

ORIGINAL ARTICLE

# Divergent structural leaf trait spectra in succulent versus non-succulent plant taxa

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- **Background and Scope** Plant functional traits are the result of natural selection to optimize carbon gain, leading to a broad spectrum of traits across environmental gradients. Among plant traits, leaf water storage capacity is paramount for plant drought resistance. We explored whether leaf-succulent taxa follow trait correlations similar to those of non-leaf-succulent taxa to evaluate whether both are similarly constrained by relationships between leaf water storage and climate.
- **Methods** We tested the relationships among three leaf traits related to water storage capacity and resource use strategies in 132 species comprising three primary leaf types: succulent, sclerophyllous, and leaves with rapid returns on water investment, referred to as fast return. Correlation coefficients among specific leaf area (SLA), water mass per unit of area (WMA), and saturated water content (SWC) were tested, along with relationships between leaf trait spectra and aridity determined from species occurrence records.
- **Results** Both SWC and WMA at a given SLA were ~10-fold higher in succulent leaves than in non-succulent leaves. While SWC actually increased with SLA in non-succulent leaves, no relationship was detected between SWC and SLA in succulent leaves, although WMA decreased with SLA in all leaf types. A principal component analysis (PCA) revealed that succulent taxa occupied a widely different mean trait space than either fast-return ( $P < 0.0001$ ) or sclerophyllous ( $P < 0.0001$ ) taxa along the first PCA axis, which explained 63 % of mean trait expression among species. However, aridity only explained 12 % of the variation in PCA1 values. This study is among the first to establish a structural leaf trait spectrum in succulent leaf taxa and quantify contrasts in leaf water storage among leaf types relative to specific leaf area.
- **Conclusions** Trait coordination in succulent leaf taxa may not follow patterns similar to those of widely studied non-succulent taxa.

**Key words:** Leaf water content, leaf succulence, functional traits, specific leaf area, saturated leaf water content.

## INTRODUCTION

Economic spectrum theory dictates that plant traits evolve to optimize carbon gain given environmental constraints such as exposure to freezing, aridity and precipitation seasonality and are fundamental for modeling plant function and survival (Wright *et al.*, 2004; Chave *et al.*, 2009). As a consequence, variation in plant structural and functional traits is coordinated across broad environmental gradients (Fonseca *et al.*, 2000; Reich *et al.*, 2003; Wright *et al.*, 2005, 2017; Violle *et al.*, 2007; Díaz *et al.*, 2016; Kunstler *et al.*, 2016;

Dong *et al.*, 2020). Among these environmental constraints, aridity often determines plant survival and distribution and these limitations are becoming even more relevant as a consequence of global climate change (Intergovernmental Panel on Climate Change, 2018). Therefore, a more robust understanding of drought-stress coping strategies (both tolerance or avoidance) in plants is of considerable importance to determine plant adaptation, occurrence and distribution in different environments. Plants adopt a complex suite of morphological, anatomical and physiological traits that underlie plant water

use, water transport and growth form that collectively govern drought resilience (Curtis *et al.*, 2012; Lopez-Iglesias *et al.*, 2014 and references in it). Among these traits, internal plant water storage capacity plays a key role in buffering plants against soil water deficits and drought stress (Hartzell *et al.*, 2017; Choat *et al.*, 2018). The occurrence of specifically dedicated water storage parenchyma tissue is a highly effective survival strategy of xeromorphic succulent taxa: a group of terrestrial plants that thrive in many arid and semi-arid regions of the globe (Graham and Nobel, 1999; Eggle and Nyffeler, 2009; Males, 2017; Grace, 2019).

Water storage in leaves is one of the most morphologically distinct traits that have evolved in leaf-bearing plants to cope with drought-related stress, with leaf succulence having been described in 36 plant families (54 when considering families where succulence is of minor importance) (Nyffeler and Eggle, 2010), comprising 8 % of all vascular plants (Chase *et al.*, 2016). Succulent tissue usually consists of large, poorly lignified, thin-walled cells in which a large water-storing vacuole occupies up to 95 % of cell volume (von Willert, 1992; Gibson, 1996). Water-storing cells buffer the surrounding tissues from excessive water loss and play a primary role in maintaining photosynthetic gas exchange during periods with low available soil moisture (Barcikowski and Nobel, 1984; Nobel, 2006). Moreover, the water buffering capacity is likely involved in maintaining near-constant plant water potential, and thus protecting the water transport system (i.e. xylem) from cavitation and subsequent loss in axial hydraulic conductivity under water stress (Herrera *et al.*, 2000; Eggle and Nyffeler, 2009; Males and Griffiths, 2018). These unique adaptive features isolate succulent plants from external water deficits during extended periods of the growing season and, in some extreme cases, internal water storage can buffer plants from drought for several years (Hultine *et al.*, 2019).

Leaf water storage capacity, size and mass can vary by orders of magnitude among plant functional types, populations, and even within plants as a function of resource gradients and stress. Water storage can be quantified using indices based on leaf mass and volume. One of the most widely used metrics is the ‘degree of succulence’ initially proposed by Delf (1912) and hereafter called water mass per area (WMA,  $\text{g dm}^{-2}$ ) (Bartlett *et al.*, 2012). The WMA is the ratio of leaf water content at saturation to the leaf surface area, and it represents an area-specific measure of maximum leaf water storage. Another water storage index is the saturated water content (SWC,  $\text{g g}^{-1}$ ), a biomass-specific measure of leaf water capacity (Ogburn and Edwards, 2012) calculated as the difference between leaf mass at saturation and leaf dry mass divided by leaf dry mass. An additional index widely used to describe leaf morphological coordination with a host of other traits is specific leaf area (SLA,  $\text{cm}^2 \text{g}^{-1}$ ), defined as the ratio between leaf surface area and leaf dry mass (Wilson *et al.*, 1999). Although all of these leaf traits are highly integrated, there remains a remarkable paucity in our understanding of how these traits are coordinated among leaf functional groups, in particular in succulent-leaf taxa, despite being widely recognized for having broad ecological and socio-economic importance.

