Physiological adjustments of a Mediterranean shrub to long-term experimental warming and drought treatments

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Abstract

Warmer temperatures and extended drought in the Mediterranean Basin are becoming increasingly important in determining plant physiological processes and affecting the regional carbon budget. The responses of plant physiological variables such as shoot water potential (Ψ), carbon-assimilation rates (A), stomatal conductance (gs) and intrinsic water-use efficiency (iWUE) to these climatic regimes, however, are not well understood. We conducted long-term (16 years) field experiments with mild nocturnal warming (+0.6 ºC) and drought (-20% soil moisture) in a Mediterranean early-successional shrubland. Warming treatment moderately influenced Ψ, A and gs throughout the sampling periods, whereas drought treatment strongly influenced these variables, especially during the summer. The combination of a natural drought in summer 2003 and the treatments significantly decreased A and iWUE. Foliar $\delta^{13}C$ increased in the treatments relative to control, but not significantly. The values of Ψ, A and gs were correlated negatively with vapor-pressure deficit (VPD) and positively with soil moisture and tended to be more dependent on the availability of soil water. The plant, however, also improved the acclimation to drier and hotter conditions by physiological adjustments (gs and iWUE). Understanding these physiological processes in Mediterranean shrubs is crucial for assessing further climate change impacts on ecosystemic functions and services.

Keywords: global warming, frequent droughts, physiological response; long-term experiments; plant acclimation

1. Introduction

Mediterranean-type shrubland ecosystems occupy large areas of the global terrestrial surface and provide important ecosystemic services, such as carbon storage, global biogeochemical cycles and the conservation of biodiversity [1–5]. The impacts of anthropogenic climate change, however, are gradually representing a prominent disturbance, affecting from individuals to ecosystems [3,5–7]. Numerous studies of Mediterranean ecosystems have reported that global warming is advancing plant spring phenology and extending growing periods [3,8–10]. In contrast, rapid changes in the patterns of temperature and precipitation have also negatively affected plant growth and survival by reducing water availability, ultimately leading to catastrophic carbon starvation and widespread mortality [11–15]. Robust climatic models have projected a continuous increase in warming and drought severity in the Mediterranean Basin for the coming decades, which could severely impact carbon sinks in shrubland ecosystems and alter regional carbon budgets [16,17].

Many studies over the latest several decades have reported physiological [18–23], morphological [3,24,25] and genetic [3,26–28] changes in Mediterranean plants in response to warming and drying conditions. Among these changes, physiological adjustments are considered the most rapid and effective, because they can increase photosynthetic rates or decrease water loss and improve intrinsic water-use efficiency (iWUE) via stomatal conductance (gs) [3,11,25,29–31]. Recent studies have also demonstrated the resistance and resilience of terrestrial biomes to climatic change by the modulation of the responses to inter-seasonal and inter-annual stresses over time [25,32–34]. Changes in iWUE regulated by gs have been widely studied by the variation of foliar carbon isotope ($\delta^{13}C$) composition [35], so WUE can be evaluated by changes in the amount of foliar $\delta^{13}C$ [36–39]. Our understanding of the effects of future scenarios of warming and drought on these physiological processes in Mediterranean shrubs, however, remains poor due to a lack of long-term data sets.

Climatic experiments have provided effective approaches for studying the potential physiological and phenological changes in plants to future climatic regimes [40–44]. Numerous field experiments manipulating in Mediterranean ecosystems have helped to identify the physiological [18–21,45], morphological [24,25] and structural [11,34,46] adjustments of plants for coping with changes in climatic regimes. Long-term precipitation-manipulation experiments in Mediterranean forests have highlighted dampened effects on carbon assimilation [45], biomass accumulation [47,48] and aboveground net primary production (ANPP) [48]. The physiological adjustments over time in response to new climatic regimes, however, are still unclear because most experimental studies have short terms (<5 years) [18–21,23]. Long-term experiments are consequently desirable for interpreting the cumulative effect of certain climatic regimes and detecting the patterns of plant physiological responses [42,45,48].

We carried out a long-term nocturnal warming and drought experiment in a Mediterranean early-successional shrubland from 1999 to 2014 (16 years), which is one of the longest climate-manipulation experiments ever conducted. Species in early-successional stages are sensitive to rapid climate change but have received little attention [3,18,20,49]. We studied the shrub *Erica multiflora*, which is widely distributed in western and central Mediterranean Basin and is one of the dominant species at the study site [18,20]. Previous experimental studies conducted in this experimental site have observed that the rates of carbon assimilation in *E. multiflora* were not affected by warming but were significantly decreased by drought throughout the first two years of treatment [18]. Prieto et al [20] reported that warming tended to increase the rate of carbon-assimilation of this species in cold seasons, but the response to drought depended on the year and season. Our sampling periods were: 1999-2001 (short-term), 2003-2005 (medium-term) and 2014 (long-term). The specific objectives of our study were to (i) verify if warming and drought exacerbate the loss of shoot water in Mediterranean shrub species, (ii) investigate the effects of experimental warming and drought on A, gs, iWUE (A gs^{-1}) and foliar $\delta^{13}C$ levels at different timescales, and (iii) determine the effect of long-term ecosystem exposure to warmer and drier conditions on plant physiological adjustments. The results will be crucial for identifying the potential physiological responses to climatic changes and will help us to understand further the effects of climate on terrestrial ecosystemic functions and services.

2. Materials and methods

2.1 Study site

We carried out a field experiment on a south-facing hill (13% slope) in Garraf Natural Park near Barcelona (northeastern Spain) (41°18´ N, 1°49´ E; 210 m a.s.l.). The climate at the experimental site is typically Mediterranean, with mild winters, dry summers and rainy springs and autumns. The mean annual air temperature during the study period was 15.8 °C, with the maximum mean summer temperature (June-August) of 23.5 °C. The mean annual precipitation was 537.3 mm, as much as 70% of which falls in spring and autumn. The soil is calcareous and composed of marls and limestone, with depths of 10-40 cm. The site suffered two large fires in summer 1982 and spring 1994, which degraded the vegetation to early-successional shrubland. Most of the current vegetation has sprouted from underground organs after the two fires. The vegetation is co-dominated by *E. multiflora*, a species widely distributed in the Mediterranean Basin. *E. multiflora* grows mainly in the spring but also in the autumn; flowering begins in late summer and ends the following spring.

