Impact of simulated changes in rainfall regime and nutrient deposition on the relative dominance and isotopic composition of ruderal plants in anthropogenic grasslands

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Abstract

Background and aims Plant productivity in drylands is frequently co-limited by water and nutrient availability, and thus is expected to be influenced by ongoing changes in rainfall regime and atmospheric nutrient deposition. Roadside grasslands are widespread worldwide, represent ecologically meaningful examples of highly dynamic anthropogenic ecosystems, and are well suited to investigate global change

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effects on plant performance. We evaluated the effects of changes in water and nutrient availability on the relative dominance and physiological performance of *Bromus rubens*, *Carduus tenuifolius* and *Melilotus officinalis*, which belong to contrasting functional groups (grasses, non-legume forbs and legumes, respectively).
Methods We conducted a factorial field experiment in two semiarid roadside grasslands in central Spain with the following factors: watering (no water addition vs. watering with 50% of the monthly total precipitation median) and fertilization (no fertilization vs. addition of 80 kg Nha^{-1} $year^{-1}$). The cover of the species evaluated, was surveyed over a 2-year period. Plant isotopic composition (leaf $\delta^{13}C$ and $\delta^{18}O$) and nutrient concentrations (foliar N, P and K) were used to assess plant ecophysiological performance.
Results Carduus was able to cope with lower water availability levels through stomatal adjustments without a significant reduction in its relative dominance. The relative dominance of *Bromus* was negatively affected by even moderate water stress, although elevated nutrient deposition buffered the adverse impact of drought through a nutrient-mediated enhancement of plant water use efficiency. Increased nutrient availability strongly decreased the relative dominance of *Melilotus,* irrespective of water availability.
Conclusions Species-specific physiological mechanisms of adjustment to treatments suggest that plant communities in roadside grasslands will not respond as a unit to global environmental change. The character-

ization of species-specific responses to major global change drivers may improve predictions about the future dynamics of plant communities in novel ecosystems such as roadside slopes.

Introduction

Most climate change models for the Mediterranean Basin forecast not only an increase in temperature, but also a decrease in the amount of annual rainfall, a lengthening of drought periods and a reduction of spring precipitation (IPCC 2007). Increases in water stress associated to these changes in temperature and rainfall regime will profoundly affect the composition and productivity of plant communities, especially in semiarid environments where soil moisture availability is critical (Miranda et al. 2009). Nitrogen (hereafter N) is the most limiting nutrient for plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991), although phosphorus (hereafter P) often plays an important role in co-limiting productivity, especially in semiarid environments (Morecroft et al. 1994; Sardans et al. 2004). The global cycles of N and P have been amplified by c. 100% and c. 400%, respectively, by post-industrial human activities (Falkowski et al. 2000), mainly due to combustion of fossil fuels in the case of N (Galloway et al. 2008) and biomass burning in the case of P (Echalar et al. 1995). At the global scale, these emissions have increased atmospheric nutrient deposition at unprecedented rates (Galloway et al. 2008). As a result, nitrophilous species (often ruderals) have increased, but other native species have declined in European grasslands since the mid-20th century (Bobbink et al. 1998; Lee and Caporn 1998). This pattern could be especially dramatic in Mediterranean grasslands, because the threshold for N deposition impacts (increase in ruderal species and decline in species non adapted to disturbed conditions) can occur at rather low loads (10–15 kg N ha^{-1} year^{-1}) in Mediterranean-type ecosystems (Ochoa-Hueso et al. 2011). Surprisingly, the effects of nutrient deposition on plant communities have rarely been studied in grasslands from the Mediterranean Basin (Bobbink et al. 2010; Ochoa-Hueso et al. 2011).

Interactions between multiple human-driven disturbances (e.g. climate change, nutrient deposition or land use changes) are especially relevant in Mediterranean ecosystems, which have been intensively transformed by humans for centuries (UNESCO 1962; Naveh and Dan 1973). Most research on the effects of ongoing global environmental change (hereafter global change) on plant species have focused on single drivers, an approach that overly simplifies the complex responses of plant species and communities to multiple interacting drivers (Matesanz et al. 2009; Maestre et al. 2005; Maestre and Reynolds 2007). Due to such complexity, and the high frequency of non-additive effects between water and nutrient availability on plant growth (Maestre and Reynolds 2007; Matesanz et al. 2009), multifactorial experiments are needed to elucidate potentially counterintuitive effects of global change drivers (e.g. climate change and nutrient deposition, Zavaleta et al. 2003) on plant performance.

Nowadays, road construction is among the most widespread and ecologically meaningful examples of land use change worldwide (Forman and Alexander 1998). Grasslands dominated by ruderal species usually establish in roadside margins (Spellerberg 1998), which cover approximately 1% of most developed countries (Forman 2000). These highly disturbed environments have been recently identified as novel and emergent ecosystems (*sensu* Hobbs et al. 2006); their artificial soils and potentially new vegetation compositions differ significantly from those of nearby natural ecosystems, and their responses to global change may differ from those of natural grasslands (Wang 2007). Plant communities in these anthropogenic grasslands are characterized by rapid structural and compositional shifts (Wali 1999). Since productivity changes occur at rather low rates in undisturbed semiarid and arid systems (Reynolds et al. 2007; Matesanz et al. 2009), highly dynamic anthropogenic grasslands represent an exceptional study system to investigate global change effects upon plant performance in semiarid regions.

Plant water use efficiency (the ratio between carbon gain and water loss, hereafter WUE) is a useful indicator of plant performance in dry environments (e.g. Tsialtas et al. 2001; Querejeta et al. 2003, 2006, 2008). It is usually modified by drought via its effects on the balance between photosynthesis and stomatal conductance (Robinson et al. 2000). The stable carbon isotope composition of plant tissues (δ^{13}C) provides a time-integrated proxy of intrinsic WUE (the ratio between

photosynthesis and stomatal conductance; Dawson et al. 2002) in C_3 plant species (Farquhar et al. 1989; Robinson et al. 2000; Dawson et al. 2002). The stable oxygen isotope composition of plants ($\delta^{18}O$) provides a time integrated proxy of stomatal conductance and transpiration rate during the growing season (Jaggi et al. 2003; Barbour 2007). The measurement of plant $\delta^{18}O$ greatly aids the interpretation of $\delta^{13}C$ data by providing information about stomatal conductance independently of the effects of photosynthetic rate on $\delta^{13}C$ (Scheidegger et al. 2000). As WUE usually increases with higher concentrations of foliar nutrients (Farquhar et al. 1989; Dawson et al. 2002), the analysis of plant nutrient status can also aid when interpreting leaf $\delta^{13}C$ data (Querejeta et al. 2006, 2008; Ramírez et al. 2009). Consequently, leaf $\delta^{13}C$, $\delta^{18}O$ and foliar nutrient concentration measurements (e.g. N, P and K) can help to evaluate how simultaneous changes in soil water and nutrient availability will modulate plant physiological performance.

