

## Photosynthetic response of *Bromus inermis* in grasslands of different altitudes

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**Abstract:** In Greece, grasslands constitute an important component of montane areas. The aim of the present study was to evaluate the photosynthetic responses of the forage species *Bromus inermis* at two montane grasslands (Filippaioi, Grammatiko) and a lowland grassland under differential irradiation and CO<sub>2</sub> concentration levels. In each area, gas exchange parameters were calculated under: a) ambient conditions along with the measurement of variable to maximal fluorescence, b) low (300 μmol m<sup>-2</sup> s<sup>-1</sup>) and high (1400 μmol m<sup>-2</sup> s<sup>-1</sup>) radiation, and c) ambient (380 μmol mol<sup>-1</sup>) and high (800 μmol mol<sup>-1</sup>) CO<sub>2</sub> concentrations. In addition, assimilation response curves to different levels of irradiation and intercellular CO<sub>2</sub> partial pressure were recorded. The photosynthetic machinery of *B. inermis* responded better to changes in irradiation in the montane area of Filippaioi, while it was more sensitive to changes in CO<sub>2</sub> concentration in the lowland area. Photosynthesis in the montane area of Grammatiko could be subject to novel ecological pressures shaped by lower grazing intensity. No effect of altitude on physiological responses of *B. inermis* was found. Instead, plants from each area may use different adaptive mechanisms to persist and thrive in the environmental conditions of each area.

**Key words:** Assimilation rate, elevation, intercellular CO<sub>2</sub> concentration, light, smooth brome, stomatal conductance

### 1. Introduction

Grassland ecosystems cover about 20% of the world's land area and about 22% of the land area of the EU-25 (EEA, 2005). In the southern Mediterranean Basin, grasslands are found on shallow soils with low fertility and poor plant cover under arid and semiarid climates, with low and unpredictable rainfall. As a result, the natural vegetation has evolved different adaptation mechanisms to survive under these conditions (Porqueddu et al., 2016). In Greece, both the proportion (40% of the total area of the country) and the importance of the grasslands are higher than the EU average (NSSG, 1991), constituting an important component of the montane, low-quality, and steep-site ecosystems, where harsh climatic conditions prevail. However, intermediate and high-elevation (pseudo-alpine) grasslands are a valuable resource for the transhumant livestock system from May to October (Karatassiou et al., 2014).

Altitudinal differences over short geographical distances are correlated with big changes in environmental conditions that take place in a predictable manner (Walther et al., 2005), subjecting plant populations to different selection pressures (Gonzalo-Turpin and Hazard, 2009). The carbon dioxide concentration and temperature generally decrease with altitude, while irradiance increases

(Kumar et al., 2005). Thus, population responses can differ at contrasting altitudes (Gimenez-Benavides et al., 2007). Light is normally not considered an important limiting resource in grasslands; nevertheless, forage species can also be subjected to limited light conditions (e.g., shading, cloud cover) (Dias-Filho, 2002). Both CO<sub>2</sub> concentration and radiation level variations influence the photosynthetic machinery and thus plant performance. Therefore, it is important to understand how plants will respond to an environment with elevated CO<sub>2</sub> concentration and radiation in order to predict grassland structure and function in the future. So far, relatively few studies have examined the photosynthetic responses of grassland species to elevated irradiation and CO<sub>2</sub> concentration (Springer and Thomas, 2007).

*Bromus inermis* Leyss. (smooth brome) is a leafy, perennial, cool-season grass species that propagates and spreads by rhizomes. It is adapted to cooler climates, resistant to drought and temperature extremes, and widely distributed over different elevations (Sather, 1987; <http://www.fs.fed.us/database/feis/plants/graminoid/broine/all.html>). The forage production of *B. inermis* is highly palatable, rich in protein content, and relatively poor in crude-fiber content (USDA NRCS, 2008). Due to its massive root system and the formation of sods it can be

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used effectively for rehabilitation and erosion control. It can also be used as a component in various upland wildlife and conservation cover mixes for nesting cover and food (USDA NRCS, 2008). However, *B. inermis* is not shade-tolerant and when it receives insufficient sunlight it produces fewer (and lighter) seeds, fewer shoots and rhizomes, and lighter dry plant weight (Sather, 1987).

In the current study, we have chosen two mountainous grasslands and one lowland grassland in Greece in which *B. inermis* grows naturally. The objectives of our study were to evaluate: a) the photosynthetic performance of *Bromus inermis* plants of grasslands from different altitudinal zones, b) the photosynthetic response of mountainous and lowland *B. inermis* plants under low and high levels of CO<sub>2</sub> concentration and c) the photosynthetic response of these plants under low and high radiation levels.

## 2. Materials and methods

### 2.1. Experimental areas

Two montane grasslands, located near the villages Ano Grammatiko (Vermio Mountain) and Filippaioi (Smolikas Mountain), approximately 125 km and 225 km west of Thessaloniki, Greece, respectively, were selected. Both areas lie at the same altitudinal zone (1200–1300 m a.s.l.) and are grazed mainly by transhumance sheep and goats. The climate of the two regions is characterized as sub-Mediterranean and belongs to the humid bioclimatic floor with severe winters. The mean annual precipitation is 1047 mm and 1079 mm while the mean annual air temperature 8.29 °C and 8.09 °C for Ano Grammatiko and Filippaioi,

respectively. In both grasslands the soil pH was about 5.5, C content 5%–6%, and N content 0.4%–0.5%. A detailed description of the vegetation of the two grasslands was given by Karatassiou et al. (2014) and Parissi et al. (2014). A third low-elevation grassland, located in the area of Thermi Thessaloniki, northern Greece, at an altitude of 85 m a.s.l., was also included in the current study. It is grazed by sheep and goats during spring and autumn. Its climate could be characterized as Mediterranean semiarid with dry summers extending from May to September. The mean annual precipitation is approximately 395 mm and the mean annual air temperature 16.5 °C. The pH of the soil was 6.5, C content 2%, and N content 0.2%. The vegetation of the area consisted mainly of forage species, such as *Agropyron intermedium* (Host) P.Beauv., *Bromus inermis* Leyss., *Phalaris aquatica* L., *Dactylis glomerata* L., *Bromus sterilis* L., *Hordeum murinum* L., *Medicago arabica* (L.) Huds., *Lotus corniculatus* L., *Vicia hybrida* L., and *Medicago minima* L. Climatic data for the year 2014 from the three areas, acquired from neighboring meteorological stations, are given in Table 1.

