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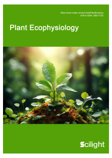

Article

Leaf Thickness as Key to the Contrasting Water and Nutrient Relations of Eight Arid-Climate Species, Including Water-Loss Resistance

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Abstract: Shrubs (families Asteraceae, Lamiaceae, Aizoaceae and Zygophyllaceae) in the succulent karoo of the southern Namib Desert survive an annual rainfall < 150 mm per annum but vary greatly in their vegetative morphology, so we hypothesised that they must possess a range of structural and physiological traits to resist perennial drought. Eight Namib species were assessed for their leaf structural properties [e.g., thickness (z), specific leaf area (SLA)], water storage capacity [e.g., relative water content (RWC)] and water potential (ψ) over 12 months or when severed from the parent plant in the field and laboratory, water-use efficiency (WUE) via $\delta^{13}C$ content, and metabolite (N, P) and osmotic ion (Na^+, K^+) contents. Four species were considered (1) orthophylls/semi-succulents, and (2) four were succulents (succophylls), (3), two of these exhibiting CAM-type photosynthesis, and (4) six with C3-type photosynthesis. Succophylls were distinguished by their thicker leaves, lower SLA, presence of water-storing parenchyma, higher levels of 'utilizable' water, slower rates of water loss, higher/less variable ψ, and higher ($Na^+ + K^+$), N and P contents/leaf-area, $\delta^{13}C$ and WUE. Water-loss resistance (WLR)—the change in RWC resulting from a given change in water potential ($\Delta RWC/\Delta\psi$) when subjected to drought conditions—was twice as high in the succophylls as the non-succulents under both laboratory and field conditions, with the latter showing twice the level of osmotic adjustment for a given drop in RWC. Leaves of the CAM species stored most water, decreased their ψ overnight, showed least rates of water loss, and had the highest N/P contents/area and WUE. Cations may serve an osmotic balancing function among succophylls, whereas high N and P/area may help maintain metabolic functions when transpiration is limited. $\delta^{13}C$/WUE relationships were functions of photosynthetic type, N/P contents/area, and (especially) leaf thickness. The opposing water relations of the four groups centre around their different trait combinations for accessing, utilizing and storing water. The special structural and physiological properties of succophylls need to be recognized when developing any general theory about the water relations of plants.

Keywords: leaf succulents; nitrogen and phosphorus contents; saturated leaf water content; SLA; water potential; water-use efficiency

1. Introduction

There is much interest in the ecomorphological properties of leaves as an adaptive response to the constraints of the plant's environment (Niinemets, 1999; Pyankov, Kondrachuk, & Shipley, 1999; Roderick et al., 1999; B. Lamont, Groom, & Cowling, 2002; B. Lamont et al., 2015; Wright & Westoby, 2002; Grubb et al., 2015; Wang et al., 2022; B. Lamont & H. Lamont 2025). With protracted hot, dry periods now occurring in the mid-latitudes due to climate change, the ecophysiological properties of sclerophylls and leaf succulents (succophylls), which enable them to cope better with drought than orthophylls (non-succulents), are receiving increasing attention (B. Lamont et al., 2015; Pérez-López, Lim, & Cushman, 2023; Yu et al., 2024). For example, succulent branches can retain water for some weeks or even months when severed from their supporting root systems (von Willert & Brinckman, 1986; Ripley et al. 2013).

Succophylls are present in all vegetated continents and are characteristic of the most seasonally hot and dry parts of Africa, the Americas and Eurasia in particular (Landrum, 2002; Males, 2017; Pérez-López, Lim, & Cushman, 2023). They have been identified in 80 families and are considered to account for ~13,000 species worldwide (Nyffeler & Eggli, 2010). However, few ecophysiological studies of leaves have included succophylls (B. Lamont & H. Lamont, 2025). This is not just unfortunate but potentially misleading, as it is usually anticipated that generalities determined for non-succulents will not apply to succulents (Roderick, Berry, & Noble, 1999b; Wilson, Thompson, & Hodgson, 1999). Vendramini et al. (2002) showed that some relationships between leaf properties depended on whether or not succulents were included in the sample, and Grubb et al. (2015) noted that the physical properties of their leaf succulents were quite different from the non-succulents.

We studied eight species, from semi-sclerophyllous to highly succulent, in the southern Namib Desert, South Africa. They were separated into four non-succulents and four succulents (succophylls) following the criteria of B. Lamont & H. Lamont (2025) and subdivided into those with C3- and CAM-type photosynthesis. The vegetation is part of the succulent karoo that Grubb et al. (2015) noted would form a useful comparison with their study in semi-arid Spain. B. Lamont & H. Lamont (2000) explored the concept of ‘utilizable’ water for the same set of species and showed that their water content on a mass basis (Q_M) at full turgor ranged over 42–93%, based on the level of bound (apoplastic) water. This approach was extended here to monitor changes in water content of severed branchlets in the laboratory and field as well as intact plants over the four seasons.

We first compare the water- and nutrient-storing properties of the eight species as a continuum, essentially as functions of their structural and physiological properties. We then compare the four succophylls with the four co-occurring orthophylls/semi-sclerophylls to determine if fundamental differences exist in their ecophysiology, as expected from Vendramini et al. (2002) and Grubb et al. (2015). Throughout, we are careful to distinguish whether traits were determined on a mass (M) or volume (V) basis (e.g., density: D_M, D_V). As two species possessed crassulacean acid metabolism (CAM) and six with C3-type photosynthesis, this created four groups based on their structural/physiological features. We were interested to see if the CAM species conformed to the expectation of greatest degree of succulence (Gilman & Edwards 2020) compared with the six species having C3-type photosynthesis and varying greatly in succulence.

While not necessarily high in inorganic cations, namely Na^+ and K^+ (a property of halophytic succulents, Winter et al., 1976), these might still have an osmotic function in promoting expansion of the cell vacuole as a structural-physiological basis for succulence, so were examined here. As a component of chlorophyll and protein, Roderick et al. (1999) also pointed to the functional value of nitrogen content (N) per unit water as a surrogate for cell volume, so we explore how N on a volume basis (N_V) varied with Q_V and z. N_A and P_A (nitrogen and phosphorus on an area basis) should indicate the assimilatory and metabolic potential of a leaf as it varies with leaf thickness (z) and have been shown to increase with increasing aridity (B. Lamont, Groom, & Cowling, 2002)—how then do these vary with leaf type? On the other hand, since increasing z in succulents, as a response to aridity, is due to the accumulation of a water-storing mesophyll rather than increased photosynthetic tissues (Nobel et al., 1994), we might expect N_A and P_A to remain stable.

Finally, extensive research shows how the carbon isotope discrimination ratio, $\delta^{13}C$, varies with rates/types of photosynthesis and transpiration that has allowed it to be used as an index of long-term water-use efficiency, WUE (Farquhar, O’Leary, & Berry, 1982, B. Lamont, Groom, & Cowling, 2002). But there is little evidence of the relevance of leaf properties (Hanba, Miyazawa, & Terashima, 1999). We therefore examine if any relationship exists between $\delta^{13}C$ and SLA, $D_{V/M}$, z, N/A, P/A and CAM. We also standardized the $\delta^{13}C$ values against an empirical data set that included CAM as well as C3 species (Winter, Aranda, & Holtum, 2005). We would expect WUE to rise as succulence increases and species adopt CAM-type photosynthesis (Teeri, Tonsor, & Turner, 1981; Maxwell, von Caemmerer, & Evans, 1997; Gilman and Edwards 2020). Overall, the aim here was to compare a wide range of ecophysiological properties of co-occurring species varying greatly in their water-storing properties to understand the various ways that shrub species may adapt to perennial aridity.

2. Theory

The volume of a leaf is made up of the product of the projected area (A) and mean thickness (z) (B. Lamont et al., 2015). Leaf turgid mass (M) consists of its dry mass (D) plus saturated water content (Q). Dry mass and water content can be related to turgid mass (D_M and Q_M respectively). They can also be related to turgid volume, V (D_V and Q_V respectively). Specific leaf area (SLA) is given by A/D and corresponds to the inverse product of D_V and z (Witkowski & B. Lamont, 1991). Here, we use an index of water-storage, $Q/A = Q_V \cdot z$ (Delf, 1911; Cowling & Campbell, 1983; B. Lamont & H. Lamont, 2025) for making comparisons. Thus, we were interested in how water-storage capacity varied between the eight species and how this related to other physical and physiological properties of the leaves in relation to drought attenuation and metabolic activity. Water retention implies that stomatal conductance is low so we concentrated on special features of succulents that should be more relevant, such as thick leaves and CAM rather than guard-cell processes (Buckley, 2017).

‘Capacitance’ can be defined, in the most general terms, as the ability of a structure to store something without a marked change in its condition. Thus, thermal capacitance among leaves refers to their ability to absorb heat without an undue rise in their temperature that might threaten their viability (Groom et al., 2004). The ease with which leaves may store water in relation to a decrease in their water potential has been called their 'bulk water-storage capacitance' (Blackman & Brodribb, 2011). These authors elaborated the formula to relativize it against leaf mass-area (D/A = LMA) and standardize by multiplying by the turgid water-dry mass ratio (Q_{100}/D), an index of succulence (Ripley et al., 2013). This reduces to $C = \Delta\text{RWC}/\Delta\psi\cdot Q_{100}/A$. That is, C is the change in relative water content (RWC) associated with the change in water potential (ψ) multiplied by the index of succulence, Q_{100}/A. Blackman & Brodribb (2011) noted that this expression was correlated with LMA, but this must be so mathematically, since LMA (D/A) is included in the formula and it is therefore a case of comparing confounded variables (M. Williams, B. Lamont, & He, 2022).

