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Title page

Plant ecophysiological responses to drought, nocturnal warming and variable climate in the Pannonian sand forest-steppe: results of a six-year climate manipulation experiment[†]

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[†] In memory of Gábor Fekete, prominent vegetation scientist, founder of plant ecophysiological research in Hungary

Running title: Ecophysiological responses of forest-steppe perennials to climate change

Abstract

The impacts of year-round nocturnal warming or late spring rain exclusion on three plant functional types were studied in a plot-scale climate simulation experiment in a semiarid sand forest-steppe of Central Hungary. Ecophysiological traits were followed through six years for the C₃ bunch grass *Festuca vaginata*, the spreading C₄ grass *Cynodon dactylon* and shrub-sized root suckers of *Populus alba*. In general, experimental treatments had slighter effects than weather fluctuations yielding extremities did. *Populus alba* responded to nocturnal warming with developing slenderer leaves. Rain exclusion reduced leaf physiological activity or growth, but only during or just after the treatment, and in certain years. When assessing treatment and background climatic variation effects together, in spring, leaf area growth was consistently stimulated by increasing temperature, but decreased with longer rainless periods for *P. alba* and *F. vaginata*. Physiological responses in spring indicated low temperature limitation for *C. dactylon*, and both low and high temperature control for *P. alba*. Longer summer droughts reduced leaf gas exchange, particularly for *F. vaginata* with substantial drop in photochemical activity and chlorophyll content. These results suggest that shallow rooted C₃ bunch grasses can be the most susceptible to climatic variation, thus their abundance is expected to decline in the Pannonian forest-steppe. In contrast, plants having deeper roots and clonal integration will probably be less affected by the projected warming and drying climate. C₄ photosynthesis or southern geographical distribution may also be beneficial, thus, the abundance of such species is expected to diminish less or even increase.

Key words: *Cynodon dactylon*; *Festuca vaginata*; forest-steppe; leaf gas exchange; perennials; plant functional types; *Populus alba*

Abbreviations: A = net photosynthetic rate; D = fraction of light absorbed in PSII antennae that is dissipated thermally; E = transpiration rate; $F_v/F_{m_{\text{dawn}}}$ and $F_v/F_{m_{\text{noon}}}$ = PSII maximum photochemical efficiency at dawn and at noon, respectively; $F_v/F_{m_{\text{depr}}}$ = dawn to midday depression of F_v/F_m ; F_v'/F_m' = antenna efficiency of PSII; g_s = stomatal conductance for water vapor; HeatSum = sum of daily mean temperature values above 5°C for the preceding 28 (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*); LD = leaf bulk tissue density; LMA = leaf mass per unit area; LT = leaf thickness; L/W = ratio of leaf length to width; NDVI = leaf normalized difference vegetation index; NPQ = non-photochemical fluorescence quenching; mND = NDVI corrected for leaf structural reflectance; PRI = photochemical reflectance index; qP = photochemical fluorescence quenching; Rainless = length of uninterrupted rainless ($\leq 4 \text{ mm day}^{-1}$) period (days; for leaf physiology) or the number of rainless days for the preceding 28 (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*; for leaf morphology and structure) before field measurement; SWC = topsoil (0-6 cm) volumetric moisture content; T_{min} and T_{max} = daily minimum and maximum temperature, respectively; VAZ pool = violaxanthin-antheraxanthin-zeaxanthin cycle pigment pool; WUE = instantaneous photosynthetic water use efficiency; ΔPRI = size of VAZ pool; ΦPSII = PSII actual quantum yield at standard irradiance.

Introduction

The ecological impacts of recent climate change, including both gradual climatic trends and extreme weather events, like summer heat waves and severe droughts, have already been detected or predicted at multiple organizational levels from individuals to ecosystems (Walther et al. 2002, Ciais et al. 2005, Peñuelas et al. 2013). These effects can be different

according to the sensitivity of ecosystems to climate variability (Seddon et al. 2016). Although climate change is likely to affect all components of plant communities, the role of dominant species in maintaining ecosystem functions under altered conditions appears to be of primary importance (Grime 1998, Smith & Knapp 2003, Grman et al. 2010). Based on shared responses to the environment or similar effects on ecosystem properties a limited number of plant functional types can be identified within ecosystems (Díaz & Cabido 1997, Hooper et al. 2002, Lavorel & Garnier 2002). This allows making investigations on one or a few, mostly dominant species as representatives of functional types. Different plant functional types, according to their life form, phenology, vegetative growth, rooting pattern or photosynthesis type, often respond differently to natural or experimentally simulated climatic stresses (Mamolos et al. 2001, Fay et al. 2002, Xia & Wan 2013, Wertin et al. 2015), which may result in changes in the dominance structure and composition of the community (Alward et al. 1999, Zavaleta et al. 2003).

Field scale climate change experiments, with manipulation of temperature, precipitation and/or air CO₂ concentration, are useful tools for assessing ecosystem responses to changing climate (Jentsch et al. 2007, Beier et al. 2012, De Boeck et al. 2015, Fu et al. 2015). In addition to studies focusing on the effects on ecosystem functions like biomass production (Jentsch et al. 2007, Wu et al. 2011, Fu et al. 2015), experiments assessing the impacts on individual plant performance, such as physiology, morphology and phenology are also necessary to identify mechanisms behind changes at community or ecosystem level. While numerous experiments were conducted on individual plant responses of species from temperature-limited cold temperate and (sub-)arctic ecosystems (e.g. Parsons et al. 1994, Suzuki & Kudo 1997, Hudson et al. 2011, Xia & Wan 2013), or Mediterranean woody species (Llorens et al. 2003, Prieto et al. 2009a, Ogaya et al. 2011, Liu et al. 2016), less attention has been paid to the components of temperate continental or dryland grasslands and

shrublands (e.g. Fay et al. 2002, Maricle & Adler 2011, Wertin et al. 2015), where soil water availability has a major role in plant responses to climate-induced changes.

The Pannonian sand forest-steppe in the Carpathian Basin is a large-scale mosaic of open sand grasslands, xerothermic sand oakwoods, and mixed juniper-poplar woodlands and shrublands (Fekete 1992, Molnár et al. 2012). This is a transitional biome between Atlantic temperate deciduous forests and European continental steppe zones with rich and unique biodiversity. In the open perennial sand grassland component of this ecosystem, regular midsummer drought is the major limitation for phytomass production (Kovács-Láng 1974). Regional climate predictions forecast increases in annual mean temperature, decreases in summer precipitation, more frequent hot and heat wave days and longer dry periods in summer for the coming decades (Bartholy et al. 2014). Increasing aridity is expected to enhance the vulnerability of open sand grasslands to other anthropogenic effects, such as the spread of invasive species and changes in land use (Molnár et al. 2012). Pannonian sand steppe belongs to the critically endangered category in the European Red List for terrestrial habitats (Janssen et al. 2016). Towards the drier and warmer end of a climatic gradient in open perennial sand grasslands of Hungary (i.e. in the same direction as the predicted climate change), Kovács-Láng et al. (2000) observed declining plant cover and species richness, and increasing abundance of annuals and sand grassland specialists.

In order to understand the mechanisms underlying growth for different plant functional types in this ecosystem, and to assess future trends in the abundance of the dominant species, vegetation composition and ecosystem functioning in response to climate change, we conducted a field scale manipulation experiment as part of a Europe-wide network VULCAN (Beier et al. 2004). We aimed at assessing the effects of elevated nighttime temperature or an extended drought in the peak vegetation period on selected plant ecophysiological traits of three dominant species representing different plant functional types in the Pannonian sand

forest-steppe. It was hypothesized that different plant functional types respond differently to the projected climatic conditions.

Materials and Methods

Study site

The field site (46°53'N, 19°23'E, 130 m a.s.l.) is located in the Danube-Tisza Interfluve, near the village Fülöpháza in the Kiskunság National Park, Hungary. The soil is coarse textured calcaric arenosol with ~11% CaCO₃ and <1% humus content in the upper 20 cm layer, low water holding and conducting capacities (Kovács-Láng et al. 2000). The climate is moderately warm semiarid temperate with continental and submediterranean influences. Annual mean temperature is 10.1°C, monthly average temperature ranges from -1.9°C (January) to 21.1°C (July). Yearly average precipitation is 505 mm with maximum in June typically followed by summer drought in July and August. During the experiment in 2001-2008, weather conditions were characterized by marked interannual variation (Fig. 1). Like throughout Europe (e.g. Ciais et al. 2005, Peñuelas et al. 2007), the summer of 2003 was exceptionally dry and hot. Compared to the eight-year (2001-2008) average, monthly mean temperature in May and June was more than 2°C higher (Fig. 1b), and monthly precipitation from April to June was 59-89% lower (Fig. 1a). This period was also drier and warmer than average in 2007. In contrast, May and June in 2004 and 2006 was relatively cool and wet, while the weather was mostly close to average in 2005 and 2008.

Insert Fig. 1 here.

The experiment was set up in the nutrient-poor, open perennial sand grassland colonized by shrub-sized root suckers of the native white poplar (*Populus alba*). For this study we chose three dominant species representing different plant functional types (Table 1).

Insert Table 1 here.

Experimental setup

A field experiment was established in 2001 as part of a network using uniform protocol in six European countries (EU FP5 VULCAN Project; Beier et al. 2004, Peñuelas et al. 2007). Nine plots of 4 × 5 m were laid out in a completely randomized design with two treatments (warming, drought) and a control, in three replicates each. Passive nighttime warming treatment was applied from dusk to dawn every night throughout the year using automatically retractable IR reflecting curtains on a light scaffolding of 1 m height. In the case of rain or strong wind, the curtains were retracted from the plot. This manipulation increased daily minimum and daily mean temperature at 20 cm height by 1.2°C and 0.6°C, respectively in average between April and August 2003-2008. The corresponding increases in mean soil temperature were 1.3°C at 5 cm depth and 0.5°C at 10 cm depth, while minimum soil temperature increased by 1.6°C and 0.8°C, respectively. Drought treatment was achieved by excluding rain from the plots using rain-activated, transparent curtains on similar scaffolding in May and June, during the peak growth of most plants. Rain exclusion was 5-29% (20-163 mm) of the yearly precipitation depending on actual weather conditions, which decreased volumetric soil moisture content (SWC, V/V%) at 20 cm depth by 23-72% in average during treatment compared to control plots. Control plots were built with scaffolding, but without any curtains. We did not apply combined (warming × drought) treatment.

Sampling and measurements

Sampling and measurements of plant traits were repeated once a month between May and August from 2003 (2005 for leaf morphology) to 2008. For leaf morphology and coarse structure, an additional sampling was made in April 2008. In some cases, data collection was not possible due to inappropriate weather or plant conditions, or equipment failure (Table S1).