*Bromus inermis*, which grows in all three areas, was used as a model species to determine whether differential CO<sub>2</sub> and irradiation affect its photosynthetic performance. For the selection of *B. inermis* plants, the transect method as described by Cornelissen et al. (2003) was used. Five transects at 5-m intervals were used; the individual that fell first on each line was chosen and one leaf was selected for measurement of its photosynthetic responses. Measurements were carried out during late spring and

**Table 1.** Monthly temperature (T) and precipitation (P) for the year 2014 in the three experimental areas.

Month	Grammatiko		Filippaioi		Thessaloniki	
	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
J	-2.25	59.45	-2.55	44.9	8.8	31.8
F	1.3	112.8	-1.55	120.35	9.0	39.6
M	2.5	65.8	3.55	150.8	14.4	56.2
A	9.35	171.3	7.9	147.8	15.5	42.2
M	13.45	136.4	11.85	111.9	21.3	11.4
J	15.3	49	16.7	22.4	26.1	39.8
J	17.6	19.5	19.25	36.8	26.7	37.0
A	21.95	50.8	19.35	38.5	28.2	11
S	14	60.9	15.1	56.6	22.3	47.0
O	0	51.5	11.35	205.2	17.9	52.2
N	7.25	164.4	6.05	240.65	11.9	43.2
D	0.2	110.3	0.7	82.5	7.4	117.6
Annual	8.4	1052.2	9.0	1258.4	17.4	529.0

early summer of 2014. Plants from the lowland area were measured earlier (first days of May) than the montane ones (middle of June) in order for all plants selected for measurement to be at the vegetative stage.

Microclimatic conditions in the study areas (Table 2) were measured when gas exchange measurements under ambient conditions were taken. Air temperature and relative humidity were acquired using the microclimatic sensor Novasima MS1 (Novatron Scientific Ltd., Horsham, UK) and vapor pressure deficit was calculated according to Abtew and Melesse (2012).

## 2.2. Gas exchange measurements

In each grassland, gas exchange parameters of *B. inermis*, such as net assimilation rate ( $A$ ), transpiration rate ( $E$ ), stomatal conductance ( $G_s$ ), and intercellular  $CO_2$  concentration ( $C_i$ ), were measured under: a) ambient conditions, b) low ( $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and high ( $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) photosynthetic photon flux density (PPFD) levels with the  $CO_2$  concentration fixed at  $380 \mu\text{mol mol}^{-1}$ , and c) ambient ( $380 \mu\text{mol mol}^{-1}$ ) and high ( $800 \mu\text{mol mol}^{-1}$ )  $CO_2$  concentrations with the PPFD level fixed at  $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ . All measurements were taken using a portable photosynthesis system (LCpro-SD, ADC Bioscientific Ltd., Hoddesdon, UK) from 1000 to 1200 hours on five mature, intact, fully expanded upper leaves. Water use efficiency (WUE) and intrinsic water use efficiency ( $WUE_i$ ) were calculated from the ratios  $A/E$  and  $A/G_s$ , respectively (Jones, 2004).

In addition, photosynthetic light response curves (A-PPFD) were recorded on at least four fully expanded leaves from each area. Each of the sampled leaves was individually placed in the leaf chamber of the above-mentioned photosynthesis system and illuminated by the built-in light unit attached to the sensor head. Leaves placed in the chamber were allowed to acclimate for approximately 10 min before gas exchange measurements were taken. Leaf gas exchange measurements at different levels of PPFD at leaf surface started at a light level of  $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD and decreased stepwise to  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD using ten different light levels of 1800, 1500, 1200, 900, 700, 400, 200, 100, 40, and  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD; at each light level leaves were equilibrated for approximately 7 min before recording photosynthesis. During the gas exchange measurements the ambient  $CO_2$  concentration in the chamber was maintained at  $350 \mu\text{mol mol}^{-1}$ , relative humidity at 45%–55%, and leaf temperature at  $25 \pm 1.0 \text{ }^\circ\text{C}$ .

The assimilation rate ( $A$ ) was also measured in response to changes in the intercellular  $CO_2$  partial pressure ( $C_i$ ). Photosynthetic  $CO_2$  response curves ( $A - C_i$ ) were recorded on at least four fully expanded leaves in each experimental area. Leaf gas exchange measurements at different levels of  $C_i$  started at a  $C_i$  concentration of  $700 \mu\text{mol mol}^{-1}$  and decreased stepwise to  $0 \mu\text{mol mol}^{-1}$  using eight different  $C_i$  levels: 700, 550, 450, 350, 200, 100, 50,

**Table 2.** Mean ( $n = 5$ )  $\pm$  SE temperature ( $T$ ), relative humidity (RH), and vapor pressure deficit (VPD) in the three experimental areas during the measurement of the gas exchange parameters under ambient conditions.

Area	T ( $^\circ\text{C}$ )	RH (%)	VPD (kPa)
Grammatiko	$25.3 \pm 0.3$	$9.3 \pm 0.8$	$2.92 \pm 0.41$
Filippaioi	$24.4 \pm 0.2$	$32.8 \pm 2.1$	$2.05 \pm 0.23$
Thessaloniki	$24.4 \pm 0.1$	$27.4 \pm 1.7$	$2.22 \pm 0.18$

and  $0 \mu\text{mol mol}^{-1}$ . At each  $C_i$  level, leaves were equilibrated for approximately 7 min before recording photosynthesis. During the gas exchange measurements the PPFD in the chamber was maintained at  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , relative humidity at 45%–55%, and leaf temperature at  $25 \pm 1.0 \text{ }^\circ\text{C}$ .

