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Altered leaf elemental composition with climate change is linked to reductions in photosynthesis, growth and survival in a semiarid shrubland

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Summary

Climate change will increase heat and drought stress in many dryland areas, which could reduce soil nutrient availability for plants and aggravate nutrient limitation of primary productivity. Any negative impacts of climate change on foliar nutrient contents would be expected to negatively affect the photosynthetic capacity, water use efficiency and overall fitness of dryland vegetation.

We conducted a four-year manipulative experiment using open top chambers and rainout shelters to assess the impacts of warming ($\sim 2^{\circ}\text{C}$, W), rainfall reduction ($\sim 30\%$, RR) and their combination (W+RR) on the nutrient status and ecophysiological performance of six native shrub species of contrasting phylogeny in a semiarid ecosystem. Leaf nutrient status and gas exchange were assessed yearly, whereas biomass production and survival were measured at the end of the study.

Warming (W and W+RR) advanced shoot growth phenology and reduced foliar macro- (N, P, K) and micronutrient (Cu, Fe, Zn) concentrations (by 8-18% and 14-56%, respectively), net photosynthetic rate (32%), aboveground biomass production (28-39%) and survival (23-46%). Decreased photosynthesis and growth in W and W+RR plants was primarily linked to enhanced nutritional constraints on carbon fixation. Poor leaf nutrient status in W and W+RR plants partly decoupled carbon assimilation from water flux and led to drastic reductions in water use efficiency (WUE_i; $\sim 41\%$) across species. The RR treatment moderately decreased foliar macro- and micronutrients (6-17%, except for Zn) and biomass production (22%). The interactive impacts of warming and rainfall reduction (W+RR treatment) on plant performance were generally smaller than expected from additive single-factor effects.

Synthesis: Large decreases in plant nutrient pool size and productivity combined with increased mortality during hotter droughts will reduce vegetation cover and nutrient retention capacity, thereby disrupting biogeochemical processes and accelerating

dryland degradation with impending climate change. Increased macro- and micronutrient co-limitation of photosynthesis with forecasted climate change conditions may offset any gains in WUE_i and productivity derived from anthropogenic CO₂ elevation, thereby increasing dryland vegetation vulnerability to drought stress in a warmer and drier climate. The generalized reduction in leaf nutrient contents with warming compromises plant nutritional quality for herbivores, with potential cascading negative effects across trophic levels.

Keywords: climate aridification, drought stress, drylands, non-stomatal limitation of photosynthesis, stoichiometric constraints, warming, water use efficiency.

Introduction

In the Mediterranean region, climate change models predict greater than global-average increases in mean and maximum temperatures and vapour pressure deficit (VPD) as a consequence of anthropogenic global warming, accompanied by substantial reductions in the amount and frequency of precipitations (Giorgi & Lionello 2008; Collins et al., 2013; Guiot & Cramer 2016). Large spatial-scale relationships between climate and plant elemental composition indicate that leaf nutrient concentrations generally decrease with mean annual temperature and increase with mean annual rainfall (Reich & Oleksyn, 2004; Sardans et al., 2017). Therefore, warmer and drier climatic conditions could be expected to cause a general decline in plant macro- and micronutrient status in drylands with potentially detrimental consequences for productivity and overall fitness, given that adequate foliar macro- and micronutrient levels are necessary for achieving and maintaining optimal photosynthetic capacity, fine-tuned stomatal control and water use efficiency in water-limited ecosystems (Wright & Westoby 2002; Sardans & Peñuelas 2013; Peñuelas et al., 2018). A poor leaf nutrient status increases non-stomatal limitations on photosynthesis through impairment of carboxylation capacity, photochemical efficiency, stomatal regulation, osmotic protection and overall photosynthetic performance under drought stress (Wright et al., 2005; Reich, Oleksyn & Wright, 2009).

There is increasing awareness that progressive atmospheric CO₂ elevation (eCO₂) with anthropogenic global change threatens plant nutrient status and nutritional quality (Elser, Fagan, Kerkhoff, Swenson & Enquist, 2010; Loladze 2014; Briat et al., 2015), given that eCO₂ decreases leaf nutrient concentrations (6.5-10%) through carbon dilution effects and reductions in plant transpiration (Sardans et al., 2012; Loladze 2014). However, the potential negative impacts of climate aridification on dryland plant nutrition remain understudied and are not well understood (Grossiord et al., 2018; Sardans et al., 2012, 2017; Sardans & Peñuelas 2012; Yuan & Chen 2015). In particular, the influence of climate warming and drying on the foliar concentrations of essential plant micronutrients such as Fe, Cu and Zn in dryland vegetation has received limited research attention despite their key roles in photosynthesis, metabolism and growth (Marschner 2011; Briat, Dubos & Gaymard, 2015; Salazar-Tortosa et al., 2018; Tian et al., 2019).

A meta-analysis of manipulative warming studies at global scale found moderate increases in foliar N concentrations (+2.9%) with elevated temperature, which is linked to increases in N mineralization, cycling rates and availability due to enhanced soil microbial activity (Bai et al. 2013). However, the enhancement of plant growth with warming can sometimes involve dilution of nutrients with reductions in elemental concentrations and increased carbon to-nutrient ratios in plant tissues (Suzuki & Kudo 2000; Sardans et al., 2012). On the other hand, the response of plant nutrient status to warming is strongly dependent on its impact on soil moisture, as warming can simultaneously exacerbate water and nutrient limitation in drylands via its negative effects on soil moisture (Peñuelas et al., 2004; Sardans & Peñuelas 2012; Sardans et al 2012; Lafuente, Berdugo, Ladrón de Guevara, Gozalo & Maestre, 2018). A recent meta-analysis found that drought generally has negative effects on plant N (-3.73%) and P concentrations (-9.18%) and a positive effect on plant N: P ratio (+ 6.98%), although these effects are alleviated with extended duration of drought (He & Dijkstra 2014). Plants decrease nutrient uptake with decreasing soil moisture and with accompanying reductions in stomatal conductance and transpiration (Cramer, Hawkins & Verboom, 2009; Sardans & Peñuelas, 2012; Salazar-Tortosa et al., 2018). Soil drying reduces plant nutrient availability and uptake by reducing mineralization and nutrient supply

(Schimel, Balsler & Wallenstein, 2007), decreasing nutrient diffusion and mass flow in the soil (Chapin 1991; Lambers et al., 2008; Gessler, Schaub & McDowell, 2017; Salazar-Tortosa et al., 2018) and reducing the production or activity of fine roots and their associated mycorrhizal fungi (León-Sánchez et al., 2018).

We conducted a 4-year manipulative field experiment in a semiarid shrubland to evaluate the impacts of climate warming and drying on the leaf nutrient status and physiological performance of a phylogenetically and functionally diverse set of six native shrub species. The responses of coexisting plant species to climate change are often idiosyncratic and species-specific (Llorens, Peñuelas, Estiarte & Bruna, 2004; Peñuelas et al., 2018), so evaluating the responses of a diverse array of species is imperative for discerning general patterns at the plant community level. Coexisting plant species could differ in their vulnerability to climate-induced nutrient deficit and imbalance due to their different nutrient requirements, mycorrhizal association types, fine root foraging strategies or rooting extent and depth, among other factors. A comprehensive assessment of plant nutrient status, isotopic composition and gas exchange rates combined with phenology, growth and survival measurements in a diverse array of species can help elucidate the physiological mechanisms underlying vegetation responses to projected climate change conditions (Salazar-Tortosa et al., 2018).

We hypothesized that warming, rainfall reduction and their interaction would H1) negatively impact soil nutrient availability and uptake by plants, thereby leading to lowered leaf nutrient concentrations across shrub species, which in turn, would, H2) impair photosynthetic capacity, stomatal control, water use efficiency and drought-stress tolerance, and, H3) drastically reduce the size of vegetation nutrient pools through mutually reinforcing reductions in foliar nutrient contents and plant growth and standing biomass. Furthermore, we expected that the interaction of warming and rainfall reduction (W+RR treatment) would cause additive or even multiplicative negative impacts on plant nutrient status, physiological performance, growth and survival in this semiarid ecosystem.

