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Lotus corniculatus L. response to carbon dioxide concentration and radiation level variations

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Abstract

Carbon dioxide concentration and light conditions may greatly vary between mountainous and lowland areas determining the photosynthetic performance of plants species. This paper aimed to evaluate the photosynthetic responses of *Lotus corniculatus*, growing in a mountain and a lowland grassland, under low and high radiation and CO_2 concentration. Net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration were measured while the water-use efficiency and the ratio of variable to maximal fluorescence were calculated. Photosynthetic response curves to different levels of radiation and intercellular CO_2 partial pressure were estimated. Our results showed that high radiation and CO_2 concentration enhanced water-use efficiency of plants at both sites, enabling them to use more efficiently the available water reserves under drought conditions. The increase of radiation and CO_2 concentration would enhance the photosynthetic performance of the mountainous population of *L. corniculatus*, which overall seems to express higher phenotypic plasticity.

Additional key words: ambient conditions; birdsfoot trefoil; elevation; light; photosynthetic rate; plasticity index.

Introduction

Grasslands represent 13.2% of the total area in 27 countries of the European Union (Trnka *et al.* 2011) including plant species with a high economic value (Allen-Diaz *et al.* 1996, Alkemade *et al.* 2013). They also cover a large area of Greece constituting an important component of the mountainous ecosystems. In addition, intermediate and high elevation grasslands represent a valuable resource for the transhumant livestock system (Karatassiou *et al.* 2014).

In mountain ecosystems, vegetation is composed of surrounding species that have dispersed to higher elevation and became capable of adapting to highland conditions (Price *et al.* 2013). In mountain areas, which are considered particularly vulnerable to climate change, the CO_2 concentration and temperature generally decrease with altitude, while irradiance increases (Kumar *et al.* 2005). Despite the sensitivity of photosynthesis to short-term variation in temperature, leaf properties minimize the differences in leaf temperature between ecosystems, and plants may become acclimated and adapted. Thus, there is no clear relationship between temperature and average photosynthetic rate in the field, when ecosystems are compared (Chapin *et al.* 2011). Since CO_2 concentration plays a crucial role in plant photosynthesis, it is important to understand plant response to elevated CO_2 concentration in order to predict grassland structure and function in the future. Light, another critical component of photosynthesis, on the other hand, is not frequently considered an important limiting resource for the photosynthetic performance of grasslands. Nevertheless, forage species are often subjected to limited irradiance conditions (*e.g.* shading by neighboring species, increased cloud cover *etc.*) (Humphreys 1991, Dias-Filho 2002), which may impact plant performance.

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Abbreviations: AQE – apparent quantum efficiency; C_i – intercellular CO_2 concentration; CE – carboxylation efficiency; CO_2 CP – CO_2 compensation point; E – transpiration rate; F_v/F_m – ratio of variable to maximal fluorescence; g_s – stomatal conductance; LCP – photosynthetic light-compensation point; LSP – photosynthetic light-saturation point; P_N – net photosynthetic rate; P_{Nmax} – light-saturated (maximum) photosynthetic rate; P_{Nsat} – photosynthesis at saturating CO_2; R_D – dark respiration rate; RDPI – relative distance plasticity index; RH – relative humidity; T_a – air temperature; VPD – vapor pressure deficit; WUE – instantaneous water-use efficiency.

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Lotus corniculatus L. (birdsfoot trefoil), a species of the Fabaceae family, grows under a wide range of environmental conditions (Escaray *et al.* 2012). It is a common herbaceous species in Greece persisting and thriving in a variety of soil conditions, including hardpan, dry, moist, acid, saline, infertile soils (Peterson *et al.* 1992, Escaray *et al.* 2012). It is agronomically important because of its high nutritive value, and the nonbloating features when grazed directly by livestock. It is a drought resistant species, well adapted to marginal environments (Carter *et al.* 1997, Frame *et al.* 1998, Escaray *et al.* 2012). Winter hardiness of *L. corniculatus* depends on cultivar provenance, while its optimum summer temperature for growth is approximately 24°C. Nevertheless, it is intolerant of shading, especially during establishment, requiring a day length of more than 16 h for assuming peak of flowering (Beuselinck and Grant 1995, Frame *et al.* 1998).

In the current study, we measured the photosynthetic response of *L. corniculatus,* growing in mountainous and lowland areas of northern Greece, to different CO_2 concentrations and irradiance level variations. We tested the hypothesis that the lowland population of *L. corniculatus* outweighs the mountainous one in terms of photosynthetic performance, exhibiting higher adaptability to an environment with elevated CO_2 concentration and radiation.

