The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought

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Climate change is expected to exacerbate drought for many plants, making drought tolerance a key driver of species and ecosystem responses. Plant drought tolerance is determined by multiple traits, but the relationships among traits, either within individual plants or across species, have not been evaluated for general patterns across plant diversity. We synthesized the published data for stomatal closure, wilting, declines in hydraulic conductivity in the leaves, stems, and roots, and plant mortality for 262 woody angiosperm and 48 gymnosperm species. We evaluated the correlations among the drought tolerance traits across species, and the general sequence of water potential thresholds for these traits within individual plants. The trait correlations across species provide a framework for predicting plant responses to a wide range of water stress from one or two sampled traits, increasing the ability to rapidly characterize drought tolerance across diverse species. Analyzing these correlations also identified correlations among the leaf and stem hydraulic traits and the wilting point, or turgor loss point, beyond those expected from shared ancestry or independent associations with water stress alone. Further, on average, the angiosperm species generally exhibited a sequence of drought tolerance traits that is expected to limit severe tissue damage during drought, such as wilting and substantial stem embolism. This synthesis of the relationships among the drought tolerance traits provides crucial, empirically supported insight into representing variation in multiple traits in models of plant and ecosystem responses to drought.

drought tolerance | stem hydraulics | leaf hydraulics | stomatal closure | turgor loss point

Plants worldwide are expected to face more frequent and severe droughts under climate change (1). Characterizing drought tolerance for diverse species is key to improved predictions of ecosystem responses to global change (2), and ecological and phylogenetic patterns have been established across many species for individual drought tolerance traits (3–7). However, plant drought tolerance is determined by multiple traits. The relationships among traits within plants and across species have not been evaluated for general patterns across global plant diversity. We synthesized the published data to elucidate global patterns in the relationships among stomatal, hydraulic, and leaf mesophyll drought tolerance traits. We evaluated the roles of functional coordination, covariance with water stress, and shared ancestry in driving trait correlations across species. Additionally, we focused on clarifying relationships among drought tolerance traits within plants of given species, i.e., determining the sequence of their water potential thresholds.

Classical drought tolerance traits quantify the water potentials that induce declines in key physiological processes, such as stomatal conductance, hydraulic conductivity, and cell turgor pressure. Previous studies have shown that these water potential thresholds are intercorrelated for small species sets (8–12). We tested these correlations for a large dataset to produce a framework for extrapolating plant responses to a wide range of water stress from one or two traits. Evaluating these correlations across a global dataset can provide additional insights into their drivers. Drought tolerance traits can be correlated across species because of (*i*) functional coordination, such as mechanistic linkages; (*ii*) concerted convergence (13), i.e., coselection by the environment, wherein traits are directionally but independently selected by water supply to optimize overall plant function; and/or (*iii*) shared ancestry. We compiled hypotheses from the literature for the drivers of each trait correlation, and evaluated these hypotheses by testing for greater coordination among traits than explained by water stress and relatedness. Water stress was measured as the minimum leaf water potential observed over the course of a year or during the dry season, at predawn ($\Psi_{\mathrm{min,\ PD}}$) and midday ($\Psi_{\mathrm{min,\ MD}}$). $\Psi_{\mathrm{min,\ PD}}$ is taken when transpiration is at its minimum and the water potential of the plant is closest to equilibration with that of the soil, whereas $\Psi_{\mathrm{min,\ MD}}$ is affected by any cuticular or stomatal transpiration and, thus, broadly captures the integrated effects of plant traits and the environment on the minimum water potential a plant reaches in natural conditions (14).

The sequence of water potential thresholds for drought tolerance traits within a plant is expected to strongly impact overall plant function under water stress (8, 15–17). Previous studies have compared values for some traits (e.g., refs. 9, 10, and 18), but have not included enough traits or species to characterize their overall sequence. We tested the degree to which plants exhibit a trait sequence that is expected to limit severe drought damage. Plants are expected to undergo stomatal closure at sufficiently high water potentials to prevent wilting and/or substantial (i.e., ≥50%) declines in stem hydraulic conductivity (6, 19, 20). Additionally, the vulnerability segmentation hypothesis predicts that plants limit stem embolism by exhibiting less negative thresholds for declines

Significance

Many plant species face increasing drought under climate change, making plant drought tolerance integral to predicting species and ecosystem responses. Many physiology traits interact to determine overall drought tolerance, but trait relationships have not been assessed for general patterns across global plant diversity. We analyzed stomatal, hydraulic, and mesophyll drought tolerance traits for 310 species from ecosystems worldwide. We evaluated the sequence of drought responses for plants under increasing water stress, and showed that coselection with environmental water stress drives most trait correlations across species, with functional coordination additionally important for some relationships. These results provide insight into how variation in multiple traits should be represented within plants and across species in models of plant responses to drought.

