Weak coordination between leaf drought tolerance and proxy traits in herbaceous plants

Maria Májeková1,2 | Tomáš Hájek3,4 | Ágnes J. Albert4 | Francesco de Bello3,5 | Jiří Doležal3,4 | Lars Götzenberger3,4 | Štěpán Janeček6 | Jan Lepš3 | Pierre Liancourt1,4 | Ondřej Mudrák4

1Institute of Evolution and Ecology, University of Tübingen, Tübingen, Germany; 2Department of Soil Science, Comenius University, Bratislava, Slovak Republic; 3Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic; 4Institute of Botany, Czech Academy of Sciences, Třeboň, Czech Republic; 5CIDE-CSIC, Valencia, Spain and 6Faculty of Science, Charles University, Prague, Czech Republic

Correspondence
Maria Majekova
Email: maria.majekova@uni-tuebingen.de

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Abstract
1. Increased drought is predicted to have a major impact on plant performance under environmental change. Yet leaf hydraulic traits directly related to drought tolerance, such as leaf turgor loss point ($\pi_{tlp}$), are under-represented in trait-based studies and have been largely overlooked within the main frameworks evaluating trait–trait coordination and trade-offs—the leaf economics spectrum (LES) and the global spectrum of plant form and function.

2. Using 122 herbaceous species from the Central European temperate grasslands, we investigated (a) the coordination between $\pi_{tlp}$ and traits often used as proxies for drought tolerance, namely SLA, leaf area (LA), leaf dry matter content (LDMC), leaf thickness (LT), plant height and intrinsic water use efficiency (iWUE); (b) whether the strength of the trait–trait relationships differed across plant functional types (PFTs: graminoids and forbs) and depended on species phylogeny; and (c) whether single or multiple traits, combined with either PFTs or phylogenetic relatedness, provide a good prediction of $\pi_{tlp}$.

3. A more negative $\pi_{tlp}$ (higher leaf drought tolerance) was coordinated with higher LDMC and higher iWUE. This pattern was consistent among PFTs and also after accounting for phylogenetic relatedness. However, the coordination of $\pi_{tlp}$ with other traits was weak. For LT and height, it was driven by the differences between PFTs. For SLA and LA, it was only observed after accounting for phylogenetic relatedness.

4. The most parsimonious model predicting $\pi_{tlp}$ as a function of other traits retained LDMC and LA (adj. $R^2 = 0.37$). Since $\pi_{tlp}$ showed a strong phylogenetic signal, accounting for the influence of phylogenetic relatedness further improved $\pi_{tlp}$ prediction by 17%.
5. In herbaceous temperate plants, there is relatively weak coordination between leaf drought tolerance ($\pi_{\text{tip}}$) and traits representing key dimensions of the LES and the global spectrum of plant form and function. None of the proxy traits considered here, alone or in combination, provided a strong prediction of $\pi_{\text{tip}}$ across a large number of grassland plant species. Therefore, our work emphasizes the need for direct measurements of leaf hydraulics when estimating plant drought responses to better understand and predict species responses to environmental change.

**KEYWORDS**
grasslands, LDMC, leaf economics spectrum, phylogeny, plant functional types, SLA, turgor loss point

## 1 | INTRODUCTION

Climate change is predicted to increase the occurrence of extreme drought events, altering the water availability for plant growth (Dai, 2013; IPCC, 2014). How plants, their geographic distribution and their communities will respond to changes in water availability can be ideally understood through physiological traits directly linked to plant water economics, also termed hydraulic traits (Engelbrecht et al., 2007; Reich, 2014). Some studies have relied directly on hydraulic traits, such as stomatal conductance or intrinsic water use efficiency, in evaluating plants’ response to climate change, species distribution and/or changes in community patterns (e.g. Bonal et al., 2007). However, the majority of trait-based research in the past decades has been based on easy-to-measure proxies such as SLA or wood density (Griffin-Nolan et al., 2018). Generally, the relationship between these proxies and precipitation (as a prevailing measure of water availability for plants) is weak, highlighting the need for a more careful trait selection (for review see Griffin-Nolan et al., 2018). Moreover, no traits derived from direct measurements of leaf hydraulics have been incorporated into the main frameworks representing plant form and function, that is, the leaf economics spectrum (LES; Wright et al., 2004) and the global spectrum of plant form and function (GSPFF; Diaz et al., 2016). Presumably, because hydraulic traits are harder to measure, they are under-represented in trait-based studies as compared to their easy-to-measure proxies.

A turning point in the use of hydraulic traits in trait-based ecology could arise from the development of a fast and feasible osmometry method to determine leaf osmotic potential at turgor loss point ($\pi_{\text{tip}}$), one of the key traits tightly associated with plant water economics (Bartlett et al., 2012b). The osmometry method requires just a single measurement of cellular solute obtained from a water-saturated leaf sample (Bartlett et al., 2012a). Originally developed on woody species (Bartlett et al., 2012a), osmometry has been subsequently validated for herbaceous species (Griffin-Nolan et al., 2019; Májeaková et al., 2019; Sun et al., 2020), and thus can be applied across a wide range of plant functional types (PFTs). Leaves exhibiting a more negative $\pi_{\text{tip}}$ (higher leaf drought tolerance) can remain turgid at lower soil moisture content, allowing plants to maintain critical physiological processes, such as growth and photosynthetic assimilation of CO$_2$, under drier environmental conditions (Kramer & Boyer, 1995). A less negative $\pi_{\text{tip}}$ causes stomata to close earlier while cells are still hydrated, resulting in the prevention of further leaf dehydration under increasing drought (Kramer & Boyer, 1995). Importantly, $\pi_{\text{tip}}$ could capture differences in plant water economics between as well as within species.