Light saturated (maximum) photosynthetic rate ( $A_{\text{max}}$ ), dark respiration rate ( $R_d$ ), apparent quantum efficiency (AQE), photosynthetic light compensation point (LCP), photosynthetic light saturation point (LSP),  $CO_2$  compensation point ( $CO_2$  CP), assimilation at saturating  $CO_2$  ( $A_{\text{sat}}$ ), and carboxylation efficiency (CE) were estimated for each area using Photosyn Assistant software, version 1.1.2 (Dundee Scientific, Dundee, UK), which determines these parameters by fitting the light response data to a model function expressed as a quadratic equation (Prioul and Chartier, 1977). The variables were estimated using least squares fitting regression and the Nelder–Mead minimization routine (Nelder and Mead, 1965).

## 2.3. Chlorophyll fluorescence measurements

In vivo chlorophyll fluorescence was measured from the same leaves used for the above-mentioned curves using a chlorophyll fluorometer (OS-30p, Opti-Sciences Inc., Hudson, NH, USA). The fluorescence parameters were measured using the saturation pulse method (Schreiber et al., 1994) with 30-min dark-adapted leaves. The ratio of variable to maximal fluorescence ( $F_v/F_m$ ), expressing the maximum quantum yield of photosystem II (Maxwell and Johnson, 2000), was measured for five plants in each experimental area.

## 2.4. Statistical analysis

To determine differences in the ecophysiological response of *B. inermis* among the three areas one-way analysis of variance (ANOVA) was performed for all parameters studied (Steel and Torrie, 1980). Means were compared using the LSD test at  $\alpha = 0.05$ . All statistical analyses were carried out with IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA).

## 3. Results

The analysis of variance revealed significant differences for  $G_s$ ,  $C_p$ ,  $A$ , WUE, and  $WUE_i$  among the three areas under ambient conditions (Table 3). However, the transpiration

**Table 3.** Mean ( $n = 5$ ) values  $\pm$  SE and level of significance for transpiration rate (E), stomatal conductance ( $G_s$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), assimilation rate (A), water use efficiency (WUE), and intrinsic water use efficiency ( $\text{WUE}_i$ ) of *Bromus inermis* plants located in montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas under ambient conditions. Means within each column accompanied with different letters differ significantly at  $P < 0.05$ .

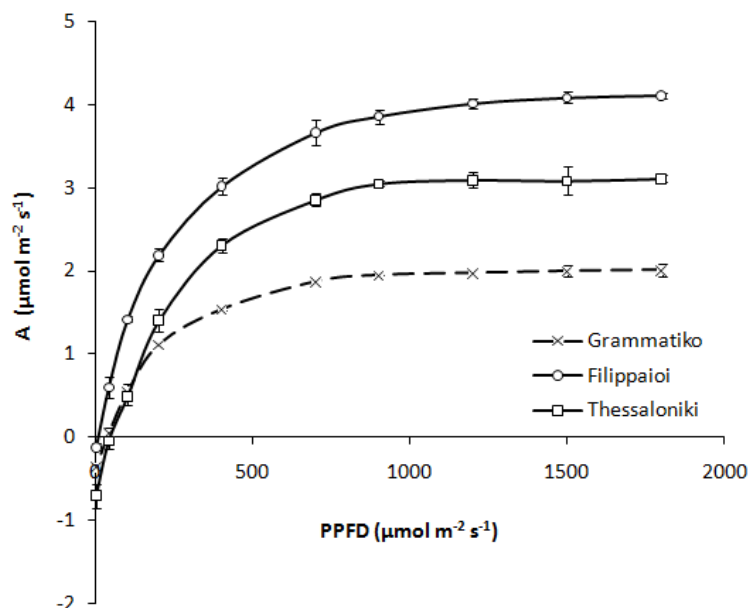
Area	E ( $\text{mmol m}^{-2}\text{s}^{-1}$ )	$G_s$ ( $\text{mol m}^{-2}\text{s}^{-1}$ )	$C_i$ ( $\mu\text{mol mol}^{-1}$ )	A ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	WUE	$\text{WUE}_i$
Grammatiko	$1.26 \pm 0.12$ a	$0.05 \pm 0.01$ a	$237.4 \pm 13.0$ a	$3.40 \pm 0.31$ a	$2.79 \pm 0.40$ a	$77.4 \pm 14.5$ a
Filippaioi	$1.41 \pm 0.17$ a	$0.02 \pm 0.01$ b	$64.6 \pm 10.1$ b	$6.03 \pm 0.37$ b	$4.53 \pm 0.57$ b	$261.4 \pm 28.6$ b
Thessaloniki	$0.99 \pm 0.07$ a	$0.04 \pm 0.01$ a	$296.2 \pm 17.7$ c	$1.12 \pm 0.43$ c	$1.25 \pm 0.46$ c	$32.0 \pm 11.7$ a
F	2.73	5.57	74.2	43.7	11.6	38.0
Significance	ns	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$

rate was similar in the three areas ( $P \geq 0.05$ ). Plants from Grammatiko and Thessaloniki exhibited higher values of stomatal conductance compared to plants from Filippaioi ( $P < 0.05$ ). On the other hand, plants from Filippaioi showed higher values of assimilation rate and water use efficiency (WUE and  $\text{WUE}_i$ ) ( $P < 0.05$ ) (Table 3).