We analysed mature leaves from 132 species growing together in a common setting to address the following questions. First, do the leaves of leaf-succulent taxa follow trait

correlations along multiple trait spectra similar to those of non-leaf-succulent plants? Second, are the leaf trait indices WMA, SWC, and SLA similarly constrained by climate in leaf-succulent and non-leaf succulent taxa? We tested three inter-related hypotheses: (1) succulent leaf taxa occupy unique leaf structural trait space compared with non-succulent taxa; (2) unlike non-succulent leaves, SWC and WMA of leaf-succulent taxa scale independently of SLA, due to broad variation in the degree of succulence that should function independently of leaf thickness; and (3) the degree of succulence and hence SWC and WMA should increase with aridity while SLA in succulent taxa is largely invariant with respect to aridity. This is among the first studies to comprehensively apply a leaf trait spectrum across a broad suite of leaf-succulent taxa. Thus, the results of this study add to our understanding of the constraints that drive plant evolution over broad taxonomic scales and in response to climate variability.

## MATERIALS AND METHODS

### *Plant material*

To test our hypotheses, we considered a wide range of leaf typologies from plants native to a worldwide range of geographic locations and habitats (Figs 1 and 2, Supplementary Data Table S1). Sample collection was carried out at the University Arboretum in Legnaro (Padova, Italy) and in the botanical gardens of the Universities of Padova and Palermo (Italy). Padova is located in north-east Italy, with a total mean annual precipitation of  $796 \text{ mm year}^{-1}$  and a mean annual temperature of  $12.8 \text{ }^\circ\text{C}$ . Palermo is located in Sicily, southern Italy, with a total mean annual precipitation of  $488 \text{ mm year}^{-1}$  and a mean annual temperature of  $17.8 \text{ }^\circ\text{C}$  (KNMI Climate Explorer, <http://climexp.knmi.nl/>). Each sampled species was classified as succulent or non-succulent. We refer to non-succulent leaves as either sclerophyllous or non-sclerophyllous, with non-sclerophyllous leaves being defined as having a fast return edna on water investment in terms of carbon uptake. Leaves were univocally considered as succulent based on their appearance as thick, fleshy and rich in water when sectioned or compressed, and on bibliographical references (Sajeva and Costanzo, 2000; Eggle, 2001, 2002, 2003). Although the sclerophyllous classification usually refers to scleromorphic leaves from the Mediterranean region, we based this classification on leaves that were evergreen, coriaceous, tough and rigid. The sampled sclerophyllous and fast-return leaf-bearing plants were growing in the ground in the arboretum or in the botanical gardens either outside or inside of greenhouses, whereas the succulent species were all pot-grown and usually placed in greenhouses during the cold season.

Overall, our dataset includes leaves collected from 132 plant species belonging to 63 families (Table 1, Supplementary Data Table S1), including gymnosperm and angiosperm species, evergreen and deciduous, monocots and dicots. Growth forms encompassed free-standing plants, climbing and epiphytes, with plant heights ranging from 5 cm to 15 m.

We collected a minimum of three leaves per plant. When available in place, we collected the leaves from different plants depending on the availability of leaves, plant size and leaf size.

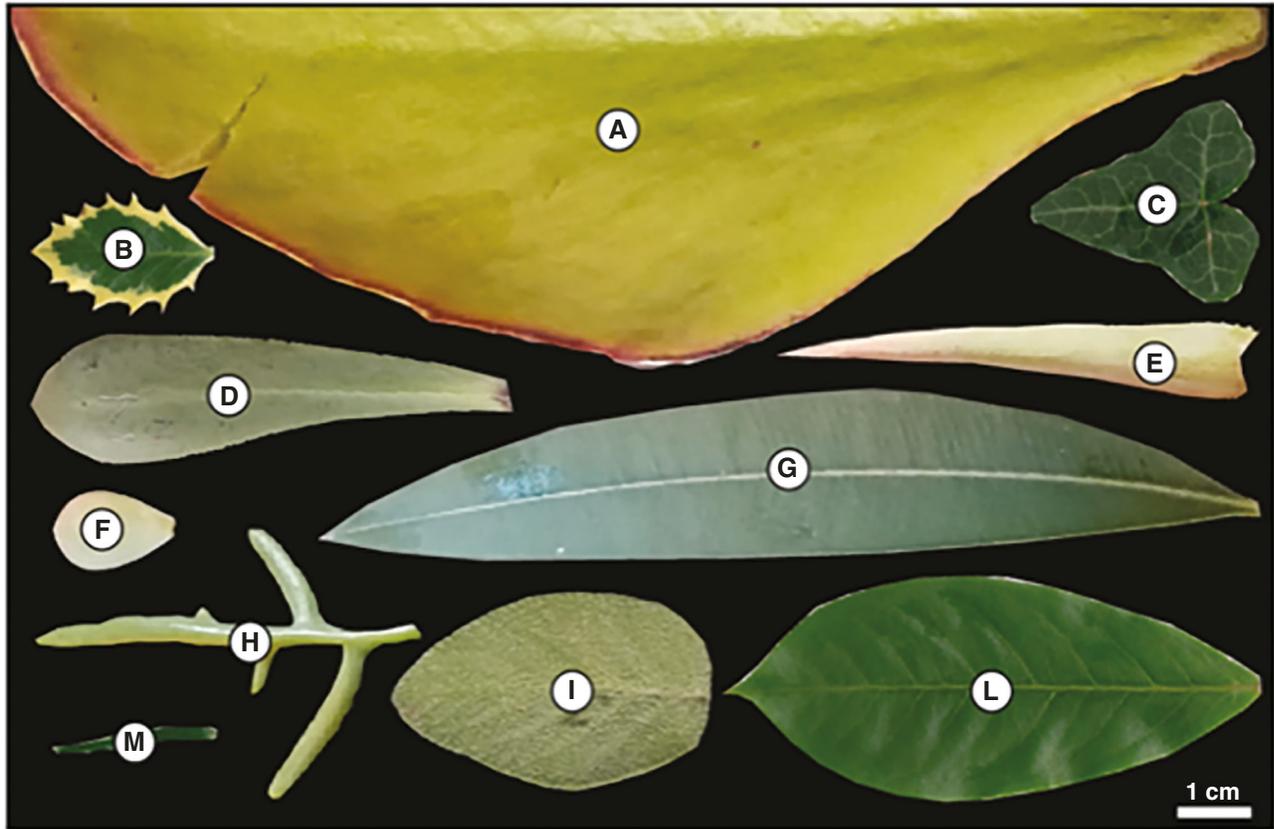


FIG. 1. Examples of leaf typology and morphology variation included in the dataset. (A) *Kalanchoe synsepala* (Crassulaceae), succulent herb. (B) *Ilex aquifolium* (Aquifoliaceae), sclerophyllous shrub. (C) *Hedera helix* (Araliaceae), fast-return climber. (D) *Aeonium percarneum* (Crassulaceae), succulent shrub. (E) *Aloe cremophila* (Xanthorrhoeaceae), succulent herb. (F) *Crassula perforata* (Crassulaceae), succulent herb. (G) *Nerium oleander* (Apocynaceae), sclerophyllous shrub. (H) *Kalanchoe laciniata* (Crassulaceae), succulent shrub. (I) *Salvia officinalis* (Lamiaceae), fast-return shrub. (L) *Prunus laurocerasus* (Rosaceae), sclerophyllous tree. (M) *Taxus baccata* (Taxaceae), sclerophyllous tree. Scale bar = 1 cm.