While $\pi_{\text{tip}}$ can be essential for understanding plant water economics, a question that remains open is the degree of coordination between $\pi_{\text{tip}}$ and traits commonly used as proxies for drought tolerance. Because the most commonly used proxy traits represent the main dimensions of plants’ form and function on the global scale (within the schemes of the LES and the GSPFF), their strength of the coordination with a hydraulic trait ($\pi_{\text{tip}}$) should reveal whether drought tolerance is well-represented within these schemes. Within the GSPFF (Diaz et al., 2016), one dimension of trait-based ecological variation among plant species is represented by the size of whole plants or their organs (plant height, leaf area (LA) and seed size). The other major dimension captures the trade-off between acquisitive and conservative resource use (SLA), which is the basic trade-off within the LES (Wright et al., 2004). SLA is also among the most commonly used proxy traits in predicting species distribution along water availability gradients (e.g. Guittar et al., 2016), as well as species response to drought events expected under climate change (e.g. Wellstein et al., 2017).

However, the evidence that the aforementioned traits are coordinated with leaf drought tolerance ($\pi_{\text{tip}}$) is so far weak and ambiguous. Moreover, most of our knowledge relating simple functional and hydraulic traits stems from woody species (Bartlett et al., 2012b and references therein; Esperón-Rodriguez et al., 2018; Griffin-Nolan et al., 2018 and references therein; Laughlin et al., 2020; Maréchaux et al., 2020) and, not surprisingly, from more arid regions of the world (Bartlett et al., 2012b; Griffin-Nolan et al., 2019; Liu & Osborne, 2015), potentially hindering a clear generalization. Multiple studies have consistently documented either no or just a weak positive relationship between $\pi_{\text{tip}}$ and SLA on both local and
global scales (Bartlett et al., 2012b; Esperón-Rodríguez et al., 2018; Liu & Osborne, 2015; Majeková et al., 2019; Maréchaux et al., 2015, 2020; Zhu et al., 2018). The results on the coordination of \( \tau_{\text{tlp}} \) with other leaf morphological traits are also not conclusive. \( \tau_{\text{tlp}} \) and LA were either found to be related positively (Maréchaux et al., 2020) or not related at all (Esperón-Rodríguez et al., 2018) in woody species, whereas no relationship was found between \( \tau_{\text{tlp}} \) and leaf thickness (LT; Esperón-Rodríguez et al., 2018). The exception of this weak evidence of coordination between leaf hydraulic and morphological traits is leaf dry matter content (LDMC). Although it is often considered interchangeable with SLA (e.g. Laughlin et al., 2020; Pierce et al., 2013, but see Garnier et al., 2001; Hodgson et al., 2011), LDMC has been consistently, though with a different strength of the relationship, found to be negatively related to \( \tau_{\text{tlp}} \) (higher LDMC related to more negative \( \tau_{\text{tlp}} \)) in different ecosystems and across different functional types (Blumenthal et al., 2020; Griffin-Nolan et al., 2019; Laughlin et al., 2020; Liu & Osborne, 2015). The scattered and often ambiguous evidence on the coordination between \( \tau_{\text{tlp}} \) and simple functional traits calls for further studies that would fill this gap.

Mesic regions dominated by herbaceous species are predicted to be severely affected by increasing drought (Easterling et al., 2000), stressing the need to understand, and potentially predict, their response to drought. Central European grasslands are ideally suited for testing relationships between the direct measurement of leaf drought tolerance (\( \tau_{\text{tlp}} \)) and proxy traits since within similar macroclimate, soil moisture varies greatly over very short spatial distances due to high heterogeneity in micro-topography and geology (Moeslund & Dalgaard, 2013). Moreover, the Central European grasslands belong to the most species-rich communities on the scales of 25 m\(^2\) and smaller (Chytrý et al., 2015). Importantly, herbaceous species might employ different mechanisms of drought resistance than woody species. A recent study on temperate grassland species (forbs and graminoids) found that a less negative \( \tau_{\text{tlp}} \) was related to higher drought survival (Sun et al., 2020). These results clearly contradict the general assumption established on woody plants that a more negative \( \tau_{\text{tlp}} \) is associated with higher drought resistance (Bartlett et al., 2012b), highlighting the need to focus more strongly on herbaceous plants when studying hydraulic traits in order to gain further insights into the different mechanisms of drought resistance.