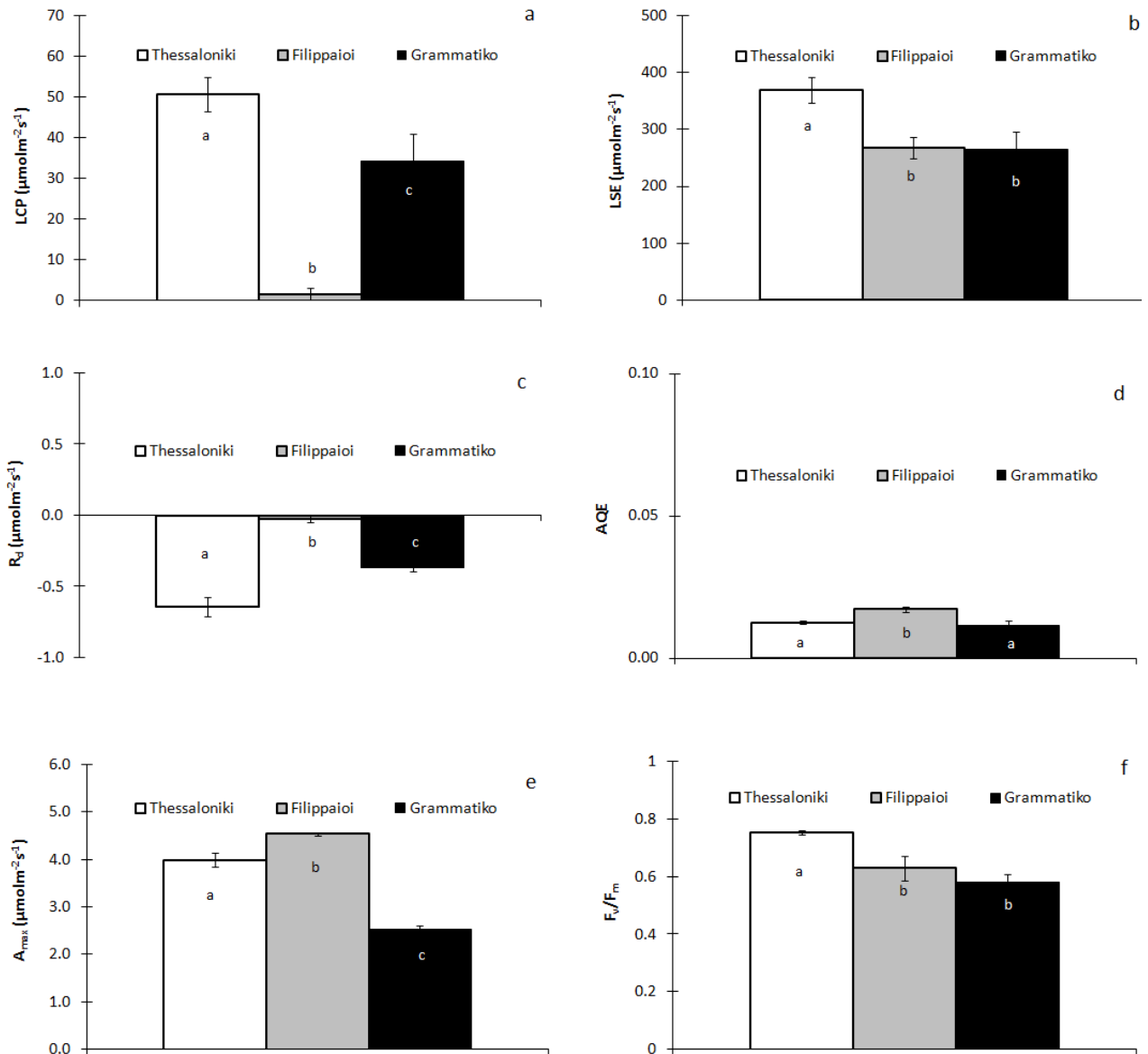
Regardless of the PPFD level, the photosynthetic light curves (A-PPFD) of *B. inermis* (Figure 1) showed higher values of net assimilation rate in Filippaioi ( $P < 0.01$ ) followed by Thessaloniki and Grammatiko. The assimilation rate of Thessaloniki and Grammatiko was similar at low levels of PPFD ( $< 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) but significantly different at higher light intensities ( $P < 0.05$ ).

The analysis of variance indicated significant differences in the maximum assimilation rate ( $P < 0.001$ ), light

compensation point ( $P < 0.01$ ), light saturation estimate ( $P < 0.05$ ), dark respiration ( $P < 0.001$ ), and quantum efficiency ( $P < 0.05$ ) among the three areas (Figure 2). The light compensation point (Figure 2a) was significantly higher in Thessaloniki ( $50.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), followed by Grammatiko ( $34.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and Filippaioi, where the lowest value was recorded ( $1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The light saturation estimate (Figure 2b) was significantly ( $P < 0.05$ ) higher in the lowland area of Thessaloniki ( $370 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); the two montane areas had similar values of LSE of about  $265 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Dark respiration ( $R_d$ ) (Figure 2c) was significantly ( $P < 0.05$ ) higher in Thessaloniki ( $-0.64 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), followed by Grammatiko ( $-0.37 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and close to zero for Filippaioi. The apparent quantum efficiency was significantly ( $P < 0.05$ ) higher in Filippaioi



**Figure 1.** Response curves of assimilation rate (A) in relation to elevated irradiation (PPFD) of *Bromus inermis* located in montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas. Points represent mean ( $n = 4$ ) values  $\pm$  SE.



**Figure 2.** Mean ( $n = 4$ ) values  $\pm$  SE of a) light compensation point (LCP), b) light saturation estimate (LSE), c) dark respiration ( $R_d$ ), d) apparent quantum efficiency (AQE), e) maximum assimilation rate ( $A_{\text{max}}$ ), and f) ratio of variable to maximal chlorophyll fluorescence ( $F_v/F_m$ ) of *B. inermis* plants located in montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas. Columns accompanied by different letters differ significantly at  $P < 0.05$ .

(Figure 2d), and similarly lower for both Grammatiko and Thessaloniki. The maximum assimilation rate (Figure 2e) was significantly ( $P < 0.05$ ) higher ( $4.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in Filippaioi, followed by Thessaloniki ( $4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), while significantly ( $P < 0.05$ ) lower values were recorded in Grammatiko ( $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The ratio of variable to maximal fluorescence  $F_v/F_m$  (Figure 2f) was significantly ( $P < 0.05$ ) higher in Thessaloniki (0.75) compared to Filippaioi (0.62) and Grammatiko (0.58).

