A safety vs efficiency trade-off identified in the hydraulic pathway of grass leaves is decoupled from photosynthesis, stomatal conductance and precipitation

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Summary

- A common theme in plant physiological research is the trade-off between stress tolerance and growth; an example of this trade-off at the tissue level is the safety vs efficiency hypothesis, which suggests that plants with the greatest resistance to hydraulic failure should have low maximum hydraulic conductance.

- Here, we quantified the leaf-level drought tolerance of nine C_4 grasses as the leaf water potential at which plants lost 50% ($P_{50 \times RR}$) of maximum leaf hydraulic conductance (K_{sat}), and compared this trait with other leaf-level and whole-plant functions.

- We found a clear trade-off between K_{sat} and $P_{50 \times RR}$ when K_{sat} was normalized by leaf area and mass ($P = 0.05$ and 0.01, respectively). However, no trade-off existed between $P_{50 \times RR}$ and gas-exchange rates; rather, there was a positive relationship between $P_{50 \times RR}$ and photosynthesis ($P = 0.08$). $P_{50 \times RR}$ was not correlated with species distributions based on precipitation ($P = 0.70$), but was correlated with temperature during the wettest quarter of the year ($P < 0.01$).

- These results suggest a trade-off between safety and efficiency in the hydraulic system of grass leaves, which can be decoupled from other leaf-level functions. The unique physiology of C_4 plants and adaptations to pulse-driven systems may provide mechanisms that could decouple hydraulic conductance from other plant functions.

Introduction

A central tenet in plant ecology is the trade-off between rapid growth and the ability to withstand stress (Grime, 1977; Chapin, 1980). Within the context of drought tolerance this theory would predict that species exhibiting rapid growth should be uncommon in water-limited ecosystems. A tissue-specific hypothesis related to the vascular system that could provide a mechanistic basis for the growth-rate/stress-tolerance trade-off is the safety vs efficiency hypothesis, which suggests a trade-off between protecting the vascular system from embolisms and the efficient transport of water through plants (Zimmermann, 1983). The safety vs efficiency hypothesis has been widely tested on the woody tissue of trees (reviewed by Meinzer *et al.*, 2010), and more recently these tests have been extended to the leaves of woody species (Johnson *et al.*, 2011; Nardini *et al.*, 2012; Blackman *et al.*, 2014; Nardini & Luglio, 2014). However, a test of this hypothesis has yet to be carried out on grasses, which is the dominant growth form in 40% of terrestrial ecosystems that span a wide range of water availabilities, from desert grasslands that typically receive < 300 mm of precipitation to tropical savannas that can receive *c.* 1300 mm. Extending our understanding of drought tolerance

and hydraulic conductance in grass leaves will be important in improving our understanding of how grassland communities respond to drought.

Based on the established understanding of xylem cavitation and maximum hydraulic conductance in woody tissue (Maherali *et al.*, 2004; Wheeler *et al.*, 2005; Hacke & Sperry, 2006), one would expect a trade-off between conductance and cavitation resistance to occur at the tissue level within the xylem resulting from two main characteristics. Cavitation of the water column occurs when air is pulled through the pores of bordered pits (Zimmermann, 1983) and is related to the pore size; larger pores are more prone to cavitation (Sperry & Hacke, 2004) but small pores reduce hydraulic conductance through these pits as water moves between cells within the vascular system. Furthermore, smaller cells (both in terms of diameter and length) have smaller total pit area on their walls reducing the chance of a large pore occurring (i.e. the pit area hypothesis), and so smaller cells are more resistant to cavitation (Hacke & Sperry, 2006). As hydraulic conductance is related to the lumen diameter to the fourth power (Zimmermann, 1983), the mechanics of the pit-area hypothesis creates a safety vs efficiency trade-off in the vascular system. In addition, cavitation-resistant vessel elements have

also been shown to have smaller diameter lumen relative to cell wall thickness (Blackman *et al.*, 2010) and building cavitation resistant cells should, theoretically, also lead to a trade-off between safety and efficiency.

The safety vs efficiency hypothesis has been supported using woody tissue of trees and shrubs (Pockman & Sperry, 2000; Maherali *et al.*, 2004; Wheeler *et al.*, 2005; Hacke & Sperry, 2006; Jacobsen *et al.*, 2007b; Gleason *et al.*, 2016), but these traits are often uncorrelated in many studies. Plants can overcome this trade-off by increasing the number of slow-conducting elements (i.e. xylem packing), and although this strategy is costly (McCulloh *et al.*, 2003) it may contribute to the weak correlations between safety vs efficiency often observed in woody tissue (Meinzer *et al.*, 2010; Gleason *et al.*, 2016). Leaves have the potential to obscure this trade-off even more because the hydraulic pathway in leaves incorporates both xylary and extra-xylary tissue (Sack *et al.*, 2004), and it is unclear where within leaves the reduction in hydraulic conductance originates in response to declining leaf water potentials (Johnson *et al.*, 2012; Scoffoni *et al.*, 2014; Buckley, 2015). The hydraulic conductance of xylary and extra-xylary tissues is not always correlated (Ocheltree *et al.*, 2013), and differential responses of these compartments to drought could decouple the resistance to hydraulic failure of leaves from their maximum hydraulic conductance. Blackman *et al.* (2010) suggested that safety and efficiency are a result of different processes in leaf tissue and showed that there was no trade-off in the species they studied. The few studies looking for this trade-off in leaves, however, produced mixed results (Blackman *et al.*, 2010; Nardini & Luglio, 2014) and the trade-off has been identified among some species (Johnson *et al.*, 2011), especially when leaf conductance is normalized by leaf mass (Nardini *et al.*, 2012). These measurements have yet to be extended to grasses, where understanding the response of leaf hydraulic conductance to drought is vital as leaves represent the majority of the aboveground hydraulic pathway.