An important factor to be considered is whether trait coordination is transferable to plants from different PFTs, or whether it is reflecting the adaptations typical for only a certain PFT. Similarly, it would be important to understand whether, in general, \( \tau_{\text{tlp}} \) carries a phylogenetic signal, that is, if closely related species do indeed have similar trait values (Crisp & Cook, 2012). It remains unclear whether a trait directly linked to a specific function, such as \( \tau_{\text{tlp}} \), would be more phylogenetically conserved than proxy traits that might reflect multiple functions. So far, most studies found that \( \tau_{\text{tlp}} \) carried a relatively weak phylogenetic signal (e.g. on woody species: Fu et al., 2012; Laughlin et al., 2020; Liu et al., 2015; and C4 grasses: Liu & Osborne, 2015). Potentially, a strong phylogenetic signal in \( \tau_{\text{tlp}} \) among species would also enable a better prediction of \( \tau_{\text{tlp}} \) than based on other traits alone, as closely related species would have similar \( \tau_{\text{tlp}} \) values (Penone et al., 2014). More accurate predictions of \( \tau_{\text{tlp}} \) would be meaningful to forecast species responses to changing climate, in particular concerning the increased number and intensity of drought events.

In this context, we screened 122 Central European temperate grassland species from different herbaceous PFTs (forbs and graminoids) across a range of soil moisture conditions. (a) We first investigated the pairwise relationships between \( \tau_{\text{tlp}} \) and the main traits representing the LES and the GSPFF under the assumption that single leaf and whole-plant traits could be used as proxies for species drought tolerance. Here we hypothesized that higher leaf drought tolerance (a more negative \( \tau_{\text{tlp}} \)) will be coordinated with a smaller size (shorter vegetative height and smaller LA) and a more conservative resource use (lower values of SLA, higher values of LDMC and/or thicker leaves). We additionally evaluated coordination between \( \tau_{\text{tlp}} \) and intrinsic water use efficiency (iWUE) measured as carbon stable isotope ratio (\( \delta^{13} \text{C} \)), expecting that higher leaf drought tolerance will be coordinated with higher iWUE. (b) We further tested whether the strength of the trait–trait relationships differed across PFTs and whether the trait–trait differences could be driven by correlated evolution across phylogeny. Here we hypothesized that if a given relationship held between and within PFTs and with and without accounting for phylogenetic relatedness, it could be considered general and transferable across PFTs and phylogenetic clades. (c) Lastly, we tested to what extent multiple traits, combined with either PFTs or phylogenetic relatedness, provide a better prediction of \( \tau_{\text{tlp}} \) than single traits.

2 | MATERIALS AND METHODS

2.1 | Plant material and localities

We evaluated 122 herbaceous species from two major PFTs (forbs, \( n = 94 \); graminoids, \( n = 28 \)) within the Central European temperate grasslands (Table S1). All plants are perennials with a C3 photosynthetic pathway. Focal plants were sampled on various species-rich grasslands belonging to habitat types with different soil moisture availability, from oligotrophic wet grasslands dominated by Molinia caerulea (Poaceae) to dry grasslands dominated by Bromus erectus (Poaceae; Table S2). The mean annual precipitation across the sampling localities ranges from 732 to 792 (±93–135) mm, classifying them into a broad category of mesic temperate grasslands (Table S2). In most of the Central and Western Europe, forest is the climax community and the open grasslands with stabilized species composition are conditioned by centuries-old management, typically grazing or mowing for hay (Bakker, 1989). In our study grasslands, species coexistence is conditioned by the traditional mowing management, which is usually applied once or twice per year, with the first mowing applied at the time of biomass peak between June and July (for more details, see Jongepierová, 2008; Lepš, 2014; Mudrák et al., 2019).
2.2 | Trait measurements

\( \pi_{tip} \) was measured for all 122 species in late May to early June 2016, that is, at the biomass peak before mowing was applied in the respective localities. Osmometry (thermocouple psychrometry) was used to measure osmotic potential at full hydration \((\pi_{o}, \text{MPa}; \text{Bartlett et al., 2012a; Majeková et al., 2019})\). Plants collected in the field were rehydrated overnight in a dark cool room. Afterward, leaf laminae were quickly cut, sealed in a 2-ml syringe with the needle stuck in a rubber stopper, frozen at \(-18^\circ\text{C}\) and then allowed to thaw for 30–60 min before measurement. About 7 μl of the solution was immediately pipetted onto a cellulose filter paper disc placed in a 1.25-mm deep sample holder and enclosed inside the C-52 sample chamber linked to a Wescor HR-33T microvoltmeter (Wescor Electronics). Measurement was taken every 3 min without opening the chamber until the equilibrium was indicated. Measurements were calibrated using 0.3 M NaCl (osmotic potential of \(-1.37 \text{MPa}\)).

\( \pi_{tip} \) was calculated based on the equation validated previously for grassland plants \( (\pi_{tip} = 0.851 \pi_{o} - 0.410; \text{Majeková et al., 2019})\).

For all other traits, sampling campaigns were run separately depending on the traits, but all followed the same standardized protocols (Pérez-Harguindeguy et al., 2013). For the 101 species collected within the six localities in the NE of the Czech Republic (locality 1–6; Table S2), SLA \((\text{m}^2/\text{kg})\), LDMC \((\text{mg/g})\), LA \((\text{mm}^2)\) and vegetative height \((\text{height; cm})\) were measured on the same rehydrated plants collected in the field as \( \pi_{tip} \). For a subset of 20 of these species (locality 1–6; Table S2), \( \delta^{13}\text{C} \) was available from a previous field campaign in autumn 2006 (for more details on the sampling design, see de Bello et al., 2012). SLA, LA, LDMC, height and \( \delta^{13}\text{C} \) for the 21 species from the locality in the SW of the Czech Republic (locality 7; Table S2) were available from a previous field campaign in June 2013 (for more details on the sampling design, see Mudrak et al., 2019).