In each plot, air temperature at 20 cm height, soil temperature at 5 and 10 cm depths and SWC at 20 cm depth were recorded hourly by installed temperature or moisture sensors connected to a data logger. Precipitation was measured with rain gauges within each plot as well as a reference gauge outside the plots. A standard meteorological station at Fülöpháza, ca. 1 km from the experimental site, also provided background data. In addition, during our monthly field campaigns, SWC was measured in a depth of 0-6 cm next to the shoots of the two grass species studied and under bare soil surface (10 measurements per plot and species) using a Delta-T Theta Kit HH2 Soil Moisture Meter (Delta-T Devices, Cambridge, UK). These 0-6 cm SWC data were used as a physical response variable to assess short-term integrated effects of experimental treatments and weather conditions on soil water regime, and were not used for characterizing soil water status under the studied species due to their deeper rooting depths (except that of *F. vaginata*, Table 1).

Leaf morphology was described using nondestructive sampling. The length and maximum width (for the filiform leaves of *F. vaginata* length only) of the youngest fully-expanded leaf were measured with a ruler (accuracy 1 mm) for 10 individuals per plot for each species. The length/width ratio was used to characterize leaf shape. Leaf area was calculated from length and width data by using species-specific regression equations ($R^2 = 0.76-0.99$, $p < 0.05$) determined each time on an additional set of 50 leaves taken on site but outside the plots.

Leaf coarse structure was described by leaf dry mass per area (LMA, g m^{-2}), and its components, i.e. leaf thickness (LT, mm) and bulk tissue density (dry mass per unit leaf volume, $\text{LD} = \text{LMA}/\text{LT}$, g cm^{-3}) on the same leaves used for chlorophyll fluorescence

measurements (see later). Leaves were taken to the laboratory in airtight plastic containers filled with humid air. We measured LT by using a thickness meter (Standard Dial Indicator, Mitutoyo, Japan; accuracy 0.01 mm) and one-sided surface area with an LI-3000A Leaf Area Meter (LI-COR Inc., Lincoln, Nebraska; accuracy 0.1 cm²). Leaves were then oven dried to constant weight at 90°C and dry weight was measured. For *F. vaginata* each sample consisted of 4 leaves for a greater accuracy. For this species, LT and LD were not determined due to a central air-filled part of variable size in its involuted leaves.

Leaf gas exchange was measured for *P. alba* by using an ADC LCA4 system (ADC Bioscientific, Hoddesdon, UK) in 2003 and 2004, and for each species by using an ADC LCA2 system between 2005 and 2008. Measurements were made at ambient conditions under clear sky during the daily peak activity (between 11:00-14:00 in May and June, and 9:00-12:00 in July and August). For each species, 2-3 samples per plot were measured (4 leaves of *F. vaginata* were inserted in the leaf chamber together). The following standard leaf gas exchange variables were calculated for unit leaf area according to von Caemmerer & Farquhar (1981): net photosynthetic rate (A), transpiration rate (E), stomatal conductance for water vapor (g_s) and instantaneous photosynthetic water use efficiency (WUE).

Leaf relative pigment content was assessed by the non-invasive technique of spectral reflectance in the 300-1100 nm wavelength range with ca. 3 nm nominal bandwidth using an UNISPECTM Spectral Analysis System (PP-Systems, Haverhill, MA, USA). Leaf reflectance spectra were recorded under clear sky between 10:00 and 14:00 hours on 1-5 leaves per plot for each species. From these field spectra two standard reflectance indices were calculated:

1) Photochemical Reflectance Index ($PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$; Peñuelas et al. 1995), developed first as 'Physiological Reflectance Index' (Gamon et al. 1992), which indicates the amount of active (photoprotective) form of the violaxanthin-antheraxanthin-zeaxanthin (VAZ) pigment pool, and

2) Normalized Difference Vegetation Index (NDVI) corrected for reflectance due to leaf structure ($mND = (R_{750} - R_{705}) / (R_{750} + R_{705} - 2 \times R_{445})$; Sims & Gamon 2002) indicating leaf chlorophyll content. (R_{nnn} in formulas refers to leaf reflectance at wavelength nnn in nanometers.)

The size of the total VAZ pool was measured on 1-4 samples per plot for each species as the difference in PRI between the dark- and the light-incubated leaf ($\Delta PRI = PRI_{\text{dark}} - PRI_{\text{light}}$; Gamon & Surfus 1999). (For the narrow-leaved *F. vaginata* each sample consisted of 4 blades.) Leaves were collected and dark-incubated for at least 12 hours at room temperature in airtight black plastic containers filled with humid air, and then PRI was determined (PRI_{dark} , total VAZ pool in inactive (epoxidated) form). Then the leaf was exposed to saturating light and PRI was measured in every 30 second until a new steady state value was reached (PRI_{light} , complete VAZ pool in photoprotective (deepoxidated) form).

Chlorophyll-a fluorescence measurements were conducted on the youngest fully-expanded leaf (*C. dactylon*, *P. alba*) or 4 fully-expanded non-senescent leaves together (*F. vaginata*) using a pulse-modulated chlorophyll-a fluorimeter (Hansatech Fluorescence Monitoring System FMS2, Hansatech Instruments Ltd., Norfolk, England). To determine the actual (light-incubated) leaf photochemical efficiency at midday (from 11:00 to 15:00 hours) leaves were first incubated to a uniform high actinic light ($1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 3 minutes, and data were measured to calculate light-incubated chlorophyll fluorescence parameters. Following a 15 min dark incubation, potential photochemical efficiency ($F_v/F_m = (F_m - F_o)/F_m$) was determined ($F_v/F_{m_{\text{noon}}}$) by applying a saturating light pulse (0.7 s, ca. $4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). F_v/F_m was also measured in the following dawn (between 3:00-6:00 hours) on the same leaves used at previous midday ($F_v/F_{m_{\text{dawn}}}$). Light-incubated chlorophyll fluorescence parameters were calculated after Schreiber et al. (1986) as follows:

1) antenna efficiency of PSII, $F_v'/F_m' = (F_m' - F_o')/F_m'$;

2) photochemical fluorescence quenching, $qP = (F_m' - F_s) / (F_m' - F_o')$;

3) PSII actual quantum yield, $\Phi_{PSII} = F_v' / F_m' \times qP$;

4) non-photochemical fluorescence quenching, $NPQ = (F_m - F_m') / F_m'$;

5) fraction of light absorbed in PSII antennae that is dissipated thermally, $D = 1 - F_v' / F_m'$.

D was calculated for *F. vaginata* and *C. dactylon* only, since in certain days $F_v / F_{m_{dawn}}$ did not recover from the previous day depression for these species, and in such cases NPQ gives a biased estimate of non-photochemical quenching (Logan et al. 2007). Daily depression of F_v / F_m was calculated as $F_v / F_{m_{depr}} = (F_v / F_{m_{dawn}} - F_v / F_{m_{noon}}) / F_v / F_{m_{dawn}} \times 100$. For each species, one sample per plot was measured. In 2005-2008, sample size for F_v / F_m , which was less time consuming to measure, was increased to 3 samples per plot at both midday and dawn for each species.

Statistical analysis

For each response variable, statistical analyses were done for each species separately, on values averaged for plots. Repeated Measures ANOVA (RMA) was used to assess variance components with a model of Treatment as fixed between effect, and Month and Year as repeated measures (within) effects. As RMA requires balanced design (e.g. Quinn & Keough 2002, Hedeker 2004), years with missing data for one of the months (Table S1, see Sampling for reasons) were omitted. Thus, the RMA dataset usually spanned four years of the study. If necessary, data were log or arcsin transformed to improve agreement with the assumptions of normality of residuals and homoscedasticity (Quinn & Keough 2002). For post-hoc comparison of means the Tukey's HSD test was used. Differences were considered significant at $p < 0.05$.

In order to assess the effect of the current and immediate past environment before measurements including both natural variations among months and years and experimental

treatments on plant response variables, multiple regression analyses were applied. These could be performed on all data including incomplete datasets of certain years. Four continuous predictor variables were used: daily minimum and maximum temperatures (T_{\min} and T_{\max} , respectively) for dates of field measurements, the sum of daily mean temperature values above 5°C in the previous 10 days for *C. dactylon* or 28 days for *F. vaginata* and *P. alba* (HeatSum), and the length of rainless (≤ 4 mm day⁻¹) period prior to the measurement (Rainless). For leaf morphology and structure, Rainless was defined as the number of rainless days during the same periods as HeatSum, which were the species-specific duration of new leaf development (Mojzes unpubl. leaf phenological observations in 2008). For leaf physiological variables, Rainless denoted the uninterrupted rainless period before field measurements. For each response variable, regression analyses were applied in two seasons separately: 1) spring (May and June, except for leaf morphology and structure of *F. vaginata* and *P. alba*, where spring was defined as April and May for leaf area, and the April-June period for the rest of variables) and 2) summer (July and August). The reason for these separate regressions was the strong effect of Month or Month \times Year interaction (i.e. seasonality) on the response variables (Tables S2, S3, S4). A General Linear Mixed Model was used, which also included Plot as a random categorical factor to meet the assumption of independence (“conditional independence”; e.g. Quinn & Keough 2002, Hedeker 2004). The significance level was set to $p < 0.01$ to avoid Type I error due to multiple comparisons. If the residuals for the dependent variable did not meet the normality assumption of the test, log or arcsin transformed data were used. For each statistical analysis, the Statistica software (version 8.0, Statsoft Inc., Tulsa, OK) was used.

Results and Discussion

Effects of experimental treatments

For each plant response variable studied, experimental treatments either had no influence or had an effect in interaction with Year and/or Month, i.e. in certain years or months only (Tables S2, S3, S4). This indicates that physiological and morphological responses of the three species to the treatments were relatively modest and mostly appeared for a limited period. Three factors may explain this. Firstly, nocturnal warming increased air temperature only moderately. In the same climate manipulation experiment as ours in a Mediterranean shrubland, a similar degree of warming treatment also imposed only moderate or season-dependent changes in the physiology of the dominant species (Prieto et al. 2009a, Liu et al. 2016). Climate change simulation studies that observed considerable warming effects on leaf growth or net assimilation rate applied greater (4-10°C) elevation of temperature (Parsons et al. 1994, Turnbull et al. 2004, Wertin et al. 2015). Secondly, drought effect on certain plant traits was detectable during or just after the two-month rain exclusion period, but after stopping this treatment no lasting influence appeared. This indicates that leaf ecophysiological traits have a relatively great ability to recover after non-drastic temporary changes in moisture conditions. Thirdly, due to the markedly changeable weather during our study, the comparison of treated and control plots could not always reflect truly the targeted difference in water or temperature conditions (e.g. the drought treatment in the dry May and June 2003, when control plots also underwent unintended water shortage). To overcome this common shortcoming, some recent climate manipulation field experiments include a historical control, in addition to the ambient control, which provides long-term average climatic conditions (Jentsch et al. 2007, Beier et al. 2012). Moderate ecophysiological responses of the three dominant species to the experimental treatments are in line with that neither total vegetation abundance nor species richness were affected by warming or drought treatment in this

experiment either in the short- or long-term (averaged across the first 5 years or 11 years (2002-2012) of manipulations, respectively; Kröel-Dulay et al. 2015). This reflects the high resilience of the system.

