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Beyond isohydricity: the role of environmental variability in determining plant drought responses

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Abstract: Despite the appeal of the iso/anisohydric framework for classifying plant drought responses, recent studies have shown that such classifications can be strongly affected by a plant's environment. Here we present measured *in-situ* drought responses to demonstrate that apparent isohydricity can be conflated with environmental conditions that vary over space and time. In particular, we (1) use data from an oak species (*Quercus douglasii*) during the 2012 – 2015 extreme drought in California to demonstrate how temporal and spatial variability in the environment can influence plant water potential dynamics, masking the role of traits, (2) explain how these environmental variations might arise from climatic, topographic, and edaphic variability, (3) illustrate, through a "common garden" thought experiment, how existing trait-based or response-based isohydricity metrics can be confounded by these environmental variations, leading to Type-1 (false positive) and Type-2 (false negative) errors, and (4) advocate for the use of model-based approaches for formulating alternate classification schemes. Building on recent insights from greenhouse and vineyard studies, we offer additional evidence across multiple field sites to demonstrate the importance of spatial and temporal drivers of plants' apparent isohydricity. This evidence challenges the use of isohydricity indices, *per se*, to characterize plant water relations at the global-scale.

Key words: isohydricity, drought responses, plant water potentials, environmental variability, intrinsic traits, classification

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The classification of plant's behavior in relation to their water status as "isohydric" or "anisohydric" was first introduced in the 1930s, and has received renewed attention in the past few decades (Berger-Landefeldt, 1936; Tardieu & Simonneau, 1998). Current interest is fueled by the need to better understand the physiological mechanisms that underpin drought-induced mortality in plants (McDowell et al., 2008). The term (an)isohydry has a varied history, having been applied to a range of phenomena over multiple timescales, notably: (i) changes in leaf water potential ψ_L relative to soil water potential ψ_S at the daily scale (Tardieu & Simonneau, 1998) and (ii) changes in midday leaf water potential ψ_{MID} relative to predawn water potential ψ_{PRE} at the seasonal scale (Martinez-Vilalta, Poyatos, Aguade, Retana, & Mencuccini, 2014); it has also been (iii) associated with plant physiological responses that regulate water potential (i.e. stomatal behavior) (Klein, 2014; Meinzer et al., 2016; Skelton, West, & Dawson, 2015; West et al., 2012) (Table 1). Regardless of the specific definition, isohydricity is interpreted as a proxy for plant water regulation and has been hypothesized to be correlated with a particular mechanism of drought mortality (McDowell et al., 2008). For example, increasing control over plant water use and an apparent decoupling of plant water potential from declining soil water potential make a plant more isohydric – this has been suggested to make a plant more likely to die from carbon limitation rather than hydraulic failure, especially during a prolonged drought (McDowell et al., 2008). In contrast, plants that exert less stomatal control over water loss allow their water potentials to fall along with soil water potential – making them more anisohydric – and are suggested to be predisposed to hydraulic failure, especially during intense droughts.

Despite the proliferation of isohydricity definitions (Table 1), conclusive evidence connecting plant (an)isohydricity with mechanisms of drought mortality remains elusive. There are a variety of reasons that may have prevented such evidence emerging. For example, different isohydricity metrics disagree about the relative degree of stomatal regulation, even when applied to the same group of plants (Martínez-Vilalta & Garcia-Forner, 2016). Perhaps more fundamentally, the classification of plant behavior as (an)isohydric has been shown to vary depending on environmental conditions (Hochberg, Rockwell, Holbrook, & Cochard, 2018),

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suggesting that (an)isohydric behavior does not emerge in isolation from a plant's soil and climatic context. Here, using a set of measured *in-situ* responses across two sites during a historic drought, we expand on these findings. We show that, beyond environmental conditions that can change over time (Hochberg et al., 2018), spatially variable site characteristics can produce very different responses (in terms of plant water potential dynamics), and lead to different interpretations of plant behaviors as (an)isohydric for the same species, across individuals whose hydraulic traits exhibit low phenotypic plasticity. This work advances the interpretation of plant water potential dynamics by highlighting the need to carefully account for both temporally and spatially variable environmental factors when interpreting water status as an indicator of a plant's drought response strategy or drought vulnerability.