Studies relating physiological performance to direct or indirect components of plant abundance and dominance within the community are biased towards greenhouse or common garden experiments, but similar studies of field populations are less frequent (Casper et al. 2005). The main goal of our study was to assess the effects of water and nutrient availability on the physiological performance and relative dominance of three ruderal plant species (*Bromus rubens*, *Carduus tenuifolius* and *Melilotus officinalis*). These species belong to distinct functional groups (grasses, non-legume forbs and legumes, respectively). The contrasting resource use strategies shown by these functional groups in semiarid roadside grasslands (García-Palacios et al. 2011a) suggest potentially distinct physiological responses to changes in soil water and nutrient availability. A 2-year field experiment was conducted in two semiarid anthropogenic roadside grasslands from central Spain (García-Palacios et al. 2010) to test the following hypotheses: i) plant species belonging to different functional groups will exhibit contrasting physiological responses to changes in water and nutrient availability, with non legumes showing more positive responses to fertilization (Lee et al. 2001), and ii) across plant functional groups, increases in nutrient availability will enhance the WUE of ruderal species, thus buffering the negative impact of reduced soil water availability on plant performance (Lee et al. 2001).

Materials and methods

Study area

The experiment was conducted at two roadside embankments located in the AP36 and R4 motorways, between Pinto (Madrid; 40º14′N, 3º43′W) and Corral de Almaguer (Toledo; 39º45′N, 3º03′W), in the centre of the Iberian Peninsula (altitude *c*. 700 ma.s.l.). The climate is semiarid, with cold winters and a severe summer drought; annual mean temperature and total precipitation are 15°C and 450 mm, respectively (Getafe Air Base climatic station 40º18′N, 3º44′W, 710 ma.s.l., 1971–2000). A meteorological station (Onset, Pocasset, MA, U.S.A.) was located in each embankment to get a more detailed description of the local climatic conditions during the study. The AP36 site was a recently built embankment, where construction was finished 3 months prior to the field surveys. The R4 site was a 3-year old embankment. The vegetation of the two study sites is dominated by an annual herbaceous community dominated by fast-growing species with C_3 photosynthetic pathways, except for two C3–C4 species (Appendix A in Supplementary Material). The artificial soils of these embankments were constructed using local parent material, gravels and components from external sources stockpiled for a while before road building (information provided by the road building company). Both sites are nutrient poor, with low levels of soil organic carbon, total N and P, scarce soil biological activity and alkaline pH (Table 1).

Table 1 Main characteristics and soil properties of the two sites studied at the beginning of the experiment (December 2006). Numerical values are means ± SE (n=30). A detailed description on the methodology used to get these data can be found in García-Palacios et al. (2010)

	R4 site	AP36 site
Initial plant cover (%)	58	12
Water holding capacity (ml water g^{-1} soil)	0.43±0.03	0.36±0.03
Total N (mg N g^{-1} soil)	0.34±0.04	0.14±0.01
Total P (mg P g^{-1} soil)	0.35±0.01	0.16±0.01
Basal respiration (mg CO$_2$-C g^{-1} soil day^{-1})	0.04±0.003	0.01±0.001
Soil organic carbon (g C kg^{-1} soil)	9.90±0.09	5.3±0.06
pH	8.06±0.15	8.35±0.14

Experimental design

We conducted a 2-year field experiment with two manipulated factors, watering and fertilization, to simulate the potential effects of changes in rainfall regime and nutrient (N, P, K) deposition, respectively. A generalized randomized block design was set up in each site, with six blocks per site. This design was chosen to reduce the effect of within-site variation because of the potential heterogeneity of materials used for embankment construction. Twelve 1×1 m plots, with at least 1 m buffer zone each, were randomly established in each block to obtain three replicates of each watering × fertilization treatment.

Two levels of watering were applied: no water addition vs. watering with 50% of the monthly total precipitation median of the 1971–2000 period. Watering was conducted in four pulses at the end of each spring month in March, April, May and June in both 2007 and 2008 (i.e. 12, 18, 22, 11 mm, respectively; Fig. 1). The non-watered plots (low water availability level) received ambient rainfall (equivalent to drier conditions), while the watered plots (high water availability level) received ambient rainfall plus the added water, irrespectively of the rainfall actually registered during the experiment (equivalent to wetter conditions). The oxygen isotopic composition of the water used for irrigation was nearly identical to that of

Fig. 1 Climatic data (mean monthly temperature and rainfall) obtained from the two meteorological stations (Onset, Pocasset, MA, USA) located in the R4 site (**a**) and AP36 site (**b**). White bars represent the increment in ambient monthly rainfall by the irrigation treatment (equivalent to a 50% increase of the monthly total precipitation median from the 1971–2000 period) applied from March to June in both 2007 and 2008. Grey bars represent the ambient rainfall recorded by the meteorological stations in both years (equivalent to a 0% increase of the monthly total precipitation median from the 1971–2000 period)
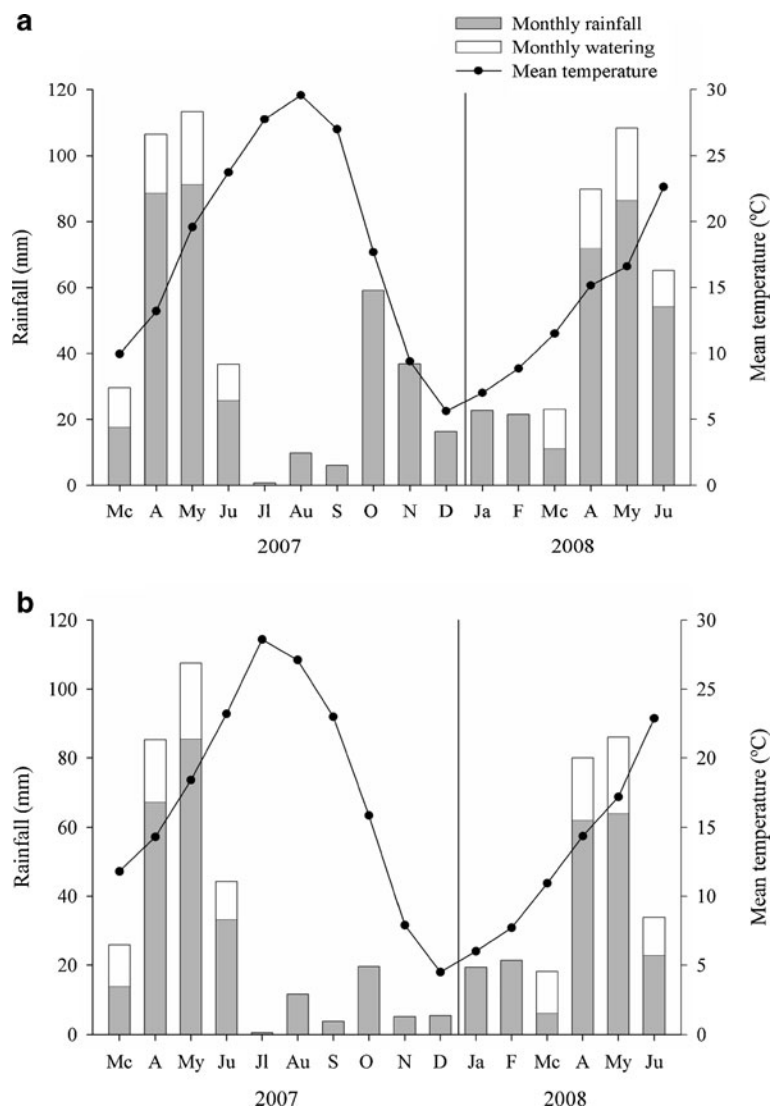
rain water at both study sites (Table 2). With this watering scheme we aimed to simulate the effects of a reduction in total annual precipitation, by decreasing water availability during spring and early summer in the non-watered plots (compared to the watered plots), as predicted by the most likely climate change scenarios for the Mediterranean Basin (IPCC 2007). Furthermore, spring is considered the period of maximum impact of weather conditions on plant physiology and isotopic composition in Mediterranean ecosystems (Damesin et al. 1997). Although our experiment did not include rainfall exclusion treatments, the approach we followed has been effectively tested to simulate climate change scenarios in Mediterranean regions (Zavaleta et al. 2003; Matesanz et al. 2009; Soliveres et al. 2011). In November 2007, we placed 16 ECH$_2$O humidity sensors (Decagon Devices Inc., Pullman, USA) in the soil at a depth of 5 cm at the R4 and AP36 sites to assess the effects of watering on soil moisture dynamics during the study period (2 sites × 2 watering levels × 4 replicates). These measurements were recorded every 90 min.