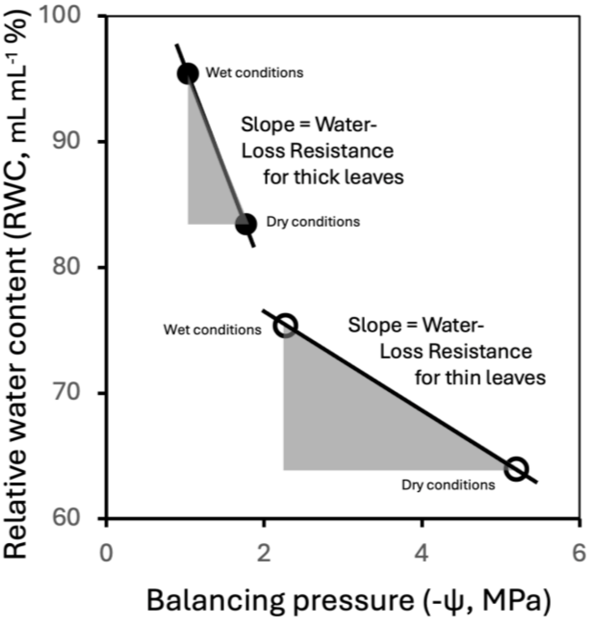

Figure 1. Expected relationship between leaf water potential (ψ) and relative water content (RWC) of branchlets under moist and dry conditions for species with thick or thin leaves, whose slope gives water-loss resistance (WLR). Note that a large decrease in ψ (osmotic adjustment) is associated with the same decline in relative water content under drought conditions by the non-succulent species (lower line) compared with the succulents (upper line).

Guo et al. (2024) expressed their index of capacitance as $C = \Delta Q/\Delta\psi\cdot D^{-1}$ based on a curve fitting technique. Compared with the Blackman & Brodribb (2011) formula, this is equivalent to $\Delta\text{RWC}/\Delta\psi\cdot Q_{100}/D$. The problem with using Q_{100} in both formulas is that two possible reasons exist for C being high: (a) the leaves are large, or (b) their relative water content is high. Dividing by A (Blackman & Brodribb 2011) or D (Guo et al. 2024) attempts to reduce that effect but this then adds a term for succulence (Q_{100}/A), making the expression no longer independent of it. So, their formulas are unsuitable for our purposes, since we were interested in how C varies with succulence via its component, leaf thickness, z. Here, we extract the term, $\Delta\text{RWC}/\Delta\psi$, from both expressions and call it *water-loss resistance* (WLR). We note that this term was also treated as an index of capacitance (C') by von Willert et al. (1992). RWC is recognized as a suitable index of the level of drought stress to which the leaf is subjected (Richards & B. Lamont, 1996). So, the extent of osmotic adjustment ($\Delta\psi$) required to produce a fixed level of water loss (ΔRWC) is a measure of the relative resistance to water loss, as the major component of water-storage capacitance. We expected that the thickest leaves would have the highest WLR (Figure 1) as they have the highest RWC (B. Lamont & H. Lamont, 2000) and highest ψ under field conditions (Eccles et al., 2001).

3. Materials and Methods

3.1. Study site and species

The study site was located at Groenriviersond, 500 km north of Cape Town, South Africa (30° 51′ S, 17° 34′ E). This location lies in the southern portion of the Namib Desert. Rainfall averages 140 mm pa but was 79 mm in the year of the study (1998), although fog and dew are regular occurrences. The vegetation is part of the succulent karoo and consists of clumps of creepers to woody shrubs up to 2 m tall (Eccles et al., 2001). The soil is red aeolian sand overlying an impenetrable silcrete hardpan at about 2 m depth.

Eight indigenous species were selected to cover the full range of apparent water-storing properties at the site, with leaf thickness (z) ranging 0.97 to 5.23 mm, SLA ranging 2 to 20 mm^2 mg^{-1}, and leaf succulence (turgid water content on a leaf area basis, Q_A) ranging 0.43 to 4.80 mg mm^{-2} (B. Lamont & H. Lamont, 2025, Tables 1 and S1). On this basis, four species were considered non-succulents ($Q_A < 0.9$ mg mm^{-2} after B. Lamont & H. Lamont 2025), with two orthophylls (lowest water content, $Q_A < 0.4$ mg mm^{-2}) and two semi-succophylls ($Q_A = 0.4$–0.9 mg mm^{-2}), and four leaf succulents (succophylls, ($Q_A > 0.9$ mg mm^{-2}) (Table 1). The numbers assigned there are used throughout and nomenclature is as given in Eccles, Esler, & Cowlinget. (1999), except that Mesembryathemaceae became Aizoaceae, and *Zygophyllum* became *Roepera* following recent taxonomic revisions. For clarity, only the genus names are used as all species are in different genera. The photosynthetic type (6 C3 species, 2 CAM species) was based on the winter δ^{13}C values (C3: $\delta^{13}\text{C} < -24$, CAM: $\delta^{13}\text{C} > -22$), of three field-collected plants per species (B. Lamont & H. Lamont, 2000), plus continual measurements of ψ over 24 h during drying of branchlets in the laboratory over three days (C3: ψ stabilizing overnight, CAM: ψ greater fall overnight). Leaves of all species, but *Salvia*, were iso(bi)lateral and sessile. Five species possessed water-storing hydrenchyma. Of most importance, the leaf properties of the eight species studied formed a well-defined gradient whose range was comparable with, and often exceeded, values previously obtained for the structural and physiological indices examined here (B. Lamont & H. Lamont, 2025).

Table 1. Leaf shape, anatomy and photosynthesis type for the eight species studied here. The associated numbers are used for identification among some graphs. Orthophyll, semi-succophyll = designated non-succulent in results, succophyll = leaf succulent. Additional references: B. Lamont & H. Lamont (2000, 2025).

Species	Family	Leaf Shape	Tissue Arrangement	Outer Mesophyll	Inner Mesophyll	Leaf Thickness (mm)	Leaf Type /Photosynthesis	References
1. *Pteronia onobromoides*	Asteraceae	Linear, subulate	Isobilateral	Double-layered palisade, both sides	Thick, cell width 5–10 µm, chloroplasts, no large airspaces	1.2	Orthophyll/C3	Hulley et al. 2010
2. *Salvia lanceolata*	Lamiaceae	Lanceolate, slightly swollen	Bifacial	Double-layered palisade, adaxial side only	Thick, cell width 20 µm, chloroplasts, large airspaces	1.0	Orthophyll/C3	Özdemir, Baran, & Aktas, 2009
3. *Eriocephalus africanus*	Asteraceae	Acicular	Isolateral	Double-layered palisade, entire perimeter	Thin, cell width 15–20 µm, chloroplasts, no large airspaces	1.3	Semi-succophyll/ C3	Jordaan and Kruger 1992
4. *Stoeberia utilis*	Aizoaceae	Linear, rounded sub-cylindrical	Isolateral	Triple-layered chlorenchyma, entire perimeter	Thick, no chloroplasts, dense hydrenchyma, cell width 45–55 µm	4.2	Succophyll/CAM	Melo-de-Pinna et al. 2014
5. *Ruschia fugitans*	Aizoaceae	Linear, trigonous	Isolateral	Triple-layered chlorenchyma, entire perimeter	Thick, no chloroplasts, dense hydrenchyma, cell width 45–55 µm	5.3	Succophyll/CAM	Melo-de-Pinna et al. 2014
6. *Roepera morgsana*	Zygophyllaceae	Orbicular, swollen	Bifacial	Unlayered chlorenchyma, adaxial side	Dense hydrenchyma-like chlorenchyma, cell width 45–55 µm	2.0	Semi-succophyll/ C3 (potentially CAM)	Lauterbach et al. 2016
7. *Othonna cylindrica*	Asteraceae	Acicular, club-shaped	Isolateral	Unlayered chlorenchyma, entire perimeter	No chloroplasts, dense hydrenchyma, cell width 100–200 µm	2.3	Succophyll/C3	Cicuzza et al. 2018
8. *Senecio* aff. *sarcoides*	Asteraceae	Acicular	Isolateral	Unlayered chlorenchyma, entire perimeter	No chloroplasts, dense hydrenchyma, cell width 100–200 µm	3.1	Succophyll/C3	Cicuzza et al. 2018

3.2. Water potential estimation

Current season's mature branchlets (100–150 mm long) were removed from side branches of 6–8 plants of each species by cutting under water predawn in late August (winter). They were transported to Stellenbosch University where they were kept in an air-conditioned laboratory with their ends in water at 17.5–20.5 °C and covered with plastic bags for 1–4 days to ensure full hydration. Half the branchlets were recut under water and their pressure-volume relations determined (following the protocol of Radford & B. Lamont, 1992). The balancing pressure (P) was achieved with a digital pressure chamber, model 1003, PMS Instruments, Corvallis, OR, USA. Wet weight values of branchlets were extrapolated to P = 0 in order to obtain saturated mass for estimates of water content at full turgidity. All leaves were removed from the stems and, together with ten mature, full-sized leaves, were weighed, frozen at −16 °C to rupture the cells and hasten drying, dried at 72 °C for 48 h and reweighed. From this, leaf turgid water content was determined on a turgid mass basis [$Q_D = Q/(Q + D)$] (see Abbreviations for meanings of symbols) after correcting for stem contribution to the values.

The remaining branchlets were allowed to dry on a bench in the laboratory at 17.5–20.5 °C and relative humidity of 40–50%, after B. Lamont & H. Lamont (2000) and Ripley et al. (2013). This was to test the hypothesis that the succulents would have superior control of water loss when severed from their root systems to highlight the differences in their water relations and for determining water-loss resistance. They received fluorescent light above and weak sunlight from large windows at the side during the day. Pressure-volume data were collected, noting the time at each assessment until the leaves had wilted and used to calculate ψ_{100} (water potential at full turgor), ψ_{tlp} (water potential at the turgor loss point, tlp) and bound water content (B, water in the apoplast no longer available to the plant and used to calculate utilizable water content, UWC_B—see B. Lamont & H. Lamont (2000). Harvest mass/dry mass (X axis) was plotted against the square root of time for each pressure-volume run (to produce a linear relationship). The best-fit equation was used to estimate time to reach B. Means ± SE were determined for ψ at 1 h after the start of a run, over the first night (12 h) and after five days (final). *Water-loss resistance* (WLR) was based on RWC at full turgor minus RWC at tlp compared with ψ_{100} minus ψ_{tlp}. It can be expected that the greater the decrease in RWC for a given decrease in ψ, the higher its WLR (see Theory section).