Water potential data were collected from adult blue oak (*Quercus douglasii*, Fagaceae) individuals occurring at two sites in Northern and Central California during the historic drought of 2012 – 2015 (Figure 1). Both sites experience Mediterranean-type climates with hot, dry summers and cold, wet winters. In the first site, where the climate has historically had higher rainfall (hereafter "wet site", Table S1), the seasonal trajectories of predawn and midday plant water potentials for each individual changed in response to climate seasonality as the weather became progressively hotter and drier from spring to late summer (Figure 1a). The drought responses at this site are calculated for each individual as the slope of the midday and predawn water potentials, or ψ_{MID} vs. ψ_{PRE} – a prevailing measure of isohydricity at the seasonal scale (Martínez-Vilalta & Garcia-Forner, 2016; Martinez-Vilalta et al., 2014),

$$\sigma = \Delta\psi_{MID}/\Delta\psi_{PRE}, \tag{1}$$

with values ranging from 0 (strictly isohydric) and 1 (strictly anisohydric). Using this definition, an average value $\sigma = 0.69$ is obtained across all individuals (considering their early and late summer water potential measurements), suggesting that these oaks are partially isohydric. At the comparatively drier second site (hereafter "dry site", Table S1), however, the same isohydricity index σ calculated for each individual is on average greater than 1, indicating that they are

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"extremely anisohydric" (Figure 1b). Additionally, within-site variability at the dry site also influenced leaf water potentials. Instead of the seasonal trajectories of water potentials exhibited by individuals at the wet site, the dry site individuals were much better differentiated based on their hillslope positions – i.e., whether they are situated on hilltops (Figure 1b, red dots) or on an alluvial channel bed (Figure 1b, black dots), with hill individuals more likely to experience lower water potentials regardless of the season compared to channel individuals (Figure 1b) (see also Figure S1 where dry site data are organized by seasonality and wet site data are organized by topography).

These variations in plant water potentials across sites cannot be chiefly attributed to differences in plant specific hydraulic traits (i.e., xylem vulnerability to embolism), as the effects of phenotypic plasticity across these blue oak populations are likely to be much lower relative to those due to site- and hillslope-level differences in climatic and edaphic factors. This is supported by the low intra-specific variability in hydraulic traits shown for blue oaks in Skelton et al. (2018) and for other Mediterranean woody species in Lamy et al. (2014). For example, variations in water potentials toward the end of the dry season – especially of dry site individuals on hills – are likely to be caused by gradual water loss from the epidermis or leaky stomata *after* declines in canopy conductance (due to stomata closure and/or decrease in canopy area, Figure S2) which are in turn induced by locally extreme drought conditions as plant water potentials decreased beyond their turgor loss points (Skelton et al., 2018). Similarly, the differences in predawn water potentials between the hill and channel populations are likely due to differences in edaphic properties (i.e., sandy vs. alluvial soils, respectively) across hillslope positions that contributed to differences in soil water potentials. These climatic and edaphic factors vary greatly across sites and hillslope positions, and have little to do with the conventional characterization of these individuals as being isohydric or anisohydric (i.e., involving stomatal regulation or xylem vulnerability to embolism). Thus, the differences between individuals in these two sites must be mainly attributed to spatially-variable differences in site conditions (McLaughlin et al., 2017) rather than differences in intrinsic plant properties, strategies, or traits.

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Furthermore, we examined within-site differences in species responses by looking at the overall range in the plant water potentials experienced over the course of the drought for each individual, ω – another common metric of isohydricity (Table 1, low ω indicates more isohydric behavior). Values of ω were calculated for individuals growing on hilltops versus in channels (Figure 2a), and correlated against the oxygen stable isotope ratio ($\delta^{18}O$) of water extracted from stems (Figure 2b), which provides an indication of the water source plants take up (Ehleringer & Dawson, 1992). Overall, the seasonal range in midday water potentials was significantly higher for individuals in the wet site (suggesting a more anisohydric response) than for those in for the dry site (more isohydric) (Figure 2a, Mann-Whitney U test p-value of 7.84e-7). This association of isohydricity with individuals at the dry site is inconsistent with what the isohydricity index of equation (1) indicated. Moreover, the channel and hill populations in the dry site showed significantly different *ranges* in the observed values of ω (Bartlett's test *p*-value of 8.57×10^{-4}). The variations in individual ω can be explained by correlation to xylem water $\delta^{18}O$ ratios (‰ VSMOW), both across sites and between hill and channels populations. In the wet site, plants relied on a similar water source (possibly shallow groundwater) throughout the season, shown by relatively limited range in the xylem water $\delta^{18}O$ signatures of all individuals. In the dry site, individuals were clearly differentiated in their water sources depending on whether they were located in a channel or on a hill. Those on a hill were drawing from shallower (e.g., soil and/or shallow subsurface) water (with more positive $\delta^{18}O$ values indicating more isotopic enrichment due to evaporation in shallower soil layers) and were more likely to experience a lower range in midday water potentials (with more negative ω values, Figure 2a) relative to their channel conspecifics (Figure 2b). They were also subject to a higher rate of drought-induced canopy damage and mortality over the course of the study, relative to other study populations (Weitz, 2018).