Insert Fig. 2 here.

The most marked effects of our experimental treatments were detectable in different leaf traits for the three species studied: morphology for *P. alba*, photochemistry and coarse structure for *C. dactylon* and gas exchange for *F. vaginata*. This suggests that ecophysiological traits most sensitive to the predicted climatic shifts may vary among the dominant species or functional types of a plant community. The leaf length to width ratio for *P. alba* was the only exception when Treatment was significant as main effect (Table S2): under nocturnal warming *P. alba* developed more elongated leaf blade ($L/W = 1.120 \pm 0.009$ averaged across years and months) than in the control (1.056 ± 0.007) or drought plots (1.065 ± 0.010 ; $p < 0.0001$). This is consistent with the marked temperature response of leaf shape for this species (discussed later). For the leaf area of *P. alba* significant Year \times Month \times Treatment interaction was found (Table S2), due to smaller leaves developed in the drought plots compared to the control or warming plots in June 2005 (Fig. 2). For *C. dactylon*, marked treatment effect was observed for leaf photochemistry in interaction with Year or Year and Month (Table S3). This was due to decreased photochemical (qP) or increased non-photochemical fluorescence quenching (NPQ) compared to control in June or July of a particular year (e.g. strong drought treatment effect on qP in June 2008: control: 0.564 ± 0.047 ; drought: 0.301 ± 0.060 ; $p = 0.0203$). Similar to our results, rain exclusion decreased photochemical fluorescence quenching and increased non-photochemical (thermal) energy dissipation in summer for two dominant perennial C₄ grasses in a mixed-grass prairie (Maricle & Adler 2011). Among the three species in our study, only *F. vaginata* exhibited pronounced treatment effects in leaf gas exchange (Table S4). For example, rain exclusion

reduced both transpiration rate (E) and stomatal conductance for water vapor (g_s) in July 2005 and 2008 compared to control or warming plots (Fig. 3; Year \times Month \times Treatment interactions). In contrast, E and g_s of the two other species did not differ significantly between treatments and control in the same periods (Fig. 3). Consistently, in a Mediterranean shrubland, the effect of drought treatment similar to ours on the actual efficiency of PSII and/or leaf gas exchange was also dependent on species, season and year (Llorens et al. 2003, Prieto et al. 2009a). However, contrasted with the unresponsiveness of leaf gas exchange for *P. alba* in our study, a similar degree of experimental warming in that Mediterranean shrubland increased the net photosynthetic rate for *Globularia alypum* in a cold winter, while decreased it for *Erica multiflora* in the dry and hot summer of 2003 (Prieto et al. 2009a). In addition, enhanced photosynthetic rate of the congeneric *Populus deltoides* was found in response to a greater (10°C) increase in night-time temperature in a short-term experiment (Turnbull et al. 2004). The responses of *P. alba* to the simulated climate change were probably underestimated in our plot-scale study as experimental treatments imposed on shrub-sized root suckers could not be extended to the connected parent tree. Nevertheless, the nature and direction of the responses observed for this species are still instructive, since the background weather fluctuation, the dominant environmental influence (see the next section) affected both the parent tree and the root suckers the same.

Insert Fig. 3 here.

Plant responses to variation in temperature and precipitation

In contrast to treatments, months and/or years or their interaction had significant effect on most variables for each species (Tables S2, S3, S4). This indicates that interannual and between-month differences in weather elicited more pronounced responses of leaf morphology and physiology of the three species than the experimental manipulations did. In

many cases, these differences accounted for the unusually warm and dry spring (April) and summer of 2007 (Fig. 1). For example, $F_v/F_{m_{\text{dawn}}}$ was lower in July 2007 (and also in August 2007 for *P. alba*) compared with the other years or months (Year \times Month interactions: Table S3, Fig. 4). The substantially lower leaf size of *P. alba* in June 2007 than in other years also reflected this effect (Fig. 2; despite Year \times Month \times Treatment interaction discussed above). Due to the high variation in background weather relative to climate manipulation during our study, species responses to climate could be obtained more realistically by examining the effects of temperature and precipitation that included both natural seasonal and interannual fluctuations and experimental treatments.

Insert Fig. 4 and Table 2 here.

Responses to temperature

Temperature during leaf development (HeatSum) in spring had strong and consistently positive partial correlations with leaf area of the species studied (Table 2). This indicates that with warmer springs temperature limitation on leaf growth was ameliorated. Leaf cell elongation is considered to be highly temperature dependent with high Q_{10} values (e.g. 1.8-6.6 for other perennial C_3 and C_4 grasses between 5 (10) °C and 20°C; Thomas & Stoddart 1995, Clifton-Brown & Jones 1997). In addition, *C. dactylon* and *P. alba* developed slenderer leaves in response to increased spring temperature, which is consistent with the greater optimum temperature for the maximum length than for maximum width of leaves, as it was documented for other grasses (Langer 1979). Similar to our results, experimental warming also increased leaf area (Parsons et al. 1994, Hudson et al. 2011) or modified leaf shape (Llorens et al. 2002) in certain dominant species of other shrubland ecosystems. In our experiment, leaf coarse structure was also highly responsive to temperature in spring, even for *C. dactylon* despite structural and functional constraints associated with C_4 leaf anatomy

(Sage & McKown 2006). LMA positively correlated with HeatSum, mostly (*P. alba*) or entirely (*C. dactylon*) due to greater density of leaves (Table 2). This relationship may possibly be the consequence of greater leaf size, as larger leaves often require a greater structural investment to build and maintain a unit of leaf area than smaller leaves (Milla & Reich 2007). However, various responses of LMA were detected in other climate warming experiments including increase, decrease or no change (Parsons et al. 1994, Suzuki & Kudo 1997, Hudson et al. 2011).

Insert Table 3 here.

Regarding leaf physiology, the temperature response of *C. dactylon* was detected most clearly in leaf gas exchange variables. Net photosynthetic rate consistently correlated positively with each temperature variable in spring (with T_{\max} also in summer; Table 3). Water transport (E and g_s) also increased with T_{\min} (i.e. after warmer nights) in both seasons, particularly in spring. These relationships suggest strong low temperature limitation on the leaf gas exchange of this warm-season C_4 grass. Simultaneously, both E and g_s declined with increasing HeatSum and T_{\max} (i.e. during unusually warm periods or hot days, respectively) in spring (Table 3). However, the positive relationships of A with these temperature variables indicate the capacity of *C. dactylon* for high photosynthetic rate at high temperatures, as it was documented for C_4 plants (Percy & Ehleringer 1984). For this grass, only spring temperature response was detected in VAZ pigments (Δ PRI and PRI) among the leaf spectral reflectance indices, while leaf photochemistry responded only to T_{\max} (Table 4). The total VAZ pool size (Δ PRI) correlated negatively with T_{\min} and T_{\max} , while VAZ pigments in photoprotective deepoxidated state (PRI) associated positively with T_{\min} . Consistently, lower daily temperature maxima in spring elicited higher NPQ. These relationships indicate enhanced photoprotection on cool days, which could efficiently protect PSII against photoinhibition during cold stress. However, Δ PRI positively correlated with HeatSum, which

suggests greater VAZ pool size also in unusually warm periods during spring. In addition, when the daily peak temperature increased, Fv/Fm showed greater diurnal amplitude in both seasons and a lower daily maximum in summer (Table 4). For *F. vaginata* in spring, E and g_s showed similar relationships with T_{\min} and T_{\max} to those observed for *C. dactylon*, but net photosynthetic rate decreased with increasing T_{\max} and did not associate with T_{\min} or HeatSum (Table 3). These results show that low daily minimum temperatures in spring restricted the water loss, but not the photosynthesis of *F. vaginata*. This is consistent with previous studies reporting that this grass exhibits a considerable net CO₂ assimilation rate even in winter (Tuba et al. 2008). As observed for *F. vaginata*, both g_s and A showed negative relationships with T_{\max} in spring also for *P. alba*. Thus, unlike *C. dactylon*, the photosynthesis of the two C₃ species was negatively influenced by high daily peak temperatures in spring. Furthermore, in *F. vaginata*, T_{\max} in spring also showed marked negative associations with maximal (Fv/Fm_{noon}) and actual photochemical efficiencies (Φ PSII, Fv'/Fm', qP), and a positive correlation with non-photochemical thermal energy dissipation (D; Table 4). Probably the unusually hot May and June of 2003 and the April-June period of 2007 (Fig. 1b) played an important role in these relationships. Strong negative correlations were found between soil moisture content and T_{\max} both under bare soil and at the base of *F. vaginata* (Table 3), which indicated that the temperature effects described above might – at least partly – be mediated by moisture limitation. These results suggest that increases in daily maximum temperatures in spring projected for the next decades (Bartholy et al. 2014) will probably restrict the carbon assimilation capacity of the cold-tolerant *F. vaginata*. In line with our findings, early-season net photosynthetic rate was also reduced by a 4°C increase in soil surface temperature for the C₃ grass *Achnatherum hymenoides*, but was unaffected for the C₄ grass *Pleuraphis jamesii* in a cool desert (Wertin et al. 2015). For *P. alba*, both low and high temperatures appeared to exert a major control on leaf physiology. Both mND (chlorophyll

content) and PRI, as well as the actual photochemical efficiency (Φ_{PSII} , F_v'/F_m') showed opposite associations with HeatSum in the two seasons: positive in spring and negative in summer (Table 4). Consistently, HeatSum negatively correlated with NPQ in spring, and with qP and $F_v/F_{m_{dawn}}$ in summer. These imply that warmer periods enhanced the photochemical performance of *P. alba* in spring, but impeded it in summer. However, positive correlation was detected between $F_v/F_{m_{dawn}}$ and T_{min} in both spring and summer, which indicated that the low temperature limitation for the recovery of F_v/F_m to the daily maximum value manifested throughout the measurement period. These results suggest that warmer periods in spring under predicted climate change will enhance the capacity of this species to convert light energy to fixed carbon. Springtime temperature limitation for *P. alba* is in line with the South Eurasian affinity of this species, and is supported by earlier budbreak in spring in warmed plots relative to control in the same experiment (Prieto et al. 2009b). Consistent with our results, the negative impact of low temperature (or the positive influence of warming treatment) on photochemical performance (F_v/F_m) was reported for other thermophilous ((sub)tropical or Mediterranean) woody species (Llorens et al. 2003, Weng et al. 2006, Ogaya et al. 2011).

