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Warming reduces both photosynthetic nutrient use efficiency and water use efficiency in Mediterranean shrubsWarming reduces nutrient use efficiency

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ABSTRACT

The ratio between net photosynthetic rates and the foliar contents of essential plant macronutrients (N, P, K) is termed photosynthetic nutrient use efficiency (PNutUE). A universal trade-off exists whereby plants cannot maximize their PNutUE and their intrinsic water use efficiency (WUEi, carbon gain per unit water spent) simultaneously, because any increase in intercellular CO_2 concentration (c_i) through a greater stomatal opening would increase PNutE but also enhances transpiration and therefore decreases WUEi. Rising temperatures associated with climate change can result in large decreases in WUEi in semiarid shrubs through photosynthetic machinery impairment and enhanced stomatal conductance and transpiration, but we know remarkably little about the influence of warming and drought on PNutUE and its interplay with WUEi in dryland vegetation. Using a 6-year (2011–2017) manipulative field experiment, we examined the effects of warming (2.5ºC, W), rainfall reduction (30%, RR), and their combination (W+RR) on the photosynthetic use efficiency of three essential nutrients (PNUE, PPUE and PKUE) and on WUEi in three shrub species growing at two semiarid shrublands for the years 2015–2017. Across species, warming (W and W+RR) reduced PNUE by 42.9%, PPUE by 43.8% and PKUE by 41.5% on average relative to shrubs growing under ambient temperatures, whereas RR did not significantly affect their PNutUE. These drastic reductions in PNutUE with warming were mainly driven by non-stomatal and largely non-nutritional decreases in net photosynthetic rates, which were almost halved in warmed shrubs. The photosynthetic use efficiencies of N, P and K were inversely related to foliar $\delta^{13}C$, a proxy for time integrated WUEi, in both ambient (control and RR) and warmed (W and W+RR) shrubs, but with significantly smaller slopes and intercepts for warmed shrubs. Thus, plants achieve a smaller gain in PNutUE for any given increase in stomatal conductance (and reduction in WUEi) under warmer climatic conditions. The strong negative impact of warming on PNutE, along with the warming-induced shift in the trade-off between PNutUE and WUEi, could be indicative of an increasing inability of native plants to cope with warmer conditions, with dire implications for dryland vegetation productivity and survival under climate change.

1. Introduction

Leaf nutrients, and especially N, are determinants of photosynthetic capacity for plant C uptake. Given the large proportion of foliar N present in chloroplasts (more than 75%), most of it allocated into the photosynthetic machinery (Evans, 1989; Evans and Seemann, 1989), net photosynthetic rates generally increase linearly with leaf N concentrations within and across species (Field and Mooney, 1986; Kattge et al., 2009). The ratio between net photosynthetic rates and the amount of nutrients in the leaf is termed photosynthetic nutrient use efficiency (PNutUE), (Hikosaka, 2010). Dryland plant species tend to have higher leaf N concentration and higher photosynthetic N use efficiency than plants from wetter ecosystems, allowing them to maintain similarly high C uptake capacities, despite tighter stomatal constraints on photosynthesis in drylands (Prentice et al., 2014; Querejeta et al., 2022; Wright et al., 2003, 2001).

Anthropogenic greenhouse gas emissions in the past century have caused surface air temperatures to rise by 1.5 °C on average across the Mediterranean region, (Ali et al., 2022; Cramer et al., 2018). Climate warming will be accompanied by substantial reductions in the amount

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and frequency of precipitation across the region in the future (Collins et al., 2013; Giorgi and Lionello, 2008; Guiot and Cramer, 2016). These projected changes in temperature and precipitation regimes will increase aridity and reduce soil moisture availability for plants (Dai, 2013; Huang et al., 2016; León-Sánchez et al., 2018; Miranda et al., 2009), and are thus expected to have a strong negative impact on the ecophysiology and nutrient status of native Mediterranean vegetation (Guiot and Cramer, 2016; He et al., 2014; León-Sánchez et al., 2020, 2018; Llorens et al., 2003; Prieto and Querejeta, 2020; Querejeta et al., 2021; Sardans et al., 2008; Wu et al., 2011). However, recent studies have shown that enhanced soil nitrogen mineralization and availability to roots under warming could buffer the negative impacts of a warmer and drier climate on dryland vegetation (Grossiord et al., 2018) and could even enhance vegetation photosynthesis and productivity in wetter Mediterranean ecosystems (Liberati et al., 2021).

Besides nitrogen (N), other macronutrients like phosphorus (P) or potassium (K) are also critical components of plant nutrition and photosynthetic processes and, along with N, are considered the most limiting nutrients for plant productivity in terrestrial ecosystems (Du et al., 2020; Elser et al., 2007; Güsewell, 2004; Marschner, 2012; Sardans and Peñuelas, 2015; Vitousek et al., 1997). Foliar P is involved in the regeneration of the ribulose 1–5 biphosphate, a key enzyme involved in photosynthesis (Reich et al., 2009; Warren, 2011) and is also a key element in nucleic acid and phospholipid synthesis and in energy transfer (ATP/NADPH) in the plant cell (Aerts and Chapin, 1999), both necessary for photosynthetic processes. Leaf K is a key element regulating stomatal conductance that influences the amount of CO_2 entering the leaf (c_i) and being transferred to carboxylation sites (c_c) for photosynthesis (Sardans and Peñuelas, 2015; Tränkner et al., 2018). Further, K plays a key role in enhancing photosynthetic CO_2 fixation since it improves the Rubisco carboxylation activity and the transport of photosynthates into sink organs (Oosterhuis et al., 2013; Tränkner et al., 2018) and inhibits the transfer of photosynthetic electrons to O_2 reducing photorespiration (Cakmak, 2005). Similar to leaf N, leaf P and K concentrations are also affected by precipitation and temperature (Crous et al., 2022; Reich and Oleksyn, 2004) and are thus sensitive to future changes in these variables with climate change (León-Sánchez et al., 2020; Prieto and Querejeta, 2020; Sardans and Peñuelas, 2015). Thus, it is also important to understand the adjustment in photosynthetic use efficiencies of foliar P and K in addition to that of N if we aim at predicting plant physiological responses under future climate scenarios (Terrer et al., 2019).

Mediterranean plant productivity and physiology are not only strongly limited by nutrients but also by soil water availability (Hooper and Johnson, 1999; Noy-Meir, 1973; Ruiz-Navarro et al., 2016). One mechanism that plants use to cope with drought stress in Mediterranean ecosystems is increasing their leaf-level intrinsic water use efficiency (WUEi, e.g. the ratio between net photosynthetic rates and stomatal conductance, A/g_s), which may confer them a competitive advantage over other plants in the community (Bussotti et al., 2013; Ehleringer and Cooper, 1988; Maseyk et al., 2011). However, increased water use efficiency can only be achieved at the cost of decreased nutrient use efficiencies since WUEi and photosynthetic NUE are generally negatively correlated (Field, 1983; Gong et al., 2011; Patterson et al., 1997; Pons and Westbeek, 2004; van den Boogaard et al., 1995; Wright et al., 2003; Zhang and Cao, 2009). This trade-off arises because leaf intercellular CO_2 concentration (c_i) is typically not saturating for carbon assimilation and any increase in c_i through a greater stomatal opening increases the photosynthetic nutrient use efficiency but also enhances transpiration and therefore decreases water use efficiency. Thus, plants cannot maximize PNutUE and WUEi simultaneously (Cai et al., 2007; Gong et al., 2011; Patterson et al., 1997; Poorter and Evans, 1998; Santiago et al., 2004). However, under future scenarios of increasing heat and drought stress, dryland plants could respond by maximizing their WUEi to cope with higher VPD and lower soil moisture conditions, induced by predicted reductions in precipitation along with warming-induced soil desiccation. Alternatively, dryland plants could respond maximizing their PNutUE to compensate for the lower nutrient availability and uptake in drier soil (Cramer et al., 2009; He et al., 2014; Matimati et al., 2014; Querejeta et al., 2021; Salazar-Tortosa et al., 2018; Wright et al., 2001). This may alter the relationship between WUEi and PNutUE but to our knowledge, this has not been investigated yet, even though understanding how this trade-off will be affected under a climate change scenario is necessary to predict the responses of plant communities to future climate warming and drying in dryland ecosystems.