Materials and Methods

Study site and experimental design

The experimental site is located in Sorbas, Southeastern Spain (37°05'32.4"N 2°05'41.5"W, 409 m.a.s.l.). The climate is semiarid Mediterranean, with mean annual temperature of 17°C and mean annual precipitation of approximately 275 mm. Soils are rich in gypsum (>50%) and are classified as Gypsic Leptosols (IUSS Working Group WRB, 2006). The poor soil physical properties, high calcium and sulphur contents and low water availability and fertility of gypsum soils impose stressful abiotic conditions for vegetation (Table S1; see also Merlo et al., 2009; Escudero, Palacio, Maestre & Luzuriaga, 2015). Vegetation is a mixed grassland-shrubland community dominated by *Stipa tenacissima* and several gypsophilous shrub species. Our six target shrub species were *Helianthemum squamatum*, *Helianthemum syriacum* (Cistaceae), *Gypsophila struthium* (Caryophyllaceae), *Teucrium turredanum* (Lamiaceae), *Santolina viscosa* (Compositae) and *Coris hispanica* (Primulaceae). This set of phylogenetically diverse species encompasses varying plant sizes, life history traits, phenology, elemental compositions, rooting depths, water use strategies and mycorrhizal association types (Brundrett 2009; Moreno-Gutiérrez, Dawson, Nicolás & Querejeta, 2012; Palacio, Montserrat-Martí, Ferrio & Michalet, 2017; Table S2). Gypsum ecosystems are widespread with over 100 million ha worldwide, and native plant communities growing on gypsum soils are rich in rare and endemic plant species with a high conservation value (Martínez-Hernández et al., 2011).

We conducted a randomized factorial experiment with two experimental factors: warming (W) and rainfall reduction (RR). Ventilated open-top chambers (OTCs) were used to achieve temperature elevation. OTCs are hexagonal chambers, with sloping slides of 40 cm x 50 cm x 32 cm (Fig. S1). They are made of methacrylate, which transmits about 92% of visible light, has a reflection of incoming radiation of 4%, and passes on ca. 85% of incoming energy (information provided by the manufacturer; Decorplax S. L., Humanes, Spain). OTCs were suspended ~3 cm above the ground level by a metal frame to allow free air circulation and exchange with the surrounding environment, thus reducing undesirable experimental artifacts (such as potential

changes in CO₂ concentration, wind speed, boundary layer conductance or transpiration demand; see Hollister & Weber 2000; Maestre et al., 2013). To simulate projected reductions in precipitation for the Mediterranean region (de Castro, Martín-Vide & Alonso, 2005), passive rainout shelters made of methacrylate troughs covering ~30% of the area of the experimental plots were installed. Rainfall reduction was achieved by suspending the methacrylate troughs over an aluminum frame above the experimental plots (height 130 cm, width 100 x 100 cm, see Figure S1). Intercepted rainwater is diverted through pipes and collected in storage tanks placed next to the experimental plots, and is thereafter removed after each rainfall event. Finally, both OTCs and rainout shelters were installed over the same experimental plot in order to achieve the combined W+RR treatment (Fig. S1). Twelve plots with OTCs (W), 10 plots with rainout shelters (RR) and 10 plots combining OTCs with rainout shelters (W+RR), along with 32 interspersed control plots, were installed in May 2011, resulting in a total of 64 experimental plots.

Air and surface soil temperatures, relative humidity and soil moisture content were continuously monitored in the climate manipulation treatments using replicated automated sensors (HOBO[®] U23 Pro v2 Temp/RH and TMC20-HD sensors, Onset Corp., Pocasset, MA, USA, and EC-5 soil moisture sensors, Decagon Devices Inc., Pullman, WA, USA, respectively).

Plant measurements

We used a LI-6400XT photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA) equipped with a LI-6400-40 Leaf Chamber Fluorometer and a LICOR 6400-01 CO₂ injector to measure net photosynthetic rate (A), stomatal conductance (gs) and quantum efficiency of photosystem II (ØPSII) throughout the study in *H. squamatum*, *H. syriacum* and *G. struthium* (8-10 measurement dates for each species, Figs. S6, S7, S8). Leaf gas exchange and fluorescence were measured on fully sun-exposed leaves that were placed in a 2 cm² leaf cuvette. During these measurements, air CO₂ concentration was controlled using the injection system and compressed CO₂-cylinders with a CO₂

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concentration of $390 \mu\text{mol mol}^{-1} \text{CO}_2$. Measurements were done at a saturating light of $1.500 \mu\text{mol m}^{-2} \text{s}^{-1}$, and at ambient air temperature and relative humidity. The air flow was set to $250 \mu\text{mol s}^{-1}$. All leaf-gas exchange and fluorescence measurements were conducted between 9:00 am and 13:00 pm (GMT), when the peak of maximum photosynthetic rates was found at each survey. These measurements were conducted under the prevailing microclimatic conditions in each experimental treatment. Thereafter, leaves measured were collected to determine their area with an image scanner program in order to correct the LICOR outputs (Image Pro Plus, Media Cybernetics, Inc. Rockville, MD, USA). Intrinsic water use efficiency (WUEi) was determined as A/g_s .

In May 2012 and 2013 and in October 2015, we collected leaf samples of all shrub species to evaluate the effects of the climate treatments on plant nutrient status (N, P, K, Fe, Zn, Cu). *Coris hispanica* leaves were only collected in 2012 due to widespread mortality across treatments in subsequent years. Leaf samples were oven-dried at 60°C and thereafter finely ground with a ball mill for elemental analyses. Leaf C and N concentrations were measured with an ANCA/SL elemental analyzer, whereas leaf P, K, Fe, Cu and Zn concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with $\text{HNO}_3:\text{H}_2\text{O}_2$ (4:1, v:v).

In early March 2012 and 2013, three terminal shoots per individual of the target species were labeled using red tape in order to track shoot elongations throughout the growing season. We measured shoot length in March, May and July/August and calculated early spring shoot elongation as the difference between shoot length in May and shoot length in March, and late spring shoot elongation as the difference between shoot length in July/August and shoot length in May. Thereafter we calculated the ratio between late/early shoot elongations as a shoot growth phenology index.

In November 2015 (i.e., 54 months after the beginning of the experiment), one 10 cm long terminal shoot was collected from one randomly chosen individual of every target species present in each experimental plot. The oven-dried biomass (60°C; leaves and stem) per unit shoot length was measured and the total shoot biomass was calculated as the sum of leaf and stem biomass. Leaf samples were also used to measure their carbon isotope composition ($\delta^{13}\text{C}$). Oven-dried leaf sub-samples were finely ground with a ball mill before being weighted and placed into tin capsules for isotopic analyses. The $\delta^{13}\text{C}$ values of leaf material were measured by elemental analyzer/continuous flow isotope ratio mass spectrometry (ANCA/SL elemental analyzer coupled with a Finnigan MAT Delta PlusXL IRMS). Isotope ratios are expressed in delta notation (‰) relative to the internationally accepted reference standard V-PDB. Long-term (3+ years) external precision for $\delta^{13}\text{C}$ measurements is 0.14‰.

The survival rate of shrub species in each climate treatment was assessed once at the end of the study period (November 2015) as the proportion of individuals that were still alive in each experimental plot in November 2015 (i.e., 54 months after the beginning of the experiment) relative to the total number of individuals present in March 2012.

