Interspecific competition changes photosynthetic and oxidative stress response of barley and barnyard grass to elevated CO_2 and temperature

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This work focuses on the investigation of competition interaction between C_3 crop barley (*Hordeum vulgare* L.) and C_4 weed barnyard grass (*Echinochloa crus-galli* L.) at 2 times higher than ambient [CO_2] and +4 ^{0}C higher ambient temperature climate conditions. It was hypothesized that interspecific competition will change the response of the investigated plants to increased [CO_2] and temperature. The obtained results showed that in the current climate conditions, a higher biomass and photosynthetic rate and a lower antioxidant activity were detected for barley grown under interspecific competition effect. While in the warmed climate and under competition conditions opposite results were detected: a higher water use efficiency, a higher photosynthetic performance, a lower dissipated energy flux and a lower antioxidant enzymes activity were detected for barnyard grass plants. This study highlights that in the future climate conditions, barnyard grass will become more efficient in performance of the photosynthetic apparatus and it will suffer from lower oxidative stress caused by interspecific competition as compared to barley.

Key words: interspecific competition, climate change, photosynthesis, JIP-test, oxidative stress

Introduction

Changes in temperature, precipitation and increasing CO_2, all have potentially important consequences for crop/weed interactions, which is evident from a consideration of the basic biology of weeds and crops. The effects of climate change on crop-weed interactions are likely to vary by region and crop. These affects can be assessed by understanding the response of the physiological mechanisms to such factors. The dynamics of competition between weed and crop plants are affected by environmental conditions and have been shown to change with CO_2 enrichment (Naidu and Murthy 2014). The interactive effect of the CO_2 enrichment will affect weed-crop competition simultaneously or sequentially in a complex manner, quite differentially from its effect on the photosynthetic pathway alone. The information regarding the interactive effects of elevated CO_2 with sub-ambient temperatures in either C4 weeds or crops is scarce. Moreover, differential stimulation of C3 crops and weeds by elevated CO_2 at sub-optimal temperatures have received an increasing attention recently (Ramesh et al. 2017).

Echinochloa crus-galli is one of the most noxious weeds in modern agriculture (Heap 2014). It has all the competitive features and adaptive characteristics which are necessary for survival and successful competition under a range of geographical and climatic conditions (Marambe and Amarsinghe 2002). Originating from Europe, it has dispersed all around the globe including Asia, Australia, and America (Bajwa et al. 2015). For instance, Zhu et al. (2008), while investigating the effect of nutrient and CO_2 on weed-crop competition using a C3 crop (rice) and a C4 weed (*E. crus-galli*) model system, found a proportionate increase in rice biomass compared with *E. crus-galli* under an optimum nitrogen supply. In contrast, at a sub-optimum nitrogen level, elevated CO_2 reduced the competitive ability of rice against *E. crus-galli* (Ramesh et al. 2017). *Echinochloa crus-galli*, being a C4 weed, has strong potential for competing with C3 plants under climate change scenarios of low water availability and higher temperature (Rodenburg et al. 2011). Because of these characteristics, *E. crus-galli* becomes more competitive than agricultural crops and can cause significant yield losses in the future climate conditions (Awan and Chauhan 2016).

Carbon dioxide (CO_2) concentration has increased since pre-industrial period from 280 to 405 µmol mol^{-1} (ppm) currently (NOAA-ESRL 2017). It is expected that CO_2 concentration could reach up to 750 – 1300 ppm by the end of this century, if no mitigation measures are taken to reduce emissions (IPCC 2014). The emissions of greenhouse gases caused by human activities have augmented 70% from 1970 to 2004. If greenhouse gases emissions continue at high levels, the temperature is predicted to increase between 1.8 and 6.0 °C (Salazar-Parra et al. 2015).

Manuscript received December 2017

If these forecasts are realized, crops and cropping systems are likely to experience significant changes and it is so for the associated weeds too. Weeds are a major threat to agriculture and biodiversity as they out-compete crops and native species and contribute to land degradation. They reduce crop productivity through yield reduction. Research data strongly suggest that geographic range transformations for agricultural weeds are highly probable outcomes from global climate change (Fuhrer 2003).

Some studies have shown that low or high temperatures reduce or eliminate the high CO_2 growth enhancement (Coleman and Bazzaz 1992), whereas others have shown that CO_2 enrichment may increase the plant tolerance to temperature extremes (Baker et al. 1989). Based on the differences in temperature optimal for physiological processes, it is predicted that C4 spp. will be able to tolerate higher temperatures than C3 spp. Therefore, C4 weeds may benefit more than the C3 crops from any temperature increases that accompany elevated CO_2 levels (Naidu and Murthy 2014), since under the conditions of elevated CO_2, reduced CO_2 solubility, and decreased affinity of ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco) for CO_2 would deter C3 photosynthesis (Patterson 1995). As a result, a variation in weed distribution will affect the world's most important cropping systems. Many C4 weeds, such as *Amaranthus retroflexus* L., *Sorghum halepense* (L.) Pers., and *Paspalum dichotomiflorum* (L.) Michx., are expected to expand further north (Clements and DiTommaso 2011), which would have a more pronounced effect in the northern Europe (Fried et al. 2010, Ramesh et al. 2017).

Since plants with C3 photosynthetic metabolism are CO_2 limited, it is expected that any CO_2 increase would lead to higher photosynthetic rates (Salazar-Parra et al. 2015) and growth, because of increased photochemical efficiency (Cordoba et al. 2017). By contrast, plants with a C4 photosynthetic pathway manifest little response to elevated CO_2 as they have an internal mechanism to concentrate CO_2 at the site of CO_2 carboxylation (Ramesh et al. 2017). However, a photosynthetic down-regulation with elevated CO_2 has also been observed, and the degree of down-regulation depends on the species and the environment (Shi et al. 2016). Although the CO_2 stimulation effect occurs regardless of water conditions in C3 species, C4 plants benefit from elevated CO_2 only under a water-deficit rather than when the water supply is sufficient (van der Kooi et al. 2016). Thus, the effects of elevated CO_2 levels on crops and weeds will alter the weed-crop competitive interactions, sometimes for the benefit of the crop and sometimes for the weeds (Korres et al. 2016).

The increase in CO_2 concentrations and temperature could also induce alterations in plant metabolism. It has been-shown that heat stress may lead to accumulation of ROS (Martins et al. 2016, Noctor and Mhamdi 2017). Reactive oxygen species (ROS), in particular H_2O_2, possibly plays a role as a signaling molecule (Baxter et al. 2013). However, overproduction of ROS leads to damaging effects on lipids, proteins and nucleic acids (Gill and Tuteja 2010). To minimize free radical damage, plants induce various defense systems (Gadjev et al. 2006). CO_2 enrichment also affects stress-induced ROS levels (Geissler et al. 2009). This is possibly achieved through enhanced antioxidant concentrations, as a consequence of increased photosynthetic carbon assimilation (Pinto-Marijuan et al. 2013). On the other hand, decreases in the antioxidant defense system under elevated CO_2 have also been reported in several species, suggesting that other mechanisms than antioxidant increases contribute to this stress reducing effect (AbdElgawad et al. 2015). As it has been presented before, barnyard grass is one of the competitive plants for agricultural crops, which causes yield losses (Awan et al. 2016). However, the studies analyzing the competitiveness of this weed and barley under future climate conditions are lacking. Therefore, the aim of this investigation was to evaluate spring barley and barnyard grass growth, photosynthetic and oxidative responses to warmed climate when crop and weed species were grown in mono cultural and mix cultural conditions.