Insert Table 4 here.

Responses to precipitation

In spring, precipitation exerted pronounced impact only on the leaf morphology of the three species studied. The number of rainless days during leaf development was negatively associated with the leaf area of *F. vaginata* and *P. alba*, or with the length to width ratio for *C. dactylon* (Table 2). These negative effects might influence the marked positive response of leaf morphology to spring temperature (described above).

In summer, the most conspicuous precipitation response of each species was that leaf gas exchange variables consistently showed negative correlations with Rainless, except for A and WUE for *P. alba* (Table 3). This reflects a pronounced and uniform water availability control over leaf water relations (for each species) and photosynthesis (for the two grasses) in summer. In spring, such control was invariably absent. Similarly, Llorens et al. (2004) reported water availability (the accumulated rainfall during the growth period) as a major factor controlling photosynthetic performance of ericaceous shrubs in a network of similar climate change experiments along a European gradient. In our study, the strongest limitation elicited by longer rainless period in summer was observed for *F. vaginata*, which manifested also in leaf pigment content and photochemistry. As the length of drought period increased, PRI was lower (more negative) and Δ PRI was larger together with an increase in D (Table 4). However, longer rainless period decreased chlorophyll content (mND), and consistently reduced both maximal ($F_v/F_{m_{noon}}$, $F_v/F_{m_{dawn}}$) and actual (Φ PSII, qP, F_v'/F_m') photochemical efficiencies. These relationships indicate that despite greater photoprotection through an increased level of VAZ pigments and thermal energy dissipation, photodamage might have appeared under serious water stress with lasting droughts in both the light-harvesting antennae and the reaction centers of PSII. The unresponsiveness of the dawn to midday amplitude of F_v/F_m ($F_v/F_{m_{depr}}$) and NPQ is probably due to that both $F_v/F_{m_{noon}}$ and $F_v/F_{m_{dawn}}$ declined with persisting drought (e.g. in July 2003; Fig. 5). In line with our results, Tuba (1984) found an increased amount of protective carotenes and the conversion of violaxanthin to antheraxanthin in *F. vaginata* in response to drought during subsequent dry and wet periods. In our study, contrasted with *F. vaginata*, there wasn't any effect of Rainless on the leaf pigment content and photochemical activity of *C. dactylon* and *P. alba* in summer (Table 4), which indicated that these two species could withstand drought periods with less restriction of metabolism.

The greatest drought sensitivity of *F. vaginata* among the species studied might be explained by its shallow and fibrous (i.e. laterally not extensive) root system (Magyar 1933, Simon & Batanouny 1971). In our experiment, topsoil water content was uniformly negatively associated with the length of rainless period in both seasons (Table 3), reflecting quick drying of soil surface between rains. Thus, the physiological activity and growth of *F. vaginata* practically relies on current precipitation. Drought responses of this grass correspond with the bimodal annual course of photosynthetic activity and phytomass production (Kovács-Láng 1974, Kovács-Láng et al. 1989) reaching maxima prior to and after the hot and dry midsummer period (in spring and early summer, and in autumn). A deeper root system and clonal integration for *C. dactylon* (through both stolons and rhizomes), and especially for *P. alba* (Magyar 1933, Forde 1966, Krízsik & Körmöczi 2000) may provide a greater ability for these species to reach water resources in deeper soil layers and exploit spatial environmental heterogeneity. Consistently, in other studies in arid or semiarid habitats, shallow rooted plants were found to be more constrained by severe drought than deep rooted species (Schwinning et al. 2005, Wertin et al. 2015), however such difference can be buffered physiologically (Fay et al. 2002). In our experiment, the effective source-to-sink assimilate transport of *C. dactylon* (Forde 1966) and the production of root suckers of *P. alba* may reduce end-product limitation on photosynthesis. Furthermore, in a pot experiment with rooted cuttings of *P. alba*, Imada et al. (2008) found high plasticity in the fine root growth in response to variations in water-table depth and soil water profile. However, lasting droughts in our study, which probably depleted water deeper in the soil, could substantially restrict leaf expansion for *P. alba* too during the peak growth period of this species. This was most pronounced in 2007 (Fig. 2), after an eight-month-long water shortage (between September 2006 and April 2007). For *C. dactylon*, higher water use efficiency due to its C₄ photosynthesis (Percy & Ehleringer 1984) might have also helped this grass to maintain high photochemical activity even during dry periods in

summer. Consistent with our results, C₃ grasses were found to be the most drought-sensitive plant functional type compared with C₄ grasses and (C₃ or C₄) shrubs experiencing a 4-year experimental press-drought during which a natural pulse-drought occurred in drylands of Colorado Plateau (Hoover et al. 2015).

Relationships between physiological variables

Leaf chlorophyll content (mND) positively correlated with A, and with leaf water loss (E and/or g_s) consistently in each species (most strongly in *F. vaginata*), and with maximal (F_v/F_m_{dawn} and/or F_v/F_m_{noon}) and actual photochemical efficiencies (ΦPSII, F_v'/F_m', qP) in *F. vaginata* and *P. alba* (Table 5). These relationships indicate that decreased chlorophyll content might have contributed to the restricted leaf photochemical performance and assimilation rate under temperature and/or water stress. Similar to our results, positive relationships were detected between leaf chlorophyll content and net photosynthetic rate for *Pinus densiflora* seedlings in an open-field climate change experiment (Yun et al. 2016) and for evergreen chaparral shrubs under contrasting seasonal temperature and moisture conditions (Stylinski et al. 2002). Also in each species studied here, higher PRI (i.e. lower level of deepoxidated photoprotective pigments) was associated with higher net photosynthetic rate and maximum photochemical efficiency (F_v/F_m_{noon} and/or F_v/F_m_{dawn}; Table 5). Similar relationships with PRI also appeared for stomatal conductance and the actual photochemical efficiency of PSII (ΦPSII, qP and/or F_v'/F_m') for *F. vaginata* and *P. alba*. Such correlations might reflect coordinated regulation of the capacity for CO₂ fixation with changes in environmental conditions, as found in other studies at different timescales and plant functional types (Peñuelas et al. 1995, Stylinski et al. 2002, Weng et al. 2006, Zhang et al. 2016). ΔPRI negatively correlated with F_v/F_m_{dawn} in *C. dactylon* and *P. alba*, and with E and g_s in *F. vaginata* and *P. alba*, which might have also contributed to this regulation. These

indicate higher photoprotective VAZ pigment pool when lower leaf water loss impends stomatal limitation of photosynthesis, or when the nocturnal regeneration of maximum photochemical efficiency is incomplete. Consistently, Δ PRI is positively associated with NPQ in *P. alba* (Table 5). Weng et al. (2006) also detected positive relationships between these two variables for tree species adapted to different temperature regimes. In addition, Φ PSII and Fv/Fm measured at leaf level for dominant species showed positive relationships with canopy level reflectance indices (PRI and NDVI) in climate simulation experiments along a north-south climatic gradient including our site (Mänd et al. 2010).

Insert Table 5 here.

Ecophysiological responses to extreme weather in 2003 summer

A rainless period lasting for 73 days in drought plots and 48 days in control and warming plots associated with high temperatures (Fig. 1b) prior to measurement in July 2003, strongly reduced the potential photochemical efficiency of PSII both at midday ($F_v/F_{m_{noon}} = 0.55-0.63$) and at dawn ($F_v/F_{m_{dawn}} = 0.63-0.71$) for *F. vaginata* in both control and treated plots (Fig. 5). This means that the daily depression of Fv/Fm did not completely recover to its optimum value (~ 0.8) during night. It could be accounted for increased photoprotection (i.e. maintenance of high levels of deepoxidated VAZ carotenoids) and/or photodamage (i.e. the accumulation of inactive PSII reaction centers). This restricted photochemical capacity might have contributed to the unprecedented dieback of this species in the area in 2003 (Kovács-Láng et al. 2006). In contrast, Fv/Fm remained high ($0.74-0.77$ at noon and $0.83-0.86$ at dawn) for this species in July 2005, when the weather conditions between April and June were close to the average (Figs. 1, 5). Resco et al. (2008) also reported low predawn Fv/Fm due to lasting photoinhibition after prolonged (a 2-month) drought, which was accompanied by $>75\%$ loss of mean maximum assimilation rate for two perennial C_4 grasses in the Sonoran

Desert. In our study, contrary to *F. vaginata*, *C. dactylon* and *P. alba* exhibited high values of $F_v/F_{m_{\text{dawn}}} (\geq 0.82)$ and $F_v/F_{m_{\text{noon}}} (> 0.72)$ even following a hot and dry period of 2003 (Fig. 5). Consistent with our result, the net photosynthetic rate of *P. alba* remained similar in the drier and warmer summer of 2003 to that in the same period of 2002 in another semiarid environment (Manzanera & Martínez-Chacón 2007). However, a marked decline in annual shoot growth was observed for *P. alba* in a simultaneous study at our experimental site in 2003 compared with previous and subsequent years (Peñuelas et al. 2007).

Insert Fig. 5 here.

Conclusions

Our six-year field experiment indicates that high seasonal and interannual variability of weather – including extremes – have more profound influence on the ecophysiology of the dominant species representing different functional types in the Pannonian sand forest-steppe than moderate nighttime warming or an extended drought in the peak vegetation period do. This could be important, as climatic irregularities and extremes will act on the top of gradual climatic changes, thus interacting with (intensifying or ameliorating) their effects. In line with our hypothesis, we could document that the three functional types in our study responded differently to such integrated changes in temperature and precipitation. Shallow rooted sclerophyllous C_3 bunch grasses adapted to regular midsummer drought (*F. vaginata* in our study) appear to be the most susceptible to irregularities of climate. Thus, the abundance of this functional type is expected to decline in the Pannonian forest-steppe under the forecasted more extreme climate (e.g. greater frequency of dry spells and heat waves). Indeed, the cover of this species has declined to half during a decade (from 66% in 2002 to 30% in 2012 in

average across treatments and control; Kröel-Dulay et al. unpubl. data). However, shading by *P. alba* suckers and trees in the natural mosaic of this vegetation may reduce the mortality of *F. vaginata* and allow it to recolonize open patches in climatically more favourable periods. (Kovács-Láng et al. 2006). Plants having deeper roots and clonal integration (*C. dactylon* and *P. alba* in our experiment) will probably be less hindered by such climatic anomalies. Moreover, C₄ photosynthesis or southern geographical distribution is at an advantage in a warming climate in the temperate zone. Thus, the relative abundance of these two functional types is anticipated to diminish to a smaller degree or even increase in the future, particularly at the expense of *F. vaginata* considering its sluggish regeneration following drought (Kovács-Láng et al. 2006). In line with this, for *C. dactylon* and *P. alba*, cover in 2012 did not differ significantly from that in 2002 (Kröel-Dulay et al. unpubl. data). The predicted changes may result in a decline in functional diversity of the community, with diminishing its stability and functions, thus enhance its susceptibility to climate change. However, at ecosystem level, fluctuations in the biomass production of dominant bunch grasses under a warmer, drier and more changeable climate might be buffered to some extent by the productivity of the two other functional types (Chapin et al. 1995, Zavaleta et al. 2003).