Statistical analyses

We used a general linear mixed model approach (LMMs) to evaluate the effects of warming (W), rainfall reduction (RR) and their interaction on plant performance across shrub species and measurement dates. The plant response variables analyzed were leaf nutrient concentrations (N, P, K, Fe, Cu, Zn), leaf gas exchange (A, g_s , E, WUE_i) and fluorescence (Φ_{PSII}), with warming (W), rainfall reduction (RR) and time (Date) as fixed factors. Plot was used as the random factor to account for the repeated measures structure of the study in which the same plot was measured in several dates. Leaf $\delta^{13}\text{C}$, shoot biomass production and shoot phenology were analyzed using general linear models (LMs) with warming (W), rainfall reduction (RR) and their interaction as factors. Linear model analyses were followed by Tukey's post hoc tests to detect significant differences among climate treatments for all the response variables. For both LMMs and LMs, residuals were assessed for normality (Shapiro–Wilks test at $P > .05$) and data were log- transformed when necessary.

We estimated the slopes of relationships between A and g_s across experimental treatments with standardized major axes (SMA) regressions and used standard slope comparison procedures to test for slope heterogeneity between treatments. We conducted a principal component analysis (PCA) of leaf nutrient concentrations (N, P, K, Fe, Cu, Zn) across shrub species and study years, in order to use the scores along PCAaxis1 as an integrative measure of overall leaf nutrient status. The influence of average leaf multi-elemental composition on average net photosynthesis and biomass production across the study period was assessed by linear mixed-model regression analyses, using shrub species as random factor. Plant survival at the end of the study period (November 2015) was analyzed using generalized linear models (GLM) with a binomial distribution where survival was the dependent variable and simulated climate warming (W), rainfall exclusion (RR) and their combination (W+RR) were the predictor factors.

We used R software (R Core Team 2019) for linear mixed-models and mixed model regressions (lme4 and lmerTest packages, Bates, Maechler, Bolker & Walker, 2015; Kuznetsova, Brockhoff & Christensen, 2017), PCA (ade4 package, Chessel, Dufour, & Thioulouse, 2004), SMA (smart package, Warton, Duursma, Falster & Taskinen, 2012) and survival analyses.

Results

Experimental treatment effects on microclimatic variables

Mean annual temperatures steadily increased from the first to the last hydrological years (October to September) of the study (from 17.6°C in 2011-2012 to 19.1°C in 2014-2015). Annual precipitation varied widely during the study period (Figure S2). In the first two hydrological years (2011-2012 and 2012-2013), rainfall was slightly below the historical average (247 and 241 mm, respectively). The third year of the study (2013-2014) was extremely dry (94 mm), and the last year (2014-2015) was also drier than average (180 mm).

Open top chambers (OTCs) increased air temperature by $\sim 2^{\circ}\text{C}$ in the W and W+RR plots, relative to control (ambient) conditions (Figure S3), with larger increments during warm periods (up to $4\text{-}5^{\circ}\text{C}$ during some hot days in Summer) than during cold periods ($\sim 1^{\circ}\text{C}$ in Winter). As a result of the air desiccating effects of temperature elevation, vapor pressure deficit (VPD) was on average 14% higher in warmed plots than in the control plots (798 Pa in W and W+RR plots versus 703 Pa in control and RR plots). The temperature of surface soil (0-5 cm) was also elevated in the warmed plots by 2.2°C on average (Figure S3). Warming reduced the moisture content of the topsoil layer (0-5 cm depth) by 16% relative to the control plots (Figure S4). Rainout shelters also reduced topsoil moisture content by 14% but did not alter mean air and soil temperatures or VPD. Reductions in topsoil moisture under the rainout shelters were greatest immediately after rainfall events, with up to 36% lower moisture in RR and W+RR plots relative to control plots. Contrary to expectations, we found no additive effects of warming and rainfall reduction on topsoil moisture in the W+RR treatment.

Impacts of climate manipulation on leaf nutrient status

The foliar concentrations of all the six essential plant macro- and micronutrients considered in this study were reduced by warming across species (Table S3), with reductions of roughly similar magnitude in the W and W+RR treatments. On average, plants in the W and W+RR treatments had 11-13% lower leaf N, 8-18% lower P and 13-15% lower K concentrations than control plants (Fig. 1), but without changes in foliar N:P ratios (as mean N:P ratios were $\sim 24\text{-}26$ across treatments). Warming-induced reductions in the foliar concentrations of micronutrients were even larger (Fig. 2), as plants in the W and W+RR treatments had on average 39-56% lower leaf Fe, 24-29% lower Cu and 25-14% lower Zn concentrations than plants in control plots (Figure 2). Plants in the rainfall reduction treatment (RR) had on average 6% lower foliar N and P, 11.5% lower Fe and 17.5% lower K and Cu concentrations than control plants (but with 13% higher leaf Zn).

A principal component analysis (PCA) of all six nutrients across species revealed a highly significant first component (PCA_{axis1}) accounting for 44.0% of the variance in foliar multi-elemental concentrations and a secondary component (PCA_{axis2}) accounting for 26.0% of the variance (Table S4). The first component of the PCA showed strong loadings for all the six macro- and micronutrients (ranging from 0.45 to 0.83 with positive signs in all cases, Table S4), which allowed us to use the individual plant scores along PCA_{axis1} as an integrative measure of overall leaf nutrient status. Plant scores along PCA_{axis1} were strongly decreased by warming (with similar reductions in the W and W+RR treatments; Table S3, $P < 0.001$; Figure S5), whereas scores along PCA_{axis2} were not significantly affected by climate manipulation ($P > 0.05$, data not shown).

Leaf carbon concentration was not affected by climate manipulation (data not shown), which implies that all carbon-to-mineral nutrient ratios increased with warming. Averaged across species, mean leaf C:N ratios increased from 28.5 in control plants to 32-33 in W and W+RR plants ($P < 0.001$).

Leaf-gas exchange and carbon isotopes

Warming strongly decreased net photosynthetic rate in the W and W+RR treatments (Figs. 3a, S6; Table S5; $P < 0.001$). Reductions in A with warming (W and W+RR) averaged 36% in *H. squamatum*, 29% in *H. syriacum* and 36% in *G. struthium*, relative to the controls. Warming also reduced the mean quantum efficiency of photosystem II (Φ_{PSII}) (Fig. 3d, Table S5; $P < 0.001$), with average reductions of 11.4% in W and 17.3% in W+RR plants relative to the controls. Warming moderately increased stomatal conductance (Table S5; $P = 0.013$), with W and W+RR plants showing on average 5% higher mean g_s values than control plants (Figs. 3b, S7). Transpiration rates followed the same pattern as g_s , with an average increase of 7% in warmed plants (W and W+RR) relative to the controls (data not shown). Intrinsic water use efficiency ($WUE_i = A/g_s$) was strongly reduced by warming, with reductions of similar magnitude in the W and W+RR treatments; Figs. 3c, S8; Table S5; $P < 0.001$). The reduction of WUE_i in warmed plants relative to control plants averaged 38% in *H. squamatum*, 33%

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in *H. syriacum* and 54% in *G. struthium*. Reductions in A and WUEi with warming were often largest during relatively wet periods at the peak of the Spring growing season (Fig. S6 and S8). Average reductions of A and WUEi with warming were quite similar between the W+RR (34.6% and 42.5%, respectively) and the W treatment (30% and 40.5%, respectively).

Net photosynthetic rate was tightly positively correlated with stomatal conductance in all the shrub species, but warming consistently reduced the slope of the A versus g_s relationship, as denoted by flatter slopes in W and W+RR plants than in Control or RR plants ($P < 0.001$; Fig. 4).

In contrast to the strong impacts of warming, the impact of rainfall reduction on leaf gas exchange rates was moderate or non-significant and varied among species (significant RR x Species interactions for g_s and WUEi in Table S5; Figs. 3, S6, S7, S8). The RR treatment decreased gas exchange rates in *H. syriacum*, which led to 9.1% and 16.6% lower mean A and g_s than in control plants in this species ($P = 0.030$ and $P = 0.016$, respectively). Rainfall reduction decreased the WUEi of *H. squamatum* by 11% in the RR treatment relative to control plants ($P = 0.004$). However, leaf gas exchange rates in the deep-rooted species *G. struthium* were unaffected by the RR treatment ($P > 0.05$).

