

Predicting plant vulnerability to drought in biodiverse regions using functional traits

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Attempts to understand mechanisms underlying plant mortality during drought have led to the emergence of a hydraulic framework describing distinct hydraulic strategies among coexisting species. This framework distinguishes species that rapidly decrease stomatal conductance (g_s), thereby maintaining high water potential (P_x ; isohydric), from those species that maintain relatively high g_s at low P_x , thereby maintaining carbon assimilation, albeit at the cost of loss of hydraulic conductivity (aniso-hydric). This framework is yet to be tested in biodiverse communities, potentially due to a lack of standardized reference values upon which hydraulic strategies can be defined. We developed a system of quantifying hydraulic strategy using indices from vulnerability curves and stomatal dehydration response curves and tested it in a speciose community from South Africa's Cape Floristic Region. Degree of stomatal regulation over cavitation was defined as the margin between P_x at stomatal closure (P_{g12}) and P_x at 50% loss of conductivity. To assess relationships between hydraulic strategy and mortality mechanisms, we developed proxies for carbon limitation and hydraulic failure using time since P_{g12} and loss of conductivity at minimum seasonal P_x , respectively. Our approach captured continuous variation along an isohydric/aniso-hydric axis and showed that this variation was linearly related to xylem safety margin. Degree of isohydric/aniso-hydric was associated with contrasting predictions for mortality during drought. Merging stomatal regulation strategies that represent an index of water use behavior with xylem vulnerability facilitates a more comprehensive framework with which to characterize plant response to drought, thus opening up an avenue for predicting the response of diverse communities to future droughts.

drought | functional traits | plant hydraulics | xylem vulnerability | stomatal response

Increased frequency of plant mortality is often predicted in response to global climate change-induced drought events (1). Such a threat has resulted in attempts to understand the precise mechanisms underlying plant mortality and to determine which species are more vulnerable than others (2, 3). One of the most promising avenues is a hydraulic framework describing the influence during drought periods of distinct hydraulic regulation strategies on carbon balance and the integrity of the soil-plant-atmosphere hydraulic pathway (2, 4, 5). The framework's success has come with the realization that two major mechanisms of mortality, carbon starvation and hydraulic failure, are intimately linked (2, 6, 7). In land plants, water loss and CO_2 uptake are closely coupled, because the vast majority of gaseous exchange occurs through a single pathway: stomatal pores on the leaf surface. This coupling sets up a long-recognized carbon assimilation vs. water conservation trade-off (8). To achieve adequate rates of assimilation and to avoid becoming carbon-limited, plants need to maintain a favorable water status that, in turn, begets high stomatal conductance (g_s) (9). High g_s also promotes high transpiration rates, which, for a given level of whole-plant hydraulic conductance, generate increasingly negative leaf and stem water potential (P_x) and greater tension in the water column, as posited by the cohesion-tension theory of sap ascent (10). Increasing tension in the water column may eventually lead to cavitation within the xylem by

promoting the seeding of air bubbles into water-filled conduits (5, 11). Because cavitation reduces total plant hydraulic conductance, it limits the ability of a plant to transport water and nutrients to sites of growth and productivity, and ultimately may lead to death (2, 6).

As one might expect, attempts have been made to categorize plant species' responses according to which process, carbon uptake or water conservation, is favored. Frequently, hydraulic strategies are classified along a continuous axis from drought avoidance (or isohydric P_x regulation) to drought tolerance (or aniso-hydric P_x regulation). Isohydric species respond to dehydration by rapidly decreasing g_s , thereby limiting excessive water loss and maintaining high plant P_x (12). Such drought avoiders may prevent cavitation occurring in xylem conduits, albeit at the cost of carbon assimilation during dry periods. Aniso-hydric species maintain relatively high g_s at low P_x , thereby maintaining CO_2 uptake, but endure the cost of possible formation of embolism within conduits (2, 12) or the cost of constructing xylem that can resist negative P_x s (13).

A major drawback of the current hydraulic framework is that it has yet to be thoroughly examined in highly diverse communities, where it is unclear to what extent species separate out into the isohydric-aniso-hydric dichotomy or form a continuum of hydraulic strategies. In functionally diverse communities, significant variability in key hydraulic functional traits, such as rooting depth (14), may produce a high diversity of drought response strategies. Much of the limitation in our understanding can be traced back to the model systems explored in the original hydraulic framework studies. These systems were often composed of relatively few codominant species, which made detailed, long-term monitoring tractable (2, 15–18). To address this shortcoming, we aimed to develop a functional traits-based framework that can be

Significance

Recent reports of widespread drought-induced plant die-back have enhanced the need to understand variation in plant hydraulic strategies and to determine which species are susceptible to drought-induced mortality. Finding meaningful ways to describe the many different types of responses plants might demonstrate in biodiverse regions can be extremely challenging. Here, we present the architecture in which functional traits can be incorporated into a known hydraulic framework to provide additional insight into the range of hydraulic strategies present in diverse communities. Merging stomatal regulation strategies that represent an index of water use behavior with xylem hydraulic strategies that represent water transport and vulnerability to water deficit behavior facilitates a more comprehensive framework to characterize plant response to drought.