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Table and figure legends

Table 1. Selected traits of the three plant species studied. References are Magyar (1933), Simon & Batanouny (1971) and Krízsik & Körmöczi (2000) for belowground structures, Euro+Med (2006-) for geographical distribution, and Forde (1966), Kalapos (1989, 1991) and Kovács-Láng et al. (1989) for the other traits.

Table 2. Partial correlations from Mixed Model Multiple Regressions for variables describing leaf morphology (Area and L/W) and coarse structure (LMA, LT and LD) with two environmental variables (HeatSum = the sum of daily mean temperature values above 5°C, and Rainless = the number of days with precipitation ≤ 4 mm day⁻¹) for the preceding 28 days (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*) before sampling. Other abbreviations as in Table S1. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

Table 3. Partial correlations from Mixed Model Multiple Regressions for leaf gas exchange parameters (E , g_s , A , WUE) and soil moisture content (SWC) with four environmental variables (HeatSum, T_{\min} , T_{\max} , Rainless). Daily minimum and maximum temperature (T_{\min} and T_{\max} , respectively, °C) for dates of field measurements, sum of daily mean temperature values above 5°C for the preceding 28 days (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*, HeatSum, °C) and the length of uninterrupted rainless (≤ 4 mm day⁻¹) period (Rainless, days) before field measurement. Other abbreviations as in Tables S1 and S4. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

Table 4. Partial correlations from Mixed Model Multiple Regressions for leaf pigment content indices (mND, PRI, Δ PRI) and chlorophyll fluorescence variables ($F_v/F_{m_{dawn}}$, $F_v/F_{m_{noon}}$, $F_v/F_{m_{depr}}$, Φ PSII, F_v'/F_{m}' , qP, NPQ, D) with four environmental variables (HeatSum, T_{min} , T_{max} , Rainless). Abbreviations as in Tables 3 and S1. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

Table 5. Correlation coefficients (Pearson r values) among physiological variables studied. Abbreviations as in Table S1. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

Figure 1. Deviation of monthly precipitation (**a**) and mean temperature (**b**) from the eight-year (2001-2008) average for the months from April to July in the years of our study (2003-2008) at the standard meteorological station near Fülöpháza, 1 km from the experimental site.

Figure 2. Leaf area of *P. alba* in early June in years from 2005 to 2008 (measurement was not made in 2003 and 2004). Different letters indicate significant ($p < 0.05$) differences: lower case letters between years within control or each treatment, upper case letters between treatments in 2005. Error bars show ± 1 SE.

Figure 3. Stomatal conductance for water vapor (g_s) in July 2005, 2006 and 2008. Different letters indicate significant ($p < 0.05$) differences for each species separately: lower case letters between years within control or each treatment, upper case letters between treatments for *F. vaginata* in 2005 and 2008. Error bars show ± 1 SE.

Figure 4. Potential photochemical efficiency of PSII (Fv/Fm) at dawn between June and August 2005, 2007 and 2008. Control and treatment values were pooled as no treatment effect was detected (Table S3). Different letters indicate significant ($p < 0.05$) differences for each species separately: lower case letters between years in a particular month, upper case letters between months in 2008 and/or 2007. Error bars show ± 1 SE.

Figure 5. Potential photochemical efficiency of PSII (Fv/Fm) at dawn and at noon in July 2003 and 2005. Error bars show ± 1 SE.

Tables

Table 1. Selected traits of the three plant species studied. References are Magyar (1933), Simon & Batanouny (1971) and Krízsik & Körmöczi (2000) for belowground structures, Euro+Med (2006-) for geographical distribution, and Forde (1966), Kalapos (1989, 1991) and Kovács-Láng et al. (1989) for the other traits.

Trait	<i>Cynodon dactylon</i> (L.) Pers.	<i>Festuca vaginata</i> Waldst. & Kit. ex Willd.	<i>Populus alba</i> L.
Life form	perennial grass, both stoloniferous and rhizomatous	perennial grass, bunch-type	deciduous tree, shrub-sized root suckers studied
Geographical distribution	worldwide from tropics to warm temperate	Pannonian subendemism	South Eurasian
Status in the studied ecosystem	frequent gap occupant	matrix forming dominant xerophyte	woodland dominant tree, encroaches grasslands
Photosynthesis type	C ₄	C ₃	C ₃
Leaf blade morphology	relatively short, flat	sclerenchymatous, narrow involute	romboid to rounded, petiolate
Belowground structures	rhizomes, fibrous root system	fibrous root system	taproot, subsurface (20- 30 cm) laterals extending up to 20 m from the tree
Main (maximum) rooting depth (cm)	20-50 (80)	5-20 (60)	60-100 (200)
Timing of growth	starts late spring, peak in summer	peak in spring and autumn, adapted to midsummer drought	foliage sustains from spring/early summer till autumn

Table 2. Partial correlations from Mixed Model Multiple Regressions for variables describing leaf morphology (Area and L/W) and coarse structure (LMA, LT and LD) with two environmental variables (HeatSum = the sum of daily mean temperature values above 5°C, and Rainless = the number of days with precipitation ≤ 4 mm day⁻¹) for the preceding 28 days (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*) before sampling. Other abbreviations as in Table S1. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

	HeatSum		Rainless	
	Spring	Summer	Spring	Summer
<i>Cynodon dactylon</i>				
Area	0.42	0.17	-0.19	0.48
L/W	0.75	0.27	-0.51	0.50
LMA	0.53	0.11	0.11	0.09
LT	-0.33	-0.03	0.14	0.16
LD	0.66	0.18	-0.13	-0.11
<i>Festuca vaginata</i>				
Area	0.65	-0.17	-0.75	0.05
L/W	NA	NA	NA	NA
LMA	0.59	0.35	0.05	-0.34
LT	NA	NA	NA	NA
LD	NA	NA	NA	NA
<i>Populus alba</i>				
Area	0.91	-0.45	-0.83	0.42
L/W	0.54	0.06	-0.14	0.00
LMA	0.76	-0.05	-0.24	-0.15
LT	0.29	0.34	-0.15	0.02
LD	0.65	-0.28	-0.12	-0.09

Table 3. Partial correlations from Mixed Model Multiple Regressions for leaf gas exchange parameters (E, g_s , A, WUE) and soil moisture content (SWC) with four environmental variables (HeatSum, T_{min} , T_{max} , Rainless). Daily minimum and maximum temperature (T_{min} and T_{max} , respectively, °C) for dates of field measurements, sum of daily mean temperature values above 5°C for the preceding 28 days (*F. vaginata*, *P. alba*) or 10 days (*C. dactylon*, HeatSum, °C) and the length of uninterrupted rainless (≤ 4 mm day⁻¹) period (Rainless, days) before field measurement. Other abbreviations as in Tables S1 and S4. Significant ($p < 0.01$ in this analysis) correlations are in boldface.

	HeatSum		T_{min}		T_{max}		Rainless	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
<i>Cynodon dactylon</i>								
E	-0.85	0.11	0.78	0.41	-0.64	-0.05	0.25	-0.37
g_s	-0.70	0.20	0.70	0.34	-0.66	-0.01	0.20	-0.47
A	0.66	0.26	0.71	-0.24	0.64	0.43	0.35	-0.45
WUE	0.62	0.26	-0.22	-0.52	-0.06	0.49	0.06	-0.35
SWC	-0.09	0.44	0.23	0.33	-0.27	-0.35	-0.39	-0.57
<i>Festuca vaginata</i>								
E	-0.18	0.08	0.59	-0.11	-0.57	0.26	-0.25	-0.56
g_s	-0.23	0.14	0.54	-0.21	-0.62	0.31	-0.36	-0.54
A	0.00	0.11	0.26	-0.39	-0.50	0.43	-0.40	-0.54
WUE	0.25	-0.01	-0.27	-0.23	-0.11	0.20	-0.31	-0.55
SWC	0.53	-0.14	-0.24	0.27	-0.57	-0.29	-0.40	-0.40
<i>Populus alba</i>								
E	0.13	-0.01	0.09	-0.14	-0.18	0.27	0.19	-0.40
g_s	0.39	-0.07	-0.05	-0.04	-0.48	0.11	0.07	-0.45
A	0.20	-0.13	0.12	0.08	-0.33	-0.01	-0.07	0.21
WUE	-0.03	-0.22	0.14	0.05	-0.07	-0.16	-0.16	0.15
SWC	0.54	-0.02	-0.36	0.26	-0.61	-0.34	-0.41	-0.58

Table 4. Partial correlations from Mixed Model Multiple Regressions for leaf pigment content indices (mND, PRI, Δ PRI) and chlorophyll fluorescence variables (Fv/Fm_{dawn}, Fv/Fm_{noon}, Fv/Fm_{depr}, Φ PSII, Fv'/Fm', qP, NPQ, D) with four environmental variables (HeatSum, T_{min}, T_{max}, Rainless). Abbreviations as in Tables 3 and S1. Significant (p < 0.01 in this analysis) correlations are in boldface.