Materials and methods

Experimental sites: A mountainous grassland and a lowland grassland were selected. The mountainous grassland (site 1) was located near the village Filippaioi (Mountain Smolikas), approximately 225 km west of Thessaloniki (Greece) (1,200–1,300 m a.s.l.). The climate of this region is characterized as submediterranean and belongs to the humid bioclimatic floor with severe winter (Mavromatis 1978). The mean annual precipitation is 1,079 mm and the mean annual air temperature 8.09°C. The low elevation grassland (site 2) was located in Kilkis, approximately 50 km north-east of Thessaloniki, northern Greece (290 m a.s.l.). The climate of this area is characterized as Mediterranean semiarid, with dry summers extending from May to September. The mean annual precipitation is approximately 585 mm and the mean annual air temperature 12.2°C. Both grasslands are grazed mainly by sheep and goats (Karatassiou *et al.* 2014, Parissi *et al.* 2014) and their soil has a pH of approximately 6. At each site, five transects at five-meter intervals were used according to Cornelissen *et al.* (2003). A plant that fell first on each line was chosen and one leaf per plant was selected for measurements. At each site, a total of five plants were used for each set of measurements.

Microclimatic conditions in the study sites were measured under ambient conditions, when the gas-exchange measurements were taken as average of five measurements. Air temperature (T_a) and relative humidity (RH) were acquired using the microclimatic sensor *Novasima MS1* (*Novatron Scientific Ltd*, Horsham, UK). Vapor pressure deficit (VPD) was calculated according to Abtew and Melesse (2012). At site 1, the T_a, RH, and VPD were 24.1 ± 0.2°C, 32.8 ± 2.1%, and 2.05 ± 0.23 kPa, respectively, while at site 2, the T_a, RH, and VPD were 25.1 ± 0.3°C, 29.2 ± 1.5%, and 2.26 ± 0.18 kPa, respectively.

Gas-exchange measurements were carried out from late spring to early summer 2014. Plants from the site 2 were measured earlier in a season than those from the site 1 in order to achieve similar phenological stage of the plants at both sites. In each grassland, gas-exchange parameters of *L. corniculatus*, such as net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i), were measured under: (*1*) ambient conditions, (*2*) low [300 μmol(photon) m^{-2} s^{-1}] and high [1,400 μmol(photon) m^{-2} s^{-1}] PPFD with the CO_2 concentration fixed at 380 μmol(CO_2) mol^{-1}, and c) under ambient [380 μmol(CO_2) mol^{-1}] and high [800 μmol(CO_2) mol^{-1}] CO_2 concentration with the PPFD fixed at 1,400 μmol(photon) m^{-2} s^{-1}. All measurements were taken using a portable photosynthesis system (*LCpro-SD, ADC Bioscientific Ltd*, Hoddesdon, UK) from 10:00 to 12:00 h on five mature, intact, fully expanded, upper leaves. Water-use efficiency (WUE) was calculated from the ratio P_N/E (Jones 2004).

Photosynthetic light-response curves (P_N-PPFD) were recorded on five fully expanded leaves (one leaf per plant) from each site. Sampled leaves were placed in the leaf chamber of the above mentioned photosynthesis system and illuminated by the built-in light unit attached to the sensor head, acclimating for approximately 10 min before gas-exchange measurements. These measurements were taken at different PPFD [1,800; 1,500; 1,200; 900, 700, 400, 200, 100, 40, and 0 μmol(photon) m^{-2} s^{-1}], following a 7-min acclimation. During the measurements the ambient CO_2 concentration in the chamber was maintained at 380 μmol(CO_2) mol^{-1} and the temperature at 25 ± 1.0°C.

P_N was also measured in response to changes in C_i. Photosynthetic CO_2 response curves (P_N-C_i) were recorded on five fully expanded leaves (one leaf per plant) in each site at different C_i [700, 550, 450, 350, 200, 100, 50, and 0 μmol(CO_2) mol^{-1}]; following a 7-min acclimation process. During the gas-exchange measurements the PPFD in the chamber was maintained at 1,500 μmol(photon) m^{-2} s^{-1} and temperature at 25 ± 1.0°C.

Light-saturated photosynthetic rate (P_{Nmax}), 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), photosynthesis at saturating CO_2 (P_{Nsat}), and carboxylation efficiency (CE) were estimated by *Photosyn Assistant*, software, version *1.1.2* (*Dundee Scientific*, Dundee, UK), that 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).

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

Plasticity index: The "relative distance plasticity index" (RDPI) (Valladares *et al.* 2006) was used in order to compare the phenotypic plasticity of the populations from the two sites under ambient and high CO_2 and PPFD conditions. RDPI was calculated as

$$RDPI = \sum(dij \rightarrow i'j'/(xi'j'+xij))/n$$

where $dij \rightarrow i'j'$ is the absolute value of the difference ($x_{i'j'} - x_{ij}$), n is the total number of distances, x_{ij} is the trait value of a given individual j (j = 1,…3) subjected to i treatment (ambient concentration of CO_2 or radiation) and $x_{i'j'}$ is the trait value of a given individual j' (j' = 1,…3) subjected to treatment i' (high CO_2 or PPFD).

