 ± 6^{ab}	118 ± 7^{ab}	524 ± 29^{bc}	460 ± 26^{bc}	0.76 ± 0.01^{a}	0.74 ± 0.01^{ab}
ET	HW	132 ± 4^{ab}	123 ± 5^{a}	612 ± 69^{ab}	506 ± 13^{b}	0.78 ± 0.02^{a}	0.76 ± 0.01^{a}
	NW	127 ± 5^{ab}	123 ± 6^{a}	587 ± 58^{b}	491 ± 50^{b}	0.78 ± 0.01^{a}	0.75 ± 0.01^{a}
	LW	118 ± 2^{b}	108 ± 4^{b}	457 ± 13^{d}	314 ± 17^{d}	0.74 ± 0.01^{ab}	0.65 ± 0.01^{b}
EC	HW	140 ± 3^{a}	135 ± 5^{a}	660 ± 90^{a}	572 ± 48^{ab}	0.79 ± 0.02^{a}	0.76 ± 0.01^{a}
	NW	138 ± 4^{a}	127 ± 2^{a}	607 ± 57^{ab}	513 ± 67^{b}	0.77 ± 0.01^{a}	0.75 ± 0.03^{a}
	LW	122 ± 6^{ab}	115 ± 6^{ab}	511 ± 67^{bc}	433 ± 54^{bc}	0.76 ± 0.02^{a}	0.73 ± 0.02^{ab}
ETC	HW	130 ± 5^{ab}	125 ± 2^{a}	605 ± 59^{ab}	447 ± 35^{bc}	0.78 ± 0.01^{a}	0.73 ± 0.02^{ab}
	NW	127 ± 5^{ab}	121 ± 5^{a}	502 ± 57^{bc}	410 ± 46^{c}	0.75 ± 0.02^{ab}	0.70 ± 0.04^{ab}
	LW	116 ± 3^{b}	105 ± 8^{b}	405 ± 58^{d}	291 ± 42^{d}	0.69 ± 0.03^{ab}	0.64 ± 0.03^{b}

q_p and NPQ: The q_p decreased with increasing PPFD (Fig. 4). In comparison, at low PPFD, the values of NPQ remained fairly constant, but increased drastically at high PPFD. In L1 leaves, the values of were higher than those of L2, regardless of climate treatment and water regime. In addition, the PPFD-response decline pattern of the q_p was nearly linear in L1 leaves and exponential in L2. ET, EC, and ETC produced a lower value of q_p, compared with CON regardless of water regime. NPQ was reduced and increased by elevated temperature and elevated CO_2 treatments, respectively regardless of water regime (Fig. 4). The values of NPQ in L1 leaves were slightly higher, when comparing EC with CON and ETC with ET, while this effect was not found in L2 leaves. LW led to lower and higher values for q_p and NPQ, respectively, compared with well-watered soil conditions. In ET, the lowest values of q_p and the highest values of NPQ were observed in LW in both layer leaves (Fig. 4).

Discussion and conclusions

During the early growth periods, elevated temperature enhanced the photosynthesis and the biomass accumulation relative to ambient temperature. However, they rapidly declined in the leaves from both layers towards the end of the growing season. This was because the developmental stages of boreal RCG are triggered by the effective temperature sum (Sahramaa and Jauhiainen 2003). The stimulation of carbon uptake was due to the high nitrogen content and Chl of the leaves during the early stages of growth (Zhou *et al*. 2011). During the later stages, the inhibition of photosynthesis and biomass growth was attributed to the acceleration of senescence with lower content of Chl *a* and N_L, which was consistent with studies on wheat of Pérez *et al*. (2007). On the other hand, the reduction of photosynthesis at high temperatures was related to a decrease in Rubisco activity and in particular to the inhibition of the light-dependent activation of Rubisco by Rubisco activase (Crafts-Brandner and Salvucci 2000).