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in hydraulic conductivity in the leaves and roots, thereby sequestering hydraulic damage in those organs (17). Plants that do not exhibit this trait sequence are expected to avoid drought damage by limiting water stress (i.e., maintaining a high $\Psi_{\mathrm{min,\ MD}}$ relative to thresholds for damage) through deep roots, capacitance from stored water, drought deciduousness, or a preference for mesic environments (21, 22), or to experience significant damage at $\Psi_{\mathrm{min,\ MD}}$ and survive through recovery processes (23).

We compiled species means from the published literature for 262 woody angiosperm and 48 gymnosperm species from 174 studies for the water potential thresholds for wilting, plant death, and declines in stomatal conductance (g_s) and hydraulic conductivity (K) of leaves, stems, and roots (trait symbols and definitions in Table 1, references in *SI Appendix*, Table S1, and ranges in *SI Appendix*, Fig. S1). Controversy has recently arisen regarding measurements of stem and root hydraulic traits (24), in particular about whether nonsigmoidal vulnerability relationships (i.e., of K vs. Ψ) are caused by methodological artifacts that overestimate vulnerability. We tested the correlations across species by using all available data (*SI Appendix*, *SI Methods*), but confirmed our conclusions for the smaller dataset derived from sigmoidal relationships (n = 285) and present these results in the main text (Dataset S1). We evaluated the drivers of the correlations and the trait sequence for the subset of species for which all traits were measured at the same site during the same ≤6 mo sampling period, to minimize intraspecific variation (n = 238) (Dataset S2). Both analyses used hydraulic traits derived from sigmoidal relationships, and the sequence analyses focused on woody dicots, because there was insufficient data to test other curve shapes or plant functional types.

Results and Discussion

Correlations Across Species in Drought Tolerance Traits. We found significant correlations among most of the drought tolerance traits, with r values ranging from 0.21 to 0.87 (Fig. 1 and *SI Appendix*, Table S2; n = 11–151). The nonsignificant correlations were between K_{stem} Ψ_{12} and g_s Ψ_{50}, and K_{leaf} Ψ_{50} and g_s Ψ_{50}, g_s Ψ_{95} and K_{stem} Ψ_{88} ($P > 0.1$, n = 11–52). These correlations were robust to vulnerability curve shape, except that K_{leaf} Ψ_{50} and K_{stem} Ψ_{88} were correlated when including data for all curves (P = 0.03, n = 61; *SI Appendix*, Table S3). The stomatal and leaf hydraulic trait correlations represent particularly small species sets, indicating a need for more measurements of these traits. Nearly all traits were correlated with $\Psi_{\mathrm{min,\ MD}}$ and $\Psi_{\mathrm{min,\ PD}}$, with r values ranging from 0.21 to 0.86 (*SI Appendix*, Figs. S2 and S3 and Table S2). $\Psi_{\mathrm{min,\ PD}}$ and K_{leaf} Ψ_{50} were not significantly correlated (P = 0.07, n = 44), and there were insufficient data to test correlations between $\Psi_{\mathrm{min,\ PD}}$ and the stomatal traits. Six of the 19 correlations with sufficient data to test ($n \geq 10$ for each functional type) were significantly different between the angiosperms and gymnosperms. K_{stem} Ψ_{12} was significantly correlated with K_{leaf} Ψ_{50} and $\Psi_{\mathrm{min,\ MD}}$ in the gymnosperms but not the angiosperms (*SI Appendix*, Table S4 and Fig. S2*E*), whereas the two functional types showed significantly different slopes for the correlations of K_{stem} Ψ_{50} with π_{tlp} and K_{stem} Ψ_{12} (Fig. 1*D*), and of $\Psi_{\mathrm{min,\ MD}}$ with K_{stem} Ψ_{50} and K_{root} Ψ_{50} (*SI Appendix*, Fig. S2 *F* and *H*).

Applying the Framework To Predict Drought Tolerance Traits. These correlations provide a framework representative of many species for extrapolating plant responses to a wide range of water stress from a small number of measured traits. Extrapolating from the correlations with K_{stem} Ψ_{50}, which has been measured for the most species (4), or π_{tlp}, which can be assessed rapidly (25), provides a reasonable estimate for less commonly measured

Table 1. The symbol, definition, and functional significance of the drought tolerance traits and the environmental water supply and general plant water status variables