Statistical analysis: In order to determine differences in the ecophysiological response of *L. corniculatus* between the two sites, one-way analysis of variance (*ANOVA*) was performed on all parameters studied (Steel and Torrie 1980). *Pearson*'s *r* was used for the correlation analysis of the physiological data under ambient conditions. All statistical analyses were carried out with the *SPSS*® statistical software *v. 22.0* (*SPSS Inc.*, Chicago, IL, USA).

Results

Ambient conditions: The *ANOVA* revealed significant differences for C_i, P_N, and WUE between the two sites under ambient conditions (Table 1). However, E and g_s were not significantly different at the two sites. Plants from the site 1 exhibited significantly higher C_i compared to plants from the site 2. On the other hand, plants from

Table 1. Mean values ± SE (n = 5) and level of significance for transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), net photosynthetic rate (P_N), and water-use efficiency (WUE) of *Lotus corniculatus* plants located in a mountainous (site 1) and a lowland (site 2) grassland under ambient conditions.

	E [mmol(H_2O) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	P_N [μmol m^{-2} s^{-1}]	WUE [mol(CO_2) mol^{-1}(H_2O)]
Site 1	2.18 ± 0.37	0.12 ± 0.03	278.8 ± 10.5	3.64 ± 0.48	1.79 ± 0.28
Site 2	1.97 ± 0.35	0.11 ± 0.03	205.8 ± 21.2	8.30 ± 0.88	4.71 ± 0.95
F	0.18	0.05	9.5	21.58	8.63
Significance	ns	ns	$P<0.05$	$P<0.01$	$P<0.05$

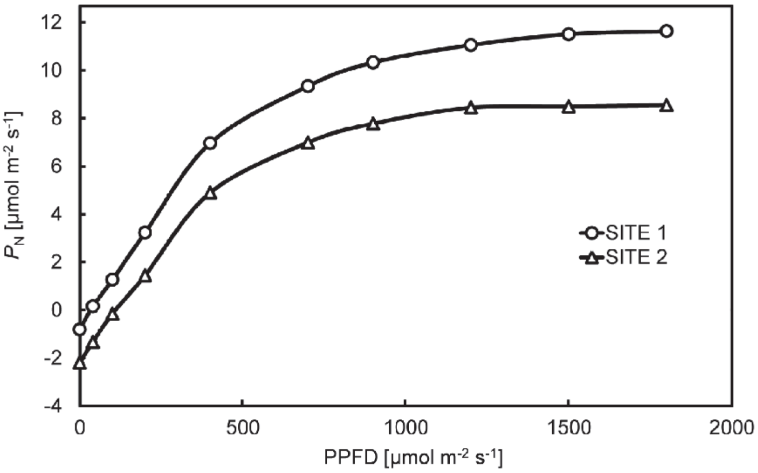

Fig. 1. Response curves of net photosynthetic rate (P_N) in relation to elevated irradiation (PPFD) of *Lotus corniculatus* plants located in a mountainous (site 1) and a lowland (site 2) grassland of northern Greece. Points represent mean values ± SE, n = 5.