These observations illustrate how the interpretation of plant responses depends on the choice of isohydricity metric: using ω or σ alternately identifies individuals at the wet site to be

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more or less isohydric. More importantly, they highlight how site-specific factors can interact with stomatal regulation to influence plant water potential dynamics, making metrics likes ω or σ ineffective at attributing observed responses to the effects of environmental variation versus stomatal regulation. In contrast to the controlled conditions in which a single (temporal) driver of plant water potential can be isolated (Collins, Fuentes, & Barlow, 2010; Hochberg et al., 2017), multiple environmental drivers (both temporal and spatial) can interact in field conditions. Thus, the effects of changing soil water potentials identified by Hochberg *et al.* (2018) – using greenhouse and vineyard experiments – can, in the field, be further mediated by seasonal and interannual variation in vapor pressure deficit (an additional temporal effect), and become fundamentally transformed by substrate type, access to groundwater, or varying level of exposure to solar radiation or vapor pressure deficit within the same site (a spatial effect due to variation in edaphic, climatic, and topographical factors). As plant responses result from the *interaction* of plant physiological strategies within a local environment, any classification of plant responses should in theory account for both the phenotypic characteristics of plants and the dynamic and spatially variable settings in which they occur. Alternative measures like the hydroscape area (Meinzer et al., 2016) offer a promising angle to synthesize temporal variations, but are likely to remain confounded by spatial variations such as the ones that prevail across our two sites. This is suggested by the fact that the seasonal trajectories of predawn and midday water potentials – which contributes to the hydroscape area – vary across the different oak populations (Figure 1), and are influenced by their landscape positioning, climatic exposure, and/or access to subsurface water.

In practice, existing metrics for classifying plant drought vulnerability through the framework of isohydricity (Table 1) fall into two categories: those formulated from plant water potentials ("response-based" e.g., Martinez-Vilalta et al. (2014)) and from plant ecophysiological traits ("trait-based", e.g., Skelton et al. (2015)). We use these two categories broadly to distinguish between metrics based on emergent response characteristics of the plant (i.e., water potentials that result from an interplay of traits and their environment) and metrics based on

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phenotypic characteristics of a plant that remain fixed (or only slowly vary) under environmental change (i.e., traits). In a modeling framework to predict drought responses, these categories are also analogous to using respectively state variables such as water potential, which are model outcomes, as opposed to parameters corresponding to specific traits, which are model inputs.

For example, the slope of the midday vs. predawn water potentials may sometimes be viewed as an individual or species-specific trait; however, this slope is itself derived from highly variable measurements (e.g., water potentials) and as such we consider it to be a response-based metric. Both response-based and trait-based metrics are often obtained and reported without considering environmental factors as covariates during experimental design, data collection, and data analysis. Thus, to the extent that plant responses are influenced by both physiological traits and environmental variations (climatic, edaphic, and topographic), the omission of the latter makes it difficult to disentangle the influences of the environment on whether a plant appears to be isohydric or anisohydric.

To illustrate the potential pitfalls of neglecting environmental variations, consider a "common garden" thought experiment: apparent species vulnerability to drought predicted using either response-based or trait-based isohydricity metrics is compared against actual drought vulnerability when plants are brought under a common environment (Figure 3). For response-based metrics, two types of false diagnostics can occur. First, plants that exhibit different responses in different environments can respond similarly when brought into the same environment (a Type-I, false positive error that suggests differences where they do not exist). This is the type of error that can be introduced through our earlier use of σ and ω. Second, plants that exhibit similar responses under different environments might respond differently under the same environment (a Type-II, false negative error in which true differences are obscured by the environment). In both cases, plant responses measured using response-based metrics are confounded by variations in their underlying environment. In the case of trait-based metrics, plants with different responses in one common environment might respond similarly in a different common environment, because the response emerges from the interaction of traits with

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environmental conditions, thus limiting the scope of prediction across multiple variable environments (another Type-I error).

Therefore, existing isohydricity metrics can fall short of effectively classifying plant drought responses – and particularly in identifying the extent of plant stomatal regulation - in one of two ways: (1) by conflating environmental influences and plant phenotypic characteristics (in response-based metrics), or (2) by ignoring the environment altogether (in trait-based metrics). Even as (an)isohydricity metrics have come under more scrutiny within the plant ecophysiology community (Hochberg et al., 2018; Martínez-Vilalta & Garcia-Forner, 2016), their adoption in other scientific fields, for example, hydrology and water resources, forest management, remote sensing, and earth systems modeling, is proliferating. Prompted by the urgency to characterize plant water relations at the biome level, the increasing availability of trait datasets, and advances in remote sensing, many of these new applications (for example Giardina et al. (2018); Konings & Gentine (2016); Li et al. (2017)) diagnose (an)isohydricity from variations in plant water potential at 100-1000 km scales – thereby encompassing an enormous range of variability. Such studies have creatively leveraged novel remote sensing products, and place important empirical constraints on predictions of the variations in plant water potentials at large scales. However, inference and attribution of the drivers of observed variability in plant water potential at such scales is fraught with the kinds of challenges outlined above. Consequently, translating observations into predictions of plant responses outside the envelope of existing observations should be approached very cautiously.