Given the positive relationship between leaf N concentration, specific leaf area (the inverse of leaf mass area: 1/LMA) and photosynthetic rates globally (Wright et al., 2004), photosynthetic nutrient use efficiency also declines as leaf mass per area (LMA) increases (i.e. SLA decreases, Poorter and Evans, 1998; He et al., 2009; Hikosaka and Shigeno, 2009). Species with low LMA generally have thinner leaves (e.g. lower leaf thickness) and lower leaf lifespan (Wright et al., 2004), which they compensate with a more efficient photosynthetic use of nutrients. Globally, the LMA of plant species is negatively correlated with mean annual temperatures and precipitation (Poorter et al., 2009). However, in Mediterranean shrubland ecosystems, *Helianthemum squamatum* shrubs subjected to warmer temperatures had lower LMA than shrubs under ambient temperatures (León-Sánchez et al., 2018) and decreases in LMA with drought have been reported for other Mediterranean species (Montserrat-Martí et al., 2009; Ogaya and Peñuelas, 2006). These trade-offs between LMA and photosynthetic nutrient use efficiency have been widely observed across species (He et al., 2009; Onoda et al., 2017; Santiago et al., 2004). However, it is not known whether the observed plasticity in leaf physiology, morphology and economics under long-term warmer and/or drier conditions in Mediterranean species (León-Sánchez et al., 2020, 2018) will also affect their photosynthetic nutrient use efficiency, and how.

We conducted a manipulative field experiment in two semi-arid shrublands (located in central and south-eastern Spain, ~500 km apart) to evaluate the impacts of climate warming and drying on the photosynthetic use of nutrients in three phylogenetically and functionally diverse native shrub species (Table S1). We focused on physiological and morphological traits that directly affect leaf C assimilation and water loss, their relationship with foliar macronutrient concentrations (N, P and K) and their use in photosynthetic processes. Since the relationships between leaf nutrients and photosynthesis are often species-specific (Hikosaka, 2010; Pons and Westbeek, 2004; Poorter and Evans, 1998; Ripullone et al., 2003; Takashima et al., 2004) we aimed to evaluate the concurrent responses of several coexisting plant species at two sites to assess the potential impacts of climate change on the PNutUE of Mediterranean plant communities more comprehensively. A comprehensive assessment of plant performance and nutrient status in a diverse array of species can help elucidate the physiological mechanisms underlying vegetation responses to projected climate change conditions in Mediterranean drylands (León-Sánchez et al., 2020; Salazar-Tortosa et al., 2018).

The present study builds upon the results of previously published studies conducted at the same sites using the same experimental setup and shrub species (León-Sánchez et al., 2016, 2018, 2020). In those earlier studies, we found that experimental warming (+2.5ºC) enhanced stomatal conductance by 5–33%, reduced leaf N and P concentrations by 5.2–13%, reduced photosynthetic rates by 29–36% and reduced leaf-level-water use efficiency by 38–55.2% in semiarid Mediterranean shrubs, overall leading to 28–39% reductions in aboveground biomass production across species and sites. We attributed these large decreases in photosynthesis, WUEi and growth in warmed shrubs to both long- and short-term mechanisms, including changes in leaf nutritional status and also to non-nutritional effects, e.g., an impairment of the Rubisco activity and regeneration, decreased mesophyll conductance to CO_2 and/or higher photorespiration with warming (Flexas and Medrano, 2002; Voss et al., 2013). In contrast to the strong negative impacts of experimental warming, plants under rainfall reduction showed more moderate decreases (*c.* 10–22%) in foliar nutrients, as well as only moderate reductions in photosynthesis, stomatal conductance and shoot growth, but with small or no changes in WUEi (León-Sánchez et al., 2018, 2020). However, the potential impacts of warming and rainfall reduction on the PNutUE of semiarid shrubs were not addressed in those earlier studies, so in the present study we specifically aimed to investigate the impacts of warming, rainfall reduction and their combination on PNutUE by using the same experimental setup, sites and shrub species used in León-Sánchez et al., (2018, 2020).

Reductions in foliar nutrient concentrations under warming could have major impacts on leaf nutrient-photosynthesis relationships depending on whether the warmed plants can increase their stomatal conductance and/or decrease their LMA to compensate for the lower nutrient uptake by maintaining or increasing their photosynthetic nutrient use efficiency (Onoda et al., 2017). Thus, climate-change induced variations in PNutUE would be expected to occur via effects on photosynthetic rates (A), stomatal conductance (g_s), leaf morphology (LMA), leaf nutrient concentrations (N, P and K) and their interplay (León-Sánchez et al., 2020, 2018). Given the large reductions in WUEi found in semiarid shrubs with warming in previous years (León-Sánchez et al., 2020, 2018, 2016) we hypothesize that 1) PNutUE would be maintained or increased in experimentally warmed plants through enhanced stomatal conductance and/or decreased LMA (Cai et al., 2007; Gong et al., 2011; Santiago et al., 2004; Wright et al., 2003); and 2) the expected adaptive trade-off between WUEi and PNutUE will be strengthened by experimental warming.

2. Material and methods

2.1. Study sites and experimental design

The study was carried out near Aranjuez, in central Spain (Madrid, 40°02′N–3°32′W, 495 m.a.s.l.) and near Sorbas (Almería, south-eastern Spain, 37°05′32.4″N, 2°05′41.5″W, 409 m.a.s.l.), both with a Mediterranean climate, during the spring of years 2015, 2016 and 2017. The study area in Aranjuez has a mean annual temperature of 15 °C and an average rainfall of 358 mm (for the period 1977–2016), concentrated mainly in the autumn and spring months (Lafuente et al., 2018). Mean annual precipitation in Aranjuez was 427.7, 325.1 and 309.8 mm for the hydrological years (October to September) corresponding to 2015, 2016 and 2017, respectively (Agencia Española de Meteorología, AEMET). The study area in Sorbas has a mean annual precipitation of 275 mm and a mean annual temperature of 17 °C (Maestre et al., 2013). Mean annual precipitation in the nearest weather station (~30 linear km, Albox, AEMET) was 177.5, 140.2 and 338.6 mm for the 2015, 2016 and 2017 hydrological years, respectively. Soils in both Aranjuez and Sorbas derive from gypsum, have pH values ca. 7 and are classified as Gypsiric Leptosols (IUSS Working Group WRB, 2006). Soils are shallow (4–10 cm deep overlying weathered gypsum bedrock) and show a thin organic topsoil horizon (1–2 cm thick). Native vegetation at both sites is a mixed grassland and shrubland community dominated by the perennial tussock grass *Stipa tenacissima* (L.) Kunth. In Aranjuez, the community is co-dominated by the gypsophilous shrub *Helianthemum squamatum* (L.) Dum. Cours. In Sorbas, the community is co-dominated by several gypsophilous shrub species such as *Helianthemum squamatum* L. Pers., *Helianthemum syriacum* (Jacq.) Dum. Cours. and *Gypsophila struthium* L. subsp *hispanica* (Wilk.) G. López, among others (León-Sánchez et al., 2020). For this study, we selected *Helianthemum squamatum* in Aranjuez and *Helianthemum squamatum, Helianthemum syriacum* (Jacq.) Dum. Cours. and *Gypsophila struthium* L. subsp *hispanica* (Wilk.) in Sorbas. These target species belong to two distant plant families and encompass a range of plant sizes, resource use strategies, mycorrhizal association types and phenology (Table S1).

A manipulative field experiment was set up in February 2011 in Aranjuez and April/May 2011 in Sorbas to examine the effects of temperature and rainfall manipulation according to predictions for the second half of the twenty-first century in the Mediterranean area (Giorgi and Lionello, 2008). Measurements for this study were taken during the spring of experimental years 4–6 (2015–2017) after experiment setup. Manipulated climate treatments were: warming (W; ~2–2.5ºC increase in mean annual temperature), rainfall reduction (RR; interception and exclusion of 30% of the incoming precipitation) and the combination of both factors (W+RR). The warming treatment simulates the predictions derived from six Atmosphere General Circulation Models for the second half of the twenty-first century (2040–2070) in the Western Mediterranean region (de Castro et al., 2005) and was achieved by installing open-top chambers (OTCs), which increase mean air and surface soil temperature through a greenhouse effect (Fig. S1). Open-top chambers were of hexagonal shape and made of transparent methacrylate panels with very high transmittance of both visible and ultraviolet wavelengths (92%) and pass on ca. 85% of incoming energy (information provided by the manufacturer; Decorplax S.L., Humanes, Spain). OTCs were suspended ~3 cm above the ground by a metal frame to allow free air circulation and exchange with the surrounding environment, minimizing undesirable experimental artifacts (Hollister and Webber, 2000; Maestre et al., 2015). Upon installation in the field, the vertical height of the suspended OTCs was 40 cm. These OTCs have been used in previous warming experiments in the field (León-Sánchez et al., 2020, 2018, 2016; Maestre et al., 2013; Prieto et al., 2019; Prieto and Querejeta, 2020, see Fig. S2 for experimental layout). In Sorbas, OTCs increased air temperature by ~2 °C in the W and W + RR plots, relative to control (ambient) conditions. Increments in temperature within the OTCs were larger during warm (up to 4–5 °C during some hot days in summer) than during cold periods (~1 °C in winter; León-Sánchez et al., 2020). The temperature of surface soil (0–5 cm) was also elevated in the warmed plots by 2.2 °C on average (León-Sánchez et al., 2020). In Aranjuez, the warming treatment (W and W+RR) increased mean air temperature within the OTCs by ~2.5 °C relative to control (ambient). Increments in temperature were greatest during summer when midday temperatures inside the OTCs were increased by up to 6–7 °C on the hottest days. The warming treatment also increased surface soil temperature by ~2.5 °C on average. Vapor pressure deficit was on average 14–20% higher in warmed plots than in ambient plots (798 Pa in W and W + RR plots vs. 703 Pa in control and RR plots in Sorbas and 1311 in W and W + RR plots vs. 1042 Pa in control and RR plots in Aranjuez). Warming also reduced the moisture content of the topsoil layer (0–5 cm depth) by ~14% relative to control plots at both sites (León-Sánchez et al., 2020, 2018).