	HeatSum		T _{min}		T _{max}		Rainless	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
<i>Cynodon dactylon</i>								
mND	-0.20	0.16	0.13	-0.14	-0.07	0.28	0.25	-0.18
PRI	-0.17	-0.01	0.49	0.00	-0.28	-0.04	-0.09	-0.20
Δ PRI	0.48	0.08	-0.62	-0.08	-0.30	0.12	-0.14	-0.03
Fv/Fm _{dawn}	-0.24	-0.14	0.16	0.00	0.25	0.07	-0.10	-0.08
Fv/Fm _{noon}	0.14	-0.13	-0.15	0.16	-0.23	-0.39	-0.03	0.10
Fv/Fm _{depr}	-0.12	0.04	0.25	-0.09	0.34	0.42	-0.13	-0.06
Φ PSII	-0.21	-0.21	-0.09	-0.13	0.09	0.04	-0.10	-0.16
Fv'/Fm'	0.03	-0.13	-0.15	-0.17	0.00	0.00	-0.01	0.06
qP	-0.29	-0.20	-0.01	-0.12	0.13	0.07	-0.11	-0.24
NPQ	0.27	-0.07	0.10	0.00	-0.44	-0.20	0.02	0.27
D	-0.03	0.13	0.15	0.17	0.00	0.00	0.01	-0.06
<i>Festuca vaginata</i>								
mND	-0.14	0.08	0.11	0.08	-0.06	0.22	-0.21	-0.55
PRI	-0.09	-0.16	0.27	0.25	-0.32	-0.04	-0.22	-0.57
Δ PRI	0.25	-0.39	-0.26	0.10	-0.37	-0.33	0.03	0.50
Fv/Fm _{dawn}	-0.05	-0.33	0.18	0.07	-0.30	-0.06	0.17	-0.48
Fv/Fm _{noon}	-0.17	-0.19	0.13	-0.09	-0.40	-0.01	0.05	-0.63
Fv/Fm _{depr}	-0.03	-0.03	0.15	0.17	0.16	0.00	0.12	0.14
Φ PSII	-0.23	0.04	0.33	-0.16	-0.57	0.24	0.00	-0.74
Fv'/Fm'	0.04	-0.14	0.10	0.02	-0.56	0.06	-0.05	-0.55
qP	-0.38	0.27	0.45	-0.31	-0.44	0.37	0.06	-0.65
NPQ	0.19	-0.21	0.00	0.06	-0.24	-0.30	-0.10	0.29
D	-0.04	0.14	-0.10	-0.02	0.56	-0.06	0.05	0.55
<i>Populus alba</i>								
mND	0.62	-0.48	-0.41	0.13	-0.06	0.15	-0.20	0.09
PRI	0.50	-0.37	-0.13	0.14	-0.43	0.00	-0.29	-0.24
Δ PRI	-0.25	-0.22	-0.04	0.15	0.19	-0.35	-0.22	0.27
Fv/Fm _{dawn}	0.00	-0.48	0.51	0.34	0.13	-0.12	0.06	-0.11
Fv/Fm _{noon}	0.07	-0.29	0.07	0.25	-0.21	-0.17	0.13	-0.10
Fv/Fm _{depr}	-0.14	0.07	0.21	-0.13	0.31	0.11	-0.13	0.00
Φ PSII	0.46	-0.44	-0.20	0.01	-0.26	0.05	-0.19	-0.08
Fv'/Fm'	0.57	-0.41	-0.24	0.01	-0.46	-0.03	-0.18	-0.20
qP	0.22	-0.32	-0.11	0.04	0.04	0.08	-0.15	0.03
NPQ	-0.47	0.08	0.52	0.25	-0.17	-0.23	0.06	0.13

Table 5. Correlation coefficients (Pearson r values) among physiological variables studied. Abbreviations as in Table S1.

Significant ($p < 0.01$ in this analysis) correlations are in boldface.

	E	g_s	A	WUE	Fv/Fm _{dawn}	Fv/Fm _{noon}	Fv/Fm _{depr}	Fv'/Fm'	Φ PSII	qP	NPQ
<i>Cynodon dactylon</i>											
PRI	0.12	0.14	0.30	0.15	0.28	-0.09	0.19	-0.07	0.13	0.23	0.01
Δ PRI	-0.14	0.13	-0.04	0.12	-0.34	0.02	-0.17	-0.17	-0.04	0.06	0.24
mND	0.20	0.33	0.34	0.08	-0.02	-0.17	0.16	-0.25	-0.02	0.16	0.05
<i>Festuca vaginata</i>											
PRI	0.24	0.31	0.29	0.25	0.19	0.37	-0.38	0.39	0.34	0.13	-0.16
Δ PRI	-0.36	-0.29	-0.15	-0.04	-0.09	0.07	-0.15	-0.11	-0.20	-0.21	0.20
mND	0.59	0.60	0.49	0.21	0.20	0.34	-0.30	0.46	0.53	0.38	-0.23
<i>Populus alba</i>											
PRI	0.08	0.22	0.41	0.18	0.28	0.44	-0.28	0.41	0.38	0.28	0.02
Δ PRI	-0.46	-0.27	0.06	0.40	-0.30	-0.20	0.02	-0.18	-0.09	-0.00	0.25
mND	0.21	0.06	0.30	0.02	0.53	0.30	0.01	0.36	0.46	0.43	-0.16

Figures

Figure 1.

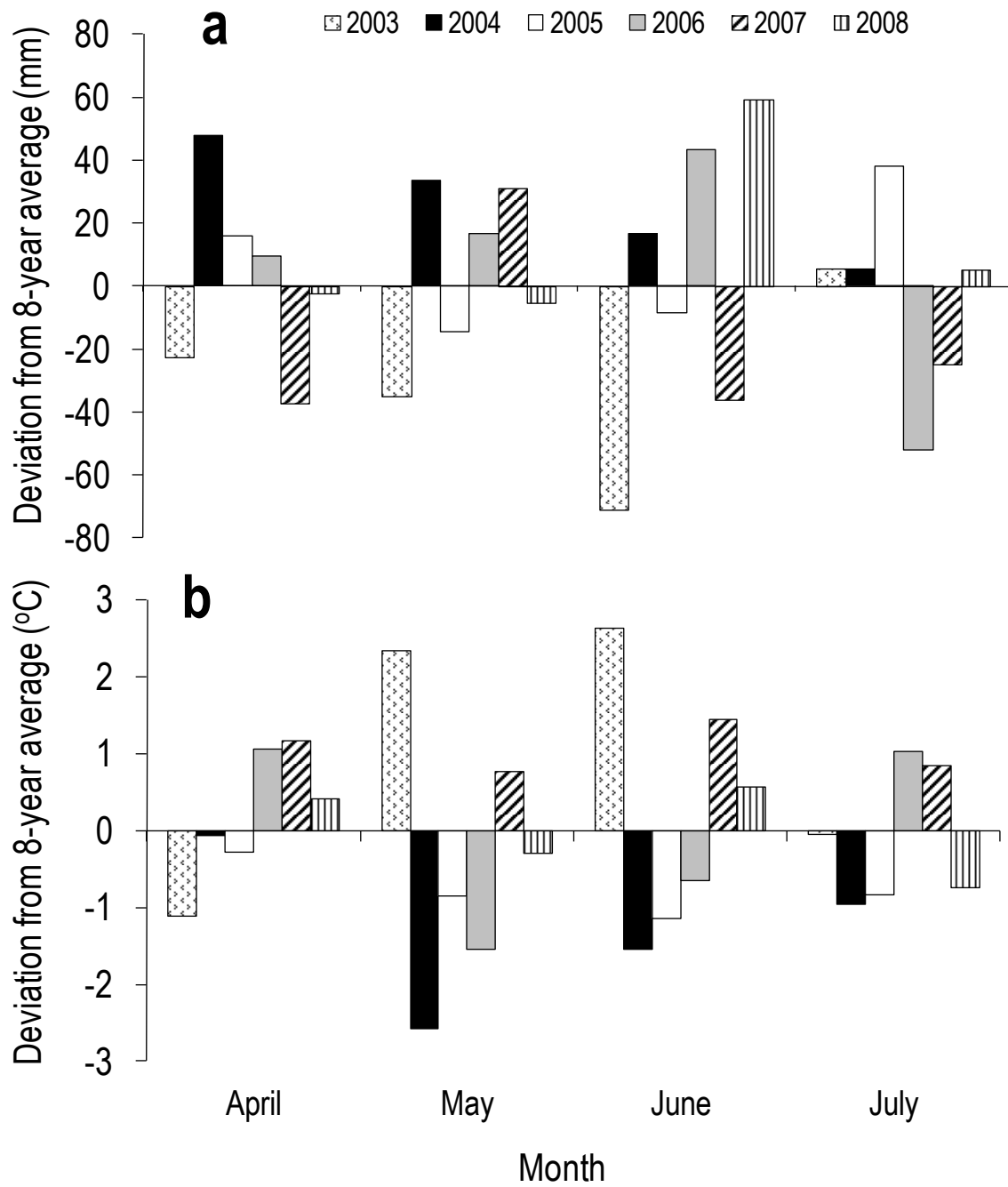


Figure 2.

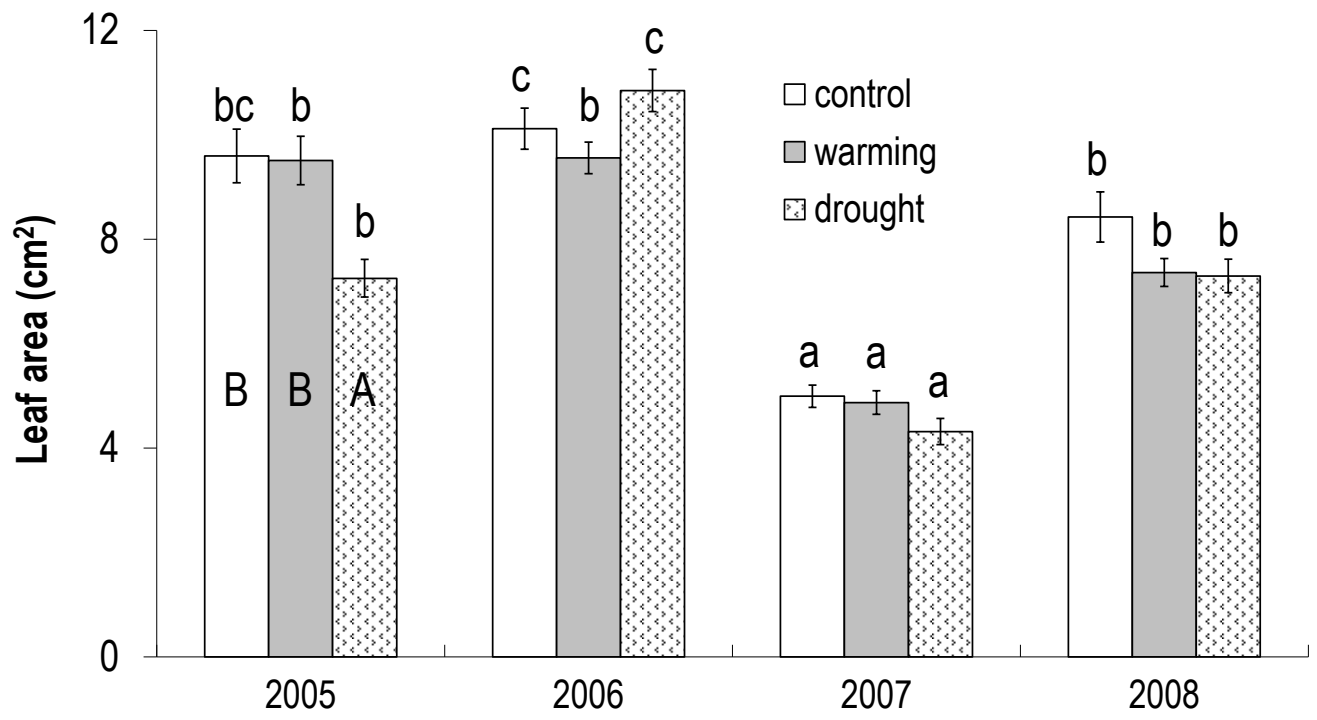


Figure 3.