2.2 Experimental manipulations

Nine plots (5 × 4 m^2) were randomly organized in three blocks, with each block having one warming, one drought and one control plot. Each treatment thus had three replicates. The experiment was maintained from 1999 to 2014.

The warming treatment consisted of a passive nocturnal warming by covering the plots with aluminum curtains. This covering system reduces the amount of long-wave infrared radiation reflected back to the atmosphere at night [40]. A light scaffold was installed in each warming plot for supporting the covering. The curtains were automatically unfolded at night and retracted during the day and were controlled by light sensors (below and above 200 lux, respectively). This passive nocturnal warming is realistic and effective, because the effects of global warming are predicted to be higher at night than during the day [40,49]. The curtains were automatically retracted during rain to avoid influencing the hydrological cycle.

The drought treatment extended the summer drought to the following spring and autumn by preventing rainwater from entering the plots using transparent waterproof roofs [40]. Scaffolds were also installed in the drought plots, but the curtain material was transparent plastic. Rain sensors activated the curtains to cover the plots whenever it rained during the treatment period and retracted them when the rain stopped. Rain was sensed by a sensitive (>5 mm rainfall) tipping-bucket rain gauge. The rainwater blocked by the waterproof plastic was drained outside the plots. The curtains were also automatically retracted to avoid damage during winds exceeding 10 $m\cdot s^{-1}$. The drought plots were treated the same as the control plots during the rest of the year. The control plots had similar scaffolds as the warming and drought plots but without curtains.

2.3 Environmental conditions

Air temperature and precipitation have been recorded at the study site since 1998. Soil temperature at -5 cm depth was recorded by temperature sensors distributed in each plot. Precipitation was recorded by a tipping-bucket rain gauge 1.5 m above the ground. Relative humidity was recorded every 30 minutes at a nearby meteorological station.

Vapor-pressure deficit (VPD) was calculated every 30 minutes from the relative humidity and air temperature. Soil moisture in the top 15 cm of soil was measured weekly by Time Domain Reflectometry (TDR) using three probes in each plot.

2.4 Measurements of shoot water potential and gas exchange

We monitored the shoot water potential (ψ), foliar carbon-assimilation rates (A) and gas exchange (gs) for 3-6 consecutive days per season during the sampling periods (spring 1999 to winter 2001, winter 2003 to summer 2005 and winter 2014 to autumn 2014). Five current-year shoots of *E. multiflora* with similar growth performance were collected in each plot to measure the changes of ψ at midday (11:00-13:00, solar time) using a Scholander-type pressure chamber (PMS Instruments, Corvallis, USA). Measured Erica plants were always the same ones, permanently marked with labels at the beginning of experiments. Foliar A and gs were measured on three consecutive days in each season with a portable gas-exchange system (an ADC4 system configured with a chamber model PLC4B (Hoddesdon, Hertfordshire, UK) from 1999 to 2001, a CIRAS2 system (Hitchin, Hertfordshire, UK) from 2003 to 2005 and an LI-6400XT system (LI-COR Inc., Lincoln, USA) from 2013 to 2014). Two to six sunny and current-year shoots with similar growth status were collected from each plot, and gas exchange was measured in triplicate. Leaf area was measured using ImageJ 1.46r (NIH, Maryland, USA) from a photocopy of all leaves on the twigs. Intrinsic water-use efficiency (iWUE) was calculated from the gas-exchange measurements as the ratio A gs^{-1}. The medium- (2003-2005) and long-term (2014) periods focused on gs and iWUE.

2.5 Isotopic and elemental analyses

Foliar $\delta^{13}C$ content was measured for current-year leaves in August 2000, 2004 and 2014. Leaves from three *E. multiflora* plants were collected in each plot and analyzed in an EA1108 elemental analyzer (Carlo Erba, Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO Π interface (Thermo Finnigan MAT, Bremen, Germany). The results were calibrated with interspersed international isotopic carbon standards (IAEA, Vienna, Austria). Values are expressed relative to Pee Dee Belemnite (PDB) standard for $\delta^{13}C$ as:

$$\delta\ ^{13}C = (R_{sample}/R_{standard} - 1) \times 1000\ (‰)$$

where ^{13}C is the heavier carbon isotope and R is the ratio of the heavier to the lighter isotope (^{13}C:^{12}C) for the sample and standard. The accuracy of the measurements was ± 0.15‰ for $\delta\ ^{13}C$.

2.6 Data analyses

For all physiological parameters (Ψ, A, gs, iWUE and $\delta^{13}C$), we calculated one value per plot as the mean of all plants measured per plot. The warming and drought treatments were always separately compared with the controls. The differences between the treatments and controls were analyzed by analyses of variance using R version 3.2.3 (R Core Development Team, 2015). The significance level was $p<0.05$ for the statistical tests within year and season.

3. Results

Climatic parameters

The climatic parameters (rainfall, temperature and VPD) varied greatly throughout the sampling periods (Fig. 1). The highest temperatures were recorded in summer (mean of June, July and August), with the highest summer temperature of 26.4 °C in 2003. The rainy seasons were usually spring and autumn. VPD was correlated with accumulated precipitation and temperature, with extremely severe deficits in summer 2003. The mean annual soil temperature at -5 cm depth was on average 0.6 °C higher in the warming than in the control plots. There were no significant differences between warming and control plots in 2014 (Fig. 2a). Soil moisture in the 0-15 cm layer fluctuated with season and year during the sampling periods, with wet springs and autumns and dry summers (Fig. 2b). Soil moisture averaged 20% lower in the drought treatment than the control plots throughout the sampling periods ($p<0.01$).

Shoot water potential (ψ)

Shoot water potential (ψ) of *E. multiflora* varied with VPD and was lowest in summer (Fig. 3). The warming treatment did not significantly affect ψ compared to the control, but ψ was significantly lower in the drought treatment throughout the sampling periods ($p<0.01$).