In short, all leaves were blotted dry, weighted for fresh mass, scanned, dried at 60°C until constant mass and then weighted. Leaf area was assessed using software ImageJ (Abramoff et al., 2004). SLA was calculated as the ratio of LA to dry mass, and LDMC was calculated by dividing leaf dry mass by the fresh mass. Leaf thickness \((\mu\text{m})\) was calculated for all species from the relationship: \( \text{LT} = 1/ (\text{SLA} \times \text{LDMC}) \) (Vile et al., 2005). To measure the carbon stable isotope ratio \( (\delta^{13}\text{C}; \%o) \), dried leaves were first homogenized and \( \delta^{13}\text{C} \) was then measured using a continuous-flow stable isotope ratio mass spectrometer. Leaf \( \delta^{13}\text{C} \) is an integrated, long-term measure of the ratio between internal and ambient \( \text{CO}_2 \) concentrations \((C_i/C_a)\) that reflects the stomatal conductance to \( \text{CO}_2 \) and thus also drought-induced stomatal closure (Farquhar et al., 1989). \( \delta^{13}\text{C} \) in leaves has often been used as a surrogate of iWUE (Farquhar et al., 1989; but see, Bowling et al., 2008; Seibt et al., 2008).

2.3 | Statistical analyses

First, we tested the coordination between \( \pi_{tip} \) and other functional traits with the standardized major axis regression (SMA), using the package SMAtr (Warton et al., 2012). The approach was chosen because, contrary to linear regression, residuals are computed for both variables, thus not assuming any directionality in which one variable affects the other one. Additionally, we performed a principal component analysis (PCA) to explore the multivariate trait variation among the grassland species. Because information on \( \delta^{13}\text{C} \) was only available for a subset of species (54 out of 122), we performed a separate PCA with all traits on this subset (Figure S1).

Second, we performed SMA separately for forbs and graminoïds to compare the trait-trait relationships across as well as within PFTs. The slopes of the trait-trait relationships between forbs and graminoïds were compared using the Bartlett-corrected likelihood ratio (Warton et al., 2012). We further considered the phylogenetic relatedness among species. For this, we used an age-calibrated phylogeny (Durka & Michalski, 2012), in which the topology of deeper nodes (up to the family level) was based on Angiosperm Phylogeny Group III (The Angiosperm Phylogeny Group et al., 2016) and nodes within families were dated based on recent molecular studies (see Durka & Michalski, 2012 for details). The tree was pruned to the studied species. Five species were missing in the phylogenetic tree and were therefore omitted from all analyses involving phylogenetic relatedness (Carex leporina, Ornithogalum orthophyllum, Plantago uliginosa, Polygala major and Veronica vindobonensis; for family names, see Table S1). Based on this phylogenetic tree, we tested whether each of the traits exhibited a phylogenetic signal by calculating Pagel’s lambda (Pagel, 1999) using the package phytools (Revell, 2012). Pagel’s lambda ranges between 0 and 1 (0 = no phylogenetic signal; 1 = phylogenetic signal following a Brownian motion model of evolution) and a significance for lambda can be calculated by a log-likelihood ratio test where the estimated lambda is compared to a null model of no phylogenetic signal (Pagel, 1999).

Since most traits (except for SLA and \( \delta^{13}\text{C} \)) showed a strong phylogenetic signal (Table S3), we applied phylogenetically independent contrasts (PIC; Felsenstein, 1995) implemented in the package ape (Paradis et al., 2004) to account for phylogenetic relatedness among species. To follow the same approach as for the across species analyses, the contrasts were then used instead of the raw data in the SMA analyses, with intercept set to 0 (Garland et al., 1992). This approach gives identical results as using phylogenetic generalized least squares (Blomberg et al., 2012), with the exception that the slope estimates differ as we used SMA instead of ordinary least square regression. The goal was not to use phylogeny as an addition to the PFTs, but rather as two alternative methods that are commonly used in trait-based ecology. Height, LA and LT were log\(_{10}\)-transformed for all SMA analyses.

Third, we tested whether multiple traits together could explain more variation in \( \pi_{tip} \) than single traits by using stepwise multiple regression. We started with a full model with either all traits and PFTs or with all traits and phylogenetic relatedness (see below). We used model selection based on the minimum Akaika information criterion corrected for a small sample size (AICc; Burnham & Anderson, 2003). Because information on \( \delta^{13}\text{C} \) was only available for a subset of species (54 out of 122), we omitted this trait from these analyses. For model construction, we assessed phylogenetic relatedness by a phylogenetic eigenvector approach (Diniz-Filho et al., 1998), that is, first
performing a principal coordinates analysis (PCoA) on the matrix of phylogenetic distances (package hierfstat; Goudet, 2005), then selecting eigenvectors that together explained more than 80% of the total variance (here, first nine eigenvectors). We then fitted these nine eigenvectors against the response variable (π_{tlp}) with stepwise multiple regression and selected the best model based on the minimum AIC. Eigenvectors retained in the best model (PCoA1, 4, 6, 8 and 9) were then used as covariates in the models involving the traits in order to consider the phylogenetic relatedness of species.