Evidence from the literature suggests that if a safety vs efficiency trade-off exists in the water transport system of plants, it should translate to a trade-off between hydraulic safety and the rates of gas exchange (i.e. growth capacity). Across a wide range of growth forms, maximum photosynthetic rates are tightly correlated with maximum leaf hydraulic conductance (Brodribb *et al.*, 2007). Combined with the safety vs efficiency trade-off these results suggest that the 'safest' leaves would have lower maximum hydraulic conductance and therefore slow photosynthetic rates, which could provide a mechanistic explanation for the growth-rate/stress-tolerance trade-off in plants. The results from Brodribb *et al.* (2007) were measured on different growth forms growing in mesic ecosystems and so it is unclear if these results would translate to species adapted to semi-arid and arid systems. Plants growing in semi-arid and arid systems are often adapted to take advantage of resources in short pulses following small rain events (Noy-meir, 1973). In order to capture these resources and maximize growth during this resource pulse, plants can attain very high photosynthetic rates (Reynolds *et al.*, 2004), and organismal-level results do not show a trade-off between drought tolerance and growth rate in grasses (Fernandez & Reynolds, 2000);

when xeric grasses were grown at different water potentials, no trade-off between growth rate and drought tolerance was found. These results suggest that even if there is a safety vs efficiency trade-off in the hydraulic pathway of leaves, it may not translate to a trade-off in whole-plant growth rates or rates of gas exchange.

Apart from the safety vs efficiency trade-off, the ability to maintain hydraulic function under water-limiting conditions has been shown to correlate with the biogeography of woody angiosperms (Blackman *et al.*, 2014; Nardini & Luglio, 2014). Plants growing in drier environments must maintain function when soil moisture is limiting, and so the maintenance of hydraulic function in leaves provides an explanation for the distribution of woody angiosperms. It is not clear how well this might explain species distribution in grasses; grasslands are characterized by temporal complementarity (Loreau, 2000) of herbaceous species, so species that contribute to ecosystem function early in the growing season when soil moisture is available may not be as cavitation-resistant as species that are active during the growing season, which may mask a relationship between precipitation and 'safety' of the hydraulic system. In addition, there is a large amount of variability of drought tolerance levels in grasslands (Craine *et al.*, 2012) that likely result from the range of microclimates within a single system. Still, it seems logical that species adapted to growing across a broad precipitation gradient, even within these diverse systems, would show differences in the ability to maintain hydraulic function within leaves, especially species that are active during the growing season when soil moisture is likely to be more limiting.

The objective of this study was to investigate if there is a trade-off between safety vs efficiency in grasses from a broad geographic and climatic range, and investigate how well the resistance to hydraulic failure (quantified as P_{50}) scales across other measures of drought tolerance and plant functions. We hypothesized that: there would be a safety vs efficiency trade-off at the leaf level between maximum hydraulic conductance (K_{sat}) and vulnerability to hydraulic failure (P_{50}), and that this trade-off in the hydraulic system will translate into a trade-off between P_{50} and rates of gas exchange. Because the leaves represent the portion of plants actively engaged in the uptake of carbon dioxide for growth, the ability to maintain function in the leaves (P_{50}) should be correlated with the ability of the entire plant to survive drought and will explain the distribution of grass species along a precipitation gradient.

Materials and Methods

We selected C_4 perennial grass species that are common in a diverse range of grassland ecosystems (Table 1) to test our hypotheses. Seeds were obtained through the Germplasm Resources Information Network (GRIN; http://www.ars-grin.gov), germinated and grown in commercial potting soil for *c.* 4 wk, and then 30–35 individuals per species were transplanted into native soil collected from the Konza Prairie Biological Station (KPBS, Manhattan, KS, USA) in 1.25-l containers ('Short One' Treepots; Stuewe & Sons Inc., Tangent, OR, USA). Plants

Table 1 Species and seed sources used in this study

Species	GRIN accession number	Seed source location	MAP (mm)	C_4 subtype
Andropogon gerardii Vitman	Rhizome	Kansas, USA	859	NADP-ME
Bouteloua gracilis Lag. ex Griffiths	PI 648364	New Mexico, USA	214	NAD-ME
Pennisetum flaccidum (Griseb.) Morrone	PI 434640	Punjab, Pakistan	946	NADP-ME
Eragrostis lehmanniana Nees	PI 410109	Thabazimbi, South Africa	574	NAD-ME
Eragrostis nigra Nees ex Steudal	W6 23585	Xizang, China	627	NAD-ME
Panicum virgatum L.	PI 657661	Oklahoma, USA	840	NAD-ME
Schizachyrium scoparium (Michx.) Nash	PI 421553	Kansas, USA	859	NADP-ME
Sorghastrum nutans (L.) Nash	PI 648380	Kansas, USA	924	NADP-ME
Spartina pectinata Bosc. ex Link	W6 30925	Wisconsin, USA	927	PCK

All plants except *Andropogon gerardii* were grown from seeds obtained through the Germplasm Resources Information Network (GRIN). An insufficient number of *A. gerardii* seeds germinated and so plants were grown from rhizomes collected at Konza Prairie Biological Station, Kansas, USA. The median of mean annual precipitation (MAP) for all reported occurrences of each species in the Global Biodiversity Information Facility (GBIF) database is also provided.

were grown in a growth chamber (Conviron PGV 36; Conviron Environments Ltd, Winnipeg, MB, Canada) with a 16 h photoperiod (PAR *c.* 1200 $\mu mol\, m^{-2}\, s^{-1}$) and daytime and night-time temperatures held at 25 and 22°C, respectively. Plants were watered daily and fertilized weekly with a Hoagland's solution until the initiation of the drought treatment.

Before the drought treatment, pressure–volume curves were measured on five leaves per species to determine leaf cellular and structural characteristics. Briefly, plants were watered to pot-holding capacity and then placed in a dark chamber overnight to allow the leaves to fully hydrate. The following morning the most recently matured leaf was wrapped in parafilm and cut near the ligule so that *c.* 75% of the leaf was used for the pressure–volume curves. The leaf and parafilm were weighed immediately on a micro-balance (± 0. 1 mg, Ohaus Pioneer; Ohaus Corporation, Parsippany, NJ, USA) and then placed in a Scholander-style pressure chamber (PMS Instruments, Albany, OR, USA) and pressurized until water was extruded from the cut surface of the leaf. The pressure was then maintained until the leaf reached equilibrium with the pressure in the chamber, taken as the point when water ceased coming out of the leaf. The leaf was removed from the pressure chamber, the cut surface was dried, and reweighed. This procedure was repeated by increasing the pressure in *c.* 0.2 MPa increments until a pressure of *c.* 3.0–3.5 MPa was reached. When the pressure–volume curve was completed the leaf was rehydrated and then scanned (at 600 dpi, Epson Perfection V500; Epson America Inc., Long Beach, CA, USA) to measure leaf area using imageJ (US National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/), and dried for 24 h at 60°C and weighed. Turgor loss point (Ψ_{TLP}), osmotic potential at full turgor (Ψ_{osm}), and leaf capacitance (C_{leaf}) were calculated for each individual leaf and averaged across species.