However, for exotic plants growing in greenhouses in the botanical gardens, often only one plant per species was available. All leaves came from plants cultivated in the arboretum and botanic gardens; therefore we collected plants native to a wide range of habitats from tropical to temperate (Fig. 2). Fast-return taxa were the most represented, with 49 different species, while 42 and 41 species were classified as succulent and sclerophyllous, respectively.

#### Leaf data collection

All collected leaves were mature and fully expanded. We only sampled sun leaves located in the outer part of the crown and generally avoided north-exposed leaves. After collection, leaves were stored in plastic bags filled with distilled water and kept at 4 °C for 48 h to achieve full hydration (Yamasaki and Dillenburg, 1999). The petiole was removed, and leaves were weighed to obtain the full hydration mass ( $M_s$ ). Next, each leaf was scanned and its projected, one-sided surface area ( $A_l$ ) measured using ImageJ imaging processing software (<https://imagej.net/ij/>, Schneider et al., 2012). Afterwards, each leaf was oven-dried at 70 °C for at least 48 h and weighed to obtain leaf dry mass ( $M_0$ ). With the collected data we calculated the SWC ( $\text{g g}^{-1}$ ) as  $(M_s - M_0)/M_0$ , WMA ( $\text{g dm}^{-2}$ ) as  $M_s/A$ , and

SLA ( $\text{cm}^2 \text{g}^{-1}$ ) as  $A/M_0$ , according to Delf (1912), Ogburn and Edwards (2012) and Pérez-Harguindeguy et al. (2016).

#### Species occurrence records and climate niche analysis

We obtained georeferenced occurrence records for the natural distribution of each from the Global Biodiversity Information Facility database (GBIF 2020). Taxonomic nomenclature was standardized using the WorldFlora package in R (Kindt, 2020). We eliminated duplicated georeferenced records, and visualized remaining records in ArcGIS to eliminate all the ones misplaced or occurring outside the coastal boundaries. The geographical distribution of each species was corroborated manually following the Kubitzki series (<https://www.springer.com/series/1306>) and online information platforms <https://tropicos.org>, <http://www.mobot.org/MOBOT/research/APweb/> and <https://powo.science.kew.org>. For 21 of the 132 species we studied, no occurrence records were located. For the remaining species, a range of a single record to >100 000 records were obtained (Supplementary Data Table S1), with a mean of 1900 records extracted for each species. Among the 111 species, all but 9 had at least 16 extracted occurrence records (Supplementary Data Table S1). Overall, succulent plants had fewer occurrence records relative to non-succulent plants,

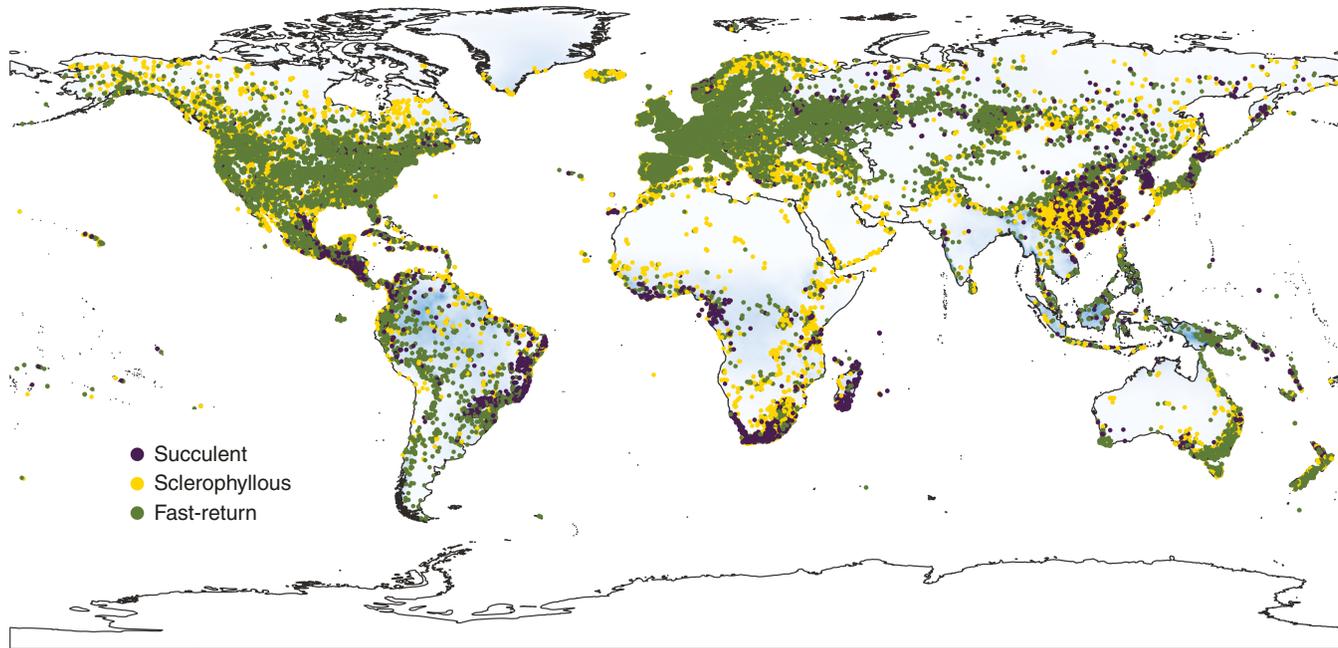


FIG. 2. Species occurrence records of all 132 species sampled for the study. Data were downloaded from the Global Biodiversity Information Facility (<http://www.gbif.org>).

TABLE 1. Number of unique families, genera and species of each leaf typology and mean values for projected leaf area ( $A_l$ ), SWC, WMA and SLA. Numbers in parentheses represent the standard error of the mean. Different letters represent different statistical groups among leaf typologies determined from Tukey's HSD tests.