Fertilization consisted of two levels of a slow-release N:P:K (16:11:11) inorganic fertilizer addition (Scott Corp.). Fertilized plots received 80 kg Nha^{-1} year^{-1} in December 2006 and January 2008. Control plots were not fertilized. Although this rate is considerably higher than the predicted scenarios of N deposition for the Mediterranean Basin in 2050 (20 kg Nha^{-1} year$^-$1; Phoenix et al. 2006), our objective was to maximize the contrast between treatments in order to obtain clear responses to nutrient deposition. Further, N deposition from vehicle emissions represents an important extra N input to the nearby roadside grasslands (Cape et al. 2004), and hence high fertilization rates are needed to get a contrast between fertilized and non fertilized

plots. As the fertilizer included N, P and K, we cannot refer exclusively to N deposition, and therefore this experimental treatment simulates a general nutrient deposition scenario. Similar rates of soil inorganic slow-release fertilization have been used to simulate nutrient deposition in grasslands worldwide (Morecroft et al. 1994; Zavaleta et al. 2003; Dukes et al. 2005).

Sample selection, isotopic composition and nutrient concentration

Plant samples were collected in June 2008, at the end of the growing season; this month correspond to the optimal phenological moment to measure the herbaceous communities studied (García-Palacios et al. 2010). The total cover of each species was visually estimated by the same observer as an indirect measure of plant biomass (Carson and Pickett 1990; Myster and Pickett 1992; Flombaum and Sala 2009). The total cover of each species was measured independently of that of other species, and the sum of the total covers of all the species in a plot can exceed 100% (Stevens and Carson 2001). This method can led to misleading conclusions when estimating community biomass, but it is a good predictor for individual plant species biomass (Stevens and Carson 2001). The most dominant species in terms of plant cover in spring 2008 were *Melilotus officinalis* at the AP36 site and *Carduus tenuifolius* and *Bromus rubens* at the R4 site (27, 32 and 24% species cover, respectively; Appendix A. See García-Palacios et al. 2010 for further information). These dominant species (*B. rubens*, *C. tenuifolius* and *M. officinalis*) belong to three distinct plant functional groups (grasses, non-legume forbs and legumes, respectively) with contrasting resource use strategies in semiarid roadside grasslands (García-Palacios et al. 2011a). The relative species cover (percent species cover relative to the total community cover) was also calculated as a surrogate of species dominance and competitive ability within the community (Sala et al. 1996).

In each 1×1 m plot, we harvested two green, fully developed leaves per individual to get a composite species sample per plot and dominant species. When no individual of these species occurred in a plot (as happened in two blocks with *M. officinalis* and in one block with *B. rubens*) the entire block was discarded for further analysis. Leaf samples were dried (80°C, 48 h) and ground to fine powder using a ball mill. All stable isotope and N concentration analyses were

Table 2 δ^{18}O of the rain water collected from March to June in 2008 at both the R4 and AP36 sites, and of the watering for the same period. The oxygen isotope signature is expressed relative to the internationally accepted standard (Vienna Standard Mean OceanicWater, VSMOW)

	Rain—R4	Rain—AP36	Watering
March	−5.58±0.11	−5.74±0.03	−5.53±0.28
April	−6.33±0.17	−6.20±0.02	−6.29±0.05
May	−6.20±0.02	−6.02±0.06	−6.25±0.06
June	−6.14±0.08	−6.54±0.03	−6.32±0.13

conducted at the Stable Isotope Facility of the University of California-Davis. Leaf $\delta^{13}C$ was analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The standard was Pee Dee Belemnite. Foliar N concentration was analyzed using the same PDZ Europa ANCA-GSL elemental analyzer. Leaf $\delta^{18}O$ was analyzed using a Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The oxygen isotope signature is expressed in $\delta^{18}O$, relative to the internationally accepted standard (Vienna Standard Mean OceanicWater, VSMOW). Foliar P and K concentration were analyzed by atomic absorption spectrometry (Perkin Elmer ICP-OES 6500, Norwalk, USA).

Statistical analyses

We evaluated the effects of fertilization and watering on leaf $\delta^{13}C$, $\delta^{18}O$, foliar N, P and K concentrations, and on the total and relative cover of each of the three species separately, using a three-way nested ANOVA model. We used block as between plot factor (random), and fertilization and watering as within plot factors (both of them fixed). Although we conducted a large number of statistical tests, P values were not adjusted for multiple testing as this approach is considered overly conservative (Gotelli and Ellison 2004). Linear regressions were used to evaluate the relationships between $\delta^{13}C$ and $\delta^{18}O$, and between plant isotopic composition and foliar N, P and K concentrations. Prior to these analyses, data were tested for assumptions of normality and homogeneity of variances, and were log-transformed when necessary. Statistical procedures were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Unfortunately, 2008 was a rainy year in the study area (Fig. 1). As a consequence, soil moisture content was maintained at relatively high levels in both watered and non-watered plots throughout the experimental period (Fig. 2). Despite this limitation, soil moisture