Symbol	Definition	n	Significance
Ψ_W	Water potential		Potential energy of water; a thermodynamically explicit and scalable index of water status
Ψ_{leaf}, Ψ_{stem}, Ψ_{root}	Ψ_W of the leaf, stem, and root		Index of hydration and the demand for water of each organ
π_{tlp}	Bulk leaf turgor loss point, the Ψ_{leaf} where turgor potential = 0	285	Point at which, on average, leaf cells lose turgor and the leaf wilts (7)
g_S Ψ_{50}	Ψ_{leaf} at 50% loss of stomatal conductance	49	Ψ_W at 50% loss is a standard and, thus, comparable measure of drought tolerance across physiological processes (6)
g_S Ψ_{95}	Ψ_{leaf} at 95% loss of stomatal conductance	49	Approximates the maximum leaf water stress a plant can tolerate while maintaining gas exchange and C uptake
K_{leaf} Ψ_{50}	Ψ_{leaf} at 50% loss of leaf conductivity	117	Hydraulic traits measure drought impacts on the water supply for transpiration, which limits gas exchange and C uptake (17). Leaf water supply is hypothesized to be the most direct hydraulic constraint on transpiration (8)
K_{stem} Ψ_{12}	Ψ_{stem} at 12% loss of stem conductivity	208	Early declines in stem water supply are expected to impact gas exchange and C uptake more directly than later declines (10)
K_{stem} Ψ_{50}	Ψ_{stem} at 50% loss of stem conductivity	286	Hypothesized to correspond closely to the maximum water stress plants tolerate in natural conditions (4)
K_{stem} Ψ_{88}	Ψ_{stem} at 88% loss of stem conductivity	204	Hypothesized to be the point of irreversible xylem damage (18)
K_{root} Ψ_{50}	Ψ_{root} at 50% loss of root conductivity	44	Roots are hypothesized to be the "weakest link" (least tolerant organ), limiting tolerance of the entire hydraulic system (45)
Plant Ψ_{lethal}	Ψ_{leaf} at plant death; here, the Ψ_{leaf} at which all leaves show tissue damage	15	Integrates physiological and metabolic drought responses and recovery and directly links drought to performance (11)
$\Psi_{\mathrm{min,\ MD}}$, $\Psi_{\mathrm{min,\ PD}}$	Seasonal minimum water potential (Ψ_{min}), the most negative Ψ_{leaf} measured in the growing season at predawn (PD) or midday (MD)	174	Midday measurements quantify the strongest water stress the leaves experience in a typical year, whereas predawn measurements characterize the most negative soil water potential (13)

n is the number of species compiled for each trait. All units are MPa.

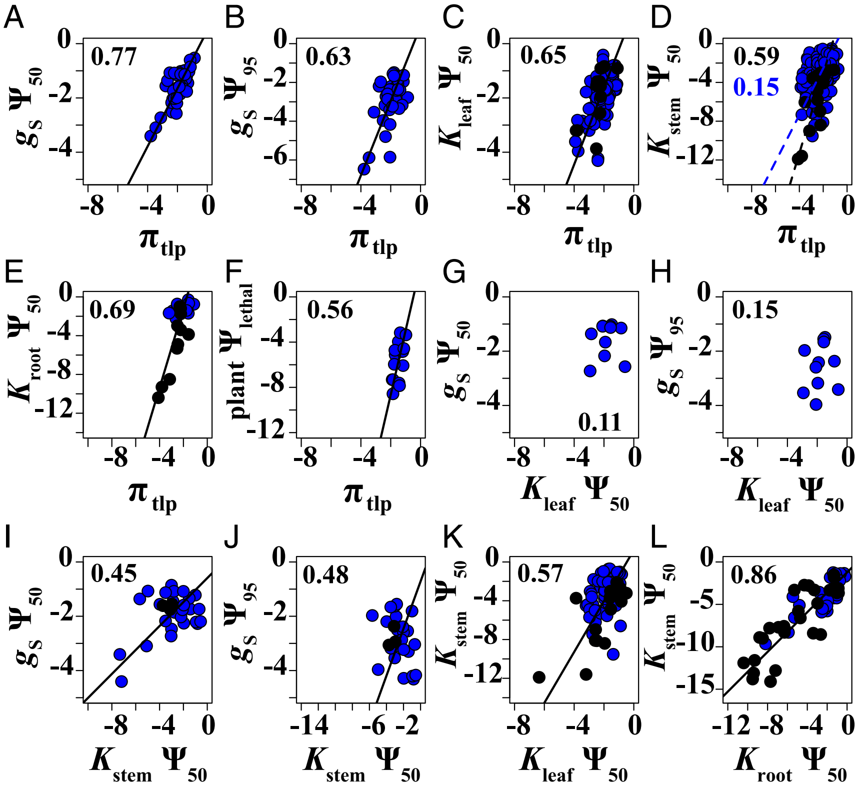

Fig. 1. Correlations among drought tolerance traits across species. Symbols follow Table 1. Blue points represent angiosperms, and black points represent gymnosperms. Solid black lines are standard major axis relationships that are significant across all species. Dashed lines are correlations that are significantly different between the gymnosperms (black lines) and angiosperms (blue lines). All significant correlations remained significant after correcting for multiple tests (46). The r values are shown on each graph, and *P* values and sample sizes are in *SI Appendix*, Table S2. All of the traits were significantly correlated (*A*–*F* and *I*–*L*), except for K_{leaf} Ψ_{50} and g_S Ψ_{50} (*G*) and g_S Ψ_{95} (*H*). For graphical clarity, correlations with K_{stem} Ψ_{12} and Ψ_{88} are not shown. All of the stem hydraulic traits showed the same correlations, except that K_{stem} Ψ_{12} was not significantly correlated with g_S Ψ_{50} and K_{leaf} Ψ_{50} was not significantly correlated with K_{stem} Ψ_{88} (*SI Appendix*, Table S2). K_{stem} Ψ_{12} was significantly correlated with K_{leaf} Ψ_{50} in the gymnosperms but not the angiosperms, whereas the two functional types showed significantly different slopes for the correlations of K_{stem} Ψ_{50} with π_{tlp} (*D*) and with K_{stem} Ψ_{12} (*SI Appendix*, Table S4). We did not compile variation in plant Ψ_{lethal} from the literature, because most published studies use different definitions for plant death, but instead show this correlation from the largest study of these traits (11) for comparison with the other correlations with π_{tlp} (*F*).