The drought was implemented when plants had at least four mature leaves; water was completely withheld from plants for the entire drought period and soil moisture declined to the point of a 'severe' drought. As soil moisture declined following the cessation of watering, plant function at different levels of soil moisture were measured over the course of *c.* 14 d at 1–2 d intervals. During each measurement period stomatal conductance (g), photosynthesis (A), leaf hydraulic conductance (K_{leaf}), and leaf water

potential (Ψ_{leaf}) was measured for approx. four individuals per species. All measurements were made between 11:00 and 14:00 h to represent mid-day plant function. After all measurements were completed on an individual, the plant was rehydrated and monitored for leaf mortality (described in more detail later). In this way an individual plant was only used for measurements at a single soil moisture level.

Rates of gas exchange were measured using a Li-6400 gas exchange system (Li-Cor Inc., Lincoln, NE, USA) and the conditions inside the leaf chamber were set to match conditions in the growth chamber (50% relative humidity, PAR = 1500 $\mu mol\, m^{-2}\, s^{-1}$, CO_2 concentration = 400 $\mu mol\, mol^{-1}$). Rates of gas exchange were monitored until they stabilized (*c.* 5–10 min) and a single point was logged. Measurements were made at the center of each leaf to minimize the effect of acropetal changes in gas exchange rates (Ocheltree *et al.*, 2012). Because gas exchange rates change linearly along the length of the leaf, measuring at the center of the leaf provides an estimate of the integrated whole-leaf gas exchange rate.

Following gas exchange measurements, leaf hydraulic conductance was measured on the same leaf using the method of relaxation by rehydration (Brodribb & Holbrook, 2003; Holloway-Phillips & Brodribb, 2011a,b). Briefly, a pair of adjacent leaves (including the one selected for gas exchange measurements) that visually appeared to be of similar condition and size was identified on each individual. The more apical leaf was removed from the plant with a razor blade, sealed in a plastic bag and placed in the dark for *c.* 30 min, to allow any water potential gradients within the leaf to equilibrate. Then the water potential of this leaf segment was measured and used as an estimate of mid-day leaf water potential (Ψ_{mid}) and as the initial leaf water potential (Ψ_0) in the calculation of K_{leaf} (Eqn 1). The more basal leaf of the pair was partially submerged in filtered and de-ionized water and recut near the base of the leaf for rehydration. The apical portion of the leaf segment was kept out of the water so that rehydration of the leaf could occur only through the exposed xylem at the cut surface. Leaves were allowed to rehydrate for 30–120 s depending on the species and hydration status of the plant and then cut again *c.* 30 mm from the basal section submerged in the water bath. The apical section of the leaf was then sealed in a plastic

bag with a moist paper towel and stored in the dark to equilibrate for c. 30 min and then leaf water potential was measured and used as the rehydrated leaf water potential (Ψ_f) in Eqn 1. K_{leaf} was calculated using the leaf water potentials and leaf capacitance (calculated from the pressure–volume curve):

$$K_{leaf} = \frac{C_{leaf} \ln\left[\frac{\Psi_0}{\Psi_f}\right]}{t} \qquad \text{Eqn 1}$$

(C_{leaf}, bulk leaf capacitance before turgor loss normalized by leaf area; t, duration of rehydration; Ψ_0 and Ψ_f, leaf water potentials before and after rehydration, respectively). We also estimated K_{leaf} based on leaf dry mass (K_{mass}) by calculating leaf capacitance on a leaf dry mass basis in addition to leaf area. In this way we could estimate K_{mass} without relying on using leaf mass area (LMA) for a conversion, which could introduce biases to mass based estimates of leaf function (Lloyd & Bloomfield, 2013).

The relationship between Ψ_{leaf} and K_{leaf} was used to generate vulnerability curves for the response of plant function to decreasing Ψ_{leaf} (Supporting Information Figs S1–S3). Each point on the curve represents the measurement of one individual and the range of Ψ_{leaf} values were generated by measuring different individuals within a species at different stages into the drought treatment (described earlier). In order to identify the 'best' vulnerability curve, four different mathematical functions were fit to the Ψ_{leaf} and K_{area} data that had been binned to 0.5 MPa increments as described by Scoffoni et al. (2012). Sigmoidal, exponential, logistic and linear curves were fitted to these data and the curve with the lowest Akaike Information Criteria (AIC) value was selected as the 'best' fit. Details of the equations used and AIC values are shown in Table 2 and the individual vulnerability curves for K_{area} of each species are shown in Fig. S1. After selecting the best fitting curve we then calculated the Ψ_{leaf} at which K_{area} was reduced by 50% relative to its maximum value; here referred to as $P_{50 \times RR}$. We include the 'RR' designation to indicate that we used the relaxation by rehydration method for this analysis, which is also referred to as the 'rehydration kinetics method'. However, any references to vulnerability curves from previous research are referred to as 'P_{50}' to describe curves developed from a range of methods. We also developed P_{50} estimates of g_{area} ($P_{50 \times g}$) and leaf survival ($P_{50 \times survival}$) in a similar

manner to K_{leaf}. To estimate a P_{50} for leaf survival the y-intercepts of the leaf survival curves were set to 1. Vulnerability curves for g_{area} and leaf survival are shown in Figs S2 and S3, respectively.

Following hydraulic measurements, each plant was rewatered and pots were kept at their maximum water holding capacity for 4 d to evaluate the mortality of each plant in response to drought (Tyree et al., 2003). After 4 d the proportion of live and dead leaves was recorded and used to generate a vulnerability curve of whole-plant mortality to drought. The leaf water potential used for $P_{50 \times RR}$ (Ψ_0) was used as Ψ_{Leaf} for the vulnerability curves of plant survival. $P_{50 \times survival}$ was then determined using the same procedure as described above for $P_{50 \times RR}$.

Climate envelopes for temperature and precipitation were determined for the geographic range of each species studied. The geographic range of each species was assumed to be represented by the global occurrence of each species reported in the Global Biodiversity Information Facility (GBIF; www.gbif.org). Bioclimatic variables were then retrieved for the nearest 0.5-km grid cell of each species occurrence using the WorldClim database (http://www.worldclim.org/bioclim). Bioclimatic variables include estimates of temperature and precipitation during different periods of time, as well as seasonality estimates, that are aimed at being more biologically relevant than annual climate statistics. The median, 5th, 10th, 90th and 95th quantiles of each bioclimatic variable was calculated from data compiled for all recorded occurrences of each species. Correlations between hydraulic and drought tolerance traits and these bioclimatic variables were investigated using the 'cor' function in the base R package.