	Succulent	Sclerophyllous	Fast return	All species
Number of unique families	10	22	30	62
Number of unique genera	20	30	41	91
Number of unique species	42	41	49	132
Leaf area ( $\text{cm}^2$ )	19.14 (5.95) <sup>a</sup>	60.98 (22.07) <sup>a</sup>	231.92 (55.24) <sup>b</sup>	111.12 (22.07)
SWC ( $\text{g g}^{-1}$ )	17.35 (1.17) <sup>a</sup>	2.01 (0.14) <sup>b</sup>	3.88 (0.18) <sup>b</sup>	7.58 (0.70)
WMA ( $\text{g dm}^{-2}$ )	10.71 (1.19) <sup>a</sup>	1.16 (0.07) <sup>b</sup>	1.03 (0.12) <sup>b</sup>	4.15 (0.54)
SLA ( $\text{cm}^2 \text{g}$ )	224 (22) <sup>a</sup>	188 (13) <sup>a</sup>	499 (33) <sup>b</sup>	315 (19)

averaging 75 records per species (Supplementary Data Table S1). For each occurrence record, we obtained the annual aridity index value as well as potential evapotranspiration sourced from the CGIAR-CSI Global Aridity and PET Database, Ver 3, constructed from monthly and yearly global hydro-climate data averaged from 1970 to 2000 with a spatial resolution of 30 arc-seconds (Zomer and Trabucco, 2022). The automatic data acquisition was performed using R package raster 3.6-26 (Hijmans *et al.*, 2015).

#### Statistical analysis

All statistical analyses were conducted in R version 4.3.0 (R Core Team, 2023), with  $P < 0.05$  considered the level of significance. Contrasts in  $A_l$ , SWC, WMA and SLA among leaf typologies were evaluated using one-way ANOVA tests followed by a Tukey's HSD tests. Leaf traits were log-transformed and

linear regression analyses were performed both among the leaf traits and between each leaf trait and the aridity index. All the relationships were fitted with a power function and plotted as a linear function along log–log axes. Regression analysis among leaf traits was first performed on each leaf class separately. If correlations between leaf traits were significant, we performed a linear model and analysis of covariance to test for differences in regression line slopes and intercepts among the different leaf classes. When leaf class regression line slopes were not significantly different, the leaf types were combined into a single linear regression. We conducted a principal component analysis (PCA) of leaf trait spectra (SWC, WMA, SLA) using the factoextra and the FactoMineR packages (Lê *et al.*, 2008; Kassambara and Mundt, 2017). We used a biplot to visualize trait representation based on the magnitude of the correlation (loadings) between traits and the given principal component. Thus, in each biplot, traits were represented as vectors with the

length and direction indicating the strength and trend of a trait's relationship among other traits. To assess the relationships in trait space among the three leaf types, we constructed 95 % confidence ellipses for each leaf type based on PCA scores of each of the leaf type means. Additionally, we performed one-way ANOVAs followed by Tukey's HSD tests to assess differences in PC axis scores among leaf types. Differences among the three leaf types and mean climate niche space, calculated from aridity index values for each species occurrence record, were evaluated with a one-way ANOVA followed by an HSD test. We also performed regression analyses testing correlations between PCA scores and leaf trait values of individual species with mean aridity index values calculated for each species.

## RESULTS

Among all species, the mean projected leaf surface area was  $111.12 \pm 22.07 \text{ cm}^2$  (Table 1). The largest leaf in our dataset ( $1422 \text{ cm}^2$ ) was from a horseradish (*Armoracia rusticana*) (Supplementary Data Table S1), while the smallest leaf ( $0.39 \text{ cm}^2$ ) was from a juniper (*Juniperus communis*) (Supplementary Data Table S1). Fast-return leaves showed the largest variability and had larger leaves than either succulent leaf or sclerophyllous leaf taxa (Table 1). Succulent leaves had a larger SWC and WMA (Table 1) and a larger variability in these traits relative to fast-return and sclerophyll species (Fig. 3A, B). Conversely, fast-return species had a larger SLA (Table 1) and showed the highest variability in SLA (Fig. 3C). The mean WMA values in fast-return and sclerophyllous species were similar (Fig. 3A). Similarly, the mean SLA values in succulent plants and sclerophyllous plants were not significantly different (Table 1, Fig. 3C). All paired comparisons among leaf types are reported in Table 1.

### SWC to WMA relationship

The relationship between SWC and WMA showed that water storage capacity per unit leaf area increased with water storage capacity per unit biomass investment in all leaf types (Table 2). Given that the relationships among leaf types were significant, we tested whether the slopes and intercepts among leaf types were independent or general. We found that the slopes were general but the intercepts differed among leaf types. We therefore pooled the regressions and found a positive and statistically significant correlation, accounting for 67 % of the variation ( $P < 0.0001$ ; Fig. 4A), suggesting there are similar constraints in water storage capacity across the different leaf types.

### SLA to SWC and WMA relationships

Specific leaf area is often used to describe gradients in plant resource use strategies and we tested the relationships between SLA, SWC and WMA (Fig. 4B, C). Saturated water content was not dependent on SLA (Fig. 4B) in succulent species. Conversely, SWC increased with SLA when considering non-succulent species ( $F = 62.3$ , d.f. = 89,  $P < 0.0001$ ,  $R^2 = 0.41$ ; Fig. 4B). Water mass per area decreased with SLA in all leaf types. However, the relationship between WMA and SLA in