Foliar gas exchange

Carbon-assimilation rates (A) was highly variable in the control plants inter-seasonally and inter-annually throughout the sampling periods. Photosynthesis was generally highest during the wet seasons (spring and autumn) and lowest during the hot and dry summers (Fig. 4). Neither warming nor drought treatment significantly decreased the values of A when compared to control, and warming treatment even had some positive effects. The values of A, however, decreased significantly under both warming and drought in the extremely hot and dry summer of 2003 (p<0.01 and p<0.05, respectively). The values of gs fluctuated greatly with season and year and was lowest in winters and highest in the wet springs and autumns (Fig. 5), but gs in the warming and drought treatments was similar to that in the controls. The iWUE in control was generally highest in summer, except for the extreme summer of 2003 (Fig. 6). The (iWUE) was generally higher in the warming and drought treatments but decreased significantly in summer 2003 due to the combination of the extremely hot summer and the experimental warming (p<0.05) and drought (p<0.05).

Shoot water potential (ψ), Carbon-assimilation rates (A) and stomatal conductance (gs) were correlated negatively with VPD and positively with soil moisture, indicating a strong dependence on soil moisture. The (iWUE), however, was not affected when soil-water potential and VPD were high (Fig. 7).

Variability of isotopic values (^{13}C)

The foliar δ ^{13}C values in *E. multiflora* varied greatly during the sampling periods (Fig. 8). The δ ^{13}C values after one year of both the warming and drought treatments were similar to those in the controls. After five years of treatment, however, were significantly higher in the warming treatment than the controls. Neither the warming nor the drought treatments had significant effects on δ ^{13}C after 15 years of treatment.

4. Discussion

Shoot water potential (ψ) in *E. multiflora* was highly variable inter-seasonally and was lowest in hot and dry summers (Fig. 3). The lower ψ in the experimental treatments was likely induced mainly by increased evapotranspiration in the warming treatment and by the decrease in soil moisture due to the exclusion of rainwater in the drought treatment [18,20]. The effects on ψ differed greatly between warming and drought treatment, consistent with the previous studies showing that the impacts of drought treatment on plant water potential were stronger than those of warming treatment [18,20]. Our results indicated that hot and dry summers accelerated the loss of water from plant shoots, which can cause large-scale crown die-off, mortality and vegetation shifts in Mediterranean ecosystems [11,14,15]. Long periods of warmer and drier climate combined with extreme conditions in the future would likely cause more severe shoot-water deficits in Mediterranean shrubland ecosystems [15,17].

Foliar photosynthesis is an important physiological process of plant life that is strongly affected by conditions of temperature and water across various types of ecosystems [23,31,34]. The moderate warming and prolonged drought treatments decreased photosynthetic rates, although the experimental warming had some positive effects in wet seasons (Fig. 4). Photosynthesis in *E. multiflora* was strongly correlated with soil moisture, and it was significantly lower in both the warming and drought treatments during the extreme summer in 2003, probably due to the lower water availability [14,17].

Stomatal adjustment is the primary regulator of foliar hydrological changes, which control the response of WUE to climatic stresses [23,31]. Photosynthesis is highly correlated with gs, although the mechanisms by which water stress limits photosynthesis by stomatal closure or metabolic impairment are controversial [23,30,31]. Most studies accept that stomatal closure is the main determinant of photosynthesis under moderate drought stress [20,23,45,50]. Foliar photosynthetic rates are also well correlated with gs in some studies of Mediterranean species [18–20,45]. Plant photosynthesis, however, may not be limited by stomatal closure but by metabolic impairment [3,20,23,50,51]. Our study indicated that foliar photosynthetic rates were significantly lower in both the warming and drought treatments in the extreme 2003 summer, but this effect was probably associated with the impairment of photosynthetic enzymes, because the treatments did not significantly affect gs [23,31,51].

Intrinsic water-use efficiency (iWUE) is an important indicator of plant physiological response to environmental changes [23,31,39]. In our study, the plants increased iWUE to reduce water loss in summer, except in the extreme 2003 summer (Fig. 6), consistent with the theory that the balance between carbon assimilation and the control of water loss is optimized in hot and dry summers [29,30,39]. Climatic extremes such as during the 2003 summer, however, can cause severe plant-water deficits that can irreversibly damage plant hydraulic systems [11,12,14,15,17]. Changes in foliar $\delta^{13}C$ during warmer and drier conditions can increase plant WUE via photosynthetic or metabolic processes [35–37,39,52]. Our results, however, showed that $\delta^{13}C$ did not change significantly in the warming and drought treatments throughout the study period, except in the warming treatment in 2004 when $\delta^{13}C$ was significantly higher, which was probably due to the higher iWUE after the extremely hot summer in 2003 (Fig. 8). Various studies have reported that ^{13}C discrimination decreases with the concentration of intercellular carbon dioxide due to stomatal closure, and ultimately WUE [3,38,39,52]. Foliar $\delta^{13}C$ was therefore higher under the warming and drought conditions, consistent with the findings of other experiments in terrestrial ecosystems where plants regulate the relationship between WUE and climate change [32,37,39].

The available information on global change indicates that long periods of warming have been impacting terrestrial ecosystems since the mid-20th century [5,16]. We have also concluded that the severe decrease in water availability had substantial effects on physiological processes, such as Ψ, A and gs, whereas the effects of the mild warming treatment were very mild (nearly no influences). These differential treatment effects indicate that low water availability may has a more direct impact on plants [23,31]. These physiological adjustments, however, are essential for adapting to changes in climatic regimes. Our study shows that the physiological responses (A, g_s, iWUE and $\delta^{13}C$) to long-term experimental warming and drought decrease over time, indicating acclimation to the climatic stresses [21,23,45,46]. Robust models, however, predict that global warming will continue to increase, accompanied by other stresses such as extreme droughts and heat waves [5,16,17]. Mediterranean species have shown some acclimation responses and strategies, but these could be potentially overwhelmed by future climate, leading to increased risks of xylem embolism, crown die-off and mortality, which would affect future ecosystemic carbon budgets.