To simulate projected reductions in precipitation, we used passive rainout shelters that intercept and exclude ~30% of the incoming rainfall from the plots. The permanent (non-moveable) rain exclusion shelters are made of transparent methacrylate troughs (same material as for the OTCs) covering ~30% of the area of experimental plots. Troughs were suspended over an aluminium frame above the experimental plots (height 130 cm, width 100 ×100 cm, Fig. S1) with a 20º inclination so that intercepted rainwater is diverted into tanks outside experimental plots and removed after each rainfall event. The RR treatment reduced mean annual topsoil (0–5 cm) water content by ~14% relative to control plots in Aranjuez and Sorbas, but did not affect air or soil temperatures (León-Sánchez et al., 2020, 2018). Finally, the combined W+RR treatment is achieved by installing both OTCs and rainfall exclusion shelters over the same experimental plot (Fig. S1).

In Aranjuez, the experiment includes 10 replicate plots per each climate manipulation treatment (warming: W, rainfall reduction: RR and their combination: W+RR) plus 30 control plots interspersed among the climate change treatments, making a total of 60 experimental ~1 m^2 plots (Fig. S2, León-Sánchez et al., 2018). In Sorbas, the experiment included 12 warming plots (W), 10 plots with rainout shelters (RR), 10 plots combining OTCs with rainout shelters (W+RR) and 32 interspersed control plots (64 plots in total; Fig. S2, León-Sánchez et al., 2020). Experimental plots (Control, OTC, RR and W+RR) were all at least 2 m distant from each other at both sites. Each study year, between 40 and

46 individuals of *H. squamatum* were measured for leaf gas exchange, leaf nutrient concentration and isotopic composition, PNutUE, leaf morphology in Aranjuez, along with 27–29 individuals of *H. squamatum*, 34–43 of *H. syriacum* and 16–21 individuals of *G. struthium* in Sorbas.

2.2. Plant physiology, morphology and nutrients

Net photosynthetic rate per area (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^2 s^{-1}), transpiration rate (E, mmol H_2O m^2 s^{-1}) and the ratio between internal and ambient CO_2 (c_i/c_a) were measured in one plant per plot in spring (early May) for *H. squamatum* in Aranjuez and for *H. squamatum* and *H. syriacum* in Sorbas and in early summer (July) for *G. struthium* in Sorbas. Leaf gas exchange was measured with a portable photosynthesis system (LI-6400, LICOR, Inc., Lincoln, NE, USA) equipped with a LI-6400–40 leaf chamber fluorometer and a LICOR 6400–01 CO_2 injector on mature, fully sun-exposed leaves. The CO_2 concentration in the cuvette (2 cm^2) was maintained at 400 μmol mol^{-1} CO_2 and measurements were performed at a saturating light intensity of 1500 μmol m^2 s^{-1} and at ambient air temperature and relative humidity. The airflow was set to 250 μmol s^{-1}. The leaves used to determine gas exchange were collected and a digital image taken with a Sony α200 DSRL camera (Sony Corp, Tokyo, Japan) to determine their area using an image software (Photoshop, Adobe Systems, San José, California, USA). Leaf gas exchange measurements were then corrected by area. We calculated the intrinsic water use efficiency as the ratio between net photosynthetic rate and stomatal conductance ($WUE_i = A/g_s$, μmol CO_2 mol H_2O^{-1}) and instantaneous water use efficiency as the ratio between net photosynthetic rate and transpiration ($WUE_t = A/E$, μmol CO_2 mmol H_2O^{-1}). For the W and W+RR treatments, all leaf gas exchange measurements were conducted under the conditions of temperature and humidity prevailing in the OTCs, rather than under controlled conditions, as customarily done in climate manipulation field studies (Gunderson et al., 2000; Reich et al., 2018; Sáez et al., 2018; Wertin et al., 2017). Given that mean air temperatures in the W and W+RR treatments averaged 2.2–2.5ºC higher than air temperatures in the ambient temperature treatment, mean leaf temperatures during measurements were correspondingly higher for leaves of shrubs in the warm OTCs (Gunderson et al., 2000). As a result, the leaf gas exchange rates of plants in the W and W+RR treatments reflected the warmer conditions, higher VPD and lower water availability prevailing in these treatments relative to the Control, as done in the previous studies conducted at the same sites (León-Sánchez et al., 2018, 2020; Querejeta et al., 2021).

Following field measurements, leaf morphology, the leaf C isotopic composition ($\delta^{13}C$) and leaf elemental composition were determined on fully mature, sun-exposed intact leaves in the lab. To characterize morphology, four leaves from the same plant where gas exchange was measured were selected to measure their leaf area (LA, cm^2) and leaf mass (mg) to calculate their leaf mass per area (LMA, mg cm^{-2}). Leaves were rehydrated under dark conditions for 24 h and weighed (saturated mass, SM; g). After weighing, a picture of the leaves was taken with a Sony α200 DSRL camera (Sony Corp, Tokyo, Japan) and the resulting image was processed using Photoshop (Adobe Systems, San José, California, USA) to determine LA (cm^2). Leaves were then oven-dried at 60 °C for 48 h and weighed again to obtain their dry mass (DM; g). Leaf mass area was calculated as LMA= (DM/LA). Leaf thickness (LT) was calculated as LT= $((1/LMA) \times LDMC)^{-1}$ following Vile et al. (2005). For Leaf $\delta^{13}C$ and leaf nutrients, leaf samples were oven-dried at 60ºC for 72 h and finely ground with a ball mill. Leaf P (mg g^{-1}) and K (mg g^{-1}) concentrations were measured using inductively coupled plasma optical emission spectrometry (ICP- OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with HNO_2: H_2O_2 (4:1, v-v) at the Ionomics facility at CEBAS-CSIC. For leaf $\delta^{13}C$ (‰) and leaf carbon and nitrogen concentrations (mg g^{-1}), samples were weighted and encapsulated into tin capsules and analyzed using an elemental analyser coupled to an isotope ratio mass spectrometer (EA/GA- IRMS, Carlo Erba, Elementar, Finnigan, Isoprime) at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley (USA). Leaf $\delta^{13}C$ is expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V- PDB).

We calculated the photosynthetic use efficiency of nitrogen, phosphorus and potassium (PNUE, μmol CO_2 mol N^{-1} s^{-1}; PPUE, μmol CO_2 mol P^{-1} s^{-1}; PKUE, μmol CO_2 mol K^{-1} s^{-1}) as follows:

$$PNutUE = A \times \frac{1}{LMA \times 10} \times \frac{Nut_{atomicmass}}{Nut_{conc}};$$

Where A is the net photosynthetic rate per area (A, μmol CO_2 m^{-2} s^{-1}), LMA is the leaf mass per area (mg cm^{-2}), $Nut_{content}$ is the concentration of the nutrient in the leaves (mg g^{-1}) and $Nut_{atomic\ mass}$ is the atomic mass of the nutrient (14.61 g mol^{-1} for N, 30.974 g mol^{-1} for P and 39.1 g mol^{-1} for K).

2.3. Statistical analyses

Linear mixed models were used to analyse differences in leaf gas exchange parameters (A, g_s, WUE_i, c_i/c_a, $\Delta c_i/c_a$ E and WUE_t), leaf nutrient concentrations (N, P, K), leaf morphology (LMA, LT and LA) and photosynthetic nutrient use efficiencies (PNUE, PPUE and PKUE). First, we built the models with warming (W), rainfall reduction (RR), Site, Year and their interactions included as fixed factors and *plot* and *species* included as random factors to account for repeated measurements taken within the same plot over time and across species variation in analysed variables. Since we did not detect any effects of site or any interactions including site effects ($P > 0.50$) for any of the variables we built the models with the same fixed structure and including *plot* and the combination between *site x species* as random factors. Air temperature inside the LICOR chamber at the time of measurement was included as a covariable in models analysing leaf gas exchange parameters (A, g_s, WUE_i, c_i/c_a, $\Delta c_i/c_a$ E and WUE_t) and photosynthetic nutrient use efficiency (PNUE, PPUE and PKUE) to account for both natural and experimental variations in temperatures at the time of measurement within and across treatments. Models for PPUE and PKUE were re-run including leaf N concentrations as an additional covariate to rule out any potential confounding effect of N on PPUE or PKUE, due to positive correlations between leaf N and P or K. PNUE, PPUE and PKUE and leaf K were squared-root transformed, whereas leaf N and P, leaf thickness and leaf area were log-transformed to comply with normality and homogeneity of variance assumptions in the models.