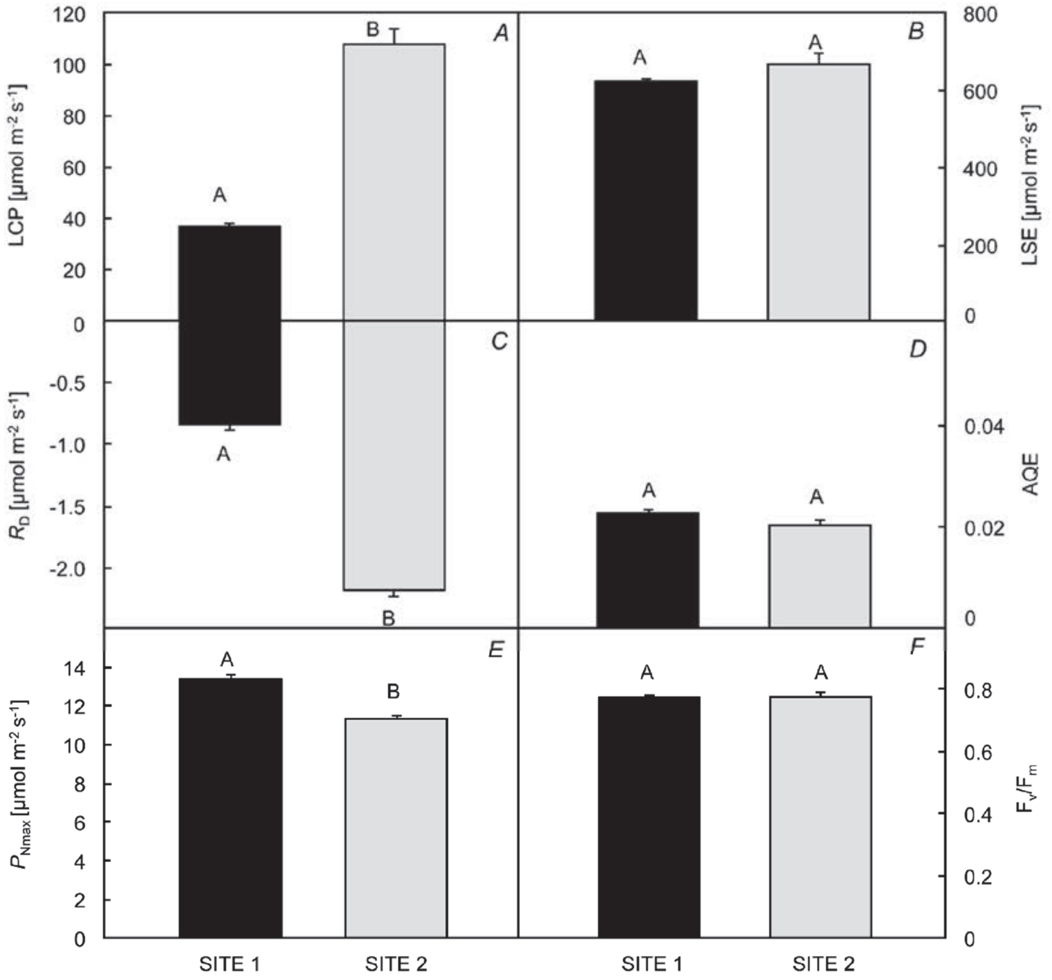

Fig. 2. Mean values ± SE ($n = 5$) of light-compensation point (LCP), light-saturation estimate (LSE), dark respiration (R_D), apparent quantum efficiency (AQE), maximum photosynthetic rate (P_{Nmax}), and ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) of *Lotus corniculatus* plants located in a mountainous (site 1) and a lowland (site 2) grassland of northern Greece. Columns accompanied by *different letters* differ significantly at $P<0.05$.

the site 2 showed significantly higher values of P_N and WUE. Correlation analysis revealed a significant association between C_i and WUE (site 1: $r = –0.948$, site 2: $r = –0.966$, $P<0.05$), g_s and E (site 1: $r = 0.991$, site 2: $r = 0.975$, $P<0.01$), and F_v/F_m and P_N only for the site 2 ($r = 0.912$, $P<0.05$).

Light curves: The P_N-PPFD curves (Fig. 1) showed significantly higher values of P_N for the site 1 compared to the site 2. P_{Nmax} (Fig. 2*E*) was 13.4 and 11.3 µmol m^{-2} s^{-1} at the site 1 and site 2, respectively. LCP (Fig. 2*A*) was significantly higher at the site 2 [108 µmol(photon) m^{-2} s^{-1}] compared to the site 1 [37 µmol(photon) m^{-2} s^{-1}]. R_D was also significantly higher in the site 2 [–2.2 µmol m^{-2} s^{-1}] compared to the site 1 [–0.8 µmol m^{-2} s^{-1}] (Fig. 2*C*), while LSE, AQE, and F_v/F_m (Fig. 2*B,D,F*) did not differ significantly between the two sites.

P_N-C_i curves: For CO_2 concentrations higher than 100 µmol mol^{-1}, the P_N of *L. corniculatus* plants was significantly higher at the site 1 than that in the site 2 (Fig. 3). Significant differences between the two sites were also found for CE and P_{Nsat}, with plants from the site 1 showing higher values (Table 2). On the contrary, plants from the site 2 had significantly higher CO_2 CP compared to those from the site 1 (Table 2).

Gas-exchange responses to elevated radiation: Gas-exchange responses of *L. corniculatus* plants under low and high PPFD (Fig. 4) demonstrated that all parameters, except C_i, increased under high PPFD. In particular, plants from the site 1 had a higher increase in E and g_s (48 and 37%, respectively) compared to the site 2, where the increase in E and g_s was 28 and 21%, respectively. Under high PPFD, plants from the site 2 showed a higher increase of P_N and WUE (446 and 325%, respectively) compared to plants from the site 1 (226% for P_N and 120% for WUE), while plants from the site 1 had higher g_s, P_N, and WUE compared to plants from the site 2 (Fig. 4). Plants from both sites had similar E and C_i.