Materials and methods

Experimental design and plants growing conditions

The experiments were conducted in two closely controlled environment plant growth chambers, located at Vytautas Magnus University, with each chamber volume of 10 m^3 in 2015. Barley (*Hordeum vulgare* L. cv. 'Aura DS') and barnyard grass (*Echinochloa crus-galli* L.) plants were grown in 3-liter plastic pots filled with a mixture of field soil, perlite and fine sand (volume ratio 5:3:2) in monoculture (Mono) (15 plants per pot) and multicultural (Mix) (9 crop plants and 6 weeds) interspecific competition conditions. A nutrient supply corresponding to 90 kg ha^{-1} of nitrogen was applied during the sowing, and the additional fertilization with the complex nutrient (NPK 12-11-18 + microelements) solution increasing N level until 150 kg ha^{-1} was applied one day before the treatment.

The duration of the photoperiod in both chambers was 14 h (8:00 a.m. to 10:00 p.m.), the relative air humidity (RH) was 65±8% during the day and 85±6% at night, and a photosynthetically active radiation (PAR), provided by a combination of six natural day-light luminescent lamps (Philips, Waterproof OPK Natural Daylight LF80 Wattage 2×58 W/TL-D 58 W) and one high-pressure sodium lamp (Philips MASTER GreenPower CG T 600 W), was about 200 µmol m^{-2} s^{-1} photon flux density. The pots with plants grown under the same growing conditions were rotated every day, in order to minimize the effects of the differences in growing conditions on plant performance within the same growth chamber. The plants were watered daily.

Imposed treatment

Initially, both plants were grown in two chambers under the conditions of current climate (400 µmol mol^{-1} CO_2 and day/night temperatures of 21/14 °C). A warmed climate (800 µmol mol^{-1} CO_2 and day/night temperatures of 25/18 °C) treatment was started/imposed in one of the chambers when both plants grown in mono and multicultural interspecific competition conditions had the second true leaf (BBCH code 12) (Meier 2001), i.e. after 14 days after germination. In the second chamber, the plants were growing further under the conditions of current climate. CO_2 and temperature exposure lasted two weeks until the plants reached the stage of four to five leaves (BBCH code 14–15) (Meier 2001). The treatments were run in three replicates (3 pots per treatment). An atmospheric concentration of CO_2 in the chambers was manipulated automatically by controlling the amounts of the injected CO_2 gases that were controlled by the IGSS 9-13175 software run on a computer.

Measurements of gas exchange

One plant per pot of each pot per treatment were used for the measurements of leaf gas exchange that were performed using a portable closed infrared gas analyzer LI-COR 6400 (LI-COR, Inc., Lincoln, NE) with randomly selected youngest fully expanded leaves on the last (14^{th}) day of the treatment between 10:00 am and 15:00 pm. The photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs, mol H_2O m^{-2} s^{-1}), transpiration rate (Tn, mmol H_2O m^{-2} s^{-1}), and the intercellular CO_2 concentration (Ci, µmol CO_2 m^{-2} s^{-1}) were recorded automatically for approximately 10 min at 5 s interval. Water use efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O) was calculated according to the manufacturer's instructions as Pn divided by Tn, and the intercellular-to-ambient CO_2 concentration (Ci/Ca) was calculated as Ci divided by respective growth Ca. The CO_2 level and temperature were set depending on the individual treatment of plants. During the measurements, the CO_2 concentration in the chamber of leaves was controlled with the LI-COR CO_2 injection system. Air flow rate through the assimilation chamber was maintained at 400 µmol s^{-1}, and PAR was about 170 µmol m^{-2} s^{-1}.

Chlorophyll *a* fluorescence

All measurements of chlorophyll fluorescence were taken with the Plant Efficiency Analyser, PEA (Hansatech Instruments, Ltd., King's Lynn, Norfolk, England). The measurements were taken on the healthy top intact leaves of the plants grown under the conditions of current climate and warmed climates. For a possible comparison of fluorescence and gas exchange parameters, the fluorescence recordings were initiated approximately at the same time and at the same canopy level as gas exchange parameters were measured. The leaves were pre-darkened with clips for 15 min prior to the measurements and later chlorophyll fluorescence transients of the dark-adapted leaves were measured. The transients were induced by 5 s illumination with an array of three light emitting diodes providing a maximum light intensity of 1800 µmol (photon) m^{-2} s^{-1} and a homogenous irradiation over a 4-mm diameter leaf area. The fast fluorescence kinetics (f_0 to f_M) was recorded from 10 µs to 1 s. The fluorescence intensity at 50 µs was considered as f_0 (Rasineni et al. 2011).

Analysis of the fluorescence transients using the JIP-test

Raw fluorescence OJIP transient, which reflects the reduction in the photosynthetic electron transport chain (Lin et al. 2009), was transferred with WINPEA 32 software and BiolyzerP3 to a spreadsheet (Strasser and Strasser 1995, Rasineni et al. 2011). The translation of the measured parameters into JIP-test parameters provided the information on the stepwise flow of energy through PSII at different levels such as specific fluxes on the level of the excited leaf cross-section (CS) (absorption [ABS/CSm], trapping [TRo/CSm], dissipation [DIo/CSm] and electron transport [ETo/CSm]). Ψo, is the probability that an electron can move further than Q_A^- and Ψ_0 was calculated according to formula: $(F_P - F_J)/(F_P - F_{50\mu s})$ where F_P = Fluorescence maximum in OJIP transient, F_J and $F_{50\mu s}$ = Fluorescence yield at point J and at 50 µs.

Normalized total complementary area above the O–J–I–P transient (reflecting multiple turnover Q_A reduction events) was calculated as Sm (Sm = [Area]/[F_P – $F_{50\mu s}$]). The density of RCs (QA- reducing PSII reaction centers) was calculated as RC/CSo = $\Phi_{Po}(V_J/Mo)$ (ABS/CSo). The detailed formulae and terms used by the JIP-test for analysis of the fluorescence transient O–J–I–P are given in Table S1 (Lin et al. 2009, Rasineni et al. 2011).

Measurements of leaf area and biomass harvest

The leaf area measurements of all leaves per plant and one plant per pot of each pot per treatment were carried out using a scanner (CanoScan 4400F, Canon, USA) on the last 14th day of the treatment and then determined by GIMP 2.8 software. Above-ground biomass was also harvested after 14 days of exposure. Some randomly selected leaves of each plant per pot per treatment were grounded with liquid nitrogen and stored at –80 °C for the lipid peroxidation and enzyme analysis.

Lipid peroxidation

Lipid peroxidation was determined measuring malondialdehyde (MDA) as an end product of lipid peroxidation using the thiobarbituric acid. Tris-HCl buffer with 1.5% (w/v) of PVPP (pH 7.4) was used for MDA extraction. The supernatant was mixed (volume ratio 1:1) with 0.5% thiobarbituric acid, diluted in 20% trichloroacetic acid (w/v). The mixture was heated at 95 °C for 30 min. After centrifugation, the absorbance was measured at 532 nm and corrected for unspecific turbidity by subtracting the value of absorbance at 600 nm (Heath and Packer 1968, Wu et al. 2003).