3.3. Leaf structure

As a key structural property, midpoint thickness of 10 leaves from three plants was determined with calipers. Projected area (A) was obtained by placing 30 leaves or more diagonally on the conveyor belt of an area meter (LI-COR 3000, Lincoln, NK, USA). Adjustments were made for the shape of leaves and their volume (V) determined geometrically (B. Lamont et al., 2015): five were cylindrical ($V = \pi/4z \cdot A$) where z is diameter, two were laminate ($V = z \cdot A$ where z is thickness) and one was subulate (V = mean $z \cdot A$), all lacking midribs or exposed veins. SLA [$A/M = 1/(D_V \cdot z)$, where D = dry leaf mass and D_V = dry leaf density on a volume basis (Witkowski & B. Lamont 1991)] was adjusted for leaf shape in the same way, and D_V and Q_V (dry matter and water mass per unit leaf volume) were based on these measurements.

3.4. N, P, K, Na and C isotope estimation

In order to examine their chemical properties, the 10 dry leaves from the physical analyses above were bulked and 0.5 mg samples analysed for total P, K and Na by digesting with nitric acid and analysing by inductively coupled plasma spectroscopy (WAITE Labs, Adelaide, South Australia). Total N was determined similarly but using standard Kjeldahl digestion and titration. Other 0.5 mg samples were analysed in duplicate for the stable isotopes ^{12}C and ^{13}C and the standardized ratio, $-\delta^{13}C$, determined (Farquhar, O'Leary, & Berry, 1982). A mass spectrometer (Finnigan MATT 252) was used after tissue combustion in a Fisons CHN analyser under continuous flow and compared against the PDB standard (Ehleringer & Osmond, 1989). The two sub-samples for each analysis were bulked for statistical purposes.

For some comparisons, $\delta^{13}C$ was inserted into an actual water-use efficiency (WUE) curve produced for 12 *Clusia* species (Winter, Aranda, & Holtum, 2005) that included a number of succulents with CAM-type photosynthesis. Two of our species possessed CAM so were compared against this part of their curve. This overcomes the problem that $\delta^{13}C$ is not only affected by stomatal conductance and leaf structure (B. Lamont, Groom, & Cowling, 2002) but also by the type of photosynthesis (Winter, Aranda, & Holtum, 2005) to produce a standardized index (SWUE). As $\delta^{13}C$ responds to growing conditions independent of photosynthesis type, this means that the SWUE data are unlikely to be identical to their actual WUE values but will be proportional to them. Thus, this served to show that a given $\delta^{13}C$ for a CAM species produces a higher WUE than a C3 species at the same $\delta^{13}C$ (Winter, Aranda, & Holtum, 2005). To enable comparisons between water status and $\delta^{13}C$ data for C3 and CAM species in Ripley et al. (2013), their Q/D values were converted to % water content $[Q/(Q + D)]$ and thence UWC_B, using the equivalent graph in B. Lamont & H. Lamont (2000), assuming that their Q was at saturation.

3.5. Fieldwork

For field work, the balancing pressure ($-\psi$) was determined predawn (3.30–5.30 am) and in the early afternoon (1–3 pm) on 10–15 cm long branchlets from three plants of each species. These were weighed at once, or placed in plastic bags and weighed within 2 h. Their dry mass was determined as noted above. Absolute water contents were calculated as Q_V or Q_M. Relative water content (RWC) was calculated as water content on a mass basis (Q_M) at harvest /Q_M at saturation, as a %. Q and ψ assessments were conducted in spring (November, 70 mm rain in the current and previous month), summer (January, no rain in the current or previous month), autumn (no rain in the current or previous month) and winter (July, 46 mm in the current and previous month). The data were divided into the four succophylls and four orthophylls for the two diurnal measurements and RWC compared against $-\psi$. Linear slopes were fitted to the 4×3 values per leaf type per time-of-day per season to give WLR under field conditions.

3.6. Statistics

Pairwise comparisons of attributes of interest were made using standard best-fit curves (linear, exponential, logarithmic, power) as calculated by the Microsoft® Excel for Mac graphing programs (Version 16.94, 2025). As directional relationships were expected from theory, a one-tailed test was used, although the type of curvilinearity was not known, allowing for choice of the one with the highest R^2 value. The R^2 outputs were converted to p values using https://www.danielsoper.com/statcalc/calculator.aspx?id=44 (6 February 2024). Solid lines were drawn to indicate significant relationships and dashed lines when the trends were not significant at $p < 0.05$. Analyses of variance, t-tests, Tukey's tests, and calculation of means and standard errors were conducted with VassarStats programs (http://vassarstats.net/, Richard Lowry 1998–2023, accessed 6 February 2024). Actual p values are stated for all correlations and the best-fit equation cited where of interest.

4. Results and Discussion

4.1. Whole leaf physical and chemical components

Figure 2 shows increasing leaf thickness (z) was associated with an escalating increase in leaf dry mass (D, weak relationship and moderate slope) and water volume per leaf (Q, strong and steep) but none with leaf area, A (Figure 2). Although starting from a high base, total cations ($Na^+ + K^+$) increased strongly with increasing z at a similar slope to Q. Total N and P increased at a rate below that of Q but above that of D. Collectively, these slopes and base values mean that the protoplasts were enlarging at 10 times the rate of the cell walls as the leaves thickened by five times (as illustrated in Figure 2). P increased at four times the rate of N that increased at a similar rate to dry mass, whereas the cations only increased by 60%.

From Table 1, the parenchyma cells of the studied succophylls are up to 20 times thicker than the sampled orthophylls/semi-succophylls, and the walls are typically 25% thinner (e.g., four times increase in volume but three times increase in wall material) (Males, 2017; B. Lamont & H. Lamont, 2025). Although the cation levels are much too low for the succophylls to be treated as halophytes (they clearly remain in the glycophyte class), they appear to have an osmotic balancing function (Winter et al. 1976) and might help to explain their ability to absorb extra water, provided the walls remain plastic enough during cell swelling. Nevertheless, they clearly became diluted as succulence increased. This contrasts with halophytes that accumulate cations to produce low ψ via osmotic adjustment that increases stomatal control and assists in soil water absorption. The raw data in Figure 2 have been relativized and grouped in Figure 3 that shows a progressive increase in z from orthophylls, C3 species, succophylls and CAM species, although the first two are grouped according to Tukey's test and the last two are grouped.

SLA (=A/D) changes in the opposite direction to z that, at face value, infers a reduction in photosynthetic potential among the succophylls, especially those with CAM. But A does not decrease among the succophylls (Figure 2), and, since the formula also corresponds to $(D_V \cdot z)^{-1}$, most of the decline in SLA must be due to increasing z (which must more than counteract the decrease in D_V, B. Lamont and H. Lamont 2000). As greater z might imply greater light-capturing ability, the net effect on photosynthesis is actually unclear. Q_A, as the best index of succulence (B. Lamont & H. Lamont, 2025), increases strongly across the four leaf types, although the orthophylls and C3 species are grouped, as are the succophylls and CAM species. Since $Q/A = Q_V \cdot z$, it can be expected that Q_V will rise as Q_A increases. This is confirmed in Figure 3d, although its slope is much lower than for z and it tends to asymptote rather than be linear. From these trends, it at first appears possible to use z as a surrogate for Q_A. The problem is that z also increases among sclerophylls (b. Lamont et al., 2015) so that inclusion of RWC, via Q_V, is essential to counteract the density effect, for sclerophylls are characterized by high dry density as well as z (Witkowski & B. Lamont, 1991; Groom & B. Lamont 1999). This paradox probably explains why Roderick, Berry, & Noble (1999b), who only examined orthophylls, instead obtained a negative relationship between Q_V and z.

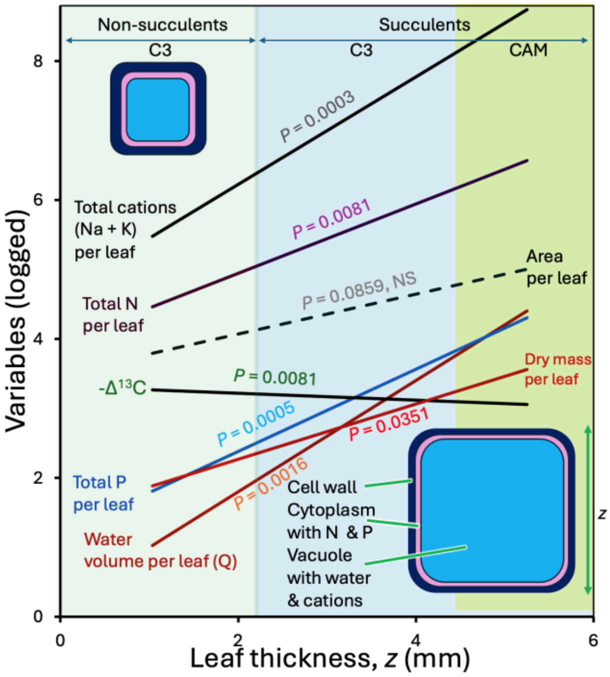

Figure 2. Total contents (logged) per leaf of physical and chemical components for eight co-occurring arid species varying in succulence in relation to leaf thickness, z. All relationships are based on linear fits to mean values for each species and their p values are given beside each line. Regions of succulence/non-succulence and C3/CAM are shown in different colours. Area per leaf has a 8.6% of occurrence by chance alone so is indicated by a broken line ($p > 5\%$). The two figures show locations of the components in relation to cell structure and highlight how they all increase exponentially with increasing z, with water and cation contents rising at a greater rate. To reduce confusion, data points have been removed but are available on request.