An alternate approach to relying on a single metric like the isohydricity index is to move toward a classification framework that can measure *multiple* dimensions of plant performance, and – crucially – do so relative to the environment in which the plants are found. Given the potentially intractably large number of traits and environmental variables that could be relevant for drought vulnerability in different biomes (O'Brien et al., 2017), the challenge of effective plant response classification will lie in the identification of a minimal set of key parameters that drive plant water relations. We have shown recently that simple plant hydraulics models – in the

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spirit of those adopted by Couvreur et al. (2018); Feng, Dawson, Ackerly, Santiago, & Thompson (2017); Manzoni, Vico, Katul, Palmroth, & Porporato (2014); and Sperry et al. (2016) – can embed the interplay of plant phenotypic traits and environmental variations in plant response dynamics and suggest a promising way forward for systematically exploring this multidimensional space (Feng et al., 2018). More complex computational models (Mackay et al., 2015; Mirfenderesgi et al., 2016) complement these simple approaches by providing more process-based descriptions that point to limitations in the simpler models (albeit at the cost of additional data requirements). Mathematical and computational models, informed by up-to-date ecophysiological paradigms, can complement greenhouse or field experiments in disentangling the roles of physiology versus environment due to their ability to: (i) survey alternate (and counterfactual) scenarios that would otherwise be difficult to experimentally impose or impossible to observe, and (ii) replicate a vast number of "virtual experiments" at relatively little cost. These advantages make them especially well-suited to hypothesis testing and sensitivity analysis (Sobol', 2001), allowing for the efficient diagnosis of key mechanisms and the selection of parameters that are most informative for predicting drought responses. These key trait and environmental parameters – used in conjunction with dimensional analysis techniques (Buckingham, 1914; Curtis, Logan, & Parker, 1982) – will reduce the complexity of the classification problem by collapsing its dimension along a few important axes of variation. For example, we have recently demonstrated the effectiveness of such a modeling approach in deriving a suite of trait-environment "groups" – or independent axes – for predicting different physiological outcomes under drought conditions (Feng et al., 2018). The most significant of the resulting axes capture the plant's aversion to hydraulic risk, the ease with which water can be extracted from the site, and the drought intensity and duration defined in terms of atmospheric water supply and demand relative to plant water uptake and soil water storage. These axes account for the combinations of traits and climatic and edaphic conditions (rather than treating them as independent drivers) that ultimately determine the dynamics of plant water potential. Moreover, they are non-dimensional in nature, which facilitates synthesis of results across

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different sites and biomes, because inter-comparisons can be made relative to a "standardized" environment along one or more of these axes.

In summary, the lack of conclusive evidence that isohydry/anisohydry are important for drought response derives from the tendency to interpret these plant behaviors as stable and independent of environmental context, when in fact they are labile and emerge from the interaction of physiology and environment. To make progress toward an effective classification scheme for plant responses, we need to account for and standardize the many sources of environmental variations that occur over space and time, and place emergent outcomes solidly within the ecohydrological contexts in which they occur.

Acknowledgements

Table 1: Common measures of isohydricity, their descriptions, and references (VPD: vapor pressure deficit). We group these measures broadly into two categories: "response-based" and "trait-based" (see text for a more extended discussion), with the former employing water potentials that may vary with the environment, and the latter based solely on invariant traits.