traits, until such data become available in the literature for more species (see Dataset S3 for estimating traits from these correlations). The correlations strongly support predicting K_{leaf} Ψ_{50} and, for the angiosperms, the stomatal traits from π_{tlp} ($r^2 = 0.40$–0.59), and π_{tlp} enabled trait estimation with considerably smaller prediction intervals than K_{stem} Ψ_{50}. π_{tlp} also enabled estimation of $\Psi_{min,\ MD}$ with smaller prediction intervals than K_{stem} Ψ_{50} in both the angiosperms and gymnosperms. These "first pass" estimates lend expediency to assessing drought tolerance for many species, and potentially enable more detailed modeling of plant drought responses, given that few species have been studied relative to the worldwide diversity of plant species, and even these have only been assessed for a few traits.

Trait Correlations with Environmental Water Supply. The significant correlations with $\Psi_{min,\ MD}$ support the selective pressure of plant water stress on all of the traits (*SI Appendix*, Fig. S2). Further, the correlations with $\Psi_{min,\ PD}$ supported the use of any of the traits but K_{leaf} Ψ_{50} to predict species distributions relative to soil water supply (*SI Appendix*, Fig. S3), although previous studies of smaller species sets have shown significant correlations between K_{leaf} Ψ_{50} and precipitation (5, 26), indicating a need to test this relationship across yet-larger species sets. Notably, $\Psi_{min,\ MD}$ was especially strongly correlated with g_S Ψ_{50} and g_S Ψ_{95} ($r = 0.76$–0.86), suggesting that these stomatal traits may be especially important influences on the maximum water stress the leaves experience (*SI Appendix*, Fig. S2 *B* and *C*), whereas K_{root} Ψ_{50} had the strongest association with minimum soil water potential ($r = 0.72$) (*SI Appendix*, Fig. S3*F* and Table S2). Testing these hypotheses requires measuring more traits for the same species, and, especially, focusing on closely related species within clades that have diversified across habitats ranging widely in water availability.

Disentangling the Basis for Trait Correlations. We found support for hypotheses from the literature (Fig. 2) that attributed drought tolerance trait correlations to functional coordination, concerted convergence (wherein water stress selects for each trait independently), and/or shared ancestry. Of the 14 correlations with sufficient data to test (n = 19–64), 4 correlations were improved beyond the correlation of each trait with $\Psi_{min,\ MD}$ alone by accounting for a trait predictor (29%), 1 by accounting for phylogeny (7%), and 1 by accounting for both (7%) (*SI Appendix*, Table S5). Thus, for a total of 43% of trait correlations, we could resolve linkages beyond simply a correlation arising from independent associations with water stress. As hypothesized, π_{tlp} improved prediction of K_{leaf} Ψ_{50}, and vice versa, whereas the stem hydraulic traits K_{stem} Ψ_{12} and Ψ_{88} were not correlated with π_{tlp} after accounting for water stress. However, contrary to prediction, K_{stem} Ψ_{50} and K_{leaf} Ψ_{50} were more related than expected from correlations with water stress alone. Further, the π_{tlp} improved prediction of $K_{stem}\Psi_{50}$, and K_{stem} Ψ_{12} improved prediction of K_{leaf} Ψ_{50}, but not vice versa.

It is well recognized that $\Psi_{min,\ MD}$ can be affected by plant traits in addition to soil dryness (14), so we verified these findings for $\Psi_{min,\ PD}$ (n = 18–40; *SI Appendix*, Table S6). The water stress variables were strongly correlated ($r^2 = 0.85$, $P < 0.001$, $n = 71$). The coordination analyses showed largely similar results, with the exceptions that K_{leaf} Ψ_{50} and K_{stem} Ψ_{12} were both more strongly related than expected from associations with $\Psi_{min,\ PD}$, whereas K_{stem} Ψ_{50} and π_{tlp} were not correlated after accounting for $\Psi_{min,\ PD}$.