Foliar $\delta^{13}\text{C}$ values were significantly lower in the W and W+RR treatments than in the Control and RR treatments at the end of the study period ($P = 0.048$; Figure S9 and Table S6), thus revealing a lowered time-integrated water use efficiency under warming.

Shoot biomass production, shoot growth phenology and plant survival

Shoot dry biomass production per unit length (leaves plus stem) was reduced by both warming ($P < 0.001$) and rainfall reduction ($P < 0.001$), with no significant interactions between both factors (Fig. 5, Table S6). W+RR plants showed the largest mean reduction in shoot biomass (39%), followed by W (28%) and RR (22%) plants. Foliar biomass production was strongly reduced by both warming and rainfall reduction ($P < 0.001$ and $P < 0.001$, respectively), whereas stem biomass production was decreased by warming only ($P < 0.001$; Tables S6, S7). For all *Species x Climate Treatment* combinations, average net photosynthetic rate and shoot dry biomass production were positively associated with average multi-elemental concentrations in leaves (scores along PCA_{axis1}) within and across shrub species (Figure 6a, b, respectively).

Concurrent reductions in leaf biomass production and foliar nutrient concentrations with warming (and to a lesser extent, rainfall reduction) led to drastic decreases in the size of foliage nutrient pools per unit shoot length in the climate manipulation treatments. Reductions in foliage nutrient pool sizes were largest in the W+RR treatment, reaching 45% for N, 41% for P, 39% for K, 70% for Fe, 51% for Cu and 41% for Zn, relative to the control treatment.

Warming consistently advanced shoot growth phenology in the W and W+RR treatments. Shoot elongation during the earlier, cooler part of the growing season (late winter and early spring) was stimulated by warming ($P = 0.003$), with average values of 7.54 ± 0.33 cm in warmed plants (W, W+RR) versus 6.22 ± 0.24 cm in ambient temperature plants (C, RR). In contrast, warming inhibited shoot elongation during late spring and early summer ($P = 0.001$), with average values of 3.21 ± 0.44 cm in warmed plants vs. 5.20 ± 0.33 cm in plants exposed to ambient temperatures. As a result of these phenological changes, the late/early season shoot elongation ratio differed markedly between climate treatments: 1.29 ± 0.12 in control, 1.30 ± 0.17 in RR, 0.90 ± 0.18 in W and 0.51 ± 0.21 in W+RR plants. Earlier shoot growth initiation with warming did not extend the vegetative growing season or enhance biomass production because it was more than offset by growth inhibition and earlier growth cessation in mid to late Spring.

Plant survival rate after 4 years of climate manipulation (November 2015) was significantly reduced by warming ($P= 0.009$, Figure 7; Table S8), with the W and W+RR treatments showing 23.5% and 45.8% lower survival, respectively, than the control treatment. However, there were some important differences among species in this respect, as *C. hispanica* showed 0% survival in the W +RR treatment, whereas *T. turredanum* showed 100% survival in this same treatment.

Discussion

Simulated climate change impact on leaf elemental composition

In agreement with our hypotheses (H1 and H2), the W and W+RR treatments lowered the foliar concentrations of six essential macro- and micronutrients while reducing aboveground biomass production by one-third or more across shrub species, thus revealing that altered leaf elemental composition was not attributable to growth dilution effects. Simultaneous reductions in both foliar nutrient concentrations and foliage biomass production drastically reduced the size of vegetation nutrient pools in the W and W+RR treatments (thus supporting H3), with potentially negative consequences for ecosystem nutrient retention capacity and increased risk of massive soil nutrient losses through runoff and erosion during rainstorms (Matías, Castro & Zamora, 2011; Peñuelas et al., 2018).

The generalized reduction of foliar nutrient concentrations in the W and W+RR treatments was not primarily driven by reductions in cumulative stomatal conductance and transpiration as previously observed in other studies (e.g. Salazar-Tortosa et al., 2018), given that g_s and E were marginally higher under warming. We propose three potential mechanisms (non-mutually exclusive) through which warming may lead to decreased nutrient availability and uptake in dryland plants: a) the nutrients concentrating in the fertile topsoil layer may become less available to plants due to the desiccating effect of warming, with nutrients becoming “locked up” in a drier topsoil more often and for longer periods due to lower mineralization, diffusion and mass flow rates (Gessler et al., 2017; Salazar-Tortosa et al., 2018); b) a shift to a deeper rooting

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pattern in response to a drier topsoil under warming may lead to proportionally greater resource extraction from deeper, moister, but less fertile soil layers where the soil solution is poorer in nutrients; c) reductions in photosynthesis with warming may lead to carbon limitation of nutrient uptake through reductions in fine root production and decreases in the abundance and activity of mycorrhizal fungi (as previously detailed in the conceptual model proposed by León-Sánchez et al., 2018), thereby compounding the effects of the two aforementioned mechanisms.

Consequences of altered leaf elemental composition for plant physiological performance under climate change conditions

In agreement with H2, the generalized reduction in the foliar concentrations of essential macro- and micronutrients with warming had mutually reinforcing and multiplicative negative impacts on the photosynthetic performance, WUEi and biomass production of W and W+RR plants across species (Bloom, Chapin & Mooney 1985; Marschner 2011; Gessler et al., 2017). As evident from Figure 4, the large reduction of photosynthesis with warming was not exclusively or even primarily driven by increased drought stress or increased stomatal limitation on carbon assimilation as previously reported in other semiarid ecosystems (Adams et al., 2015; García-Forner et al 2016). Instead, reductions in photosynthesis and growth were consistently linked to increased nutritional (non-stomatal) constraints on carbon assimilation within and across species (Figs. 4, 6; León-Sánchez et al., 2018; Dusenke et al., 2019). Reductions of A in the W and W+RR treatments relative to the control were large (>30%) even during wet periods in Spring when ambient temperature and soil moisture were optimal for photosynthetic activity (Fig. S6). Nonetheless, in addition to enhanced nutrient limitation, decreased topsoil water availability relative to the control plots (Fig. S6) likely contributed further to reduce biomass growth over the growing season in all the climate manipulation treatments (W, RR, W+RR). Contrary to expectations, the interactive impacts of warming and rainfall reduction on plant nutrient status, physiological performance and growth in the W+RR treatment were generally smaller than expected from additive single-factor effects, as previously encountered in other dryland ecosystems (García-Forner et al., 2016; Grossiord et al., 2017b). As a result, reductions in leaf nutrient

concentrations, photosynthesis, water use efficiency, growth and survival were rather similar between the W and W+RR treatments.

We found that the quantum efficiency of photosystem II (Φ_{PSII}) was reduced by warming in the W and W+RR treatments across shrub species, which suggests downregulation of photosystem II in response to a reduced carboxylation capacity (Sage & Kubien 2007). Plant photosynthetic capacity scales tightly with leaf N content because nitrogen plays a fundamental role in protein synthesis and enzymatic activity and is the major component of RuBisCO, which is the enzyme responsible for photosynthetic CO₂ fixation (Evans 1989; Ellsworth & Reich 1992; Wright et al., 2005; Kattge et al., 2009). A poor leaf P status in warmed plants further constrained the A_{max} -N relationship, given that P-deficient plants have limited RuBP regeneration capacity, photochemical efficiency of photosystem II and mesophyll conductance to CO₂ (Reich et al., 2009; Thomas, Montagu & Conroy, 2006; Warren 2011). Moreover, P is involved in nucleic acid and phospholipid synthesis and in energy transfer (ATP/NADPH) in the plant cell (Aerts & Chapin, 1999). Poor leaf K status in the W and W+RR treatments likely increased plant vulnerability to drought stress, given that K plays a key role in plant osmoregulation and in stomatal functioning, and is particularly important for controlling plant water fluxes and achieving photosynthetic acclimation to drought (Cakmak 2005; Nardini, Lo Gullo, Trifiló & Salleo, 2014; Sardans & Peñuelas 2015).

