

THESIS

RELATIONSHIPS BETWEEN ANATOMY AND CLIMATE IN MEMBERS OF THE  
SUBFAMILY *POOIDEAE*

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## ABSTRACT

### RELATIONSHIPS BETWEEN ANATOMY AND CLIMATE IN MEMBERS OF THE SUBFAMILY *POOIDEAE*

As climate continues to change, it will become increasingly important to accurately predict the landscape-level response of plant functional groups to climatic shifts. Relationships between vascular anatomy and climate distribution have been revealed in woody plant species, but little research has been done on this subject in the *Poaceae* family. These relationships were studied in 21 grass species from 5 genera in the subfamily *Pooideae* by analyzing their anatomical traits and climate data from each species' range. Analysis showed multiple correlations among anatomical traits, such as a positive correlation between the ratio of vessel wall thickness to vessel diameter (t/b ratio) with vein density. These relationships suggest that anatomical traits are closely linked to each other and to vein density, which may be confer some advantage or be in response to selective pressure. Some correlations between climate traits and anatomical traits were found, but the strength of these relationships was less than expected and were often the result of the interaction of multiple climate variables with a single anatomical variable. This may be because of broad, multi-year averaged data obscuring important temporal and spatial factors, as well as phenological variation across species not being accounted for. Future research should focus on microclimatic conditions species occupy and noting phenology for each species to better identify the climate conditions species are adapted to.

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## OVERVIEW OF RESEARCH CONDUCTED ON PLANT VASCULAR ANATOMY IN RELATION TO CLIMATIC VARIABLES

There is a theoretical link between the anatomical traits observed in plants and the climate variables within which these plants exist. We would expect to see this relationship because both anatomical traits and climate variables are linked to plant physiology. Vascular anatomy is the means by which water is conducted through plants along the Soil-Plant-Atmosphere continuum, and the size of the conduits dictates how much water can be moved through this system per unit time (conductance), which is tied to physiological traits such as photosynthetic rate (Hemsley and Poole 2004). Climate is connected to physiological traits because plant physiology is adapted and responds directly to both temperature and precipitation variables (Lambers *et al.* 2008). Reduced water availability in the soil increases the tension in the xylem sap column, which may introduce air bubbles into the water column that rapidly expand (cavitation), resulting in embolism of xylem vessels that prevent the transport of water along the Soil-Plant-Atmosphere continuum (Lambers *et al.* 2008). Xylem vessel wall thickness, xylem conduit width, and other vascular anatomical traits have been shown to be linked to xylem vulnerability to hydraulic failure (Hemsley and Poole 2004; Blackman *et al.* 2018). Hydraulic failure can also occur as a result of xylem sap freezing and thawing, due to cavitation from gases that were dissolved out of the xylem sap solution as the xylem sap froze, then expanded as the xylem thawed (Lambers *et al.* 2008).

The relationship between anatomy and climate has been studied in three ways:

- 1) Examining the influence anatomy has on physiology, which interacts directly with

climate variables. 2) Studying variation in plant anatomical traits across space, particularly across varying temperature and precipitation regimes. 3) Applying direct manipulations of water availability or temperature to simulate climate variables, and then measure the impact these manipulations have on anatomy.

### **Relationships that anatomical traits have with physiology**

Climate variables directly impact the physiological traits of plant species, and so it is important to understand the relationship between anatomy and physiology in plants, to better understand the way anatomy influences physiology as a part of adaptation to climate. Analysis of vascular anatomy has often been tied to an interest in understanding the functional traits of xylem in relation to plant physiology, especially conductance capacity at various scales, hydraulic vulnerability as represented by P50 (the pressure of xylem water when hydraulic conductance is reduced by 50% relative to maximum conductance). Blackman *et al.* (2018) found that across 26 Australian woody angiosperms, P50 values were highly correlated with the vessel thickness to vessel span (t/b) ratio, an important indicator of vessel reinforcement against collapse, and that vessel wall thickness increased more in smaller vein orders. This same study suggested that P50 values were not related to vein density or leaf size. Bachle and Nippert (2018) studied the anatomical and physiological traits of four C4 grass species across a precipitation gradient on the Konza prairie and found that anatomical traits such as vein bundle sheath area were significantly different in plants from the highest-precipitation area. This indicates a direct link between anatomy and climate, but the study points out the need for more evidence to be collected using more phylogenetically diverse assemblages. In a study on the physiological and anatomical attributes of *Festuca*