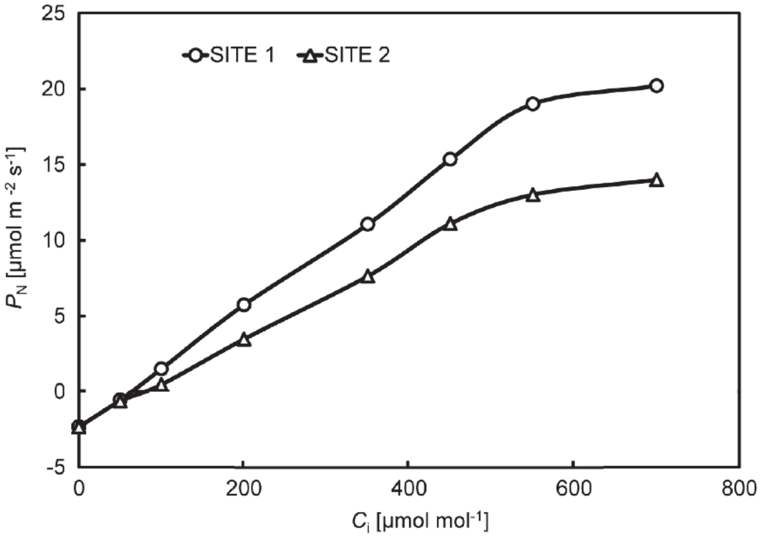

Fig. 3. Response curves of net photosynthetic rate (P_N) in relation to elevating CO_2 concentration of *Lotus corniculatus* plants located in a mountainous (site 1) and a lowland (site 2) grassland of northern Greece. Points represent mean values ± SE, *n* = 5.

Table 2. Mean values ± SE (*n* = 5) of carboxylation efficiency (CE), photosynthesis at saturating CO_2 (P_{Nsat}) and CO_2 compensation point (CP) for *Lotus corniculatus* plants located in a mountainous (site 1) and a lowland (site 2) grassland.

	CE	P_{Nsat} [μmol m $^{-2}$ s $^{-1}$]	CO_2 CP [μmol(CO_2) mol $^{-1}$]
Site 1	0.516 ± 0.013	74.12 ± 5.18	6.08 ± 0.14
Site 2	0.389 ± 0.009	49.19 ± 1.07	7.93 ± 0.29
Significance	$P<0.01$	$P<0.01$	$P<0.001$

Gas-exchange responses to elevated CO_2: Plants of *L. corniculatus* from both sites expressed slightly reduced *E* under an elevated CO_2 [800 μmol(CO_2) mol $^{-1}$] concentration (Fig. 5) (site 1: 15%, site 2: 3%), while g_s decreased by 4% at the site 1 and increased by 14% at the site 2. From 380 to 800 μmol(CO_2) mol $^{-1}$, C_i increased by 90% at the site 1 and by 122% at the site 2. A similar increase of P_N (about 80%) was found for plants from both sites under elevated CO_2 concentration, while WUE increased by 116% at the site 1 and 85% in the site 2. Under elevated CO_2 concentration (Fig. 5) plants from the site 1 demonstrated higher values of g_s, P_N, and WUE. On the contrary, plants from the site 2 showed higher C_i, while plants from both sites showed similar values of *E*.

Phenotypic plasticity index: The comparison of plant phenotypic plasticity of the two populations between ambient and high PPFD level (Fig. 6*A*) showed significant differences of RDPI only for P_N, which was higher for the site 1 compared to site 2. No significant differences were found between the two sites for the other gas-exchange parameters. The comparison of plant phenotypic plasticity of the two populations between ambient and high CO_2 (Fig. 6*B*) showed significant differences of RDPI for C_i, P_N, and WUE; higher values of RDPI were found at the site 1 for P_N and WUE, while the site 2 had higher RDPI for C_i.

Discussion

The results of the current study suggest that under ambient conditions, *L. corniculatus* plants from both sites demonstrated similar values of g_s and *E*. However, those from the site 2 expressed 2-fold higher P_N, and consequently also much higher WUE compared to plants from the site 1. Nevertheless, no significant correlation between P_N and g_s was found at both sites. Probably at high elevation sites under natural rainfall conditions other attributes (*e.g.* the allocation pattern, particular environmental conditions) and/or the previous grazing history often play a more important role than the photosynthetic mechanism alone (Pearcy and Ehleringer 1984, Karatassiou and Noitsakis 2010).

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. The photosynthetic light-response curves from both sites saturated at irradiances of about 650 μmol (photon) m $^{-2}$ s $^{-1}$. Saturation at higher irradiances is usually reported for sun-adapted, early successional species (Bazzaz and Carlson 1982, Awada *et al.* 2003). Plants grown under high light show some ability for photosynthetic acclimation as evidenced by the higher

