

# Co-ordination between xylem anatomy, plant architecture and leaf functional traits in response to abiotic and biotic drivers in a nurse cushion plant

Ana I. García-Cervigón<sup>1\*</sup>, María A. García-López<sup>2</sup>, Nuria Pistón<sup>3</sup>, Francisco I. Pugnaire<sup>4</sup> and José Miguel Olano<sup>2</sup>

<sup>1</sup>Department of Biology and Geology, Physics and Inorganic Chemistry, Rey Juan Carlos University, c/Tulipán s/n, ES-28933 Móstoles, Spain, <sup>2</sup>iuFOR-EiFAB, University of Valladolid, Campus Duques de Soria s/n, ES-42004 Soria, Spain,

<sup>3</sup>Department of Ecology, Center for Health Sciences, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho, 373, CEP-21941-902, Rio de Janeiro – RJ, Brazil and <sup>4</sup>Estación Experimental de Zonas Áridas, EEZA-CSIC, Ctra. de Sacramento s/n, ES-04120 La Cañada, Almería, Spain

\*For correspondence. E-mail [ana.garciacervigon@urjc.es](mailto:ana.garciacervigon@urjc.es)

Received: 1 December 2020 Returned for revision: 12 February 2021 Editorial decision: 19 February 2021 Accepted: 24 February 2021  
Electronically published: 27 February 2021

• **Background and Aims** Plants in dry Mediterranean mountains experience a double climatic stress: at low elevations, high temperatures coincide with water shortage during summer, while at high elevations temperature decreases and water availability increases. Cushion plants often act as nurses by improving the microclimate underneath their canopies, hosting beneficiary species that may reciprocally modify their benefactors' microenvironment. We assess how the nurse cushion plant *Arenaria tetraquetra* subsp. *amabilis* adjusts its hydraulic system to face these complex abiotic and biotic constraints.

• **Methods** We evaluated intra-specific variation and co-ordination of stem xylem anatomy, leaf functional traits and plant architecture in response to elevation, aspect and the presence of beneficiary species in four *A. tetraquetra* subsp. *amabilis* populations in the Sierra Nevada mountains, southern Spain.

• **Key Results** Xylem anatomical and plant architectural traits were the most responsive to environmental conditions, showing the highest mutual co-ordination. Cushions were more compact and had smaller, more isolated conductive vessels in the southern than in the northern aspect, which allow minimization of the negative impacts of more intense drought. Only vessel size, leaf mass per area and terminal branch length varied with elevation. Nurse cushions co-ordinated plant architecture and xylem traits, having higher canopy compactness, fewer leaves per branch and fewer, more isolated vessels than non-nurse cushions, which reflects the negative effects of beneficiary plants on nurse water status. In non-nurse cushions, plant architecture co-ordinated with leaf traits instead. The interacting effects of aspect and elevation on xylem traits showed that stress due to frost at high elevation constrained xylem anatomy in the north, whereas stress due to drought had a parallel effect in the south.

• **Conclusions** Trait co-ordination was weaker under more demanding environmental conditions, which agrees with the hypothesis that trait independence allows plants to better optimize different functions, probably entailing higher adjustment potential against future environmental changes.

**Key words:** *Arenaria tetraquetra* subsp. *amabilis*, drought stress, intra-specific trait variation, leaf functional traits, Mediterranean high mountains, nurse cushion plant, plant architecture, quantitative wood anatomy, trait co-ordination.

## INTRODUCTION

Climatic change is the most relevant environmental challenge in alpine habitats. Limited availability of high elevation areas constrains altitudinal migration of species (Rumpf *et al.*, 2018), and this limitation is particularly severe in dry Mediterranean mountains, where the increase in mean air temperature is faster than average (Giorgi and Lionello, 2008). In addition, plants in Mediterranean mountains experience a double climatic stress: freezing winter temperatures coincide with water shortage during summer, defining complex altitudinal gradients of temperature and aridity (Schöb *et al.*, 2013; Pescador *et al.*, 2016). Mortality events and population declines derived from these limitations have been documented for endemic alpine species (Pauli *et al.*, 2012; Olano *et al.*, 2013), as have

been phenological responses that allow some species to remain stable within their current ranges (Arzac *et al.*, 2016).

Cushion plants are often engineers in alpine habitats, as their dense and compact canopies create a microhabitat underneath with increased availability of water and nutrients and higher soil temperature, improving the microclimate over that of surrounding areas (Cavieres *et al.*, 2007; Mihoč *et al.*, 2016; Pistón *et al.*, 2016; Pugnaire *et al.*, 2020). This is crucial for many other species that find shelter in cushion canopies (Cavieres *et al.*, 2007; Schöb *et al.*, 2013; Pistón *et al.*, 2016). The facilitation effect of nurse plants is determined by their vigour and morphology in such a way that plants with better physiological status are stronger facilitators (Michalet *et al.*, 2011; Schöb *et al.*, 2013). However, this is not always a neutral interaction

(e.g. García *et al.*, 2017). Depending on the context, beneficiary species may have reciprocal effects on benefactors that can be either negative (e.g. beneficiaries may impoverish nurse water status or diminish their reproductive output; Schöb *et al.*, 2014; García *et al.*, 2016) or positive (e.g. increasing nurse nitrogen content or shoot water potential; Pugnaire *et al.*, 1996). In addition, the specific identity of beneficiary species and their associated traits may cause contrasting effects on benefactors depending on environmental conditions (Schöb *et al.*, 2012, 2013; Pistón *et al.*, 2016). For instance, a water-demanding beneficiary species might compete for water with the nurse under drought conditions, but might not have a significant effect when water is not a limiting factor. Since functional responses of nurse plant species to environmental variability may determine the response of the whole community (Schöb *et al.*, 2012), understanding how cushion plants respond to environmental constraints is thus paramount to anticipate potential responses of alpine plant communities to climatic change.

In Mediterranean mountains, physiological risks derived from drought and frost episodes can be minimized by adjusting the plants' hydraulic system. At the xylem level, plants in dry or cold environments tend to have smaller xylem conduits than in moister or warmer sites to minimize the cavitation risk derived from soil water depletion or freezing temperatures (Tyree and Sperry, 1989; Schreiber *et al.*, 2015; Olano *et al.*, 2017; García-Cervigón *et al.*, 2020). This reduction in conduit diameter is usually compensated by an increase in the number of conduits per unit area so that the xylem hydraulic efficiency might increase without compromising the safety of the hydraulic system (Sperry *et al.*, 2008). The degree of vessel clustering in angiosperms may also contribute to increase or reduce safety without the need for varying vessel size, either by increasing hydraulic redundancy or by enhancing the risk of cavitation spreading between contiguous vessels (Robert *et al.*, 2009; Brodersen *et al.*, 2013; Trifilò *et al.*, 2014). However, since plant hydraulic pathways go from roots to leaves, adjustments in water transport at the xylem level are expected to be co-ordinated with other functional and structural adjustments (Castro-Díez *et al.*, 1998; Martínez-Vilalta *et al.*, 2009). For instance, the absence of a steady water input replacing water losses by evapotranspiration may force leaves to close their stomata to avoid desiccation at the expense of reductions in carbon uptake (Mencuccini, 2003; Martínez-Vilalta and García-Forner, 2016). Production of smaller leaves or architectural adjustments such as reductions of canopy surface or leaf area index (LAI) may therefore contribute to maintaining water balance at the whole-plant level. In cushion plants, shifts in canopy compactness allow for additional modifications in canopy transpiration: more compact cushions increase soil moisture and buffer temperature beneath them (Schöb *et al.*, 2013), influencing their own micro-environment and thus protecting themselves from desiccation (Michalet *et al.*, 2011).

