

## Plant Ecophysiology

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# **Supplementary Figures**

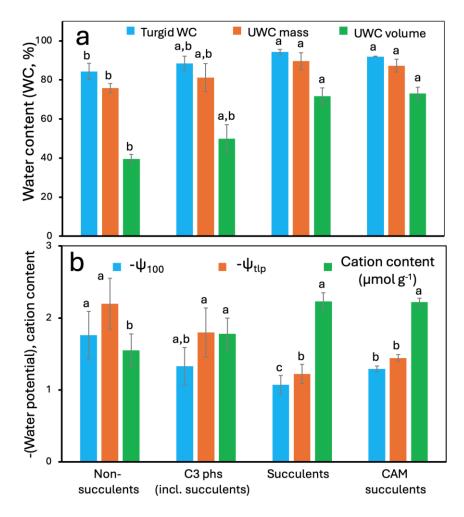


Figure S1. (a) three indices of (utilizable) water contents [(U)WC] and (b) water potential at saturation  $(\psi_{100})$  and the turgor loss point  $(\psi_{tlp})$  and cation  $(Na^+ + K^+)$  content separated into the four leaf types recognized here4 non-succulents (C3 orthophylls, semi-succophylls), 6 C3 non-succulents and succulents, 4 succulents (C3, CAM succophylls), and 2 CAM succulents. phs = photosynthesis. Leaf types are given in order of increasing leaf thickness. 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.

#### Comments on Figure S1:

Figure S1 describes the contrasting trends in Figure 4 in terms of the four leaf types, especially as the values are not logged and so the slopes are steeper. Although WC and UWC at turgidity on a mass basis are significantly higher among the succophylls than orthophylls, it is only in the order of 10%. The difference rises to 30% when UWC is placed on a volume basis, that has more physiological significance, since water acts volumetrically rather than gravimetrically. Figure S1b shows that  $\psi_{100}$  and  $\psi_{tlp}$  for the succophylls are only 50–60% of the orthophylls/semi-succophylls and they tend to merge among the succophylls. The reason for the poor fits to the overall data in Figure 4 is clearly due to the CAM species not continuing the trend of increasing  $\psi$  with increasing  $\psi$ , and shows that the highest  $\psi$  among the sampled species must lie with the succulent C3 species. The 60% increase in cation concentration, on a mass, but not volume, basis, among the succophylls is consistent with the interpretation of Figure 2 that they serve as osmotic balancing ions in promoting water absorption during cell expansion.



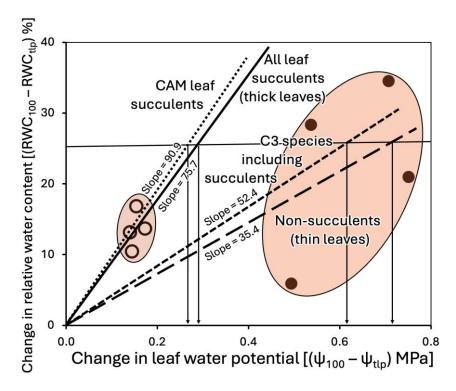


Figure S2. Water-loss resistance (WLR) for branchlets of eight Namib Desert species allowed to dry out under laboratory conditions from relative water content (RWC<sub>100</sub>) or water potential ( $\psi_{100}$ ) at full turgidity to those at the turgor loss point (RWC<sub>tlp</sub>,  $\psi_{tlp}$ ), which occupies 0,0 on the graph. They have been separated into four leaf types with WLR for the succophylls over twice that of the orthophylls/semi-succophylls. The horizontal line at a difference of 25% between RWC<sub>100</sub> and RWC<sub>tlp</sub> shows the large difference in the associated  $\Delta\psi$  between the succulent/CAM species and orthophylls/C3 species.

#### Comments on Figure S2:

WLR was determined as the change in RWC from full turgor (RWC<sub>100</sub>) to the turgor loss point (RWC<sub>tlp</sub>) in the laboratory study relative to change in water potential at full turgor ( $\psi_{100}$ ) to the turgor loss point ( $\psi_{tlp}$ ) (Figure S2). The slopes represent WLR of the four leaf types and show that WLR of the succophylls is 2.14 times that of the non-succophylls. WLR for the CAM species is 2.57 times higher but is not statistically significant from all succophylls by Tukey's test (no doubt because so few CAM species were analysed). Similarly, WLR is 1.73 times higher than for the C3 species, which is significant. We conclude that succophylls, especially CAM species, are most efficient at storing water in relation to the supply of osmotically active solutes in the protoplast. This has been interpreted as enhancing drought resistance of the leaves by promoting water storage and reducing hydraulic conductance in the transpiration stream (B. Lamont & H. Lamont, 2000; Blackman & Brodribb, 2011).

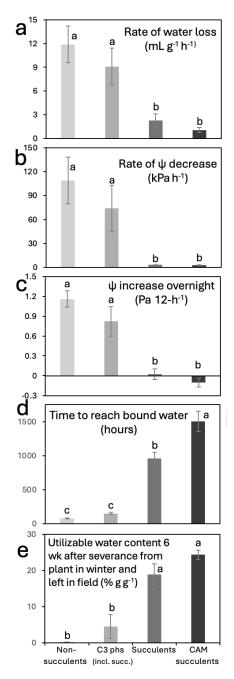
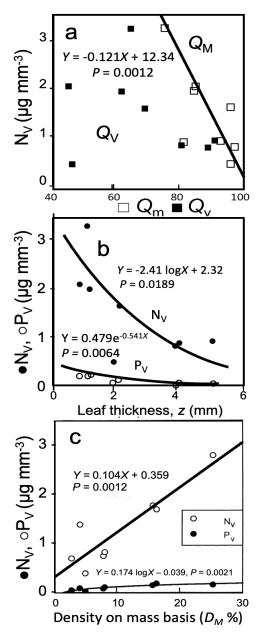