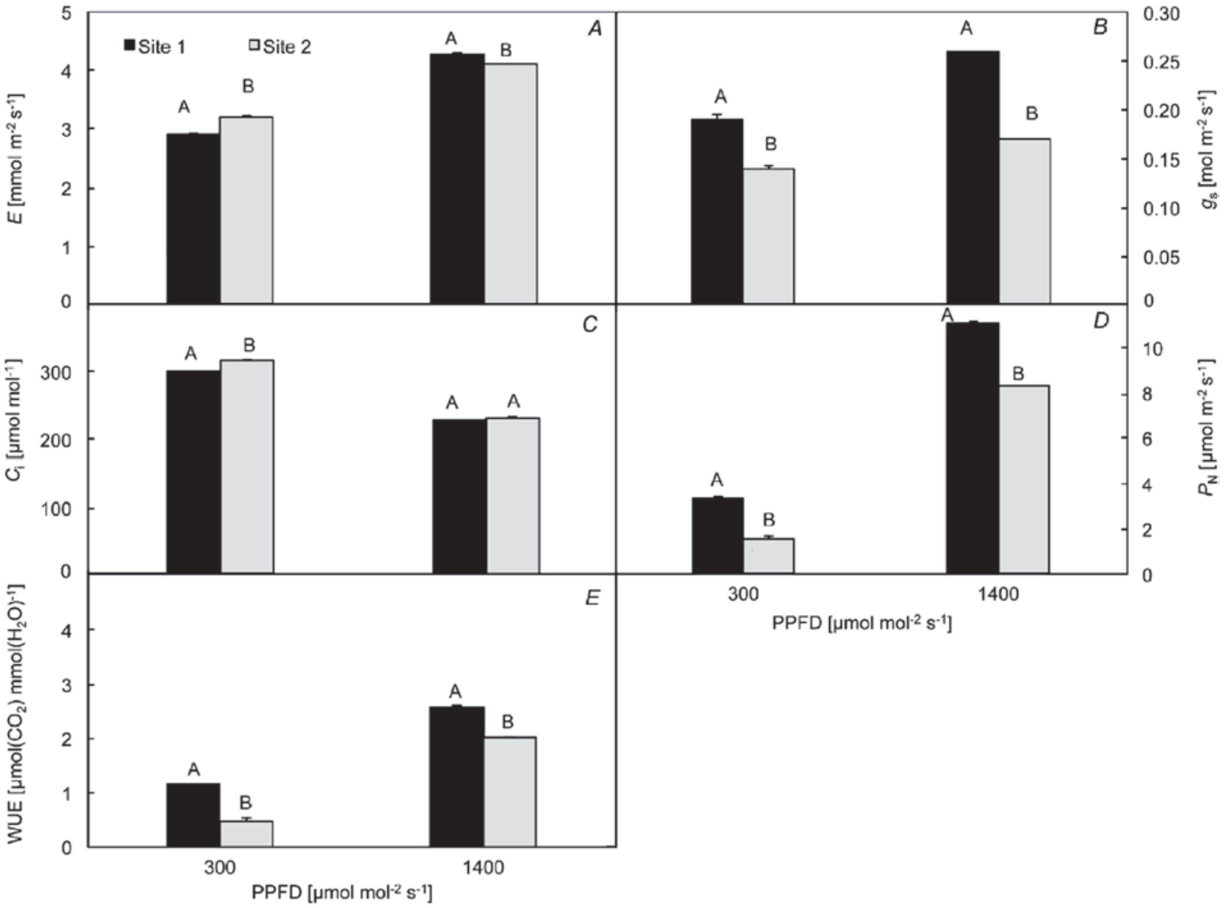

Fig. 4. Mean values ± SE ($n = 5$) of *A*: transpiration rate (E), *B*: stomatal conductance (g_s), *C*: internal CO_2 concentration (C_i), *D*: net photosynthetic rate (P_N), and *E*: water-use efficiency (WUE) of *Lotus corniculatus* plants from a mountainous (site 1) and a lowland (site 2) grassland under low [300 µmol(photon) m^{-2} s^{-1}] and high [1,400 µmol(photon) mol^{-1}] radiation (PPFD).

light-saturated rate of CO_2 assimilation (Horton and Neufeld 1998). On the other hand, plants from the site 1 expressed lower LCP and R_D values compared to plants from the site 2, which is typical of 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 the site 2 expressed also significantly higher values of P_{Nmax}. Maximum photosynthetic capacity reflects the rate of diffusion of CO_2 to Rubisco, the activity of Rubisco and/or the rate of ribulose-1,5-bisphosphate (RuBP) regeneration (Farquhar *et al.* 1980). Differences in P_{Nmax} have been attributed to differences in the biochemical efficiency of carboxylation, rather than to differences in CO_2 supply to the intercellular spaces (Reich *et al.* 1998, Shangguan *et al.* 2000). In addition, F_v/F_m, which is proportional to the quantum yield of photosynthesis, was similar at both sites. This ratio is usually ranging from 0.79 to 0.84 in nonstressed terrestrial leaves (Bjorkman and Demmig 1987). Changes in F_v/F_m are very sensitive and inversely proportional to physiological stress and might indicate the beginning of a slowly reversible photoinhibition (Osmond and Grace 1995). Since F_v/F_m and P_N showed a significant correlation in the site 2, the lower photosynthetic performance of plants from the site 2, as expressed by the lower P_N values in the P_N-PPFD curve and the lower P_{Nmax} values, is pointing towards the existence of additional factors that inhibit photosynthesis of *L. corniculatus* plants in this particular site compared to the mountainous one.

The increase in the PPFD exerted a positive effect in all measured physiological parameters, except C_i. Generally in grasslands, both P_N and g_s have been found to parallel PPFD (Bollig and Feller 2014). Interestingly, plants from both sites increased their WUE at the higher PPFD. Working with different cultivars of *L. corniculatus*, Inostroza *et al.* (2015) demonstrated that the highest WUE was also accompanied by the highest dry matter yield under water stress conditions. Therefore, the increment of WUE under high radiation could be beneficial to the plants, enabling them to use more efficiently the available water reserves and to express higher performance under drought conditions, which are very common in the Mediterranean area.