Fig. 2 Effects of the watering treatment on soil moisture dynamics during the study period in the R4 (a) and AP36 sites (b). Data represent daily means in four randomly selected watered and non-watered plots at each site
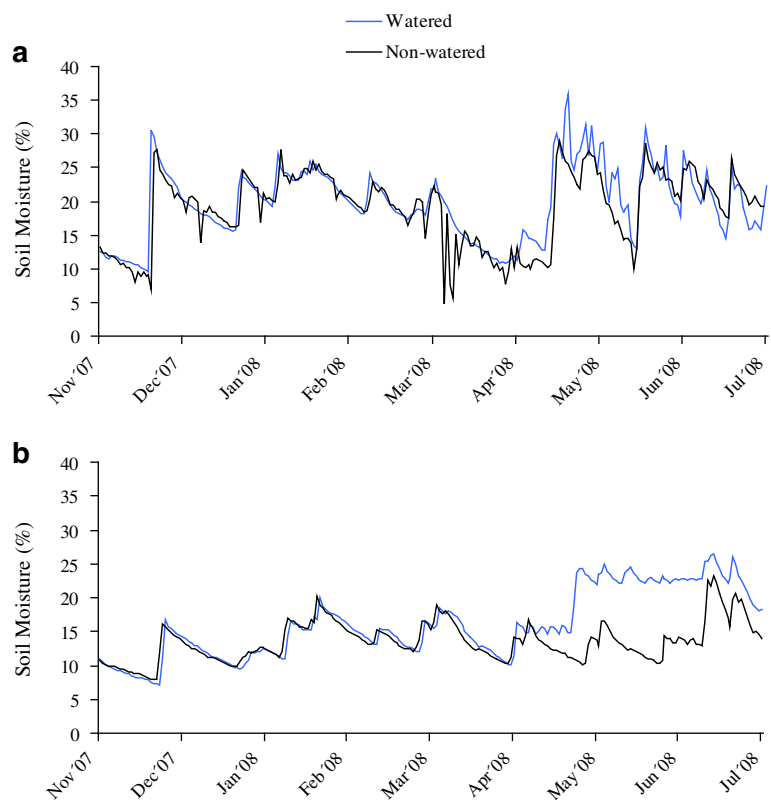
content during the spring months was indeed higher in watered than in non-watered plots at both sites, although this difference was larger at the AP36 site than at the R4 site.

Total plant community cover was significantly affected by the watering treatment at the R4 site ($F_{1, 5}=15.53$; $P=0.011$), being higher in the watered ($71\%\pm4.3$, mean$\pm$SE, $n=36$) than in the non-watered plots ($66\%\pm4.2$, mean$\pm$SE, $n=36$), but was not affected by the fertilizer addition treatment. Neither watering, nor fertilizer addition or their interaction, affected total plant community cover at the AP36 site.

Whereas the total cover of *Bromus* at the R4 site was not affected by the experimental treatments, the relative cover of this species was about 12% higher in the watered plots and 11% higher in the fertilized plots. However, only the effect of watering was statistically significant (Table 3; Fig. 3). Fertilization significantly increased the leaf $\delta^{13}C$ of this grass species, but not its leaf $\delta^{18}O$ (Table 3; Fig. 4). We did not find any significant treatment effects on the foliar N, P or K concentrations in *Bromus* (Table 3; Fig. 5). Foliar $\delta^{13}C$ was related with neither $\delta^{18}O$ nor leaf N concentration in this species (Figs. 6a and 7a, respectively).

Neither the total nor the relative cover of *Carduus* were affected by the experimental treatments at the R4 site (Table 3). The leaf $\delta^{13}C$ of this species was not significantly influenced by the experimental treatments, but leaf $\delta^{18}O$ was higher in the non-watered plots (Table 3; Fig. 4). We did not find any significant treatment effects on the foliar N, P or K concentrations of *Carduus* (Table 3; Fig. 5). Across experimental treatments, leaf $\delta^{13}C$ of this species was strongly and positively related to both leaf $\delta^{18}O$ and foliar N concentration (Figs. 6b and 7b, respectively).

While the total cover of *Melilotus* was not significantly affected by the experimental treatments at the AP36 site (Table 3), the relative cover of this legume was about 57% higher in non-fertilized than in fertilized plots (Fig. 3). Leaf $\delta^{13}C$, $\delta^{18}O$ and foliar N, P and K concentrations in this species were not significantly affected by the experimental treatments (Table 3). Across treatments, leaf $\delta^{13}C$ of *Melilotus* was not related to $\delta^{18}O$ (Fig. 6c), but leaf $\delta^{13}C$ was strongly and negatively associated with foliar N, P and K (Fig. 7c, $r^2=0.102$; $P=0.037$, $r^2=0.235$; $P=0.001$, respectively).

Discussion

The relative dominance of three key plant species belonging to contrasting functional groups (grasses, non-legume forbs, legumes) was affected very differently by the simulated changes in rainfall regime and

Table 3 Summary of the three-way nested ANOVA model for main treatment effects and interactions on the total (TSC) and relative (RSC) species cover, leaf $\delta^{13}C$, $\delta^{18}O$ and foliar N, P and K concentrations of *Bromus rubens*, *Carduus tenuifolius* and *Melilotus officinalis* in June 2008. Values represent the F statistic. * P values <0.05. Block (random factor) was not included in the table as it only has interest for F calculations. *Bromus rubens* and *Carduus tenuifolius* were located in the R4 site and *Melilotus officinalis* in the AP36 site

Source of variation (d.f.)	TSC	RSC	^{13}C	^{18}O	Foliar N	Foliar P	Foliar K
Bromus rubens							
Watering (1, 4)	0.89	9.65*	0.88	0.92	0.3	0.19	1.41
Fertilization (1, 4)	0.03	1.17	14.01*	0.27	0.18	0.15	0.47
Irrigation x fertilization (1, 4)	0.43	0.21	0.42	0.85	0.38	2.43	4.09
Carduus tenuifolius							
Watering (1, 5)	4.51	0.57	3.901	14.41*	5.68	0.56	0.04
Fertilization (1, 5)	0.19	4.92	0.235	4.06	2.08	1.51	0.76
Irrigation x fertilization (1, 5)	1.05	1.58	0.27	0.05	0.88	4.57	5.32
Melilotus officinalis							
Watering (1, 3)	0.03	0.01	0.01	0.72	0.01	0.56	0.99
Fertilization (1, 3)	2.51	12.43*	3.83	1.98	0.58	0.5	0.44
Irrigation x fertilization (1, 3)	3.51	2.8	3.65	0.45	2.93	6.31	4.98