Figure S3. Some water-relation properties (mean  $\pm$  standard error) of the four leaf types recognized here: 4 non-succulents (C3 orthophylls, semi-succophylls), 6 C3 non-succulents and succulents, 4 succulents (C3, CAM succophylls), and 2 CAM succulents, in relation to a) rate of water loss, b) rate of water potential ( $\psi$ ) decline over some days, c)  $\psi$  decline overnight, and d) length of time to reach B after leaving branchlets in an air-conditioned laboratory, and e) UWC<sub>B</sub> six weeks after severing branchlets and leaving in the field attached to the parent. Increasing depth of shading corresponds to increasing leaf thickness. 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.

### Comments on Figure S3:

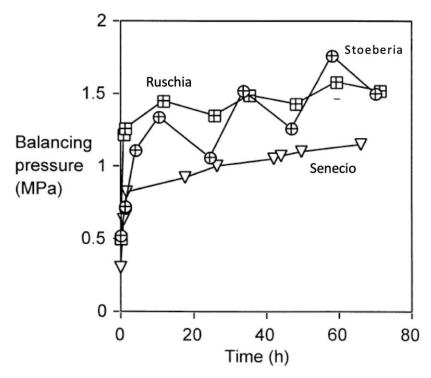
The pattern for UWC<sub>B</sub> after severed branches were left under field conditions for 6 weeks from winter to spring exactly mimics the pattern for time to reach B in the laboratory for similarly detached branchlets (Figure S3d vs Figure S3e). The orthophylls/semi-succophylls lacked any utilizable water by 6 weeks with, at the other extreme, CAM species retaining a mean UWC<sub>B</sub> of 25%. This means that ecophysiological properties determined under laboratory conditions can be applied to the same plants under natural conditions. Martin et al. (2004) were puzzled that the succulent epiphytes, which they studied and collated from elsewhere, had ambient  $\psi$  values only around -1 MPa whereas drought-tolerant species are renowned for their ability to reach exceptionally low  $\psi$  values. The answer is provided by Figure S3d where the succophylls averaged 13.4 times as long to reduce their water content to the B point as for the orthophylls (also see Borland et al. 2009). The orthophylls/semi-succophylls took 1.4–8.7 days and the succophylls, 10-93.4 days, to lose all of their utilizable water. The strongly increasing overall slope is consistent with Ripley et al. (2013) who compared time to lose 10% water content vs degree of succulence rather than the more complete approach used here.



**Figure S4.** Relationship of nitrogen and phosphorus on a leaf volume basis ( $N_V$  and  $P_V$ ) with a) water content on a volume ( $Q_V$ ) and mass ( $Q_M$ ) basis, b) with leaf thickness (z), and c) with dry density on a mass basis ( $D_M$ ). P values of equations for the best-fit curves are highly significant.

#### Comments on Figure S4:

Comparing the data of Roderick, Berry, & Noble (1999b) and Ripley et al. (2013) with ours shows that no fixed relationship is present generally between  $N_V$  and  $Q_V$  (Figure S4a). Since  $N_V = N/V$  and  $Q_V = Q/V$ , the V terms cancel out when they are compared, so that, in the absence of any obvious reasons why N should increase as Q increases, the lack of a correlation between  $N_V$  and  $Q_V$  is not unexpected. By placing z and  $D_V$  together we get SLA that is uncorrelated with  $N_V$  and  $P_V$  (not shown), as here z and  $D_V$  cancel out each other because their slopes are in opposing directions (Figure S4b,c). We also got no relationship between SLA and  $N_D$  or  $P_D$ , unlike Roderick, Berry, & Noble (1999a) but agreeing with B. Lamont, Groom, & Cowling (2002) for LMA (1/SLA) based on 165 species which, however, did not include succulents. This time, the D divisors cancel so that no logical reason exists for why high A should imply high N or P. Clearly, low SLA due to high levels of sclerophylly or succulence is not necessarily a function of N or P contents, and must be assessed on a case-by-case basis before such claims can be made, as the trends (for non-succulents) vary greatly (B. Lamont, Groom, & Cowling, 2002; Wright et al. 2004).



**Figure S5.** Diurnal fluctuations in  $\psi$  (negative balancing pressure) for the two CAM species, Stoeberia and Ruschia, compared with the C3 species, Senecio, the three species with the thickest leaves.

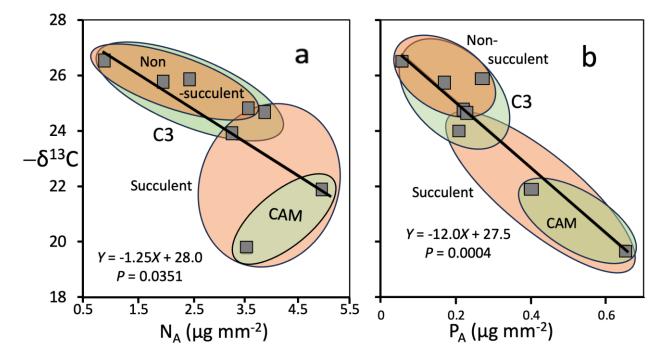


Figure S6.  $\delta^{13}$ C  $\nu s$  a) N and P on an area basis (N<sub>A</sub>, P<sub>A</sub>) for the eight co-occurring species. The ringed points highlight positions of the C3/CAM (green) and non-succulent/succulent species (orange).