Results

We selected the mathematical function that best fit the vulnerability curve data for K_{area}, g_{area}, and survival using four possible equations: linear, exponential, sigmoidal and logarithmic. Five of the nine species had linear curves for K_{area}, but all of the plant survival curves ($P_{50 \times survival}$) exhibited logarithmic shaped curves as selected by low AIC values. Because we measured K_{area} at 'midday' rather than when plants were fully hydrated (i.e. first thing in the morning), our Ψ_{leaf} values never got above -0.5 MPa,

Table 2 Results from the vulnerability curve fitting procedure
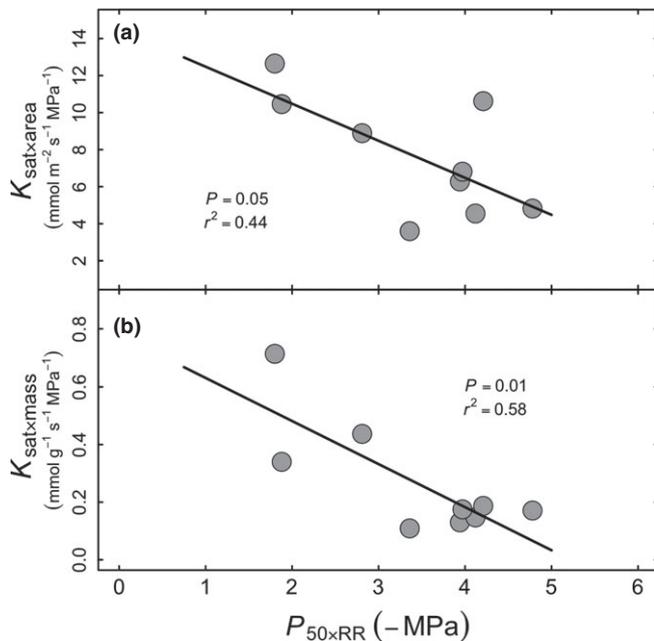
Species	$P_{50 \times RR}$	$K_{sat \times area}$	Selected curve	Linear AIC	Sigmoidal AIC	Logistic AIC	Exponential AIC
Andropogon gerardii	3.78	6.81	Linear	32.38	32.47	33.53	36.34
Bouteloua gracilis	3.69	3.27	Linear	19.22	26.86	27.73	27.04
Eragrostis lehmanniana	4.12	4.55	Linear	22.08	25.18	25.62	25.15
Eragrostis nigra	4.78	4.82	Linear	16.50	16.94	17.10	18.56
Panicum virgatum	2.72	9.72	Sigmoidal	13.77	0.88	3.51	3.85
Pennisetum flaccidum	1.88	10.46	Exponential	11.38	11.43	11.50	11.34
Schizachyrium scoparium	3.94	6.81	Linear	5.43	5.84	5.49	7.42
Sorghastrum nutans	4.21	10.62	Sigmoidal	11.67	11.47	11.58	12.08
Spartina pectinata	1.80	12.65	Logistic	17.40	15.80	26.37	16.02

The Akaike Information Criteria (AIC) values from all four mathematical functions are shown and the 'best' fit is indicated. Additionally, the $P_{50 \times RR}$ value calculated from the 'best' fitting function is shown.

which makes it more difficult to estimate K_{sat}. The absence of K_{area} values at high water potentials could bias our results toward linear or exponential curves because we do not know if K_{area} is asymptotic at high Ψ_{leaf}, or continues to increase. However, when K_{sat} and $P_{50 \times RR}$ are compared between the 'best' curve (Table 2) and a logarithmic curve the correlations are high (0.94 and 0.82, respectively), suggesting that our data are not sensitive to the type of curve selected. All subsequent data presented are based on the 'best' curve as selected by their low AIC values from the curve-fitting algorithm (shown in Figs S1–S3).

The nine species selected for this experiment are common in regions with a wide range of climates and spanned a precipitation range of 214–946 mm mean annual precipitation (MAP; Table 1), and are generally characterized as having a wide range of drought tolerances (plants.usda.gov). Leaf hydraulic conductance when leaves were fully hydrated ($K_{sat \times area}$) ranged by nearly three-fold among this set of species, ranging from 3.54 to 11.78 mmol m^{-2} s^{-1} MPa^{-1}. When curves were fit to the relationship between Ψ_{leaf} and K_{leaf}, five species showed a linear decline in K_{leaf}, three species were logistic and one exponential (Table 2). The $P_{50 \times RR}$ values determined from the best-fitting curve for each species ranged from −1.13 (MPa) for *Spartina pectinata*, which is a lowland species in the tallgrass prairie, to −4.78 (MPa) for *Eragrostis nigra*, which is often found on dry mountain slopes.

$P_{50 \times RR}$ values calculated from the selected curve were negatively correlated with $K_{sat \times area}$ values (Fig. 1a), and this relationship was even stronger when K_{sat} was calculated on a leaf mass basis ($K_{sat \times mass}$) rather than leaf area (Fig. 1b). This strong

correlation remained when $K_{sat \times area}$ and $P_{50 \times RR}$ values estimated from logistic curves were used instead of allowing the shape of the curves to vary ($P = 0.05$, $r^2 = 0.49$). When comparing $P_{50 \times RR}$ to traits not directly related to hydraulic transport, we did not find the same trade-off. There was no significant correlation between $P_{50 \times RR}$ and maximum rates of g_{area} ($P = 0.59$) or A_{area} ($P = 0.14$, Fig. 2). A moderately significant relationship between $P_{50 \times RR}$ and A_{area} ($P = 0.08$, Fig. 2b) was present when a parabolic function was fit, but not for g_{area} ($P = 0.48$). Although not highly significant, the general trend was for plants with high $P_{50 \times RR}$ values (greater resistance to hydraulic failure) to have a capacity for higher rates of gas exchange under well-watered conditions when calculated on a leaf-area basis. However, when g and A were calculated based on leaf mass, a trade-off became apparent ($P = 0.002$ and 0.004, respectively), but only when the two *Eragrostis* species were removed from the analysis (Fig. S4).

Among the species studied we also found no correlation between maximum leaf hydraulic conductance (K_{sat}) and leaf gas exchange when these variables were normalized by leaf area (Fig. 2c,d) and leaf mass (Fig. S5). Again, the two *Eragrostis* species were outliers relative to the other species, but removal of these species from the analysis did not result in a significant correlation among the remaining species.

