ARTICLE

Physiological responses of two co-existing oak species in years with contrasting climatic conditions

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ABSTRACT   In this study we investigated how the intra- and inter-annual fluctuations of weather conditions influenced the physiological traits of sessile oak (*Quercus petraea* (Matt) Liebl.) and turkey oak (*Quercus cerris* L.*)* at Síkfőkút LTER site, Hungary, which is considered to be a peripherial habitat for *Quercetum petraeae-cerris* forest community. Seasonal changes in pigment composition and chlorophyll fluorescence parameters of upper (sun) and lower (shade) leaves of mature trees were studied during two consecutive growing seasons (2007, 2008). During the growing season of 2007 higher annual mean temperature and summer heat waves were recorded as compared to 2008. Leaves of both tree species had smaller chlorophyll content and higher total carotenoid content and xanthophyll cycle pool in summer of 2007 than in 2008. Changes of latter leaf traits under stress conditions in summer of 2007 were indicative for appearance of photoinhibition under heat stress. In 2007 lower F_v/F_o values also reflected more severe heat-induced decrease in shade leaves of both species compared to 2008. Recovery of F_v/F_o took place after the hot days of 2007 only in leaves of turkey oak
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According to the IPCC Report (IPCC 2007) global climate change has become real in the past century as the global mean temperature has increased by 0,76°C since 1850. Further warming (from 2 to 5°C depending on scenarios) and more frequent extremities (heat waves, and severe droughts) are predicted within the next decades, especially over Europe. Climate change and climatic extremes may have significant influence on forest communities by inducing change in vigour of dominant tree species and in longer term it may result shift in their distribution range (Geßler et al. 2007; Mátyás 2010). Alteration in tree vitality under changing climatic conditions has a serious consequence in source/sink function of these ecosystems in global carbon cycle. This study aimed to investigate how contrasting climatic conditions in growing seasons of 2007 and 2008 influence the canopy physiological traits of sessile oak and turkey oak co-existing at the same forest stand.

Materials and methods

Experimental site

The experimental site belongs to the Síkfőkút Project LTER forest area (47°90'N, 20°46'E, elevation 320-340 m), Bükk Mountains, north-eastern Hungary. The area is covered by a mixed forest stand dominated in the canopy layer by two oak species, sessile oak (*Quercus petraea* 46.9%) and turkey oak (*Quercus cerris* 22.8%) (Mészáros et al. 2010). The soil of the site is a deep brown forest soil formed on miocenic pebble (Jakucs 1985).

The research site could be characterized with 613 mm annual total rainfall and 11.3°C annual mean temperature as an average of the years between 2000 and 2009. The growing season lasts from mid-April to the end of October. The annual total global radiation in the region ranges between 4300 and 4400 MJ m^{-2} (Jakucs 1985).

Monitoring of environmental characteristics

During the study period air temperature and precipitation were recorded automatically in every 30 min by Hobo Pro-Series RH&Temp sensor (Onset Computer Corporation, Pocasset, USA) and Hobo Micro Station (Onset Computer Corporation, Pocasset, USA) with external sensors at the top of a meteorological tower at 25 m.

Ecophysiological measurements

Trees of sessile oak (4) and turkey oak (2) (100-105 years old, with a height of 20-22 m and DBH=28-36 cm) were selected in close vicinity to each other in the forest stand for this study. Leaf samplings were performed from the meteorological tower between 11 a.m. and 15 p.m. on sunny days from leaf

flush till autumn: in 2007 on 24th April (DOY 114), 16th May (DOY 136), 7th June (DOY 158), 9th July (DOY 190), 19th July (DOY 200) and 29th August (DOY 241), in 2008 on 24th April (DOY 115), 9th May (130 DOY), 27th May (DOY 148), 24th June (DOY 176), 28th July (DOY 210), and 3rd September (DOY 247) (Fig. 1). Leaves were sampled and measured in the upper (20 m) and lower (10 m) canopy positions which are considered in this paper as "sun" and "shade" leaves of trees, respectively.

Chlorophyll fluorescence measurements were performed with a PAM 2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) on 10-15 leaves from each sampling point of trees. In this study the ratio of variable (F_v) to minimal (F_o) fluorescence ($F_v/F_o = (F_m − F_o)/F_o$) measured after 30 min dark adaptation of leaves was used for assessment of maximum (or potential) quantum yield of PSII photochemistry of leaves. F_v/F_o fluorescence parameter may indicate more sensitively and with higher amplitude the effects of photoinhibition under stress conditions than F_v/F_m (Lichtenthaler et al. 2005).