Co-ordination between different sets of traits implies the existence of trade-offs and covariation patterns that are indicative of diverse plant strategies at global (e.g. Wright *et al.*, 2004; Chave *et al.*, 2009; Díaz *et al.*, 2016) or regional scales (e.g. Baraloto *et al.*, 2010; Méndez-Alonzo *et al.*, 2012; Silva *et al.*, 2017). However, and despite the fact that single traits may vary with environmental conditions, studies assessing shifts in trait

co-ordination in response to environmental drivers are particularly scarce. These studies show that trait coordination may respond to aridity gradients (Dwyer and Laughlin 2017), elevation (Carvalho *et al.*, 2020) or light availability (Trueba *et al.*, 2016), and suggest that trait co-ordination is stronger under harsher conditions (Dwyer and Laughlin, 2017; Zeballos *et al.*, 2017). Since strong co-ordination implies higher trait covariation and as this reduces potential combinations of trait values, the existence of strong co-ordination might imply low adjustment potential against a sharp shift in environmental conditions, entailing a potential compromise for population viability. Nevertheless, other studies that have detected independence between different sets of traits interpret this lack of co-ordination as an opportunity to better optimize plant survival and growth by investing differently in their form and function (Baraloto *et al.*, 2010; Fortunel *et al.*, 2012). Trait independence might also imply that responses in different sets of traits are driven by different environmental constraints. In Mediterranean mountains, where harshness is defined by frost in winter and high temperatures and drought in summer, it is thus plausible that some traits are more responsive to variations in temperature, whereas others are more responsive to variations in moisture. In this sense, we might specifically expect that canopy compactness positively covaries with leaf mass per area and negatively with vessel size to face colder temperatures, whereas co-ordinated adjustments to face drought might include reductions in leaf size, vessel size and LAI, as well as increases in canopy compactness.

In this study, we evaluated the role of elevation, aspect and the presence of beneficiaries on the intra-specific variation of stem xylem anatomical traits, leaf functional traits and plant architecture in *Arenaria tetraquetra* subsp. *amabilis*, an endemic nurse cushion from the Sierra Nevada mountains in southern Spain. Specifically, we wanted to test (1) whether single-trait variations are driven by aspect, elevation and biotic interactions; (2) whether trait variation is co-ordinated; and (3) whether simultaneous gradients of moisture and temperature related to aspect and elevation, as well as the effect of beneficiaries, result in stronger or weaker trait co-ordination. We hypothesize that traits will respond to environmental gradients in a co-ordinated fashion. According to mainstream theory, we expect that cushions in the southern (i.e. drier and hotter) aspect will have a safer conductive system formed by smaller vessels but at higher density, smaller and denser leaves, and more compact canopies than in the northern aspect. Similar adjustments are expected at higher (i.e. colder) elevations. Also, we expect that the reported negative effects of beneficiary species on nurse water status (Schöb *et al.*, 2014) will be reflected in co-ordinated responses of xylem, leaf and architectural traits of nurse cushions, and that trait co-ordination will be stronger under harsher conditions.

## MATERIALS AND METHODS

### *Species and study area*

*Arenaria tetraquetra* subsp. *amabilis* (Bory) H. Lindb. Fil (Caryophyllaceae) is a cushion plant species endemic to the Sierra Nevada mountains, south-east Spain. It appears

associated with perennial pastures on poorly developed soils on mica–schist bedrock between 2400 and 3300 m a.s.l. (Blanca *et al.*, 2009). Despite its overall high elevation, the range occupied by this species experiences a strong summer drought, characteristic of Mediterranean climate. Mean annual temperature at 2500 m a.s.l. is 4.4 °C, and mean annual rainfall is 720 mm, concentrated between October and June mostly as snow. Summer months are rather dry, with rainfall average slightly above 2 and 5 mm in July and August, respectively, when temperatures are the highest (mean approx. 10 °C, Pugnaire *et al.*, 2020). In the northern aspect of the Sierra Nevada, temperature decreases by 0.61 °C and precipitation increases by 33 mm every 100 m, while in the southern aspect these rates are 0.59 °C and 28 mm, respectively (Delgado Calvo-Flores *et al.*, 1988; Supplementary data Fig. S1). Such changes in abiotic factors with elevation result in a crossed gradient in which temperature and soil organic matter decrease whereas precipitation and soil water increase with elevation (Schöb *et al.*, 2013).

The role of *A. tetraquetra* subsp. *amabilis* as a nurse plant in these mountains has been widely documented. Variability in its functional and morphological traits has been shown to mediate plant interactions along stress gradients (Schöb *et al.*, 2013), and to affect trait-based community assembly (Schöb *et al.*, 2012). Reciprocally, negative effects of beneficiaries on nurse cushions include reductions in their water status, flower density and seed set (Schöb *et al.*, 2014).

### Sampling design

We selected four populations of *A. tetraquetra* at contrasting aspects and elevations near the Sierra Nevada summits (Fig. 1A). Two populations were located in the northern aspect of the Veleta Peak, at 2680 m (37°04'57"N, 3°22'50" W) and 3240 m a.s.l. (37°03'30"N, 3°22'06"W), respectively. In the northern aspect, snow cover persists in some areas until summer, providing a permanent source of humidity for soils (Pugnaire *et al.*, 2020) which sustains a herbaceous community including *Eryngium glaciale*, *Jasione amethystina*, *Leucanthemopsis pectinata*, *Lotus corniculatus* subsp. *glacialis* and narrow endemics such as *Plantago nivalis* or *Armeria splendens*. The other two populations were located in the southern slope of the Mulhacén Peak (Fig. 1B), at 2565 m (37°00'51"N, 03°18'45"W) and 2830 m a.s.l. (37°01'35"N, 03°18'30"W). In the southern aspect, soils are much drier and reach higher temperature than in the northern aspect (see figure 2 in Schöb *et al.*, 2012). The plant community here is made up of herbs and perennial grasses (*Herniaria boissieri*, *Festuca indigesta*, *Poa ligulata* and *Leontodon boryi*) and shrubs (*Thymus serpylloides* and *Cytisus galianoi*), also with endemics such as *Trisetum glaciale* or *Nevadensia purpurea*.

In July 2015, we selected ten pairs of cushions per population, each pair comprising a cushion with herbaceous species within its canopy (Fig. 1C) – the nurse plant – and the other without beneficiary species. For each cushion, we took zenith