We used standardized major axis regressions (SMA) to test for differences in the slope of the relationship between time-integrated water use efficiency ($\delta^{13}C$), leaf mass area (LMA) and PNutUE under warming and ambient temperature; i.e. difference in slopes between plants within plots with ambient temperatures (Control and RR) and within warmed plots (W and W+RR). We also applied SMA to test for differences in the slope of the relationships between leaf nutrient concentrations (N, P and K) and net photosynthetic rates (A) with the slope test in the *smatr* package (Warton et al., 2012). These tests were carried out using the whole dataset (i.e., across sites and species) and within species.

To investigate the effects of climate change on photosynthetic nutrient use efficiency (PNUE, PPUE and PKUE) and the existence of nutritional or non-nutritional limitations to photosynthesis and PNutUE in these semiarid ecosystems, we built an a-priori set of hypothesis and relationships among variables and analysed them using structural equation modelling (SEM). Based on this previous knowledge, we proposed an a priori model of hypothesized relationships within a path diagram (Fig. S3), allowing a causal interpretation of the model outputs (Grace, 2007). This approach allows evaluating the effects of climate change (W and RR) on nutrient use efficiency (PNUE, PPUE and PKUE). We assessed the goodness of fit of the SEM models using the traditional $\chi 2$ goodness-of-fit test, but because of its sensitivity to sample size, the Bentler comparative fit index (CFI) and the Tucker-Lewis index (TLI) and Root Mean Square Error of Approximation (RMSEA) were also considered (Grace, 2007). For SEM analyses, contrary to other statistical analyses, p-values higher than 0.05 in the $\chi 2$ and RMSEA indices, respectively (Schermelleh-Engel et al., 2003), and values greater than 0.90 for CFI and TLI indices are required to guarantee an acceptable fit (Hu and Bentler, 1999).

Calculations and statistical analyses were performed with the R software (v 4.0.4, R Core Team 2022) using the packages lme4 (Bates et al., 2015) and nlme (Pinheiro et al., 2014), effects (Fox et al., 2014), Hmisc (Harell Jr, 2015) and lavaan (Rosseel, 2012). Data shown throughout the text are mean ± standard error (SE).

3. Results

3.1. Impact of warming and rainfall reduction on leaf gas exchange, leaf nutrients and leaf morphology

In the present study (2015–2017), experimental warming decreased net photosynthetic rate (A) drastically in W and W+RR plots compared to Control plots (45.5% reduction on average; Table S2 and Fig. S4a) across sites and species. Warming moderately enhanced stomatal conductance (g_s) with a 16.9% increase in W and W+RR plots on average compared to controls across species and sites (Fig. S4b). Transpiration (E) followed a similar pattern with an average increase of 32.0% with warming across species and sites (W and W+RR; Fig. S4c). Overall, this resulted in considerable reductions in both intrinsic ($WUE_i = A/g_s$) and instantaneous ($WUE_t = A/E$) water use efficiency with warming across species and sites (on average 58.1% and 58.4% lower WUE_i and WUE_t, respectively in warmed plots compared to controls, Fig. S4d,e). The ratio between internal and ambient CO_2 in the leaves (c_i/c_a) increased with warming by 63.5% on average compared to controls across species and sites (Fig. S4f). There was no consistent effect of the rainfall reduction factor on any of the leaf gas exchange parameters evaluated across sites and species ($p > 0.40$, Table S2). The effect of warming on leaf $\delta^{13}C$ depended on the year (significant *warming* x *year* interaction, Table S4) with leaf $\delta^{13}C$ being marginally lower in warmed (W and W+RR) than in non-warmed (Control and RR) plots in 2016 across sites and species (F= 3.381, $p = 0.07$), but with no significant effects on the other years. Rainfall reduction had a moderate effect on leaf $\delta^{13}C$ (F = 4.779, $p = 0.03$, Table S4) that was slightly lower on average in treatments with rainfall reduction than in ambient rainfall treatments ($\delta^{13}C$, −29.66‰ ± 0.17 in RR and W+RR vs. −29.22‰ ± 0.13 in control and W plots).

Leaf N concentrations were marginally lower (4.9% reduction) in warmed plots relative to controls across sites and species, but, in contrast to earlier years, we did not find any significant reductions in leaf P or K concentrations under warming or rainfall reduction (Tables S3 and S4, F = 0.005, $p = 0.942$ for leaf P and F = 0.067, $p = 0.796$ for leaf K). Moreover, foliar P concentrations increased on average by 12.2% under rainfall reduction (RR and W+RR) relative to ambient rainfall (Tables S3 and S4, F = 4.797, $p = 0.030$) across sites and species. Across sites, species and years, foliar N, P and K concentrations were positively correlated with net photosynthetic rates on a mass basis (R^2 =0.32, R^2 =0.24 and R^2 =0.11 for leaf N, P and K, respectively, $p < 0.001$; Fig. S5a,b,c), as well as on an area basis (R^2 =0.75, R^2 =0.72 and R^2 =0.68 for leaf N, P and K, respectively, $p < 0.001$; Fig. S5d,e,f). Leaf mass per area (LMA) was not significantly affected by either warming or rainfall reduction, but leaves were on average 11.8% smaller in size (leaf area) and 6.1% thinner in warmed plots (W and W+RR) compared to controls (F = 4.000, $p = 0.040$ for leaf area and F = 5.823, $p = 0.016$ for leaf thickness, Tables S3 and S4,) across sites, years and species.

3.2. Impact of warming and rainfall reduction on plant nutrient use efficiency

Experimental warming consistently decreased the PNutUE of all the shrub species, with roughly similar reductions in the W and W + RR treatments for all three nutrients (Fig. 1a,b,c, Table 1; $p < 0.001$). Across sites and species, warming (W and W+RR) reduced PNUE by 42.9% on average compared to controls (Fig. 1a) across years, sites and species. Warming (W and W+RR) also reduced PPUE and PKUE on average by 43.8% and 41.5%, respectively (Fig. 1b,c) across years, sites and species. Although there was some year-to-year variation in PNUE, PPUE and PKUE values, which were slightly higher in 2017 than in other years (Table 1), none of the interactions in these models were significant, indicating very consistent effects of warming across species, sites and years (Table 1). When we included the effect of leaf N as a covariable to account for the potential covariation of nutrients within leaves,

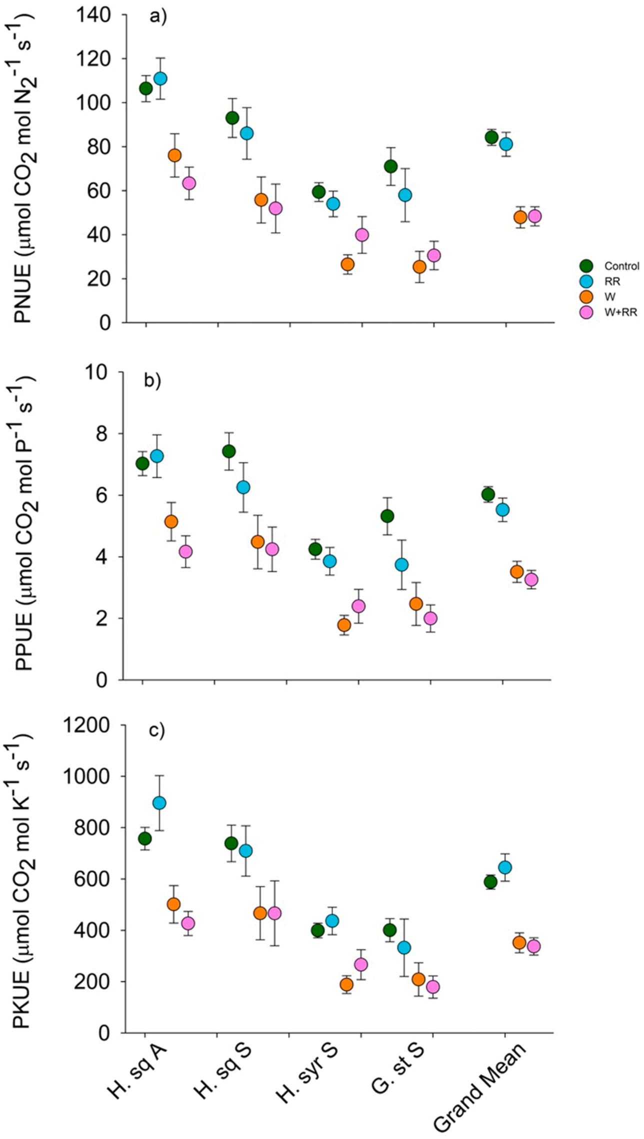

Fig. 1. Photosynthetic nutrient use efficiency of leaf N, P and K (PNUE, PPUE and PKUE) in *Helianthemum squamatum* in Aranjuez (H. sq A) and *Helianthemum squamatum* (H. sq S), *Helianthemum syriacum* (H. syr S) and *Gypsophila struthium* (G. st S) in Sorbas measured in spring. Values for each species are mean (± SE) of three consecutive growing seasons (2015–2017) and across years and species (Grand mean). Experimental treatments are ambient conditions (Control), warming (W), rainfall reduction (RR) and warming and rainfall reduction (W+RR). See Table S2 for statistical results.

leaf N was not a significant covariable either for PPUE ($p = 0.97$) or for PKUE ($p = 0.90$), indicating that warming effects on PPUE or PKUE were independent of the N concentration in the leaves. In contrast to the strong effects of warming, there was no consistent effect of rainfall reduction on plant photosynthetic nutrient use efficiency across years, sites and species (Table 1).