Activity of antioxidant enzymes

Potassium phosphate buffer (pH 7.8, 0.1 M), containing 2 mM dithiothreitol, 0.1 mM EDTA, 0.5% of PEG 4000 and 1% of PVPP was used for proteins and enzymes extraction. The extracts were centrifuged at 14,000 x g for 15 minutes at 4 °C. The supernatant was filtered through Sephadex G-25 PD10 columns. The soluble protein concentration in the supernatant was determined by the dye-binding method using bovine serum albumin as standard (Bradford 1976). For the estimation of superoxide dismutase (SOD) activity, the reaction mixture, containing potassium phosphate buffer (pH 7.8, 0.1 M), protein extract, 13 µM riboflavin, 13 mM methionine and 63 µM NBT, was incubated for 5 min at 25°C. One unit of SOD was defined as the enzyme activity that inhibited photoreduction of NBT by 50% (Giannopolitis and Ries 1977, Bailly et al. 1996). Catalase (CAT) activity was estimated by measuring the consumption of H_2O_2 at 240 nm. The reaction mixture contained potassium phosphate buffer (pH 7, 50 mM), protein extract and 3.125 mM H_2O_2. The rate of reduction in light absorbance was measured for 30 seconds (Clairbone 1985, Bailly et al. 1996). Glutathione reductase (GR) activity was determined by measuring the decrease in the absorbance (340 nm) during NADPH oxidation and expressed as nmol NAPH oxidized mg protein^{-1} min^{-1} (Bailly et al. 1996).

Statistical analysis

The data were first tested using a three-way ANOVA (three factors): 1) P – plant: barley and barnyard grass; 2) In. comp. – interspecific competition, plants grown in monoculture and mix culture; 3) C – climate, current and warmed, in order to determine the effects of the treatments and their possible interactions. The Least Significant Differences (LSD) test procedure was applied to estimate the difference between different treatment values in all parameters. This test was used when the effects of the treatments were statistically significant or when the interaction between the factors was detected (not allowing to conclude about the main effects). In all the treatments p value < 0.05 was the threshold for significance. All the analyses were performed by STATISTICA, and the results were expressed as the mean values and their confidence intervals (p<0.05) (±95% CI).

Results

The fluctuations of all morphometric indicators followed the same tendency (Fig. 1). Under the warmed climate conditions, the biomass of monoculture grown barley and barnyard grass increased by 29% and 78% respectively, as compared to the current climate conditions. The competition led to changes in plants' dry weight under both investigated climates, but statistically significant only under the current climate conditions. The biomass of barnyard grass decreased by 48% (p<0.05) under the current and 26% (p>0.05) under the warmed climate. While the changes in barley biomass under the competition conditions were different, i.e. under the current climate, it in-

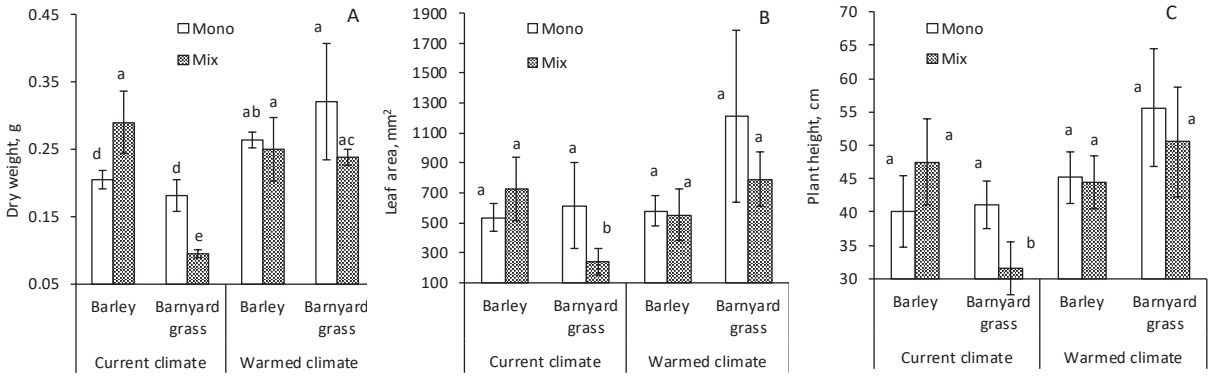

Fig. 1. The changes in dry weight (A), leave area (B) and plant height (C) of barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plants grown in monoculture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) under the current climate (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and the warmed climate (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at p<0.05 between the treatments as determined by Least Significant Differences (LSD) test.

Interspecific competition has affected the photosynthetic rate of both investigated plants (Fig. 2A). Under both investigated climate conditions, photosynthetic rate of mono cultured barley and barnyard grass was at the same range, and this parameter was higher under the warmed climate (p<0.05). The competition had no statistically significant effect on photosynthetic rate of barley under the current climate, while the decreases in photosynthetic rate of barnyard grass were statistically significant (34%, p<0.05). Under the warmed climate, photosynthetic rate of both plants decreased when they were grown under interspecific competition. The decreases were more pronounced for barley (40% vs. 17% (p<0.05)). The changes in transpiration rate and stomatal conductance followed the same tendency (Fig. 2 B and D).