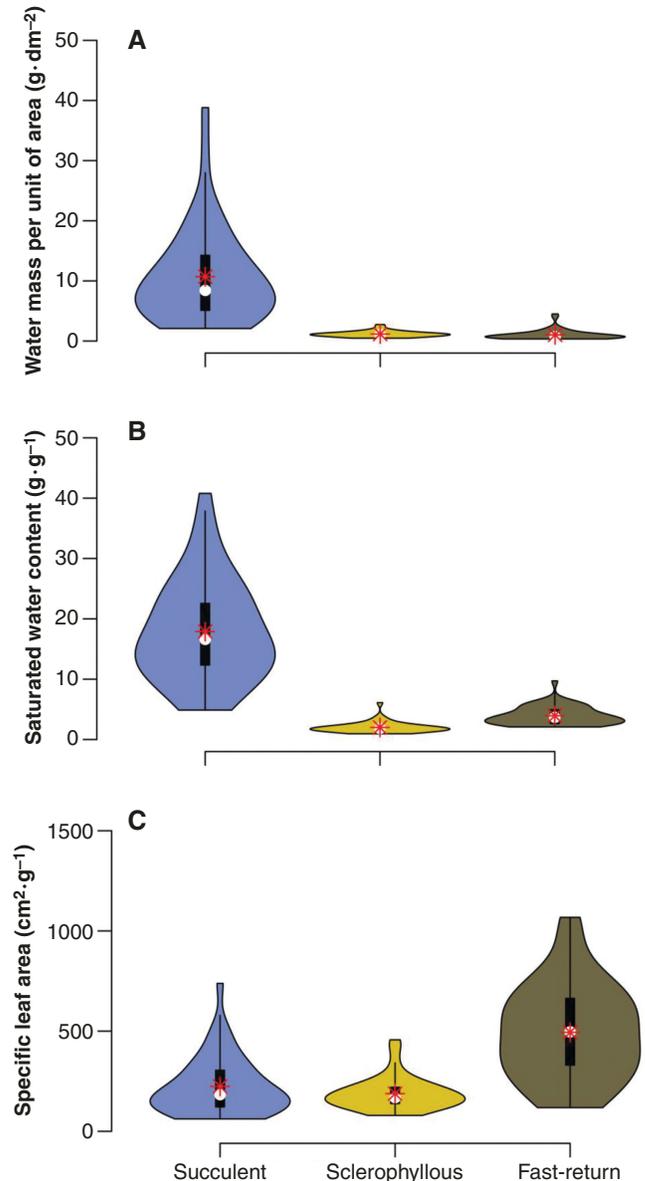


FIG. 3. Distribution of leaf trait indices for taxa with succulent, sclerophyllous and fast-return leaves. The red asterisk in each violin plot shows the index mean value per leaf class while the white dot represents the median value.

TABLE 2. Results from statistical models describing the relationship between WMA and SWC.

Leaf type	<i>F</i> -statistic	Degrees of freedom	<i>P</i> -value
Sclerophyllous	11.54	39	<b>&lt;0.005</b>
Fast-return	7.39	48	<b>&lt;0.01</b>
Succulent	14.83	40	<b>&lt;0.0005</b>
All leaves	271.00	131	<b>&lt;0.0001</b>

Bold text represents *P*-values of  $<0.05$ .

succulent species was independent of the other two leaf types, with 52 % of the variation explained ( $F = 43.3$ ,  $P < 0.0001$ ; Fig. 4C). The relationship between WMA and SLA was similar

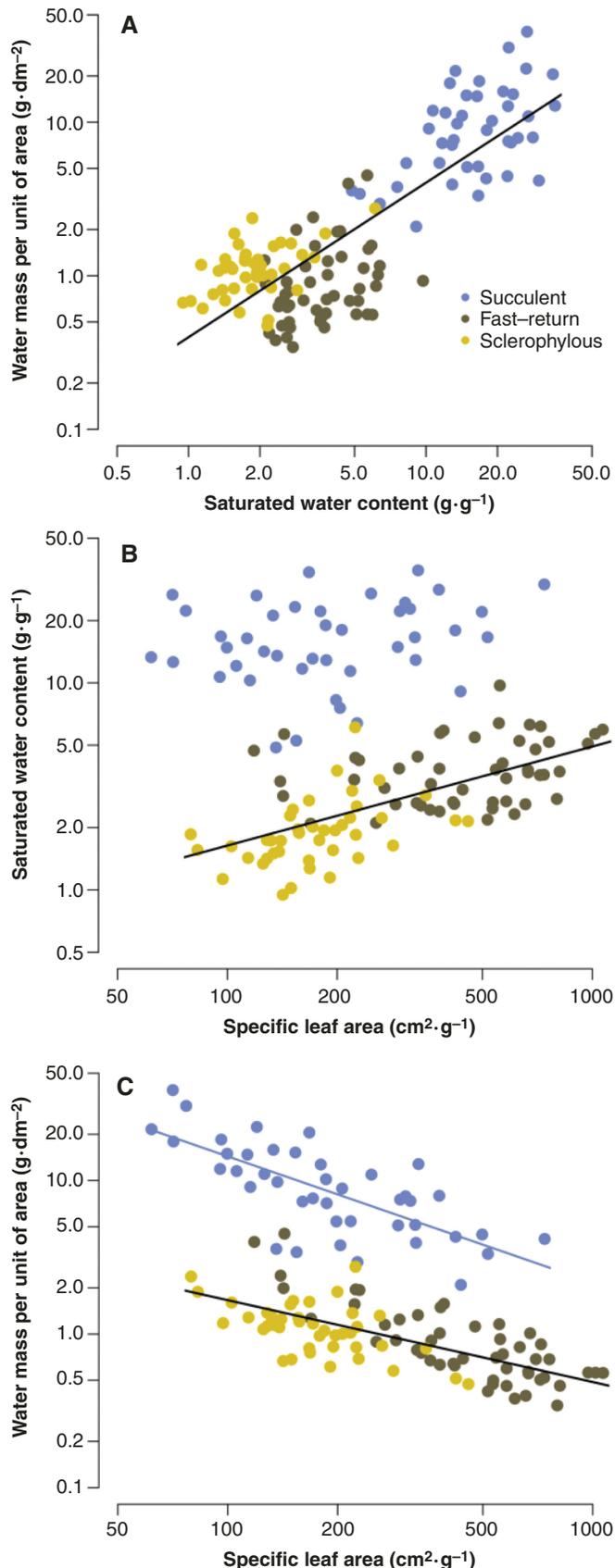


FIG. 4. Relationships between morphological leaf metrics in taxa with succulent, sclerophyllous and fast-return leaves fitted on log–log axes. (A)

in sclerophyllous and fast-return species, explaining 47 % of the variation ( $F = 79.2$ ,  $P < 0.0001$ ; Fig. 4C).

#### Principal component analysis

The first principal component (PC1) explained 63.1 % of the variation in leaf traits while PC2 explained 30.7 % of the variation in leaf traits (Fig. 5). There were sharp differences in leaf trait space along PC1 between plants with succulent leaves and plants with either sclerophyllous or fast-return leaves (Table 3), largely due to sharp contrasts in WMA and SWC among leaf types (Fig. 5). No differences in leaf trait space were detected along PC1 between sclerophyllous and fast-return leaves ( $P = 0.71$ ; Fig. 5, Table 3). Likewise, no contrasts in trait space were detected among leaf types along PC2, owing to the broad similarities in SLA among leaf types (Fig. 5, Table 3). Only one species, *Kalanchoe synsepala*, fell outside the 95 % confidence ellipses, owing to its very high WMA relative to other species ( $38.81 \text{ g dm}^{-2}$ ; Supplementary Data Table S1).