Measures	Timescale	Type	Description	References
Response-based				
ψ_{MID} vs. t $[ABA]_{xyl}$ vs. ψ_s g_s vs. $[ABA]_{xyl}$	Diurnal	Qualitative	Daytime fluctuation of leaf water potential in response to soil water deficit and VPD; sensitivity of ABA concentration to soil water decay, sensitivity of stomatal conductance to ABA concentration	(Tardieu & Simonneau, 1998)
g_s vs. t Sapflow vs. t ψ_L vs. t ψ_X vs. t	Diurnal	Qualitative	Diurnal time series of stomatal conductance, sapflow, leaf and stem water potentials	(Fisher et al., 2006; McDowell et al., 2008)
ψ_{MID} vs. t $\psi_L - \psi_s$ vs. t	Seasonal	Qualitative	"Isohydrodynamic" denotes stable water potential gradient from root to shoot, despite variations in midday leaf water potential	(Franks, Drake, & Froend, 2007)
ψ_{MID} vs. t	Seasonal	Qualitative	Midday leaf water potential over time	(McDowell et al., 2008; West, Hultine, Jackson, & Ehleringer, 2007)
g_s vs. ψ_L	Seasonal	Qualitative	Stomatal conductance vs. leaf water potential	(McDowell et al., 2008)
g_s vs. ψ_{MID}	Seasonal	Quantitative	Parameters of sigmoidal curve between stomatal conductance and midday leaf water potential	(Klein, 2014)
$\sigma =$ slope of the ψ_{MID} vs. ψ_{PRE} relation	Seasonal	Quantitative	Slope of the linear relation between midday and predawn leaf water potentials at plant scale (Figure 1)	(Martinez-Vilalta et al., 2014)
	Diurnal to seasonal	Quantitative	As above, but estimated from remotely-sensed variations in canopy water content (ecosystem scale)	(Konings & Gentine, 2016)
Minimum ψ_{MID}	Seasonal	Quantitative	Minimum seasonal midday leaf water potential	(Martínez-Vilalta & Garcia-Forner, 2016)
$\omega =$ range of ψ_{MID}	Seasonal	Quantitative	Seasonal range in midday leaf water potential (Figure 2)	(Martínez-Vilalta & Garcia-Forner, 2016)
Hydroscape area	Seasonal	Quantitative	The area on a plot of ψ_{min} vs. ψ_{PRE} over which stomata were able to exert effective control of plant water status	(Meinzer et al., 2016)
$\psi_{PRE} - \psi_{MID}$ vs. ψ_{PRE}	Seasonal	Quantitative	Slope of daily range of leaf water potentials with respect to predawn water potential	(Meinzer et al., 2016)
$g_s(t), A(t)$	Dry-down	Quantitative	Optimal use of limited soil water to maximize productivity while avoiding mortality during dry periods of random duration; no explicit	(Cowan, 1982)

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| | | | | plant hydraulic parameterization | |

Trait-based

Sapwood to leaf area ratio; leaf area specific xylem hydraulic conductivity	Seasonal	Qualitative	Higher mortality in pine populations with lower sapwood to leaf area ratio; leaf area specific xylem hydraulic conductivity	(Martínez-Vilalta & Piñol, 2002)
ψ_{g50}, ψ_{X50}, $g_{s,max}$, $k_{X,max}$	Dry-down	Quantitative	Combinations of trait values and environmental conditions (VPD, root water potential) leading to hydraulic failure, defined as lack of stable equilibrium along the SPAC	(Manzoni, Katul, & Porporato, 2014)
Stomatal sensitivities to changes in plant and soil water contents	Diurnal to seasonal	Quantitative	Mortality occurs by hydraulic failure or C starvation when a threshold plant water or carbohydrate content (respectively) is reached	(Parolari, Katul, & Porporato, 2014)
Sensitivity of foliar abscisic acid levels to leaf water potential	Dry-down	Quantitative	Iso/anisohydric responses based on contrasting dynamics of stomatal closure during a dry-down due to different levels of foliar abscisic acid levels.	(Brodribb & McAdam, 2013)
ψ_{g50}	--	Quantitative	Leaf water potential corresponding to 50% loss in stomatal conductance	(Klein, 2014)
$\psi_{g12} - \psi_{X50}$	--	Quantitative	Difference between stomatal closure point and xylem water potential at 50% of cavitation	(Skelton et al., 2015)
Sensitivities of xylem and stomatal conductance, and xylem capacitance to plant water potential	Diurnal to seasonal	Quantitative	Fully resolved tree hydrodynamics model; iso/anisohydricity emerge from combinations of all plant hydraulic traits	(Mirfenderesgi et al., 2016)

Symbol definitions

ψ_{PRE}, ψ_{MID}	Predawn and midday plant water potentials
ψ_s, ψ_L	Soil and leaf water potentials
ψ_{X50}	Stem water potential corresponding to 50% loss in maximum xylem conductivity
ψ_{g12}, ψ_{g50}	Leaf water potential corresponding to 88% and 50% loss in maximum stomatal conductance
$[ABA]_{xyl}$	Abscisic acid concentration in the xylem
g_s, $g_{s,max}$	Stomatal conductance, actual and maximum
$k_{X,max}$	Maximum conductivity in the xylem