We compared the observed values of π_{tlp} with the predicted values of π_{tlp} from the best models using the SMA. Within the SMA, we then tested the regression slopes of predicted versus observed π_{tlp} for their difference from the 1:1 line to evaluate the under or over-estimation of π_{tlp} at either end of the slope.

All analyses were performed in the R software (R Core Team, 2016).

3 | RESULTS

The first, second and third PCA explained 36.2%, 25.9% and 16.6%, respectively, of the total variance in the traits among the 122 grassland species (Figure 1; Figure S1). π_{tlp} was considerably lower in forbs (\(-1.16 \pm 0.02\) MPa) than in graminoids (\(-1.37 \pm 0.03\) MPa); π_{tlp} showed the strongest phylogenetic signal (Pagel’s λ = 0.79, \(p < 0.001\)), together with LDMC (Pagel’s λ = 0.83, \(p < 0.001\)) and LT (Pagel’s λ = 0.75, \(p < 0.001\)). Other traits tended to show a lower phylogenetic signal, particularly SLA and δ^{13}C (for detailed information on PFT differences and phylogenetic signal in all traits, see Figure S2 and Tables S3–S4).

Individual traits explained between zero and 31% of the variation in π_{tlp} (Table 1). A more negative π_{tlp} (higher leaf drought tolerance) was related to higher LDMC and less negative δ^{13}C (higher IWUE) on all levels: (a) when considering all species together, (b) within the PFTs with the slopes of the relationships being not significantly different, (c) as well as after accounting for phylogenetic relatedness (Table 1, Figure 2).

Lower SLA was weakly related to a more negative π_{tlp}, explaining 4% of the variation in π_{tlp} within forbs and 10% after accounting for phylogenetic relatedness (Table 1, Figure 2). π_{tlp} was significantly, but weakly (7% of the variation explained), positively related to LT when all species were considered together (Table 1, Figure 2). However, this relationship seemed to be driven by the differences between graminoids and forbs (Figure S1, Figure 2) and was not detectable within PFTs and after accounting for phylogenetic relatedness (Table 1, Figure 2). π_{tlp} was not related to LA, and even though the test was significant after accounting for phylogenetic relatedness, LA explained only 4% of the variation in π_{tlp} (Table 1, Figure 2). When all species were considered together, height explained 3% of the variation in π_{tlp} with a more negative π_{tlp} associated with taller species (Table 1, Figure 2). However, the relationship was neither significant within PFTs, nor after accounting for species phylogenetic relatedness (Table 1, Figure 2).

From the combination of all traits and PFTs, the most parsimonious model explained 37% of variation in π_{tlp} (AICc = -88.41, RMSE = 0.165, adj. \(R^2 = 0.369\), \(p < 0.001\)). It combined a negative relationship with LDMC (slope ± SE = -0.002 ± 0.000, \(t = -8.34\), \(p < 0.001\)), which explained the dominant proportion of this variation, with a negative relationship with LA (slope ± SE = -0.003 ± 0.000, \(t = -3.22\), \(p = 0.002\)). A model with LDMC, LA and PFT yielded similar result (AICc = -87.85, ΔAICc = 0.56, RMSE = 0.164, adj. \(R^2 = 0.377\), \(p < 0.001\)); however, the effect of PFTs on π_{tlp} was not significant (\(t = 1.25\), \(p = 0.212\)).

From the combination of all traits and phylogenetic relatedness, the most parsimonious model explained 54% of variation in π_{tlp} (AICc = -112.15, RMSE = 0.144, adj. \(R^2 = 0.541\), \(p < 0.001\)). It combined LDMC (slope ± SE = -0.001 ± 0.000, \(t = -3.44\), \(p = 0.001\)) with the five PCoA axes that together represent the most variation in species phylogenetic relatedness (all \(p < 0.05\)). For both best models, the regression lines between predicted and observed π_{tlp} were significantly different from the 1:1 line (in both models \(p < 0.001\); Figure 3). Both models tended to overestimate π_{tlp} in the more drought-tolerant species (more negative π_{tlp} values) and underestimate it in the less drought-tolerant species (less negative π_{tlp} values; Figure 3). The 10 best models of each of the model selection are reported in Table S5.

4 | DISCUSSION

In this study, on an extensive set of 122 herbaceous species from the temperate Central European grasslands, we documented the strength of the coordination between the π_{tlp} and traits frequently considered proxies of drought tolerance and also associated with the LES (Wright et al., 2004) and the global spectrum of plant form and function (Díaz et al., 2016). Our results demonstrate three key findings. (a) We found only moderate-to-weak coordination between π_{tlp} and single traits. (b) Some relationships were rather general since their direction and strength were consistent across and within PFTs as well as after accounting for phylogenetic relatedness (π_{tlp}–LDMC and π_{tlp}–IWUE). Other π_{tlp}–trait relationships were less general. For LT and height, the relationship was driven by the differences between PFTs. For SLA and LA, it was only observed after accounting for phylogenetic relatedness. (c) The most parsimonious model predicting π_{tlp} combined LDMC and LA and explained a relatively low amount of variation (37%) in π_{tlp}. It overestimated π_{tlp} in the more negative π_{tlp} values and underestimated it in the less negative values. π_{tlp} showed a strong phylogenetic signal and accounting for the influence of phylogenetic relatedness improved π_{tlp} prediction by an additional 17%.