Turgor loss point (Ψ_{TLP}) and osmotic potential at full turgor (Ψ_{osm}) both characterize drought tolerance in leaves, and these variables were strongly correlated among the species we studied

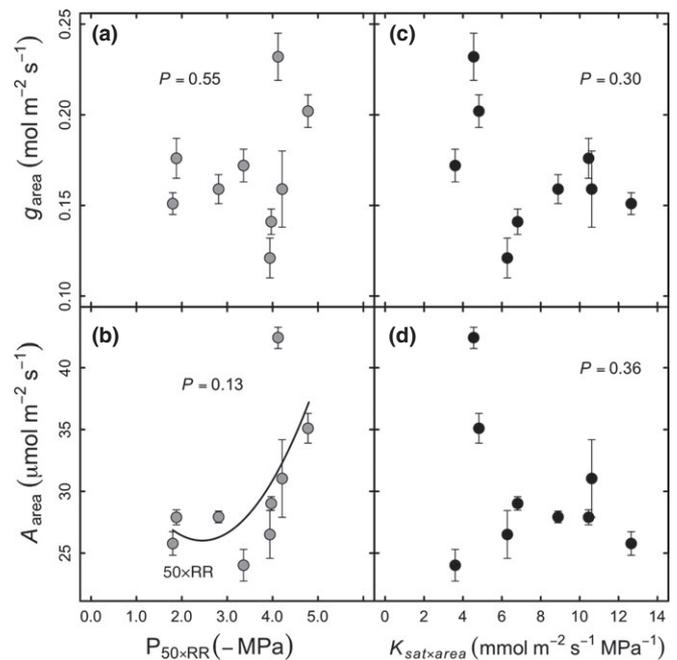**Fig. 1** The relationship between the resistance to hydraulic failure ($P_{50 \times RR}$) and the hydraulic conductance of leaves when fully hydrated (K_{sat}). Symbols represent the K_{sat} of a single species calculated from vulnerability curves (shown in Supporting Information Fig. S1). $P_{50 \times RR}$ was correlated with K_{sat} when it was estimated on (a) a leaf area and (b) leaf mass basis. See Table 1 for a list of included species.


Fig. 2 Relationship between resistance to hydraulic failure ($P_{50 \times RR}$) and leaf-level gas exchange. $P_{50 \times RR}$ was not significantly correlated with either (a) stomatal conductance or (b) photosynthesis. Although, the relationship between $P_{50 \times RR}$ and photosynthesis was parabolic, such that plants with higher $P_{50 \times RR}$ values also had higher maximum photosynthetic rates. Leaf hydraulic conductance normalized by leaf area ($K_{sat \times area}$) was not correlated with either (c) stomatal conductance or (d) photosynthesis normalized by leaf area. Error bars represent the $\pm$ SE of measurements made on the most recently matured leaf of five individuals ($n = 5$). See Table 1 for a list of included species.
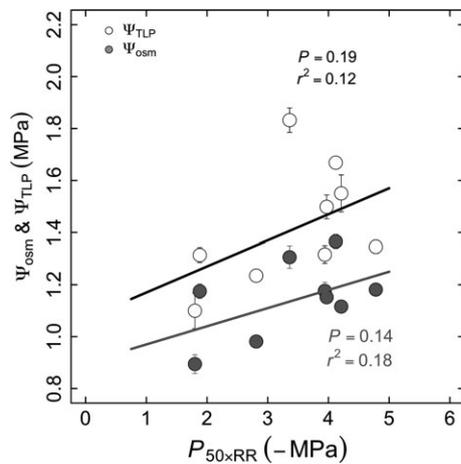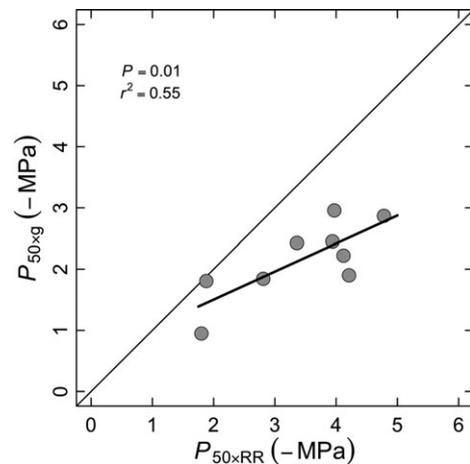
Fig. 3 Relationship between parameters obtained from pressure–volume curves, which are indicative of drought tolerance on a cellular level, and $P_{50 \times RR}$. There was a weak positive correlation between $P_{50 \times RR}$ and both the turgor loss point and osmotic potential when plants were growing under well-watered conditions. Error bars represent the $\pm$ SE of measurements made on the most recently matured leaf of five individuals ($n = 5$). See Table 1 for a list of included species.

Fig. 4 Correlation between the resistance to hydraulic failure ($P_{50 \times RR}$) and the decline in stomatal conductance ($P_{50 \times g}$). The difference between the slope of this correlation and the 1 : 1 reference line is an estimate of the 'safety margin' for when stomatal conductance begins to decline relative to when the hydraulic system would lose 50% of its maximum conductance. $P_{50 \times g}$ is always lower than $P_{50 \times RR}$, indicating that the stomatal conductance is reduced at lower leaf water potentials relative to the decline in hydraulic conductance, possibly to protect the hydraulic system from failure. See Table 1 for a list of included species.

($P = 0.006$, $r^2 = 0.68$). $P_{50 \times RR}$ and Ψ_{TLP} or Ψ_{osm} were only weakly correlated (Fig. 3); there was generally a positive correlation between $P_{50 \times RR}$ and both of these variables that was not significant at the $P = 0.05$ level. There was, however, a strong correlation between $P_{50 \times RR}$ and the leaf water potential when stomatal conductance was reduced by 50% relative to maximum ($P_{50 \times g}$, Fig. 4). The difference between this slope and a 1 : 1 reference line can be considered an estimate of the safety margin with which these species operate. Among the species measured, the safety margin increased for species with larger $P_{50 \times RR}$ values (Fig. 4).