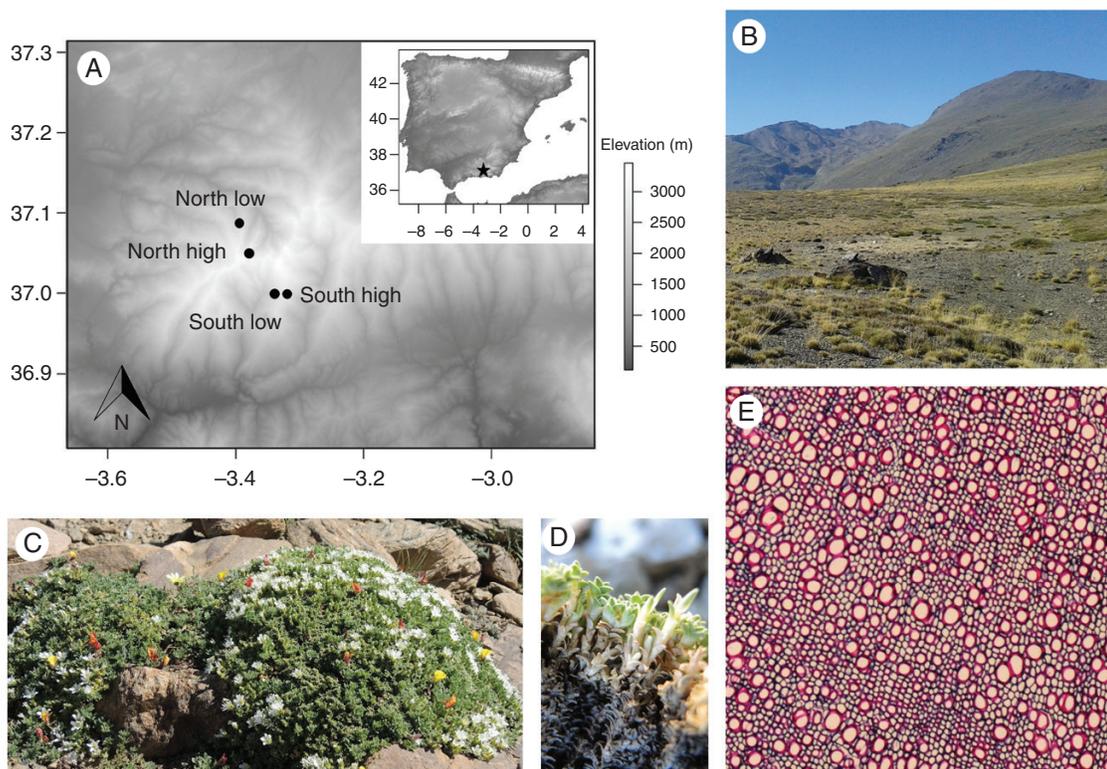


FIG. 1. (A) Location of the study populations in the Sierra Nevada mountains (southern Spain) with  $x$ - and  $y$ -axes displaying longitude and latitude in degrees (0.2° equals approx. 22.2 km). The location of the study area in the Iberian Peninsula is indicated by a star. (B) Population on the southern aspect and at high elevation (2830 m a.s.l., 37°01'N, 03°18'W). (C) *Arenaria tetraquetra* subsp. *amabilis* nurse cushion with some individuals of *Lotus corniculatus* subsp. *glacialis* (yellow and orange flowers) growing within its canopy. (D) View of *A. tetraquetra* terminal branches. (E) Microsection of the root collar of an *A. tetraquetra* individual growing in the south–high population. Vessels are identified as larger white circles with thick pink walls. The whole microsection comprises a surface of 0.5 mm<sup>2</sup>.

photographs using a scale and collected the aerial biomass and the root collar. Root collars were preserved in formalin (a solution with ethanol, formaldehyde and acetic acid at 90:5:5) until processed. The aerial biomass was stored under cold conditions and transported to the laboratory for further analyses.

#### Trait data

We processed plant material to obtain a set of variables related to (1) cushion architecture, (2) leaf functional traits and (3) xylem anatomical traits (Table 1; Supplementary data Table S1). Architectural traits included terminal branch density (BD), branch length (BL), number of leaves per terminal branch (LTB) and LAI. BD is a proxy of the degree of cushion compactness (Schöb *et al.*, 2013). To measure this trait, we delimited three areas of 1 cm<sup>2</sup> in each of the zenith photographs with the software ImageJ version 1.51j8 (available at <http://rsb.info.nih.gov/ij>; developed by W. Rasband, NIH, Bethesda, MD, USA) and recorded the number of terminal branches in each square. The BL was measured with a calliper in four branches per plant, on which we also recorded the number of leaves (Fig. 1D). For these three traits (BD, BL and LTB), we used the mean values per plant. Mean BD values were multiplied by LTB and by average leaf area (see below) to obtain the LAI. Additionally, we measured plant cover from the zenith photographs using the software ImageJ.

Regarding leaf functional traits, we measured leaf area (LA) and leaf mass per area (LMA). We selected between seven and 13 leaf pairs per cushion that were processed following standard protocols (Cornelissen *et al.*, 2003). Fresh leaves were scanned with an Epson Perfection V750 Pro scan (Seiko Epson, Japan) at 300 dpi resolution, and their area was measured using the ImageJ software, obtaining LA as the average value per individual. Scanned leaves were then oven-dried at 80 °C for 48 h and weighed together with an accuracy of 0.1 mg. The LMA was obtained as the ratio between total dry leaf mass and the sum of fresh leaf area of all selected leaves per cushion.

#### Quantitative xylem anatomy

Xylem anatomical traits were quantified following the protocol proposed by von Arx *et al.* (2016; see Supplementary data Method S1). Since xylem in this species is located in lobes that occupy the outer part of the root collar, whereas the central part is often rotten or twisted (Supplementary data Fig. S2), pith could not be identified and, consequently, xylem anatomy could not be accurately measured in the whole root collar. Therefore, we selected between one and three square sub-images of 0.5 mm<sup>2</sup> area per section, depending on the available surface of xylem lobes and selecting those parts of the image located close to the bark with higher quality and without tissue fractures (Fig. 1E). In this way, we quantified xylem traits regardless of growth rings, ensuring that individuals with no clear ring boundaries could be considered in the study. On each sub-image, we measured five anatomical traits: (1) vessel density (VD; no. mm<sup>-2</sup>), which is the number of vessels per mm<sup>2</sup>; (2) mean vessel lumen area (MVA; µm<sup>2</sup>), which is the average lumen area of vessels; (3) percentage of conductive area (CA),

TABLE 1. Coefficients of variation (CV) and coefficients of linear mixed-effects models fitted for architectural, leaf functional and xylem anatomical traits measured on four *A. tetraquetra* subsp. *amabilis* populations in the Sierra Nevada mountains (Spain)

Trait	Acronym	CV	Aspect	Elevation	Aspect × elevation	Plant size	Facilitation role	R <sup>2</sup> m (R <sup>2</sup> c)
<b>Architectural traits</b>								
Terminal branch density	BD	0.318	<b>5.778*** (1.197)</b>	2.276 (1.198)	-0.621 (1.699)	<b>-0.016*** (0.003)</b>	1.259 (0.852)	0.518 (0.518)
No. of leaves per terminal branch	LTB	0.232	<b>-1.702*** (0.296)</b>	-0.033 (0.303)	0.552 (0.422)	-0.001 (0.001)	2.225 (0.178)	0.444 (0.541)
Terminal branch length	BL	0.355	<b>-0.644** (0.228)</b>	<b>0.522* (0.232)</b>	-0.127 (0.325)	-1.36·10 <sup>-4</sup> (4.95·10 <sup>-4</sup> )	0.081 (0.122)	0.315 (0.511)
Leaf area index	LAI	0.372	-0.201 (0.962)	1.302 (0.963)	0.782 (1.365)	<b>-0.007** (0.002)</b>	<b>1.300* (0.529)</b>	0.267 (0.468)
Leaf area	LA	0.191	-0.008 (0.007)	0.003 (0.007)	-1.02 × 10 <sup>-4</sup> (9.78 × 10 <sup>-5</sup> )	<b>3.19 × 10<sup>-5</sup>*</b> (1.47 × 10 <sup>-5</sup> )	0.003 (0.003)	0.108 (0.399)
Leaf mass per area	LMA	0.206	-2.79 × 10 <sup>-4</sup> (1.73 × 10 <sup>-4</sup> )	<b>-4.11 × 10<sup>-4</sup>*</b> (1.77 × 10 <sup>-4</sup> )	4.97 × 10 <sup>-4</sup> (2.46 × 10 <sup>-4</sup> )	3.48 × 10 <sup>-7</sup> (4.01 × 10 <sup>-7</sup> )	-3.89 × 10 <sup>-5</sup> (1.11 × 10 <sup>-4</sup> )	0.090 (0.184)
<b>Xylem anatomical traits</b>								
Vessel density	VD	0.215	<b>-228.864* (87.392)</b>	164.289 (96.887)	-30.893 (129.427)	-0.002 (0.246)	-50.976 (53.664)	0.258 (0.410)
Percentage of conductive area	CA	0.317	<b>-4.433*** (1.018)</b>	-1.685 (1.138)	<b>3.853* (1.511)</b>	<b>0.007* (0.003)</b>	-1.117 (0.745)	0.304 (0.304)
Mean vessel area	MVA	0.308	<b>-21.447* (9.207)</b>	<b>-21.982* (10.204)</b>	<b>30.229* (13.634)</b>	<b>0.079*** (0.026)</b>	-6.761 (5.618)	0.258 (0.415)
Vessel grouping index	VGI	0.148	<b>-0.174* (0.074)</b>	0.060 (0.082)	0.114 (0.109)	2.26 × 10 <sup>-4</sup> (2.18 × 10 <sup>-4</sup> )	-0.044 (0.052)	0.166 (0.201)
Vessel solitary fraction	VSF	0.196	<b>9.609*** (2.897)</b>	-0.906 (3.241)	-6.971 (4.301)	-0.007 (0.009)	1.906 (2.120)	0.214 (0.214)
Xylem-specific hydraulic conductivity	K <sub>s</sub>	0.470	<b>-5.94 × 10<sup>-8</sup>*** (1.53 × 10<sup>-8</sup>)</b>	-3.01 × 10 <sup>-8</sup> (1.71 × 10 <sup>-8</sup> )	<b>5.83 × 10<sup>-8</sup>*</b> (2.27 × 10 <sup>-8</sup> )	<b>1.34 × 10<sup>-10</sup>** (4.49 × 10<sup>-11</sup>)</b>	-1.56 × 10 <sup>-8</sup> (1.06 × 10 <sup>-8</sup> )	0.313 (0.361)