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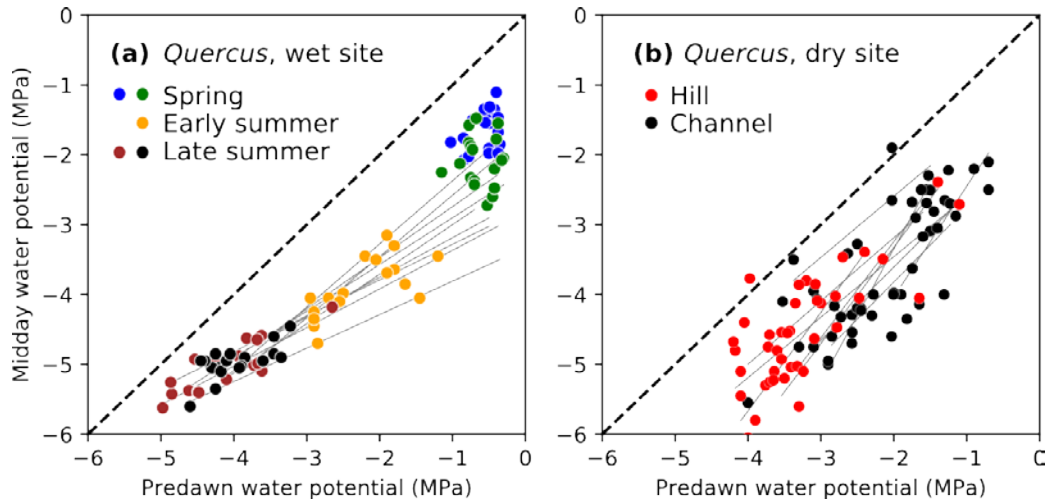| *A*	Assimilation rate

Figure 1: Variations in shoot water potential across **(a)** seasonal soil moisture and/or VPD gradients and **(b)** topographic gradients. Data collected from blue oaks (*Quercus douglasii*) in California at the Blue Oak Ranch Reserve (wet site) and a private ranch in San Luis Obispo County (dry site), over five campaigns from 2014 – 2016 (C1: Jun/July 2014, yellow dots; C2: Aug/Sept, 2014, brown dots; C3: Mar/Apr 2015, blue dots; C4: Aug/Sept 2015, black dots; C5: Apr/May 2016, green dots). Data at the wet site track the seasonal drought progression, whereas data at the dry site are separated by hill (red dots) and channel locations (black dots). Grey lines show the linear regression between midday and predawn water potentials for each individual (with statistically significant relationships) used to calculate their degree of isohydricity (Equation 1). The slope of the relationship between midday and predawn water potentials (an index of isohydricity based on equation 1) is calculated to be 0.69 for all individuals at the wet site (using only early and late summer measurements; left panel), for the dry site, 1.24 for hill individuals and 1.25 for channel individuals (right panel), suggesting that their apparent isohydricity is dependent on site conditions and hillslope position. The study design and data collection methods are described in Weitz (2018) and summarized in the Supporting Information.
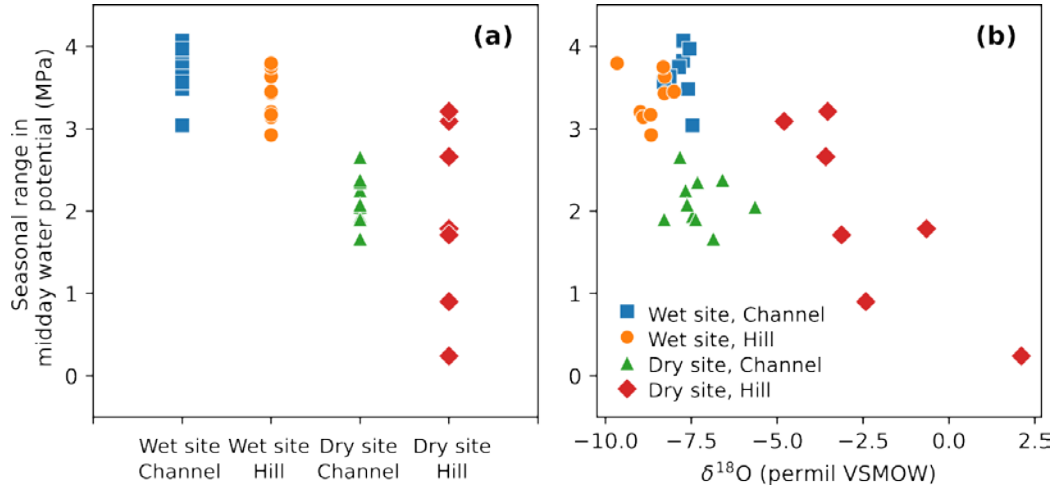
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Figure 2: **(a)** Seasonal range in the measured midday water potential (across 5 campaigns) for each individual at the two sites, divided between those located on a hill or in a channel. Higher seasonal variations as a metric suggests increased "anisohydricity." **(b)** Negative correlation ($r = -0.674$) between seasonal range in midday water potential and δ^{18}O (averaged across all campaigns, ‰ VSMOW) in the stemwater of each individual, showing plants with more variable water potentials across seasons (more "anisohydric") behave as such due to access to deeper and more stable water sources.