PNutUE was positively related to both net photosynthetic rates (A) and stomatal conductance (g_s) across sites, species and years ($R^2 = 0.82$, $R^2 = 0.70$ and $R^2 = 0.65$, $p < 0.001$ for A; Fig. 2a,c,e and $R^2 = 0.34$, $R^2 = 0.29$ and $R^2 = 0.27$, $p < 0.001$ for g_s; Fig. 2b,d,f). These relationships were also consistent within each species separately (R^2 between 0.12 and 0.48 for PNUE, $R^2 = 0.07–0.40$ for PPUE and $R^2 = 0.05–0.42$ for PKUE; $p < 0.05$). Moreover, these relationships held both within non-warmed and warmed plots separately (Fig. 2). The slopes for PNutUE vs. A were not significantly different between warmed and non-warmed shrubs (slope tests, LR=2.36, LR=2.18 and LR= 0.45, $p > 0.08$ for PNUE, PPUE and PKUE, respectively, Fig. 2a,c,e). In contrast, the slopes of the PNutUE vs g_s relationships were significantly different between warmed and non-warmed shrubs (LR=135.4, LR=136.6 and LR= 120.1, all $p < 0.001$ for PNUE, PPUE and PKUE, respectively, Fig. 2b,d,f).

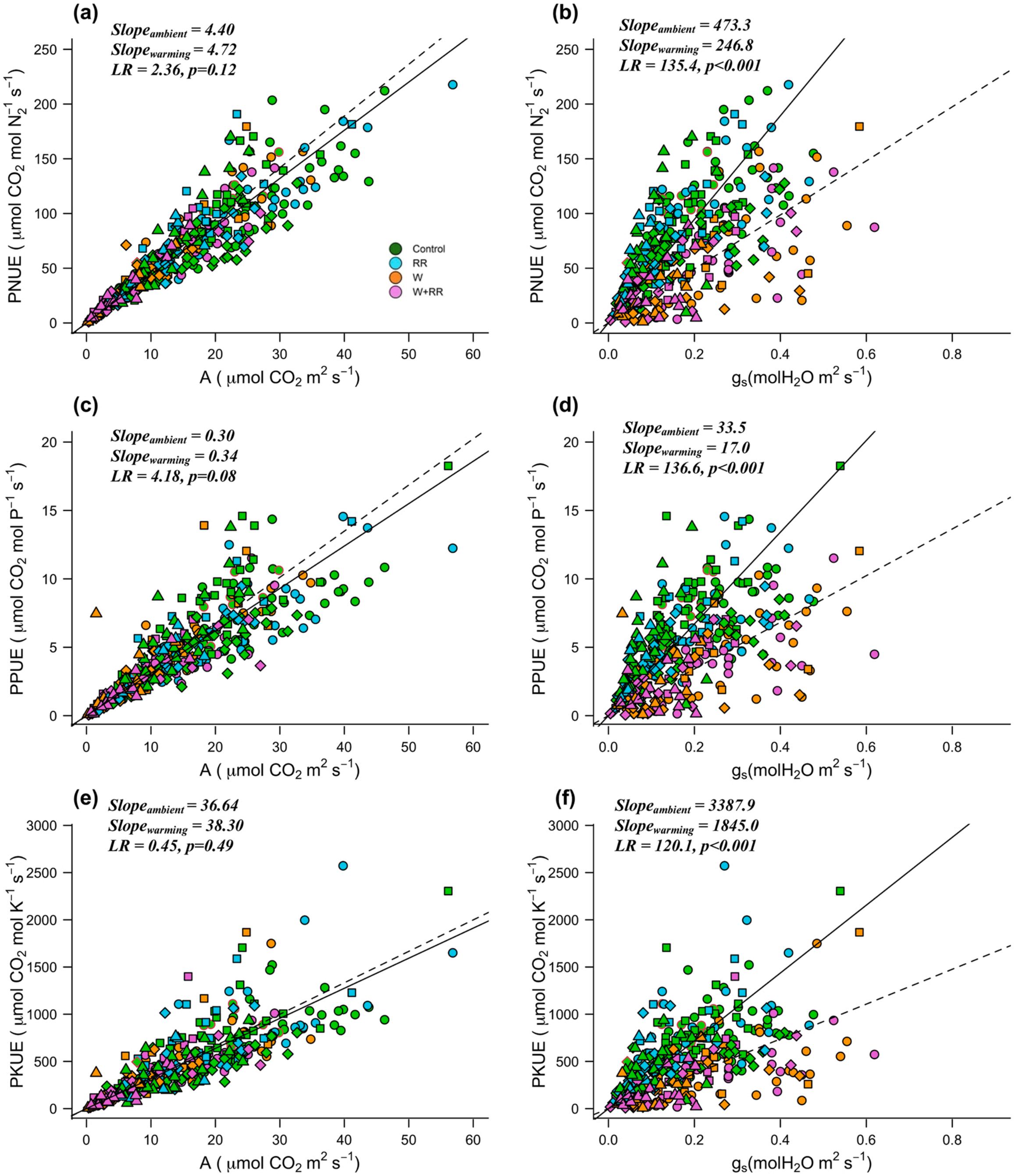

Fig. 2. Relationships between photosynthetic nutrient use efficiency (PNUE, PPUE and PKUE) and net photosynthetic rates (A) and between nutrient use efficiency and stomatal conductance (g_s) across sites and species. Regression lines are drawn for warmed (dashed line, W and W+RR) and non-warmed (solid line, Control and RR) plots. Corresponding slopes for ambient (Control and RR) and warmed plots (W and W+RR) and results from slope tests (Likelihood ratio, LR and p-values are shown within each panel. Dot color codes are: Green: ambient conditions (Control), cyan: rainfall reduction (RR), orange: warming (W) and violet: warming and rainfall reduction (W+RR). Dot shape codes are: Circle: *Helianthemum squamatum* (Aranjuez); Squares: *H. squamatum* (Sorbas); Diamonds: *H. syriacum* (Sorbas); Upper triangles: *G. struthium* (Sorbas).

Similar slopes for the relationships between PNUE, PPUE, PKUE and net photosynthetic rate indicate similar gains in photosynthetic nutrient use efficiency with increasing A across climate treatments. Conversely, the much lower slopes in warmed shrubs compared to non-warmed shrubs for PNutUE vs g_s indicate that shrubs under elevated temperature conditions need greater stomatal aperture and transpiration water loss to achieve any given gain in photosynthetic nutrient use efficiency than do control shrubs under ambient temperature.

Considering the time-integrated water use efficiency over the entire growing season (foliar δ^{13}C), we found the expected trade-off and negative relationship between photosynthetic nutrient use efficiency and foliar δ^{13}C/WUEi ($R^2 = 0.07$, $R^2 = 0.08$ and $R^2 = 0.10$ for PNUE, PPUE and PKUE, respectively, $p < 0.001$; Fig. 3d,e,f). These relationships held both within non-warmed and warmed plots separately, but the intercepts were smaller and slopes were significantly flatter for warmed shrubs (slope tests, LR=7.45, $p < 0.01$; LR=4.75, $p < 0.05$ and LR= 8.73, $p < 0.01$ for PNUE, PPUE and PKUE, respectively, Fig. 3d,e,f). This indicates that any given reduction in WUEi through greater stomatal aperture results in a smaller gain in PNutUE in warmed shrubs than in shrubs growing under ambient temperature.