4.1 | π_{tlp} and LDMC

In line with our hypothesis, a more negative π_{tlp} (higher leaf drought tolerance) was coordinated with higher LDMC, and this pattern was consistent within and across PFTs. One explanation is that because a more negative osmotic potential represents a response to drought stress (e.g. Májeková et al., 2019), water limitation simultaneously
**FIGURE 1** Principal component analysis representing the overall trait-trait relationships of the 122 herbaceous species from mesic temperate grasslands: turgor loss point ($|\pi_{tlp}|$; arrow direction indicates higher leaf drought tolerance), leaf dry matter content (LDMC), SLA, leaf thickness (LT), leaf area (LA) and vegetative height (Height). Forbs are indicated in purple and graminoids in green (for full species name, see Table S1).
results in smaller cell wall expansion, smaller cells and/or relatively thicker and more lignified secondary cell walls, that is, greater LDMC (Poorter et al., 2009). Our results are consistent with previous evidence on coordination between these two leaf-level traits—on C4 grasses from different habitats (Liu & Osborne, 2015), on prairie species including C3 and C4 graminoids, forbs and shrubs (Blumenthal et al., 2020; Griffin-Nolan et al., 2019) and on woody temperate rainforest angiosperms (Laughlin et al., 2020).

The fact that we found the coordination between $\pi_{\text{tlp}}$ and LDMC to be consistent on all levels, together with other evidence including also woody angiosperms and C4 herbaceous species, suggests that this coordination might be biophysically based and transferable to other systems, PFTs and plant families. However, further research insofar under-represented PFTs, such as conifers or annual plants, are needed to fully validate this hypothesis. An absence of such a relationship in temperate rainforest conifers (possibly due to smaller sample size—on C4 grasses from different habitats (Liu & Osborne, 2015), on prairie species including C3 and C4 graminoids, forbs and shrubs (Blumenthal et al., 2020; Griffin-Nolan et al., 2019) and on woody temperate rainforest angiosperms (Laughlin et al., 2020).

### 4.2 $\pi_{\text{tlp}}$ and iWUE

In line with our hypothesis, a more negative $\pi_{\text{tlp}}$ (higher leaf drought tolerance) was coordinated with higher iWUE (less negative $\delta^{13}$C) on all levels considered. A possible explanation would be that an increased iWUE and leaf drought tolerance via a more negative $\pi_{\text{tlp}}$ might be complementary strategies to cope with drought in temperate herbaceous plants. Our results are consistent with the results on C4 grasses from different habitats (Liu & Osborne, 2015), but not with the results from tropical trees where no relationship was found (Maréchaux et al., 2020). The inconsistency of this trait–trait relationship across different systems could reflect different levels of osmotic adjustment that results in stomatal responses.

We should, however, consider this relationship critically, as we did not explicitly measure iWUE but used instead leaf $\delta^{13}$C as a surrogate and factors other than iWUE may affect leaf $\delta^{13}$C. For example, shifts in the relative abundance of cellulose and lignin or other leaf constituents could influence leaf $\delta^{13}$C independently of photosynthetic WUE (Bowling et al., 2008; Seibt et al., 2008). That said, $\delta^{13}$C of cellulose is only ~5% greater than that of lignin (Bowling et al., 2008) and lignin represents only 5%–10% of dry biomass of green leaves in grassland plants (Fukushima & Hatfield, 2004). A realistic difference in leaf lignification of 5% would affect the bulk $\delta^{13}$C by 0.25‰, representing only 5% of our $\delta^{13}$C gradient (Figure 2). This is an acceptable difference, which justifies using bulk leaves instead of isolated cellulose for $\delta^{13}$C analysis.

### 4.3 $\pi_{\text{tlp}}$ and SLA

Although SLA seems more strongly related to nutrient and light availability (e.g. Hodgson et al., 2011; John et al., 2017), it has become the most commonly used proxy for adaptations to water availability (Griffin-Nolan et al., 2018 and references therein; Westoby et al., 2002; Wright et al., 2004), and as a response to drought under climate change scenarios (e.g. Wellstein et al., 2017). However, mounting evidence suggests that low SLA and more negative $\pi_{\text{tlp}}$ have evolved rather independently under different selection pressures (Bartlett et al., 2012b; Esperón-Rodriguez et al., 2018; Liu & Osborne, 2015; Májeková et al., 2019; Maréchaux et al., 2015, 2020; Zhu et al., 2018), and our results from temperate grassland species further support this pattern. The relationship between $\pi_{\text{tlp}}$ and other

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**Table 1** Results of the standardized major axis regression of the relationships between turgor loss point (_tlps, n = 122) and leaf dry matter content (LDMC, n = 122), intrinsic water use efficiency measured by carbon stable isotopes (δ13C, n = 54), SLA (n = 122), leaf thickness (LT, n = 122), leaf area (LA, n = 122) and vegetative height (Height, n = 122) in models (a) with traits, (b) for the plant functional types and (c) based on data after accounting for phylogenetic relatedness. In (b) `LR` denotes the Bartlett-corrected likelihood ratio for slopes comparison between the PFTs under the null hypothesis that slopes are equal. Height, LA and LT were log10-transformed. Significant relations ($\alpha = 0.05$) are highlighted in bold.