Interestingly, $P_{50 \times RR}$ determined at the leaf level did not correlate with $P_{50 \times survival}$ ($P = 0.75$), which is an estimate of plant-level response to drought, suggesting that leaf-level drought responses were decoupled from survival of the whole plant. Ψ_{TLP} and Ψ_{osm} were better predictors of whole-plant survival ($P = 0.06$ and 0.09, respectively). Furthermore, the best correlation between any drought tolerance trait and precipitation was the relationship between Ψ_{osm} and precipitation during the driest month (Fig. 5a); plants growing in drier regions generally had lower Ψ_{osm} ($P = 0.06$) values. $P_{50 \times RR}$ was not well correlated with MAP (Fig. 5b inset), but was correlated with temperature of the wettest quarter of the year (Fig. 5b).

Discussion

The safety vs efficiency hypothesis applies directly to the hydraulic pathway of plants, but the tight linkage between hydraulic conductance and other plant functions would suggest that this trade-off translates to other leaf- and plant-level processes. As theory would predict, our data show a trade-off between hydraulic efficiency and the ability to maintain hydraulic function during drought among nine C_4 grass species that occur in ecosystems spanning a large rainfall gradient. Grass species that

had high leaf-specific hydraulic conductance (K_{sat}) lost 50% of their ability to conduct water at higher leaf water potentials than grasses with low K_{sat}. However, we did not find evidence that this trade-off was coupled to other leaf-level functions or whole-plant mortality in response to drought. For example, there was no correlation between $P_{50 \times RR}$ and leaf-level gas exchange and, in fact, the most drought-tolerant species in our study tended to have the highest rates of gas exchange. We also found no correlation between $P_{50 \times RR}$ and the leaf water potential that resulted in a 50% loss of leaf area; suggesting that maintenance of the hydraulic pathway in leaves does not necessarily translate to whole-plant drought survival among the species we studied and the conditions imposed by our drought. Alternatively, it was osmotic potential at full turgor (Ψ_{osm}) that best correlated with whole-plant mortality and species distributions across a rainfall gradient. This decoupling of leaf-level functions may be important in understanding different plant growth strategies, especially considering several of the species studied here are common in plant communities that rely on short-term pulses of resource availability.

Safety vs efficiency trade-off

The $P_{50 \times RR}$ values measured here fall within the range reported for leaves of both woody-species and other grass species. Two forage grass species from Tasmania had P_{50} values of only $c.$ -1 MPa (Holloway-Phillips & Brodribb, 2011a,b); one was a drought-sensitive cultivar and the other was considered 'drought-resistant', but had a deep root system that may have allowed it to avoid low leaf water potential values during drought (Holloway-Phillips & Brodribb, 2011a). A grass common in the marshlands of eastern US (*Spartina patens*) had P_{50} values ranging between

Fig. 5 Relationship between drought tolerance traits and bioclimatic variables. The best correlation between the leaf-level drought tolerance traits and bioclimatic variables as the relationship between the 5th quantile for precipitation of the driest month and osmotic potential at full turgor (a). The $P_{50 \times RR}$ of leaves did not correlate well with MAP (b, inset), but did correlate with the temperature of the wettest quarter (b). See Table 1 for a list of included species.
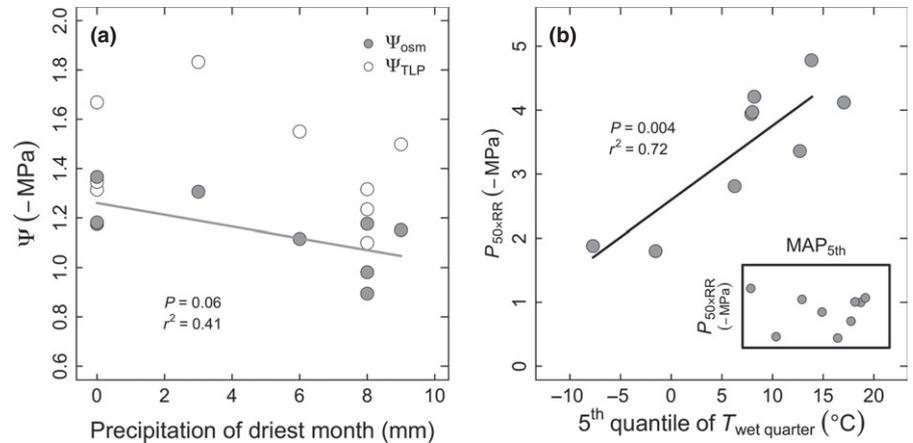
−2.9 and −3.5 MPa depending on whether it grew in marsh or dune regions, which shows plasticity in P_{50} at the species-level (Casolo *et al.*, 2015). In a recent meta-analysis, P_{50} values of leaves in woody-angiosperms ranged from −0.5 down to −4.5 MPa among species occurring in dry sclerophyllous, temperate and tropical forests (fig. 1 in Nardini & Luglio, 2014). However, woody angiosperms across Australia had P_{50} values from −1.03 to −8.35 MPa, with many species with values below −4 MPa (Blackman *et al.*, 2014). Currently we have very few data on the hydraulic vulnerability of grasses and a larger body of data is required to fully understand how P_{50} values are distributed within the *Poaceae* family and how P_{50} values compare to those found in woody angiosperms.

Support for the safety vs efficiency hypothesis has come from research on the stems of woody plants within species (Jacobsen *et al.*, 2007a; Meinzer *et al.*, 2010) and across species (Jacobsen *et al.*, 2007b; Johnston *et al.*, 2007; Gleason *et al.*, 2016), but the correlation is often weak when looking for an interspecific trade-off at the tissue-scale (Maherali *et al.*, 2004; Hacke & Sperry, 2006). Similarly, data has not consistently supported this trade-off when investigating leaf tissue of woody angiosperms (Blackman *et al.*, 2010; Nardini & Luglio, 2014), although among a small set of Mediterranean trees a safety vs efficiency trade-off was apparent (Nardini *et al.*, 2012). Blackman *et al.* (2010) suggested that this trade-off should not be expected in leaves because K_{sat} and P_{50} are the result of different processes; P_{50} is related to the structure of individual vessel elements (Wheeler *et al.*, 2005; Hacke & Sperry, 2006; Blackman *et al.*, 2010) where K_{sat} is more closely related to vein density (Scoffoni *et al.*, 2011), mesophyll hydraulic architecture (Brodribb *et al.*, 2007; Ocheltree *et al.*, 2012) and aquaporin regulation (Cochard *et al.*, 2007).