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Figures

Fig. 1 Seasonal variation of mean air temperature, precipitation and vapor-pressure deficit (VPD) during the sampling periods 1999-2001, 2003-2005 and 2014. Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 2 (a) Changes of seasonal soil temperature in the control and nocturnal warming treatment during the sampling periods. (b) Changes of seasonal soil moisture in the control and drought treatment during the sampling periods. Significant differences between the control and treatment plots are indicated by asterisks (**, $p<0.01$). Each treatment had three replicates (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 3 Changes in shoot water potential in *Erica multiflora* during the sampling periods in the control and warming treatment and drought treatment. Significant differences between the control and treatment plots are indicated by asterisks (*, $p<0.05$; **, $p<0.01$). Each treatment had three replicates (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 4 Seasonal variation of net photosynthetic rates in *Erica multiflora* in the control and treatment plots during the experimental periods. Error bars indicate the standard errors of the means (n=3 plots). Significant differences between the control and treatment plots are indicated by asterisks (*, $p<0.05$; **, $p<0.01$). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 5 Seasonal stomatal conductance in *Erica multiflora* in the control and the warming and drought treatments during the medium- and long-term study periods. Error bars indicate the standard errors of the means (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 6 Intrinsic water-use efficiency (iWUE, A g^{-1}) in *E. multiflora* leaves in the control and the warming and drought treatments during the medium- and long-term sampling periods. Error bars indicate the standard errors of the means (n=3 plots). Significant differences are indicated by asterisks (*, $p<0.05$). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Fig. 7 The relationships of shoot water potential (SWP), net photosynthetic rate, stomatal conductance and intrinsic water-use efficiency (iWUE) with vapor-pressure deficit (VPD) and soil moisture during the sampling periods.

Fig. 8 Foliar $\delta^{13}C$ in *E. multiflora* in summer 2000, 2004 and 2014. Error bars indicate the standard errors of the means (n=3 plots). Significant differences with control are indicated by asterisks (*, $p<0.05$).

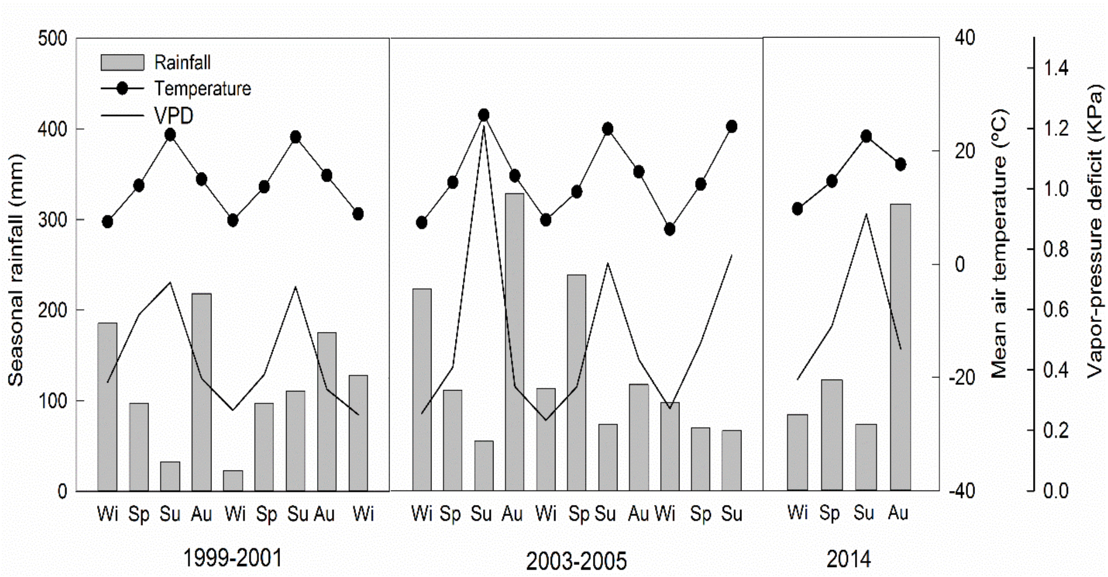

Rainfall
Temperature
VPD
Seasonal rainfall (mm)
500
400
300
200
100
0
Mean air temperature (°C)
40
20
0
-20
-40
Vapor-pressure deficit (KPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Wi Sp Su Au Wi Sp Su Au Wi
1999-2001
Wi Sp Su Au Wi Sp Su Au Wi Sp Su
2003-2005
Wi Sp Su Au
2014