The leaf senescence was also reflected in the increased ratio of Cars/Chl (*a*+*b*) due to the progressive loss of Chl coinciding with the partial retention of carotenoids. In our case, the leaves from the L2 layer turned yellow and senesced earlier than those from the L1 layer, as indicated by higher Cars/Chl (*a*+*b*). In L2 layer leaves, the Φ_{PSII} declined sharply under low light density, and the sensitivity of Φ_{PSII} and ETR to high level of PPFD was much less than in L1 layer leaves. The down-regulation of PSII photochemistry observed in the senescent leaves was in line with the studies on wheat (Lu *et al*. 2003), as reflected in decreases in the efficiency of excitation energy capture by open PSII center, concurrent with decreases in q_p. Under heat stress, this is a key biochemical protection mechanism against photo-damage through a process of carotenoid-mediated dissipation of excess energy in the antennae complexes of PSII (Lu *et al*. 2003).

Higher temperature only significantly reduced the F_m under dark-adaption in both layer leaves, indicating an occurrence of enhanced nonradiative energy loss and inactivation and/or damage in PSII center complex (Genty *et al*. 1989). Another interpretation for the decrease in F_m is the changes in photosynthetic pigment content, *i.e*. decreases in Chl and increases in Cars/Chl (*a*+*b*). The reduced sensitivity of F_v/F_m to temperature and age classes was due to the conserved F_0 in relation to lower Chl content, indicating a low-level functioning of PSII with a low absorption rate of light in the leaves.