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applied to the world's biodiversity hotspots, where knowledge of the vulnerability to future drought events might be particularly urgent. Functional traits are often well-coupled to environmental stresses, are less time-consuming to measure, and have been used widely in plant ecology to explore functional strategies of different species (19–22). Provided that we can develop a system whereby plant functional traits capture a hydraulic strategy of a species, they may also afford rapid comparisons between multiple species (21). Here, we propose a framework that uses several commonly measured traits to define a hydraulic response and show that it provides insight into potential mechanisms of mortality within diverse communities.

To define a hydraulic strategy, it is necessary to include some measure of xylem vulnerability (2, 21). The resistance of a plant to embolism propagation is quantified by describing the loss of conductivity with declining P_x : so-called vulnerability curves (4, 23). Although several standardized indices of vulnerability can be taken from these curves, the most commonly used is the P_x at 50% loss of conductivity (P_{50}). Species have been shown to vary considerably in P_{50} , ranging from -0.18 MPa (highly vulnerable) to -14.1 MPa (highly resistant) (24). Much of this variation has been explained by a safety-efficiency trade-off, because xylem conduits (mostly vessels in angiosperms or tracheids in conifers) with greater resistance to embolism and implosion usually [but not always (25)] have lower maximum hydraulic conductance (24, 26–28). However, in isolation, xylem vulnerability to cavitation does not provide a robust measure of hydraulic conservatism or drought tolerance, as shown by the lack of a clear relationship between P_{50} and aridity (19, 24). As previously mentioned, it is essential to account for the CO_2 uptake-water loss trade-off, in addition to the safety-efficiency trade-off of xylem conduits, to define a hydraulic strategy (29–32).

A way to quantify these trade-offs emerges from the concept of safety margins, usually described as the difference between a critical point on the vulnerability curve and the minimum seasonal P_x , referred to as P_{\min} . Safety margins are used to describe the degree of conservatism in a plant's hydraulic strategy (19, 30, 33). In this context, P_{\min} is often assumed to provide a measure of stomatal regulation of P_x , where large positive differences imply a relatively conservative response and small differences (or even negative differences) suggest a hydraulically risky response (19). Although this assumption is true for many species, many other traits may also influence P_{\min} , causing it to either underestimate or overestimate the influence of stomatal regulation on P_x . For example, shallow-rooted species that close stomata early in response to drought to avoid hydraulic failure may still reach low P_x as the soil dries out. Other species are able to maintain high P_x independent of stomatal regulation, such as through access to permanent (deep) moisture sources (14, 34, 35) or regular uptake of atmospheric moisture (36, 37). These factors make P_{\min} a potentially unreliable measure of stomatal regulation of P_x .

To assess stomatal response directly during desiccation, we propose defining standardized critical points from P_g P_x response curves (Fig. 1A). One such point, which we term P_{g12} , is particularly useful as a proxy of the point of stomatal closure (Fig. 1A). P_{g12} is defined as the P_x at which the tangent to the response curve at 50% of maximum stomatal conductance (g_{\max}) intersects with the P_x axis (Fig. 1A). The term “ P_{g12} ” is derived from the observation that the g_s at this P_x is $\sim 12\%$ of g_{\max} (Fig. 1A and Methods). The difference between P_{g12} and P_{50} (Fig. 1B) can then be used as a safety margin for determining stomatal regulation strategy (Fig. 1C), because neither point in isolation provides a reliable measure of hydraulic conservatism or drought tolerance. Large positive differences (e.g., the point labeled “2 & 3” in Fig. 1C) indicate that stomatal closure occurs before large losses of conductivity, whereas negative differences (e.g., the point labeled “1 & 4” in Fig. 1C) indicate stomatal closure subsequent to P_{50} . We used these safety margins as a proxy for the degree of hydricity of a species, with positive values being defined as isohydric and negative values being defined as anisohydric. By defining a measure of hydricity, we were then able to test hypotheses relating to the trade-offs between

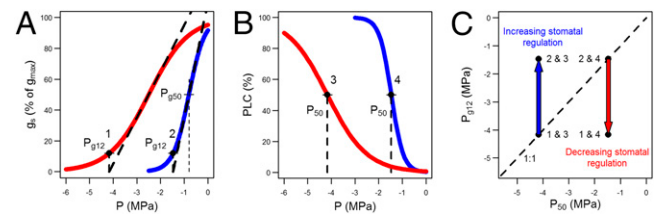


Fig. 1. Functional traits can be used to describe the hydraulic regulation strategy of a species. (A) g_s response curves provide a measure of the sensitivity of stomata to desiccation: P_{g12} . (B) Vulnerability curves provide a measure of xylem resistance to embolism: P_{50} . The colored curves illustrate two extreme responses for each: early (blue) and late (red) stomatal response in A and vulnerable (blue) and resistant (red) xylem in B. (C) These traits can be combined to define a safety margin capturing the degree to which stomatal closure regulates cavitation.