*arundinacea* Schreb., Martre and Durand (2001) found that both the number and diameter of vessels decreased acropetally along the leaf blade, and that this reduction also resulted in a reduction of theoretical hydraulic conductivity. Cochard *et al.* (2004) conducted a study on leaves of *Laurus nobilis* L. and *Juglans regia* L. and found that measurements of hydraulics in leaves treated to eliminate extra-vasculature resistance, as well as models of theoretical hydraulic resistance, indicated that most of the hydraulic resistance (88%) in the leaf is driven by the mesophyll, not the leaf vein architecture. Willigen *et al.* (2000) grew four different, unrelated tropical evergreen tree species in identical climate conditions, and subsequent examination of their hydraulic and vascular traits revealed that conductivity correlated well with conduit diameter and conduit density across all species, but hydraulic vulnerability as expressed by P50 varied widely across species. The researchers concluded that while xylem anatomy traits were driven by environmental conditions, cavitation vulnerability was genetically determined. Volaire *et al.* (2018) found that in three *Dactylis glomerta* L. populations from three distinct climates, p50 was highly correlated with metaxylem vessel wall thickness, and that P50 values were also highly correlated with the precipitation values of the driest 3 months of each specie's home range. Sack and Frole (2006) studied 10 Panamanian tropical tree species, measuring hydraulic resistance of leaves and 24 traits related to leaf venation and mesophyll structure. They found that hydraulic resistance was positively related to leaf hydraulic resistance of the midrib, and negatively related to vein density. However, numerous other leaf traits they tested appeared to be independent of resistance, suggesting that a large amount of variability in leaf morphology is possible without physiological trade-offs. There has also been



some interest in the relationship between xylem anatomy and plant morphology and phenology. Gorusch *et al.* (2001) conducted a comparison of vessel characteristics of six deciduous and six evergreen arctic dicots with both erect and prostrate growth forms and found that vessel diameters were larger in deciduous species than in evergreen species. This difference between deciduous and evergreen species was also true when shrub- and tree-form species were compared with cushion- and mat-form species (species characterized as cushion- or mat-form had growth horizontally from meristems low to the ground). The authors postulated that this was due to the need for the deciduous species to maximize growth during conducive conditions. They also discovered that vessel density was greater in cushion- and mat-type species than tree-shrub species and theorized that these species were therefore less vulnerable to freezing-induced cavitation. Yin *et al.* (2016) found that among 82 native and non-native understory deciduous forest species, those with higher percentages of total vessel area in metaxylem and earlywood had earlier bud and leaf emergence. The researchers suggested that this indicated a need to transport water efficiently to fuel earlier growth, even if it put the plants at higher risk for freezing-induced cavitation. Woodruff *et al.* (2008) researched *Pseudotsuga menziesii* (Mirbel) Franco leaf tracheids and determined that the t/b ratio increased as height on the tree increased, but tracheid density, tracheid diameter, and total tracheid number declined. These adjustments were theorized to be coping mechanisms to reduce xylem tension along the vertical gradient, at the expense of reduced water transport capacity and efficiency. Ocheltree *et al.* (2012) found that along the leaf blades of five grass species, stomatal conductance and photosynthesis increased, without a similar increase in vascular hydraulic efficiency.

However, interveinal distance decreased along the leaf blade, reducing the path length for water moving from xylem to stomata. The authors postulated that this served to minimize water potential gradients as water loss increased acropetally. Examining physiological relationships to anatomy can provide us an indirect view to potential relationships between climate and anatomy.