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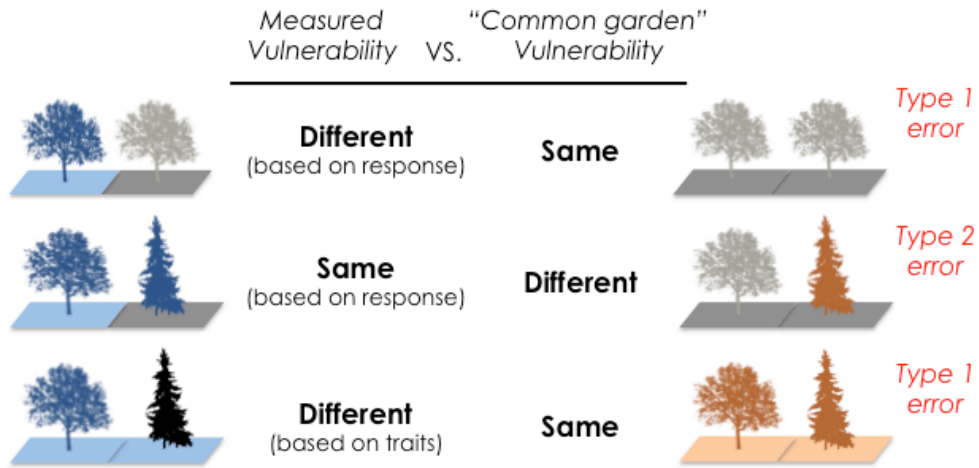
Figure 3: Different cases for which measured plant responses diverge from the actual responses. "Measured vulnerability" shows the apparent similarities or differences in responses measured using response- (or ψ-) based metrics or trait-based metrics (see also Table 1). They are contrasted with "intrinsic vulnerability," or the plant responses when they are brought under a common environment. The discrepancies demonstrate the inadequacy of metrics to accurately capture drought vulnerability under certain scenarios, when these metrics are based solely on plant water potentials or traits.