The  $A - C_i$  curves of *Bromus inermis* showed that for values of  $\text{CO}_2$  lower than  $350 \mu\text{mol mol}^{-1}$  plants from

Filippaioi had significantly ( $P < 0.05$ ) higher assimilation rates, while for values of  $\text{CO}_2$  above  $350 \mu\text{mol mol}^{-1}$  plants from Thessaloniki had significantly ( $P < 0.05$ ) higher assimilation rates, followed by plants from Filippaioi and Grammatiko (data not shown). Significantly ( $P < 0.05$ ) higher carboxylation efficiency (CE) (Table 4) was found in Filippaioi, followed by Thessaloniki and Grammatiko. The lowland area of Thessaloniki had significantly ( $P < 0.05$ ) higher  $A_{\text{sat}}$  and  $\text{CO}_2$  compensation point compared to the other two areas. Plants from Filippaioi and Grammatiko did not differ significantly in terms of  $A_{\text{sat}}$ . On the contrary,

**Table 4.** Mean ( $n = 4$ ) values  $\pm$  SE of carboxylation efficiency (CE), assimilation at saturating  $\text{CO}_2$  ( $A_{\text{sat}}$ ), and  $\text{CO}_2$  compensation point (CP) of *Bromus inermis* plants located in montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas. Means within each column accompanied with different letters differ significantly at  $P < 0.05$ .

Area	CE	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$\text{CO}_2$ CP ( $\mu\text{mol mol}^{-1}$ )
Grammatiko	$0.113 \pm 0.014$ a	$10.93 \pm 2.24$ a	$6.31 \pm 0.25$ a
Filippaioi	$0.245 \pm 0.041$ b	$7.94 \pm 0.54$ a	$1.40 \pm 0.54$ b
Thessaloniki	$0.203 \pm 0.025$ ab	$17.81 \pm 1.84$ b	$8.06 \pm 0.30$ c

plants from Filippaioi presented significantly ( $P < 0.05$ ) lower  $\text{CO}_2$  compensation points.

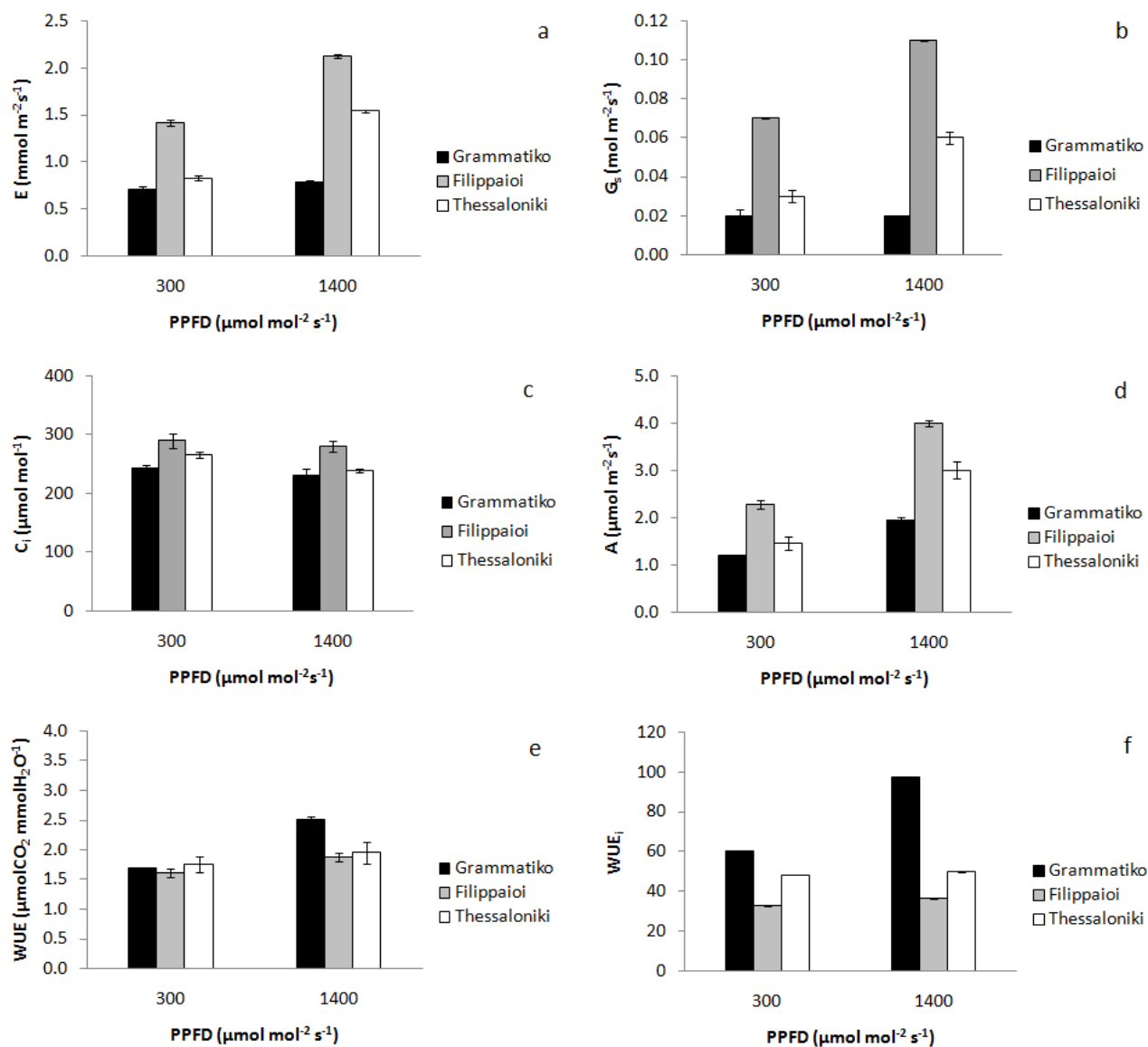
Comparison of the gas exchange responses of *B. inermis* under low and high PPF levels demonstrated that all parameters, except  $C_p$ , increased under high levels of PPF (Figure 3). Although this increase was very negligible in Grammatiko, plants from Filippaioi and Thessaloniki increased their transpiration rate (Figure 3a), stomatal conductance (Figure 3b), and assimilation rate (Figure 3d) at high PPF levels. On the other hand, plants from Grammatiko had higher WUE values at high levels of PPF, while plants from Thessaloniki had higher WUE under low PPF levels (Figure 3e). The intrinsic WUE ( $\text{WUE}_i$ ) was almost two times higher in Grammatiko compared to the other two areas, under both high and low PPF (Figure 3f).