Fig. 1

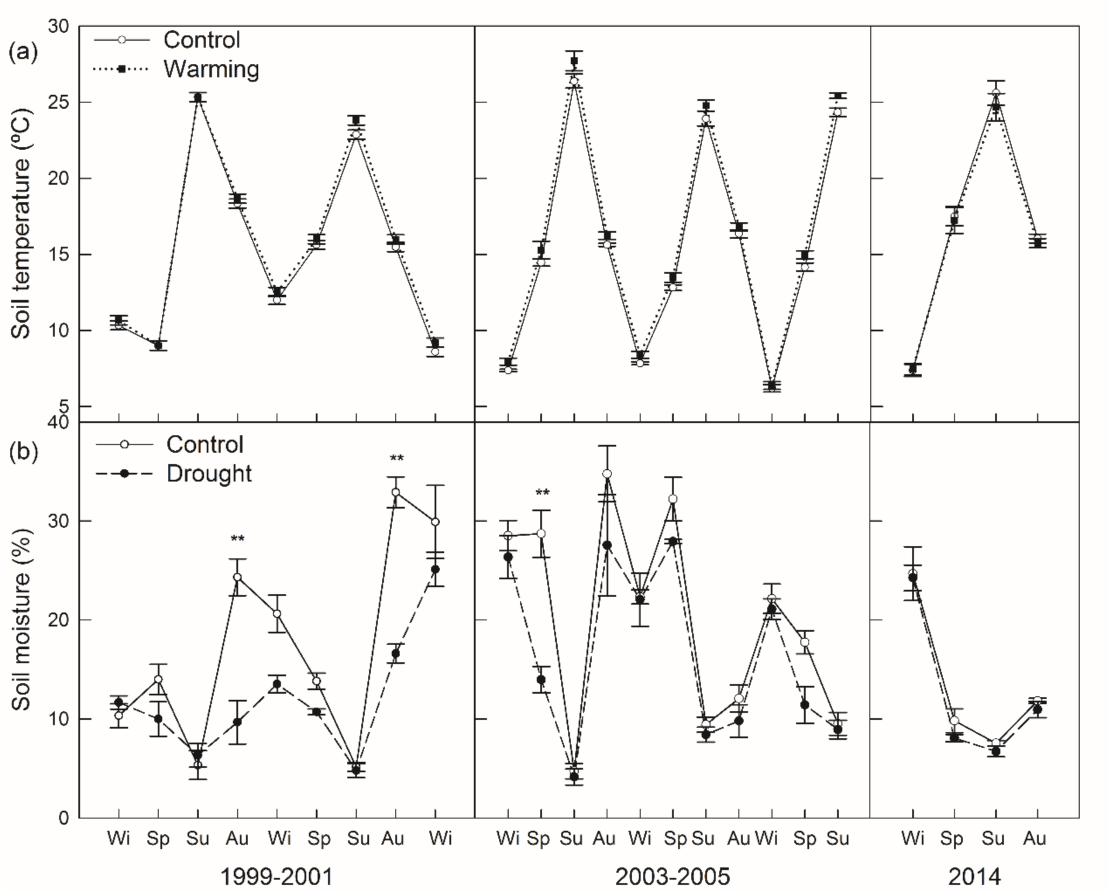

(a)
Control
Warming
Soil temperature (ºC)
30
25
20
15
10
5
(b)
Control
Drought
Soil moisture (%)
40
30
20
10
0
**
**
**
Wi
Sp
Su
Au
Wi
Sp
Su
Au
Wi
Wi
Sp
Su
Au
Wi
Sp
Su
Au
Wi
Sp
Su
Wi
Sp
Su
Au
1999-2001
2003-2005
2014

Fig. 2

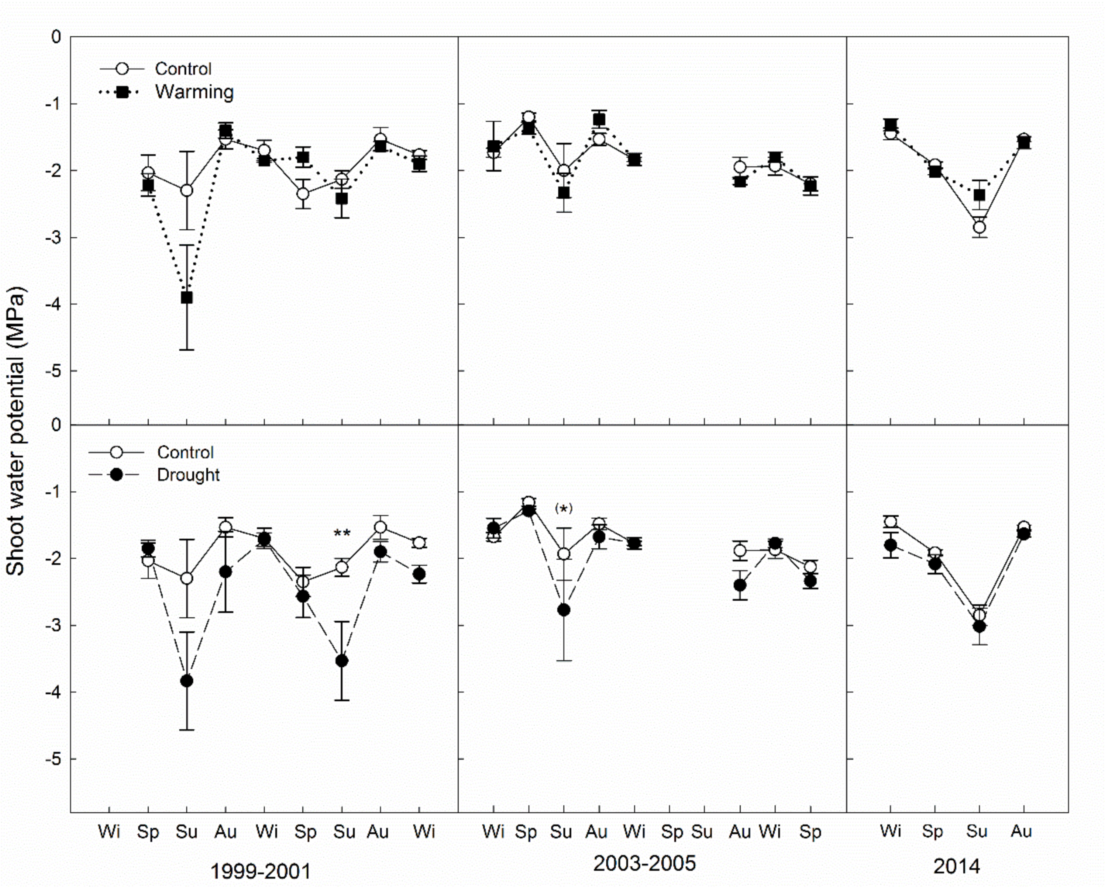

Shoot water potential (MPa)
0
-1
-2
-3
-4
-5
Control
Warming
Control
Drought
**
(*)
Wi
Sp
Su
Au
1999-2001
2003-2005
2014