The large reductions in leaf micronutrients with warming likely compounded the negative impacts of poor leaf N-P-K status in the W and W+RR treatments. Of particular concern is the drastic reduction in leaf Fe with warming (39-56%), as this micronutrient activates many metabolic pathways and thus plays a critical role in metabolic processes such as photosynthesis, respiration and DNA synthesis. Fe is a component of multiple enzymes such as the cytochromes of the photosynthetic electron transport chain, is involved in the synthesis of chlorophyll and is essential for the maintenance of chloroplast structure and function (Briat, Curie & Gaymard, 2007, Briat et al., 2015; Marschner, 2011). Zn has a stabilizing role on the chlorophyll molecule, and is necessary as a catalyst for the activity of several enzymatic systems in

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photosynthesis; and Cu is indispensable for photosynthetic electron transfer as part of the proteins plastocyanin and cytochrome c oxidase (Marschner, 2011). On the other hand, Fe, Cu and Zn play key roles in plant protection against the oxidative damage induced by heat and drought stress (e.g. chloroplastic and cytosolic Fe, Cu and Zn-superoxide dismutases; Gill & Tuteja 2010), so severe micronutrient deficiency likely reduced the ability of W and W+RR plants to cope with increased abiotic stress.

In addition to multiple nutritional constraints on carbon assimilation, other non-stomatal (biochemical and metabolic) mechanisms may have further contributed to reduce net photosynthetic rates, WUE_i and growth in the W and W+RR treatments, especially during hot and dry periods (Flexas & Medrano, 2002; Zhou, Prentice & Medlyn, 2019). These include downregulation of photosynthesis (Flexas et al., 2004), reduced electron transport capacity (Sage & Kubien 2007); decreases in Rubisco regeneration and activation state (Rennenberg et al., 2006; Galmés, Flexas, Medrano, Niinemets & Valladares, 2012); decreased mesophyll conductance to CO₂ (Galmés et al., 2007; Dewar et al., 2018); enhanced photorespiration and dark respiration (Dusenge et al., 2019); and/or carbon sink-limitation of photosynthesis by heat- and drought- induced inhibition of growth (Grassi & Magnani 2005).

In terrestrial vegetation, progressive stomatal closure with increasing soil water limitation and/or VPD causes a proportionally smaller decrease in photosynthesis than in stomatal conductance and thus generally increases WUE_i (Flexas et al., 2004; Limousin, Yopez, McDowell, Pockman & Tjoelker, 2015; Wertin, Belnap & Reed, 2017). However, we found that the combination of strongly reduced A with moderately enhanced g_s resulted in a drastic reduction in the leaf-level WUE_i of W and W+RR plants (Fig. 3c), which was further confirmed by their lowered foliar δ¹³C values relative to the control treatment (Cernusak et al., 2013; León-Sánchez et al., 2016, 2018). Carbon assimilation was thus partly decoupled from water flux under warming (Fig. 4) which led to drastic reductions in leaf-level WUE_i. This unexpected result suggests a warming-induced reduction in the sensitivity of stomata to air dryness and stomatal acclimation to elevated temperature and VPD in the W and W+RR treatments

(Grossiord et al., 2017a; Urban, Ingwers, McGuire and Teskey, 2017). Moderately increased stomatal conductance under warming may represent an adaptive physiological mechanism aimed at enhancing evaporative cooling to prevent leaf overheating and damage of the photosynthetic machinery (Drake et al., 2018) and/or may be aimed at enhancing nutrient harvesting in nutrient-stressed plants through increased mass flow to roots (Cramer et al., 2009). It should be noted, though, that transpiration at whole-plant level (not measured) surely decreased in the W and W+RR treatments through the drastic reduction of foliage standing biomass (and hence whole canopy leaf area) relative to the controls in all the shrub species (Limousin et al., 2009). Potential reductions in wind speed and thus boundary layer resistance within the OTCs might also have influenced whole-plant transpiration (which could not be assessed as the fan speed in the LICOR chamber is adjusted to constant values during leaf gas exchange measurements).

Warming significantly increased plant mortality (Table S8, Fig. 7), especially in the W+RR treatment, as expected from increased risk of hydraulic failure during hotter droughts due to enhanced soil drying combined with the greater evapotranspiration demand of warmer air (Adams et al., 2009, 2017; McDowell & Allen 2015; Williams et al., 2013). However, there were differences in warming-induced mortality among shrub species (Fig. 7), which might be linked to interspecific variation in rooting patterns and water uptake depths, iso/anisohdry (stomatal behavior and water potential regulation), vulnerability to embolism and hydraulic failure, heat-stress tolerance and/or vulnerability to carbon starvation (McDowell 2011).

Implications for dryland responses to climate change

The results of this study indicate that large decreases in plant nutrient pool size and growth combined with increased mortality during hotter droughts will lead to drastic reductions in vegetation cover and nutrient retention capacity, thereby disrupting biogeochemical processes and accelerating dryland degradation and desertification with forecasted climate change (Vicente-Serrano, Zouber, Lasanta & Pueyo, 2012; Morillas

et al., 2017). In more mesic Mediterranean ecosystems, coexisting plant species often show different responses and vulnerability to warming and rainfall reduction, which buffers the impacts of climate change on overall vegetation productivity through shifts in species dominance in diverse plant communities (Llorens et al., 2004; Peñuelas et al., 2004, Peñuelas et al., 2018). However, in our semiarid ecosystem, six contrasting shrub species which together account for 69% of the total plant cover exhibited remarkably similar reductions in foliar nutrient concentrations and aboveground productivity in the W+RR treatment (although with interspecific differences in survival), which indicates a much more limited potential for mitigation of the negative impacts of climate change by shifts in species dominance.

Generalized reductions in leaf multi-elemental concentrations with warming will slow down litter decomposition and carbon and nutrient cycling through cross-species increases in leaf litter recalcitrance (Prieto, Almagro, Bastida & Querejeta, 2019). Increased plant C:nutrient ratios compromise plant nutritional quality for both specialist and generalist herbivores, with cascading negative impacts on trophic and detrital food webs (Cebrian et al., 2009; Finzi et al., 2011). The warming-induced reductions in foliar nutrients reported in this study are of similar or greater magnitude than those induced by eCO₂, especially for essential micronutrients like Fe, Cu and Zn (Loladze, 2014). Moreover, additive or synergistic interactions between climate warming, increasing aridity and eCO₂ could amplify the observed reduction in leaf nutrients in the future (Sardans et al., 2012; Moreno-Jiménez et al., 2019), with cascading negative effects across trophic levels. Of special concern is the drastic decrease in foliar Fe concentration in the W+RR treatment (56%), given its key importance for photosynthetic efficiency and its low bioavailability and widespread deficiency in calcareous and high pH soils (Briat et al., 2015).

In conclusion, lowered foliar macro- and micronutrient content under simulated climate change was linked to severely impaired photosynthetic performance and water use efficiency, which led to large decreases in productivity and nutrient pool size in dryland vegetation. In contrast to other dryland studies (Grossiord et al., 2017a, b, 2018), we

found that warming had a stronger detrimental impact on plant physiological performance and growth than rainfall reduction, and this impact was primarily driven (rather than mitigated) by shifts in foliar elemental composition that increased non-stomatal constraints on photosynthesis. The large warming-induced reduction in leaf nutrient contents encountered in this study, and its negative impact on A and WUE_i, would be expected to offset any increases in WUE and productivity derived from atmospheric CO₂ elevation during coming decades (Norby, Warren, Iversen, Medlyn & McMurtrie, 2010; Morgan et al., 2011; Dijkstra et al., 2012), thereby augmenting vegetation vulnerability to drought stress in a warmer and drier climate. Increased anthropogenic nutrient deposition (especially N) could mitigate the negative impacts of warming on dryland plant performance to some extent, although sustained N deposition will eventually exacerbate nutrient imbalances by further aggravating P and micronutrient deficiency in the longer term (Peñuelas et al., 2012).