CO_2 uptake and water loss. In particular, we hypothesized that if a trade-off exists between CO_2 uptake and water loss, we might expect isohydric species (drought avoiders) to have greater xylem safety margins in comparison to anisohydric species (drought tolerators).

An appreciation of why knowledge of the hydraulic strategy of a species is essential emerges from the logical conclusion to the CO_2 uptake-water loss trade-off. Under severe drought events, plants may die from two mechanisms that are not mutually exclusive: carbon starvation or hydraulic failure (38). Although the precise details of these mechanisms are intensely debated (6, 39), there is evidence that both have contributed to recent mortality events (16, 39, 40). The intensity and duration of a drought are critical to understanding which species are likely to succumb to either hydraulic failure or carbon starvation (2, 16, 40). Isohydric species are more likely to succumb to carbon starvation during protracted mild drought, whereas anisohydric species are more likely to die from hydraulic failure during short but severe droughts (2, 40). A further test of our traits-based approach is whether it fits into this predictive framework: Can we use our assessment of hydraulic strategy to assess the likely mechanism of drought-induced mortality for each species in a diverse community?

To answer this question, we developed proxies for the likelihood of suffering carbon starvation or hydraulic failure using combinations of functional traits. We used the number of days since P_x in the field surpassed the point of stomatal closure (P_{g12}) as a novel proxy for the degree of carbon limitation. We reasoned that the longer a species spends with its stomata closed, the more likely it is to incur leaf-level carbon limitation, because assimilation rate is linearly related to g_s (9, 41). To assess the degree to which a species suffers hydraulic failure during drought conditions, we calculated the percent loss of conductivity at P_x . The two proxies can also be envisaged as corresponding to axes of drought intensity and duration, which are considered crucial to predictions of drought mortality of distinct hydraulic strategies (2). We then hypothesized that species defined as more isohydric by our traits-based system (i.e., species with more positive $P_{g12} - P_{50}$ values) would tend to be more carbon-limited during a drought, whereas anisohydric species would tend to incur hydraulic failure.

We tested our standardizable traits-based framework system in highly diverse mountain fynbos communities located within South Africa's Cape Floristic Region (CFR). The CFR presents an excellent system in which to explore these questions and examine the suitability of the hydraulic framework in understanding how complex communities might respond to drought events. Much of the CFR is exposed to hot, dry summers typical of Mediterranean-type climate regions, ensuring that the plants are regularly exposed to drought events (42). In addition, strong associations between climatic variables and diversity within the region suggest that climate might be an important driver of functional diversity (14, 43). Although previous long-term rainfall manipulation or in situ studies have shown the presence of contrasting drought response strategies within mountain fynbos

communities, these studies have only been able to capture the response of a handful of species (14, 44, 45). Given the exceptionally diverse nature of the flora (42), a more rapid assessment of the response of a wider range of species is desirable. This capacity may be particularly pressing, considering that the CFR is predicted to become hotter and drier with future climate change (46, 47). We quantified the response of g_s to changes in P_x and related the point of stomatal closure to standardized measures of vulnerability for a wide range of species. Here, we show that our traits-based framework assesses the degree of stomatal regulation of cavitation of a species and allows direct comparison between species within this global biodiversity hotspot. We also show how this system can be extended beyond the CFR to allow predictions about likely mechanisms of mortality within highly diverse communities elsewhere.

Results and Discussion

Here, we have extended previous research by presenting the architecture in which functional traits can be incorporated into a known hydraulic framework to provide additional insight into the range of hydraulic strategies present in diverse communities (Fig. 1). The ability to quantify the range of hydraulic regulation strategies rapidly within a single, highly diverse ecosystem is critically important in being able to assess vulnerability to future drought (22). Merging stomatal regulation strategies, which represent an index of water use behavior, with xylem hydraulic strategies, which represent water transport and vulnerability to water deficit behavior, facilitates a more comprehensive framework with which to characterize plant response to drought. This architecture may prove to be most valuable in biodiverse regions, where finding meaningful ways to determine the diversity of plant responses to drought can be extremely challenging.