### **Variability of anatomy across climate gradients**

One way to study these relationships is to observe variation in anatomical traits over broad spatial scales with differing climate variables. A study of *Ilex aquifolium* L. populations in two contrasting sites (mesic vs drought-prone) conducted by Rita *et al.* (2015) reported that vessel size and vessel frequency were greater at the mesic site, but vulnerability to cavitation was higher at the drought-prone site. Schuldt *et al.* (2018) found that in a study on *Fagus sylvatica* L., along a decreasing precipitation gradient, there was a decline in vessel size and pit membrane thickness but not an increase in vessel density. Verheyden *et al.* (2005) collected data during a time-series study of *Rhizophora mucronata* Lam. tree populations in Kenya, and found intra-annual variations in vessel anatomy, with smaller, more numerous vessels appearing in the dry season and larger, less numerous vessels growing during the wet season. Spectral analysis revealed a potential direct relationship between relative humidity and precipitation with the vessel features studied. In a study of *Betula utilis* D. populations located in the Himalayas, Pandey *et al.* (2018) found that mean vessel area positively correlated with summer temperature in the previous and current year, as well as early spring precipitation. A study by Bourne *et al.* (2017) focused on six eucalyptus species from a variety of climate ranges grown in a common garden and discovered a

correlation between xylem diameter vessel and climate parameters from the species native range, especially maximum mean monthly vapor pressure deficit and mean annual precipitation. These tree species, despite being grown in the same location, exhibited xylem anatomy more closely adapted to conditions of their home ranges. Jordan *et al.* (2013) found that climate traits of more than 50 species in the family *Proteaceae* were the best predictor of the ratio of vessel wall thickness to vessel diameter (t/b), especially mean annual precipitation. Vein density was weakly related to t/b but unrelated to leaf area or climate. They also found that variation among species in t/b was driven by variability in both vessel wall thickness and vessel diameter, suggesting that these things could be actively controlled. Lens *et al.* (2004) found in a study of wood samples from 115 species in the subfamily *Vaccinoideae* that vessel diameter was negatively correlated with increasing latitude, while vessel density was positively correlated. They also found that some of the anatomical traits they examined were correlated with each other, such as tracheary length with vessel density and diameter, and vessel density with vessel diameter. A large amount of plasticity was found in the root xylem of the perennial herb *Potentilla diversifolia* Lehm. that occurred in response to available soil moisture. The anatomical traits in these studies is related to both broad, coarse climate variables and more specific temporal variables in both temperature and precipitation. Medek *et al* (2011) found that in two *Poa* species (*P. foliosa* Hook. f. and *P. hothamensis* Vickery.), *P. foliosa* Hook. f. had smaller conduit radii, consistent with being from a colder environment if smaller conduit size is related to freezing-induced cavitation. *P. foliosa* Hook. f. also exhibited greater hydraulic plasticity than *P. hothamensis* Vickery. when grown in a warmer environment, with conduits

growing in both size and density. In two populations of *Sesleria nitida* Scop. along an altitude gradient on Mount Terminillo, Gratani *et al.* (2014) reported a 31% increase in leaf thickness on trees growing at higher elevation. Variance in physiological traits of the two sites measured, such as transpiration and stomatal conductance, were found by two-way ANOVA to be driven primarily by monthly changes that keep carbon balance steady. Blonder *et al.* (2018) studied reticular venation traits for 136 dominant angiosperms along an elevation gradient in south-eastern Peru, and based on principal component analysis found three primary axes driving variation in venation architecture traits; reconnecting vs. branching veins, elongated vs. compact areoles and low vs. high-density veins. The first two axes were predicted by traits related to mechanical strength, while the last was predicted by site temperature. The authors postulated that vein reticulation was driven mostly by species interactions and less by climatic factors. Anatomy-climate relationships may exist within grasses, but significant differences in morphology and phenology prevents a broad assumption of similarity between woody plant species and herbaceous monocots.