Data Availability

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.66j8343> (León-Sánchez et al., 2019).

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Author contribution

JIQ and FTM conceived and designed the experiment; LLS, EN, IP, PN and JIQ conducted all the field and laboratory work and collected the data; LLS, IP and JIQ analysed and interpreted the data; JIQ and LLS wrote the paper, with substantial contribution from all the co-authors.

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Figure legends

Figure 1. Mean leaf N, P and K concentrations (\pm SE) in *H. squamatum* (n= 33 in 2012, 26 in 2013, 32 in 2015), *H. syriacum* (n= 41, 40, 41), *G. struthium* (n= 22, 28, 30), *T. turredanum* (n= 27, 24, 24), *S. viscosa* (n= 32, 26, 30) and *C. hispanica* (n= 33 in 2012 only) in the control (C), rainfall reduction (RR), warming (W), and warming + rainfall reduction (W+RR) treatments. Average leaf N, P and K concentrations across species and years are also shown for each climate treatment.

Figure 2. Mean leaf Fe, Cu and Zn concentrations (\pm SE) in *H. squamatum* (n= 33 in 2012, 26 in 2013, 32 in 2015), *H. syriacum* (n= 41, 40, 41), *G. struthium* (n= 22, 28, 30), *T. turredanum* (n= 27, 24, 24), *S. viscosa* (n= 32, 26, 30) and *C. hispanica* (n= 33 in 2012 only) in the control (C), rainfall reduction (RR), warming (W), and warming + rainfall reduction (W+RR) treatments. Average leaf Fe, Cu and Zn concentrations across species and years are also shown for each climate treatment.

Figure 3. Mean (\pm SE) net photosynthetic rate (A), stomatal conductance (g_s), intrinsic water use efficiency (WUE_i) and quantum efficiency of photosystem II (Φ_{PSII}) between 2012 and 2015 in each climate treatment in *Helianthemum squamatum*, *Helianthemum syriacum*, and *Gypsophila struthium* (8-10 measurement dates per species). Average leaf gas exchange rates across species and measurement dates are also shown for each climate treatment. Climate treatments are denoted by colors as in Figures 1, 2: control (C, green), rainfall reduction (RR, blue), warming (W, orange), warming + rainfall reduction (W+RR, pink).

Figure 4. Fitted linear regressions between net photosynthesis rates (A) and stomatal conductance (g_s) across measurement dates in each climate treatment for *H. squamatum* (a), *H. syriacum* (b) and *G. struthium* (c). Climate treatments are denoted by colors: Control (green), Rainfall Reduction (RR, blue), Warming (W, orange), Warming + Rainfall Reduction (W+RR, pink). The A: g_s regression lines had consistently flatter slopes in the W and W+RR treatments than in the Control or RR treatments for all the three shrub species ($P= 0.001$).

Figure 5. Mean shoot dry biomass production (leaves plus stems) per unit shoot length (\pm SE) in *Helianthemum squamatum* (n= 32), *Helianthemum syriacum* (n= 41), *Gypsophila struthium* (n= 31), *Teucrium turredanum* (n= 24) and *Santolina viscosa* (n= 30) at the end of the study period (November 2015). Average shoot dry biomass across species is also shown for each climate treatment, with different letters above columns indicating significant differences ($P<0.05$) between them according to Tukey's post hoc tests. W: warming; RR: rainfall reduction; W+RR: warming plus rainfall reduction.

Figure 6. Relationship between average leaf nutrient status (average score along the first axis of a PCA conducted with leaf N, P, K, Fe, Cu and Zn concentrations) and mean photosynthetic rate (a) and mean shoot biomass (b) across all shrub species \times climate treatment combinations integrated over the duration of the experiment (2012-2015). Significant regression lines across (solid line; ** $P<0.01$) and within species (dashed lines; $P<0.05$) are shown. Shrub species are denoted by symbols: *Helianthemum squamatum* (circles), *Helianthemum syriacum* (triangles), *Gypsophila struthium* (downward triangles), *Santolina viscosa* (squares) and *Teucrium turredanum* (diamonds). Climate treatments are denoted by colors: Control (green), Rainfall Reduction (blue), Warming (orange), Warming + Rainfall Reduction (pink). Data are means \pm SE.

Figure 7. Mean plant survival rate (\pm SE) at the end of the study period (November 2015) in *Helianthemum squamatum* (n= 42), *Helianthemum syriacum* (n= 56), *Gypsophila struthium* (n= 31), *Teucrium turredanum*, (n= 26), *Santolina viscosa* (n= 35) and *Coris hispanica* (n= 41). Average plant survival rates across species in each climate treatment are also shown, with different letters above columns indicating significant differences ($P<0.05$) between them according to Tukey's post-hoc tests. W: warming; RR: rainfall reduction; W+RR: warming plus rainfall reduction. No surviving

Coris hispanica individuals were found in the W+RR treatment at the end of the study period.

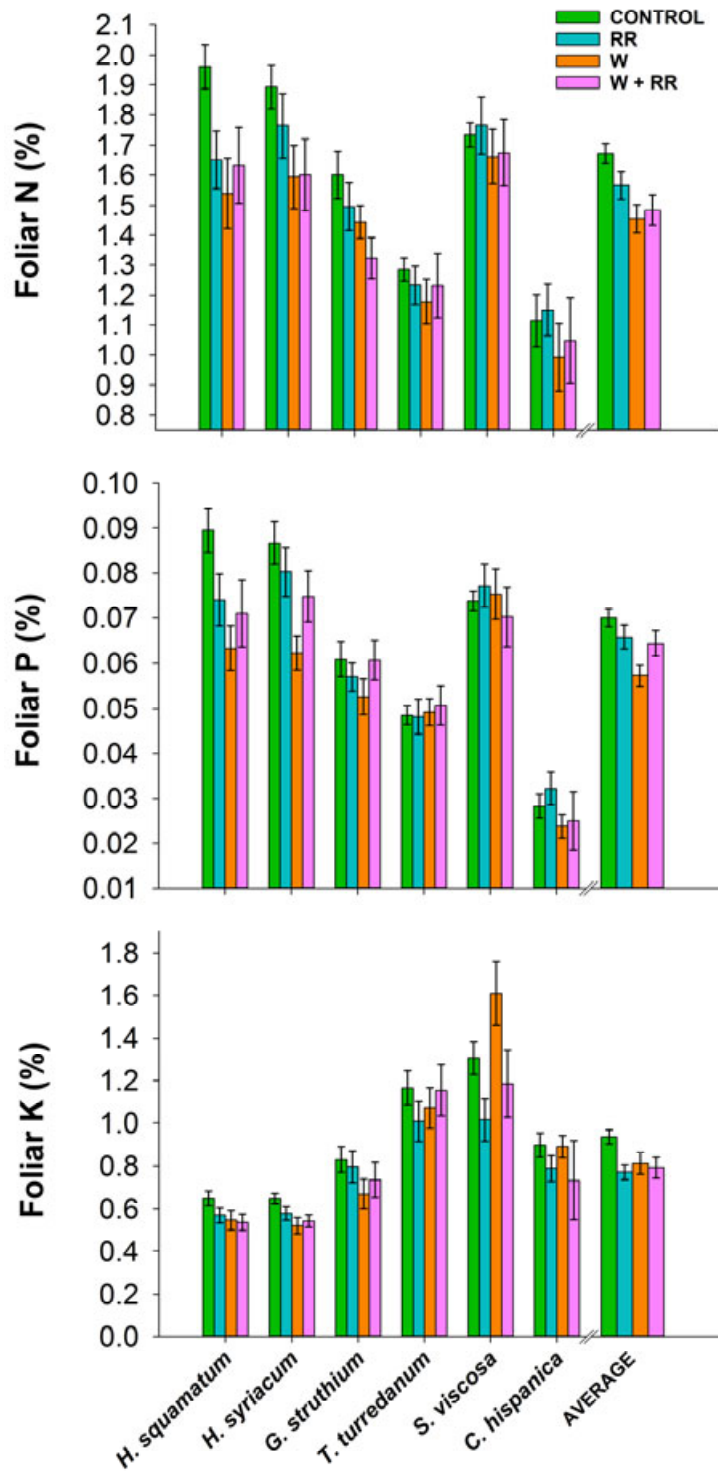


Figure 1.