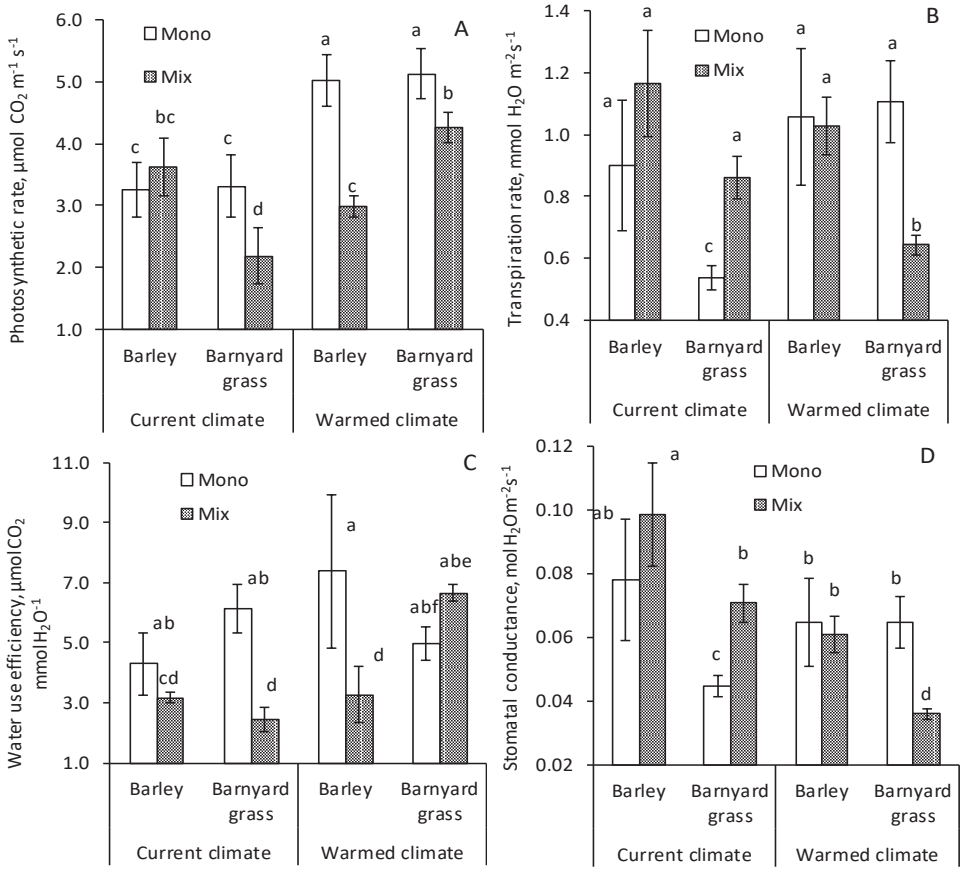

Fig. 2. The changes in photosynthetic rate (A), transpiration rate (B), water use efficiency (C), and stomatal conductance (D) in barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plant leaves grown in mono culture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) at current (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and a warmed (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) climate conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at p<0.05 between the treatments as determined by Least Significant Differences (LSD) test.

Under the current climate conditions, transpiration rate of barley and barnyard grass grown under competition increased by 29% (p>0.05) and 60% (p<0.05), respectively, as compared to the monoculture. Under the warmed climate conditions, an opposite effect was detected: the transpiration rate of the investigated plants decreased insignificantly for barley and significantly by 42% for barnyard grass. Interspecific competition reduced water use efficiency, and the effect was more pronounced for barnyard grass (60% [p<0.05]) than for barley (26% [p<0.05]), under the current climate (Fig. 2 C). Under the warmed climate, water use efficiency of barley was by 56% lower under interspecific competition (p<0.05), whereas this parameter of barnyard grass was by 34% higher as compared to the monoculture (p<0.05).

A higher intercellular CO_2 concentration (Ci) was detected under the warmed climate conditions for both investigated plants (Fig. S1 A). Under the competition conditions, it was increasing, comparing to the monoculture plants, except for barnyard grass under the warmed climate, when Ci of plans grown in mixture culture decreased by 8% (p<0.05), comparing to the monoculture ones. Higher Ci/Ca ratio was detected for both plants grown under the competition conditions, except for barnyard grass under the warmed climate, when it decreased by 8%, compared to the monoculture (Fig. S1 B).

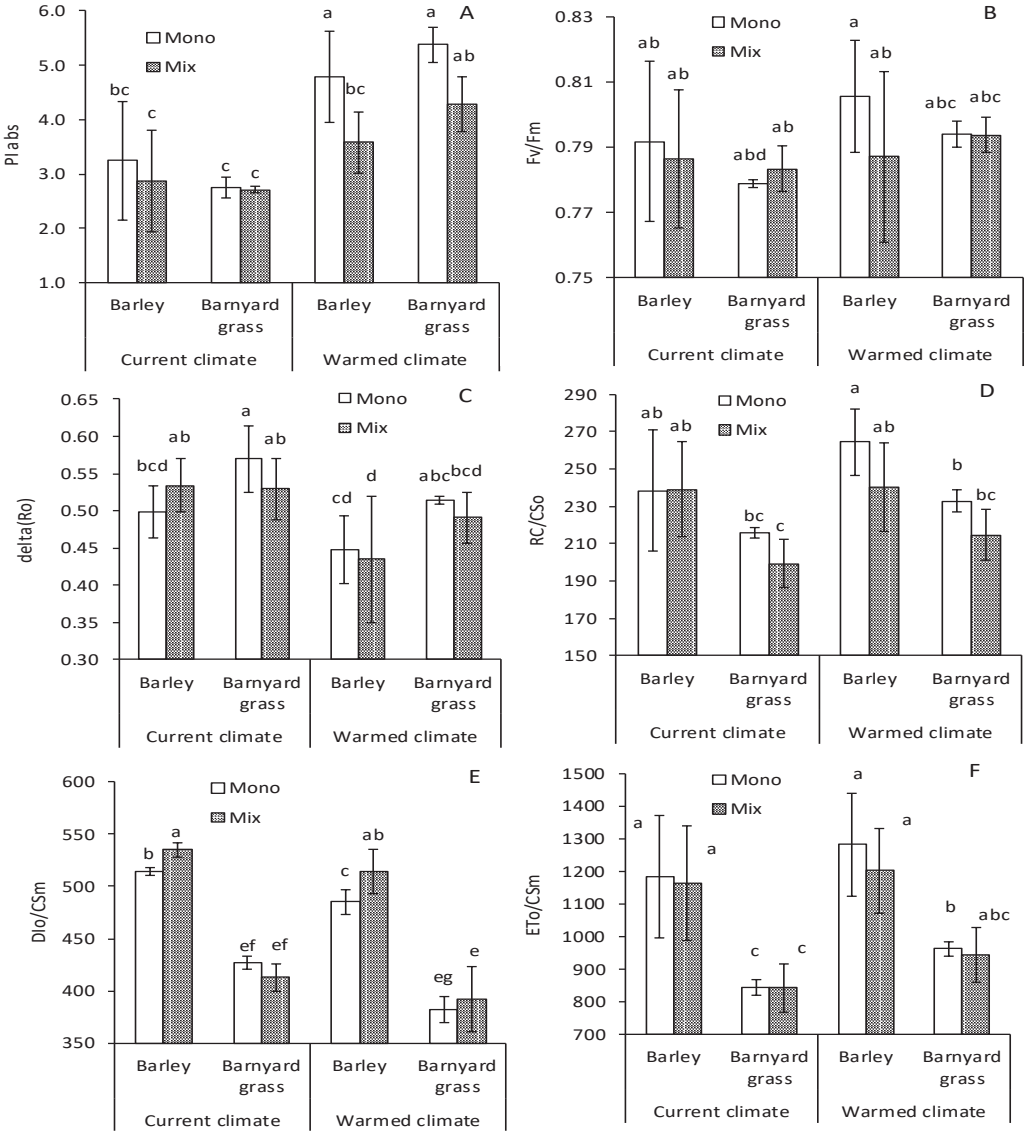

Fig. 3. The changes in performance index (PI_{abs}) on absorption basis (A), Fv/Fm ratio (B), efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (delta(Ro)) (C), density of RCs (QA-reducing PSII reaction centers) RC/CSo (D), dissipated energy flux per CS (DIo/CSm) (E), electron transport flux (ETo/CSm) (F) in barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plant leaves grown in monoculture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) at current (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and a warmed (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) climate conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at p<0.05 between the treatments as determined by Least Significant Differences (LSD) test.

PI_{abs} index of barnyard grass at warmed climate increased in both monoculture and competition conditions, compared with the plants under the current climate and monoculture (p<0.05) (Fig. 3 A). Significant differences in PI_{abs} of barley and barnyard grass under interspecific competition under the current climate were not found. Furthermore, under the warmed climate, lower PI_{abs} was detected for both plants, when it decreased by 25% (p<0.05) and 20% (p<0.05) for barley and barnyard grass respectively. The changes in Fv/Fm ratio of both investigated plants were not so pronounced as for PI_{abs} (Fig. 3 B). Fv/Fm ratio of barnyard grass under the warmed climate conditions increased in monoculture and competition conditions, compared with the plants under the current climate and monoculture conditions (p<0.05). The changes in Fv/Fm ratio of barley were statistically insignificant.

The efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI and electron acceptors (delta(Ro)) was slightly higher under the current climate conditions (Fig. 3 C). However, neither changing climate nor interspecific competition had statistically significant effects on delta(Ro). A higher density of reaction centers (RC/CSo) was detected for barley plants (Fig. 3 D). Under the warmed climate, this parameter increased, but statistically insignificantly, as compared to current climate for both investigated plants. Under interspecific competition conditions, the density of reaction centers decreased but statistically insignificantly under both investigated climates.

Higher spatial fluxes per CS approximated by F_m were detected for barley. Dissipated energy flux per CS (DIo/CSm) decreased under the warmed climate conditions for both plants (p<0.05) (Fig. 3 E). Interspecific competition increased DIo/CSm of barley plants by 4% (p<0.05) and 6% (p<0.05) under the current and warmed climates, respectively. And the changes in this parameter of barnyard grass were statistically insignificant. Electron transport flux (ETo/CSm) was also increasing under the warmed climate conditions for both plants, but was statistically significant (by 14%, p<0.05) only for barnyard grass grown in monoculture conditions (Fig. 3 F). Interspecific competition had no statistically significant effect on electron transport flux, trapped energy flux (TRo/CSm) and reduction of end acceptors at PSI electron acceptor side (REo/CSm) (Fig. S2 A and B).

Competition had a significant effect on the SOD activity in the both plants under the current and warmed climate conditions (Fig. 4 A). Elevated temperature and CO_2 decreased the SOD activity, and this decrease was strengthened by interspecific competition. Under the current climate conditions, the activity of SOD in the barley grown with barnyard grass was 4.28-fold higher than in barley grown in weed free conditions. The same tendency was observed under the warmed climate conditions, though the increase in SOD activity was slightly lower (2.55 times). The changes in SOD activity of barnyard grass were opposite: it decreased under competition effect under both investigated climates, and this decrease was more pronounced and statistically significant under the warmed climate (23% [p>0.05] vs. 38%, [p<0.05]).

The effect of barnyard competition on barley had a similar impact on the activity of CAT as well (Fig. 4 B). Barnyard competition significantly increased CAT activity in barley (p<0.05) under the current and warmed climate conditions. CAT activity in mixture culture under the current and warmed climate conditions was 6.42 and 4.86 times higher than that in monoculture, respectively. CAT activity in barley was higher under the warmed climate conditions, although the changes were insignificant (p>0.05). The response of barnyard to elevated CO_2 and temperature was different depending on if they were grown in monoculture or in mixture culture. In monoculture, a slight, although not significant increase in CAT activity in barnyard was recorded. On the contrary, the CAT activity in barnyard in mixture culture was decreased under elevated CO_2 and temperature, and the effect was close to significant (p=0.058).

GR activity in barleys grown in mixture culture with barnyard grass was 3-fold higher (p<0.05) than in monoculture under both current and warmed climate conditions (Fig. 4 C). An increased temperature and CO_2 concentrations led to a reduction in GR activity in both species, although the response of barnyard grass was more pronounced and significant (p<0.05).

As the barnyard competition led to an increase in antioxidative enzymes in barley leaves, the level of lipid peroxidation did not change, or it decreased under the current and warmed climate conditions (Fig. 4 D). The MDA concentration of barnyard grass grown in competition conditions decreased significantly by 34% at elevated temperature and CO_2, while under the current climate the changes were insignificant (Fig. 4 D).

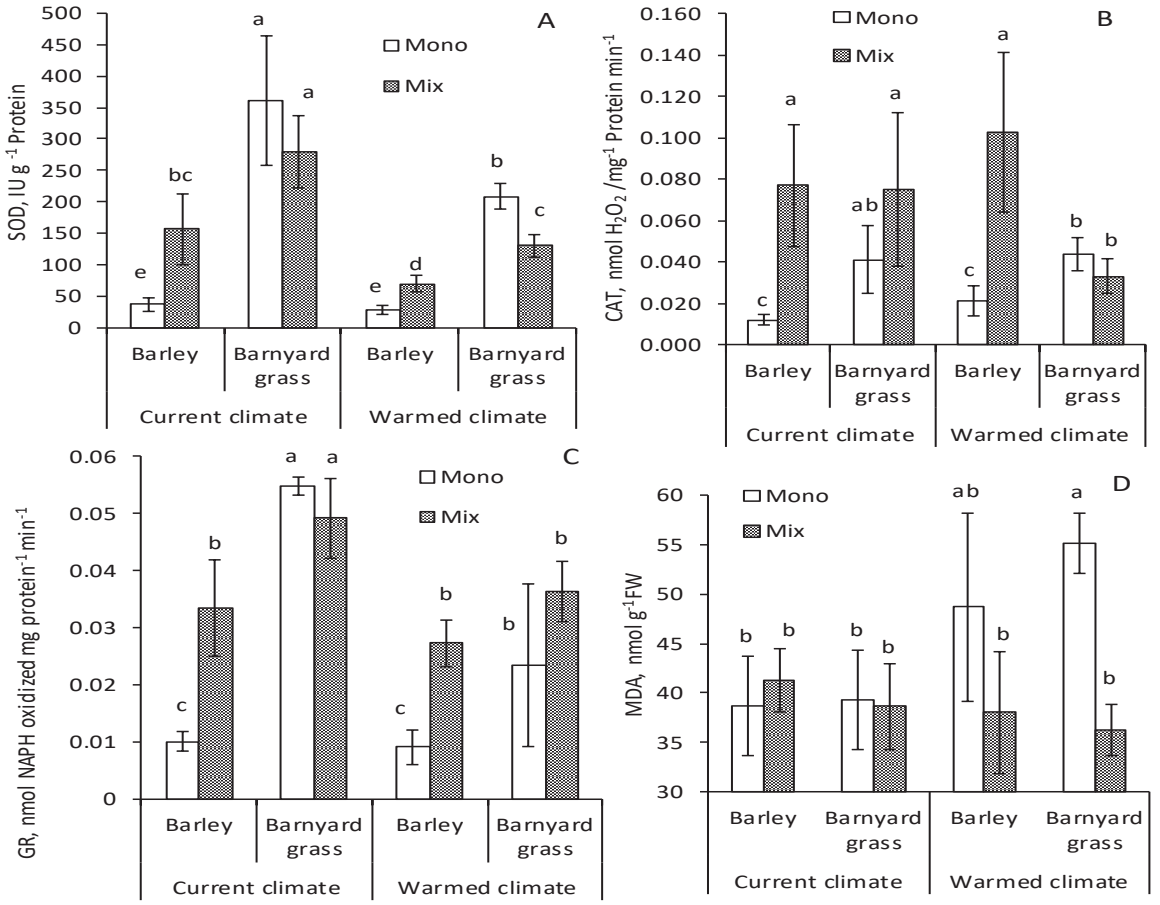

Fig. 4. The changes in the activity of superoxide dismutase (SOD) (A), catalase CAT (B) and glutathione reductase (GR) (C), and the concentration of malondialdehyde MDA (D) in barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plant leaves grown in mono culture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) at current (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and a warmed (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) climate conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at p<0.05 between the treatments as determined by Least Significant Differences (LSD) test.