### **Anatomical response to manipulations of temperature and precipitation**

Relationships between anatomy and climate variables can also be studied by exposing plants to manipulations of temperature and precipitation, then measuring changes to anatomical traits. A study in the southwestern United States by Hudson *et al.* (2017) focused on the anatomical and physiological adjustments of *Pinus edulis* Engelm. and *Juniperus monosperma* (Engelm.) Sarg. to either water addition or partial precipitation exclusion. This study found no significant changes to vascular traits in either treatment of either species, although several morphological and physiological

responses were reported. Arx *et al.* (2012) found that root xylem in this species shifted toward wider, more conductive elements (up to 54% more efficient) when water was supplemented, only in years in which water supplementation occurred. Additionally, aboveground growth parameters exhibited only insignificant or inconsistent responses to water supplementation. A study by Bacelar *et al.* (2006) found that water stress induced an increase in xylem vessel frequency in three cultivars of *Olea europaea* L., which had variable physiological responses to the water stress treatment. Heinrich and Banks (2006) subjected specimens of *Toona sinensis* M.Roem. and *Toona ciliata* M.Roem. to various treatments of temperature, nutrient levels and moisture. They found that in *T. ciliata* M.Roem., groups with low water treatments tended to have more numerous and smaller vessels than the same species in the 'optimum' treatment groups. This was not the case in *T. sinensis* M.Roem., which generally had smaller conduits across treatment groups. The authors postulated that this was because *T. sinensis* M.Roem. was from and was adapted to a more arid habitat. In a 24-month precipitation exclusion experiment conducted by Tng *et al.* (2018), the hydraulic-related wood anatomy and leaf traits of four tropical tree species were collected and compared against a control group. They showed a tendency for narrower or fewer vessel, reduced vessel groups and more heavily reinforced or occluded vessels. While the studies indicate that anatomy responds to climate-related manipulations, the degree and nature of response varies with phenology, functional group, and treatment type, and this trend will likely be true among grass species as well.

## Summary

We would expect to see relationships between plant anatomical traits and climate variables due to a mutual connection to physiology (book source 1 and 2), but interest in studying direct anatomy-climate relationships has only increased the past 20 years, with many studies now citing climate change as a primary justification for examining anatomy-climate interactions (Von Arx *et al.* 2012; Gratani *et al.* 2014; Pandey *et al.* 2018; Hudson *et al.* 2017). These relationships have been studied indirectly, through examination of how anatomy influences physiology and how anatomical traits might therefore respond to climate variables. Climate-anatomy relationships have also been observed by simple measurement of variation in anatomy across differing climate regimes, but focus has been primarily on woody plant species, and differences in both morphology and phenology in herbaceous monocots prevents broad assumptions of similar relationships. More direct manipulations of water availability and temperatures and the effects of these manipulations on anatomy have also been performed, but the revealed relationships between anatomy and climate have varied widely by functional group, morphology, phenology, and treatment type. This sort of variability is likely to also be found among herbaceous monocots. While previous research might inform what kinds of relationships between climate and anatomy we would expect to see in grasses, wide morphological and phenological differences makes it important to study anatomy-climate relationships among grass species specifically and to test these expectations, rather than make assumptions about these relationships based on past studies.

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# RELATIONSHIPS BETWEEN ANATOMY AND CLIMATE IN MEMBERS OF THE SUBFAMILY *POOIDEAE*

## INTRODUCTION

Grass-dominated ecosystems compromise approximately 40% of all land area on Earth, excluding Antarctica and Greenland. Grass species constitute some of the most economically important agricultural crops on the planet, accounting for 3.6 billion tons of annual seed harvest in sugar cane, maize, and rice alone (FAO 2017) and over one-fifth of the total calories consumed by the global human population (Smith 1998). While the importance of studying the physiological and anatomical characteristics of grasses is well-accepted, the bulk of research conducted to date has been focused on species with agricultural importance. Improving our understanding of climate-anatomy relationships in grasses is vital in predicting future population changes, but comparatively little research is being directed towards non-domestic grass species, and even less research has examined the anatomical characteristics of grass venation, their significance in individual plant function and their specific relationship with climatic distribution. Broadening our understanding of the characteristics and relationships of grass vein anatomy has several potential benefits in the fields of ecology, rangeland management and phylogeny.