The value of direct quantification of stomatal closure dynamics is shown by a comparison of the relationships between P_{50} and P_{g12} , P_{50} and P_{min} , and P_{g12} and P_{min} (Fig. 2). Although the range of P_x over which stomata closed was less than the range of P_{50} , it was large enough in magnitude to separate the different species. Some species displayed stomatal closure at P_x higher than -1 MPa, and others displayed stomatal closure at P_x at lower than -3 MPa (Fig. 2A). This variation of 2 MPa shows that a varied, continuous range of stomatal responses to declining P_x can exist within species-rich communities. However, unlike the relationship between P_{min} and P_{50} (Fig. 2B), P_{g12} and P_{50} were not linearly related (Fig. 2A). There exist at least two possible alternative explanations for this observation. On the one hand, stomatal closure might be entirely unrelated to xylem vulnerability. This explanation is contrary to a well-supported functional role for stomatal closure in avoiding xylem cavitation and is also inconsistent with several recent findings showing coordination between stomatal closure and xylem vulnerability for a range of species (28, 48, 49). A more plausible alternative explanation is that there exists a nonlinear relationship between these two variables that is influenced by multiple trade-offs (28, 32). As we show below, species that close their stomata relatively early (in terms of P_{50}) might well be favoring CO_2 uptake during wet

conditions while avoiding loss of hydraulic conductivity during dry periods (hydraulic plasticity). Conversely, species that close their stomata relatively late might be playing a riskier hydraulic game to maintain carbon uptake later into a drought.

Comparison of P_{g12} and P_{min} allowed us to identify species in which P_{min} overestimated or underestimated stomatal response to declining P_x (Fig. 2C). Over 90% of the species in which P_{min} was lower than P_{g12} were ericoid (i.e., small- to medium-sized shrubs with small leaves). Species of this functional type occurring in mountain fynbos generally have shallow rooting depths (14), which, although promoting the uptake of episodic rainfall, may also expose them to low soil P_x s during seasonal drought periods (11). Our results are consistent with the notion that reduced leaf size may be adaptive under these hot, dry conditions, because it decreases the boundary layer thickness, promoting convective heat loss without a further loss of water (50). The majority of species that experienced higher P_{min} than P_{g12} were from the Proteaceae and Restionaceae (Fig. 2C). The Proteaceae within the CFR are commonly deep-rooted (51), consistent with the notion that they are able to avoid desiccation partly by having access to moist soil layers (14). However, this explanation does not fit with the Restionaceae, which are composed of shallow-rooted, reed-like species. Here, low P_{g12} and high P_{min} are consistent with previous suggestions that species of this functional type are able to take up cloud moisture or dew (14, 52). Taken together, these findings suggest an additional utility of our traits-based approach: It highlights unusual combinations of functional traits and reveals alternative physiological strategies to coping with drought stress. Applying our system within diverse biomes should produce testable hypotheses regarding the functionality of species.

Returning to the question of potential trade-offs captured by the relationship between P_{50} and P_{g12} , it is significant that species within mountain fynbos communities separated out along a continuous range of stomatal regulation strategies or degree of isohydr/anisohydr (Fig. 3). The large amount of variation in $P_{g12} - P_{50}$ is consistent with a recent global comparison of xylem safety margins (measured as $P_{min} - P_{50}$), which revealed that species within Mediterranean and woodland biomes displayed the highest range of values (19). Given a potential trade-off between carbon assimilation and hydraulic safety, one might propose that these species are occupying distinct water regulation niches. We provide further support for this notion by showing a linear relationship between degree of isohydr/anisohydr ($P_{g12} - P_{50}$) and xylem safety margin ($P_{min} - P_{50}$) (Fig. 4). Species that displayed a conservative xylem safety margin (i.e., had a low risk of experiencing major xylem cavitation in situ) were also those species that closed their stomata relatively early in response to desiccation (Fig. 4). On the other hand, those species that closed their stomata relatively late were also those species that displayed a relatively risky xylem safety margin (Fig. 4). Our interpretation of these results is that isohydric species are more likely to maximize carbon uptake when conditions are favorable, but to do so and to avoid hydraulic failure, they also need to maintain larger safety margins. Anisohydric species, on the other hand, tend to favor lower carbon uptake over longer

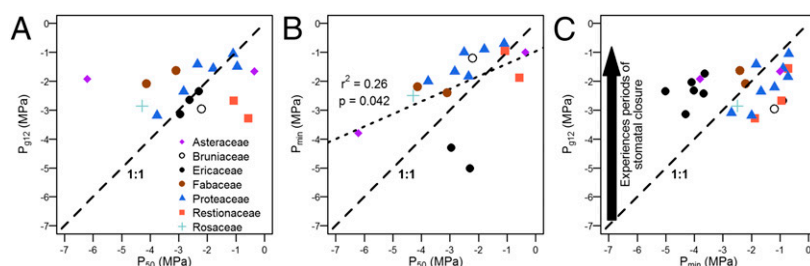


Fig. 2. Relationships between xylem vulnerability (P_{50}) and P_x at stomatal closure (A; P_{g12}) and minimum P_x (B; P_{min}), and between P_{g12} and P_{min} for fynbos species (C). These data highlight the inadequacy of P_{min} for capturing the point of stomatal closure.