Parallel to chlorophyll fluorescence measurements discs of 7 mm diameter were cut from leaves. Discs were pooled into three mixed samples from each sampling position of tree crown. Leaf samples were immediately frozen in liquid nitrogen and stored at -70°C until analysis. Leaf discs were powdered in liquid nitrogen and photosynthetic pigments were extracted with 80% acetone (with 0.1% NH_4OH). Absorbance of leaf extract was measured at 470, 646.8 and 663.2 nm with UV/VIS 1601 spectrophotometer (Shimadzu, Japan). Absorbance values were used for calculating the concentrations of chlorophyll *a* (CHL *a*) and chlorophyll *b* (CHL *b*) as suggested by Wellburn (Wellburn et al. 1994). Carotenoid (CAR) components including xanthophyll cycle (VAZ) pigments, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) were determined in the same extract by means of the reversed phase HPLC method modified after Goodwin and Britton (Goodwin et al. 1988) with Jasco UV/VIS HPLC system (Láposi et al. 2009). Chlorophyll content was expressed on a dry weight basis (mg g^{-1}), total carotenoid content and total VAZ pigment pool were calculated on CHL basis (mmol mol^{-1} CHL *a*+*b*).

Data processing and statistical analyses

Evaluations of data were performed by MS Excel 2003. Cumulative values of daily mean temperature and daily rainfall from 1st April till 10th September were calculated for comparing the weather conditions of growing seasons in the two years. These cumulative values were plotted against day of the year (DOY). Physiological parameters from different layers of tree crowns and sampling dates were compared by two-tailed, unpaired Welch's *t*-tests using Past v2.0 statistical software (Hammer et al. 2001). Means of respective parameters were considered as significantly different at p<0.05, and at higher p-values as non significant (NS).
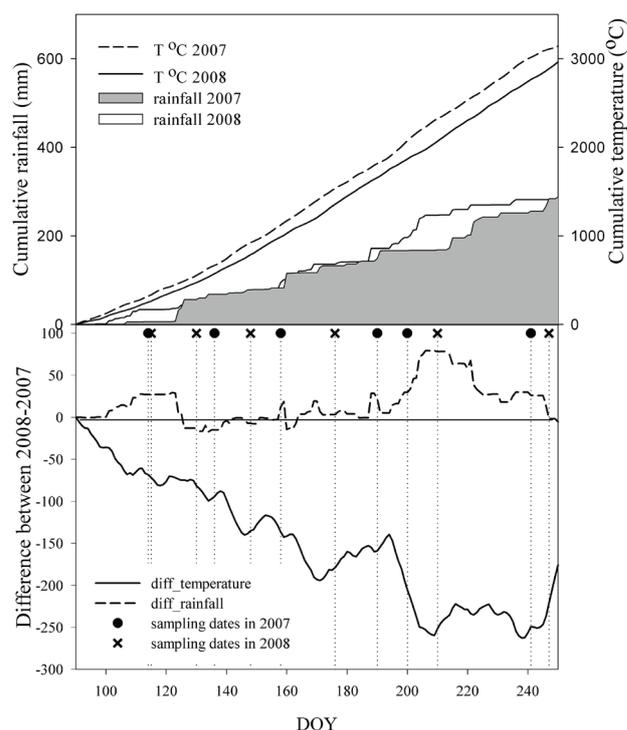

Figure 1. Cumulative values of rainfall (mm) and cumulative daily mean temperature (°C) during the growing seasons of 2007 and 2008 (upper figure) and their inter-annual differences (lower figure). Cumulative data are processed for period between 1st April and 10th September.

Results and discussion

Weather conditions during the study years

The two study years differed in the annual mean of temperature. 2007 was nearly 1°C warmer than 2008 (12.2 and 11.3°C, respectively). Higher daily mean temperature were characteristic all over the growing season in 2007, which resulted in a 235°C difference of cumulative temperatures by the last sampling dates (DOY 241 in 2007 and DOY 247 in 2008) between the two years (Fig. 1). There were prolonged heat waves in 2007 and the mean daily temperature exceeded 25 °C on 29 days in July and August. However, in the same period of 2008 similar conditions were recorded only for 6 days.