#### Correlations with climatic variables

The distribution of plants with succulent leaves ranged across aridity indices from an extremely arid value of 0.080 (unitless) in the succulent shrub *Aeonium percarneum* to a relatively mesic value of 1.826 in the vine *Hoya lacunosa*. The mean aridity index of all succulent plants in the survey was 0.623 (s.e.  $\pm 0.077$ ,  $n = 28$ ). The distribution of plants with sclerophyllous leaves ranged across aridity indices from 0.408 in the evergreen scrub oak *Quercus coccifera* to 2.076 in the evergreen shrub *Viburnum propinquum*. The mean aridity index of all sclerophyllous plants in the survey was 0.969 (s.e.  $\pm 0.056$ ,  $n = 39$ ). The distribution of plants with fast-return leaves ranged across aridity indices from 0.124 in the thorn-shrub *Fouquieria splendens* to 1.673 in the epiphytic fern *Asplenium nidus*. The mean aridity index of all fast-return plants in the survey was 0.887 (s.e.  $\pm 0.045$ ,  $n = 44$ ). Relatively strong contrasts in mean aridity scores were detected between plants with succulent leaves and plants with sclerophyllous leaves ( $F = 5.73$ ,  $P = 0.0003$ ), and between plants with succulent leaves and plants with fast-return leaves ( $F = 4.47$ ,  $P = 0.0058$ ). Conversely, no contrasts were detected in mean aridity scores between sclerophyllous and fast-return plants ( $F = 1.54$ ,  $P = 0.52$ ).

Within individual leaf types, no relationships were detected between either PC1 or PC2 scores and species mean aridity index. When all leaf types were pooled, PC1 scores were correlated with aridity index ( $F = 14.25$ ,  $P = 0.0003$ ), but only explained 12 % of the variation in PC1 scores (Fig. 6A). Conversely, no relationship was detected between the pooled PC2 scores and aridity index ( $F = 0.31$ ,  $P = 0.58$ , data

Relationship between WMA and SWC. The regression line was fitted for all the leaf types ( $F = 271$ ,  $P < 0.001$ ,  $R^2 = 0.67$ ,  $\text{WMA} = 0.3944 \text{ SWC}^{1.1015}$ ). (B) Relationship between leaf SWC and SLA of succulent, sclerophyllous and fast-return leaf types. The regression line was fitted for sclerophyllous and fast-return species pooled ( $F = 62.3$ , d.f. = 89,  $P < 0.0001$ ,  $R^2 = 0.41$ ,  $\text{SWC} = 0.1932 \text{ SLA}^{0.4658}$ ). (C) Relationship between mean leaf WMA and SLA of succulent, sclerophyllous and fast-return leaf types. The regression line was fitted for sclerophyllous and fast-return species pooled ( $F = 79.2$ , d.f. = 89,  $P < 0.0001$ ,  $R^2 = 0.47$ ,  $\text{WMA} = 19.418 \text{ SLA}^{-0.553}$ ) and for succulent species ( $F = 43.3$ , d.f. = 40,  $P < 0.0001$ ,  $R^2 = 0.52$ ,  $\text{WMA} = 633.3 \text{ SLA}^{-0.822}$ ).

not shown). When all data were pooled among leaf types, WMA and SWC were correlated with aridity index:  $F = 14.02$ ,  $P = 0.0003$ , Fig. 6B;  $F = 9.85$ ,  $P = 0.0022$ , Fig. 6C for WMA and SWC, respectively. However, the regressions only explained 11 and 8 % of the variation in WMA and SWC, respectively. No relationships were detected between SLA and aridity index, either within individual leaf types, or when all data were pooled (data not shown).

## DISCUSSION

Decades of research has illustrated that plant leaves operate within a spectrum of coordinated traits that are governed by environmental constraints on resource acquisition (Wright *et al.*, 2004, 2005; Reich, 2014; Dong *et al.*, 2020). Among these traits, SLA is widely recognized as a key indicator of resource acquisition and optimization strategy due to its correlation with

leaf longevity, construction costs and resource acquisition efficiency (Wilson *et al.*, 1999; Vendramini *et al.*, 2002; Poorter *et al.*, 2009; Grubb *et al.*, 2015; De La Riva *et al.*, 2016). However, results from this study indicate that succulent-leaved taxa do not follow constraints similar to those of non-succulent leaf taxa (supporting hypothesis 1), and are instead governed by biophysical constraints on leaf water storage as opposed to fast returns on resource acquisition. Specifically, across a wide range of succulent-leaf taxa, we found that traits related to leaf water storage, including SWC and WMA, did not follow relationships with SLA similar to those of non-succulent leaf taxa. As a consequence, succulent-leaved taxa may not follow classic leaf economic theory in ways other leaf-bearing plants appear to follow. To our knowledge, this study is among the first to compare trait correlations between succulent-leaf and non-succulent-leaf taxa, and thus provides new headway for understanding evolutionary constraints on the expression of leaf traits.

Along with providing a normalized quantification of succulence, leaf indices can be interpreted in terms of efficiency in carbon assimilation relative to water loss via transpiration (i.e. water use efficiency), sunlight interception and excess transpiration avoidance. Water mass per unit of leaf area (WMA) describes the geometric trade-off between leaf volume and its surface area, underlining leaf water storage capacity in relation to maximum transpiration rates. Saturated water content (SWC) quantifies succulence based on leaf dry mass and is closely correlated with leaf density and leaf thickness (Wilson *et al.*, 1999; Vendramini *et al.*, 2002). Saturated water content informs on carbon and water use strategies, quantifying the relationship between the amount of carbon invested in leaf construction and its capacity to store water (Vendramini *et al.*, 2002; Vile *et al.*, 2005). Specific leaf area (SLA) describes the expected return on previously allocated resources in leaf construction. Specific leaf area is well correlated with leaf photosynthetic capacity, leaf longevity and growth habit in non-succulent plant taxa (Wilson *et al.*, 1999; Wright *et al.*, 2004; De La Riva *et al.*, 2016). Combined, WMA, SWC and SLA describe the interaction among resource allocation, leaf morphology and water storage strategies and may be used to infer drought coping strategies.