Plants of *Bromus inermis* from all three areas reduced their transpiration rate under elevated  $\text{CO}_2$  ( $700 \mu\text{mol mol}^{-1}$ ) concentration (Figure 4a) at differential rates (Grammatiko 47%, Thessaloniki 29%, and Filippaioi 14%). A higher transpiration rate was found in plants from Filippaioi and lower in plants from Grammatiko. Stomatal conductance decreased by 50% in Grammatiko and 40% in Thessaloniki and increased by 14% in Filippaioi under elevated  $\text{CO}_2$  concentration (Figure 4b). At  $350 \mu\text{mol mol}^{-1} \text{CO}_2$  plants from Thessaloniki had higher  $G_s$ , while at  $700 \mu\text{mol mol}^{-1} \text{CO}_2$  concentration plants from Filippaioi showed higher  $G_s$  values. The  $C_i$  was higher in Filippaioi and Thessaloniki and lower in Grammatiko under both  $\text{CO}_2$  concentrations (Figure 4c). From  $350$  to  $700 \mu\text{mol mol}^{-1} \text{CO}_2$  concentration,  $C_i$  increased by 17% in Grammatiko, 117% in Filippaioi, and 66% in Thessaloniki. The assimilation rate was higher in plants from Thessaloniki both under  $350$  and  $700 \mu\text{mol mol}^{-1} \text{CO}_2$  concentrations, followed by Filippaioi and Grammatiko (Figure 4d). Under elevated  $\text{CO}_2$  concentration,  $A$  increased by 76% in Grammatiko and 62% and 67% in Filippaioi and Thessaloniki, respectively. Plants from the montane area of Grammatiko showed the highest values of WUE (Figure 4e) and  $\text{WUE}_i$  (Figure 4f) under both  $\text{CO}_2$  concentrations. In particular, WUE increased dramatically in Grammatiko under elevated  $\text{CO}_2$  (230%) and moderately in Filippaioi

(87%) and Thessaloniki (135%). On the contrary, under elevated  $\text{CO}_2$  concentration the  $\text{WUE}_i$  in both Thessaloniki and Grammatiko increased by 184% and 187%, respectively, while this increase in Filippaioi was only 54%.

#### 4. Discussion

*Bromus inermis*, a common species in Mediterranean grasslands, is widely adapted to both a variety of sites and light environments and has a wide elevation range. However, in the current study, under ambient conditions, *B. inermis* expressed great differentiation in its physiological response in the three experimental areas. Although plants from Filippaioi presented significantly lower values of  $G_s$  compared to the other areas, their assimilation rate was significantly higher, suggesting that the stomatal aperture is not the only factor affecting photosynthesis in these plants. On the contrary, plants from Grammatiko and Thessaloniki showed higher values of  $G_s$  under ambient conditions; nevertheless, the higher  $G_s$  did not have the expected impact on the assimilation rate, which presented low values in both areas. Stomatal responses could increase the supply of  $\text{CO}_2$  to the intercellular spaces and, consequently, an increase in assimilation rate is usually expected (Chen et al., 2005). Interestingly, in the current study, the differentiation in assimilation rate in the above-mentioned areas is pointing towards the existence of additional factors that inhibit the photosynthetic performance. It is well known that the photosynthetic rate, responsible for dry matter production, is controlled by, among others, both stomatal ( $G_s$ ) and mesophyll ( $G_m$ ) conductance (Karatassiou et al., 2009), with the stomatal limitations comprising less than 20% of the total limitations of photosynthesis (Jones, 1998). In Grammatiko the lower assimilation rate could also be attributed to the low values of the ratio of variable to maximal fluorescence ( $F_v/F_m$ ). This ratio is proportional to the quantum yield of photosynthesis and in nonstressed terrestrial leaves an average value of  $F_v/F_m$  ranging from 0.79 to 0.84 has been estimated (Maxwell and Johnson, 2000). Changes in  $F_v/F_m$  are very sensitive and inversely proportional to physiological stress and might indicate the beginning of a nonrapidly reversible photoinhibition (Osmond and Grace, 1995; Flexas et al., 2012.).