During the study period (from DOY 90 to DOY 250) the total rainfall was only 287 mm and 290 mm in 2007 and 2008, respectively, 79 mm and 76 mm less than the longer-term average (1999-2009) of total rainfall (366 mm). These rainfall conditions suggested that growing season of both study years was relatively dry. Although the cumulative rainfall was nearly the same in the two years other conditions resulted in more severe drought by mid summer of 2007 compared to the respective period of 2008: i) There was a long-lasting dry winter period in the previous year (2006) with total precipitation
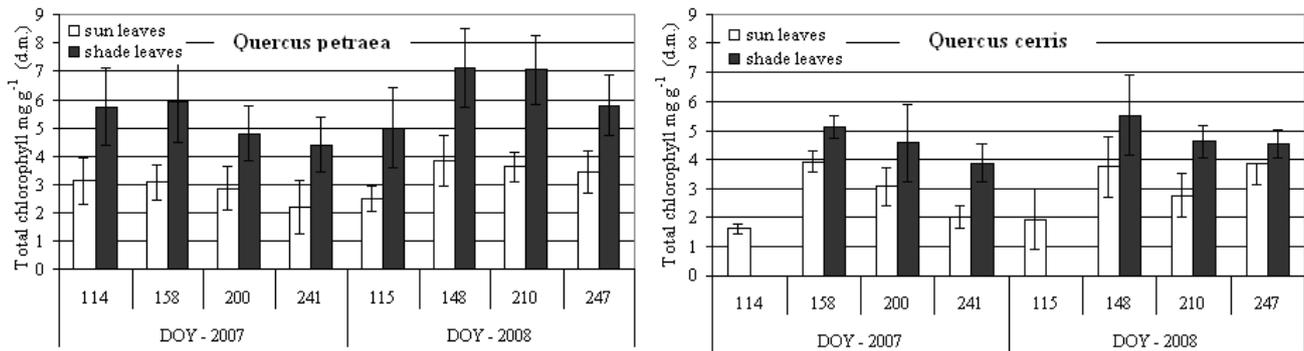
Figure 2. Seasonal changes in total chlorophyll content (mg CHL *a+b* g^{-1} leaf dry mass) of sun leaves and shade leaves of *Quercus petraea* and *Quercus cerris*. Means ±S.E.

of 28 mm from 1 September to 31 December which was the lowest in the past 35 years and reflected "rainfall-deficiency" of 130 mm considering the mean of the respective period between 1999-2009. ii) The higher daily mean temperature in 2007 (Fig. 1) induced stronger evapotranspiration rate and consequently faster drying of the soil (data not shown). iii) The seasonal distribution of rainfall was more extreme in 2007 with short and intense rain events and longer dry periods. In growing season of 2008 small rains were recorded with even seasonal distribution (Szőllősi et al. 2010).

Leaf pigment content

Leaf CHL showed larger inter-annual and within-canopy variability in sessile oak trees than in turkey oak (Fig. 2). Flushing of turkey oak began 1-2 weeks later than that of sessile oak in both study years. Because of its earlier flushing sun leaf CHL of the latter species was significantly higher (by 49 and 23% in 2007 and 2008 respectively, p<0.001 in both years) on the first sampling dates (DOY 114 and 115) compared to the former species.

Mature leaves in lower canopy of both oak species invested more in synthesizing chlorophylls per unit of dry mass (CHL *a+b*) than leaves in the upper canopy (Fig. 2). This divergence in leaf pigmentation within the tree crown coincides with previous observations for other forest tree species (Thayer et al. 1990; Faria et al. 1996; Closa et al. 2010). However, there was a considerable difference in the magnitude of within-crown variation of CHL between the studied tree species. CHL content exhibited larger differences between sun and shade leaves of sessile oak than in those of turkey oak (Fig. 2). CHL content of shade leaves of sessile oak was 187±17% and 183±13% compared to sun leaves in summer of 2007 and 2008, respectively (as means of three summer sampling dates) while the comparable data for the turkey oak were only 158±32% and 145±25% in 2007 and 2008, respectively. This can be explained by the different crown architecture of the two species. Lower leaves of sessile

oak trees receive weaker irradiance than those of turkey oak due to the denser canopy structure and develop high CHL content as a response to light conditions.