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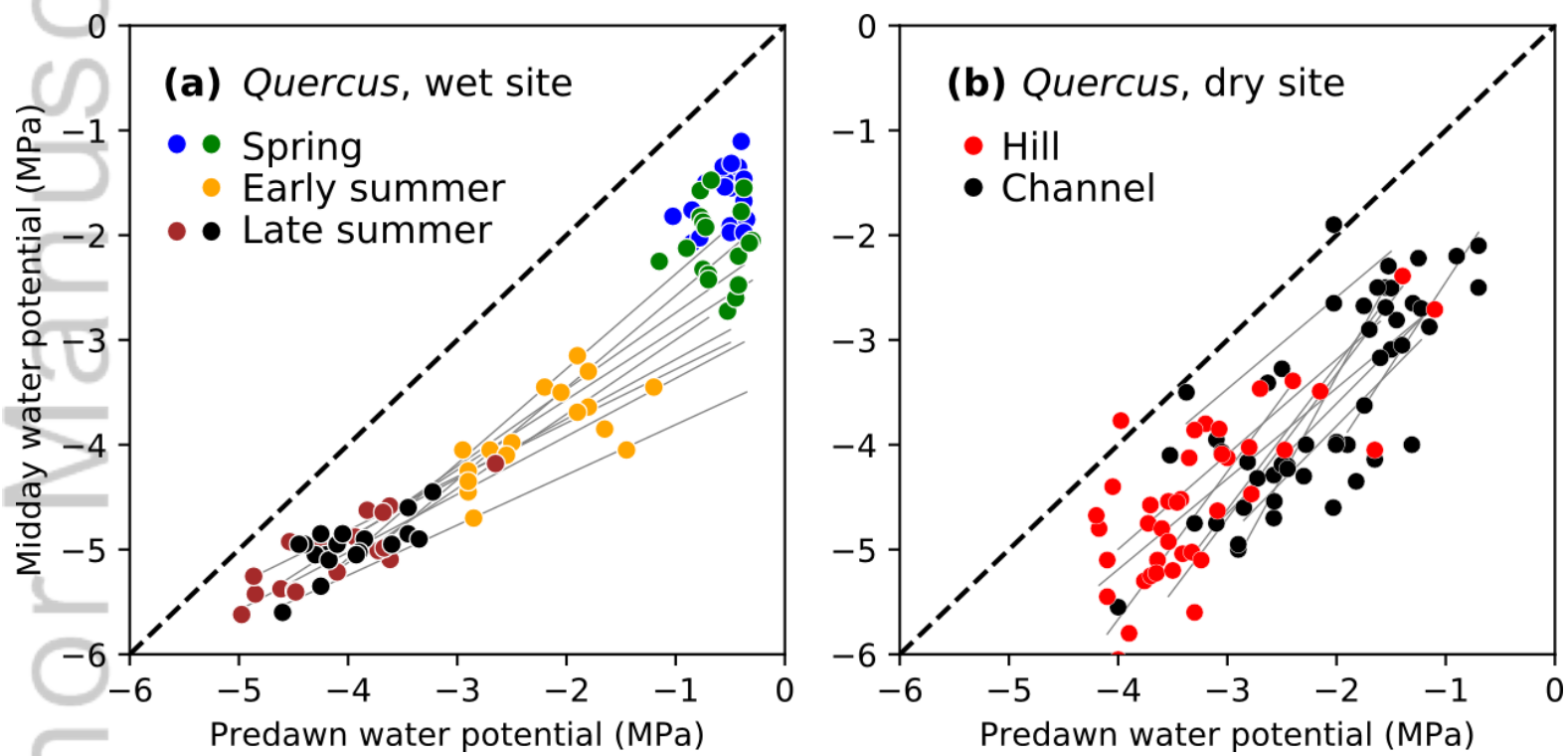
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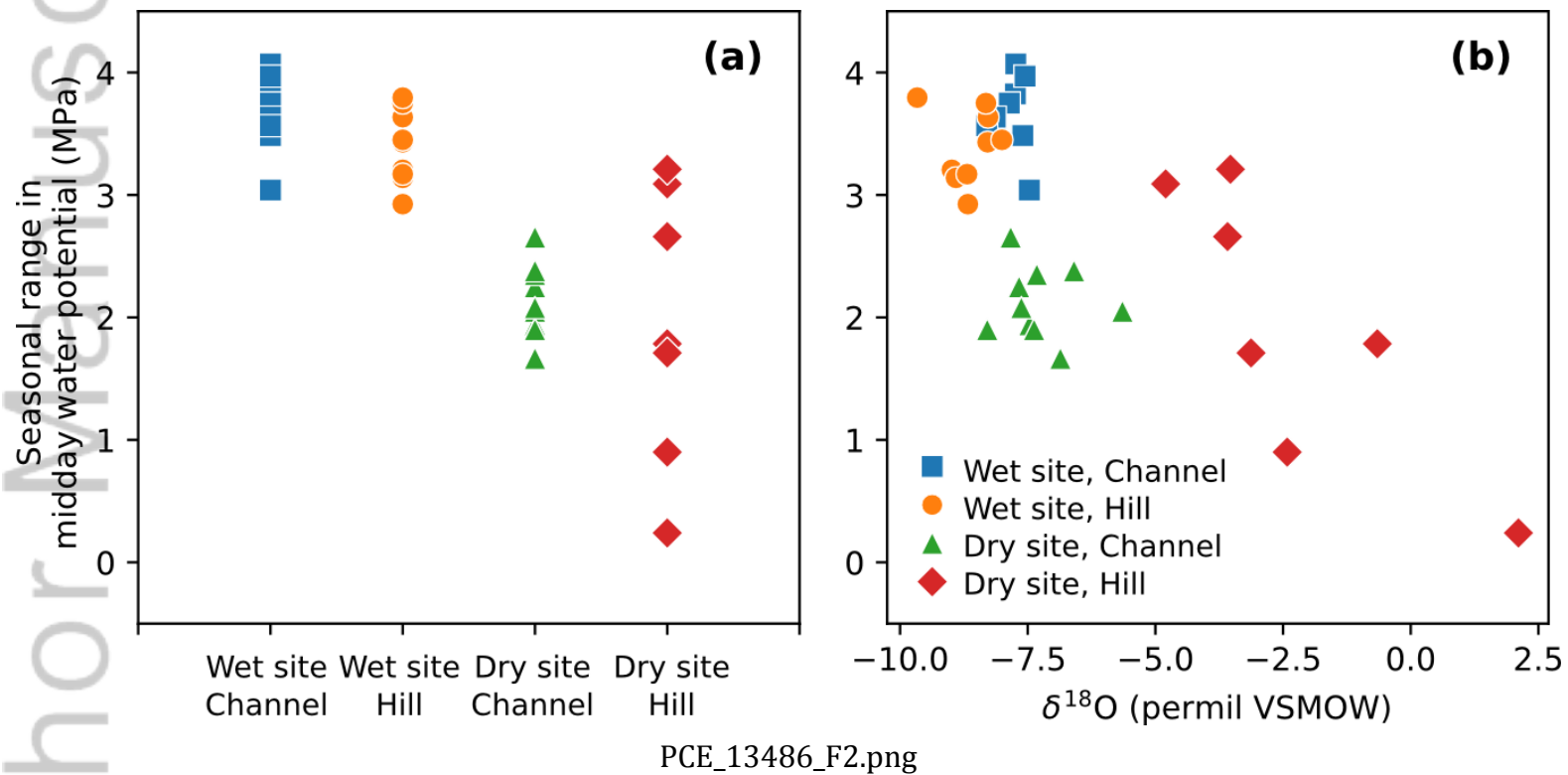
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