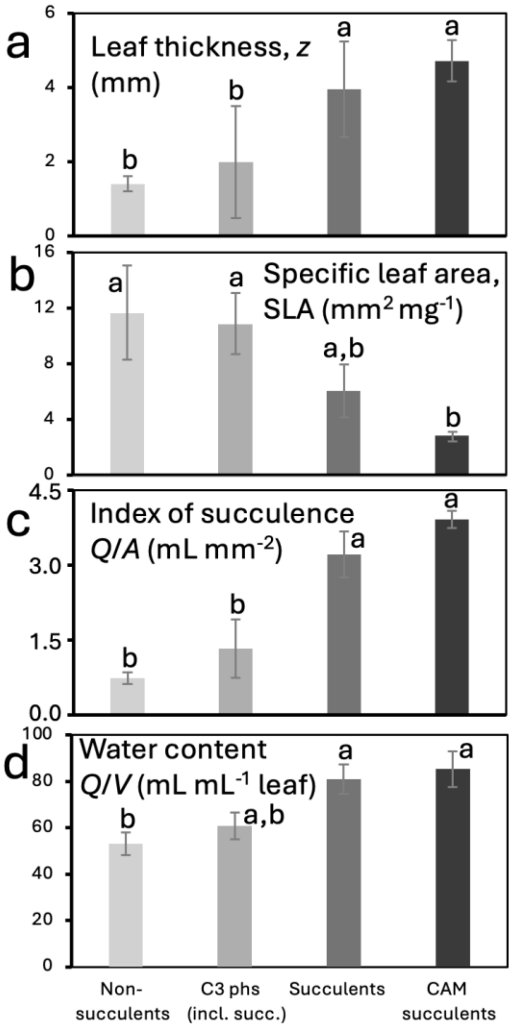

Figure 3. Some physical properties (mean ± standard error, SE) separated into the four leaf types recognized here: 4 non-succulents (C3 orthophylls, semi-succophylls), 6 C3 non-succulents and succulents, 4 succulents (C3, CAM succophylls), and 2 CAM succulents in relation to (**a**) leaf thickness, z, (**b**) specific leaf area (SLA), (**c**) Q_A at full turgor, and (**d**) Q_V at full turgor as a %. Bars are placed in order of increasing z indicated by their increasing darkness. Letters attached to SEs for each column are the result of Tukey's multiple-range test, different letters indicating differences significant at $p < 0.05$.

4.2. Water relations relative to leaf thickness, *z*

Figure 4 highlights the strong and steep relationship between Q_A and z, although logarithmic, i.e., Q_{100}/A at full turgor tends to asymptote as z continues to increase, essentially because Q_V approaches 100% water. As expected from Figure 2, a strong logarithmic increase in Q_V exists with increasing z. Although equally significant, water content on a mass basis Q_M is much less steep, essentially because of a trade-off between water and dry matter. The steepest and most significant increase is with UWC_B. This is likely because more water is bound to the thicker cell walls of the thinner leaves, so reducing available water content (B. Lamont & H. Lamont, 2000). As the leaves thicken, the water-storing cells of the parenchyma (especially hydrenchyma) become engorged with water and the apoplast and air spaces becomes relatively small

(B. Lamont & H. Lamont 2025), ensuring more water is available for physiological processes. All four indices of water content are strongly correlated with *z* and hence with each other, and agree with the results for nine halophytic, semi-succulent shrubs studied by Guo et al. (2024). This means that any differences in unavailable (apoplastic) water between species play little role in affecting the general patterns. Although ψ_{tlp} increased exponentially with increasing *z*, $p = 0.0975$ means that some doubt exists about its statistical significance. ψ at full turgor also tended to increase exponentially and the difference between the two declined with increasing *z*, but the slope was not statistically significant.

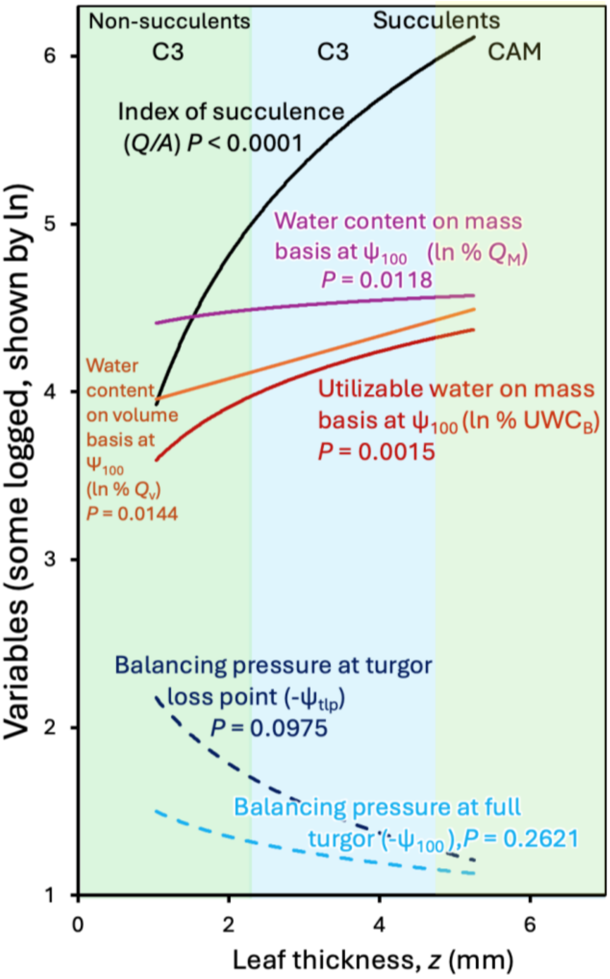

Figure 4. Three indices of water contents (Q_V, Q_M, UWC_B), some logged (shown by ln), at full turgor (ψ_{100}), and ψ at turgor loss point (tlp), for eight co-occurring arid species varying in degree of succulence ($Q/A = Q_A$) in relation to leaf thickness, *z*. All relationships are based on the best curvilinear fits to mean values for each species and their *p* values are given beside each line. Regions of succulence/non-succulence and C3/CAM are shown in different colours. The broken line for ψ_{tlp} is because of the 9.8% probability that the relationship occurs by chance alone, while that for $-\psi_{100}$ has a 26% probability of non-significance.

Figure 5 shows the consequences of severing branchlets from the parent, leaving them on the laboratory bench for some days and monitoring their water relations over that time. A steep exponential decline in rate of water loss with increasing *z* is clear. This was accompanied by an equally steep and significant rate of decline in ψ, but tending to asymptote more sharply. As a result, time to reach B increased strongly but tended towards an asymptote with increasing *z*. ψ declined at a much lower rate overnight and less steeply. This was not because temperatures were lower, as the laboratory was air-conditioned, but more likely because stomatal conductance was reduced. The exception were the species with thickest leaves that gained -ψ overnight (the CAM species, discussed further under Section 4.7).

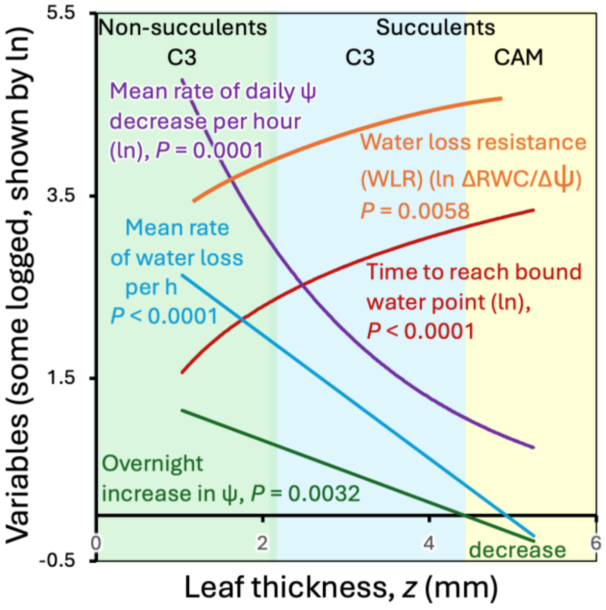

Figure 5. Water relations of eight co-occurring arid species varying in succulence in relation to leaf thickness, *z*, and time. Three branchlets per species left on the laboratory bench were monitored for up to five days. All relationships are based on the best curvilinear fits to mean values for each species and their *p* values are given beside each line. Regions of succulence/non-succulence and C3/CAM are shown in different colours. Rates of water loss and decrease in ψ, time to reach bound water content (B) and overnight change in water potential (ψ) were all highly significant. Note that the CAM species could be identified by their decrease in ψ overnight. See Figure S2 for the units applicable to each variable.

4.3. Water-loss resistance (WLR)

As the major component of capacitance (*C*) and not confounded by structural properties (see Theory section), WLR increased logarithmically (when unlogged the slope was steeper) with increase in leaf thickness, *z* (Figure 5). Had the terms Q_A or Q_D been retained, then the outcome would have been confounded by the fact that each of these contains the variable *z* so that they would have been correlated by definition (M. Williams, B. Lamont, & He, 2022). WLR had a similar steep logarithmic slope to *z* as the index of succulence, Q_{100}/A (Figure 4), and justifies its exclusion from the formula. The slope of WLR is opposite to the rate of water loss but almost parallel with time to reach bound water, B. To the extent that comparisons are possible, *C* for the nine halophytic, semi-succulent shrubs studied by Guo et al. (2024) were not correlated with time to reach turgor loss point, which is the equivalent range for our calculations of WLR, but the sclerophyllous trees studied by Gleason et al. (2014) in temperate climates were so correlated. Guo's index of succulence, Q_{100}/A (the same as ours), was also correlated with their index of C, but this is not unexpected because of the confounding effects already noted. Despite their conclusion to the contrary, we show that such relationships are not confined to non-arid environments—in fact, it is not surprising that arid species with the highest WLR (CAM succophylls) take the longest time to desiccate.

4.4. Water relations in the field over four seasons

Figure 6 highlights how the water relations of the four succulents (succophylls) contrast with those of the four non-succulents (orthophylls) under their natural arid environment. Key features are (a) the wide range in relative water content (RWC) and water potential (ψ) over the four seasons for the orthophylls compared with the succophylls, (b) the wide diurnal range for the orthophylls compared with the succophylls that declines from winter-spring to autumn among the former and is negligible among the latter, and (c) both leaf types remain below the turgor loss point during summer-autumn. These results are consistent with marked water loss by the orthophylls compared with succophylls in the laboratory study (Figure 5). As expected, the dry summer-autumn is most 'stressful' for both leaf types but far less so for the succulents. As a result, a strong interaction effect is expressed, with the winter-spring orthophyll data tending to group just beneath the succophyll data (see Table S2 for the ANOVA results). That is, the strong decline in ψ and RWC among the orthophylls in summer-autumn dictates the pattern.

Slopes of the relationships for the two main leaf types (ΔRWC/Δψ) in Figure 6 correspond to water-loss resistance (WLR). Their difference (9.58 vs. 4.90) is the same (twice) as for the laboratory droughting study so that their relative properties are maintained under field conditions. However, values are 7.2–7.9 times lower (Figure S2). This means that WLR greatly depends on the environmental circumstances under which it is assessed. The slowly applied drought conditions of summer-autumn appears to have allowed substantial osmotic adjustment to occur, resulting in a fall in ψ > 7 times greater than in the five-day laboratory study, for a given drop in RWC. Richards & B. Lamont (1996) observed a similar pattern among deep-rooted seedlings under severe summer-autumn drought in Australia. Thus, succophylls also adjust osmotically to drought but only in a minor way. Although removed from the parent plant, the retention of utilizable water among the succulents in contrast to the orthophylls over 6 weeks in the field (Figure S3e) is consistent with the differences in WLR between the two groups (Figure 6) and implies a much slower rate of water loss, as also recorded under laboratory conditions.