Fig. 3 Total (**a**) and relative (**b**) species cover of *Bromus rubens* (Br), *Carduus tenuifolius* (Ct) and *Melilotus officinalis* (Mo) in June 2008. Values are means±1 SE, *n*=60 in *Bromus rubens*, *n*=72 in *Carduus tenuifolius* and *n*=48 in *Melilotus officinalis*. Watered and non-watered levels correspond to the addition of the 50% and 0% of the monthly median precipitation conducted in four pulses from March to June in 2007 and 2008, respectively. Non-fertilized and fertilized levels correspond to 0 and 80 kg N ha^{-1} year^{-}1, respectively, of a N:P:K (16:11:11) fertilizer, applied in December 2006 and January 2008. *Bromus rubens* and *Carduus tenuifolius* were located in the R4 site and *Melilotus officinalis* in the AP36 site
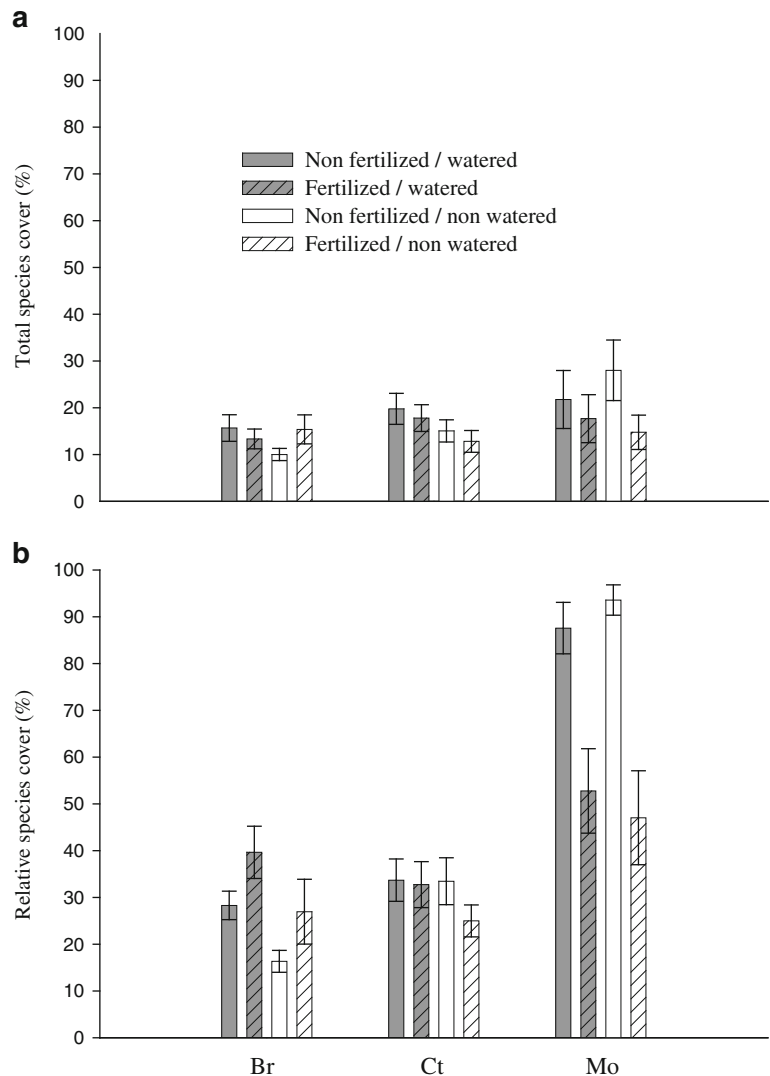
nutrient deposition, suggesting species-specific responses to the ongoing global change. In addition, species-specific physiological mechanisms of adjustment to simulated changes in rainfall and nutrient deposition further suggest that plant communities in anthropogenic roadside grasslands will not respond as a unit to global change, as suggested by previous studies (Zavaleta et al. 2003; Maestre et al. 2005; Maestre and Reynolds 2007).

Interestingly, total plant community cover was significantly enhanced by watering only at the R4 site, probably because much lower soil fertiliy at the AP36 site (Table 1) may have constrained the vegetation growth response to watering. Species-specific responses to watering may have also contributed

to this differential response, as the plant community was dominated by different species at each site (i.e., *Bromus* and *Carduus* at R4 vs. *Melilotus* at AP36 site). Higher plant cover in watered than in non-watered plots at the R4 site may have accelerated depletion of surplus soil moisture after irrigation through enhanced transpiration, which may explain the smaller differences in soil water content between watered and non-watered plots (compared to the AP36 site where plant cover was unaffected by the watering treatment).

The higher (isotopically enriched) leaf $\delta^{18}O$ values of *Carduus* in the non-watered treatment strongly suggest that even moderate moisture stress caused a reduction of stomatal conductance in this species. Numerous studies have shown that leaf $\delta^{18}O$ provides
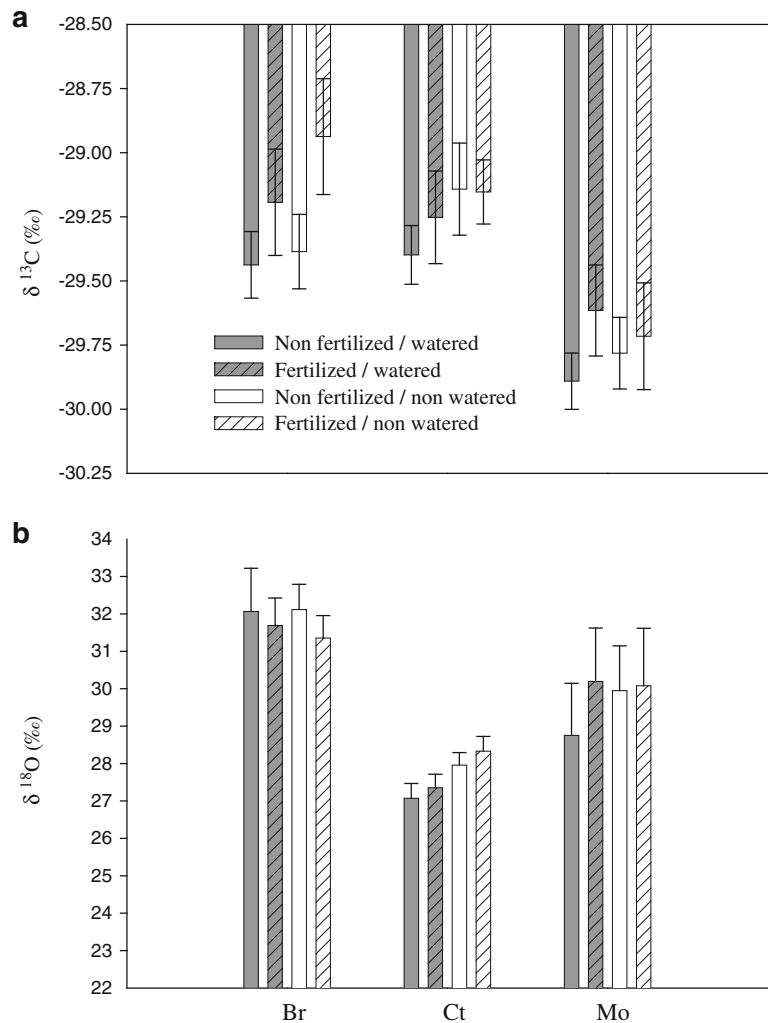
a time-integrated proxy for stomatal conductance and cumulative transpiration over the entire growing season, with higher leaf δ^{18}O values indicating lower stomatal conductance (e.g. Barbour et al. 2000; Grams et al. 2007; Cabrera-Bosquet et al. 2009a, 2011). In this regard, it is important to note that the oxygen isotopic composition of the water used for irrigation in this study was nearly identical to that of rain water (Table 2), so differences in plant δ^{18}O between treatments cannot be plausibly attributed to lack of similarity in the isotopic composition of source water (Barbour 2007). Variations in the degree of evaporative isotopic enrichment of soil water between treatments are likewise unlikely, because environmental conditions (aspect, slope, radiation levels) and plant cover were quite similar in all the treatments.