Significant coefficients (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001) are highlighted in bold. Marginal (R<sup>2</sup>m, fixed effects only) and conditional (R<sup>2</sup>c, including both fixed and random effects) coefficients of determination are shown

obtained as the cumulative lumen area of all vessels divided by total xylem area; (4) vessel grouping index (VGI), which is the mean number of vessels with contiguous cell walls (von Arx *et al.*, 2013); and (5) the fraction of solitary vessels (VSF), i.e. the percentage of isolated vessels with respect to all vessels in the sub-image. In addition, we obtained (6) an anatomy-based estimate of specific hydraulic conductivity per unit area ( $K_s$ ), where the hydraulic conductance of each vessel is calculated following Nonweiler (1975) and considering the ovality of vessels (see equations in Olano *et al.*, 2017). We used MVA instead of hydraulic diameter to avoid redundant parameters as far as possible and to separate physical (MVA) from functional ( $K_s$ ) parameters. Data from the three sub-images of a given individual were averaged in order to include a single value per xylem trait and cushion in further analyses. Xylem anatomical traits were measured using ROXAS v3.0 (von Arx and Dietz, 2005), a specific image analysis tool based on Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA).

#### Age estimation

Since ontogeny is a potential source of intra-specific variability in xylem and leaf traits (Fonti *et al.*, 2010; Dayrell *et al.*, 2018) and is independent from environmental conditions, it was included in our analyses. For this, we estimated the age of each cushion by counting the number of growth rings present in the root collar. This counting was performed on images where ring identification was possible (i.e. 66.7 % of samples, Supplementary data Fig. S3). Given the problems referred to above, the ring count only gave an estimation of the minimum age reached by each plant. Since we needed some quantitative assessment of the ontogenetic stage of each plant, and rings could not be counted on all cushions, we related the estimated age to plant cover in order to search for a proxy. The linear relationship between canopy size and estimated age was strongly significant ( $t = 14.12$ ,  $P < 0.001$ ,  $R^2_{\text{adj}} = 0.826$ , Supplementary data Fig. S4), so plant size was used in subsequent analyses as a surrogate of age.

#### Data analysis

We used linear mixed-effects models (LMMs) to test the effects of aspect, elevation and facilitation on architectural, leaf and xylem traits of *A. tetraquetra* subsp. *amabilis*. We performed a separate model for each response variable, including aspect, elevation, their interaction and their role in facilitation (i.e. nurse vs. non-nurse plants) as fixed factors, and plant size as covariate to control for ontogeny. The pair of cushions (see ‘Sampling design’) was included as a random factor. Models were fitted using restricted maximum likelihood (REML; Zuur *et al.*, 2009), and normalized residuals were extracted and checked for normality and homoscedasticity, with model assumptions being met in all cases. We used the ‘nlme’ package (Pinheiro *et al.*, 2019) in the R environment (R Core Team, 2019) to fit LMMs, and the function ‘r.squaredGLMM()’ from the ‘MuMIn’ package (Barton, 2019) to obtain marginal (fixed effects only) and conditional (including both fixed and random effects)  $R^2$  per model.

Multivariate analyses were used to assess trait co-ordination and to evaluate the effect of explanatory factors on the co-ordinated variation of traits. We excluded from multivariate analyses several traits (i.e. CA, VSF and  $K_s$ ) that were highly correlated (pairwise Pearson’s  $r > 0.70$ ) with each other (Supplementary data Table S2). We first used canonical ordination techniques to evaluate the effects of aspect, elevation, facilitation and ontogeny on the co-ordinated variation of all measured traits. Specifically, we performed a redundancy analysis (RDA), which combines multiple regression with principal component analysis, to relate the dependent matrix (individuals  $\times$  traits) to an explanatory matrix with the same structure as the fixed part of LMMs (i.e. aspect  $\times$  elevation + facilitation role + plant size). The significance of the model and that of explanatory variables was evaluated by Monte Carlo tests with 9999 permutations. The RDA was performed with the ‘vegan’ (Oksanen *et al.*, 2019) package and adjusted  $R^2$  was obtained with the function ‘RsquareAdj()’ from the ‘r2glmm’ package (Jaeger, 2017). Then, we used multiple factor analysis (MFA) to assess the co-ordination between architectural, leaf and xylem traits (see Supplementary data Method S1 for a description of this technique). The degree of co-ordination between leaf, xylem and architectural traits was measured by RV coefficients, which are a multivariate generalization of the Pearson’s  $r$  coefficient and vary between 0 (for total independence) and 1 (for total similarity). The significance of RV coefficients was tested with permutations (Josse *et al.*, 2008). We computed a general MFA including all *A. tetraquetra* subsp. *amabilis* cushions, and additional MFAs on sub-sets of cushions divided by aspect, elevation and facilitation role. To perform MFAs, we used ‘MFA()’, ‘coeffRV()’ and ‘dimdesc()’ functions from the ‘FactoMineR’ package (Le *et al.*, 2008).

## RESULTS

Architectural and xylem traits of *A. tetraquetra* differed between aspects (Table 1). In the northern aspect, cushions were less compact (i.e. had 28.0 % lower terminal branch density) and had 40.3 % longer branches, with more leaves than in the southern aspect (Fig. 2; Supplementary data Table S3). Plants in the north also showed 5.9 % larger conductive vessels that were more numerous and more grouped than in the south, which led to 24.8 % more conductive area and a 29.8 % increase in estimated hydraulic conductivity ( $K_s$ ), this being the trait with the highest variation (Table 1). Leaf traits did not differ between aspects, but LMA was 6.1 % higher at lower elevations (Table 1; Fig. 2). Terminal branch length and MVA also varied with elevation, with cushions at higher elevations having a 24.1 % increase in terminal branch length and a 7.6% decrease in conductive vessels. The interaction between aspect and elevation only affected xylem traits. In the northern aspect, cushions had larger vessels at lower elevations, which translated into a higher percentage of conductive area and higher hydraulic conductivity at low elevations in the north. In the southern aspect, the pattern was reversed and cushions had larger vessels at higher elevation. Only LAI differed between nurse and non-nurse plants, the former having 19.1 % lower LAI than the latter (Fig. 2; Supplementary data Table S3). Finally, larger (thus older) plants were less compact (i.e. had lower branch density) and