The analysis of variance of all the investigated factors has shown that the highest impact on all the parameters was detected for climate (F = 976247; p<0.0001) (Table S2). Interspecific competition as a factor alone was weak and statistically insignificant. While under its interaction with climate the impact increased significantly, and this combination had become the strongest one (F = 10941; p<0.01).

Discussion

An intraspecific variability in growth and yield response to higher carbon dioxide concentration was found for rice (Shimono 2011), soybean (Sicher et al. 2010), and some other plants (Tausz et al. 2013). However, there have only been a few studies that have examined the impact of rising CO_2 on crop:weed interaction and/or yield loss when the photosynthetic pathways of the weed and the crop differed. For sorghum (*Sorghum bicolor*), a C4 crop, competition from two C3 weeds, cocklebur (*Xanthium strumarium*) and velvetleaf (*Albutilon theophrasti*) was enhanced under elevated CO_2 (Ziska 2003); conversely, for C3 crops soybean and rice, competition from C4 weeds was reduced in response to elevated CO_2 (Ziska 2000). However, the limited number of weed:crop competition studies at elevated CO_2 have been consistent with the kinetics of the C3 and C4 pathway (Valerio et al. 2013). Even fewer studies can be found about the combined effect of CO_2 and temperature on plants competition interactions. Elevated CO_2 and temperature influenced physiological traits and had effect on biomass accumulation of barley and barnyard grass. Many recent papers and reviews emphasize how the enhancement of atmospheric CO_2 directly impacts the physiology of plants and generally accelerates the photosynthetic rate and increases plant growth and

yield (Chang et al. 2016). Under the current climate conditions, the highest biomass accumulation was detected for barley grown in mixed culture conditions (Fig. 1), i.e. a higher accumulation might be caused by a higher photosynthetic rate (Fig. 2) and a higher efficiency of electron movements (Fig. 3) and, of course, by a higher activity of antioxidant enzymes (Fig. 4). It is also possible that at the early stage of the development, interspecific competition for barley had a less competitive effect than intraspecific competition between barley itself. Under the warmed climate conditions, the changes in the growth parameters of barley under the competition stress effect were statistically insignificant (Fig. 1), and the efficiency of photosynthetic performance, according to the gas exchange and JIP-test results, became lower compared to barnyard grass (Figs. 2 and 3). The data from the results of the experiments by Alberto et al. (1996) suggest that competitiveness could be enhanced in C3 crop (rice) relative to a C4 weed (*Echinochloa glabrescens*) with elevated CO_2 alone but simultaneous increases in CO_2 and temperature still favour C4 spp. (Naidu and Murthy 2014). In this research, the suchlike results were obtained, the photosynthetic rate of barnyard grass was more intensive at warmed climate, and interspecific competition did les harm for barnyard grass then for barley. The increase in photosynthetic rate matched up the increased water use efficiency, higher photosynthetic performance and lower dissipated energy of barnyard grass plants grown under the warmed climate conditions and interspecific competition, compared to the current climate and competition effect (Figs. 2 and 3). The response of photosynthesis to climate change is obviously complex; our study revealed that under higher CO_2 concentration and temperature conditions, photosynthetic rate was higher than that at ambient CO_2. Increasing the temperature increases the ratio of the photo-respiratory loss of carbon to photosynthetic gain, whilst elevated CO_2 has an opposite effect. The combined effects of elevated CO_2 and temperature on plant growth and photosynthesis may be increased through increases in Rubisco carboxylation and the decrease in photorespiration under elevated CO_2 (Ainsworth and Rogers 2007). Obviously, the rise in atmospheric CO_2 results in the increase of leaf intercellular CO_2 (Ci). Therefore, there was more internal CO_2 available to plants grown under elevated CO_2, thereby enhancing their growth and development as it was mentioned above (Figs. 1 and 2). However, higher temperatures may inhibit carboxylation and, in contrast, promote the oxygenation reaction and photorespiration (Yu et al. 2012). WUE may be increased by CO_2 enrichment, through decreased stomatal conductance (Long et al. 2004) and density (Teng et al. 2006), which would be negated by warming (Xu et al. 2013).

The JIP-test proposes equations to convert experimental fluorescence signals into biophysical and bioenergetic meaning (Albert et al. 2005). This approximation was performed by assuming that either F_o or F_m are reasonable measures of the absorption energy flux per excited CS of leaf sample (ABS/CS) in arbitrary units of a particular leaf sample in the dark-adapted state (Strasser and Strasser 1995). The translation of the measured fluorescence parameters into JIP-test parameters provides information on the stepwise flow of energy through PSII at different levels (Rasineni et al. 2011). A higher RC/CSo (of monoculture grown barnyard grass) induced by CO_2 and temperature suggests a larger proportion of active photosystem II (PSII) centers. This increased the proportion of available excited energy used for photochemistry, because electron transport through active RCs in PSII cross-section (ETo/CSm) of both investigated plants increased (Fig. 3). And significant decrease in dissipation of the untrappedexciton (DIo/CSm) in monocultural barnyard plants grown in warmed climate conditions depicts that effective electron transport and carbon assimilation decreased the energy dissipation non-photochemically but increased the photochemical quenching (ETo/CSm and Fv/Fm increased). In other words, these results elucidate that PSII was enhanced by the warmed climate, and thus increased the rate of electron transport and PI_{abs} under the warmed climate conditions (Fig. 3). The higher performance indices in plants grown in the elevated CO_2 and temperature also suggest an efficient overall performance of PSII photosynthetic machinery in these plants (Rasineni et al. 2011). Interspecific competition decreased PI_{abs} and increased DIo/CSm of barley under elevated CO_2 and temperature conditions. The losses were bigger and significant for barley than for barnyard grass (when only PI_{abs} decreased significantly), which possibly indicates that elevated climate stimulated the photosynthetic capacity of barnyard grass at a higher rate, and interspecific competition stress under the warmed climate became lower.

One of the most intimately related processes with the photosynthesis are the redox reactions and especially the redox status of the chloroplasts. Cellular redox in plants is related to control of the photosynthesis, stomatal closure, signal transduction, hormone signaling, vegetative and reproductive growth, and stress related gene expression (Potters et al. 2010). Also, another important relationship between photosynthesis and oxidative load is photorespiration. During photorespiration, a great amount of H_2O_2 is produced in C3 plants due to the oxidation of glycolate to glyoxylate in peroxisomes; however, in C4 plants, this process is nearly completely inhibited (Uzilday et al. 2018). To avoid net photoinhibition, plants have developed diverse photoprotection mechanisms such as reactive oxygen species scavenging systems, dissipation of absorbed light energy as thermal energy or cyclic electron flow around photosystem I, and others (Takahashi and Badger 2011). Dissipated energy flux per cross section (DIo/CSm, which also represents energy flux for non-photochemical quenching) of barley grown under competition conditions significantly increased (Fig. 3 E), the same tendency was detected for the activity of all

enzymatic antioxidants of barley grown under both investigated climate conditions (Fig. 4 A–C). Meanwhile, the electron transport through active RCs in PSII cross section (ETo/CSm) of barley under the abovementioned conditions did not change significantly, and photosynthetic rate decreased, which confirms that the received energy was moving to non-photochemical quenching and stress defense. However, the energy fluxes of barnyard grass (C4) were not related to photorespiration.