According to current conceptual models for the joint interpretation of plant δ^{13}C and δ^{18}O data (Scheidegger et al. 2000; Grams et al. 2007), increased leaf δ^{18}O combined with a smaller (non significant) increment in leaf δ^{13}C observed in *Carduus* under a reduced rainfall scenario (Fig. 4) indicate a decrease in stomatal conductance with a small decrease or no change in photosynthetic activity. The slope of the δ^{13}C–δ^{18}O relationship indicates to what extent stomatal limitation is driving shifts in δ^{13}C (Scheidegger et al. 2000). A positive relationship means that the ratio of intercellular to ambient CO_2 concentration decreases as a result of reduced stomatal conductance, while photosynthesis remains relatively less affected. The strong positive association between leaf δ^{13}C and δ^{18}O observed in *Carduus* across treatments (Fig. 6b) suggests that tight stomatal control of both transpira-

Fig. 5 Foliar N (**a**), P (**b**) and K (**c**) concentrations of *Bromus rubens* (Br), *Carduus tenuifolius* (Ct) and *Melilotus officinalis* (Mo) in June 2008. Values are means±1 SE, $n=60$ in *Bromus rubens*, $n=72$ in *Carduus tenuifolius* and $n=48$ in *Melilotus officinalis*. Rest of legend as in Fig. 3
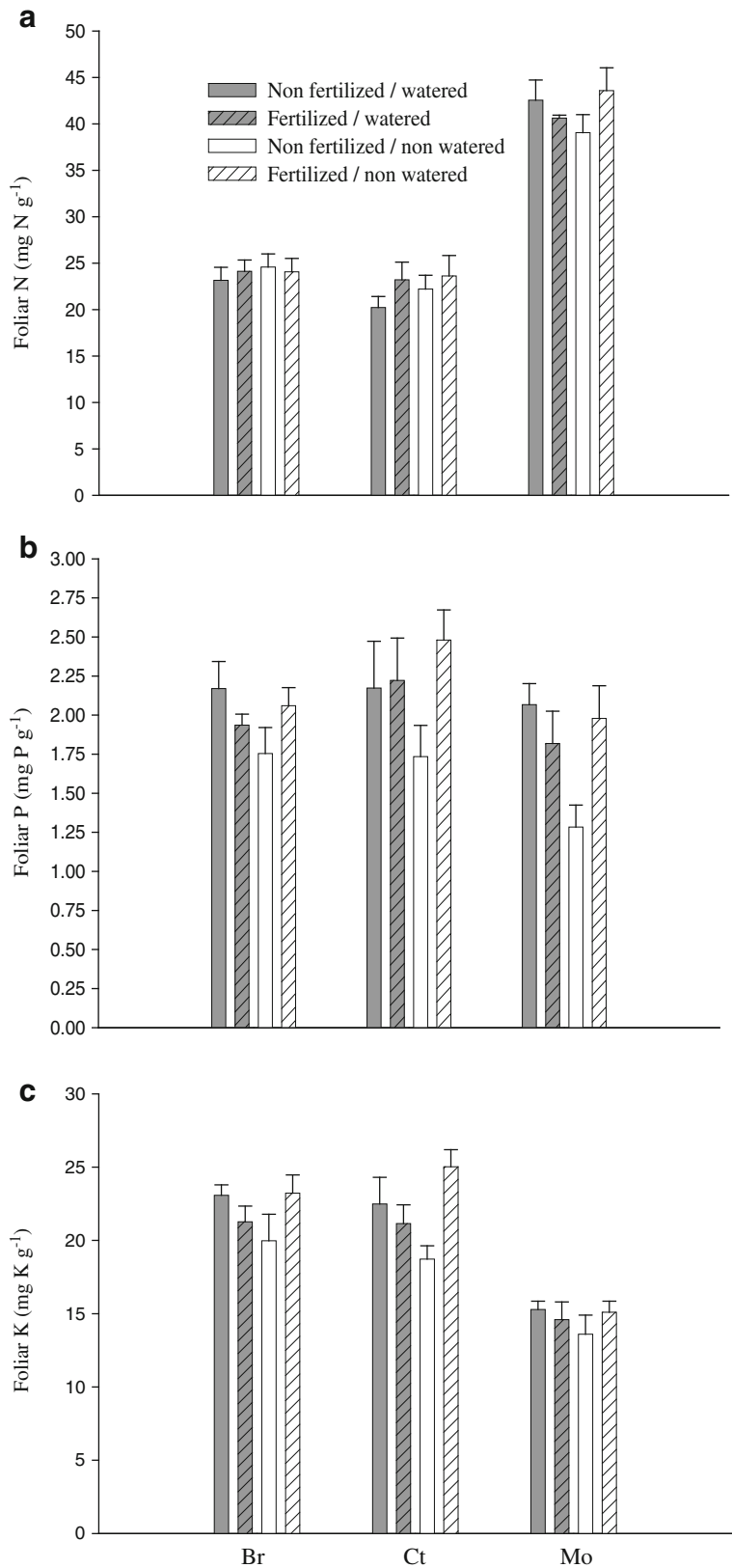
Fig. 6 Relationships between leaf $\delta^{13}C$ and $\delta^{18}O$ of *Bromus rubens* (**a**), *Carduus tenuifolius* (**b**) and *Melilotus officinalis* (**c**) in June 2008. Regression lines are shown when significant ($P<0.05$). $n=60$ in *Bromus rubens*, $n=72$ in *Carduus tenuifolius* and $n=48$ in *Melilotus officinalis*. Rest of legend as in Fig. 3
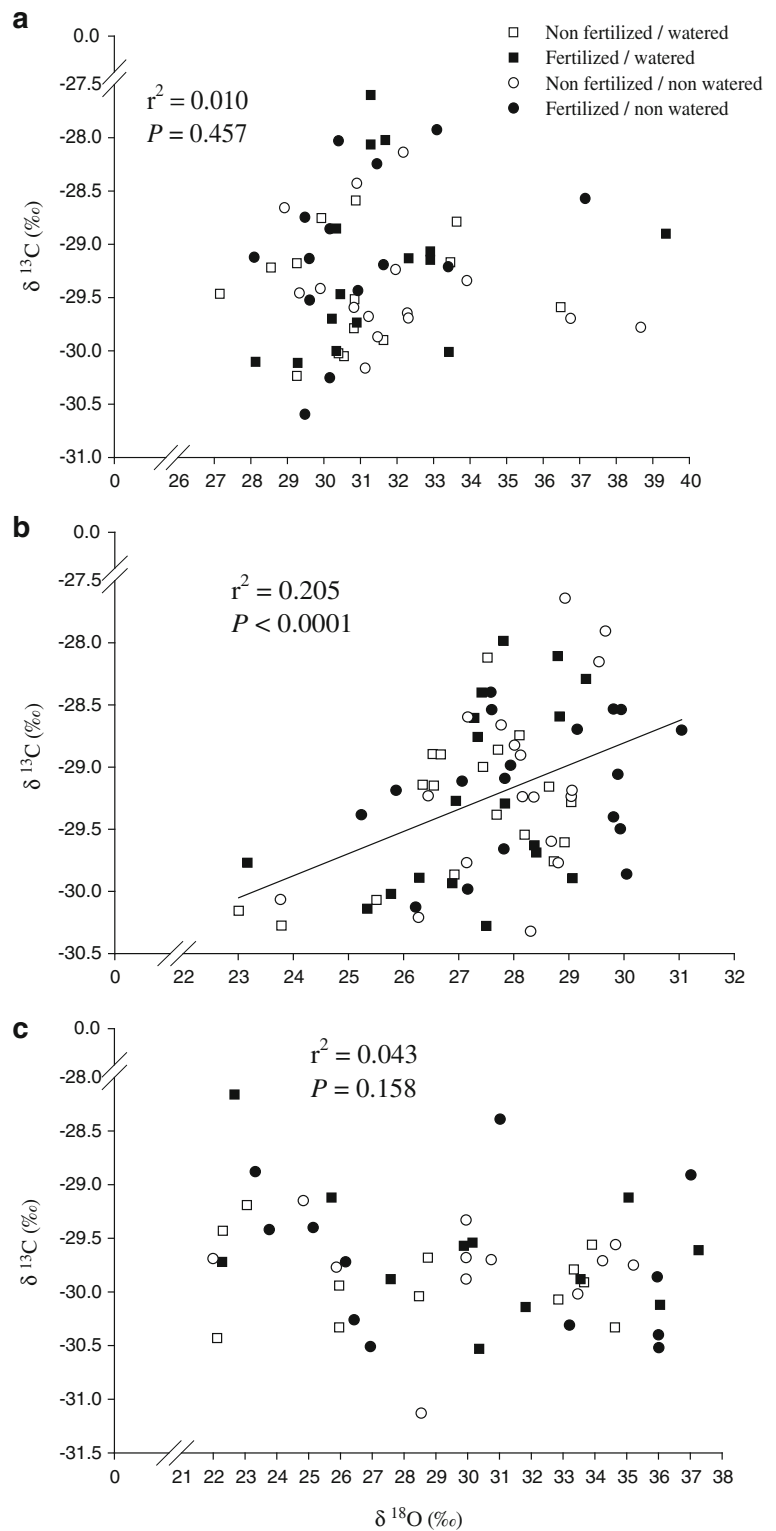
Fig. 7 Relationships
between leaf $\delta^{13}C$ and foliar
N concentration of *Bromus
rubens* (**a**), *Carduus tenuifo-
lius* (**b**) and *Melilotus
officinalis* (**c**) in June 2008.
Regression lines are shown
when significant ($P<0.05$).
$n=60$ in *Bromus rubens*,
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and $n=48$ in *Melilotus
officinalis*. Rest of legend
as in Fig. 3