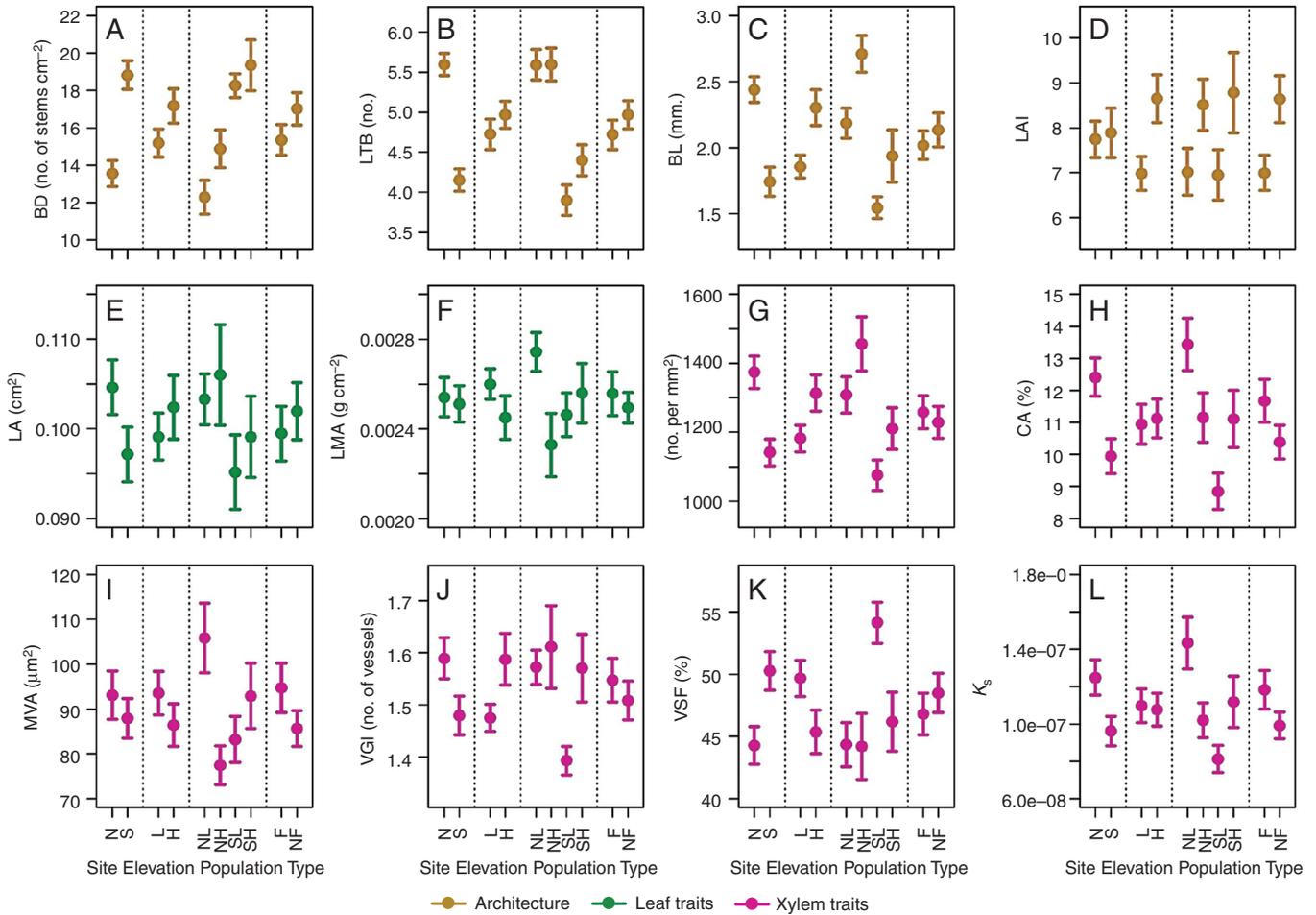


Fig. 2. Plant architecture (A–D), leaf functional traits (E and F) and xylem anatomical traits (G–L) of *Arenaria tetraquetra* subsp. *amabilis* in the Sierra Nevada mountains (Spain). Mean  $\pm$  s.e. per site (N, north; S, south), elevation (L, low elevation; H, high elevation), population (NL, north low; NH, north high, SL, south low; SH, south high) and facilitation role (F, nurse cushion; NF, non-nurse cushion) are shown. See trait abbreviations in Table 1.

had lower LAI, larger leaves, larger conductive vessels and higher estimated hydraulic conductivity (Table 1).

The RDA was significant ( $F = 4.486$ ,  $P = 0.001$ ), with the explanatory matrix explaining 21.7 % of the variance of the response matrix, and aspect and elevation having the strongest effects (Table 2). The MFA showed that xylem anatomy was linked to plant architecture, which in turn was linked to leaf traits (Table 3). The first axis showed positive loads for LA, BL, LTB, VD and VGI (Fig. 3), reflecting that plants with larger leaves also had larger branches and more leaves per branch, with vessels more grouped and at higher density. The second axis showed positive loads for MVA and negative for BD and LAI, suggesting that plants with larger vessels were less compact (Supplementary data Table S2). When analysing trait co-ordination separately per aspect, the general pattern was marginally retained in the north (Table 3), whereas in the south the three trait sub-sets were independent. The co-ordination between xylem traits and architecture, and between architecture and leaf traits, was retained at low elevation. At high elevation, however, only xylem traits were marginally co-ordinated with architectural traits, while leaf traits were independent. Striking differences in trait co-ordination were observed for cushions depending on their facilitation role.

TABLE 2. Coefficients of the redundancy analysis (RDA) fitted to assess the effects of aspect, elevation, facilitation role and plant cover on the global variation of architectural, leaf functional and xylem anatomical traits measured on four *A. tetraquetra* subsp. *amabilis* populations in the Sierra Nevada mountains (Spain)

Explanatory variable	<i>F</i>	<i>P</i>
Aspect	15.013	<b>&lt;0.001</b>
Elevation	6.131	<b>0.017</b>
Aspect $\times$ elevation	0.058	0.829
Facilitation role	1.050	0.310
Plant size	0.181	0.690

Model  $R^2 = 0.279$ , model  $R^2_{\text{adj}} = 0.217$ . Significant coefficients ( $P < 0.05$ ) are highlighted in bold

In nurse cushions, xylem traits co-ordinated with plant architecture as above, i.e. plants with more leaves per terminal branch had more grouped vessels in higher density. In contrast, architecture in non-nurse cushions was independent from xylem traits, but co-ordinated with leaf traits, i.e. plants with more

TABLE 3. *RV coefficients and their significance for the co-ordination of different paired combinations of trait sub-sets in all A. tetraquetra subsp. amabilis and in different sub-groups of cushions depending on aspect, elevation and facilitation role*

Sub-group of individuals	<i>n</i>	Xylem traits vs. architecture		Xylem traits vs. leaf traits		Architecture vs. leaf traits	
		RV	<i>P</i>	RV	<i>P</i>	RV	<i>P</i>
All cushions	64	<b>0.123</b>	<b>0.004</b>	0.038	0.318	<b>0.080</b>	<b>0.040</b>
North aspect	29	<i>0.151</i>	<i>0.085</i>	0.038	0.756	<i>0.164</i>	<i>0.057</i>
South aspect	35	0.084	0.242	0.091	0.175	0.087	0.199
High elevation	30	<i>0.161</i>	<i>0.052</i>	0.055	0.545	0.078	0.398
Low elevation	34	<b>0.324</b>	<b>0.000</b>	0.031	0.775	<b>0.171</b>	<b>0.018</b>
North at high elevation	13	0.186	0.537	0.068	0.880	0.221	0.362
South at high elevation	17	<i>0.244</i>	<i>0.081</i>	0.152	0.269	0.077	0.668
North at low elevation	16	0.165	0.368	0.079	0.696	<b>0.308</b>	<b>0.040</b>
South at low elevation	18	0.093	0.795	0.165	0.268	0.173	0.249
Nurse cushions	32	<b>0.158</b>	<b>0.045</b>	0.063	0.400	0.060	0.504
Non-nurse cushions	32	0.127	0.115	0.075	0.363	<b>0.161</b>	<b>0.036</b>

Coefficients with  $P < 0.05$  and  $P < 0.1$  are highlighted in bold and italics, respectively.

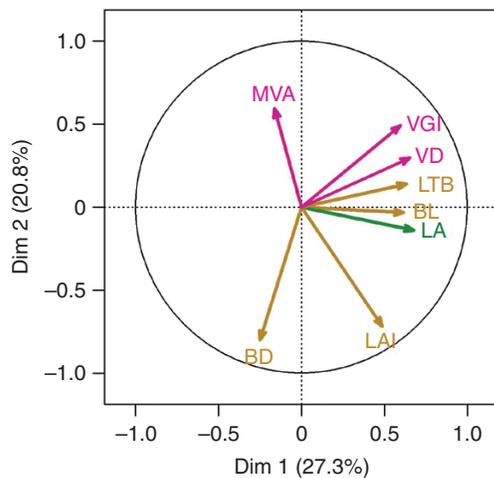


FIG. 3. Correlations (i.e. normalized vectors, represented by arrows) between the variables of each sub-set (plant architecture, leaf functional and xylem anatomical traits) and the MFA site scores on axes (dimensions; Dim) 1 and 2. Brownish, green and pink colours correspond to architectural, leaf functional and xylem anatomical traits, respectively. Only significant correlations at  $P < 0.0001$  are shown. The percentage of the total variance represented by each axis is indicated. See trait abbreviations in Table 1.

leaves per terminal branch and longer branches had larger leaves (Supplementary data Fig. S5). Xylem traits were independent from leaf traits in all cases (Table 3).