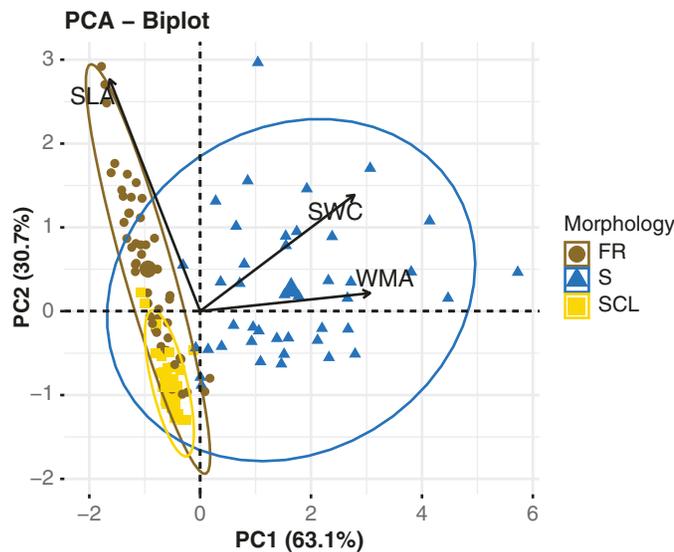


FIG. 5. PCA summarizing leaf trait spectra of taxa with succulent (S), sclerophyllous (SCL) and fast-return (FR) leaves. Ellipses represent the 95 % confidence intervals of each leaf type for PCA axes 1 (PC1) and 2 (PC2).

TABLE 3. Results of Tukey's HSD tests of trait scores from the PCA shown in Fig. 4, including differences in mean trait scores with 95 % lower and upper confidence intervals among leaf types.

Leaf type	Difference in means	Lower interval	Upper interval	<i>P</i> -value
Principal component 1				
Succulent–sclerophyllous	2.44	2.06	2.82	<b>&lt;0.0001</b>
Succulent–fast-return	2.32	1.95	2.68	<b>&lt;0.0001</b>
Sclerophyllous–fast-return	0.12	–0.25	0.49	0.71
Principal component 2				
Succulent–sclerophyllous	0.39	–0.10	0.89	0.39
Succulent–fast-return	0.39	–0.11	0.89	0.40
Sclerophyllous–fast-return	0.004	–0.51	0.52	0.99

Bold text represents *P*-values of <0.05.

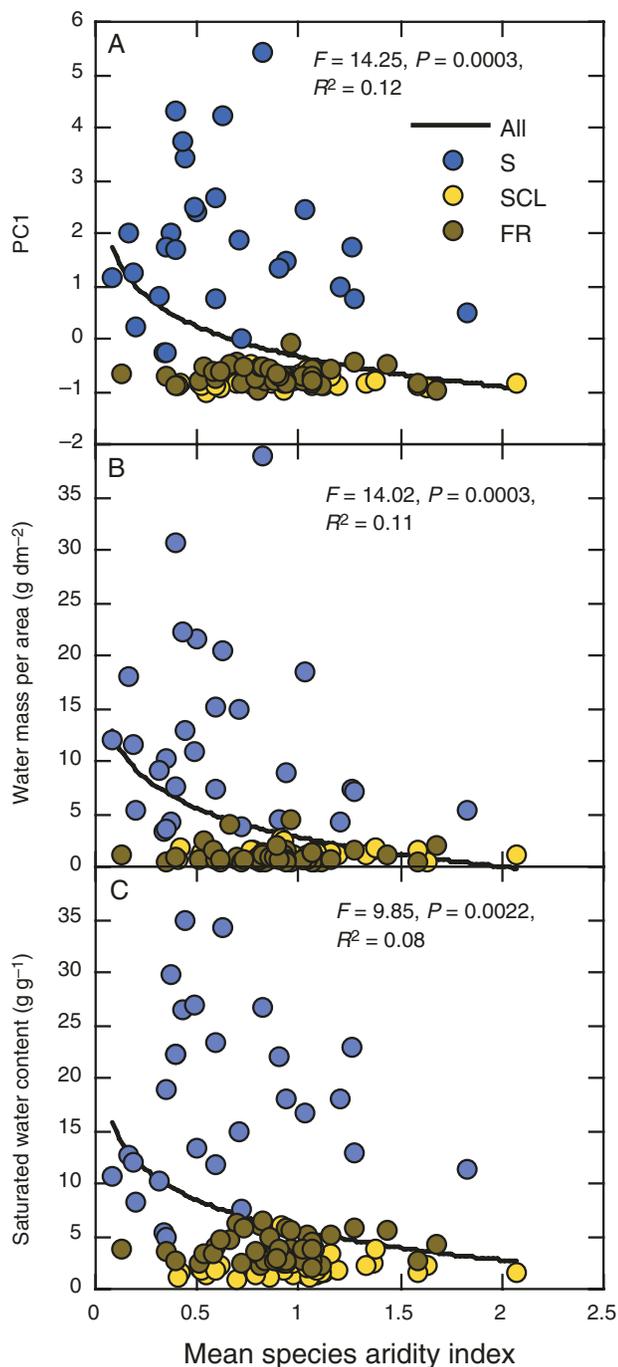


FIG. 6. Relationship between species mean aridity index and the distribution of leaf morphological traits in taxa with succulent (S), sclerophyllous (SCL) and fast-return (FR) leaves. (A) Relationship between PC1 scores (Fig. 5) and aridity index. (B) Relationship between leaf WMA and aridity index. (C) Relationship between leaf SWC and aridity index. All regression lines represent log-scale relationships between leaf traits and aridity index.

In ecological–evolutionary studies, the interplay of two or more organismal traits defines a morphospace that illustrates the shape and structure of an organism (Olson, 2012). The interplay between SWC and WMA in leaves is defined by the water storage capacity in relation to biomass allocation, light interception and transpiration avoidance. Succulents

occupy a unique region of morphospace relative to other leaf types (Figs 4 and 5), having very high water storage capacity corresponding with reduced surface area. At the opposite extreme of the morphospace are sclerophyllous leaves that also have reduced leaf area but with much lower water storage capacity. The patterns illustrated in Figs 4 and 5 are likely the result of two primary factors: mechanical constraints on leaf structure and selection of traits in response to environmental stress. Mechanical constraints (to maximize leaf toughness, for example) limit the possible combination of traits and thus limit the possible regions of morphospace that can be occupied (Olson, 2012; Olson and Arroyo-Santos, 2015). Likewise, adaptive pressures in response to environmental stress may select against specific trait combinations and as a consequence limit some regions of morphospace that a plant can occupy. We suggest that the empty regions in Fig. 4A, where no species are present, mainly represent developmental constraints, while the distribution of the different trait combinations in the occupied regions are mainly the results of trait selection in response to stress. According to this interpretation, the accessible region of the morphospace is well modelled by the regression line in Fig. 4A and the unique set of morphological and anatomical features allowed succulents to mainly occupy a unique morphospace, perhaps driven by selection to cope with seasonal water deficits.