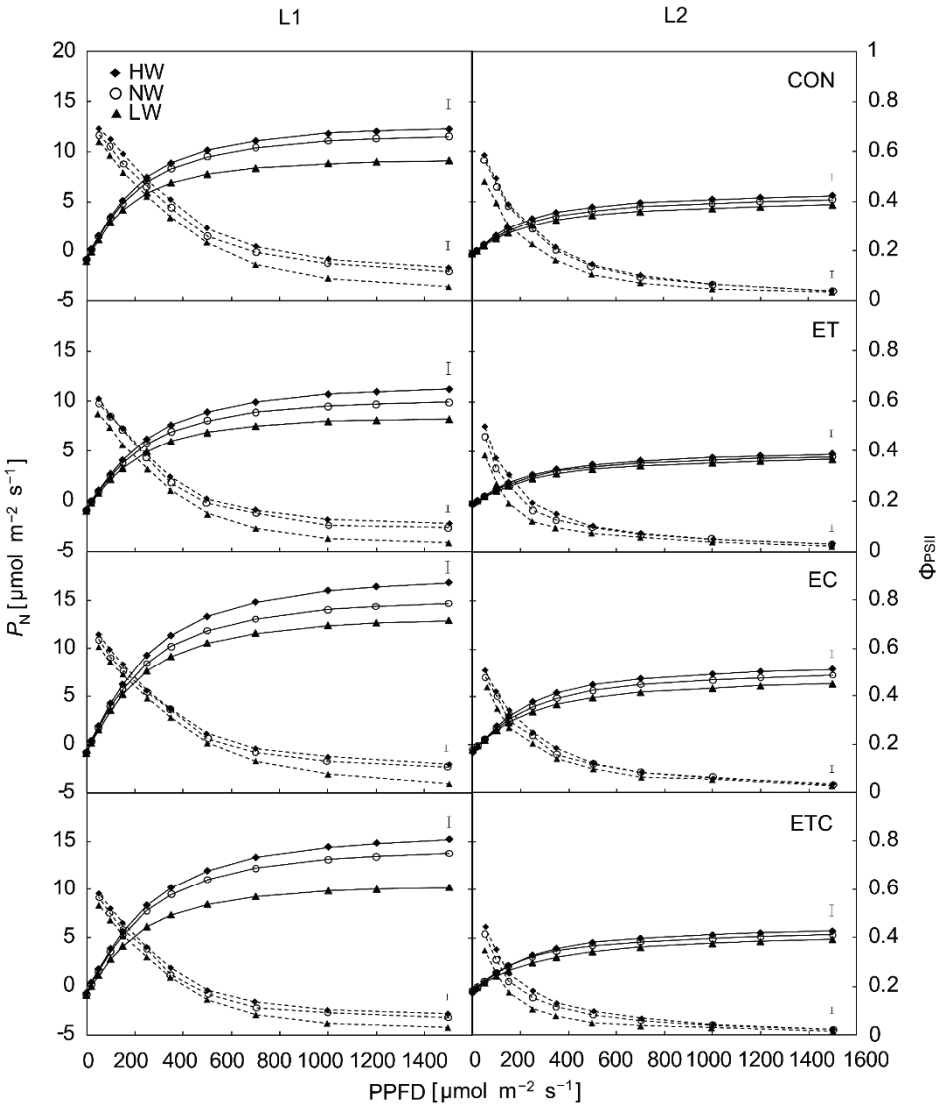

Fig. 2. PPFD-response of the net photosynthetic rates (P_N, *solid lines*) and the effective photochemical efficiency (Φ_{PSII}, *dashed lines*) in L1 and L2 leaves in control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC) chambers combined with high water table level (HW), normal water table level (NW) and low water table level (LW). The values were measured during period V (seed ripening), based on four replicates in each chamber. Mean and average SE (*bars*) are presented.

In a different manner, the stimulation of photosynthesis with higher biomass accumulation under CO_2 enrichment is mostly related to the increased availability of CO_2 for Rubisco and inhibition action for the oxygenation of ribulose-1,5-bisphosphate (RuBP) (Drake *et al*. 1997). However, at the stage of plant maturity, elevated CO_2 caused a decline in N_L and Chl content in leaves from both layers of RCG, in agreement with many acclimation studies (Del Pozo *et al*. 2007, Pérez *et al*. 2007). The content of Rubisco and Chl is strongly related to nitrogen in leaves and re-allocation of N_L under elevated CO_2 (Wullschleger *et al*. 2002, Pérez *et al*. 2005). Drake *et al*. (1997) demonstrated that the shift of nitrogen from Rubisco towards RuBP regeneration would enable plants to reduce Rubisco content at the elevated CO_2 and to optimize their investment in photosynthetic machinery. Another cause can be attributed to the increased leaf area of RCG plants under elevated CO_2 (Zhou *et al*. 2011), resulting in dilution of nitrogen according to leaf area.

As reported in the literature (Rogers *et al*. 1998, Stitt and Krapp 1999), nitrogen availability plays an important role in the maintenance of photosynthetic capacity under CO_2 enrichment. Due to the fertilized peat soil and adequate nitrogen supply for RCG cultivation, we found that the CO_2-induced decline of N_L and Chl content was less pronounced in L1 young leaves, but significant in L2 older leaves, in agreement with the results on wheat (Del Pozo *et al*. 2007, Pérez *et al*. 2007). The variations of vertical distribution of the nitrogen content might be related to the fact that plants change their nitrogen allocation in order to optimize energy costs under