## DISCUSSION

Plant architecture, leaf functional traits and xylem anatomy in *Arenaria tetraquetra* subsp. *amabilis* varied depending on aspect, elevation and the facilitation role they had. Plant architecture and xylem anatomy showed both the strongest responses to environmental conditions and the highest mutual co-ordination, albeit plant architecture was also co-ordinated with leaf traits. Interestingly, trait co-ordination varied with the facilitation role and was strong at low elevation. Our data show that both biotic and abiotic factors drove intra-specific variations in single traits

and intra-specific trait co-ordination in our cushion species, providing evidence that different strategies may exist within a single species in response to environmental constraints (García-Cervigón et al., 2015; Trueba et al., 2016).

### Co-ordination in architectural and xylem traits to face drought

As expected, cushions had more compact canopies and smaller conductive vessels, fewer vessels per area unit and lower estimated hydraulic conductivity in the southern than in the northern aspect. These trait changes are in line with intra-specific adjustments to minimize the negative impacts of drought in the south, which was exacerbated by drier and hotter soils (Schöb et al., 2012). Compactness and vessel size covaried positively with LAI, but higher LAI enhances canopy transpiration (Balocchi et al., 2002), which would be detrimental under dry conditions. The higher LAI values in the southern aspect were more likely to be a consequence of higher canopy compactness, which led to the presence of more leaves per unit area, than a direct response to environmental drivers. Given the highly compact structure of cushion plants, it seems plausible that LAI becomes a poor proxy for canopy transpiration in this particular growth form.

The fraction of solitary vessels also increased in the southern aspect, in what may be interpreted as a response to increase xylem safety (Scholz et al., 2013), since a reduction of contact surface may reduce the risk of cavitation spreading between contiguous vessels (Brodersen et al., 2013). Cushions with more grouped vessels also had a higher vessel density, longer branches and more leaves per terminal branch, but these four traits defined an axis of variation that was independent (orthogonal) from the one defined by vessel size, canopy compactness and LAI (see Fig. 3). This may suggest that hydraulic efficiency (defined by vessel size) varies independently from safety, resulting in a weak trade-off between them. However, considering that both groups of traits are differentially related to ontogenetic effects (i.e. vessel size, canopy compactness and LAI varied with plant size, whereas the other traits did not, Table 1), we may hypothesize that intrinsic factors such as

plant size might control hydraulic efficiency at the individual level, whereas traits related to hydraulic safety may be more constrained by environmental conditions.

The independence of terminal branch length and the number of leaves per terminal branch from canopy compactness suggests that different architectural traits might be involved in different trade-offs, as proposed by Messier *et al.* (2017): compactness might be more involved in hydraulic aspects (Michalet *et al.*, 2011; Schöb *et al.*, 2013), whereas branch length and the number of leaves per branch might be more related to light interception and carbon economy (Díaz *et al.*, 2016). The lower number of leaves per terminal branch and the shorter terminal branches in the southern aspect support this interpretation, since higher compactness of plants in the southern aspect implies a more solid obstacle for light penetration into deeper canopy layers. As a result, leaves might need to be closer to the cushion surface in order to capture the right amounts of light. Altogether, our results support the idea that plant traits are multidimensional and might be involved in multiple trade-offs with contrasting effects on physiological rates (Messier *et al.*, 2017; Pistón *et al.*, 2019).

#### *Response to crossed climatic gradients of temperature and moisture*

The lack of response to elevation in most single traits may be related to the complex nature of our environmental gradient, since elevation implies increases in precipitation but also simultaneous decreases in temperature (Delgado Calvo-Flores *et al.*, 1988; Schöb *et al.*, 2013). Because xylem and architectural adjustments to drought are quite similar to adjustments to frost (e.g. Olano *et al.*, 2013; Schöb *et al.*, 2013; Schreiber *et al.*, 2015; García-Cervigón *et al.*, 2020), the effects of higher thermal stress at high elevation might have been compensated by higher water availability, cancelling each other out and resulting in neutral effects of elevation. However, we did detect significant effects of the interaction between aspect and elevation on xylem traits. On the northern side, cushions had larger vessels, higher percentage of conductive area and higher estimated conductivity at low elevation, whereas the inverse occurred on the southern side, where cushions were more hydraulically efficient at high elevation. This clearly suggests a response of vessel traits to the double climatic stress of Mediterranean dry mountains: stress by frost on the northern side constrained vessel size at high elevation, whereas stress by drought on the southern aspect led to cushions with smaller vessels at low elevation. Interactive effects of temperature and moisture in determining vessel size have been previously reported in a widespread tree species (García-Cervigón *et al.*, 2020) but, given their strong implications for potential shifts in species' distribution ranges, it deserves further exploration.

The effects of the interaction between aspect and elevation on single traits also suggest that the harshest sites for *A. tetraquetra* subsp. *amabilis* were the low site on the southern aspect (due to water limitation) and the high site on the northern aspect (due to frost). According to available evidence, we expected stronger trait co-ordination under these conditions (Dwyer and Laughlin, 2017; Zeballos *et al.*, 2017).

However, we found the opposite. Plant architecture, leaf traits and xylem anatomy varied independently in the most stressful sites, whereas we observed co-ordination between plant architecture and xylem traits at high elevation in the southern aspect and co-ordination between architecture and leaf traits at low elevation in the northern aspect (Supplementary data Fig. S6). Instead of supporting our hypothesis, our results seem in line with the idea that trait independence allows plants to better optimize survival and growth by investing differently in their form and function, as hypothesized for tropical trees (Baraloto *et al.*, 2010; Fortunel *et al.*, 2012). In our case, trait independence under harsher conditions might also imply that each subset of traits is controlled by different environmental drivers, which might allow for higher adjustment potential against future changes in environmental conditions. This hypothesis, however, needs to be further investigated.

#### *Independent variation of leaf traits*

Leaf traits did not differ between aspects, probably due to a compensation from shifts in plant architecture (see Table 3). Plants with more leaves per terminal branch, longer branches and higher LAI had larger leaves, and this covariation may explain the lack of response of leaf area on its own to environmental factors. The LMA, on the other hand, varied with elevation. This trait is related to maximum photosynthetic rates, stomatal conductance, leaf construction costs and leaf life span (Wright *et al.*, 2004). Higher LMA at low elevation might suggest that *A. tetraquetra* subsp. *amabilis* is following a more conservative strategy concerning resource uptake and use by producing leaves with higher construction costs but more resistance to drought (Schöb *et al.*, 2013). Otherwise, LMA was not correlated with the other traits related to xylem anatomy or plant architecture (see Supplementary data Table S2) and, in general, there was a lack of co-ordination between leaf and xylem traits. Co-ordination between leaf and stem traits has been documented among species (e.g. Méndez-Alonzo *et al.*, 2012) and within species (e.g. Trueba *et al.*, 2016), but the weakness or even the lack of co-ordination between them has also been reported (Baraloto *et al.*, 2010; Fortunel *et al.*, 2012; Silva *et al.*, 2017). Most of these studies focus on economic spectra, which define the variety of plant strategies aimed at optimizing resource acquisition and investment (Wright *et al.*, 2004; Chave *et al.*, 2009). In our case, LMA was a trait indicative of resource economy at the leaf level (the well-known leaf economics spectrum, Wright *et al.*, 2004), but the other traits we selected were more related to the hydraulic balance or to mechanical protection against physiological damage caused by high insolation, drought or freezing temperatures.