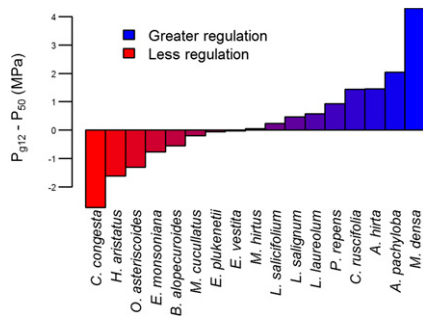


Fig. 3. Stomatal safety margin ($P_{g12} - P_{50}$) of species from mountain fynbos showing the range of stomatal regulation strategies within a single community. We propose that $P_{g12} - P_{50}$ provides a proxy for degree of isohydric/anisohydric. The full names for all species shown are as follows: *Cannomois congesta*, *Hypodiscus aristatus*, *Osmitopsis asterisoides*, *Erica monsoniana*, *Brunia alopecuroides*, *Mimetes cucullatus*, *Erica plukenetii*, *Erica vestita*, *Mimetes hirtus*, *Leucadendron salicifolium*, *Leucadendron salignum*, *Leucadendron laeolum*, *Protea repens*, *Cliffortia ruscifolia*, *Aspalathus hirta*, *Aspalathus pachyloba*, *Metalasia densa*.

periods, but with lower rates of water loss. As a result, these species can afford to have smaller safety margins. Our interpretation is consistent with previous observations showing that species with higher maximum g_s displayed greater sensitivity and an increased rate of stomatal closure in response to changes in vapor pressure deficit (53). It is also supported by observations that isohydric species are able to recover gas exchange rates more rapidly following a drought compared with anisohydric species (18, 54).

Combinations of functional traits also allowed us to explore how potential mechanisms of mortality might differ between species (Fig. 5). We selected three fynbos species with contrasting hydraulic regulation strategies for which we could obtain long-term in situ P_x data from a previously published source (44). *Protea repens*, *Erica monsoniana*, and *Cannomois congesta* displayed significant variation in the degree to which stomata regulate xylem cavitation (measured by $P_{g12} - P_{50}$ as shown in Fig. 3), and thus are likely to have contrasting hydraulic strategies. These species displayed significant variation in the degree to which they were likely to suffer carbon limitation and hydraulic failure during drought periods in the field (Fig. 5). Significantly, there appeared to be a strong association between hydraulic strategy and likely mechanism of mortality. Of the three species selected, *P. repens* had the most conservative stomatal response, closing rapidly in response to desiccation (Fig. 3). In our predictive framework, the trajectory that *P. repens* displayed indicated that it was likely to suffer from carbon limitation if the drought persisted. In contrast, *C. congesta* displayed a relatively risky stomatal strategy (Fig. 3), and the trajectory of this species indicated that it was likely to suffer from hydraulic failure (Fig. 5). The degree of stomatal regulation of cavitation in *E. monsoniana* was intermediate between the previous two species (Fig. 3). Remarkably, *E. monsoniana* appears to be vulnerable to both carbon starvation and hydraulic failure (Fig. 5), making this species highly susceptible to drought mortality.

These results are consistent with previous findings in fynbos communities (14), which have shown *Erica* species to be particularly sensitive to drought. In a rainfall exclusion study, anisohydric *Erica* species displayed the most amount of drought-induced die-back (14). Deep-rooted proteoid species displayed little drought mortality and were considered to be isohydric (14). Restioids (i.e., *Cannomois*) also exhibited patchy drought-induced die-back within individuals, consistent with hydraulic failure (14).

As an independent test of our framework, we explored how well we could categorize the well-documented drought responses of piñon and juniper from the southwestern United States. Using data from a previous study (17), we were able to show that our

trait combinations predicted outcomes consistent with the reported mechanisms of drought mortality in these species. The isohydric *Pinus edulis* incurred increasing carbon limitation during a seasonal drought period (Fig. 5), consistent with the hypothesis that this species suffers drought-mediated carbon starvation in the field (17, 18, 38, 40, 55). The anisohydric *Juniperus osteosperma* appeared to incur increasing hydraulic failure, as well as a protracted period of stomatal closure (Fig. 5), consistent with field observations (43) and the hypothesis that this species is likely to incur hydraulic failure resulting from declining P_x during intense drought (2). Taken together, the findings presented here suggest that expanding upon previous research that contrasted isohydric (avoidance) and anisohydric (tolerance) hydraulic strategies in plants by including additional traits (P_{g12} , P_x , and $P_{g12} - P_{50}$) has substantial merit because using our trait combinations has allowed us to predict the likely mechanism of mortality within biodiverse regions.