| Model                  | LDMC | δ13C | SLA  | LT   | LA   | Height |
|------------------------|------|------|------|------|------|--------|
| (a) Model with traits  |      |      |      |      |      |        |
| $R^2$                   | 0.314| 0.125| 0.011| 0.074| 0.003| 0.032  |
| $p$                     | <0.001| 0.009| 0.239| 0.003| 0.547| 0.046  |
| (b) Models for the plant functional types |      |      |      |      |      |        |
| Forb                   |      |      |      |      |      |        |
| $R^2$                   | 0.168| 0.130| 0.042| 0.008| 0.026| 0.000  |
| $p$                     | <0.001| 0.036| 0.046| 0.397| 0.120| 0.978  |
| Graminoid              |      |      |      |      |      |        |
| $R^2$                   | 0.241| 0.082| 0.028| 0.411| 0.695| 0.232  |
| $p$                     | 0.027| 0.231| 0.743| 0.397| 0.120| 0.978  |
| Slope comparison       |      |      |      |      |      |        |
| $LR$                   | 0.004| 1.436| 1.427| 0.107| 2.903| 0.143  |
| $p$                     | 0.950| 0.231| 0.743| 0.397| 0.120| 0.978  |
| (c) Model with phylogenetic independent contrasts |      |      |      |      |      |        |
| $R^2$                   | 0.197| 0.165| 0.102| 0.002| 0.000| 0.000  |
| $p$                     | <0.001| 0.003| <0.001| 0.605| 0.782| 0.782  |
traits representing the LES has also been found to be weak (e.g. between $\pi_{\text{tlp}}$ and $A_{\text{max}}$ in grassland plants from mixed-grass prairie; Ocheltree et al., 2020). Additionally, in many studies, no or only weak relationship has often been found between SLA and water availability gradients at different spatial and taxonomical scales (Bartlett et al., 2012b; Griffin-Nolan et al., 2018 and references therein). This together suggests that SLA should be considered very carefully as a proxy reflecting the direct response of plants to water availability both in predicting the future distribution of species as well as when evaluating their response to drought.

4.4 | $\pi_{\text{tlp}}$ and plant height

The relationship between $\pi_{\text{tlp}}$ and plant height represents an example of how coordination between two traits could be misinterpreted without a further stratification by PFTs or accounting for plant phylogenetic relatedness. When considering all species without any stratification, a weak, but marginally significant, negative relationship appears between $\pi_{\text{tlp}}$ and plant height, suggesting that a more negative $\pi_{\text{tlp}}$ is coordinated with a taller stature. One potential explanation, for example, could be that shorter species growing under taller species would be sheltered from direct irradiance and therefore experience less dehydration (e.g. Wright et al., 2015).

However, a closer examination reveals that, in our case, the relationship is purely driven by the pronounced differences between forbs and graminoids in both traits considered and was not detectable within the PFTs and after accounting for plant phylogenetic relatedness. It seems that the risk of embolism, driving the relationship between plant height and hydraulic traits in woody species (Ryan et al., 2006), does not play a major role in the shorter, relatively to trees, herbaceous species. Indeed, a height of 1 m generates only $-0.01$ MPa of gravitational potential, which is negligible when compared with osmotic potential. Rather, plant height in grassland plants seems to be under different and

FIGURE 2 Standardized major axis regression of the relationships between turgor loss point ($\pi_{\text{tlp}}$) and leaf dry matter content (LDMC), intrinsic water use efficiency measured as carbon stable isotope ratio ($\delta^{13}$C), SLA, leaf thickness (LT), leaf area (LA) and vegetative height (Height). Left two panels present actual data where purple dots and lines denote forbs, green triangles and lines denote graminoids, and grey line is based on all data. Right two panels present data after accounting for the phylogenetical relatedness (PIC; phylogenetically independent contrasts). Lines represent significant relationships ($\alpha = 0.05$). In d) and g), empty dots represent outliers. Height, LA and LT were log$_{10}$-transformed.
potentially more important selection pressures than water availability, such as the competitive ability for light (Keddy & Shipley, 1989), thus resulting in little coordination between height and $\pi_{\text{tlp}}$.

### 4.5 | Prediction of $\pi_{\text{tlp}}$

Considering multiple traits did not improve $\pi_{\text{tlp}}$ prediction as compared to prediction by single traits. The best model with LDMC and LA explained 37% of the variation, compared to 31% explained by the best single-trait model with LDMC. Leaf area represents the evaporative surface of a plant (Poorter et al., 2009), therefore it necessarily plays a role in water loss. Alone, LA was not coordinated with $\pi_{\text{tlp}}$, potentially because there are factors more important than water availability shaping its evolution in grassland plants, such as temperature (Givnish, 1987). For instance, a recent global study demonstrated a contingent effect of precipitation on LA, depending on temperature (Wright et al., 2017). However, considering LDMC and LA together offers further insights into the relationship between LDMC and $\pi_{\text{tlp}}$. Specifically, for a given LDMC value, plants with larger leaves (bigger evaporative surface and higher water loss) need to have a more negative $\pi_{\text{tlp}}$ to achieve the same drought tolerance. Overall, our results are consistent with results on leaf morphological and chemical traits being poor predictors of $\pi_{\text{tlp}}$ from Amazonian rainforest tree and liana plants (Maréchaux et al., 2020).

