Plant Science 252 (2016) 53–61

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

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

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ARTICLE INFO

Article history:
Received 7 May 2016
Received in revised form 8 July 2016
Accepted 10 July 2016
Available online 11 July 2016

Keywords:
Global warming
Frequent droughts
Physiological response
Long-term experiments
Plant acclimation

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 °C) 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.

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

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http://dx.doi.org/10.1016/j.plantsci.2016.07.004
0168-9452/

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 ($5 \times 4\,m^2$) 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 lx, 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 $10\,m\,s^{-1}$. 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 min at a nearby meteorological station. Vapor-pressure deficit (VPD) was calculated every 30 min 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 sta-