Several mechanisms could potentially drive the observed trait coordination. The coordination between K_{leaf} Ψ_{50} and π_{tlp} supports the hypothesized mechanistic effect of turgor loss in the mesophyll on declines in K_{leaf} via the extraxylary pathway (20). As a leaf dries, and the mesophyll cells lose turgor, the cells shrink, and aquaporin activity and abscisic acid levels can shift rapidly, affecting water transport (20). The extraxylary pathway accounts for a significant proportion of overall leaf hydraulic resistance (~25–70%) (27), and the vulnerability of this pathway strongly impacts K_{leaf} Ψ_{50} (20). Indeed, species with more negative π_{tlp} values undergo less cell shrinkage under dehydration and have slower declines in K_{leaf} with leaf water potential (20). The coordination between K_{leaf} Ψ_{50} and K_{stem} Ψ_{50},

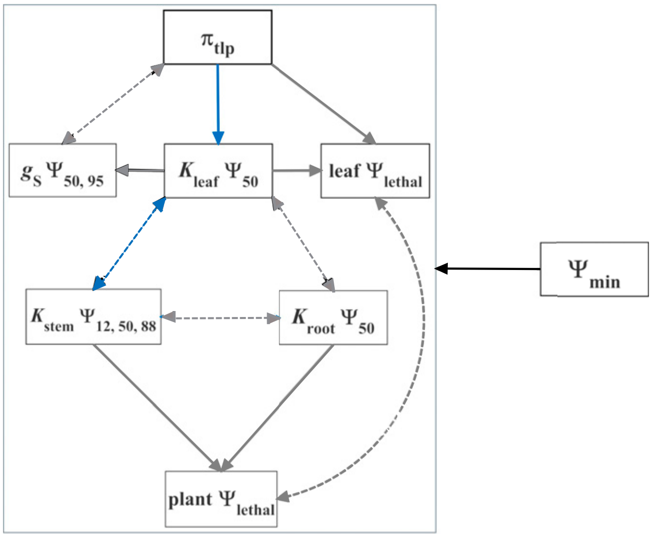

Fig. 2. Testing hypotheses for the drivers of the correlations among the drought tolerance traits. Most of the trait correlations are predicted to be driven by concerted convergence, wherein the selective pressure of water stress ($\Psi_{min,\ MD}$ or $\Psi_{min,\ PD}$) acts independently on each trait to optimize overall plant function during drought (10, 17, 28). These hypotheses are indicated with dashed lines. Additionally, π_{tlp} was hypothesized to influence K_{leaf} Ψ_{50} mechanistically (20). K_{leaf} Ψ_{50}, in turn, would influence g_S Ψ_{50} and Ψ_{95} and the threshold Ψ_{leaf} for leaf death (leaf Ψ_{lethal}) (30, 31), and the stem and root hydraulic traits would influence the plant mortality threshold (plant Ψ_{lethal}) (19). These hypotheses are indicated with solid lines. As predicted, π_{tlp} and K_{leaf} Ψ_{50} were more correlated than expected from water stress and relatedness alone. Functionally coordinated traits are indicated with blue lines. Other correlations were best explained by the independent relationship of each trait with water stress. Concerted convergence is indicated with black lines. Conversely, K_{stem} Ψ_{50} was also more strongly correlated with K_{leaf} Ψ_{50} and, when characterizing water stress with $\Psi_{min,\ MD}$, with π_{tlp} than expected from concerted convergence, consistent with strong functional coordination within the hydraulic system across organs (*SI Appendix*, Tables S5 and S6). The remaining hypotheses had insufficient data to test (indicated with gray lines).

and potentially, K_{stem} Ψ_{12}, might arise because hydraulic function in these organs is closely linked. At a given transpiration rate, K_{stem} influences Ψ_{leaf}, and K_{leaf} impacts the gradient between Ψ_{leaf} and Ψ_{stem} (17, 27). Further, many other extrinsic factors beyond Ψ_{min} (e.g., vapor pressure deficit, light exposure) may directionally select for stem and leaf hydraulic traits, producing correlations among these traits within habitats with similar soil water supply. Conversely, independent linkages with K_{leaf} Ψ_{50} may partly drive the correlation between K_{stem} Ψ_{50} and π_{tlp}. Sampling these traits across a wider range of species and environments has the potential to resolve the coordination between π_{tlp} and K_{stem} Ψ_{50} after accounting for their linkages with K_{leaf} Ψ_{50} and water stress.

Linkages Between the Stomatal and Hydraulic Traits. The correlations of stomatal and hydraulic traits can provide insight into their functional linkages. Whereas the drivers of stomatal closure are not fully resolved, the hydromechanical model predicts that guard cells regulate their aperture in response to the water status at the stomatal evaporation site; this water status, in turn, is influenced by the hydraulic conductivity of the stems, leaves, and roots (8, 28, 29). Further, declines in stomatal conductance have been hypothesized to respond more directly to K_{leaf} than K_{stem} (30, 31). Our analyses instead showed that across species, the stomatal traits were significantly correlated with stem but not leaf vulnerability. The statistical independence of g_S Ψ_{50} and Ψ_{95} and K_{leaf} Ψ_{50} is consistent with previous studies, showing wide species variation in the safety margins between stomatal closure and leaf hydraulic dysfunction (32), wherein species vary between "isohydry," which maintains high Ψ_{leaf} and K_{leaf} via early stomatal closure, and "anisohydry," which maintains gas exchange to low Ψ_{leaf} at the expense of hydraulic function. The correlation between the stomatal traits and K_{stem} Ψ_{50} and Ψ_{88} corroborates a previous metaanalysis of species from ecosystems worldwide (6), but contradicts two studies within specific ecosystems (10, 33). Thus, the coordination of stomatal sensitivity with stem vulnerability across species appears to be related to their independent roles in drought tolerance rather than to coordinated function, with stomatal responses affecting carbon uptake during mild and moderate drought, and vulnerability affecting the ability of stems to survive strong drought (2, 15).