In sun and shade leaves of sessile oak CHL content was 25% (**NS**) and 14% (**NS**) higher, respectively on the first sampling date (24th April) in 2007 than in 2008 (Fig. 2) which was due to the earlier bud burst of this species in 2007. In summer, by period between DOY 200 and 247, however, the inter-annual difference changed to the opposite, such that CHL contents were significantly lower in both sun leaves (by 27%) and shade leaves (by 28%) of sessile oak in 2007. CHL contents in comparable leaves of turkey oak exhibited significant differences between the years only by the last sampling dates of the growing seasons. In 2007 CHL decreased from 3.92±0.37 to 1.99±0.38 and from 5.16±0.39 to 3.87±0.66 mg g^{-1} (d.m.) in sun and shade leaves of turkey oak, respectively, by DOY 241 in 2007 (Fig. 2) due to the extreme weather conditions. In that period CHL content was 49% lower in sun leaves and 15% lower in shade leaves of turkey oak in 2007 compared to 2008.

In contrast to CHL per leaf dry mass sun leaves of both species had higher total carotenoid content per CHL (Fig. 3) and developed larger xanthophyll cycle (VAZ) pigment pool than shade leaves (Fig. 4). These traits of tree crown are frequently associated with sun exposed leaves to enhance photoprotection against the high irradiance (Lichtenthaler et al. 1981; Young et al. 1997; Demmig et al. 2006). Flushing leaves (DOY 114 in 2007 and 115 in 2008) of both oak species exhibited significantly higher total carotenoid (CAR) and VAZ content per CHL than mature ones which indicated an increased need for photoprotection in the tree crown during spring time.

Smaller seasonal decrease of CAR and VAZ in leaves of both species took place with maturation by summer of 2007 than in 2008 which reflected the effective maintanance of the photoprotective carotenoid pool in the former year. During heat stress in July of 2007 both tree species exhibited
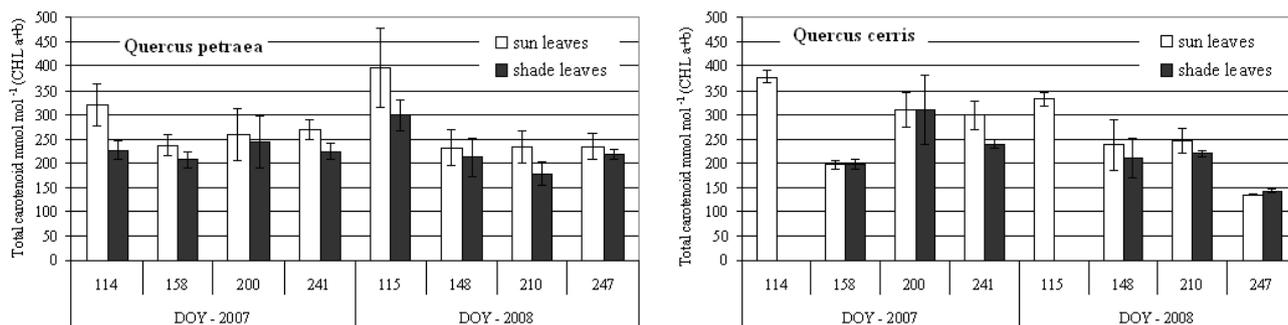
Figure 3. Seasonal changes in total carotenoid pool (CAR) (mmol mol^{-1} CHL_*a+b*) of sun and shade leaves of *Quercus petraea* and *Quercus cerris*. Means ±S.E.
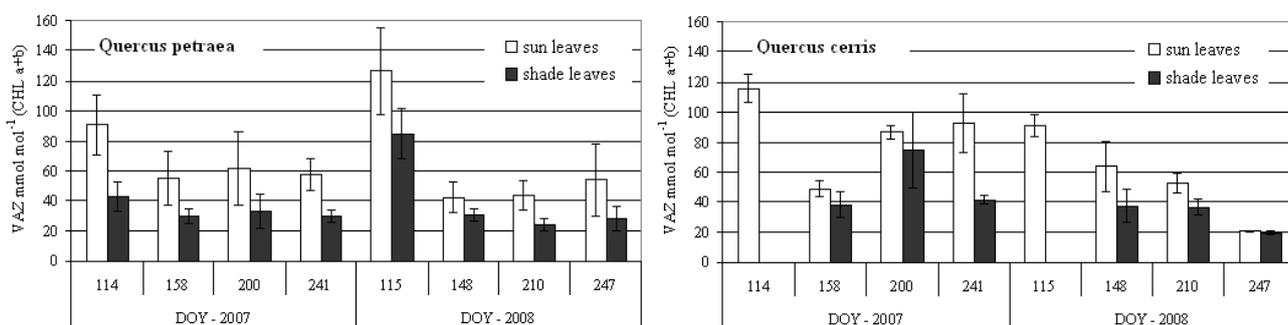
Figure 4. Seasonal changes in xanthophyll cycle pool (VAZ) (mmol mol^{-1} CHL_*a+b*) of sun and shade leaves of *Quercus petraea* and *Quercus cerris*. Means ±S.E.