Fig. 3

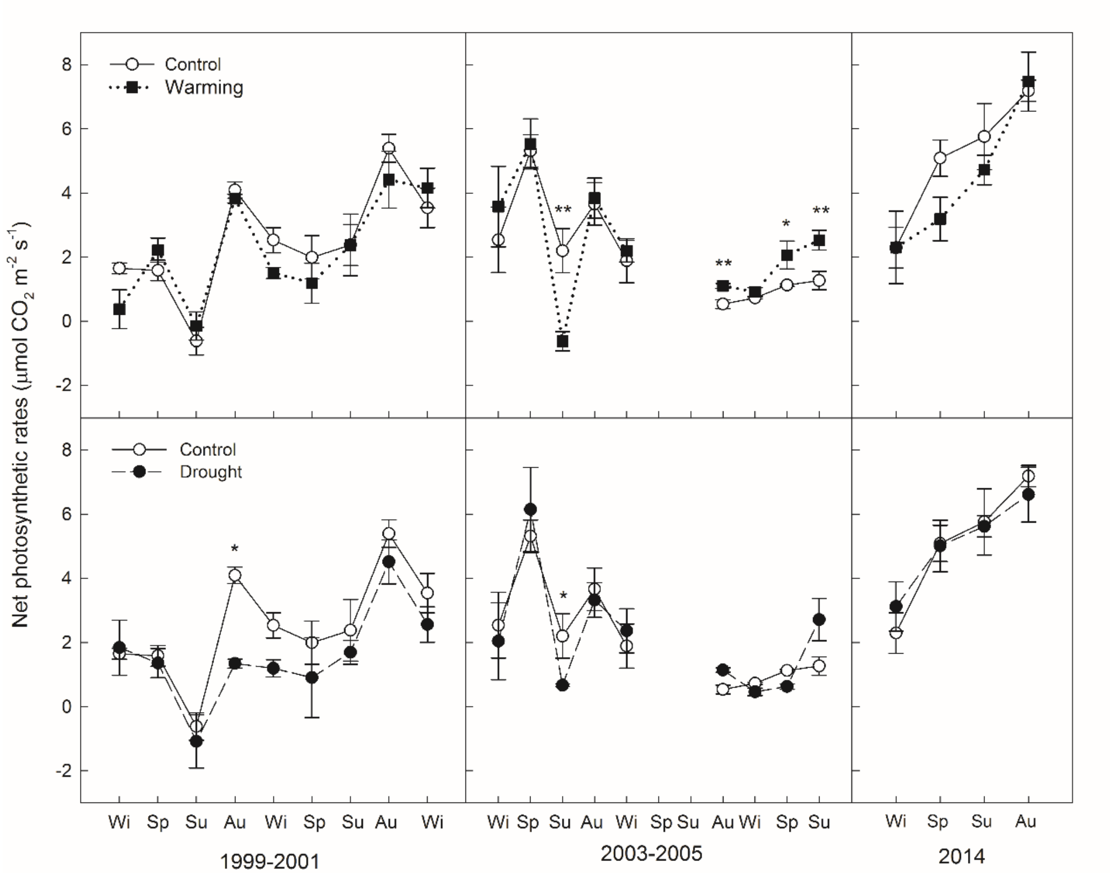

Control
Warming
Control
Drought
Net photosynthetic rates (μmol CO$_2$ m^{-2} s^{-1})
8
6
4
2
0
-2
**
**
*
**
*
*
Wi
Sp
Su
Au