Despite the potential decoupling of P_{50} and K_{sat} in leaves, we show a strong trade-off between these traits in our set of species (Fig. 1). Previous studies that have looked for this trade-off have utilized similar methods, and so it is unlikely to be a methodological difference among studies. Nardini *et al.* (2012) identified a trade-off in leaves among regionally important species, but only when K_{leaf} was normalized by leaf mass rather than area. Johnson *et al.* (2011) found a trade-off in the leaves of a broad range of tree species by combining their own results with data from the literature. Here, we selected nine C$_4$ grass species that are common in a range of grasslands, so the distribution is global in scope (Table 1) but within a single functional group. Although we had little *a priori* knowledge of the hydraulics of the plants that we selected, the systematic selection, small dataset and phylogenetically constrained set of species (all from within a single family) could have contributed to the results shown here. Our results, along with those of Nardini *et al.* (2012) and Johnson *et al.* (2011) suggest that a safety vs efficiency trade-off exists at some scales which may not be detectable when comparing across a broader range of species (Blackman *et al.*, 2014; Nardini & Luglio, 2014).

As described earlier, the safety vs efficiency trade-off is based on cellular characteristics of the xylem, and although we found a trade-off that is consistent with this theory, reductions in hydraulic conductance outside the xylem could contribute to the P_{50} values measured here. Unfortunately, we did not make anatomical measurements on the individuals that we sampled and so we cannot directly link the observed $P_{50 \times RR}$ values we measured with cellular characteristics. In other recent work on leaf hydraulic vulnerability, P_{50} values were correlated to embolism formation in the leaves of three out of four woody species tested (Johnson *et al.*, 2012), suggesting that the reduction in hydraulic conductance is a result of embolism formation in the xylem. However, modeling efforts based on leaf-shrinkage have suggested that the decline in conductance in extra-xylary tissue could be just as important as embolism formation in the xylem (Scoffoni *et al.*, 2014; Buckley, 2015). The decline in extra-xylary conductance has not been studied as intensely as embolism formation in xylem and so it is unclear if a safety vs efficiency trade-off would be expected in extra-xylary tissue.

Hydraulic vulnerability and press-volume traits

Identifying the fundamental trade-offs of plant function (e.g. the safety vs efficiency trade-off) is important for understanding evolutionary patterns, community assembly and biogeography in plants (Reich, 2014), but assessing how these trade-offs are coupled with other plant traits and functions is required to reveal plant growth strategies. With this goal in mind we compared

$P_{50 \times RR}$ with other leaf-level traits to investigate the coordination among different physiological functions. Among the species we measured, $P_{50 \times RR}$ was only weakly correlated with the turgor loss point (Ψ_{TLP}) and osmotic potential at full turgor (Ψ_{osm}). Previous research has shown these variables to be tightly correlated in other species (Blackman *et al.*, 2010; Scoffoni *et al.*, 2011, 2012; Villagra *et al.*, 2013; Nardini & Luglio, 2014), and have suggested that cellular-level traits relating to drought tolerance are well coordinated within leaves of woody angiosperms. Although there was a positive trend between these traits, the poor correlation we found in grasses is somewhat puzzling.

Considerable investment is required to construct tissue that would be considered drought-tolerant based on $P_{50 \times RR}$, Ψ_{TLP} and Ψ_{osm}, so it seems logical, then, that the ability of all cells within a tissue a tissue would be coordinated, although our data do not provide strong support for this idea. It is possible that an investigation over a larger set of species would produce a stronger correlation. It is important to note, however, that we measured Ψ_{TLP} and Ψ_{osm} in leaves that were well-watered and had been rehydrated overnight, but P_{50} curves were developed by measuring K_{leaf} of plants as soil moisture declined in a simulated drought. We did not account for any adjustment of Ψ_{TLP} or Ψ_{osm} that may have occurred in response to drought, which generally doesn't shift > 0.5 MPa (Bartlett *et al.*, 2014), but can shift by as much as 2 MPa depending, at least in part, on the water-use strategy employed by plants (Meinzer *et al.*, 2014).

Hydraulic traits and rates of gas exchange

Although there was not a strong correlation between $P_{50 \times RR}$ and Ψ_{TLP} or Ψ_{osm}, there was a correlation between $P_{50 \times RR}$ and Ψ_{leaf} when stomatal conductance reached 50% of maximum ($P_{50 \times g}$). If we consider the difference between the slope of the regression and a 1 : 1 reference line as an estimate of the operational safety margin, species with larger $P_{50 \times RR}$ values operated with a greater safety margin. Estimates of safety margins typically compare the minimum seasonal Ψ_{leaf} to P_{50}, but despite the difference in our metric of 'safety margin', it still agrees well with previous work on woody angiosperms at both local and global scales (Pockman & Sperry, 2000; Meinzer *et al.*, 2009; Choat *et al.*, 2012), suggesting that grass species also operate in a manner to minimize loss of hydraulic conductance in leaf tissue.

Previous work has shown a strong linkage between maximum leaf hydraulic conductance (K_{sat}) and maximum photosynthetic rates (A_{max}) of plants, suggesting the coordinated evolution of these processes (Brodribb & Feild, 2000; Brodribb *et al.*, 2007), but this research did not include any graminoids. Among the nine species of C_4 grasses studied here we did not find a significant correlation between K_{sat} and leaf-level gas exchange, including photosynthesis (Fig. 2). It is unlikely that the lack of relationship between K_{sat} and A_{max} shown here results from our smaller set of species because we found much larger variability in A_{max} among our species than in the previous studies. For example, species with low K_{sat} values (defined here as plants with $K_{sat} < 5$ mmol m^{-2} s^{-1} MPa^{-1}) had a range of A_{max} *c.* 20 µmol m^{-2} s^{-1} in our study compared with only *c.*

5 µmol m^{-2} s^{-1} found by Brodribb *et al.* (2007). Given the large range of A_{max} measured here, it is unlikely that expanding the range of species would improve the correlation with K_{sat}. The lack of a correlation may be explained by two biological mechanisms that could decouple K_{sat} from A_{max} in the species we studied; the unique photosynthetic pathway shared by the species in our study and water-use strategies of plants adapted to ecosystems with pulse-driven rainfall patterns.