Sequence of Drought Response Traits. On average, the woody dicots exhibited a typical trait sequence that is expected to limit severe tissue damage during drought, such as wilting and substantial stem embolism (Fig. 3). The 12% declines in stem conductivity (K_{stem} Ψ_{12}) occurred at the least negative water potentials, followed sequentially by K_{leaf} Ψ_{50}, wilting (π_{tlp}), and 50% and 88% declines in stem conductivity (K_{stem} Ψ_{50} and Ψ_{88}) (Fig. 3*B*). The positions of these traits in the sequence were clearly resolved by mixed effects models, which showed significant differences between all of these traits (*SI Appendix*, Table S7. Wilting (π_{tlp}) occurred after g_S Ψ_{50}, as predicted, but before g_S Ψ_{95}, contrary to the expectation that plants would undergo stomatal closure at sufficiently high water potentials to prevent wilting. Placing $\Psi_{min,\ MD}$ in this sequence indicated the drought responses that plants experience under seasonal water stress in natural conditions. $\Psi_{min,\ MD}$ occurred at similar water potentials as K_{leaf} Ψ_{50}, and significantly before wilting and K_{stem} Ψ_{50}, but after K_{stem} Ψ_{12} (*SI Appendix*, Table S7). The water potential at plant death (plant Ψ_{lethal}) was the most negative trait. There were insufficient data to compare g_S Ψ_{50} and g_S Ψ_{95} to traits besides π_{tlp}, or to place K_{root} Ψ_{50} in the sequence.

Phenology significantly affected one comparison (*SI Appendix*, Table S8). K_{leaf} Ψ_{50} occurred after $\Psi_{min,\ MD}$ in evergreen but not deciduous species, consistent with previous studies of smaller species sets showing that deciduous species undergo greater leaf hydraulic dysfunction to maximize carbon uptake, because the leaves are replaced annually (16). More studies are needed to characterize the variation in the sequence across leaf functional types within ecosystems and across ecosystems relative to water supply.

We applied additional statistics to confirm that the mean trait differences are robust to measurement uncertainty, and to evaluate the degree to which plants conform to the average trait sequence. We compared the 95% confidence intervals around mean trait values for each species for all traits for which SEs were provided (i.e., g_S Ψ_{50}, K_{leaf} Ψ_{50}, K_{stem} Ψ_{50}, π_{tlp}, and $\Psi_{min,\ MD}$). Across all comparisons, 42–82% of the species significantly supported the findings for the mean trait differences shown in the general sequence (*SI Appendix*, Figs. S4–S6), confirming that these results were largely robust to measurement uncertainty. Vulnerability segmentation was strongly supported, with K_{stem} Ψ_{50} significantly more negative than K_{leaf} Ψ_{50} for 82% of the species, and no species significantly showing the opposite pattern (*SI Appendix*, Fig. S4). Plants showed the most variation in the order of π_{tlp} and K_{stem} Ψ_{50}, with the finding that π_{tlp} occurs at a less negative water potential significantly supported by 33% of the species and opposed by 21% (*SI Appendix*, Fig. S5). Notably, the low sample size at the ends of the stomatal response and hydraulic vulnerability curves and the nonlinear curve shapes suggest that g_S Ψ_{95}, K_{stem} Ψ_{12}, and K_{stem} Ψ_{88} will tend to have much larger errors. Further, these traits are typically estimated from nonlinear regressions with organ water potential as the independent variable and extrapolated as *x* values from the regression at given *y* values. This convention precludes estimating SEs for these traits. Thus, strongly resolving the certainty of the position of these traits in