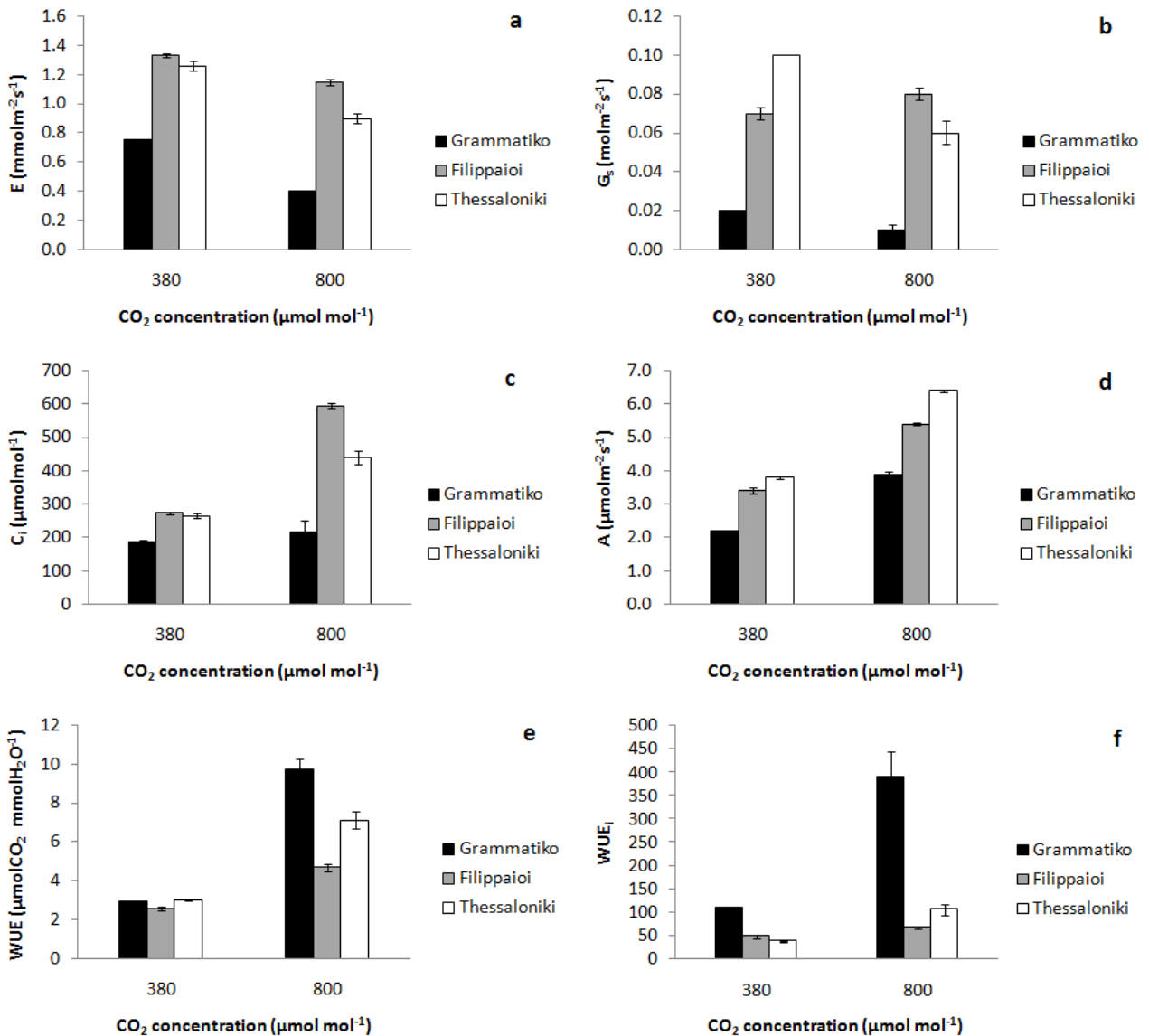


**Figure 3.** Mean ( $n = 4$ ) values  $\pm$  SE of a) transpiration rate (E), b) stomatal conductance ( $G_s$ ), c) internal  $CO_2$  concentration ( $C_i$ ), d) assimilation rate (A), e) water use efficiency (WUE), and f) intrinsic water use efficiency ( $WUE_i$ ) of *Bromus inermis* plants from montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas under low (300  $\mu mol m^{-2} s^{-1}$ ) and high (1400  $\mu mol m^{-2} s^{-1}$ ) irradiation (PPFD).

Another important finding was that, although the three areas differed in terms of stomatal conductance, no differences in their transpiration rate were found. It seems that in this species other factors, both plant and/or environmental, play a crucial role in the regulation of transpiration rate (Larcher, 2003; Karatassiou et al., 2009). In addition, the higher values of WUE in Filippaioi could be attributed to the higher values of assimilation rate in this area and, for the case of  $WUE_p$ , also to the lower values of  $G_s$ . Therefore, *Bromus inermis* probably uses not only phenological

but also physiological mechanisms in order to control its transpiration and assimilation rate under different environmental conditions.

To our knowledge there are scarce reports concerning the photosynthetic responses of grassland species to elevated irradiation and  $CO_2$  concentration, making comparisons within and between taxonomic groups rather difficult. Nevertheless, the photosynthetic performance of *B. inermis* was found to be similar to that reported for *Stipa grandis* and lower than that reported for *Leymus chinensis* (Chen et al., 2005).



**Figure 4.** Mean ( $n = 4$ ) values  $\pm$  SE of a) transpiration rate (E), b) stomatal conductance (G<sub>s</sub>), c) internal CO<sub>2</sub> concentration (C<sub>i</sub>), d) assimilation rate (A), e) water use efficiency (WUE), and f) intrinsic water use efficiency (WUE<sub>i</sub>) of *Bromus inermis* plants from montane (Grammatiko, Filippaioi) and lowland (Thessaloniki) areas under ambient (380  $\mu\text{mol mol}^{-1}$ ) and elevated (800  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> concentrations.

The photosynthetic light response curves from the two montane areas of Grammatiko and Filippaioi saturated at lower irradiances (about 265  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to the nonmontane area (lowland) of Thessaloniki (about 370  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Saturation at higher irradiances is usually reported in sun-adapted, early successional species (Awada et al., 2003). Plants grown in high light levels show some ability for photosynthetic acclimation as evidenced by the higher light saturated rate of CO<sub>2</sub> assimilation (Horton and Neufeld, 1998). On the other hand, plants from the montane area of Filippaioi had lower light compensation points and lower dark respiration values compared to the

other two areas. Low light compensation point and low dark respiration rate characterize plants exposed to shade (Dalmolin et al., 2013), allowing them to maintain a small but positive carbon gain during long periods of low light in the understory (Horton and Neufeld, 1998). Plants from Filippaioi also expressed significantly higher values of A<sub>max</sub>. Maximum photosynthetic capacity reflects the rate of diffusion of CO<sub>2</sub> to RuBisCo, the activity of RuBisCo, and/or the rate of RuBP regeneration (Farquhar et al., 1980). Differences in A<sub>max</sub> have been attributed to differences in the biochemical efficiency of carboxylation rather than to differences in CO<sub>2</sub> supply to the intercellular spaces



(Shangguan et al., 2000). In our case, however, the higher photosynthetic capacity of *B. inermis* in Filippaioi could also be due to the higher supply of CO<sub>2</sub> to the intercellular spaces as a result of the much higher stomatal conductance evidenced under both low and high PPFD levels. The lower photosynthetic performance of the plants from the other montane area of Grammatiko, as expressed by the lower A values in the A-PPFD curve (Figure 1) and the lower A<sub>max</sub> and AQE values, could be attributed to the observed low values of the maximum quantum yield of photosystem II (F<sub>v</sub>/F<sub>m</sub>), as has been pointed out earlier.