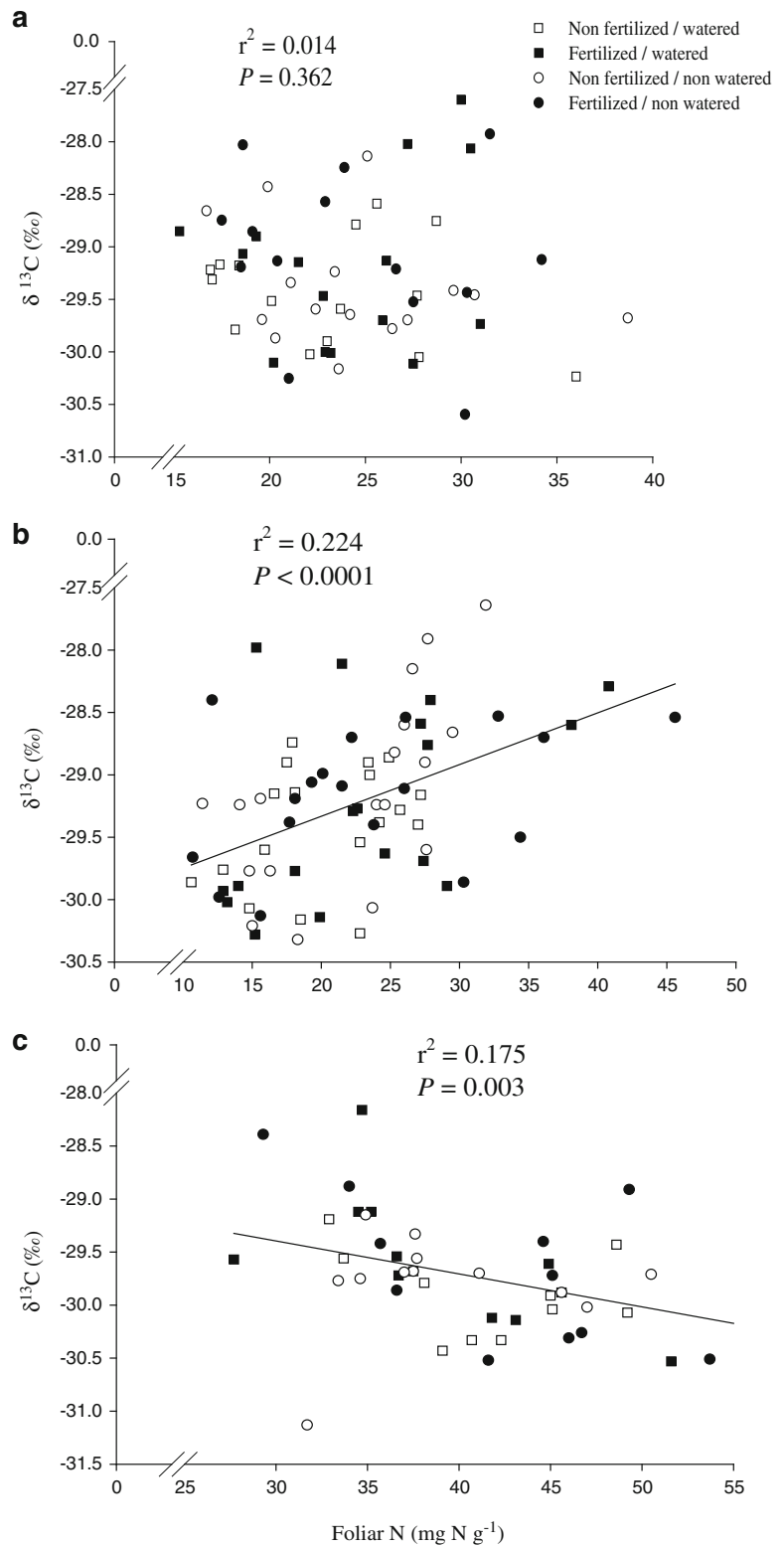
tion and photosynthesis may allow this forb species to readily adjust to moderately different water availability scenarios through effective fine tuning of WUE. Further, we found positive associations of foliar N concentration with leaf $\delta^{13}C$ (Fig. 7b) and of foliar N and P concentrations with $\delta^{18}O$ ($r^2=0.160$; $P<0.0001$ and $r^2=0.113$; $P=0.011$, respectively) across treatments. These results suggest that increasing foliar N and P concentrations to achieve enhanced WUE under drier scenarios may help *Carduus* to counterbalance the negative effects of decreased stomatal conductance on photosynthetic activity, which is in accordance with the leaf economics spectrum hypothesis (Wright et al. 2004; Prentice et al. 2011). This nutrient-mediated mechanism of adaptation to drought is widespread among C3 plant species, which often increase their foliar N concentrations in response to low moisture availability (Prentice et al. 2011). The positive associations between leaf $\delta^{13}C$ and total and relative *Carduus* cover across treatments ($r^2=0.162$; $P<0.0001$, $r^2=0.074$; $P=0.021$, respectively) further support an effective physiological adjustment (through enhanced WUE) to moderate water stress in this species. The total and relative cover of *Carduus* were unaffected by the experimental treatments, suggesting that this species has the ability to successfully cope with moderate moisture stress through effective stomatal adjustments, which translates into a maintenance of its relative dominance within the plant community. In addition, it should be noted that this species has an earlier phenology than the majority of co-occurring species in this anthropogenic grassland (García-Palacios, *personal observation*), which may help it gain further competitive advantage in annual plant communities (Grime 2001). This advantage could be especially relevant under scenarios of reduced spring rainfall predicted by climate change models for the Mediterranean Basin (IPCC 2007).