increased leaf CAR in both canopy layers (Fig. 3). Between DOY 200 and 247 the CAR was 13-17% higher in both canopy layers of sessile oak (for sun leaves p<0.01 and for shade leaves p<0.001) in 2007 compared to 2008. In the same period shade leaves of turkey oak exhibited 52% and 60% higher CAR in shade (p<0.01) and sun laves (p<0.001) in 2007.

VAZ pool of both trees was also very responsible to the contrasting weather conditions of the consecutive years (Fig. 4). In the warmer summer period of 2007 (from DOY 200 to 247) sessile oak had 20-21% higher VAZ in both canopy layers (for sun leaves p<0.05 and for shade leaves p<0.01) while turkey oak had 14% and 46% higher VAZ in shade (p<0.01) and sun leaves (p<0.001), respectively as compared to that of 2008. Between the two species VAZ pool was especially large (86-92 mmol mol^{-1} CHL_*a+b*) in sun leaves of turkey oak in that period of 2007.

Chlorophyll fluorescence parameters

The main trends of changes in F_v/F_o were similar at the beginning of leaf development of two species with rapid increases after flushing. However, the different weather conditions of

the two years influenced the seasonal course of F_v/F_o in both species (Fig. 5).

As a result of higher spring temperature in 2007 and consequent earlier maturation of leaves F_v/F_o values of both sun and shade canopy layers of sessile oak were significantly higher on the first sampling date in 2007 compared to those of 2008. From that time, along with the leaf maturation, F_v/F_o of sessile oak increased first rapidly and then moderately to values between 4.0 and 4.5 and between 4.9 and 5.1in sun and shade leaves, respectively by nearly the same dates in both years (DOY 148 and 157 in 2007 and 2008 respectively, Fig. 5). Leaf flush of sessile oak delayed in 2008 but it was compensated by a twice as fast F_v/F_o increase of both canopy layers as that in 2007. This is reflected in the slope values of linear regression lines fitted to the initial phase of seasonal F_v/F_o curves (Table 1).

In the period from early June to mid July (DOY 158-190) the average F_v/F_o values of sun and shade leaves did not differ in the two years. The extremely high temperatures in July of 2007 induced a rapid decrease in F_v/F_o of sessile oak by DOY 200. This down-regulation of potential photochemical quantum yield of PSII was significant both in sun and shade leaves compared to the previous sampling dates and
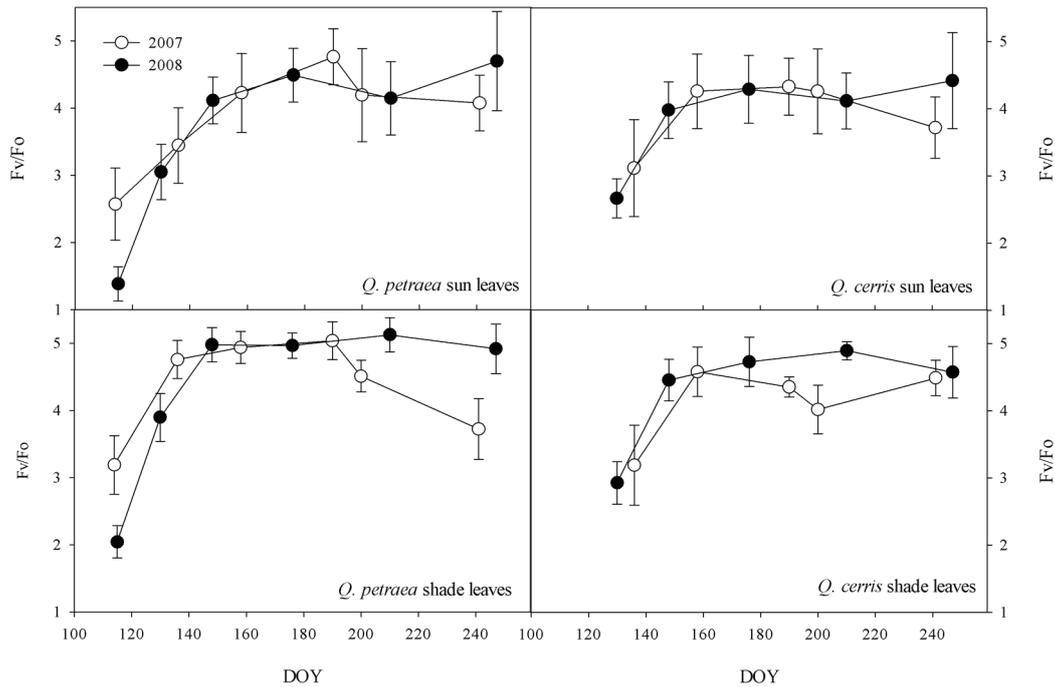
Figure 5. Seasonal changes in potential photochemical quantum yield (F_v/F_o) of sun and shade leaves of *Quercus petraea* and *Quercus cerris*. Means ±S.E.