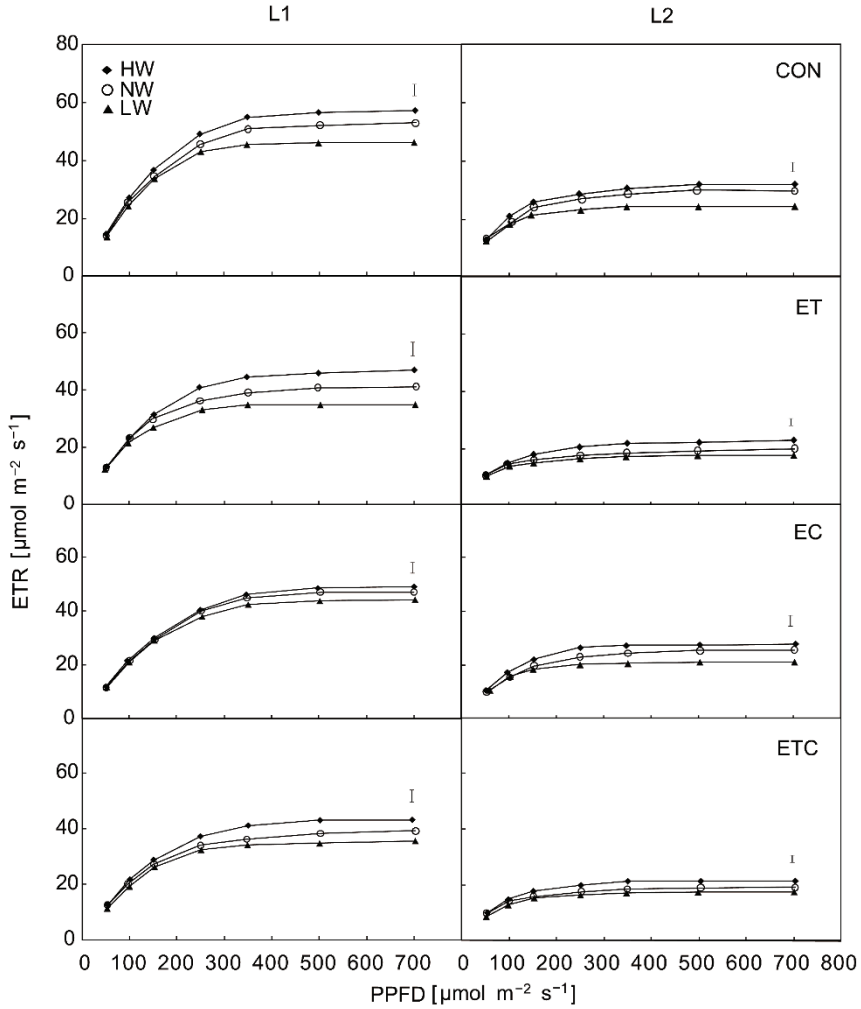

Fig. 3. PPFD-response of the electron transport rate (ETR) in L1 and L2 leaves in control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC) chambers combined with high water table level (HW), normal water table level (NW) and low water table level (LW). The values were measured during period V (seed ripening), based on four replicates in each chamber. Mean and average SE (*bars*) are presented.

elevated CO_2 (Osborne *et al*. 1998, Stitt and Krapp 1999), reflecting a strategy of nitrogen allocation to young sunlit leaves for the maintenance of photosynthesis in the later growth stage.

In line with the studies on wheat (Pérez *et al*. 2005) and rice (Kurasová *et al*. 2003), our results showed that the values of Φ_{PSII}, ETR, and q_p across the PPFD gradient were lower under elevated CO_2 in both layer leaves during the later stage, while Pérez *et al*. (2007) suggested an increase in flag leaf of wheat. The reason might be that the flag leaf generally keeps active photosynthesis and assimilation capability even during grain filling (Araus and Tapia 1987). As presented by Martínez-Carrasco *et al*. (2005), elevated CO_2 not only decreased Rubisco activity, but also ETR and q_p. The depression of Φ_{PSII} under CO_2 enrichment increased the probability of excitation energy being dissipated by increased NPQ in the antenna of PSII at high PPFD (Hymus *et al*. 2001). The modification of NPQ occurred in L1 leaves (Kurasová *et al*. 2003, Martínez-Carrasco *et al*. 2005, Pérez *et al*. 2007), but not in low canopy leaves, which was probably due to the offset effects of ageing and shading.

In ETC, the modification of photosynthetic pigments and fluorescence parameters were almost the same as those brought about by ET in leaves from both layers, due to the accelerated ageing as mentioned above. The value of P_{max} was higher in ETC than EC, but ETC resulted in a further down-regulation or decline of Φ_{PSII}, ETR and q_p, compared with ET. As reported by Martínez-Carrasco *et al*. (2005), no CO_2-temperature interaction was observed on fluorescence parameters of wheat. The diversity in the results could be related to study site. The lower ambient temperatures in our experiment site compared to other study sites may imply that an equal rise in growth ambient temperature will have greater physiological and ecological consequences for plants in the boreal zone than in the other zones.