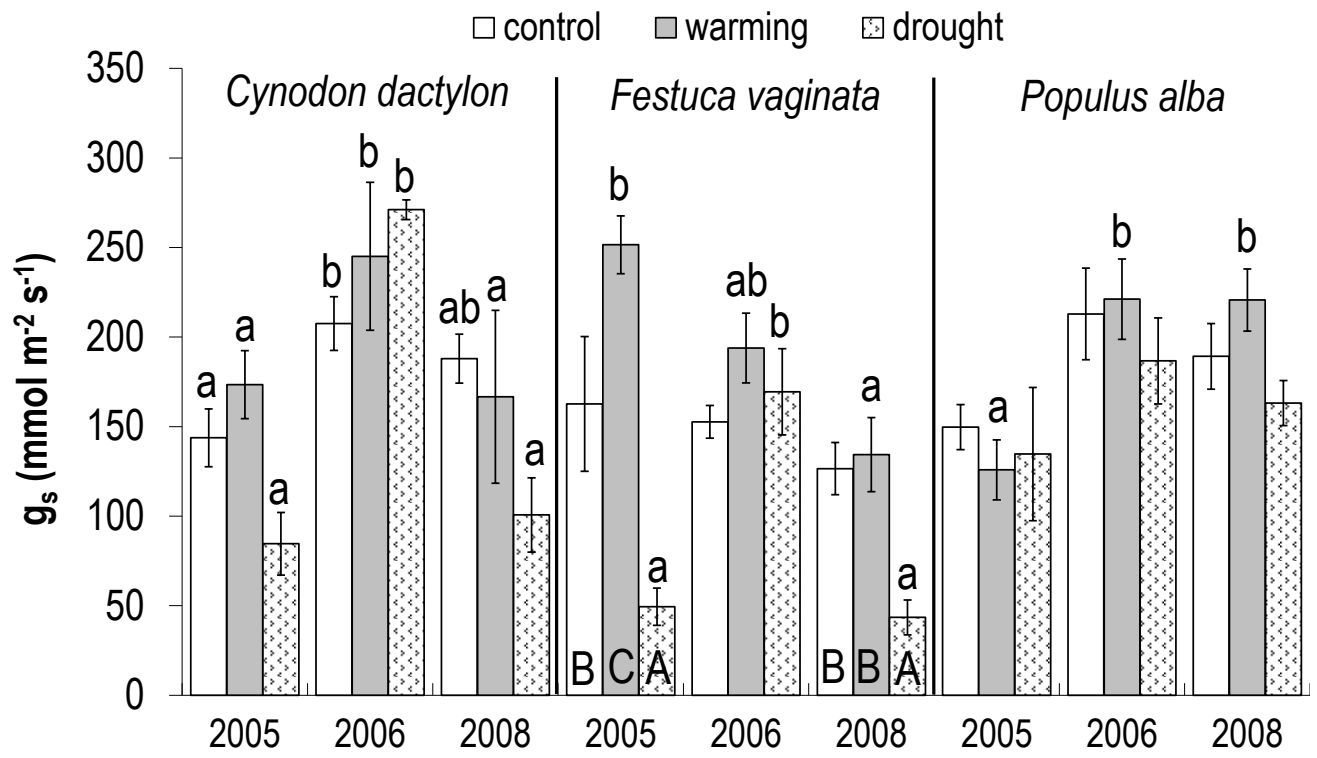


Figure 4.

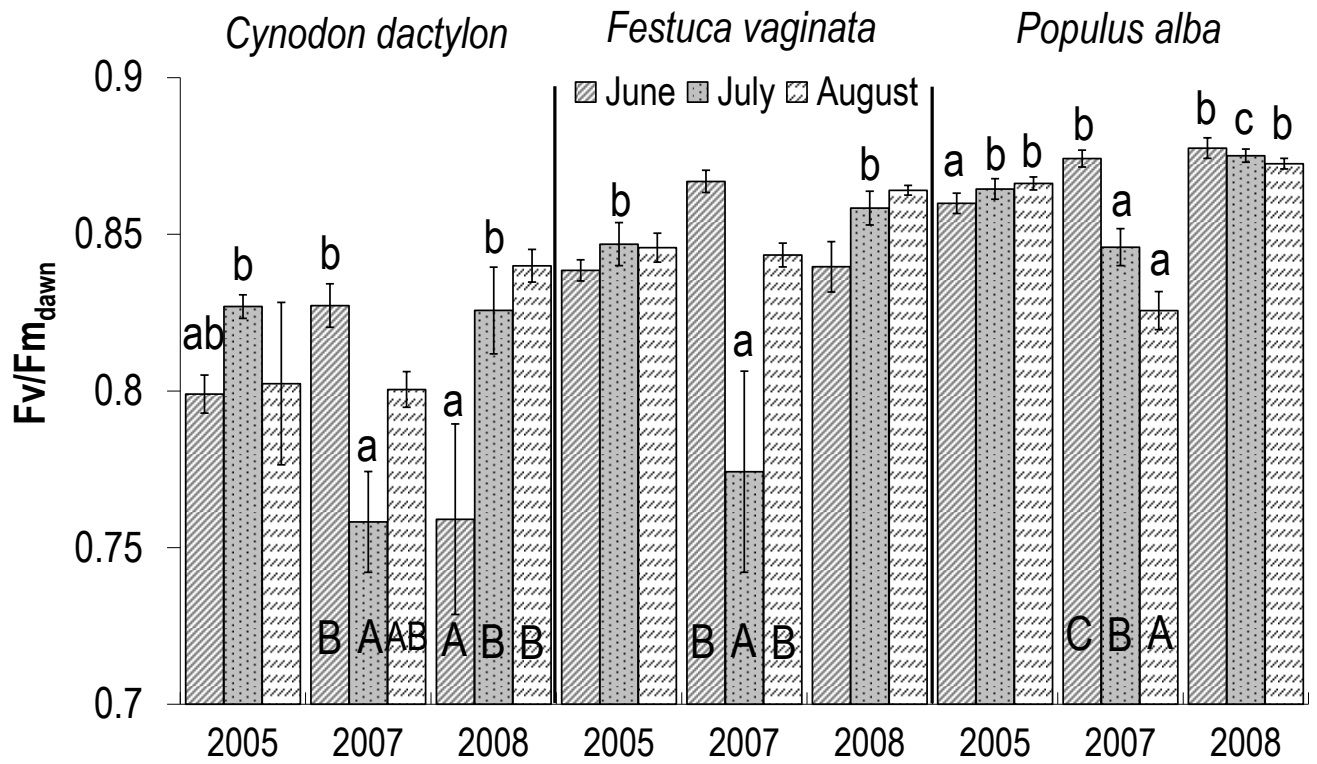
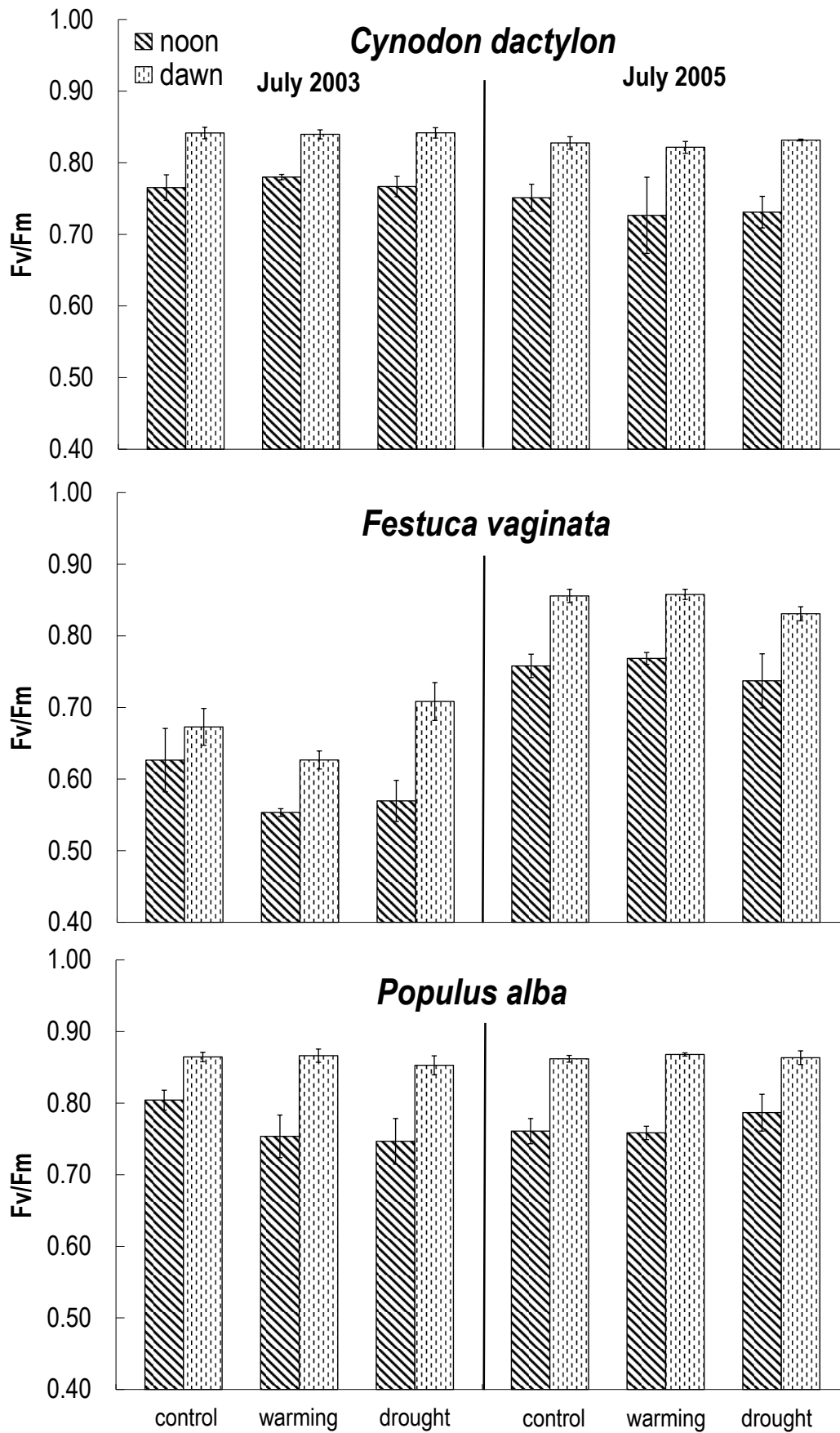


Figure 5.