#### *Effects of beneficiary species on nurse trait variation and co-ordination*

Only LAI differed between cushions hosting or not hosting beneficiary species. The LAI results from the combination of

canopy compactness (branches per unit area), number of leaves per branch and leaf size. Although none of these traits varied significantly with the facilitation role of cushions, multiplying them to obtain LAI resulted in significantly lower LAI in nurse cushions, suggesting that lower canopy compactness might allow beneficiaries to establish and grow within the dense canopies of cushion plants. This is in agreement with previous studies showing that nurse traits underlie the magnitude of its facilitative effect (Michalet *et al.*, 2011; Schöb *et al.*, 2013) which, in our case, was reduced to a simple categoric classification (i.e. cushions were either nurses or not). Whether observed variations in LAI are a cause or a consequence of the presence of beneficiaries remains unknown, since our data did not allow us to test for it. It is probable that increased sample size would have resulted in significant differences in single traits other than LAI, but, since a destructive sampling was needed to access the root collar, we considered it inappropriate in this protected, endemic species. The most plausible explanation, however, for the lack of differences in single traits with a facilitation role is that climatic factors were much stronger than biotic factors as determinants of intra-specific variations in plant architecture, leaf function and xylem anatomy, as evidenced by the RDA (Table 2). Despite this lack of single-trait variation, differences in trait co-ordination did emerge between nurse and non-nurse cushions. Leaf traits and plant architecture were co-ordinated in non-nurse cushions, since plants with larger leaves also had larger branches, more leaves per branch and higher LAI. In nurse cushions, instead, architectural traits were co-ordinated with xylem traits in such a way that more compact cushions also had fewer leaves per terminal branch and more solitary vessels that appeared in lower density. This combination of traits (higher canopy compactness, fewer leaves per branch and fewer, more isolated vessels) is indicative of higher water stress and may suggest that some of the species growing within *A. tetraquetra* subsp. *amabilis* canopies are actually competing species, instead of beneficiaries. Another possibility is that beneficiaries are altering nurse water status, as previously documented for our cushion species (Schöb *et al.*, 2013, 2014), and this alteration may be constraining the possible combinations of values for architecture and xylem traits (Dwyer and Laughlin, 2017). In any case, these data support our hypothesis that the negative effects of beneficiaries (or competitors) on nurse water status are also reflected in nurse hydraulic traits.

### Conclusions

The crossed climatic gradients in Mediterranean dry mountains were strong drivers of functional responses in *A. tetraquetra* subsp. *amabilis* cushions. The crossed gradient drove variations in single traits and in the degree of co-ordination between different sets of traits, which also responded to biotic interactions. The fact that different sets of traits were more weakly co-ordinated in the harshest situations suggests a promising opportunity for this species to face the upcoming changes in climate. This might also enable Mediterranean alpine plant communities to persist under future increases in temperature and in drought intensity and frequency.

### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Method S1: details of processing of root collars and multiple factor analyses. Figure S1: climatic diagrams of the study area. Figure S2: cross-section of the root collar of an *Arenaria tetraquetra* subsp. *amabilis*. Figure S3: detailed cross-sections of the root collar of four *A. tetraquetra* subsp. *amabilis* individuals. Figure S4: age–size relationships in *A. tetraquetra* subsp. *amabilis* cushions. Figure S5: correlations between the variables of each sub-set and the MFA site scores on axes 1 and 2. Plots are displayed for individuals separated by aspect, elevation and facilitative role. Figure S6: correlations between the variables of each sub-set and the MFA site scores on axes 1 and 2. Plots are displayed for individuals separated by elevation at each aspect. Table S1: sample size for architectural, leaf functional and xylem anatomical traits of *A. tetraquetra* subsp. *amabilis*. Table S2: correlation matrix among architectural, leaf functional and xylem anatomical traits of *A. tetraquetra* subsp. *amabilis*. Table S3: average  $\pm$  s.e. values of architectural, leaf functional and xylem anatomical traits measured in four populations of *A. tetraquetra* subsp. *amabilis*.

### ACKNOWLEDGEMENTS

We thank the Sierra Nevada National Park for granting the sampling permissions, Adrián Escudero for useful comments on a previous draft, and Miguel García-Hidalgo for his invaluable help in processing root collars to analyse xylem anatomy.

### FUNDING

This work was supported by the Spanish Ministry of Economy and Competitiveness [projects CGL2012-34209 and CGL2017-87309-P to A.I.G.C. and J.M.O., project CGL2017-84515-R to F.I.P.]; Spanish Ministry of Science, Innovation and Universities [project PID2019-109906RA-I00 to A.I.G.C. and J.M.O., Juan de la Cierva-Incorporación grant IJCI-2017-34052 to A.I.G.C.]; Comunidad de Madrid [project REMEDINAL TE-CM (S2018/EMT-4338) to A.I.G.C.] and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) [Finance Code 001 to N.P.].

### LITERATURE CITED

- von Arx G, Dietz H. 2005. Automated image analysis of annual rings in the roots of perennial forbs. *International Journal of Plant Sciences* **166**: 723–732.
- von Arx G, Kueffer C, Fonti P. 2013. Quantifying plasticity in vessel grouping – added value from the image analysis tool ROXAS. *IAWA Journal* **34**: 433–445.
- von Arx G, Crivellaro A, Prendin AL, Čufar K, Carrer M. 2016. Quantitative wood anatomy – practical guidelines. *Frontiers in Plant Science* **7**: 781.
- Arzac A, García-Cervigón AI, Vicente-Serrano SM, Loidi J, Olano JM. 2016. Phenological shifts in climatic response of secondary growth allow *Juniperus sabina* L. to cope with altitudinal and temporal climate variability. *Agricultural and Forest Meteorology* **217**: 35–45.
- Baldocchi DD, Wilson KB, Gu L. 2002. How the environment, canopy structure and canopy physiological functioning influence carbon, water