Across species and years, PNUE, PPUE and PKUE were negatively correlated with both LMA and LT ($R^2 = 0.07$, $R^2 = 0.08$ and $R^2 = 0.10$ for LMA and $R^2 = 0.14$, $R^2 = 0.03$ and $R^2 = 0.02$ for LT; Fig. 4a-f, $p < 0.001$), as predicted by theory. The negative relationships between PNutUE and LMA and LT held both within non-warmed and warmed plots separately. However, the slopes were significantly flatter in warmed than in non-warmed shrubs (slope tests, LR=8.93, LR=5.92, and LR=5.69, $p < 0.05$ for PNUE, PPUE, PKUE and LMA, respectively, Fig. 4a-c; LR=5.15, $p < 0.05$; LR=3.00, $p = 0.08$ and LR= 5.74, $p < 0.01$ for PNUE, PPUE, PKUE and LT, Fig. 4d-f). On the other hand, PNUE, PPUE and PKUE were positively related to leaf area, ($R^2 = 0.14$, $R^2 = 0.12$ and $R^2 = 0.13$, all $p < 0.001$; Fig. 4g-i,) across sites and species. The positive relationships between PNutUE and leaf area held both within non-warmed and warmed plots separately, but slopes were significantly flatter in warmed shrubs (slope tests, LR=5.49, $p = 0.002$, LR=3.50, $p = 0.06$ and LR=7.00, $p = 0.008$ for PNUE, PPUE and PKUE, respectively, Fig. 4g-i). Across species and years, LMA was negatively correlated with leaf nutrient concentrations ($p < 0.001$ for leaf N, P and K) but in these cases the slopes of the relationships were not different between warmed and non-warmed shrubs (Fig. S6, slope tests, LR= 3.009, $p = 0.083$; LR=0.067, $p = 0.79$ and LR= 0.014, $p = 0.91$ for PNUE, PPUE and PKUE, respectively).

3.3. *Key drivers of simulated climate change effects on plant nutrient use efficiency*

The use of SEM analyses further supported the strong negative effect of warming on PNutUE (Fig. 5). When evaluating the effects of climate change on PNUE, we found three supported pathways to explain the decreased PNUE in warmed (W) shrubs. In two of the pathways, warming has moderately positive indirect effects on PNUE; the first pathway through an increase in stomatal conductance ($\beta = 0.20$, $p < 0.001$), which also enhances photosynthetic rates ($\beta = 0.64$, $p < 0.001$) and in turn PNUE ($\beta = 1.00$, $p < 0.001$, Fig. 5a and S7). Leaf thickness and leaf area had positive effects on stomatal conductance ($\beta = 0.11$, $p < 0.001$ and $\beta = 0.32$, $p < 0.001$) but this pathway was controlled via negative effects of warming on both leaf thickness ($\beta = -0.14$, $p < 0.001$) and leaf area ($\beta = -0.11$, $p < 0.001$). The second positive pathway acted through reductions in foliar N concentrations with warming ($\beta = -0.11$, $p < 0.001$) given that lower leaf N stimulates PNUE ($\beta = -0.26$, $p < 0.001$; Fig. 5a and S7). Within this second pathway, leaf thickness had negative effects on leaf N concentration ($\beta = -0.31$, $p < 0.001$) but leaf area had positive effects on leaf N ($\beta = 0.42$, $p < 0.001$). The third, and main pathway, involves a strong direct negative effect of warming on photosynthetic rates ($\beta = -0.52$, $p < 0.001$) that far outweighs the abovementioned indirect pathways, given the heavy dependence of PNUE on A ($\beta = 1.00$, $p < 0.001$, Fig. 5a and S7). Furthermore, given the positive effect of foliar N concentration on A ($\beta = 0.15$, $p < 0.001$), reductions in foliar N with warming, and via leaf morphology (i.e. decreases in leaf thickness and leaf area with warming), also contributed indirectly to reducing PNUE through subsequent reductions in photosynthetic rates. Overall, these pathways resulted in a strong negative net effect of experimental warming on the PNUE of native shrubs (41.3% reduction, Fig. S7).

For both P and K, warming also had a strong negative net effect on their respective photosynthetic nutrient use efficiencies (41.0% and 40.0% for PPUE and PKUE, respectively, Fig. S7). Compared to PNUE, similar pathways were observed for leaf P and K concentrations on PPUE and PKUE, respectively (Fig. 5) with the exception that, in these cases, warming did not directly affect leaf nutrient concentrations (P or K). Warming had a small indirect positive effect on PNutUE via the negative effect of leaf thickness on leaf P and leaf K concentrations ($\beta = -0.23$, $p < 0.001$ and $\beta = -0.27$, $p < 0.001$, respectively). This resulted in an indirect negative effect of warming on PPUE and PKUE, respectively, given the negative relationships between leaf P concentration and PPUE ($\beta = -0.39$, $p < 0.001$) and between leaf K concentration and PKUE ($\beta = -0.42$, $p < 0.001$, respectively, Figs. 5 and S7). The only other pathways through which warming affected the PPUE and PKUE of native shrubs were indirectly via moderate increases in stomatal conductance ($\beta = 0.19$, $p < 0.001$ for both P and K) or more directly through strong decreases in net photosynthetic rates ($\beta = -0.54$, $p < 0.001$ for both leaf P and K; Figs. 5 and S7).

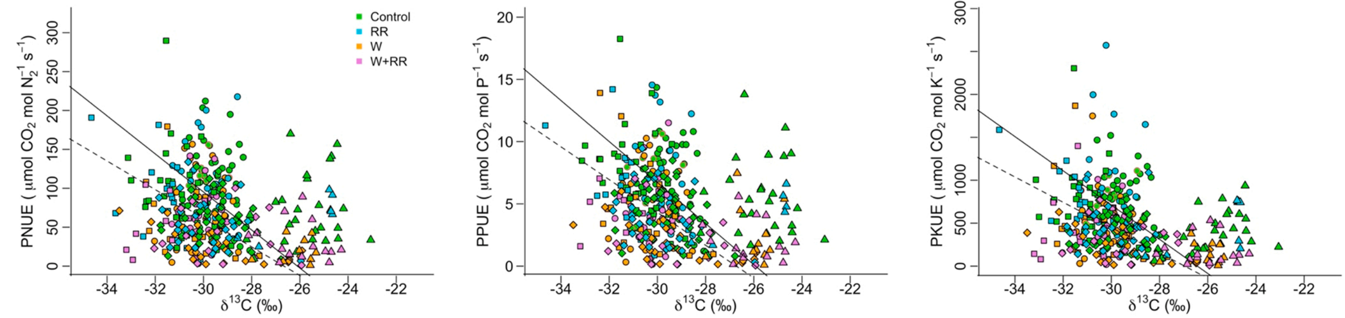

Fig. 3. Relationships between time-integrated water use efficiency (foliar δ^{13}C) and the photosynthetic nutrient use efficiency of a) leaf N (PNUE), b) leaf P (PPUE) and c) leaf K (PKUE). Regression lines are drawn for warmed (dashed line, W and W+RR) and non-warmed (solid line, Control and RR) plots. Dot color codes are: Control: ambient conditions, RR: rainfall reduction, W: warming and W+RR: warming and rainfall reduction. Dot shape codes are: Circle: *Helianthemum squamatum* (Aranjuez); Squares: *H. squamatum* (Sorbas); Diamonds: *H. syriacum* (Sorbas); Upper triangles: *G. struthium* (Sorbas).