Our results show that *Bromus* is highly vulnerable to even moderate reductions in soil water availability, as indicated by significantly lower relative cover in the non-watered plots, thus supporting the results found by Kardol et al. (2010) for the same species. On the other hand, *Bromus* increased its leaf $\delta^{13}C$ while keeping its $\delta^{18}O$ constant in the fertilized plots, which suggests a sharp increase in the WUE of this grass species under an elevated nutrient deposition scenario (Lee et al. 2001). According to current conceptual models (Scheidegger et al. 2000; Grams

et al. 2007), the observed increase in WUE in fertilized plots must be the result of a strong nutrient-mediated stimulation of photosynthetic capacity in *Bromus,* with little or no change in stomatal conductance (Dawson et al. 2002; Wright et al. 2004). An increase in WUE with fertilization might explain the observed trend towards higher relative cover by this species, and hence dominance, in the fertilized plots (Fig. 3b; Ehleringer et al. 1992; Tsialtas et al. 2001). Interestingly, the negative effect of lower soil water availability on the relative cover and dominance of *Bromus* in non-watered plots was largely counterbalanced by this nutrient-mediated stimulation of WUE in fertilized non-watered plots (Fig. 3b). These results strongly suggest the existence of counteracting effects of two major global change drivers (namely, reduced rainfall and increased nutrient depositon; Sala et al. 2000) on the performance of *Bromus*.

Fertilizer addition caused a 40% decrease in the relative species cover of *Melilotus* at the AP36 site, irrespective of soil water availability (Table 3; Fig. 3b). It is widely acknowledged that the competitive advantage of legumes decreases sharply when high soil fertility precludes the benefits of N fixation (Bobbink et al. 1998; Suding et al. 2005; Skogen et al. 2011), as is the case under elevated nutrient deposition scenarios. Lee et al. (2001) reported that leaf N content, photosynthesis and water use efficiency actually decreased in response to high soil N treatments in several legume species. Although the concentrations of foliar nutrients or the carbon and oxygen isotopic values of *Melilotus* were not significantly affected by the experimental treatments, leaf $\delta^{13}C$ was strongly and negatively associated with foliar N, P and K concentrations across treatments, suggesting that high leaf $\delta^{13}C$ may be an indication of nutritional or physiological stress in this species. In sharp contrast to non-fixer plant species, N-fixing species (legumes or actinorhizal) in semiarid ecosystems often show unchanged or even decreased $\delta^{13}C$ and WUE in response to improved nutrient availability or status (Lee et al. 2001; Querejeta et al. 2003, 2007). Our results indicate that *Melilotus* may experience a dramatic decrease in relative cover and dominance in these semiarid anthropogenic grasslands under enhanced nutrient deposition. In this scenario, the competitive ability of this early colonizer species (Merlin et al. 1999) decreases sharply, which may accelerate species replacement and enhance plant

community diversity during the early-successional stages of semiarid roadside grasslands. This interpretation is supported by the strong positive effect of fertilizer addition upon the Shannon's diversity index (67% increase) in this roadside grassland dominated by *Melilotus* (García-Palacios et al. 2010).

Our study is not without limitations, mainly the indirect simulation of drier conditions used to approach changes in rainfall regime predicted by global change in Mediterranean semiarid areas (IPCC 2007). Soil moisture content was relatively high in both watered and non-watered plots throughout the experimental period due to unusually rainy conditions during 2008, thus reducing the effectiveness of our experimental approach. Nevertheless, the relative dominance and the isotopic composition of the target plant species did respond to the watering treatments evaluated, suggesting that even stronger responses could be expected with larger differences in water availability between treatments, as found for instance by Peñuelas et al. (2000) in Mediterranean woody ecosystems subjected to partial rainfall exclusion. As in other recent studies (e.g. Bassin et al. 2009), our interpretation of $\delta^{13}C$ and $\delta^{18}O$ data relies on current conceptual models (Scheidegger et al. 2000; Grams et al. 2007) and empirical studies establishing relationships between changes in plant isotopic composition and corresponding changes in leaf-level photosynthesis, stomatal conductance and water use efficiency in similar herbaceous species (e.g. Barbour et al. 2000; Jaggi and Fuhrer 2007; Cabrera-Bosquet et al. 2009b, 2011). However, as these relationships have not been directly evaluated for the particular set of species included in this study, our interpretation of isotopic data should be taken with some caution (e.g. Cernusak et al. (2009) found strong, weak or even no relationships between isotopic and leaf gas exchange variables in different tropical tree species). Finally, additional studies including more than one species of each plant functional group should be conducted in order to evaluate the magnitude of between vs. within functional group variation in plant physiological responses to global change drivers.

Collectively, our results indicate that three dominant ruderal species belonging to contrasting plant functional groups showed strongly species-specific responses to changes in soil water and nutrient availability. Whereas the lower soil water availability levels predicted by climate change

models for the Mediterranean region will likely increase vegetation water stress and decrease net primary productivity (Miranda et al. 2009), some dominant ruderal species (e.g. *Carduus*) may be able to cope with this limitation without a significant reduction in their competitive ability and relative dominance. However, other species (e.g. *Bromus*) are strongly negatively affected by even moderate water stress, and could significantly decrease its relative dominance under low water availability scenarios. Increased nutrient (particularly N) deposition could decrease the competitive ability of currently dominant legume species in many anthropogenic ruderal grasslands. By contrast, elevated atmospheric nutrient deposition could buffer or even counterbalance the negative effects of decreased soil water availability on the performance of some non-legume species through a nutrient-mediated enhancement of water use efficiency (Lee et al. 2001). The three species evaluated in this study are dominant in the early-successional stages of semiarid roadside grasslands, promote both positive and negative effects upon plant community diversity (García-Palacios et al. 2010), and can cause profound changes in soil nutrient cycling (García-Palacios et al. 2011b).

In conclusion, the characterization of species-specific responses to major global change drivers may improve predictions about the future composition and dynamics of plant communities in novel ecosystems, as well as the potential effects of global change upon ecosystem functioning. The results of this study also highlight the importance of simultaneously considering several relevant global change drivers, such as rainfall regime and nutrient deposition, to better understand the net effects of global change on human-disturbed ecosystems.

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