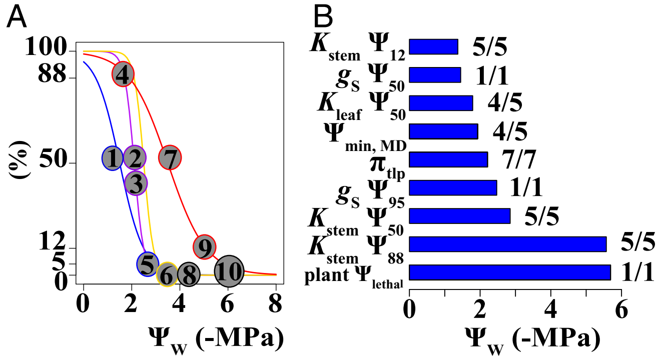

Fig. 3. The hypothesized (*A*) and observed (*B*) sequence of water potential values for the drought tolerance traits within individual plants. *A* shows the relationship between organ water potential (Ψ_W) and the percent decline in stomatal conductance (g_S, blue), hydraulic conductivity in the leaves, roots and stems (K_{leaf} and K_{root}, purple; K_{stem}, red), and turgor pressure (Ψ_P, yellow). The numbered circles show the order in which given declines in function will occur if plants generally follow a trait sequence that is expected to limit tissue damage during drought. In this sequence, 50% declines in stomatal conductance (g_S Ψ_{50}, #1) are expected to occur at the least negative water potentials to slow transpiration (37), followed by moderate (50%) declines in K_{leaf} and K_{root} (K_{leaf} and K_{root} Ψ_{50}) and minor (12%) declines in K_{stem} (K_{stem} Ψ_{12}), if leaf and root dysfunction protects the stem from embolism, as predicted by vulnerability segmentation (17). (These traits are labeled #2–4 but shown in the same position, because their order is not hypothesized). Stomatal closure, or g_S Ψ_{95} (#5), would occur before potentially major damage, including loss of turgor pressure in the leaf cells, or wilting (π_{tlp}, #6), and 50% declines in K_{stem} (K_{stem} Ψ_{50}, #7) (6, 10). K_{stem} Ψ_{50} is hypothesized to limit the water stress that plants tolerate, and thus, we expected the most negative Ψ_{leaf} values plants reach under natural growing conditions ($\Psi_{min, MD}$, #8) to be near K_{stem} Ψ_{50} (4). Eighty-eight percent declines in K_{stem} (K_{stem} Ψ_{88}, #9) have been hypothesized to induce irreversible xylem damage and, thus, to occur somewhat before plant death (plant Ψ_{lethal}, #10) (19), which we estimated as the Ψ_{leaf} at which all leaves showed tissue damage (11). The sequence is determined from pairwise comparisons between all of the traits (*SI Appendix*, Table S7), but, for clarity, *B* shows the mean of each trait from its pairwise comparison with the trait immediately after (i.e., more negative than) it in the sequence. The traits generally followed this sequence, with the order of K_{stem} Ψ_{12} > K_{leaf} Ψ_{50} & $\Psi_{min, MD}$ > π_{tlp} > K_{stem} Ψ_{50} > K_{stem} Ψ_{88} supporting the hypothesized sequence, with the exception that K_{leaf} Ψ_{50} and $\Psi_{min, MD}$ were not significantly different. π_{tlp} occurred after g_S Ψ_{50}, as hypothesized, but before g_S Ψ_{95}, contrary to prediction. There were insufficient data to test K_{root} Ψ_{50}, or to compare the stomatal traits to any other trait. For each trait, the number to the left is the number of other traits it was significantly different from, and the number to the right is the total number of trait comparisons with sufficient data to test. Notably, the sequence is shown with respect to organ-specific water potentials; in the transpiring plant, the high resistance of the hydraulic pathway produces a gradient of increasingly negative water potentials from the root to the leaf. Thus, the stem may undergo less embolism than suggested by this sequence.

the sequence will require the further development of statistical and computational methods to estimate these uncertainties (34).

The sequence provides several key insights into plant responses to drought. First, the occurrence of K_{stem} Ψ_{50} at lower water potentials than $\Psi_{min, MD}$ is generally consistent with the "high embolism resistance" paradigm, wherein plants are predicted to prevent substantial (i.e., 50%) declines in K_{stem} over the course of typical variation in water supply, and contrary to the "high embolism repair" paradigm, which expects plants to typically reach such declines and maintain function through recovery mechanisms (15, 23, 35). However, $\Psi_{min, MD}$ was more negative than K_{stem} Ψ_{50} for nearly one-fifth of the species (*SI Appendix*, Fig. S4), consistent with a previous metaanalysis of data for stem hydraulic dysfunction that were also included in this study (4). These species may experience substantial embolism during drought and depend strongly on recovery mechanisms to survive, such as refilling embolisms from stored water and/or growing new xylem in branching patterns that circumvent embolized conduits (36). However, when inferring K_{stem} responses to drought, it is important to note that, during transpiration, the leaf experiences more negative water potentials than the stem, given the high resistance of the leaf hydraulic pathway (27). This water potential difference protects the stem and, especially, the roots from extreme tension that would drive embolism during dehydration; thus, for a plant experiencing a Ψ_{leaf} equal to $\Psi_{min, MD}$, the actual Ψ_{stem} should be less negative. Therefore, these species could potentially experience less severe embolism than expected from this sequence of organ-scale water potential thresholds. Under drought, the water potentials across organs are expected to be highly variable, depending on hydraulic conductivity and influx from water storage. Thus, either in situ psychrometer measurements or a modeling approach is needed to quantify the impact of the trait sequence on the actual organ water potentials and conductivities that the plant experiences at a given soil water potential and transpiration rate.