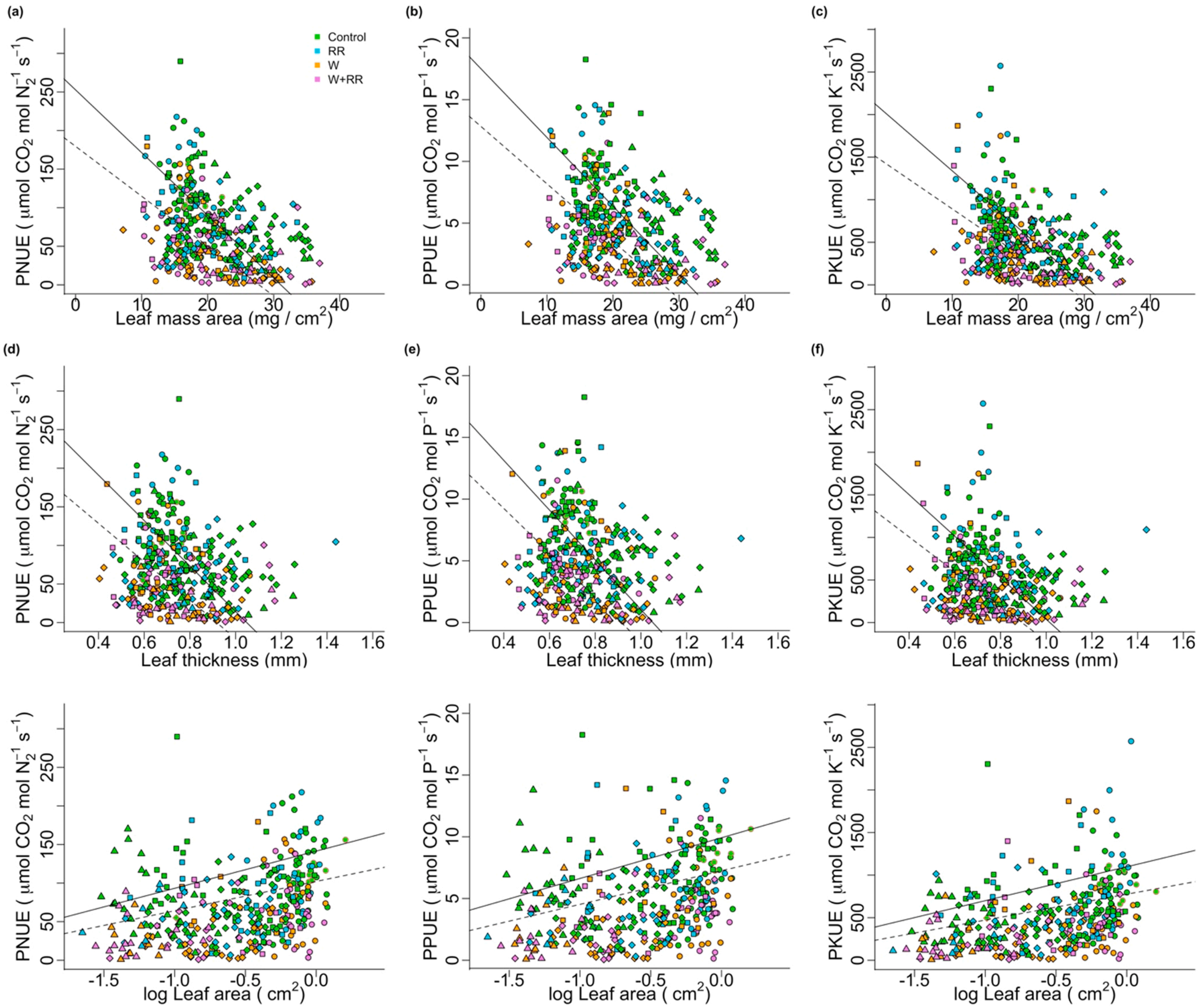

Fig. 4. Relationships between leaf mass per area (LMA) and the nutrient use efficiency of a) leaf N (PNUE), b) leaf P (PPUE) and c) leaf K (PKUE), between leaf thickness (LT) and d) PNUE, e) PPUE and f) PKUE and between Leaf area (log-scale) and g) PNUE, h) PPUE and i) PKUE. Regression lines are drawn for warmed (dashed line, W and W+RR) and non-warmed (solid line, Control and RR) plots Dot color codes are: Green: ambient conditions (Control), cyan: rainfall reduction (RR), orange: warming (W) and violet: warming and rainfall reduction (W+RR). Dot shape codes are: Circle: *Helianthemum squamatum* (Aranjuez); Squares: *H. squamatum* (Sorbas); Diamonds: *H. syriacum* (Sorbas); Upper triangles: *Gypsophila struthium* (Sorbas).

4. Discussion

4.1. Simulated climate change impact on the photosynthetic use efficiency of N, P and K

We found consistent and large reductions in the photosynthetic use efficiency of N, P and K (PNUE, PPUE and PKUE) under warming across three shrub species in two distant semiarid shrublands. This is in contrast to our first hypothesis that PNutUE would be maintained or increased under warming conditions, given the large decreases in WUEi previously observed in warmed plants in these experiments (León-Sánchez et al., 2020, 2018) and in other studies conducted in temperate and subtropical ecosystems (Ran et al., 2013; Wu et al., 2020; Zhang and Cao, 2009). The key novel finding of our study is the strong consistency of the negative effects of warming on the PNutUE of the three essential macronutrients (N, P and K) across three woody shrub species and across sites and years (2015–2017). This points to a strong direct influence of warming by itself on the PNutUE of semi-arid shrubland communities and suggests a limited ability of these shrubs to cope with and endure rapid climate warming. Although we accounted for temperature differences within and across treatments in all the statistical analyses of leaf gas exchange variables by including temperature as a covariable, we acknowledge that our study cannot fully distinguish the extent to which the observed reductions in photosynthesis and PNutUE were primarily driven by long-term, cumulative plant responses to warming (e.g. changes in integrative leaf traits or in carboxylation capacity) or by short-term responses to warmer temperatures (e.g. immediate changes in photosynthetic enzymatic reactions under warmer and drier conditions). However, it should be noted that the smaller and thinner leaves of warmed plants compared to control plants indicate lower time-integrated, cumulative plant productivity over the entire growing season (as previously reported for the earlier 2011–2015 years of the experiments; León-Sánchez et al., 2016, 2018, 2020). We interpret this as an indication of consistent long-term reductions in both photosynthesis and PNutUE leading to decreased plant productivity under warming (León-Sánchez et al., 2018, 2020).

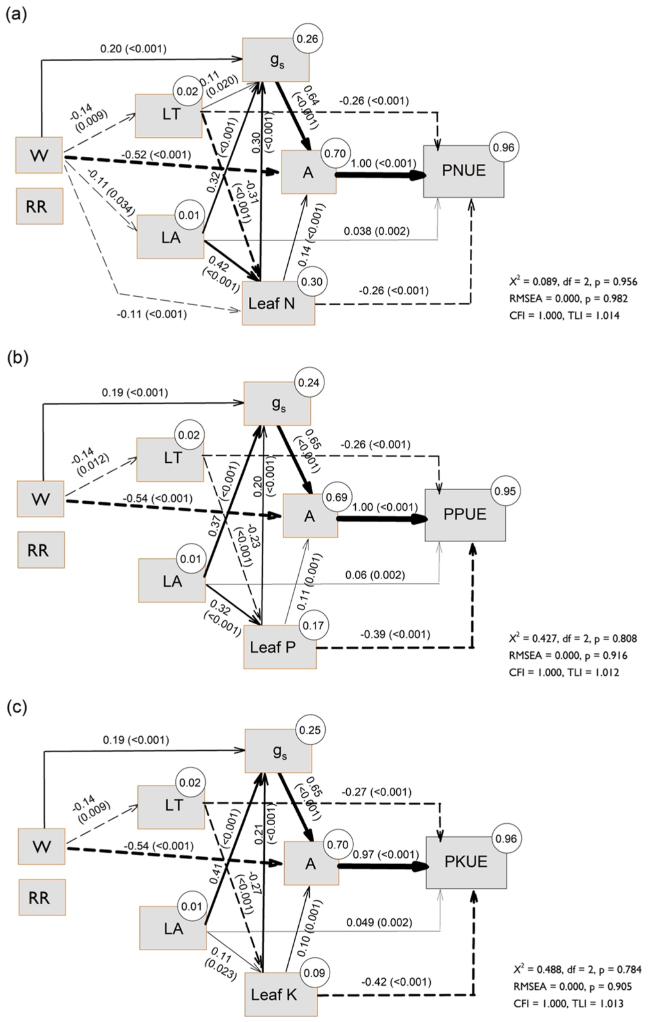

Fig. 5. Indirect effects of warming (W) and rainfall reduction (RR) on the photosynthetic use of a) N (PNUE), b) P (PPUE) and c) K (PKUE) in semi-arid Mediterranean shrubs through effects on leaf gas exchange (photosynthetic rates: A and stomatal conductance: g_s), leaf morphology (leaf thickness: LT and leaf area: LA) and leaf nutrient concentrations (leaf N, leaf P and leaf K). The model was fitted with experimental data collected in two sites across shrub species (*Helianthemum squamatum* in Aranjuez and *Helianthemum squamatum, Helianthemum syriacum and Gypsophila struthium* in Sorbas). Overall goodness-of-fit tests ($\chi 2$, CFI, TLI and RMSEA) are shown at the bottom right of the model. Continuous and dashed black arrows indicate positive and negative relationships, respectively. Numbers adjacent to arrows indicate the effect size (standardized path coefficients, analogous to partial regression weights) and significance (p-value, in brackets) of the path; arrow thickness is proportional to the effect size. Numbers within circles indicate squared multiple correlations for the variables.