The time-to-lose-all-water trend was essentially a function of leaf thickness (Figure 5) rather than whether the species was designated as succulent or not, or CAM or not, although the CAM succophylls had the thickest leaves. Linton and Nobel (2001) showed that, as they were deprived of water, the thicker-leaved *Agave* species was more conservative in its ψ drop, hydraulic conductance and transpiration than the thinner-leaved species. Similar results were obtained by von Willert et al. (1992) for severed shoots left in the field that they attributed to lower xylem conductivity of the succulents. These studies are consistent with ours for the WLR data under field conditions (Figure 6). Thus, succophylls achieve drought attenuation via efficient control of water loss (drought avoidance) not by possessing tissues that can tolerate extremely low ψ levels (drought tolerance), as, say, in halophytes (Guo et al. 2024).

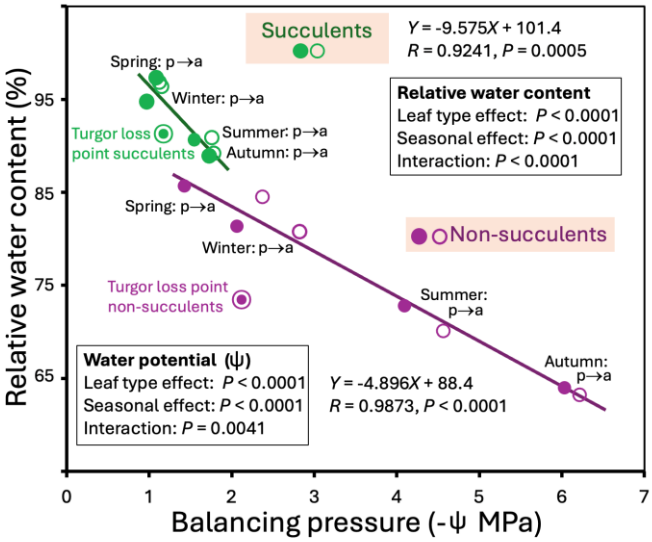

Figure 6. Relative water content versus balancing pressure (negative ψ) for the four succulent and four non-succulent species grouped separately (see Table 1 for identity), for field measurements on intact plants taken over the four seasons. Each point is the mean for 12 values (4 species × 3 replicates) with each plant assessed predawn (filled circles, p) and again (→) in the early afternoon (open circles, a), as shown. The turgor loss points (where cell wall pressure = 0) for both groups are also indicated. The slope of the best-fit lines gives water-loss resistance (WLR), and the equations show that WLR for the succulents is twice that for the non-succulents. The boxed data refer to the results for 2 leaf types × 2 seasons (winter-spring combined, summer-autumn combined) × 2 diurnal measurements interaction ANOVA with 4 replicates per treatment. Note for both leaf types that they spend summer-autumn below the turgor loss point as determined in the laboratory study, giving it little survival significance.

4.5. Water relations relative to photosynthesis type

Figure S3 separates these data into their four leaf types and shows that it is the orthophylls/C3 species which have the greatest rates of water loss (Figure S3a), reduction in ψ, both overall (Figure S3b) and overnight (Figure S3c), compared with the succophylls/CAM species. Of particular interest are the contrasting times to reach B between the orthophylls/semi-succophylls (~100 h to reach B), succulents (including the CAM species, ~1000 h) and just the CAM species (~1500 h) (Figure S3d). Clearly, by opening their stomata during the cool, more humid nights rather than during the hot, dry daylight hours (Figures S1 and S3c), this increases the water-retentive capacity of these species enormously (Gilman & Edwards 2020). The severe restriction on whole leaf conductance under the increased drought stress expected by severance from the parent plant casts a different, and arguably more critical, perspective on the water relations of succulents than concluded by Ripley et al. (2013) for a similar assemblage of species from a related succulent flora. Instead, they emphasised how mesophyll conductance of succulents was little affected by similar severance of branches from the parent plant. That might be so, but it is the observed low rates of loss of stored water that is the critical trait for survival (Figure S3). This is shown by the data on water-loss resistance (Figure S2). In support, Miller (1985) showed that one of the non-succulent C3 species studied here, *Eriocephalus africanus*, failed to close its stomata fully until ψ fell to −7 MPa.

That *Stoeberia* and *Ruschia* (Aizoaceae) exhibited CAM was demonstrated independently of their δ^{13}C values (Section 4.7) by their ψ decreasing at a faster rate overnight (Figure 7c), especially *Stoeberia* which cycled over a range of 0.5 MPa for each of the three nights as ψ declined gradually from −0.9 MPa to −1.6 MPa (Figure S5). Similar diurnal fluctuations among CAM species were observed by Winter et al. (1976). This confirms that the stomata were closed during the day but open at night with the concentration of osmotic molecules increasing as CO_2 fixation proceeded (via HCO_3^- and thence malate$^-$, Gilman & Edwards 2020). Bowie (1999) showed how this species can switch from C3 to CAM photosynthesis after 14 days of no watering (i.e., facultatively CAM). Interestingly, the decrease in ψ among three C3 species at night (*Pteronia*, *Salvia* and *Eriocephalus*, unpublished) indicated that their stomata were at least partly open then (assuming cuticular transpiration was not significant) but that this was not counterbalanced by any net CO_2 fixation. All four succulent species have abundant hydrenchyma (Table 1) consistent with species capable of CAM, but in the case of the C3 species, CO_2 uptake might have been so low that isotopic analyses could not detect this type of photosynthesis (Winter and Holtum 2002). On ψ-cycling grounds, and higher -δ^{13}C (Section 4.7), no signs existed of CAM among the other two succophylls, even though they were droughted in the laboratory over five days, but had we examined these physiological properties in summer-autumn rather than winter then CAM might have been expressed.

Ripley et al. (2013) found that none of their nine Aizoaceae species exhibited CAM and attributed this to the confinement of photosynthetic tissues to just beneath the epidermis such that mesophyll conductance was not reduced under water stress. The two highly succulent Asteraceae examined here, *Othonna* and *Senecio*, also have a similar anatomy (Table 1) but did not show nightly pulses of decrease in ψ that signal the presence of CAM (stomata open, fixation of CO_2). It is worth noting that the plants used by Ripley et al. (2013) were well-watered nursery stock and that their mean δ^{13}C values were 4‰ lower than ours (greater discrimination against the heavier isotope). Despite their conclusion to the contrary, it appears that growing conditions were not stressful enough to induce CAM in their Aizoaceae species (except for one species not mentioned by them). CAM appears to be confined to species with a middle cortex of achlorophyllous parenchyma with large water-storing cells (hydrenchyma) (Tables 1 and 2; Herrera, 2020). Even so, whether such leaves exhibit CAM depends on how water-stressed the plants are, and (possibly) independently, their degree of succulence.

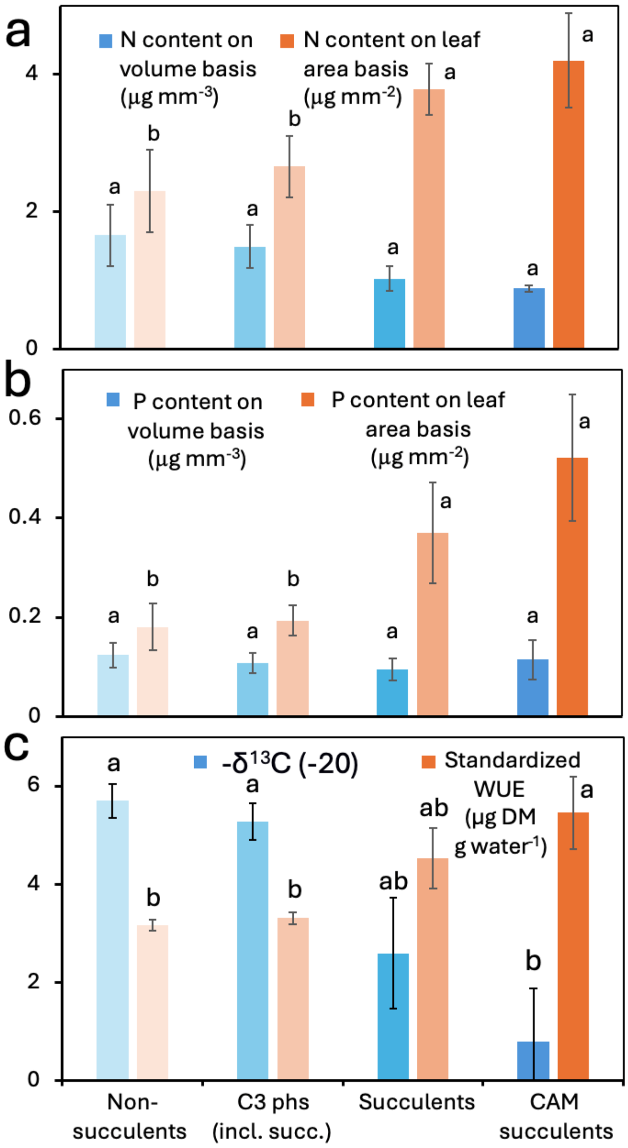

Figure 7. Metabolite contents (mean ± standard error, SE) on volume and area bases of the four leaf types recognized here: 4 non-succulents (C3 orthophylls, semi-succophylls), 6 C3 non-succulents and succulents, 4 succulents (C3, CAM succophylls), and 2 CAM succulents for (**a**) N and (**b**) P, and (**c**) intrinsic (-δ^{13}C) and standardized water-use efficiency (SWUE). Letters attached to SEs for each column are the result of Tukey's multiple-range test, different letters indicating differences significant at $p < 0.05$. Increasing colour intensity indicates increasing z.

Table 2. Examples of genera in the eight possible combinations of leaf anatomy, growing or imposed environmental conditions, and relative leaf water content (RWC) as related to the type of photosynthesis (C3, CAM). Collated from Ripley et al. (2013) and this paper where the actual species are given for each nominated genus. − means no examples could be located among these data sets but they might exist elsewhere.