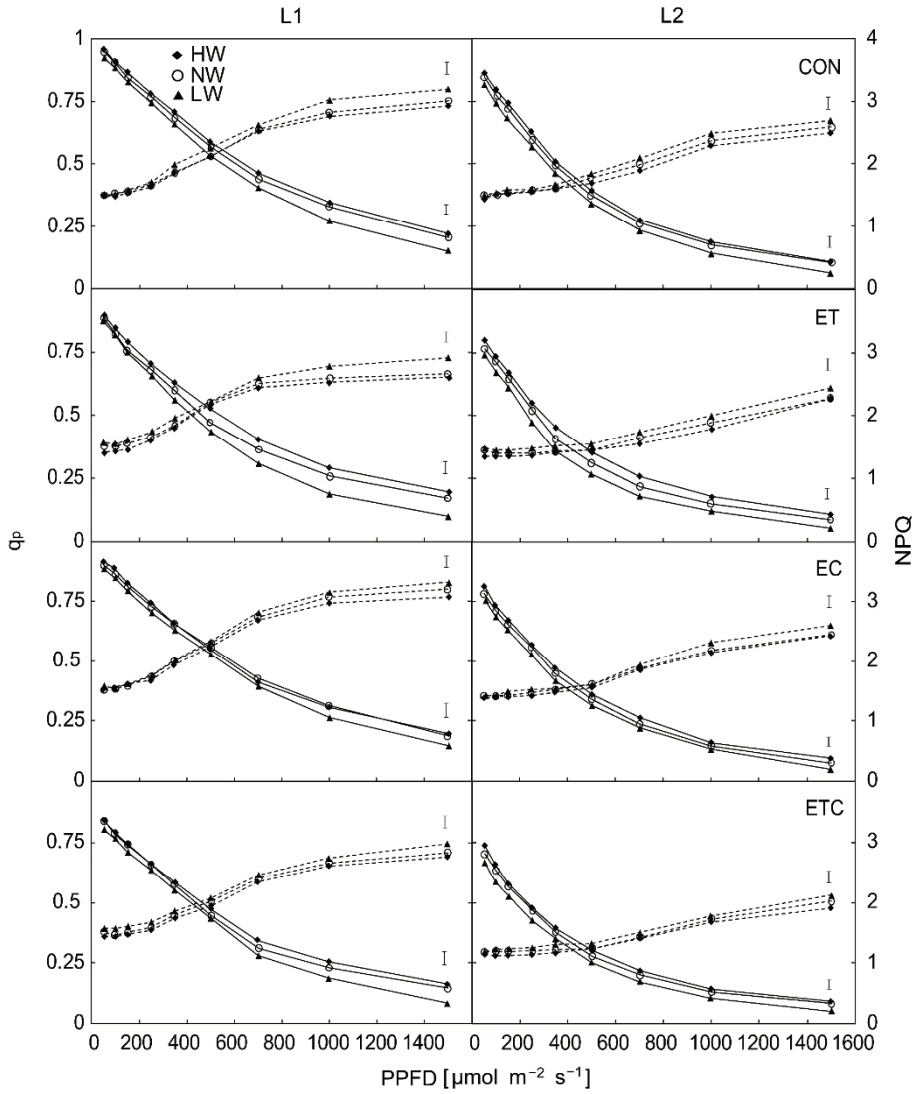

Fig. 4. PPFD-response of the photochemical quenching (q_P, *solid lines*) and the nonphotochemical quenching (NPQ, *dashed lines*) in L1 and L2 leaves in control (CON), elevated temperature (ET), elevated CO_2 (EC) and elevated temperature + CO_2 (ETC) chambers combined with high water table level (HW), normal water table level (NW) and low water table level (LW). The values were measured during period V (seed ripening), based on four replicates in each chamber. Mean and average SE (*bars*) are presented.