The photosynthetic P_N-C_i response curves of *L. corniculatus* revealed a photosynthetic upregulation by elevated

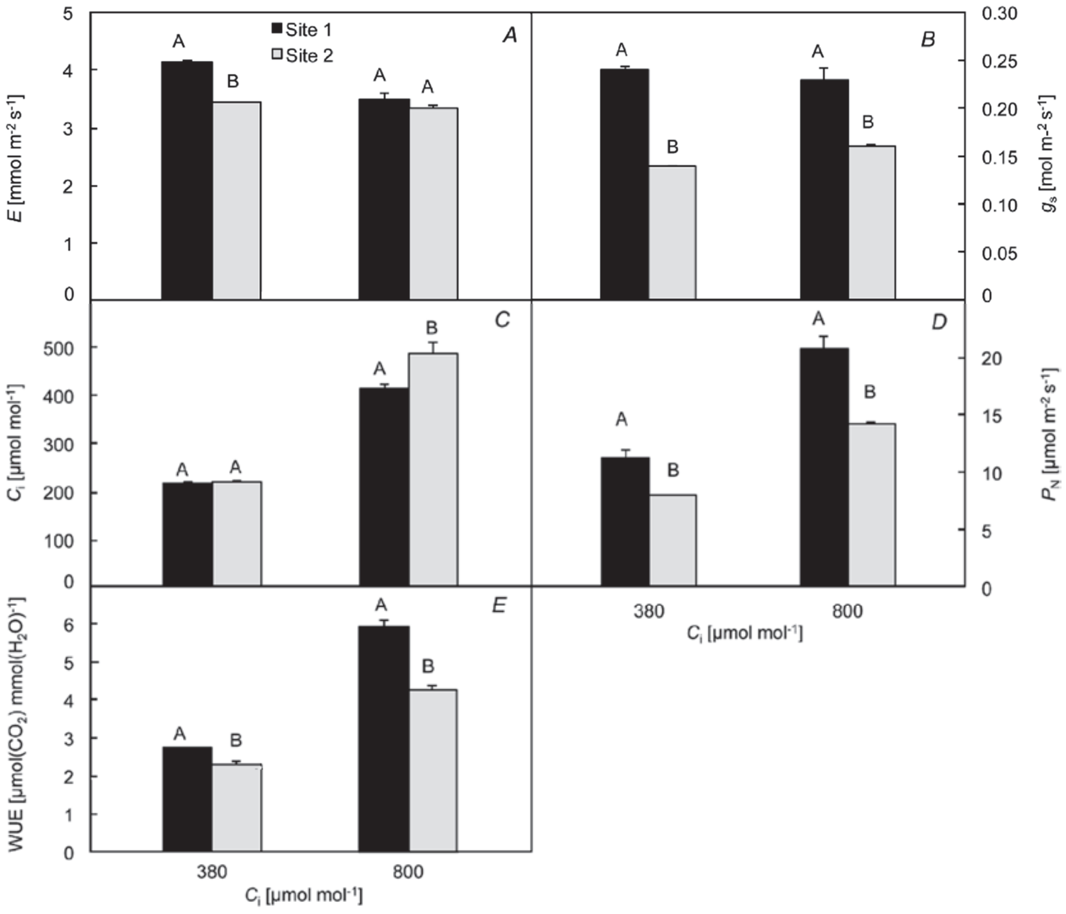

Fig. 5. Mean values ± SE ($n = 5$) of *A*: transpiration rate (*E*), *B*: stomatal conductance (g_s), *C*: internal CO_2 concentration (C_i), *D*: net photosynthetic rate (P_N), and *E*: water-use efficiency (WUE) of *Lotus corniculatus* plants from a mountainous (site 1) and a lowland (site 2) site under ambient [380 µmol(CO_2) mol^{-1}] and elevated [800 µmol(CO_2) mol^{-1}] CO_2 concentration.

CO_2 at both sites. C_3 plants show a significant positive response to photosynthetic acclimation to elevated CO_2 (Bernacchi *et al.* 2003, Reddy *et al.* 2010). Elevated CO_2 concentration increases the carboxylation efficiency and, therefore, reduces photorespiration (Reddy *et al.* 2010). Working with *L. corniculatus*, Carter *et al.* (1997) found that doubling of CO_2 concentration [up to 700 µmol(CO_2) mol^{-1}] resulted in the increase of the growth rate, shoot biomass, and WUE, and in the reduction of the flowering time, specific leaf area, and Chl content of plants.

On the other hand, elevated CO_2 concentration did not affect the g_s of plants from the site 1, while it resulted in the slight increase of g_s of plants from the site 2. Variable effects of elevated CO_2 concentration on g_s have been previously reported, most likely depending on plant species (Medlyn *et al.* 2001, Reich *et al.* 2001, Lewis *et al.* 2002, Maherali *et al.* 2002, Ainsworth *et al.* 2004, Long *et al.* 2004).