	Non-Dedicated Storage Tissue (Chlorophyll Present)				**Dedicated Storage Tissue (Chlorophyll Absent)**			
Growing conditions:	Control		Droughted		Control		Droughted	
RWC as Q_V:	<95%	>95%	<95%	>95%	<95%	>95%	<95%	>95%
C3-type photosynthesis	*Tetragonia*	*Galenia*?	*Salvia*	*Roepera*	*Antimima*	*Delosperma*	−	*Senecio*
CAM-type photosynthesis	unlikely	unlikely	unlikely	unlikely	*Sedum*?	*Crassula*	*Stoeberia*	−

4.6. Metabolic compounds (N and P)

The volume of water per leaf increased at a steeper rate than total N and P as the leaves thickened (Figure 2). This means that N_V and P_V will decrease, which is supported at the overall level (Figure S4b). Although their z values stopped where ours started, the downward trend in N_V and P_V with increasing z agrees with Garnier et al. (1999) for N_v in grasses, whereas Roderick, Berry, & Noble (1999b) obtained no relationship for woody plants. Since N_V can be expressed as $N/(A \cdot z)$, a negative curvilinear relationship with z is mathematically prescribed (M. Williams, B. Lamont, & He, 2022) so that lack of such an empirical relationship can be attributed to inadequate sample size or extreme variation in the data. As water content on a mass basis (Q_M) increased, N_v decreased strongly in a linear manner, whereas, on a volume basis (Q_V) it was uncorrelated (Figure S4a). Since N resides within the cytoplasm, it is part of the dry matter content of cells, and, as $D_M = (1 - Q_M)$, this can explain the negative relationship with Q_M. Thus, N_V and P_V should correlate positively with D_M and these relationships were strongly supported by our data (Figure S4c).

Although N and P concentrations on a volume basis decreased overall with increasing z, differences across the four leaf types were not significant at $p < 0.05$ with Tukey's test (Figure 7) due to low values for several orthophylls (large variance) and higher values among the CAM species. However, N and P on an area basis (N_A, P_A) were well correlated with z and increased strongly among the leaf types, even exponentially for P_A (Figure 7b). Since $N_A = N_V \cdot z$ this trend must be due to an increase in z that overrides the associated decrease in N_V, and is another example of confounded variables (M. Williams, B. Lamont, & He, 2022). Making this comparison is acceptable, as here we are attempting to identify which of the two component variables best explains the trends. The same applies to P_A. Garnier et al. (1999) also obtained a weak positive relationship between N_A and z. This means that net metabolic activity (photosynthesis, respiration) of the more succulent species should be somewhat more efficient on an area basis. B. Lamont, Groom, & Cowling (2002), supported by Wright & Westoby (2002), observed a marked increase in N_A and P_A with declining rainfall in their studies that they also attributed to increasing z. Thus, increasing drought attenuation as gauged by increasing z (B. Lamont et al., 2015, Figure 5), implies increased metabolic efficiency on a leaf area basis.

4.7. Water-use efficiency

Overall, a small but highly significant increase in $\delta^{13}C$ values (less negative, reduced discrimination against the heavier isotope) is evident when correlated against z (Figure 2). This parameter is often used as an index of (long-term) water-use efficiency (WUE, B. Lamont, Groom, & Cowling, 2002, Swanborough, B. Lamont, & February, 2003). But it is confounded by photosynthesis type (where it varies) as well as leaf tissue properties as they control relative water loss and carbon fixation. Here, $-\delta^{13}C$ values ranged 24.1–26.6‰ for the C3 species and 19.6–21.8‰ for the CAM species. When standardized against the best-fit equation for actual water-use efficiency (SWUE) of 12 *Clusia* species (Winter, Aranda, & Holtum, 2005), the data produced two relationships: SWUE of the two CAM species was 70% higher and twice as steep as for the six C3 species (Figure 8a). The CAM species were largely responsible for the exponential rise in SWUE as SLA declined (Figure 8b). Thus, $\delta^{13}C$ and SWUE run in parallel, with orthophylls at one extreme and CAM species at the other. Reverting to $\delta^{13}C$, the CAM species also formed a separate relationship with utilizable water content (UWC, as % turgid mass) such that the CAM species had significantly higher (less negative) $\delta^{13}C$ for equal UWC (Figure 8c). As UWC increased (species became more succulent), $\delta^{13}C$ became less negative for both C3 and CAM species, consistent with the WUE pattern in Figure 8a. However, based on their slopes, $\delta^{13}C$ of the CAM species increased six times faster (higher WUE) relative to UWC than the C3 species.

Bearing in mind that only two CAM species were studied, that the results still have generality is confirmed by superimposing the results from Ripley et al. (2013) (Figure 8c). These show the same trends, with CAM species 3.5 times the slope of the C3 species. Also noteworthy is that, whereas the Ripley species have almost the same values of $\delta^{13}C$ at 90% UWC, this diverges by 1.7‰ for C3 and 3.2‰ for CAM species at 65% UWC. This difference (less discrimination against the heavier isotope) can be attributed to the more stressful growing conditions encountered by the field plants used in our study compared with the nursery plants used by Ripley et al. (2013)—see Table 2. It is intriguing that the Aizoaceae used by Ripley et al. (2013) were C3 whereas the only CAM plants in our study *were* in the Aizoaceae. This points to the overriding effect of leaf anatomy and growing conditions rather than taxonomy in determining the type of photosynthesis and WUE (Table 2). The increasing WUE with declining SLA (Figure 8b) can be attributed to increasing z rather than increasing D_V, its other component, as the latter falls as Q_V increases ($D_V = 1 - Q_V$) (Figure 3d).

WUE, via rising δ^{13}C, increased weakly ($p = 0.0351$) with rising N_A but strongly ($p = 0.0004$) with rising P_A (Figure S6a, b). The weaker relationship with N was due to N_A of *Stoeberia* being anomalously lower than expected from its δ^{13}C value so that the CAM species were less different compared with P_A that continued to diverge at high UWC (Figure 8c). That the upward trend is strong, especially for P_A, again points to a dominant z, over D_V, effect. Our results highlight how WUE is a response to both the chemical and physical properties of leaves (Figures 2, 7, 8, S4 and S6). While *Stoeberia* and *Ruschia* (the most succulent taxa) have a CAM photosynthetic pathway and, by far, the highest WUE, they largely remain within the same best-fit line as the six C3 species such that the overall relationship is controlled by thickness, z, independent of photosynthetic type (Figure 8d).

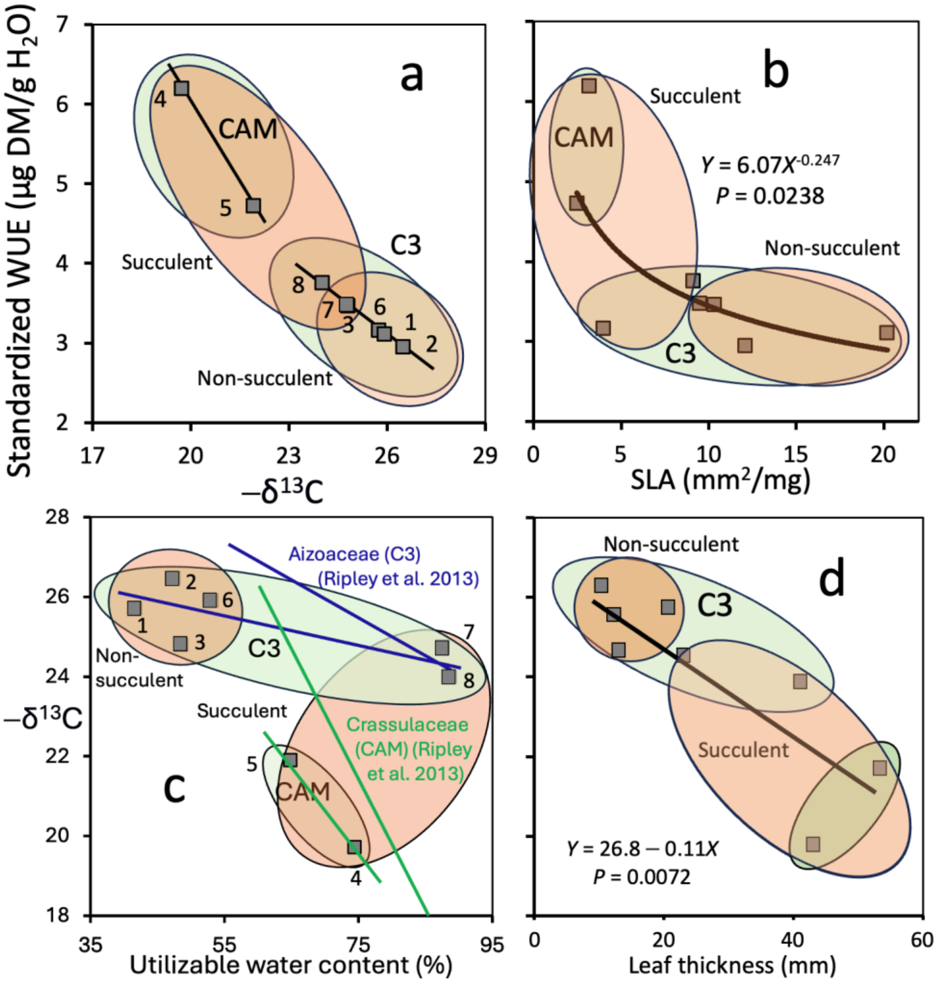

Figure 8. WUE vs. leaf traits of the eight co-occurring species. (**a**) δ^{13}C standardized against the actual water-use efficiency (WUE) curve for *Clusia* species of Winter, Aranda, & Holtum (2005), DM = dry matter, (**b**) relationship between standardized WUE and specific leaf area (SLA), (**c**) relationship between δ^{13}C (‰) and utilizable water content (UWC_B) that shows different trends between CAM and C3 species, with comparative data from Ripley et al. (2013) added as separate lines, (**d**) relationship between δ^{13}C and leaf thickness, z, with CAM and C3 species showing much the same trend. All relationships are based on the best curvilinear fits to mean values for each species, with C3 and CAM species fitted separately for comparative purposes in (**a**,**c**). The ringed points highlight positions of the C3/CAM (green) and succulent/non-succulent species (orange). Numbers correspond to species given in Table 1 and apply across the two graphs at each level.