4.2. Consequences of altered plant physiological performance on the photosynthetic use of nutrients under warming

Experimental warming drastically reduced net photosynthetic rates while also increasing stomatal conductance and transpiration, thereby inducing a large decline in both the intrinsic and instantaneous water use efficiency (WUE_i and WUE_t, respectively) of semiarid shrubs, as also observed in the earlier years of the experiments (León-Sánchez et al., 2020, 2018, 2016). The reduced photosynthetic rates of warmed shrubs along with their higher stomatal conductance also explain their much higher ratios between leaf internal and external CO_2 concentrations (c_i/c_a) relative to control plants (Wright et al., 2003, 2001). In warmed plants (W and W+RR treatments), increases in g_s (greater potential CO_2 uptake) led to much smaller increments in PNutUE than in control plants. One key finding in this study is that the warming-induced reduction of photosynthetic rates had even stronger effects on the photosynthetic use efficiency of N, P and K than did leaf nutrient status. Reductions in A under warming were accompanied by only moderate reductions in leaf N concentrations across species, and with no significant changes in P or K concentrations. The higher plant mortality observed in the W and W+RR treatments relative to the control treatment in the earlier years of the experiments (2011–2015; León-Sánchez et al., 2018, 2020) may have reduced inter-plant competition for P and K for the surviving warmed shrubs, which could explain the contrasting patterns observed in the two periods regarding leaf nutrient status. Nonetheless, poorer leaf N status in warmed plants in the present study should still increase their PNUE according to physiological theory (Cordell et al., 2001; Guilherme Pereira et al., 2019; Karlsson, 1994), which is indeed supported by the negative relationships between leaf nutrient concentrations and PNutUE in our SEM analysis. However, the slopes of the A-N, A-P and A-K relationships were consistently lower in warmed (W and W+RR) than in non-warmed shrubs (Control and RR) across species, indicating that, in the former, any given increase in leaf nutrient concentrations results in a smaller gain in photosynthetic rate. In contrast to our findings, previous studies assessing the effects of warming on PNUE (and PPUE) in subtropical (Ran et al., 2013; Wu et al., 2020), temperate (Lewis et al., 2004, 2001) and Mediterranean (Liberati et al., 2021) ecosystems generally reported unchanged or even enhanced net photosynthetic rates associated to higher stomatal conductance (i.e. higher c_i) and/or higher leaf nutrient contents in warmed plants. However, these other studies were carried out in much wetter ecosystems (mean annual precipitation over 500 mm) where water limitation of photosynthesis is not as severe as in our semi-arid ecosystems (Querejeta et al., 2021), which may explain the contrasting pattern encountered in our study.

To our knowledge, ours is the first study reporting strong negative effects of warming on the PNutUE of other essential plant macronutrients aside from N, such as P and K, which is opposite to what has been found in other studies (e.g. Niu et al., 2011). One could argue that the results for PPUE and PKUE are driven by the strong homeostasis between P, K and N in terrestrial plants (Sterner and Elser, 2002). However, when leaf N was included as a covariable in the model to control for this potential bias, results remained highly consistent for both PPUE and PKUE indicating that warming effects for these nutrients were independent of the concentration of N in the leaves and that leaf P and K were also regulating photosynthetic processes either directly or indirectly (Elser et al., 2007; Fernández-Martínez et al., 2016; Sardans and Peñuelas, 2015; Warren, 2011). In severely P-limited ecosystems, such as ours [i.e. foliar N:P and N:K ratios ranged from 27 to 39 and 1.8–3.11, respectively (Güsewell, 2004; Sardans and Peñuelas, 2015)], this element becomes strongly limiting for photosynthesis and plant productivity (Vance et al., 2003). Using N efficiently in terrestrial vegetation (i.e. functioning at low leaf protein contents) could reduce the amount of P needed for rRNA synthesis (Guilherme Pereira et al., 2019; Veneklaas et al., 2012). Thus, it is not surprising that photosynthetic N- and P-use efficiencies showed similar responses in our study given that N is not the most limiting nutrient in this and other dryland ecosystems (Delpiano et al., 2020; Du et al., 2020; Hartley et al., 2007; Schlesinger et al., 1996). In contrast to the strong negative impacts of experimental warming, PNUE, PPUE and PKUE were not significantly affected by rainfall reduction, which is in agreement with the moderate or negligible RR impacts on A, g_s or WUEi observed in the present and previous studies (León-Sánchez et al., 2020, 2018; Prieto et al., 2019; Prieto and Querejeta, 2020; Querejeta et al., 2021).

4.3. Alteration of the universal trade-off between photosynthetic nutrient use and water use efficiency with climate change

In terrestrial plants, there is a strong trade-off between water and nutrient use efficiency whereby plants cannot maximize PNutUE and WUE simultaneously (Patterson et al., 1997), so that the resource efficiency of the most limiting resource is improved at the expense of the less limiting one (Gong et al., 2011; Pons and Westbeek, 2004; Prentice et al., 2014; Wright et al., 2003). In the long term, we observed the expected trade-off between time-integrated WUEi (inferred from leaf δ^{13}C) and the PNutUE, which is in line with previous observations for PNUE in other tropical trees (*Bauhinia sp., Simarouba amara, Nectandra purpurascens, Anisoptera costata, Dipterocarpus alatus*, Santiago et al., 2004; Zhang and Cao, 2009) and dryland herbaceous species *(Leymus chinensis, Agropyron cristatum, Stipa grandis, Artemisia frigida*, Gong et al., 2011). This trade-off between time-integrated WUEi and PNutUE appears to be a common adaptive strategy in dryland species whereby plants maximize the use of the most limiting resource (Gong et al., 2011; Prentice et al., 2014; Wright et al., 2003; Querejeta et al., 2022). In our study, the slopes of the relationships between leaf δ^{13}C and the photosynthetic use efficiency of N, P and K were always flatter for warmed plants across species and years indicating that any given increase in WUEi will lead to a larger drop in PNutUE under warming than under ambient temperature conditions. Thus, minimizing the amount of water spent per unit C gained under stressful conditions, i.e. maximizing WUEi under the lower water availability in warmed plots, comes at a larger cost in the use of nutrients for photosynthetic processes in warmed shrubs (León-Sánchez et al., 2020, 2018; Querejeta et al., 2021). This impaired ability of native shrubs to adequately respond to warming conditions in a dryland ecosystem where primary productivity is already strongly co-limited by water and nutrients under current ambient conditions is a sobering finding in the face of global warming. An altered WUEi/PNutUE tradeoff in warmed plants could be a so-far overlooked mechanism that may contribute to explain their lower productivity and higher mortality rates compared to plants growing under ambient temperature in dryland plant communities (Grossiord et al., 2018; León-Sánchez et al., 2020, 2018; Peñuelas et al., 2018, 2017).

Trade-offs exist in plants between traits involved in resource uptake, including C and nutrients, and traits that enable longer leaf life span (Reich, 2014; Wright et al., 2004). Many studies have found a broad trade-off between leaf lifespan (which is linked to LMA; Onoda et al., 2017; Reich and Flores-Moreno, 2017; Wright et al., 2004) and PNutUE (Harrison et al., 2009; He et al., 2009; Reich et al., 1998; Wright et al., 2005). We also found this universal trade-off between LMA/leaf lifespan and PNutUE across our shrub species for all three nutrients (N, P and K), but the slopes of these relationships were flatter when shrubs were subjected to warming. The lower intercepts and slopes of the PNutUE-LMA relationships in warmed shrubs indicate that lower photosynthetic nutrient use efficiencies are achieved at any given LMA under warming. These altered relationships between WUEi, leaf anatomy/lifespan (LMA) and PNutUE in warmed plants are probably driven by other physiological constrains such as the need to avoid leaf overheating through enhanced evaporative leaf cooling, or by high photorespiration rates inhibiting C fixation under warming (Cakmak, 2005; Drake et al., 2018; León-Sánchez et al., 2016, 2018; Peguero-Pina et al., 2020).

4.4. Conclusions

We report large decreases in PNUE, PPUE and PKUE linked to strong reductions in net photosynthetic rates of semiarid shrubs under simulated climate warming. When exposed to the warmer climate forecasted for future decades, semiarid shrubs will thus likely trespass their optimum temperature threshold for carbon assimilation, and photosynthesis will probably become increasingly decoupled from plant respiration and increasingly vulnerable to photorespiration, degrading the ecosystem's capacity to act as a C sink (Bussotti et al., 2013; Reich et al., 2015; Saxe et al., 2001). The universal adaptive trade-offs between PNutUE and WUEi (and LMA) were also altered in warmed plants, which could be indicative of an increasing inability to allocate nutrients to the leaf photosynthetic machinery and cope with climate warming. The correlation between leaf N concentration and photosynthesis is very strong and consistent within species (Hikosaka, 2010) and has been used in mathematical models to predict carbon gain of leaves, individual plants, and plant canopies (Hikosaka, 2003; Hirose, 2005; Hirose and Werger, 1987). However, given the large reductions in photosynthetic N, P and K use efficiencies observed under simulated climate warming in our study, these modelling approaches may need to be revisited if we aim at predicting C uptake under future climate change scenarios in dryland ecosystems (Rogers et al., 2017).

CRediT authorship contribution statement

José Ignacio Querejeta designed, conceived and obtained funding for the long-term field experiments. Iván Prieto and José Ignacio Querejeta conceived and designed the present study. Iván Prieto, José Ignacio Querejeta, Lupe León-Sánchez, Pedro Nortes and Emilio Nicolás collected the data and Iván Prieto assembled the data with help of Lupe León-Sánchez. Iván Prieto and José Ignacio Querejeta analysed and interpreted the data and wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2023.105331.

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