Furthermore, the P_N-C_i response curves of plants in the site 1 saturated at higher values of CO_2 and demonstrated higher CE and lower CO_2-compensation point compared to the site 2. Other studies have also demonstrated increased CE at higher altitudes (Körner *et al.* 1988, Kumar *et al.* 2005). In addition, the rise in CO_2 concentration led to the increase of WUE in both sites; the higher increase was found in plants from the site 1. Elevated CO_2 concentration can increase WUE, in part by decreasing g_s and *E* (Ainsworth *et al.* 2002). In the current study, a decrease of *E* under elevated CO_2 concentration was also observed at least for plants from the site 1. Lower *E* along with increased WUE can support plants to overcome water stress conditions and become more antagonistic in drought periods that are usually experienced in the Mediterranean zone during the summer.

Our results clearly showed that plants of *L. corniculatus* originating from the mountainous site had higher photosynthetic performance under elevated radiation and CO_2 concentration. In a number of studies, a decrease in plant performance with elevation in altitude was found, mainly as a result of lower temperature and a shorter growing season (Koerner 2003). To the best of our knowledge there are no studies addressing the performance of *L. corniculatus* plants at different altitudes. Working with different species, Frei *et al.* (2014) found that *Ranunculus bulbosus*, *Trifolium montanum,* and *Briza media* responded differently with respect to their

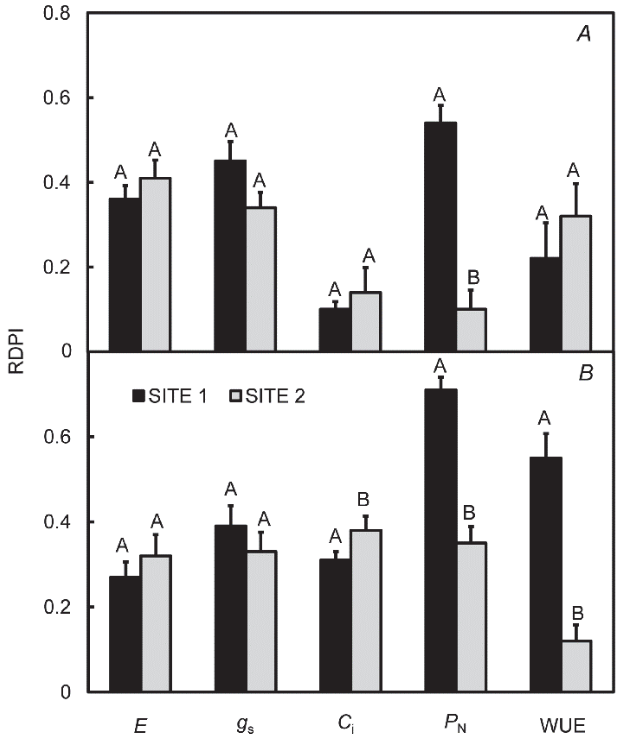

Fig. 6. Mean relative distance plasticity index (RDPI) of gas-exchange parameters of *Lotus corniculatus* plants from a mountainous (site 1) and a lowland (site 2) site between: *A*: ambient and high [1,400 μmol(photon) mol^{-1}] radiation (PPFD) and *B*: ambient and elevated [800 μmol(CO$_2$) mol^{-1}] CO$_2$ concentration. (*n* = 5).

altitude of origin indicating genetic differentiation. Other studies report that high-altitude plants have higher photosynthetic efficiency than the low-altitude ones (Körner and Diemer 1987, 1994; Friend and Woodward 1990). Kumar *et al.* (2005) reported that P_N was not significantly different between altitudes, while g_s and *E* increased with altitude. Interestingly, they also found that plants from different altitudes, when grown at 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. The photosynthetic machinery of *Bromus inermis* plants from a mountainous site responded better to changes in irradiation, while that of lowland plants expressed higher sensitivity to changes in CO$_2$ concentration as it has been demonstrated by recent studies (Kostopoulou and Karatassiou 2016). However, no effect of altitude on the physiological responses of *B. inermis* was found, indicating that plants may have employed different plastic and/or adaptive mechanisms to persist and thrive in the environmental conditions of each site (Kostopoulou and Karatassiou 2016). According to our findings plants from the mountainous site (site 1) showed higher phenotypic plasticity for P_N both under different CO$_2$ and PPFD levels. Plants from the site 1 also showed higher phenotypic plasticity for WUE under different CO$_2$.

In conclusion, our results showed that the increase of CO$_2$ concentration and radiation enhanced the photosynthetic performance of the mountainous population of *L. corniculatus*. Nevertheless, further studies using plants from both sites growing under the same environmental conditions, are needed in order to investigate whether the differentiation of *L. corniculatus* photosynthetic performance found in the present study derived from genetic differentiation between the two populations and/or from their phenotypic adaptation to the local environment of each site.

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