Roderick et al. (1999) noted that, even among non-succulents, gas exchange (rate of photosynthesis) would rise with increase in z, due to the expected increase in N_A (Wright & Westoby, 2002). Here, the positive relationship between δ^{13}C and P_A was stronger (Figure S6). If P (and possibly N) limit photosynthesis in this environment (P increased at four times the rate as N with increasing z, Figure 2), then increased photosynthesis may contribute to rising WUE with increasing succulence as well as declining water loss (since it is the ratio between the two). Both can be attributed to increasing z—thicker leaves are capable of greater photosynthesis if it implies more chloroplasts and reduced transpiration if it implies greater resistance to water movement. The lack of any relationship between δ^{13}C and dry density (D_M, D_V) (not shown) indicates that no increasing demand exists for carbon per unit area of leaf as succulence increases.

The declining WUE with increasing SLA, and rising WUE with increasing *z*, agrees with Schulze, R. Williams, & Farquhar. (1998) and B. Lamont, Groom, & Cowling. (2002) for sclero-orthophylls. With increasing *z*, transpirational water loss of our species decreases (Figure 5), probably controlled by reduced internal conductance as cellular water replaces air space and distances between the xylem and stomata increase (Figure 3c; von Willert et al., 1992; Hanba, Miyazawa, & Terashima, 1999; B. Lamont, Groom, & Cowling, 2002; Borland et al., 2009; B. Lamont & H. Lamont, 2025). This interpretation disagrees with the results of Ripley et al. (2013) who noted that mesophyll conductance was maintained with drought stress, although they were surprised by this result given their awareness of these same physical constraints. Clearly, more work is required on exactly where most resistance to water loss resides in succulents.

5. Conclusions

As a group, the succophylls ($Q_A > 0.9$ mg mm^{-2}) have unique structural and physiological properties: thick leaves and water-storing parenchyma, lower SLA and rates of water loss and ψ change diurnally and seasonally, and when severed from the parent plant, but higher levels of 'utilizable' water, ($Na^+ + K^+$) on a leaf-mass basis, N/P contents on a leaf-area basis, $\delta^{13}C$, WUE and WLR, and higher/less variable ψ than other leaf types (Figure 9). This syndrome of adaptive responses to permanent drought could be further divided into species with CAM- or C3-type photosynthesis. Leaves of the two CAM species possessed the most extreme levels of these traits: thickest leaves, lowest SLA, greatest water storage, least rates of water loss diurnally, seasonally and when severed from the parent plant, and highest N and P contents/area and $\delta^{13}C$ and WUE. Uniquely, their ψ decreased markedly overnight compared with diurnally, supporting the $\delta^{13}C$ evidence of their ability to absorb and fix CO_2 in the dark. Although still trending upwards when plotted against leaf thickness, ψ_{100}, ψ_{tlp}, utilizable water, and cations for the CAM species were not numerically different from the succophylls collectively, possibly attributable to the small number of CAM species examined as a limitation of this study.

By contrast, the orthophylls/semi-succulents ($Q/A < 0.9$ mg mm^{-2}) were characterized by strong diurnal and seasonal fluctuations in water content, and little ability to store water, especially when severed from the parent plant (Figure 9). Since all these species co-occur under extreme aridity, non-succulent shrubs must also possess special features, here centered on their strong osmotic-adjustment ability combined with deep root systems accessing soil moisture throughout the year (von Willert et al. 1992; B. Lamont & H. Lamont, 2000; Guo et al. 2024). The eight species studied are linked by a gradient in leaf thickness, *z*, ranging from the thin-leaved C3 species, to thick-leaved C3 species, to thickest-leaved CAM species that correlates extremely well with all the structural and physiological traits examined here. Demonstrating their functional link with *z* would require anatomical and internal labelling techniques beyond the scope of this study.

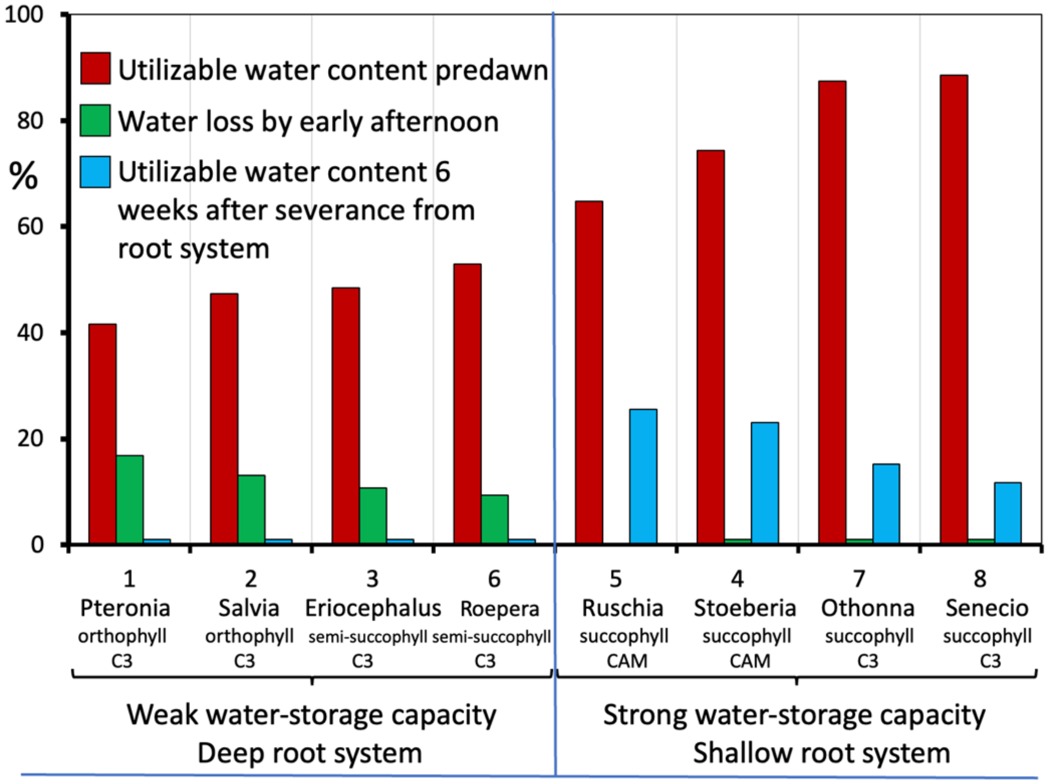

Figure 9. The eight species studied here placed in two groups having contrasting water relations with the relatively thin-leaved species with deep root systems on the left, and relatively thick-leaved species with shallow root systems on the right. Values are means for three plants per species.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2508201004201665/plantecophys-2508000095-SI-final.pdf.

Author Contributions

BBL conceived and managed the project, undertook most field and laboratory work, liaised with other researchers, wrote the manuscript, prepared the figures and oversaw the publication process. NSE undertook the water-relations fieldwork in the spring-summer-autumn seasons and approved of the submitted manuscript. HCL participated in all field, and some laboratory, work and approved of the submitted manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Data are available from BBL on request.

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Conflicts of Interest

We declare no conflict of interest.

Peer Review Statement

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Abbreviations

$\delta^{13}C$, discrimination ratio between ^{12}C and ^{13}C; ψ_{100}, water potential at full turgor; ψ_{tlp}, water potential at the tlp; A, leaf projected area; CAM, crassulacean acid metabolism, that involves absorption of carbon dioxide overnight and storage as malate to be used as a substrate in photosynthesis during the day; C3, standard photosynthetic pathway where the first product is a C_3-type compound; D_M, dry density on a turgid leaf mass basis; D_V, dry density on a leaf volume basis; D, dry leaf mass; M, turgid mass ($D + Q_{100}$); N_A, nitrogen content on a leaf area basis; N_D, nitrogen content on a dry mass basis; N_V, nitrogen content on a leaf volume basis; P_A, phosphorus content on leaf area basis; P_D, phosphorus content on a dry mass basis; P_V, phosphorus content on a volume basis; RWC, relative water content, observed Q relative to Q at full turgor (Q_{100}); SLA, specific leaf area ($A/M = D_V^{-1}\cdot z^{-1}$); Q, water mass per leaf; tlp, turgor loss point (wall pressure = 0); $Q_A = Q/A$, Q on an A basis as an index of leaf succulence; Q_B, apoplastic water unavailable for use by the tissues; Q_M, water content on a turgid mass basis; Q_V, water content on a volume basis; SWUE, standardized water-use efficiency ($\delta^{13}C$ compared with actual WUE relationships for *Clusia* species with C3/CAM in Winter, Aranda, & Holtum, 2005); UWC_B, utilizable water content = observed Q_M minus Q_B on a mass basis; water-loss resistance (WLR) = $\Delta RWC/\Delta\psi$; z, adjusted (actual) leaf thickness (after B. Lamont et al., 2015).

References

References marked with an asterisk indicate studies included in the meta-analysis.

Blackman CJ, & Brodribb TJ. (2011). Two measures of leaf capacitance: Insights into the water transport pathway and hydraulic conductance in leaves. *Functional Plant Biology*, *38*(2), 118–126.

Borland AM, Griffiths H, Hartwell J, & Smith JAC. (2009). Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *Journal of Experimental Botany*, *60*(10), 2879–2896.

Bowie MRA. (1999). Ecophysiological Studies on Four Selected Succulent Karoo Species. Master's Thesis. University of Stellenbosch.

Buckley TN. (2017). Modeling stomatal conductance. *Plant Physiology*, *174*, 572–582.

Cicuzza D, Stäheli D, Nyffeler R, & Eggli U. (2018). Morphology and anatomy support a reclassification of the African succulent taxa of *Senecio* sl (Asteraceae: Senecioeae). *Haseltonia*, *2017*, 11–26.

Cowling RM, & Campbell BM. (1983). The definition of leaf consistence in the fynbos biome and their distribution along an altitudinal gradient in the southeastern Cape. *Journal of South African Botany*, *49*, 87–101.

Delf EM. (1911). Transpiration and behaviour of stomata in halophytes. *Annals of Botany*, *25*, 485e505.