The modified photosynthetic performances in the investigated plants have severed consequences on the cellular redox state and thus, had its feedback reflected at the oxidative-stress enzymes level. The antioxidant system, controlling oxidative damage in plants, is a prime candidate for the role of molecular stress-modulator in a warmed climate conditions (Farfan-Vignolo and Asard 2012). In this research, the warmed climate increased lipid peroxidation levels in barnyard grass grown under monoculture conditions (p<0.05), but not in mixture or both conditions of barley (Fig. 3). Elevated CO_2 and temperature had no statistically significant impact on the activity of enzymatic antioxidants in spring barley leaf tissues, while on barnyard grass the activity of SOD and GR even decreased both mono and mix culture conditions (p<0.05). Interspecific competition increased enzymatic activity of barley, as compared to the monoculture in both investigated climates, while the reaction of barnyard grass was slightly different and in mostly cases insignificant. It is known that the enzymatic antioxidant defense system in plants has four cycles. The major Reactive Oxygen Intermediate scavenging (ROI-scavenging) pathways of plants include SOD, found in almost all cellular compartments, the water–water cycle in chloroplasts, the ascorbate–glutathione cycle in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes, glutathione peroxidase (GPX), and CAT in peroxisomes (Mittler et al. 2004). In this research, the activities of SOD and CAT in barely leaves increased under the effect of interspecific competition, which indicates that the water–water and catalase cycles were involved. Another well-characterized antioxidant defense system is the ASC/GSH cycle and involves reduced ascorbate (ASC) and glutathione (GSH), ascorbate peroxidase (APX), and ASC and GSH regenerating (i.e. re-reducing) enzyme GR (Farfan-Vignolo and Asard 2012). In this research, it was observed that the activity of GR in barley also increased under interspecific competition effect at both investigated climates. So, the increase in enzymatic activity of antioxidant defense system of barley caused the level of lipid peroxidation to decrease, which indicated the decreased concentration of MDA (Fig. 3 D). The reaction of barnyard grass to interspecific competition under different climate conditions was slightly different and not as pronounced as for barley. Interspecific competition even decreased the activity of SOD (p<0.05) and CAT (p>0.05) but increased the activity of GR (p>0.05), as compared to monoculture conditions. The result of that was a very high decrease of lipid peroxidation level in barnyard grass leaves, which indicates that the interspecific competition for barnyard grass under the elevated climate was very low.

Conclusion

Interspecific competition changed the response of the investigated plants to elevated temperature and increased CO_2 concentration in air. Under the current climate conditions, higher biomass and gas exchange parameters as well as lower antioxidant activity were detected for barley grown under interspecific competition effect. While under the warmed climate and under competition conditions the opposite results were detected: barnyard grass plants had a higher water use efficiency, a higher photosynthetic performance, a lower dissipated energy flux and a lower antioxidant enzymes activity as compared to barley. Thus, it might be concluded that in the future climate conditions, barnyard grass will become more efficient in the performance of photosynthetic apparatus and suffer from a lower oxidative stress caused by interspecific competition than barley. This novel finding disputes the general presumption of improved C3 plants competitiveness over C4 plants under the future air CO_2 concentration, and highlights the importance of concomitant increase in air temperature for the overall shift in weed:crop interaction. Considering other natural factors interfering with the changes of interspecific competition in the face of the climate change, the field experiments remains of high importance in the future researches.

Acknowledgements

This research was supplemented in the frame of the support from the Research Council of Lithuania (grant number: SIT-8/2015; research project “Composite impact of climate and environmental change on productivity, biological diversity and sustainability of agro-ecosystems” in the frame of National Research Program “Sustainability of Agro, Forest and Water Ecosystems”.

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Table S 1. Data extracted from the recorded fluorescence transients O–J–I–P

F_0	Minimal fluorescence, when all PSII RCs are open (at t = 0)
$F_m = F_P$	Maximal fluorescence, when all PSII RCs are closed
$V_J \equiv (F_J - F_0)/(F_m - F_0)$	Relative variable fluorescence at the J-step
$M_0 \equiv (\Delta V/\Delta t)_0 \equiv 4\ (F\ 300\ \mu s - F_0)/(F_m - F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient $V = f(t)$
$\Phi_{Po} \equiv TR_0/ABS = [1 - (F_0/F_m)]$	Maximum quantum yield of primary photochemistry (at t = 0)
$\Psi_0 \equiv ET_0/TR_0 = (1 - V_J)$	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond QA-
$delta(Ro) = RE_o/ET_o = (1 - V_I)/(- V_J)$	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
$\Phi_{Eo} \equiv ET_0/ABS = [1 - (F_0/F_m)]\ \Psi_0$	Quantum yield of electron transport (at t = 0)
$ABS/CS_m \approx F_m$	Absorption flux per CS, approximated by F_m
$TRo/CSm = \Phi_{Po}\ (ABS/CS_m)$	Trapped energy flux per CS
$ETo/CSm = \Phi_{Eo}\ (ABS/CS_m)$	Electron transport flux per CS
$DIo/CSm = (ABS/CS_m) - (TR_0/CS_m)$	Dissipated energy flux per CS
$RC/CSo = \Phi_{Po}(V_J/M_0)\ (ABS/CS_0)$	Density of RCs (QA- reducing PSII reaction centers)
$PI_{abs} = (RC/ABS) \times (\phi_{Po}/(1 - \phi_{Po})) \times (\psi_o/(1 - \psi_o))$	Performance index (PI) on absorption basis

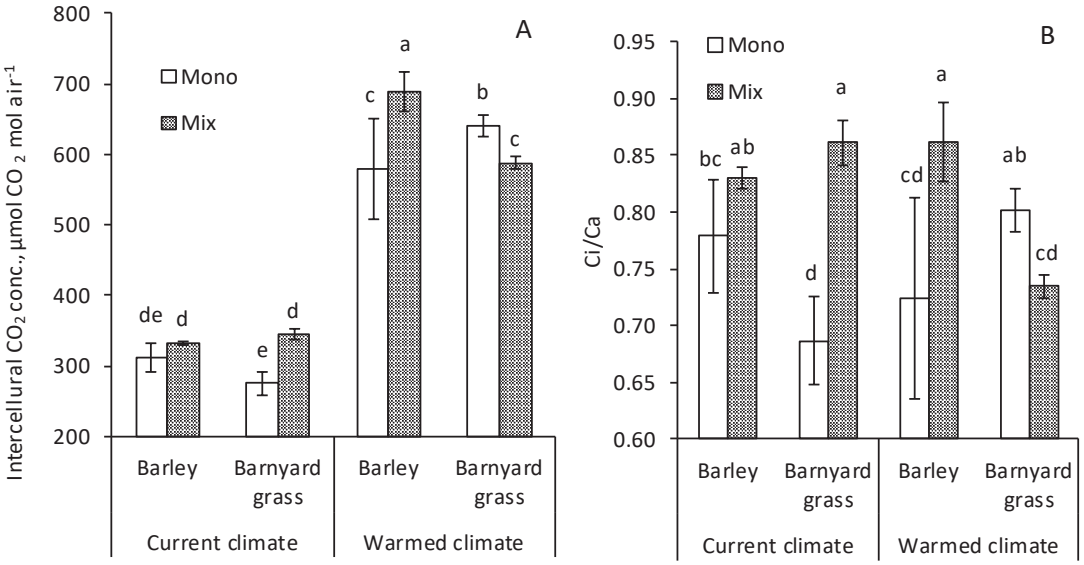

Fig. S 1. The changes of intercellular CO2 concentration (A) and the intercellular-to-ambient CO_2 concentration Ci/Ca ratio (B) in barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plant leaves grown in mono culture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) under the current climate (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and the warmed climate (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at $p<0.05$ between the treatments as determined by Least Significant Differences (LSD) test.