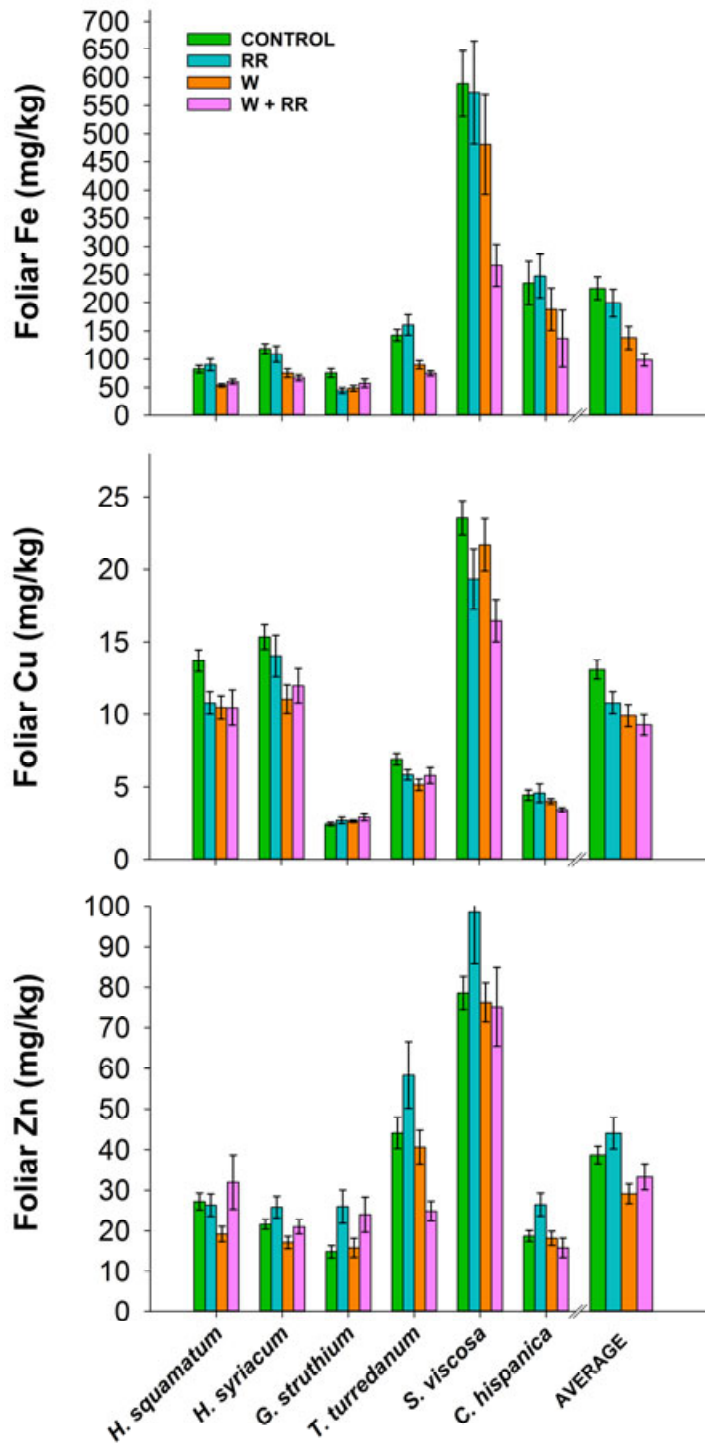


Figure2.

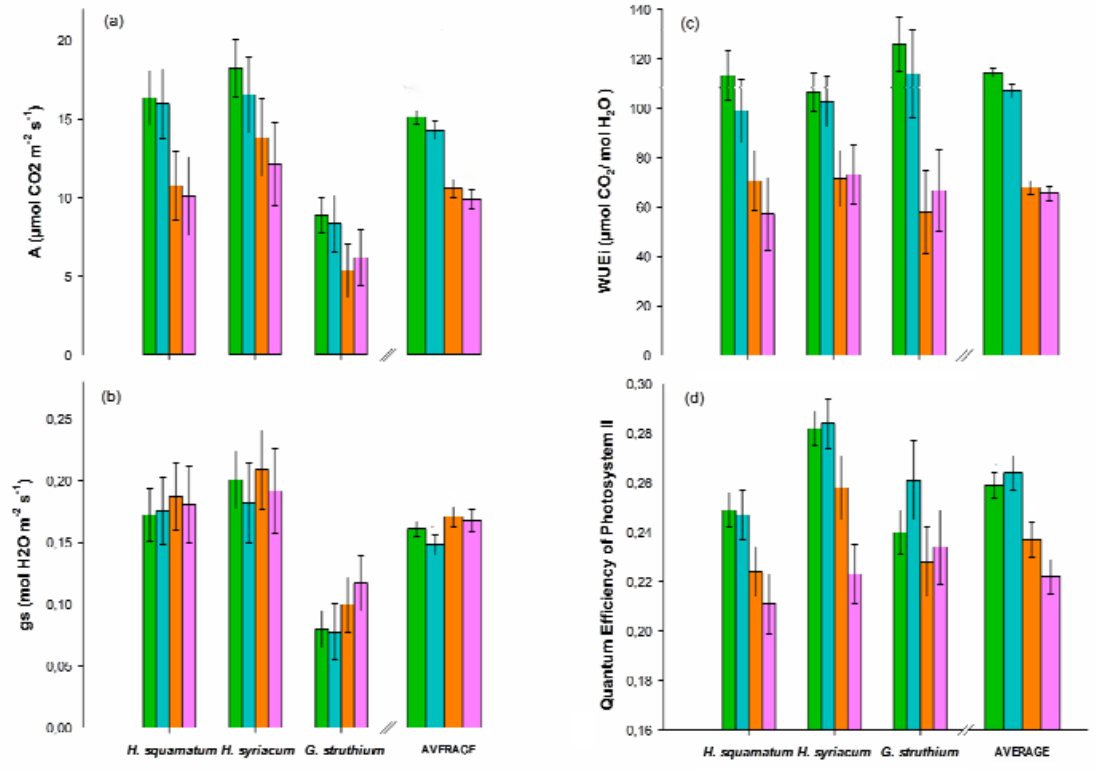


Figure 3.

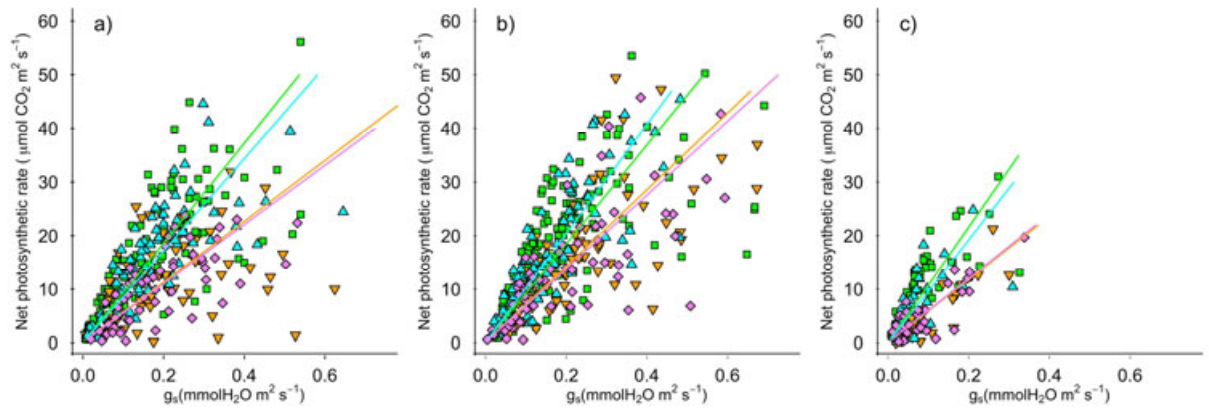


Figure 4.