The approach of using functional traits to infer the hydraulic strategy of a species has tremendous potential. Our system provides a standardizable means of identifying variation in hydraulic regulation strategies based on trait combinations and enables comparison both within and between highly diverse communities. Our examples emphasize the importance of explicitly assessing coordination between stomatal and xylem traits, as well as P_{min} , and the benefit of standardized measures for comparison between multiple species. We have also shown how variation in trait combinations may assist in explaining potential mechanisms of mortality. This approach may prove valuable for rapid assessment of the vulnerability of communities to future drought events, particularly in species-rich communities where detailed, long-term physiological studies are unfeasible.

Methods

Study Sites and Species. Species were sampled from mountain fynbos communities located within the southwest CFR (a full list of species is provided in Table S1). Specific sites were located at Jonaskop in the Riviersonderend mountain range (lat 33° 15' 48.34" S, long 19° 31' 33.97" E, elevation of 981 m), the Kogelberg Biosphere (lat 34° 19' 02.57" S, long 18° 57' 39.74" E, elevation of 61 m), and the Silvermine section of the Table Mountain National Park (lat 34° 06' 26.45" S, long 18° 26' 31.37" E, elevation of 375 m). These study sites were chosen because they contain particularly diverse floral communities that have been the focus of previous ecophysiological studies, including assessments of the variation in vulnerability of stem xylem to cavitation and long-term drought response investigations (14, 56–58).

Stomatal Response to Dehydration. The relationship between g_s and P_x was examined using a benchtop dehydration method. Stems or branches were cut under water in the field and subsequently transported back to the

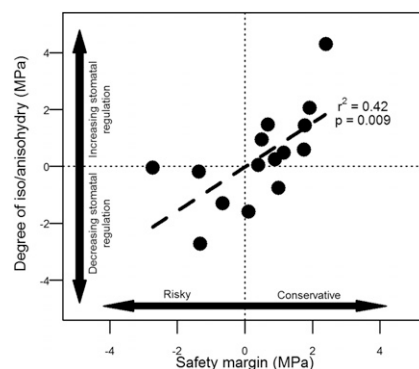


Fig. 4. Degree of isohydric/anisohydric (defined as the degree of stomatal regulation of cavitation or $P_{g12} - P_{50}$) varies with the stem-level hydraulic safety margin ($P_{min} - P_{50}$) within mountain fynbos communities. More isohydric species tend to have larger safety margins, whereas more anisohydric species tend to have smaller (or negative) safety margins.

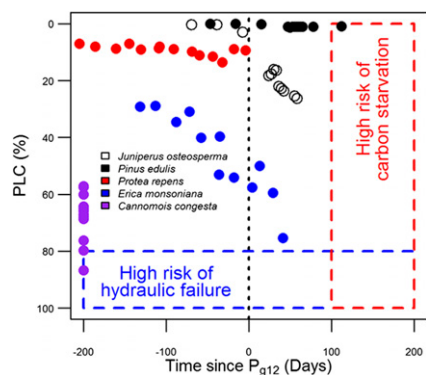


Fig. 5. Likely mechanism of mortality experienced by a species can be assessed by plotting seasonal PLC against the time since stomatal closure. Time since stomatal closure (defined as time since P_{g12}) provides an indication of the duration of the drought, and hence the degree of carbon limitation that a species endures. Negative values are produced when P_x is less negative than P_{g12} (i.e., stomata remain open) and the plant is unlikely to be carbon-limited. Positive values are produced when P_x is below P_{g12} and plants are more likely to be carbon-limited. PLC is used as a proxy for risk of hydraulic failure. Trajectories for all species were created using previously published seasonal P_x measurements and vulnerability curves (17, 44, 55).