Eccles NS, Esler KJ, & Cowling RM. (1999). Spatial pattern analysis in Namaqualand desert plant communities: Evidence for general positive interactions. *Plant Ecology*, *142*, 71–85.

Eccles NS, Lamont BB, Esler KJ, & Lamont HC. (2001). Relative performance of clumped vs. experimentally isolated plants in a South African winter-rainfall desert community. *Plant Ecology*, *155*, 219–227.

Ehleringer JR, & Osmond CB. (1989). Stable isotopes. In *Plant physiological ecology: Field methods and instrumentation*. (Piercy RW, Ehleringer J, Mooney HA, Rundel PW, Eds.) (pp. 281–300). Chapman & Hall.

Farquhar GD, O'Leary MH, & Berry JA. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, 9, 119–129.

Garnier E, Salager J-L, Laurent G, & Sonié L. (1999). Relationship between photosynthesis, nitrogen and leaf structure in 14 grass species and their dependence on the basis of expression. *New Phytologist*, 143, 35–47.

Gilman IS, & Edwards EJ. (2020). Crassulacean acid metabolism. *Current Biology*, *30*, R57–R62.

Gleason SM, Blackman CJ, Cook AM, Laws CA, & Westoby M. (2014). Whole-plant capacitance, embolism resistance and slow transpiration rates all contribute to longer desiccation times in woody angiosperms from arid and wet habitats. *Tree Physiology*, *34*, 275–284.

Guo B, Arndt SK, Miller RE, Szota C, & Farrell C. (2024). Does succulence in woody plants delay desiccation, and is stored water used to maintain physiological function during drought conditions? *Physiologia Plantarum*, *176*, e14616.

Groom PK, & Lamont BB. (1999). Which common indices of sclerophylly best reflect differences in leaf structure? *Ecoscience*, *6*, 471–474.

Groom PK, Lamont BB, Leighton S, Leighton P, & Burrows C. (2004). Heat damage in sclerophylls is influenced by their leaf properties and plant environment. *Ecoscience*, *11*, 94–101.

Grubb PJ, Marañón T, Pugnaire FI, & Sack L. (2015). Relationships between specific leaf area and leaf composition in succulent and non-succulent species of contrasting semi-desert communities in south-eastern Spain. *Journal of Arid Environments*, *118*, 69–83.

Hanba YT, Miyazawa SI, & Terashima I. (1999). The influence of leaf thickness on the CO_2 transfer conductance and leaf stable carbon isotope ratio for some evergreen tree species in Japanese warm-temperate forests. *Functional Ecology*, *13*, 632–639.

Herrera A. (2020). Are thick leaves, large mesophyll cells and small intercellular air spaces requisites for CAM? *Annals of Botany*, *125*, 859–868.

Hulley IM, Viljoen AM, Tilney PM, Van Vuuren SF, Kamatou GPP, & Van Wyk BE. (2010). The ethnobotany, leaf anatomy, essential oil variation and biological activity of *Pteronia incana* (Asteraceae). *South African Journal of Botany*, *76*(4), 668–675.

Lamont BB & Lamont HC. (2000). Utilizable water in leaves of eight arid species as derived from pressure-volume curves and chlorophyll fluorescence. *Physiologia Plantarum*, *110*, 64–71.

Lamont BB, & Lamont HC. (2025). Contrasting water, dry matter and air contents distinguish orthophylls, sclerophylls and succophylls (leaf succulents). *Oecologia*, *207*, 54. https://doi.org/10.1007/s00442-025-05686-4

Lamont BB, Groom PK, & Cowling RM. (2002). High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Functional Ecology*, *16*, 403–412.

Lamont BB, Groom PK, Williams MR, & He T. (2015). LMA, density and thickness: Recognizing different leaf shapes and correcting for their non-laminarity. *New Phytologist*, *207*, 942–947.

Landrum JV. (2002). Four succulent families and 40 million years of evolution and adaptation to xeric environments: What can stem and leaf anatomical characters tell us about their phylogeny? *Taxon 51*, 463–473.

Lauterbach M, van der Merwe PDW, Keßler L, Pirie MD, Bellstedt DU, & Kadereit G. (2016). Evolution of leaf anatomy in arid environments–a case study in southern African *Tetraena* and *Roepera* (Zygophyllaceae). *Molecular Phylogenetics and Evolution*, *97*, 129–144.

Linton MJ, & Nobel PS. (2001). Hydraulic conductivity, xylem cavitation, and water potential for succulent leaves of *Agave deserti* and *Agave tequilana*. *International Journal of Plant Sciences*, *162*(4), 747–754.

Males J. (2017). Secrets of succulence. *Journal of Experimental Botany*, *68*(9), 2121–2134.

*Martin CE, Lin TC, Lin KC, Hsu CC, & Chiou WL. (2004). Causes and consequences of high osmotic potentials in epiphytic higher plants. *Journal of Plant Physiology*, *161*(10):1119–1124.

Maxwell, K, von Caemmerer, S, & Evans, JR. (1997). Is a low internal conductance to CO_2 diffusion a consequence of succulence in plants with crassulacean acid metabolism? *Functional Plant Biology*, *24*(6), 777–786.

Melo-de-Pinna GF, Ogura AS, Arruda EC, & Klak C. (2014). Repeated evolution of endoscopic peripheral vascular bundles in succulent leaves of Aizoaceae (Caryophyllales). *Taxon*, *63*(5), 1037–1052.

Miller G. (1985). Plant-Water Relations along a Rainfall Gradient, between the Succulent Karoo and Mesic Mountain Fynbos, in the Cedarberg Mountains near Clanwilliam, South Africa. Master's Thesis. University of Cape Town.

Milton SJ, Yeaton RI, Dean WRJ, & Vlok JHJ. (1997). Succulent karoo. In *Vegetation of southern Africa.* (Cowling RM, Richardson DM, Pierce SM, Eds.) (pp. 131–166). Cambridge University Press.

Niinemets Ü. (1999). Components of leaf dry mass per area — thickness and density — alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist*, 144, 35–47.

Özdemir C, Baran P, & Aktas K. (2009). Anatomical studies in *Salvia viridis* L. (Lamiaceae). *Bangladesh Journal of Plant Taxonomy*, *16*(1), 65–71.

Pérez-López AV, Lim SD, & Cushman JC. (2023). Tissue succulence in plants: Carrying water for climate change. *Journal of Plant Physiology*, *289*, 154081.

Pyankov VI, Kondrachuk AV, & Shipley B. (1999). Leaf structure and specific leaf mass: The alpine desert plants of the Eastern Pasmirs, Tadjikistan. *New Phytologist*, *143*, 131–142.

Radford S, & Lamont BB. (1992). *An instruction manual for 'Template'–A rapid, accurate program for calculating and plotting water relations data obtained from pressure-volume curves.* Environmental Biology, Curtin University. (no longer available)

Richards MB, & Lamont BB. (1996). Post-fire mortality and water relations of three congeneric shrub species under extreme water stress—a trade-off with fecundity? *Oecologia*, *107*, 53–60.

Ripley BS, Abraham T, Klak C, & Cramer MD. (2013). How succulent leaves of Aizoaceae avoid mesophyll conductance limitations of photosynthesis and survive drought. *Journal of Experimental Botany*, *64*(18), 5485–5496.

*Roderick ML, Berry SL, & Noble IR. (1999a). The relationship between leaf composition and morphology at elevated CO_2 concentrations. *New Phytologist*, *143*, 63–72.

Roderick ML, Berry SL, & Noble IR. (1999b). On the relationship between leaf composition, morphology and function of leaves. *Functional Ecology*, *13*, 696–710.

Roderick ML, Berry SL, Noble IR, & Farquhar GD. (1999). A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology*, *13*, 683–695.

Schulze E-D, Williams RJ, & Farquhar GD. (1998). Carbon and nitrogen discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology*, *25*, 413–425.

Swanborough PW, Lamont BB, & February EC. (2003). $\delta^{13}C$ and water-use efficiency in Australian grasstrees and South African conifers over the last century. *Oecologia*, *136*, 205–212.

Teeri JA, Tonsor SJ, & Turner M. (1981). Leaf thickness and carbon isotope composition in the Crassulaceae. *Oecologia*, *50*(3), 367–369.

Vendramini F, Diaz S, Gurvich DE, Wilson PJ, Thompson K, & Hodgson JG. (2002). Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytologist*, *154*, 147–157.

von Willert DJ, & Brinckman E. (1986). Sukkulenten und ihr Überleben in Wüsten. *Naturwissenschaften*, *73*, 57–69.

von Willert DJ, Eller BM, Werger M, Brinckman E, & Ihlenfeldt H-D. (1992). *Life strategies of succulents in deserts: With special reference to the namib desert*. Cambridge University Press.

Wang Z, Huang H, Wang H, Peñuelas J, Sardans J, Niinemets Ü, & Wright IJ. (2022). Leaf water content contributes to global leaf trait relationships. *Nature Communications*, *13*(1), 1–9.

Wilson PJ, Thompson K, & Hodgson JG. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist*, *143*, 155–162.

Winter K, Aranda J, & Holtum JA. (2005). Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology*, *32*(5), 381–388.

Winter K, & Holtum JAM. (2002). How closely do the δ^{13}C values of Crassulacean acid metabolism plants reflect the proportion of CO_2 fixed during day and night? *Plant Physiology*, *129*, 1843–1851.

Winter K, Troughton JH, Evenari M, Läuchli A, & Lüttge U. (1976). Mineral ion composition and occurrence of CAM-like diurnal malate fluctuations in plants of coastal and desert habitats of Israel and the Sinai. *Oecologia*, *25*, 125–143.

Witkowski ETF, & Lamont BB. (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, *88*, 6–493.

Williams MR, Lamont BB, & He T. (2022). Forum: Dealing with “the spectre of ‘spurious’ correlations”: Hazards in comparing ratios and other derived variables with a randomization test to determine if a biological interpretation is justified. *Oikos*, *2022*, e08575.

*Wright IJ, Groom PK, Lamont BB, Poot P, Prior LD, Reich PB, Schulze E-D, Veneklaas VJ, & Westoby M. (2004). Leaf trait relationships in Australian plant species. *Functional Plant Biology*, *31*, 551–558.

Wright IJ, & Westoby M. (2002). Leaves at low versus high rainfall: Coordination of structure, lifespan and physiology. *New Phytologist*, *155*, 403–426.