Table 1. Slope of linear regression line fitted to the initial phase of seasonal F_v/F_o curve for sun and shade leaves of oak species between DOY 114-158 and 115-148 in 2007 and 2008 respectively.

| | Q. petraea | | | | Q. cerris | | | |
| | 2007 | | 2008 | | 2007 | | 2008 | |
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
Sun leaves	0.038	0.999	0.082	0.968	0.063	only 2 points	0.052	only 2 points
Shade leaves	0.040	0.826	0.088	0.959	0.067	only 2 points	0.060	only 2 points

no recovery was measured later in the season. Significantly lower F_v/F_o values were measured in the end of August 2007 compared to the same period of 2008 (Fig. 5). Adverse effects of high temperature were more pronounced in shade leaves of sessile oak and resulted in gradually decreasing F_v/F_o values during the second half of the vegetation period. In 2007 on DOY 241 midday F_v/F_o value of shade leaves was even lower than that of sun leaves (3.73±0.45 and 4.07±0.41 respectively, p=0.0287).

The main trends in seasonal change of F_v/F_o in sun and shade leaves of turkey oak were basically similar to those of sessile oak (Fig. 5). However, higher temperatures during spring of 2007 did not affect the rate of F_v/F_o increase in young leaves of turkey oak. Both the Fv/Fo values and the steepness of regression line fitted to first section of F_v/F_o curve in Fig. 5 (Table 1) was not different on the comparable dates in 2007 and 2008 (Fig. 5).

After the low spring-time F_v/F_o values of turkey oak reached the characteristic range of mature leaves in early summer: 4.1-4.4 and 4.5-4.9 for sun and shade leaves, respectively. Although the heat wave in July of 2007 decreased photochemical efficiency of Q. cerris too, sun leaves exhibited significant depression of F_v/F_o only by DOY 241 (14% decrease) compared to DOY 190 and also to values of the same period in 2008 (Figure 5). F_v/F_o in shade leaves of turkey oak, similarly to sessile oak, proved to be more sensitive to high temperature than sun leaves during this period. Heat-induced inhibition of photochemistry as reflected by decrease of F_v/F_o took place earlier in shade leaves than in sun leaves (Fig. 5). The decrease of F_v/F_o in shade leaves was 5% (NS) and 12% (p=0.002) by DOY 190 and DOY 200, respectively as compared to the previous sampling date, which significantly recovered (by F_v/F_o=4.49±0.26) after the heat wave by 241 DOY (Fig. 5).

Acknowledgements

Abbreviations

CHL, chlorophyll; CAR, carotenoids, F_m, maximum fluorescence in the dark adapted state; F_o, minimum fluorescence in the dark adapted state; F_v, variable fluorescence in the dark adapted state (F_m-F_o); F_v/F_o, variable to minimum fluorescence ratio in the dark adapted state, it is considered to be proportional to maximal quantum yield of PSII; DOY, day of year; VAZ, xanthophyll cycle pigment pool; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin.

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