laboratory in water. Each stem was placed on a benchtop in a temperature- and light-controlled chamber and allowed to dry to a range of P_x values. We measured g_s using an IR gas analyzer (IRGA; Li-Cor 6400) and expressed per leaf area. Light intensity was kept constant at $1,500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and humidity was maintained slightly below ambient using a desiccant. Immediately following the g_s measurement, the target shoot was cut and P_x was measured using a Scholander pressure chamber (PMS Instrument Company). A Weibull function was fitted to the g_s response curve for each species (Fig. 1A):

$$g_s(\% \text{ of } g_{\text{max}}) = 100 - \left(100 / \left(1 + e^{a(x-b)} \right) \right), \quad [1]$$

where a is a fitted parameter describing the slope of the curve and b is the P_x at 50% loss of g_{max} (P_{g50}). Similar to the analysis of Domec and Gartner (59), we parameterized this model and extracted several standardized values describing the sensitivity of g_s to P_x . Specifically, we found the derivative of Eq. 1:

$$d \text{ PLC}_g / d P = -a \left(e^{a(P-b)} \right) 100 / \left(1 + e^{a(P-b)} \right)^2, \quad [2]$$

which provides the slope of the tangential line to Eq. 1 at each P_x . We used this derivative to find the slope of the tangential line at P_{g50} (Fig. 1A). We then calculated the P_x intercept of this tangential line, which we termed P_{g12} , using the following equation:

$$P_{g12} = 2/a + b. \quad [3]$$

We used P_{g12} as a proxy for the P_x at stomatal closure (points 1 and 2 in Fig. 1A).

Gas Exchange and P_x of Plants in Situ. To assess the efficacy of our reasonably simple benchtop dehydration technique, we compared laboratory-based measurements of P_x and g_s with those measurements taken in the field for six species over a period of three consecutive years as part of two separate long-term monitoring studies. We will briefly report the important details of these measurement campaigns as they have been reported elsewhere (14, 44). The g_s was measured using an IRGA (Li-Cor 6400) at midday during spring and late summer over 3 y. Light intensity in the cuvette was set at $1,500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, humidity was maintained just below ambient, and CO_2 was held constant at 400 ppm. The g_s was expressed on a per-leaf-area basis. The P_x of the same shoot was measured immediately after gas exchange using a Scholander pressure chamber. There was generally very good agreement between g_s response curves obtained using the benchtop dehydration method and data collected from long-term field measurements (Fig. S1).

Vulnerability Curves. We obtained the PLC in response to declining P_x (i.e., vulnerability curves) for 18 species (Table S1). To obtain vulnerability curves for as many species as possible, we measured vulnerability curves using both

the benchtop dehydration method (60) and the air injection method (61), and we also gathered data from published studies (the studies in which vulnerability curve data were presented are listed in Table S1). We assessed the similarity of these measures by comparing the vulnerability curve of *P. repens* obtained using the benchtop dehydration method with the vulnerability curve of *P. repens* obtained using the air injection method and found strong agreement.

We used the benchtop dehydration method for *P. repens*, *E. monsoniana*, and *C. congesta*. For each species, we first determined the maximum vessel length to inform the minimum stem length needed to avoid any open vessels (62). To do this measurement, we cut five stems under water in the field and then placed tubing connected to an air tank on one end and made successive cuts from the opposite end. The mean length at which bubbles first emerged was taken as the maximum vessel length for each species. For the vulnerability curves, branches were cut under water in the field and subsequently dried to a range of P_x values. Branches were then sealed in plastic bags for at least 1 h to equilibrate. The P_x was measured from a shoot from each stem using a Scholander pressure chamber. The remaining branch was placed under water and cut into an unbranched segment. Several cuts were made at each end to ensure that the xylem tension relaxed (63). Native flow rate was measured by connecting each stem to tubing attached to a pressure head containing KCl solution (20 mM). Water was collected from the distal end of the stem and weighed on a balance. Once a consistent flow rate had been achieved and measured, the stems were removed and immediately connected to tubing connected to a high-pressure, filtered KCl solution for at least 1 h to remove any embolism. After flushing, the stems were reconnected to the pressure head and the flow rate was remeasured. Native stem-specific conductivity (K_n) and flushed stem-specific conductivity (K_{max}) were calculated by expressing flow rate per pressure head per stem length per second. The PLC at each P_x value was calculated as:

$$\text{PLC} = 100 \left((K_{\text{max}} - K_n) / K_{\text{max}} \right). \quad [4]$$

We quantified vulnerability curves of two species, *Hypodiscus aristatus* and *Leucadendron lauroolum*, using the air injection method (60). Stems of these two species were collected in the field and flushed in the laboratory with filtered 20 mM KCl solution using high pressure (150 kPa) to remove any native embolism. Flushed stems were subsequently placed in air sleeves connected to a pressure tank after being scored slightly with a razor blade. The distal end of each stem was connected to tubing connected to a pressure head containing filtered 20 mM KCl solution. Flow rate was measured by periodically measuring the weight of vials connected to the axial end of the stem. Embolism was induced in the stem by applying different pressures to the sleeve for 10 min at a time. After each pressure treatment, the pressure was released and the stable flow rate was determined. The PLC was calculated as before using Eq. 4.

Vulnerability curves were modeled for each species using the Weibull function:

$$\text{PLC} = 100 / \left(1 + e^{a(P-b)} \right), \quad [5]$$

where a and b were parameters found using least-squares regression analysis.