Wi
Sp
Su
Au
Wi
Wi
Sp
Su
Au
Wi
Sp
Su
Au
Wi
Sp
Su
Wi
Sp
Su
Au
1999-2001
2003-2005
2014

Fig. 4

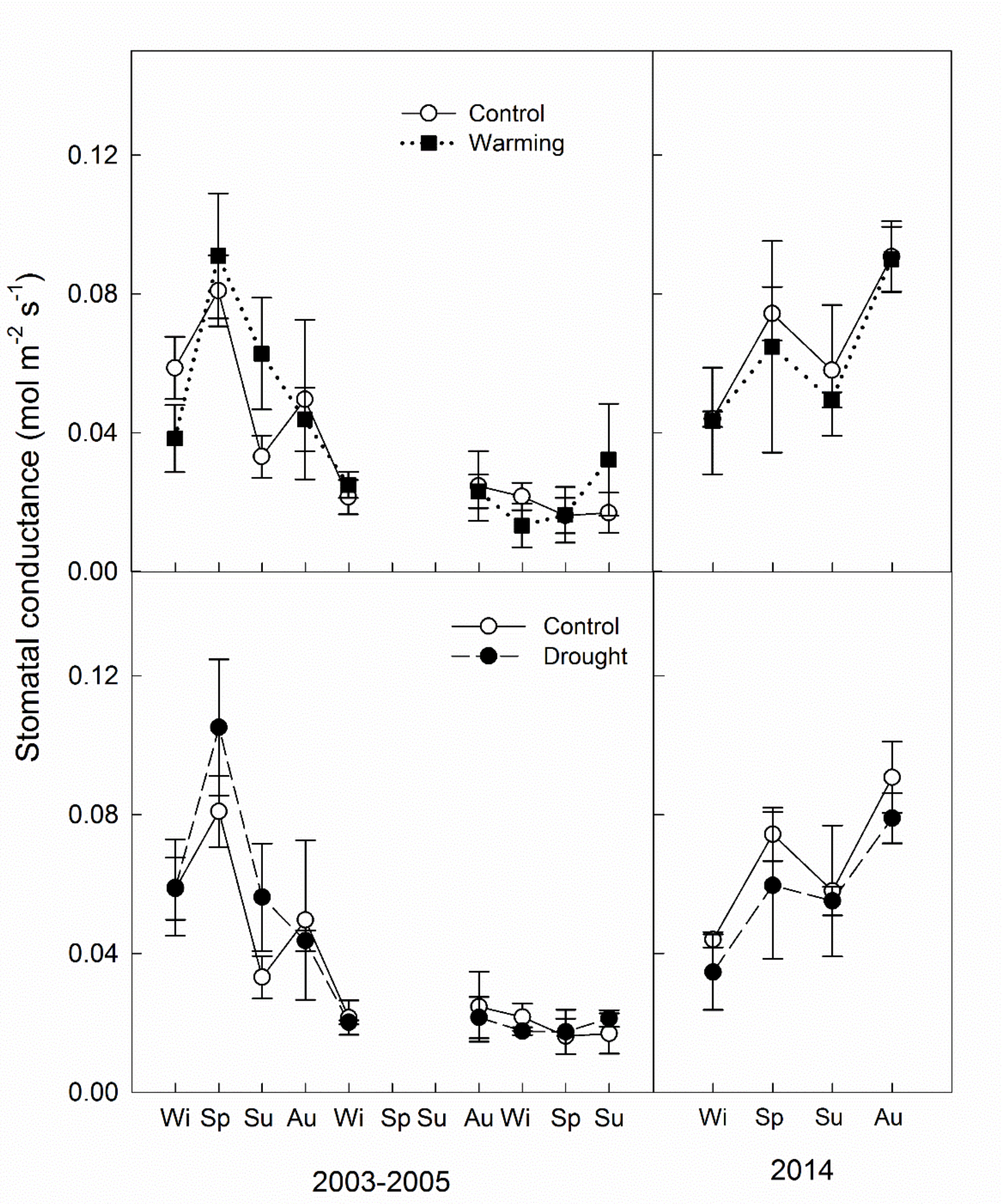
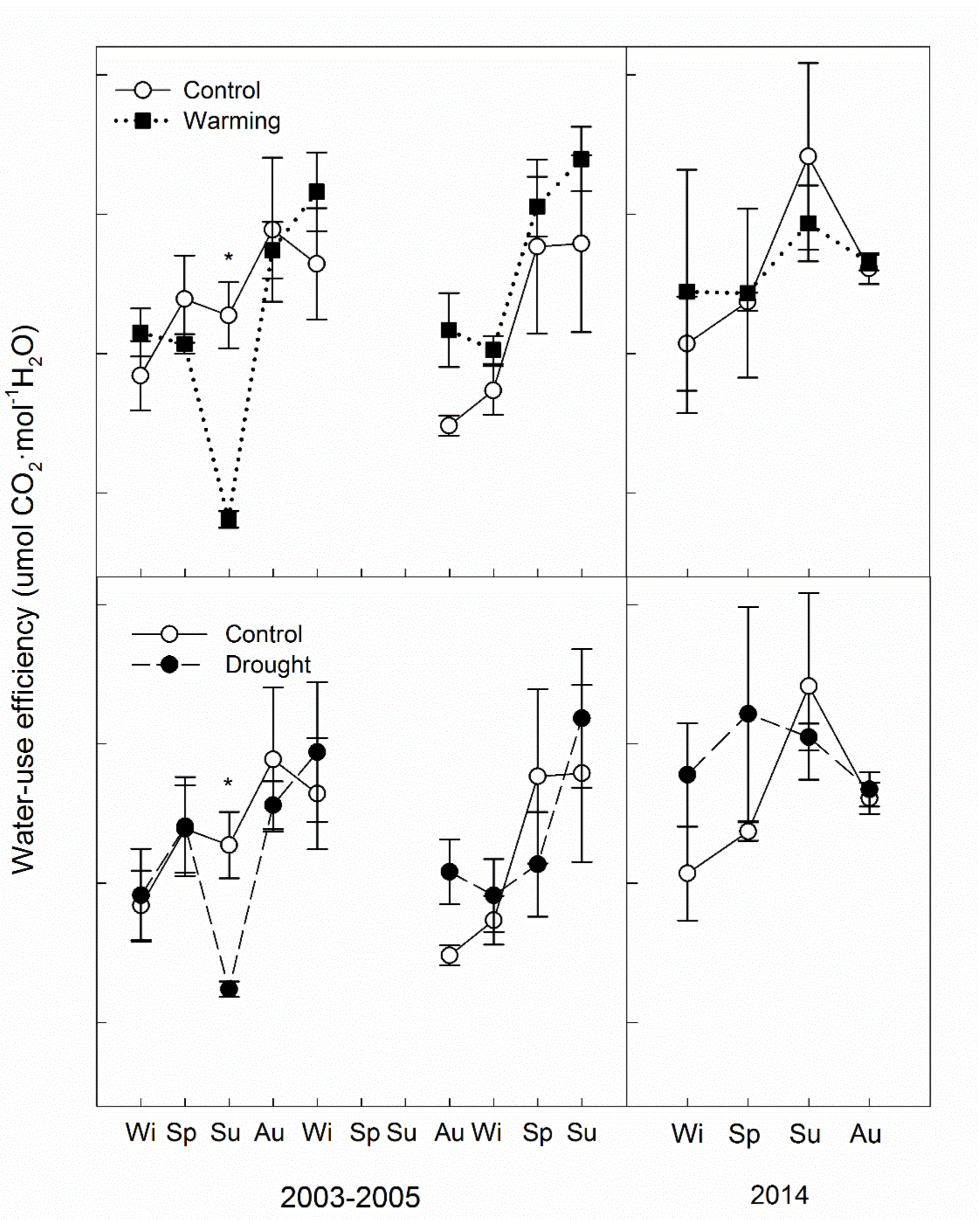