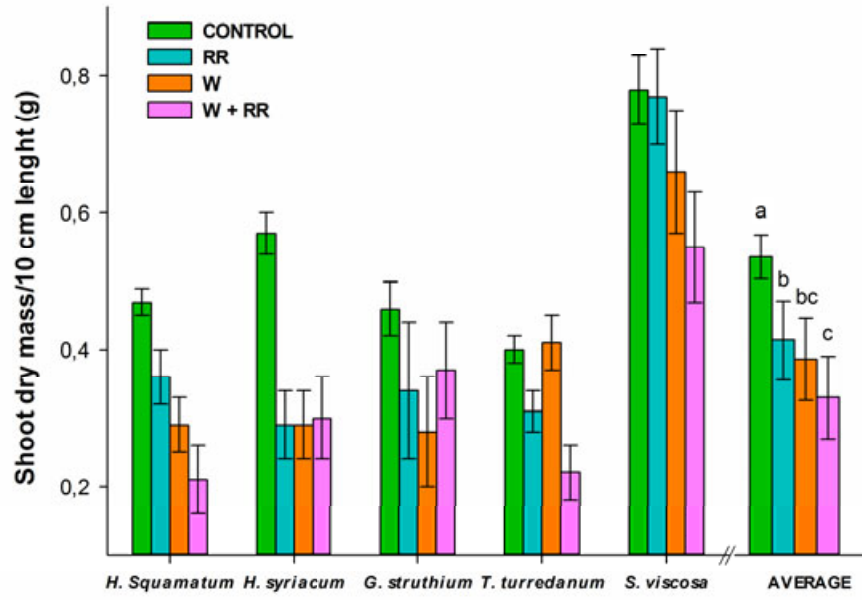


Figure 5.

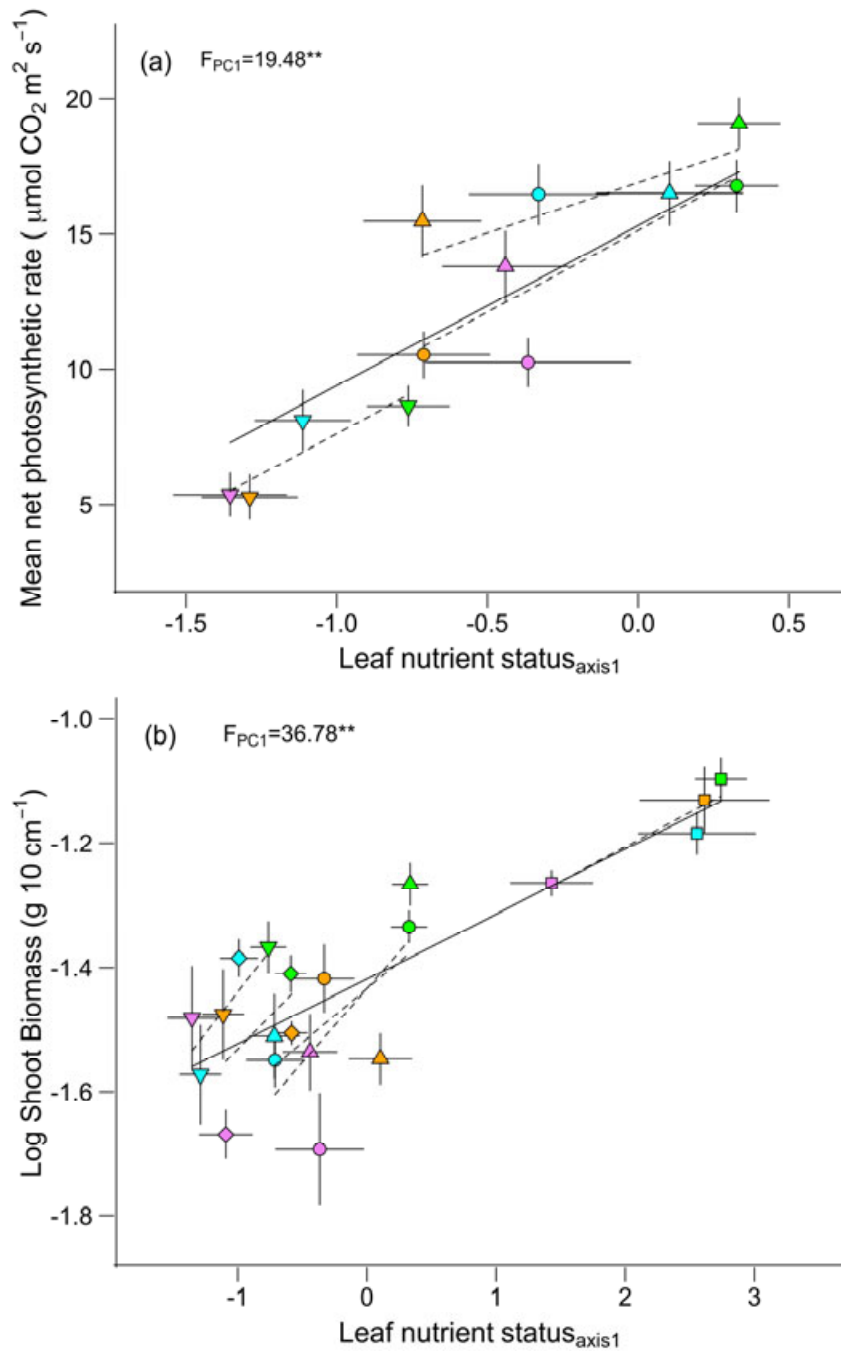


Figure 6.

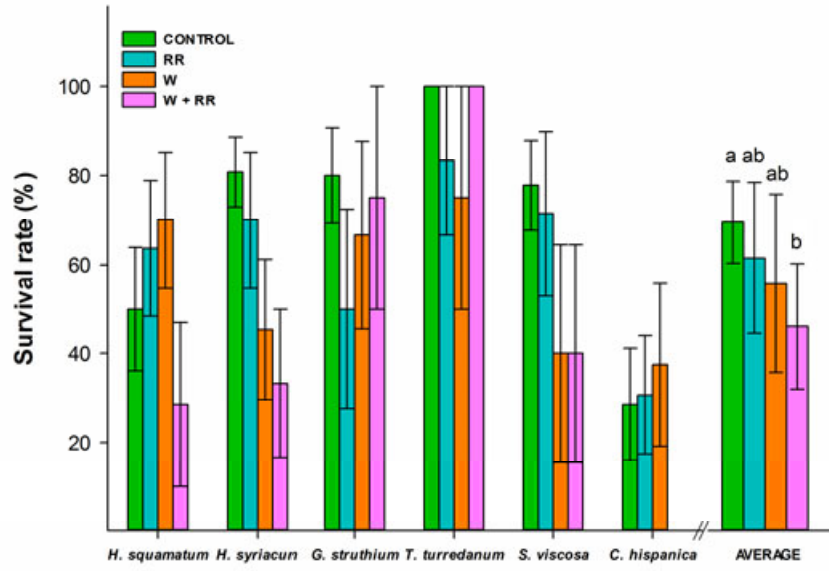


Figure 7.