P_{min} . The P_{min} in the field was collected for nine species during summer dry periods. Because these data were from long-term datasets, we have confidence that they represent close to actual P_{min} values in the absence of extreme drought. For each species, predawn and midday shoot water potential (P_{shoot}) of at least five individuals was sampled using a Scholander pressure chamber. For the remaining species, we extracted P_{min} values from published sources (17, 19, 56, 58). A potential limitation of using midday P_{shoot} values to estimate PLC is that midday P_{shoot} will be more negative than stem P_x , resulting in an overestimation of the amount of cavitation in stems (64). To reduce this error, we estimated PLC based on predawn P_{shoot} as opposed to midday values. Because the difference between stem P_x and leaf P_x is expected to be greatest under high transpiration rates, predawn measures will tend to reduce any difference between P_x and P_{shoot} . Second, segmentation of xylem vulnerability often tends to make shoots more vulnerable than stems (33), which might counteract the difference created by sampling P_{shoot} .

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Supporting Information

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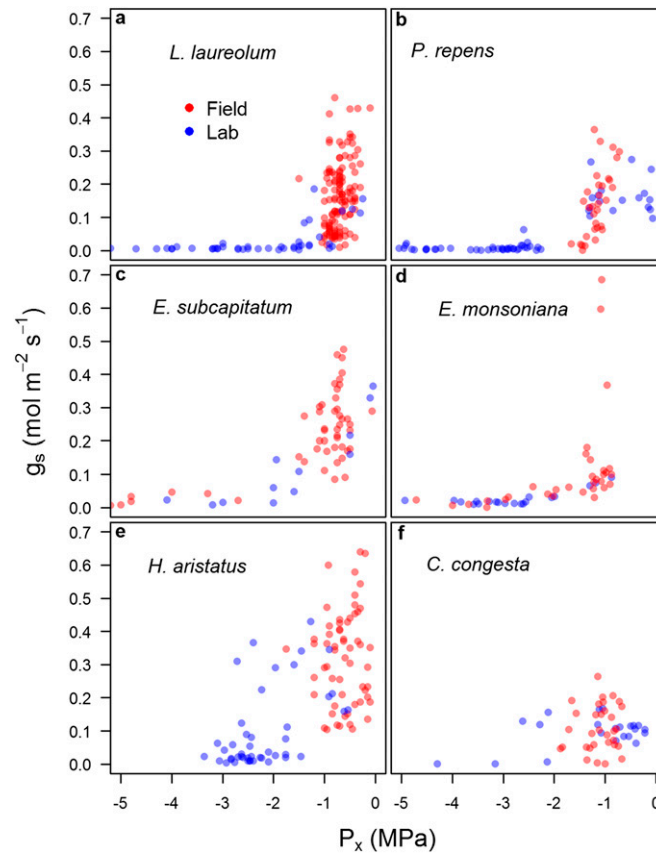


Fig. S1. Response of g_s to changes in P_x for six species obtained from long-term field studies (red) or laboratory dry-downs (blue). The full names for all species in A–F are shown in Table S1.

Table S1. List of species for which the P₅₀ has been examined and the study in which it was reported

Family	Species
Asteraceae	<i>Metalasia densa</i> (Lam.) P. O. Karis (1) <i>Osmitopsis asteriscoides</i> (P. J. Bergius) Less (2)
Bruniaceae	<i>Brunia alopecuroides</i> Brongn (2)
Cupressaceae	<i>Juniperus osteosperma</i> (Torr.) Little (3)
Ericaceae	<i>Erica plukenetii</i> E. G. H. Oliv. and I. M. Oliv (1) <i>Erica subcapitata</i> (N. E. Br.) E. G. H. Oliv <i>Erica vestita</i> Thunb (1) <i>Erica monsoniana</i> L. f. (4)
Fabaceae	<i>Aspalathus hirta</i> E. Mey (1) <i>Aspalathus pachyloba</i> Benth (1)
Pinaceae	<i>Pinus edulis</i> Engelm (3)
Proteaceae	<i>Leucadendron laureolum</i> (Lam.) Fourc (1) <i>Leucadendron salignum</i> P. J. Bergius (1) <i>Leucadendron salicifolium</i> (Salisb.) I. Williams (2) <i>Mimetes cucullatus</i> (L.) R. Br (2) <i>Mimetes hirtus</i> (L.) Salisb. ex Knight (2) <i>Protea repens</i> (L.) L. (1, 4)
Restionaceae	<i>Hypodiscus aristatus</i> (Thunb.) C. Krauss <i>Cannomois congesta</i> Mast (4)
Rosaceae	<i>Cliffortia ruscifolia</i> L. (1)

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