Electronic Supplementary material

Table S1. Treatment means \pm SD for plant response variables measured during the study period; $n = 3$ (occasionally 2 or 1 due to outlier exclusion). Leaf morphological and coarse structural variables: leaf area (Area, cm^2), length to width ratio (L/W), leaf mass per area (LMA, g m^{-2}), lamina thickness (LT, mm) and leaf bulk tissue density (LD, g cm^{-3}). Spectral reflectance indices of leaf pigment content: index of chlorophyll content (mND), VAZ cycle pigments in photoprotective (deepoxidated) form (PRI) and total VAZ pool (Δ PRI). Chlorophyll fluorescence parameters: potential photochemical efficiency of PSII at dawn ($F_v/F_{m_{\text{dawn}}}$), at midday ($F_v/F_{m_{\text{noon}}}$) and its diurnal depression ($F_v/F_{m_{\text{depr}}} = (F_v/F_{m_{\text{dawn}}} - F_v/F_{m_{\text{noon}}})/F_v/F_{m_{\text{dawn}}} \times 100$, %), actual photochemical efficiency of PSII (Φ PSII), PSII antenna efficiency (F_v'/F_m'), photochemical fluorescence quenching (qP), non-photochemical fluorescence quenching (NPQ) and thermally dissipated fraction of light absorbed in PSII antennae (D). Leaf gas exchange parameters: transpiration rate (E, $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), stomatal conductance for water vapor (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), net photosynthetic rate (A, $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and instantaneous photosynthetic water use efficiency (WUE, $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$). CD: *Cynodon dactylon*, FV: *Festuca vaginata*, PA: *Populus alba*. NM: not measured, ND: no data due to incomplete nocturnal regeneration of maximum photochemical efficiency ($F_v/F_{m_{\text{dawn}}} < 0.78$) for missing $F_v/F_{m_{\text{depr}}}$ values, or $n \leq 1$ for the other missing values.

Table S2. Results (p values) of Repeated Measures ANOVA for variables describing leaf morphology (Area and L/W) and coarse structure (LMA, LT and LD). Abbreviations as in Table S1. Significant ($p < 0.05$) effects are in boldface. For filiform leaves of *F. vaginata* only Area and LMA could be determined reliably (NA = not applicable).

	Area	L/W	LMA	LT	LD
<i>Cynodon dactylon</i>					
Treatment	0.7474	0.1353	0.7178	0.5845	0.4585
Year	< 0.0001	0.0299	0.0047	0.0424	0.0033
Year × Treatment	0.3917	0.3962	0.3824	0.8223	0.2179
Month	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Month × Treatment	0.0193	0.5140	0.0004	0.0303	0.0621
Year × Month	< 0.0001	0.0306	0.0183	< 0.0001	< 0.0001
Year × Month × Treatment	0.4156	0.6850	0.1745	0.0282	0.0011
<i>Festuca vaginata</i>					
Treatment	0.8519	NA	0.8897	NA	NA
Year	0.0007	NA	< 0.0001	NA	NA
Year × Treatment	0.0693	NA	0.4932	NA	NA
Month	< 0.0001	NA	< 0.0001	NA	NA
Month × Treatment	0.5764	NA	0.6225	NA	NA
Year × Month	0.0005	NA	0.0370	NA	NA
Year × Month × Treatment	0.2298	NA	0.1661	NA	NA
<i>Populus alba</i>					
Treatment	0.3452	0.0067	0.9492	0.8303	0.7489
Year	< 0.0001	0.0938	0.0013	< 0.0001	< 0.0001
Year × Treatment	0.0584	0.5481	0.4897	0.3829	0.5173
Month	< 0.0001	0.3622	< 0.0001	< 0.0001	< 0.0001
Month × Treatment	0.0819	0.6700	0.0067	0.9799	0.1530
Year × Month	0.0647	0.1002	0.0021	0.0537	0.0260
Year × Month × Treatment	0.0219	0.5127	0.0705	0.3122	0.2461

Table S3. Results (p values) of Repeated Measures ANOVA for spectral reflectance indices of leaf pigment content (mND,

PRI, ΔPRI) and chlorophyll fluorescence variables (Fv/Fm_{dawn}, Fv/Fm_{noon}, Fv/Fm_{depr}, ΦPSII, Fv'/Fm', qP, NPQ).

Abbreviations as in Table S1. Significant (p < 0.05) effects are in boldface.

	mND	PRI	ΔPRI	Fv/Fm _{dawn}	Fv/Fm _{noon}	Fv/Fm _{depr}	ΦPSII	Fv'/Fm'	qP	NPQ
<i>Cynodon dactylon</i>										
Treatment	0.6321	0.9048	0.7447	0.2244	0.5666	0.7989	0.2471	0.8046	0.2888	0.3918
Year	0.3016	0.0290	<0.0001	0.0569	0.0645	0.0001	0.0191	0.5547	0.0021	0.0003
Year × Treatment	0.1704	0.0862	0.2294	0.2010	0.5391	0.2458	0.1541	0.1531	0.0041	0.0064
Month	<0.0001	0.0660	<0.0001	0.0525	0.7836	0.9937	0.0542	0.1551	0.0374	0.4743
Month × Treatment	0.1701	0.3338	0.3555	0.4595	0.4972	0.9953	0.9585	0.3549	0.7603	0.9874
Year × Month	0.0079	<0.0001	0.0012	0.0028	0.0395	0.0167	0.0209	0.2604	0.0323	0.0845
Year × Month × Treatment	0.7669	0.3941	0.2929	0.3056	0.1370	0.3936	0.1343	0.8244	0.0213	0.0923
<i>Festuca vaginata</i>										
Treatment	0.6216	0.3814	0.0378	0.2271	0.6025	0.9022	0.3436	0.2934	0.2367	0.1819
Year	<0.0001	0.0315	0.0092	0.0142	0.0030	0.0189	0.0596	0.0254	0.8415	0.2185
Year × Treatment	0.5362	0.0230	0.8712	0.4833	0.3549	0.4913	0.1089	0.8604	0.4670	0.6116
Month	0.0001	<0.0001	<0.0001	0.2046	0.0240	0.0454	0.0024	0.0025	0.0004	0.2175
Month × Treatment	0.0424	0.0719	0.0465	0.1244	0.0561	0.9851	0.2587	0.2641	0.1929	0.0376
Year × Month	0.0016	0.0042	0.0001	0.0048	< 0.0001	0.2390	0.0132	0.0613	0.3195	0.1849
Year × Month × Treatment	0.1745	0.3618	0.5669	0.7756	0.4630	0.4754	0.7314	0.1053	0.5572	0.3639
<i>Populus alba</i>										
Treatment	0.5978	0.1466	0.0663	0.8208	0.2530	0.5428	0.6399	0.3700	0.9187	0.3840
Year	<0.0001	0.0049	0.0741	< 0.0001	0.1857	0.3712	0.0013	0.0007	0.0142	0.0468
Year × Treatment	0.8307	0.6876	0.9685	0.4450	0.8137	0.5530	0.5901	0.5941	0.5773	0.5508
Month	<0.0001	<0.0001	<0.0001	0.0052	0.0596	0.9150	< 0.0001	0.0230	0.0001	0.7383
Month × Treatment	0.0318	0.0311	0.0527	0.9718	0.7863	0.7013	0.1473	0.9734	0.0346	0.6095
Year × Month	<0.0001	<0.0001	0.0018	< 0.0001	0.0250	0.0915	0.0001	< 0.0001	0.0078	0.1239
Year × Month × Treatment	0.4875	0.9633	0.2894	0.8125	0.4026	0.2464	0.5005	0.4074	0.2802	0.1136

Table S4. Results (p values) of Repeated Measures ANOVA for leaf gas exchange parameters (E, g_s , A, WUE) and topsoil (0-6 cm) volumetric water content (SWC) at plant base (for *P. alba* under bare soil surface). Other abbreviations as in Table S1. Significant ($p < 0.05$) effects are in boldface.

	E	g_s	A	WUE	SWC
<i>Cynodon dactylon</i>					
Treatment	0.3794	0.3714	0.4611	0.8377	0.0013
Year	<0.0001	<0.0001	0.8672	0.0010	<0.0001
Year \times Treatment	0.3934	0.7969	0.5686	0.3878	0.0343
Month	0.0121	0.0287	0.1293	0.1428	<0.0001
Month \times Treatment	0.1433	0.0449	0.1418	0.2500	<0.0001
Year \times Month	0.0025	0.3908	0.0099	<0.0001	<0.0001
Year \times Month \times Treatment	0.2805	0.0544	0.8192	0.9320	<0.0001
<i>Festuca vaginata</i>					
Treatment	0.0727	0.0504	0.1710	0.6117	0.0645
Year	0.0006	0.0040	<0.0001	0.0271	<0.0001
Year \times Treatment	0.4160	0.3489	0.0369	0.0764	0.0027
Month	<0.0001	<0.0001	0.0068	<0.0001	<0.0001
Month \times Treatment	0.0338	0.0818	0.0115	0.5453	0.0016
Year \times Month	<0.0001	0.0006	<0.0001	0.0009	<0.0001
Year \times Month \times Treatment	0.0377	0.0375	0.4842	0.8346	0.0001
<i>Populus alba</i>					
Treatment	0.6797	0.4599	0.1562	0.3048	0.0004
Year	0.0003	<0.0001	<0.0001	0.0002	<0.0001
Year \times Treatment	0.3971	0.7517	0.8577	0.7553	0.0031
Month	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
Month \times Treatment	0.2546	0.0789	0.6993	0.3987	<0.0001
Year \times Month	<0.0001	0.0015	<0.0001	0.3250	<0.0001
Year \times Month \times Treatment	0.6060	0.9201	0.8348	0.5789	<0.0001