Stomatal conductance (mol m^{-2} s^{-1})
0.00
0.04
0.08
0.12
Control
Warming
Control
Drought
Wi Sp Su Au Wi Sp Su Au Wi Sp Su
Wi Sp Su Au
2003-2005
2014

Fig. 5



Water-use efficiency (umol $CO_2 \cdot mol^{-1} H_2O$)
Control
Warming
Control
Drought
*
*
Wi
Sp
Su
Au
Wi
Sp
Su
Au
Wi
Sp
Su
Wi
Sp
Su
Au
2003-2005
2014

Fig. 6

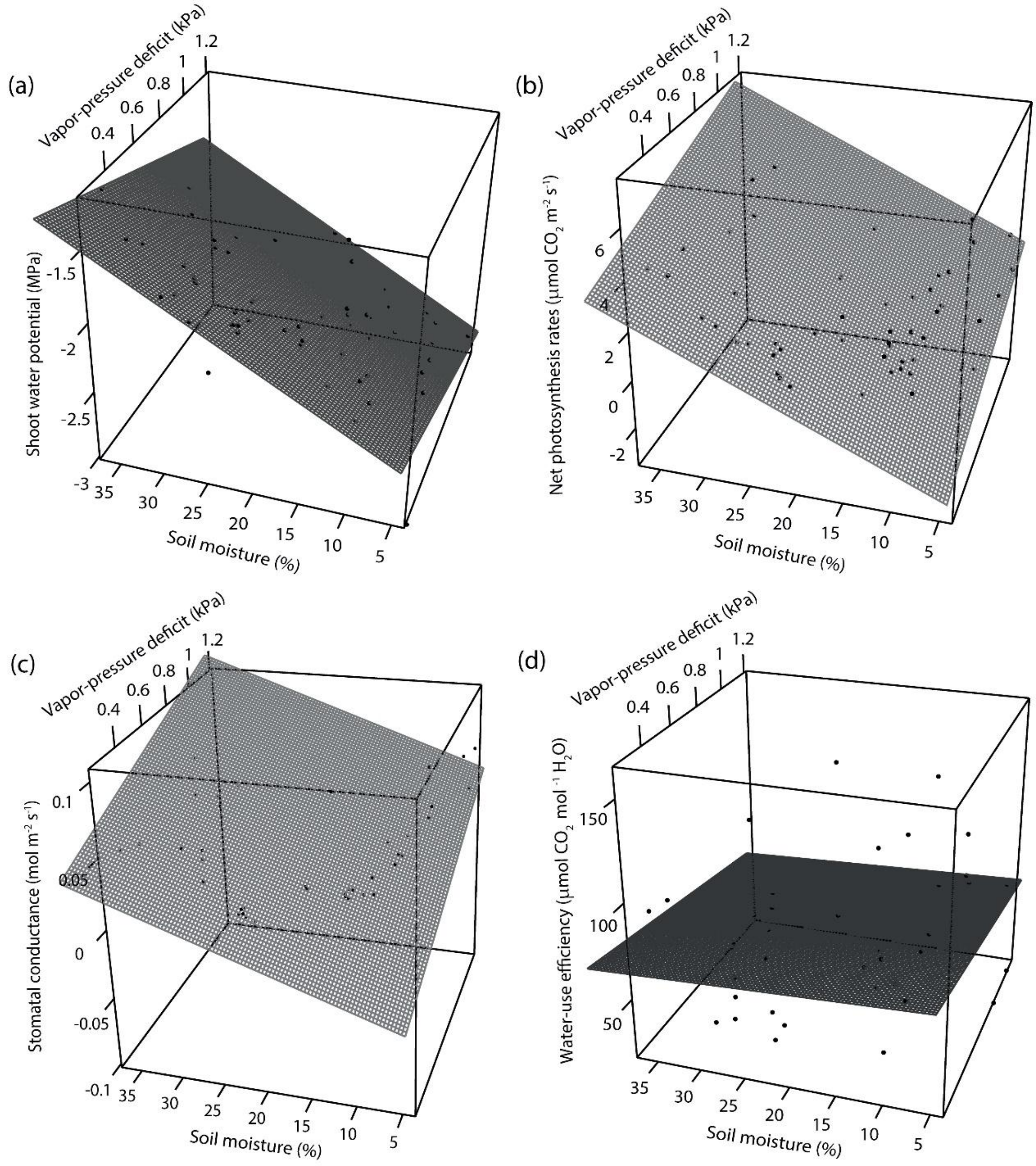

(a)
Vapor-pressure deficit (kPa)
0.4
0.6
0.8
1
1.2
Shoot water potential (MPa)
-1.5
-2
-2.5
-3
Soil moisture (%)
35
30
25
20
15
10
5
(b)
Vapor-pressure deficit (kPa)
0.4
0.6
0.8
1
1.2
Net photosynthesis rates (μmol CO$_2$ m^{-2} s^{-1})
6
4
2
0
-2
Soil moisture (%)
35
30
25
20
15
10
5
(c)
Vapor-pressure deficit (kPa)
0.4
0.6
0.8
1
1.2
Stomatal conductance (mol m^{-2} s^{-1})
0.1
0.05
0
-0.05
-0.1
Soil moisture (%)
35
30
25
20
15
10
5
(d)
Vapor-pressure deficit (kPa)
0.4
0.6
0.8
1
1.2
Water-use efficiency (μmol CO$_2$ mol^{-1} H$_2$O)
150
100
50
Soil moisture (%)
35
30
25
20
15
10
5

Fig. 7

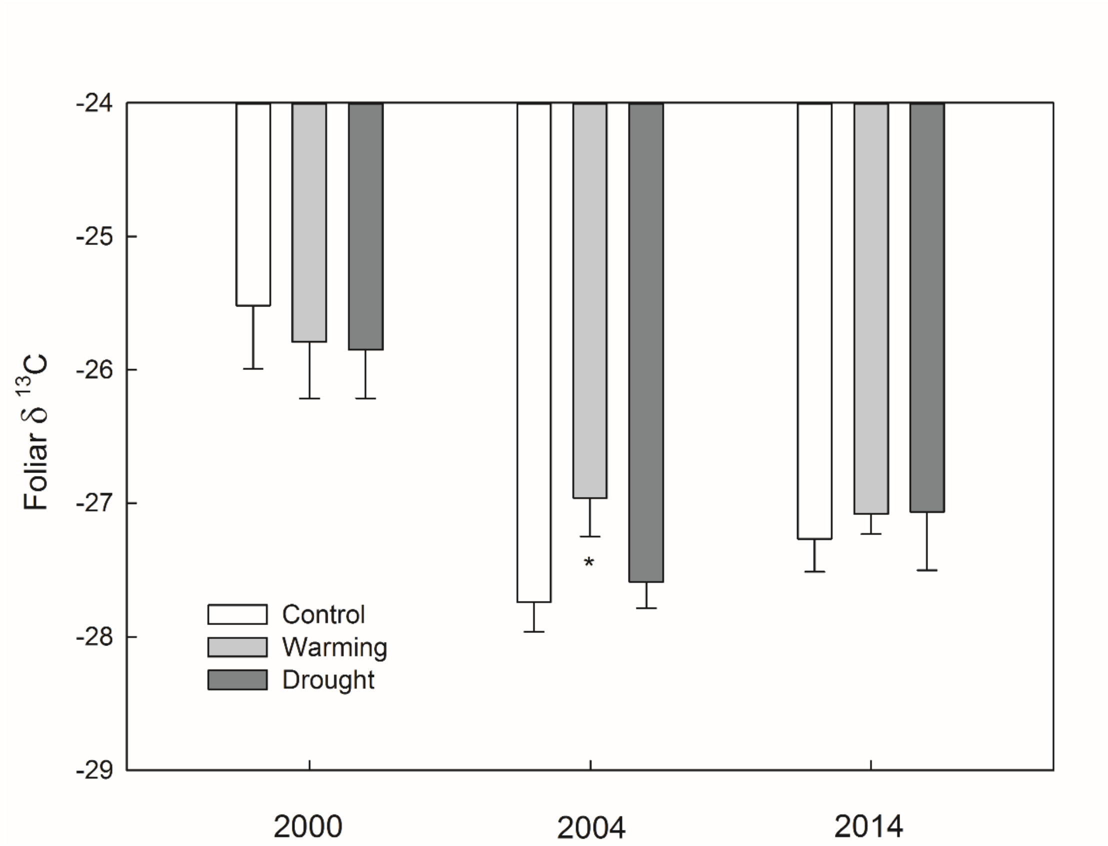

-24
-25
-26
-27
-28
-29
Foliar δ ^{13}C
*
Control
Warming
Drought
2000
2004
2014

Fig. 8