During C_4 photosynthesis inorganic carbon is concentrated in the bundle sheath (BS) cells by PEP-carboxylase to decrease photorespiratory carbon losses due to oxygenation by RuBisCO. The high inorganic carbon concentration inside the BS cells creates a concentration gradient driving diffusion out of the BS cells; the rate of diffusion out of the BS cells relative to PEP-carboxylase activity has been termed the BS 'leakiness' (Farquhar, 1983), and can contribute to the photosynthetic efficiency of plants. Best estimates of leakiness ranges from 8 to 30% (von Caemmerer & Furbank, 2003) and *Eragrostis lehmanniana*, in particular, was found to have higher photosynthetic rates and lower BS leakiness than other C_4 subtypes tested and was less affected by drought and other environmental conditions (Fravolini *et al.*, 2002). *Eragrostis lehmanniana*, along with another species in this genus (*Eragrostis nigra*), are the species that deviated the most from the expected K_{sat}–A_{max} relationship. However, even excluding these species in the analysis does not produce a significant relationship between K_{sat} and A_{max} ($P = 0.34$). If the conductance of CO_2 and H_2O are coupled in BS cells of C_4 species, then low BS leakiness to inorganic carbon would also result in low conductance to water (low K_{sat}) through these cells, providing a mechanism to decouple K_{sat} from A_{max} in species with low BS leakiness. This hypothesis relies on maximum rates of hydraulic conductance being controlled by extra-xylary tissue (i.e. BS cells) rather than in the xylem, which is an ongoing area of research (Johnson *et al.*, 2012; Ocheltree *et al.*, 2013; Scoffoni *et al.*, 2014; Buckley, 2015).

Another possible explanation is that some of the species we investigated are adapted to grow in pulse-driven ecosystems. Resource availability (i.e. soil moisture) in pulse-driven systems is characterized by a period of relatively high availability during 'pulses' followed by an 'interpulse' period where resources are extremely limited (Reynolds *et al.*, 2004). Ecological theory of semi-arid and arid systems suggests that the biodiversity and coexistence of plant species in these systems relies on plants using a range of drought resistance strategies to survive and grow during these pulse–interpulse cycles (Chesson *et al.*, 2004). One successful strategy in these systems is to survive and maintain leaf area during the dry interpulse periods and then rapidly capture resources during pulse periods (Goldberg & Novoplansky, 1997; Chesson *et al.*, 2004). For example, *Schizachyrium scoparium* was able to approach maximum photosynthetic rates and fix carbon more rapidly in response to a precipitation pulse than the co-existing woody species (Volder *et al.*, 2010). And *Eragrostis lehmanniana* can reach maximum photosynthetic rates within 7 d of a precipitation pulse, but will return to near zero rates within 2 wk (Ignace *et al.*, 2007). This strategy would require drought tolerance tissues (e.g. low P_{50} values) and high growth potential

(e.g. A_{max}). Studies investigating the photosynthetic capacity of species from different systems have found species occupying water-limited systems have higher %N than species from more mesic sites, which could allow them to attain higher photosynthetic rates during pulse periods (Cunningham, 1999; Wright *et al.*, 2001, 2005). However, not all of our species with low $P_{50 \times RR}$ values had high photosynthetic rates, and so a closer examination of the relationship between A_{max} and K_{sat} is needed across a wider range of species adapted to a range of precipitation regimes to fully understand the apparent decoupling of hydraulics and gas exchange in this set of species.

Drought tolerance traits and species distributions

Leaf-level traits are often used to infer organismal-level growth strategies, but direct comparisons of traits across these scales are difficult, especially in larger woody species. One advantage of our study is that we can compare the resistance to hydraulic decline in leaves to organismal-level drought survival. We did not find a significant correlation between vulnerability of leaf hydraulic conductance ($P_{50 \times RR}$) and whole-plant survival of drought ($P_{50 \times survival}$), suggesting that the ability to maintain K_{leaf} during drought is not a prerequisite for drought survival, as shown in gymnosperms growing in different moisture conditions (Pinol & Sala, 2000). Similarly, we did not find a good correlation between MAP and $P_{50 \times RR}$, which differs from recent work on woody angiosperms (Blackman *et al.*, 2012, 2014; Nardini & Luglio, 2014). Even when more biologically relevant estimates of precipitation were used (including estimates of precipitation seasonality), no strong correlations were identified (data not shown).

The strongest correlation between $P_{50 \times RR}$ and climate was with the temperature of the wettest quarter, suggesting that it may be the evaporative demand for water during the growth period that explains the $P_{50 \times RR}$, which is consistent with results from woody species (Maherali *et al.*, 2004). This metric may better capture the conditions during the growth period, especially in pulse-driven systems that grasslands often occupy (Woodward *et al.*, 2004). The precipitation during the driest month best explained the distribution of plants according to their Ψ_{osm} at full turgor, which suggests it may not be the failure of the hydraulic pathway within the vasculature that limits the ability of grasses to grow in semi-arid to arid systems, but rather a larger suite of traits that would represent a range of drought-resistant strategies, including both drought tolerance and avoidance.

Conclusions

We have shown that a safety vs efficiency trade-off in the hydraulic pathway exists in grass leaves, but that this trade-off does not extend to other leaf- and plant-level processes. Several relationships previously shown in woody angiosperms were not consistent among this set of C_4 grasses. For example, maximum rates of gas exchange were not well correlated with the hydraulic supply to leaves, nor was the vulnerability of the hydraulic system ($P_{50 \times RR}$) coordinated with extra-xylary drought tolerance traits. Identifying correlations between hydraulic traits and climate may

be more difficult in grass species for multiple reasons: because grass-dominated systems often occur in semi-arid and arid climates (Woodward *et al.*, 2004), which are occupied by plants with a wide range of drought resistance strategies, simple correlations between climate and plant traits may be more difficult to identify; and the unique physiological processes of the C_4 photosynthetic pathway, which is most common in the *Poaceae* family, could cause hydraulic traits to be decoupled from other plant functions and climate (Kocacinar & Sage, 2003). In order to advance our understanding of how grass hydraulics are related to species distributions, research must focus on increasing the number of species tested focusing on species adapted to different climate regimes and representing both C_3 and C_4 species.

Author contributions

T.W.O., J.N. and P.V.V.P. planned and designed the research; T.W.O. and J.N. collected data; T.W.O. performed data analysis and interpretation; and T.W.O., J.N. and P.V.V.P. wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Vulnerability curves generated for leaf hydraulic conductance of each species.

Fig. S2 Vulnerability curves generated for stomatal conductance to water vapor of each species.

Fig. S3 Leaf survival curves generated for each species.

Fig. S4 Correlations between $P_{50 \times RR}$ and mass-based rates of gas exchange.

Fig. S5 Correlations between saturated leaf hydraulic conductance ($K_{sat \times mass}$) and stomatal conductance (g_{mass}).

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