The strong support for vulnerability segmentation and for leaf hydraulic decline under mild drought indicates that hydraulic redundancy (i.e., excess hydraulic capacity) and/or the capacity for hydraulic recovery in the leaf is crucial to drought tolerance for many plants (12, 16, 37). These findings point to the importance of elucidating the leaf traits that determine this capacity (20). Although contrary to our hypotheses, the occurrence of g_S Ψ_{95} at more negative water potentials than π_{tlp} is consistent with previous findings that the guard cells that control stomatal aperture (38) are largely isolated from bulk leaf turgor (28). Notably, many species are known to adjust π_{tlp} under water stress to improve drought tolerance (39), but only a few species were assessed for drought response traits during the dry season. Although moderate plastic shifts would tend to be toward the direction of greater tolerance and, thus, unlikely to affect the sequence of traits, further studies are needed to evaluate the degree to which plasticity in π_{tlp}, or in other traits, impacts this sequence. Greater sampling is also required to characterize the role of stomatal closure in preventing damage to the hydraulic system.

Future Directions To Improve the Predictive Capacity of Drought Tolerance Traits. This synthesis provides insight into the roles of trait coordination, coselection with water stress, and shared ancestry in the correlations of stomatal, hydraulic, and mesophyll drought tolerance traits, as well as the average trait sequence within plants.

This perspective also points to key developments needed to improve the predictive capacity of trait-based approaches for plant drought tolerance. More measurements are needed for the stomatal and root hydraulic traits, especially because these traits were the strongest correlates of environmental water stress. More data are also needed for gymnosperms, which have a lower capacity for recovery and may thus depend more strongly on the trait sequence (4, 40). Further, 70% of the species were represented in more than one comparison in the sequence analysis, but most of this overlap is accounted for by $\Psi_{min, MD}$, with only 30% of species assessed for more than two plant traits. It is thus critical that the general sequence be verified by sampling more traits within given species, with this sequence serving as a "first-pass" approximation until such data are more widely available. In addition, many physiological processes contribute to growth and survival during drought. Capacitance, embolism recovery, and metabolic synthesis of abscisic acid and nonstructural carbon reserves have all been predicted to influence drought survival, but the roles of these traits and their interactions with the classical drought tolerance traits, or their influence on plant Ψ_{lethal}, are not well understood (15, 23). Indeed, measurements of plant Ψ_{lethal} are sparse in the literature, and most studies use different definitions for plant death (11, 41). These values correlate with π_{tlp} (11), as shown here, and with leaf and stem hydraulic traits across small species sets ($n \leq 5$) (19, 37, 41), and it is increasingly critical for further studies to determine how these traits interact to influence plant mortality during drought.

Methods

To compile the drought tolerance trait dataset, we drew on references from several recent metaanalyses of variation in individual drought tolerance traits (4, 6, 7, 26) and conducted Web of Science and Google Scholar searches by using the keywords "turgor loss point," "wilting point," "stomatal closure," "stomatal conductance," "lethal leaf water potential," and "hydraulic vulnerability" or "cavitation" paired with "leaf," "stem," or "root." These studies measured traits with standard methods (detailed in the *SI Appendix, SI Methods*). To minimize ontogenetic and methodological variation, we included only studies that met the following criteria. For all traits, we included only studies that sampled (*i*) mature plant organs from (*ii*) sapling or adult plants, and not seedlings, growing in (*iii*) natural ecosystems or urban conditions for wild species, or typical agricultural conditions for crop species. For π_{tlp} values, we selected only studies that measured (*iv*) leaves that were rehydrated ≥6 h before measurement, unless the study reported no significant effect of a shorter rehydration time. We included g_S Ψ_{50} and Ψ_{95} values only from studies that (*v*) measured Ψ_L and g_S for leaves collected at the same time and (*vi*) included Ψ_L values that were less negative than −1.5 MPa to capture early declines in g_S.

We evaluated the correlations among traits with standard major axis regressions by using the *smatr* package for R software (version 3.3.0) (42). We present the correlations for untransformed data and confirmed these findings for log-transformed values. We identified the drivers of the trait correlations by fitting regression models predicting each trait as a function of (*i*) $\Psi_{min,\ MD}$ or $\Psi_{min,\ PD}$, and (*ii*) $\Psi_{min,\ MD}$ or $\Psi_{min,\ PD}$ and one trait variable. To account for relatedness, we constructed a phylogeny with Phylocom (43) and fitted phylogenetic least-squares regression relationships with the *caper* package (44). We used Aikake Information Criteria corrected for small sample sizes (AICc) to evaluate model support, with $\mathrm{AICc}_{nested} - \mathrm{AICc}_{full} \geq 2$ supporting the full model. We tested the trait sequence by fitting a mixed-effects model to the trait differences to calculate the mean trait difference while accounting for study effects. We constructed 95% confidence intervals (CI) for the mean differences with 1,000 nonparametric bootstraps to correct for nonnormality. To confirm these results were robust to measurement uncertainty, we constructed 95% CI around the mean trait values for the species with available data (n = 182) (*SI Appendix, SI Methods*).

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