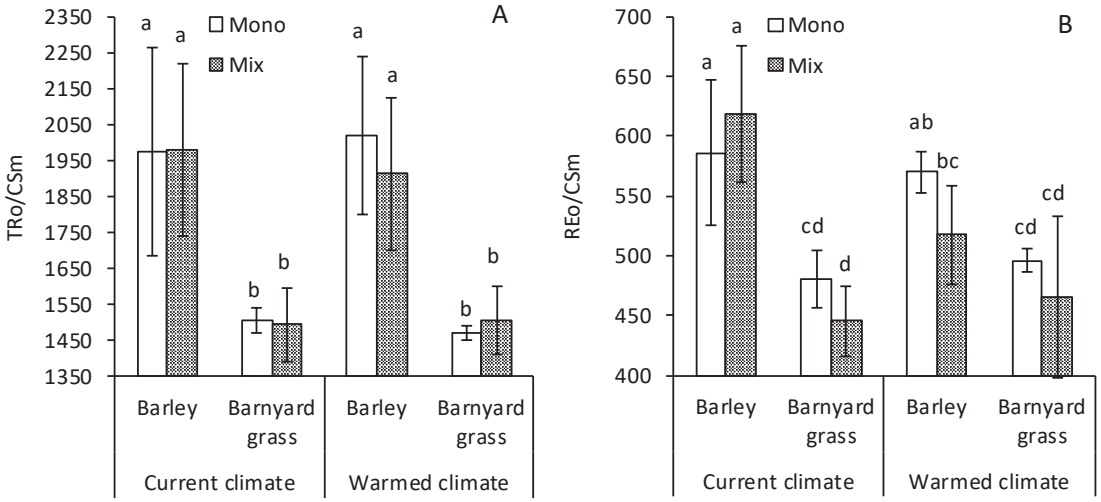

Fig. S 2. The changes of trapped energy flux (TRo/CSm) (A) and reduction of end acceptors at PSI electron acceptor side (REo/CSm) (B) in barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) plant leaves grown in mono culture (Mono; 15 plants per pot) and mix culture (Mix; 9 crop plants and 6 weeds) under the current climate (400 µmol mol^{-1} CO_2; day/night temperatures 21/14 °C) and the warmed climate (800 µmol mol^{-1} CO_2; day/night temperatures 25/18 °C) conditions. Each value represents the mean ±CI (n=3). Different letters indicate significant differences at $p<0.05$ between the treatments as determined by Least Significant Differences (LSD) test.

Table S 2. *F* value and significance of the source of variation

Parameters	Plant (P)		Interspecific competition (In. comp.)		Climate (C)		P x In. comp.		P x C		In. comp. x C		P x In. comp. x C	
Pn	0.002	N.s.	36.479	***	69.03	***	0.296	N.s.	20.903	***	12.337	***	19.374	***
Ci	1.839	N.s.	9.572	**	676.67	***	5.786	**	0.104	N.s.	0.498	N.s.	20.085	***
Gs	27.792	***	0.747	N.s.	16.14	***	1.487	N.s.	4.900	*	23.414	***	3.499	N.s.
Tn	21.717	***	0.190	N.s.	3.104	N.s.	3.029	N.s.	2.337	N.s.	25.385	***	5.311	*
Ci/Ca	2.879	N.s.	20.472	***	0.28	N.s.	1.535	N.s.	0.031	N.s.	5.571	**	25.014	***
WUE	1.458	N.s.	16.895	***	12.29	***	3.404	N.s.	0.009	N.s.	1.809	N.s.	22.417	***
All gas exchange parameters	32	***	50	***	9740	***	6	***	20	***	42	***	8	***
Fv/Fm	0.77	N.s.	0.68	N.s.	2.89	N.s.	1.42	N.s.	0.23	N.s.	0.59	N.s.	0.12	N.s.
PIabs	0.331	N.s.	6.162	**	35.45	***	0.161	N.s.	3.247	N.s.	3.027	N.s.	0.048	N.s.
delta(Ro)	8.311	**	0.385	N.s.	13.65	***	1.781	N.s.	0.741	N.s.	0.243	N.s.	0.979	N.s.
RC/CSo	18.453	***	4.387	N.s.	4.647	*	0.148	N.s.	0.033	N.s.	0.908	N.s.	0.700	N.s.
DIo/CSm	369.11	***	4.24	N.s.	26.51	***	5.68	**	0.50	N.s.	2.08	N.s.	0.50	N.s.
ETo/CSm	48.458	***	0.439	N.s.	4.103	N.s.	0.194	N.s.	0.214	N.s.	0.191	N.s.	0.060	N.s.
TRo/CSm	54.639	***	0.078	N.s.	0.029	N.s.	0.233	N.s.	0.000	N.s.	0.060	N.s.	0.372	N.s.
REo/CSm	41.985	***	1.908	N.s.	1.735	N.s.	0.550	N.s.	5.940	*	1.679	N.s.	2.058	N.s.
All fluorescence parameters	37	***	2	N.s.	22	***	1	N.s.	9	***	3	N.s.	1	N.s.
SOD	72.335	***	0.0013	N.s.	24.30	***	16.02	***	6.434	**	0.850	N.s.	1.021	N.s.
CAT	0.224	N.s.	16.115	***	0.012	N.s.	8.418	**	3.100	N.s.	0.439	N.s.	2.055	N.s.
GR	40.733	***	13.705	**	15.13	**	6.731	*	7.917	*	1.037	N.s.	3.245	N.s.
MDA	0.117	N.s.	12.342	***	6.773	**	2.118	N.s.	0.731	N.s.	16.104	***	0.409	N.s.
Antioxidante and MDA	64.330	***	5.5422	**	9.214	**	2.287	N.s.	4.476	*	10.492	***	6.355	**
Dry biomass	8.840	**	2.799	N.s.	27.228	***	17.264	***	20.62	***	2.685	N.s.	2.994	N.s.
Leaf area	1.5445	N.s.	2.7543	N.s.	7.1957	**	6.3867	**	11.26	***	0.4872	N.s.	0.206	N.s.
Plant height	0.026	N.s.	0.894	N.s.	18.59	***	6.641	**	14.436	***	0.197	N.s.	2.329	N.s.
Biomass, leaf area and hight	3.8116	*	1.3913	N.s.	9.354	***	5.7275	**	7.639	**	0.9346	N.s.	0.8821	N.s.
All investigated parameters	2172	***	104	N.s.	97625	***	3007	**	3101	**	10941	**	1492	*