The increase in the PPFD level exerted a positive effect on all measured physiological parameters; this increase was more obvious in Filippaioi and Thessaloniki. Generally in grasslands, both assimilation rate and stomatal conductance have been found to parallel PPFD (Bollig and Feller, 2014). Although showing low values of assimilation rate, plants in Grammatiko showed high values of both WUE and WUE<sub>i</sub>. This suggests that plants in this area, although achieving lower assimilation rates, have the capacity to balance their transpiration losses as well and therefore use the available water reserves more efficiently, enabling them to express higher survival rates under drought conditions, which are common in the Mediterranean area. Differential response in the two montane areas may be attributed to rangeland composition (grasslands and woodlands prevail in Filippaioi and shrublands in Grammatiko) posing a pressure for differential response to light conditions. Therefore, it is plausible to hypothesize that plants in Filippaioi may have been adapted to higher light intensity since they are growing mainly in grasslands.

The photosynthetic A – C<sub>i</sub> response curves of *B. inermis* revealed a photosynthetic upregulation by elevated CO<sub>2</sub> in all three areas. On the other hand, elevated CO<sub>2</sub> concentration also resulted in the decrease of stomatal conductance in plants from Grammatiko and Thessaloniki and the increase of G<sub>s</sub> in plants from Filippaioi. C<sub>3</sub> plants show a significant positive response to photosynthetic acclimation to elevated CO<sub>2</sub> (Bernacchi et al., 2003; Reddy et al., 2010). Effects of elevated CO<sub>2</sub> on stomatal conductance, though, are not so clear. In previous studies, elevated CO<sub>2</sub> concentration was found to decrease or have no effect on stomatal conductance (Reich et al., 2001; Maherali et al., 2002; Ainsworth et al., 2004; Long et al., 2004), although these responses were generally small or variable, depending on plant species and functional type (Medlyn et al., 2001; Lewis et al., 2002). RuBisCo is substrate limited by the current atmospheric CO<sub>2</sub> levels; this enzyme has the potential to respond to an increase in CO<sub>2</sub> concentration. Elevated CO<sub>2</sub> increases the velocity of carboxylation and competitively inhibits the oxygenase reaction. In other words, elevated CO<sub>2</sub> concentration increases the carboxylation efficiency and therefore reduces photorespiration (Reddy et al., 2010).

In addition, the A – C<sub>i</sub> response curves of plants in the lowland area of Thessaloniki saturated at higher values of CO<sub>2</sub> compared to the other areas. The higher values of A<sub>sat</sub> in plants coming from the lowland area of Thessaloniki could be due to the fact that in this area higher temperatures exist throughout the year. According to Sage (2002), the CO<sub>2</sub> saturation point increases with rising temperature. On the other hand, the rise in CO<sub>2</sub> concentration led to the increase of water use efficiency (both WUE and WUE<sub>i</sub>) in all three areas. The increase of WUE could be due to the decrease of stomatal conductance and hence transpiration under elevated CO<sub>2</sub> concentration (Ainsworth et al., 2002).

Mountain ecosystems are characterized by differences in important abiotic factors, such as temperature, atmospheric pressure, UV radiation, precipitation, snow duration, and length of the growing season, which change with elevation (Frei et al., 2014). CO<sub>2</sub> concentration and temperature generally decrease with altitude, while irradiance increases (Kumar et al., 2005). Apart from the climate parameters, other environmental factors, such as soil parameters (soil depth and fertility for example), soil fauna, and neighbors' identity, might also be variable at a more local scale, limiting plant performance (Frei et al., 2014). In a number of studies, a decrease in plant performance with elevation was found, mainly as a result of lower temperature and a shorter growing season (Koerner, 2003). Photosynthesis has long been recognized as one of the most temperature-sensitive processes in plants (Yamori et al., 2014). To our knowledge there are no studies that evaluate the performance of *B. inermis* at different altitudes. Only Grassein et al. (2014), working on the sibling species *Bromus erectus* Huds. (erect brome), reported decreased photosynthetic performance with elevation. Frei et al. (2014) found that *Ranunculus bulbosus*, *Trifolium montanum*, and *Briza media* responded differently according to their elevation of origin, indicating genetic differentiation. Other studies report that plants at high altitudes have higher photosynthetic efficiency than plants at low altitude (Friend and Woodward, 1990; Körner and Diemer, 1994). In Switzerland, Bollig and Feller (2014) found different reactions considering photochemistry and net assimilation in grass species at the highest elevation site under drought conditions. In their study, Kumar et al. (2005) reported that the assimilation rate was not significantly different between altitudes, while G<sub>s</sub> and E increased with altitude. Interestingly, they also found that plants from different altitudes, when grown under the same conditions, no longer displayed differences in gas exchange parameters, suggesting that the effect of altitude is mainly due to environmental modifications rather than genotypic adaptation.

Although both montane areas are characterized by similar climatic conditions, the photosynthetic

response of *B. inermis* from Filippaioi was better to changes in irradiation compared to Grammatiko, while photosynthesis in the lowland area of Thessaloniki responded more to changes in CO<sub>2</sub> concentration. The two montane areas are subjected to different grazing pressures, mainly by sheep and goats from May to October (Karatassiou et al., 2014; Parissi et al., 2014). Therefore, in Filippaioi other factors apart from climate, such as high grazing pressure over the last 30 years (Karatassiou et al., 2014), may have played a more important role in the plants' physiological adaptation to elevated irradiation and high CO<sub>2</sub> level. Thus, it seems that there are no clear effects of elevation on the photosynthetic responses of *B. inermis*. Plants from each area may have employed different mechanisms to adapt to local environmental and ecological conditions. Plant species with wide altitudinal ranges face different environmental conditions across the elevation gradients, which may lead to phenotypic variation or genetic differentiation among populations

(Pluess and Stöcklin, 2005; Ohsawa and Ide, 2008; Haider et al., 2012). Phenotypic plasticity has been recognized as an important alternative mechanism in plant responses to climate change (Matesanz et al., 2010; Nicotra et al., 2010).

In conclusion, our results showed that the local environmental conditions and the grazing history of each area may have influenced the physiological mechanisms used by plants in order to adapt and thrive in each environment, rather than the altitude alone. Therefore, within the same species, plants could employ different mechanisms in order to adapt in the special environmental conditions of each area.

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