- and energy fluxes of a temperate broad-leaved deciduous forest – an assessment with the biophysical model CANOAK. *Tree Physiology* **22**: 1065–1077.
- Baraloto C, Timothy Paine CE, Poorter L, et al.** 2010. Decoupled leaf and stem economics in rain forest trees. *Ecology Letters* **13**: 1338–1347.
- Barton K.** 2019. *MuMIn: multi-model inference. R package version 1.43.6*. <https://CRAN.R-project.org/package=MuMIn>.
- Blanca G, Baltasar C, Cueto M, Morales Torres C, Salazar C.** 2009. *Flora Vascular de Andalucía Oriental*. Sevilla, Spain: Consejería de Medio Ambiente, Junta de Andalucía.
- Brodersen CR, McElrone AJ, Choat B, Lee EF, Shackel KA, Matthews MA.** 2013. In vivo visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant Physiology* **161**: 1820–1829.
- Carvalho B, Bastias CC, Escudero A, Valladares F, Benavides R.** 2020. Intraspecific perspective of phenotypic coordination of functional traits in Scots pine. *PLoS One* **15**: e0028539.
- Castro-Díez P, Villar-Salvador P, Pérez-Rontomé C, Maestro-Martínez M, Montserrat-Martí G.** 1998. Leaf morphology, leaf chemical composition and stem xylem characteristics in two *Pistacia* (Anacardiaceae) species along a climatic gradient. *Flora* **193**: 195–202.
- Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA.** 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high Andes of central Chile. *Arctic, Antarctic, and Alpine Research* **39**: 229–236.
- Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE.** 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.
- Cornelissen JHC, Lavorel S, Garnier E, et al.** 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**: 335–380.
- Dayrell RLC, Arruda AJ, Pierce S, et al.** 2018. Ontogenetic shifts in plant ecological strategies. *Functional Ecology* **32**: 2730–2741.
- Delgado Calvo-Flores R, Delgado Calvo-Flores G, Párraga Martínez J, Gámiz Martín E, Sánchez Marañón M, Tenorio Urríos MA.** 1988. *Proyecto LUCDEME Mapa de Suelos, Güejar-Sierra 1027*. Granada, Spain: Universidad de Granada.
- Díaz S, Kattge J, Cornelissen JH, et al.** 2016. The global spectrum of plant form and function. *Nature* **529**: 167–171.
- Dwyer JM, Laughlin DC.** 2017. Constraints on trait combinations explain climatic drivers of biodiversity: the importance of trait covariance in community assembly. *Ecology Letters* **20**: 872–882.
- Fonti P, von Arx G, García-González I, et al.** 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* **185**: 42–53.
- Fortunel C, Fine PVA, Baraloto C.** 2012. Leaf, stem and root tissue strategies across 758 Neotropical tree species. *Functional Ecology* **26**: 1153–1161.
- García MC, Bader MY, Cavieres LA.** 2016. Facilitation consequences for reproduction of the benefactor cushion plant *Laretia acaulis* along an elevational gradient: costs or benefits? *Oikos* **125**: 434–442.
- García-Cervigón AI, Linares JC, Aibar P, Olano JM.** 2015. Facilitation promotes changes in leaf economics traits of a perennial forb. *Oecologia* **179**: 103–116.
- García-Cervigón AI, Fajardo A, Caetano-Sánchez C, Camarero JJ, Olano JM.** 2020. Xylem anatomy needs to change, so that conductivity can stay the same: xylem adjustments across elevation and latitude in *Nothofagus pumilio*. *Annals of Botany* **125**: 1101–1112.
- García Lino MC, Cavieres LA, Zotz G, Bader MY.** 2017. Carbohydrate reserves in the facilitator cushion plant *Laretia acaulis* suggest carbon limitation at high elevation and no negative effects of beneficiary plants. *Oecologia* **183**: 997–1006.
- Giorgi F, Lionello P.** 2008. Climate change projections for the Mediterranean region. *Global and Planetary Change* **63**: 90–104.
- Jaeger B.** 2017. *r2glmm: computes R squared for mixed (multilevel) models. R package version 0.1.2*. <https://CRAN.R-project.org/package=r2glmm>.
- Josse J, Pagès J, Husson F.** 2008. Testing the significance of the RV coefficient. *Computational Statistics and Data Analysis* **53**: 82–91.
- Le S, Josse J, Husson F.** 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* **25**: 1–18.
- Martínez-Vilalta J, García-Fórner N.** 2016. Water potential regulation, stomatal behavior and hydraulic transport under drought: deconstructing the iso/anisohydric concept. *Plant, Cell & Environment* **40**: 962–976.
- Martínez-Vilalta J, Cochard H, Mencuccini M, et al.** 2009. Hydraulic adjustment of Scots pine across Europe *New Phytologist* **184**: 353–364.
- Mencuccini M.** 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**: 163–182.
- Méndez-Alonzo R, Paz H, Cruz Zuluaga R, Rosell JA, Olson ME.** 2012. Coordinated evolution of leaf and stem economics in tropical dry forest trees. *Ecology* **93**: 2397–2406.
- Messier J, Lechowicz MJ, McGill BJ, Violle C, Enquist BJ.** 2017. Interspecific integration of trait dimensions at local scales: the plant phenotype as an integrated network. *Journal of Ecology* **105**: 1775–1790.
- Michalet R, Xiao S, Touzard B, et al.** 2011. Phenotypic variation in nurse traits and community feedbacks define an alpine community. *Ecology Letters* **14**: 433–443.
- Mihoč MAK, Giménez-Benavides L, Pescador DS, Sánchez AM, Cavieres LA, Escudero A.** 2016. Soil under nurse plants is always better than outside: a survey on soil amelioration by a complete guild of nurse plants across a long environmental gradient. *Plant and Soil* **408**: 31–41.
- Nonweiler TRE.** 1975. Flow of biological fluids through non-ideal capillaries. In: Zimmermann MH, Milburn JA, eds. *Encyclopaedia of plant physiology, New Series, Vol 1. Transport in plants. I. Phloem transport*. Berlin: Springer, 474–477.
- Oksanen J, Blanchet FG, Friendly M, et al.** 2019. *vegan: community ecology package. R package version 2.5-4*. <https://CRAN.R-project.org/package=vegan>.
- Olano JM, Almería I, Eugenio M, von Arx G.** 2013. Under pressure: how a Mediterranean high-mountain forb coordinates growth and hydraulic xylem anatomy in response to temperature and water constraints. *Functional Ecology* **27**: 1295–1303.
- Olano JM, González-Muñoz N, Arzac A, et al.** 2017. Sex determines xylem anatomy in a dioecious conifer: hydraulic consequences in a drier world. *Tree Physiology* **37**: 1493–1502.
- Pauli H, Gottfried M, Dullinger S, et al.** 2012. Recent plant diversity changes on Europe's mountain summits. *Science* **336**: 353–355.
- Pescador DS, Sierra-Almeida Á, Torres PJ, Escudero A.** 2016. Summer freezing resistance: a critical filter for plant community assemblies in Mediterranean high mountains. *Frontiers in Plant Science* **7**: 194.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team.** 2019. *nlme: linear and nonlinear mixed effects models. R package version 3.1-140*. <https://CRAN.R-project.org/package=nlme>.
- Pistón N, Schöb C, Armas C, Prieto I, Pugnaire FI.** 2016. Contribution of co-occurring shrub species to community richness and phylogenetic diversity along an environmental gradient. *Perspectives in Plant Ecology, Evolution and Systematics* **19**: 30–39.
- Pistón N, de Bello F, Dias ATC, et al.** 2019. Multidimensional ecological analyses demonstrate how interactions between functional traits shape fitness and life history strategies. *Journal of Ecology* **107**: 2317–2328.
- Pugnaire FI, Haase P, Puigdefabregas J.** 1996. Facilitation between higher plant species in a semiarid environment. *Ecology* **77**: 1420–1426.
- Pugnaire FI, Pistón N, Macek P, Schöb C, Estruch C, Armas C.** 2020. Warming enhances growth but does not affect plant interactions in an alpine cushion species. *Perspectives in Plant Ecology, Evolution and Systematics* **44**: 125530.
- R Core Team.** 2019. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Robert EMR, Koedam N, Beeckman H, Schmitz N.** 2009. A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*. *Functional Ecology* **23**: 649–657.
- Rumpf SB, Hübler K, Klöner G, et al.** 2018. Range dynamics of mountain plants decrease with elevation. *Proceedings of the National Academy of Sciences, USA* **115**: 1848–1853.
- Schöb C, Butterfield BJ, Pugnaire FI.** 2012. Foundation species influence trait-based community assembly. *New Phytologist* **196**: 824–834.
- Schöb C, Armas C, Guler M, Prieto I, Pugnaire FI.** 2013. Variability in functional traits mediates plant interactions along stress gradients. *Journal of Ecology* **101**: 753–762.
- Schöb C, Prieto I, Armas C, Pugnaire FI.** 2014. Consequences of facilitation: one plant's benefit is another plant's cost. *Functional Ecology* **28**: 500–508.
- Scholz A, Rabaey D, Stein A, Cochard H, Smets E, Jansen S.** 2013. The evolution and function of vessel and pit characters with respect to cavitation resistance across 10 *Prunus* species. *Tree Physiology* **33**: 684–694.

- Schreiber SG, Hacke UG, Hamann A. 2015.** Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree. *Functional Ecology* **29**: 1392–1401.
- Silva JLA, Souza AF, Caliman A, Voigt EL, Lichston JE. 2017.** Weak whole-plant trait coordination in a seasonally dry South American stressful environment. *Ecology and Evolution* **8**: 4–12.
- Sperry JS, Meinzer FC, McCulloh KA. 2008.** Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell & Environment* **31**: 632–645.
- Trifilò P, Barbera PM, Raimondo F, Nardini A, Lo Gullo MA. 2014.** Coping with drought-induced xylem cavitation: coordination of embolism repair and ionic effects in three Mediterranean evergreens. *Tree Physiology* **34**: 109–122.
- Trueba S, Isnard S, Barthélémy D, Olson ME. 2016.** Trait coordination, mechanical behavior and growth form plasticity of *Amborella trichopoda* under variation in canopy openness. *AoB Plants* **8**: plw068.
- Tyree MT, Sperry JS. 1989.** Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 19–38.
- Wright IJ, Reich PB, Westoby M, et al. 2004.** The worldwide leaf economics spectrum. *Nature* **428**: 821–827.
- Zeballos SR, Giorgis MA, Cabido M, Gurvich DE. 2017.** Unravelling the coordination between leaf and stem economics spectra through local and global scale approaches. *Austral Ecology* **42**: 394–403.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009.** *Mixed effects models and extensions in ecology with R*. New York: Springer.