Regardless of climatic treatment, the parameters of photosynthesis, fluorescence, pigments and biomass growth of RCG showed strong responses to water shortage, which agreed with many studies for different plants (Sayed 2003, Bota *et al*. 2004, Flexas *et al*. 2006, Zhou *et al*. 2007, Hu *et al*. 2010). In our measurements, the photosynthetic capacity and biomass of RCG in HW was slightly and significantly higher than in NW and LW, respectively, indicating that high soil moisture is favourable to plant growth. Under water shortage, the limited stomatal conductance led to diffusion limitations, which decreased the intercellular CO_2 concentration and amounts and activities of key photosynthetic enzymes such as Rubisco (Bota *et al*. 2004, Flexas *et al*. 2006, Hu *et al*. 2010).

The content of Chl and N_L in RCG leaves was much lower in LW than in higher soil moisture in leaves from both layers, which might reflect a cooperated adjusting mechanism on self-protection of enzyme and nitrogen allocation under drought conditions (Hu *et al*. 2010). Another strategy under water stress is the protection mechanism against excess light, as higher NPQ showed. Such protection is achieved by the regulated thermal dissipation in light-harvesting complexes, somehow involving the xanthophyll cycle (Demmig-Adams and Adams 1992, Zhou *et al*. 2007). This photoprotective mechanism competes with photochemistry for the absorbed energy, leading to a decrease in quantum yield of PSII (Genty *et al*. 1989). The Φ_{PSII}, ETR, and q_p exhibited negative responses to water shortage, indicating a reduced efficiency of excitation energy capture of open PSII or a damage in PSII center (Genty *et al*. 1989).

In this study, we set up a moderate drought (~30%) in LW, which did not result in significantly lower F_v/F_m. Additionally, sufficient nitrogen supply (similar to our case) has been shown to alleviate photodamage to PSII caused by medium water stress to some extent (Wu *et al*. 2008).

As expected, higher temperature exacerbated the impact of water shortage, as demonstrated in many studies (Hamerlynck *et al*. 2000, Xu and Zhou 2006). Nevertheless, the drought-induced impacts on photosynthesis of RCG were slightly alleviated by CO_2 enrichment. This result was similar to the works of Hamerlynck *et al*. (2000), Wullschleger *et al*. (2002) and Robredo *et al*. (2007), who reported that the low stomatal conductance produced by elevated CO_2 led to lower water loss (transpiration) and higher water-use efficiency under water shortage. In our measurements, elevated CO_2 also slightly increased NPQ in the top layer leaves under drought, as reported by Kitao *et al*. (2007). However, the protection mechanism of PSII under interaction of water stress and CO_2 enrichment is not well understood, and more investigations are still required.

In summary, this study aimed at understanding of the acclimation response to long-term climate treatments and water regimes during the whole vegetation period. Higher growth temperature accelerated the plant development and enhanced the photosynthesis and biomass growth of the boreal RCG grass at the early stage. However, at the stage of plant maturity, elevated temperature resulted in earlier leaf senescence and a significantly lower photosynthetic capacity in leaves from both layers, as well as limited fluorescence kinetic properties and Chl content. CO_2 enrichment caused a down-regulation in N_L and Chl content and fluorescence parameters of Φ_{PSII}, ETR, and q_p, and the CO_2-induced declines were alleviated slightly in the leaves in the top canopy position due to the ample availability of nitrogen. CO_2 enrichment also slightly mitigated the drought-induced adverse effects on leaf photosynthesis. The PPFD response of Φ_{PSII} and ETR, being more sensitive to leaf age classes, reflect the photosynthetic responses to climatic treatments and drought stress better than the fluorescence parameters under dark-adaption.

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