### 4.6 | $\pi_{\text{tlp}}$, PFTs and phylogeny

In our dataset, $\pi_{\text{tlp}}$ showed a strong phylogenetic signal. Our result contradicts most of the previous studies that documented a relatively weak phylogenetic signal in $\pi_{\text{tlp}}$ (e.g. Fu et al., 2012; Laughlin et al., 2020; Liu & Osborne, 2015; Liu et al., 2015). This opposing evidence on the phylogenetic signal in leaf drought tolerance highlights a research gap. Filling this gap could lead to a better understanding of the mechanisms driving hydraulic and morphological trait co-evolution as well as potentially enable a better prediction of $\pi_{\text{tlp}}$ if closely related species had similar $\pi_{\text{tlp}}$ values. Indeed, in our study, accounting for the influence of phylogenetic relatedness improved $\pi_{\text{tlp}}$ prediction by an additional 17%. A more accurate prediction of $\pi_{\text{tlp}}$ would be meaningful to forecast species responses to changing climate, in particular concerning increased number and intensity of droughts.

Trait–trait coordination can be better understood by coupling the quantitative measures with the PFTs and/or phylogeny. Indeed, if a relationship (with another trait or environmental factor) is driven only by the differences in functional types or between phylogenetic clades, but does not hold within the types or clades, one might ask whether the trait considered reflects the function being investigated. For instance, here we showed that stratification by simple PFTs and/or accounting for species phylogeny might help to identify relationships that are biophysically based, and therefore potentially better transferable among different systems. Such would be the coordination between $\pi_{\text{tlp}}$ and LDMC, where the strength of the relationship holds within PFTs, after accounting for phylogenetic relatedness, as well as in other functional groups such as C4 grasses (Liu & Osborne, 2015) and tropical trees (Laughlin et al., 2020). On the other hand, the need for verifying the relationship is in our case highlighted on the relationships between $\pi_{\text{tlp}}$ and height and $\pi_{\text{tlp}}$ and LT, which were not detectable when further stratifying by PFTs or accounting for phylogenetical relatedness.
4.7 | Critical remarks: The use of non-hydraulic traits as proxies of drought tolerance

One needs to carefully consider that the leaf is a multifunctional organ, and thus leaf traits might not always capture exactly the response to a single abiotic factor in question, such as water use. For instance, a plant’s response to water availability measured through SLA, a very important LES trait, can be simultaneously influenced by light availability, nutrient availability or herbivory resistance (John et al., 2017; Poorter et al., 2009). As advocated by Hodgson et al. (2011), one needs to carefully consider how valid the use of such a trait is as a stand-alone proxy of a single function.

Here, we would like to reinforce this view by advocating the use of traits known to be directly linked to a particular environmental factor in question (e.g. review by Griffin-Nolan et al., 2018). In the case of water availability, we suggest using $\pi_{\text{tLP}}$, which is a hydraulic trait feasible to measure on a large number of individuals of different PFTs. Furthermore, if leaf traits that could reflect multiple functions of the leaf are measured, we suggest coupling them with traits known to be directly linked to the given environmental factor in question. In case of water availability, this would be $\pi_{\text{tLP}}$ or any other hydraulic trait, such as for instance WUE or stomatal conductance. This should be done on at least a subset of species to validate that the morphological traits reflect the function in question. We advise adopting these approaches especially in designing future field studies aiming at understanding the distribution of plant populations and communities along water availability (i.e. precipitation/aridity) gradients as well as their response to increased drought stress under the scenarios of climate change.

5 | CONCLUSIONS

In this study, we addressed an issue of trait-based ecology that is likely to grow in importance in the coming years—the importance of hydraulic traits in assessment of plant drought tolerance (Brodribb, 2017; Griffin-Nolan et al., 2018; Volaire, 2018). In herbaceous temperate plants, we found a poor coordination between leaf drought tolerance, assessed via $\pi_{\text{tLP}}$, and traits representing the main dimensions of the LES and the global spectrum of plant form and function. This might suggest that the existing spectra may only coarsely approximate plant drought tolerance. Therefore, our work emphasizes the need for direct measurements of leaf hydraulics when estimating plant drought responses to better understand and predict species responses to environmental change.

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AUTHORS’ CONTRIBUTIONS

M.M., O.M. and P.L. conceived the ideas; M.M., O.M., T.H. and P.L. designed methodology; M.M., A.J.A., F.d.B., J.D., S.J., P.L. and O.M. collected the data; M.M. and L.G. analysed the data; M.M. led the writing of the manuscript. All the authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data deposited in Dryad Digital Repository https://doi.org/10.5061/dryad.hqbzk1h1fs (Májeková et al., 2021).

ORCID
Maria Májeková https://orcid.org/0000-0001-6892-5462
Tomáš Hájek https://orcid.org/0000-0002-5981-5840
Francesco de Bello https://orcid.org/0000-0001-9202-8198
Jiří Doležal https://orcid.org/0000-0002-5829-4051
Lars Götzberger https://orcid.org/0000-0003-3040-2900
Štěpán Janeček https://orcid.org/0000-0003-1285-6490
Jan Lepš https://orcid.org/0000-0002-4822-7429
Pierre Liancourt https://orcid.org/0000-0002-3109-8755
Ondřej Mudrák https://orcid.org/0000-0001-7775-0414

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