Our results also show that SWC and WMA vary continuously, suggesting that criteria based on the two indices to define succulence may be difficult when considering contrasting taxonomically, morphologically and ecologically different species. The spectrum of these traits and the resulting morphospace are only visible when succulents are considered (Grubb *et al.*, 2015), highlighting the need to consider succulents when establishing a global leaf economic spectrum.

We found a positive and significant correlation between SLA and SWC (Fig. 4B) in non-succulent taxa. Among non-succulent species, fast-return leaves had higher water saturation per unit of mass and higher efficiency in light harvesting than sclerophyllous leaves. Sclerophyllous taxa often invest resources in defensive structures and transpiration-limiting traits, such as leaf pubescence, abundant sclerification, high cell-wall to cytoplasm ratio, heavily lignified tissues, thick epidermal cell walls and cuticles, epicuticular wax layer and dense vein networks (Bacelar *et al.*, 2004; Brodribb *et al.*, 2010; De Micco and Aronne, 2012; Peguero-Pina *et al.*, 2017). The combination of these traits increases resistance to leaf water deficits, making them more tolerant to dry periods and to drought events, and increases leaf carbon cost but reduces the water storage capacity per unit dry mass. On the other hand, SWC and SLA were not correlated in succulent leaves, meaning that leaf surface area does not constrain water storage capacity when normalized by leaf dry mass, similar to results reported by Grubb *et al.* (2015). For many succulent species, the main environmental limiting factor is water availability rather than competition for light. Therefore, succulent-leaved taxa maximize water storage capacity per unit carbon cost such that water-storing tissue construction is maximized regardless of the carbon cost for light interception.

Contrary to our expectations (hypothesis 2), we found that WMA was correlated to SLA in succulent taxa with a slope that paralleled relationships between WMA and SLA in

non-succulent taxa (Fig. 4C). In contrast to what we observed between SLA and SWC in succulents, water storage capacity normalized by leaf area decreased as the leaves became more acquisitive (higher SLA), having higher surface area, lower thickness and presumably lower carbon investment. However, succulents had approximately a 10-fold higher intercept than non-succulent taxa, illustrating that per unit SLA, WMA was ~10-fold higher compared with non-succulent taxa (Fig. 4C), underscoring the importance of water storage strategy and drought-related stress avoidance in succulents with respect to other leaf morphologies. The high WMA relative to sclerophyllous leaves is the result of succulent leaves having a higher water storage capacity relative to leaf size and morphology. Increased leaf thickness, low surface area to volume ratio and poorly lignified, highly elastic cell walls facilitate highly effective water storage in succulent-leaf taxa (Ogburn and Edwards, 2010; Griffiths and Males, 2017). The high cellular elasticity, particularly within the large vacuole in cells of succulent leaves is likely why relationships among SLA, WMA and SWC do not mirror relationships among these traits in non-succulent taxa.

Across all taxa, WMA and SWC were weakly correlated with increased aridity (Fig. 6), partially supporting hypothesis 3. This result was not surprising given that succulent-leaf taxa occupied a drier climate space than either sclerophyllous or fast-return plants. At first glance, it would appear that plants with a high degree of succulence are well adapted to prolonged water scarcity and may thrive in areas faced with increasing rainfall seasonality. However, no relationships were detected within the three functional groups between aridity and any of the leaf traits we measured. These results suggest that the degree of succulence in many taxa evolved independently from aridity exposure, and may instead be linked to a host of other factors, such as salinity (Ogburn and Edwards, 2010), the expression of CAM photosynthesis (Leverett *et al.*, 2023) or cell turgor regulation to support growth (von Willert, 1992). We caution that because, on average, succulent-leaf taxa had far fewer available occurrence records relative to non-succulent taxa (Supplementary Data Table S1), it is plausible that relationships between leaf succulence and aridity were either under- or overestimated.

In contrast to either WMA or SWC, no relationships were detected between SLA and aridity. These results stand in contrast with many classic economic trait spectra studies showing that SLA is constrained by climate across broad taxonomic groups. The lack of relationship between SLA and species climate envelopes likely illustrates the limits of common garden studies to evaluate functional trait niche space measured from a single location. Many leaf traits, including SLA, are highly plastic, with large spatial and seasonal variations often being detected within species and genotypes (Messier *et al.*, 2010; Cooper *et al.*, 2022). Despite these limitations, common garden studies, such as the one described in the present study, provide a robust approach to evaluate contrasts in trait expression among taxa in ways that can otherwise be confounded by a wide range of environmental factors.

In conclusion, to our knowledge, this study is among the first to explicitly compare leaf economic traits in succulent versus non-succulent taxa in a common garden setting. We found that leaf-succulent plants likely follow a different

economic spectrum that prioritizes water storage over optimizing an immediate return on leaf water investment. These results improve our understanding of the constraints that drive plant evolution and distribution and will ultimately improve predictions on how leaf-succulent taxa will respond to future climate conditions. Future studies should focus on exploring leaf traits in a larger and more diverse spectrum of succulent taxa and morphologies, including plants adapted to warm and cold arid regions of the globe, to further explore leaf succulence *per se* and its relationship with other leaf functional groups.

## SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following. Table S1: list of species, authority, family, leaf morphology, projected leaf area ( $A_l$ , cm<sup>2</sup>), leaf saturated water content (SWC, g g<sup>-1</sup>), leaf water mass per area (WMA, g dm<sup>-2</sup>), specific leaf area (cm<sup>2</sup> g<sup>-1</sup>), principal components scores along the first (PC1), and second (PC2) axes, total number of extracted occurrence records (OR), and mean aridity index (AI) of each species analysed.

## AUTHOR CONTRIBUTIONS

A.C. and G.M. designed the study; G.M. carried out the data collection; G.M. D.E.B. and M.V.C. carried out the data analysis; G.M., A.C., T.H.H., and K.R.H. contributed to the interpretation of results; and G.M., A.C. and K.R.H. wrote the manuscript with support from all other authors.

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