The relationship between woody plant vascular anatomy and climate traits has been the subject of study for more than 50 years (Carlquist 1966). There is evidence of significant interaction between climate of origin and a species' general vascular

characteristics (Heinrich and Banks 2006; Bourne *et al.* 2017). The size of vessel elements increases with water availability, but the number of vessels decline (Verheyden *et al.* 2005; Bourne *et al.* 2017; Arx *et al.* 2012; Tng *et al.* 2018). Small-diameter vessels, which are also shorter, are thought to be more resistant to hydraulic failure (Hacke and Sperry 2006), although the direct link between embolism formation and vessel size is unclear (Schenk *et al.* 2015). Blackman *et al.* (2018) additionally links the ratio of conduit wall thickness over conduit diameter with P50, a metric of drought tolerance which estimates the xylem pressure that causes a 50% reduction in maximum hydraulic conductivity. A study by Jordan *et al.* (2013) indicated that the best predictor of the t/b ratio was climate, and particularly mean annual precipitation. That study also found that t/b was weakly related to vein density. Temperature has also been reported to have an impact on vascular anatomy; Pandey *et al.* (2018) found a positive correlation between mean vessel area in *Betula utilis* D. trees and summertime temperatures in the previous and current year. Blonder *et al.* (2018) also found a relationship to temperature in their analysis of reticulate leaf venation traits in 136 Peruvian trees, but they determined that those traits were more strongly predicted by species interactions than climate traits.

Understanding the relationship between climate and anatomy is important to increasing our ability to predict species- and population-level responses to changes in the environment. If we understand how grass species respond to certain climatic variables, we can better understand how their populations might grow or shrink on the landscape in response to environmental changes. Grasses have a unique morphology that makes it worthwhile to investigate their anatomical traits separately from those of

different plant families. Grasses possess parallel venation, and while this venation pattern is common among monocots, the implication on the plant hydraulic function are not well understood. In dicots, reticulate venation has been characterized as providing redundancy to the hydraulic network, and thus strengthening leaf resistance to embolism-induced loss of conductance (Sack *et al.* 2008). While carbon-cost efficiency in venation systems has been found to be highly selected-for in numerous woody species (Gleason *et al.* 2018), research into similar selective pressures on hydraulic efficiency for members of the *Poaceae* family is made difficult by the fact that grasses have parallel venation. Because the effects of parallel venation on hydraulic conductance and safety are not known, it is difficult evaluate the efficiency of the system, or even to compare the two types of venation architecture. Grasses also have a drastically different carbon storage and utilization strategy from other growth forms, focusing most carbon resources underground, in storage of nonstructural carbohydrates in the crown tissue at or just below the soil surface, as well as root production (Derner *et al.* 2006).

Anatomical responses to environmental changes may have important impacts for livestock management on the landscape, as plant population shifts might increase or decrease the overall palatability of plant life on the landscape. Additionally, if anatomical changes are linked to environmental conditions, environmental changes could result in changes to palatability in individual plants, such as through increased abundance of structural carbohydrate or changes to nitrogen storage and demands (Batistoti *et al.* 2011). Describing the interaction between leaf anatomy and climate variables in C3 grasses also might broaden our understanding of the evolution of the C4 photosynthetic

pathway in grasses. The development and variation of some C3 leaf anatomical traits are associated with the evolution of the C4 photosynthetic pathway in the *Poaceae* family (Griffiths *et al.* 2013; Christin *et al.* 2012). Establishing what relationship, if any, these traits have with climate variables may help us understand underlying climatic drivers towards C4 evolution in grasses, and it may also allow us to identify other anatomical traits in C3 genera of *Poaceae* that contributed to the evolution of a C4 pathway.

Our objective in this study was to expand our knowledge the anatomical, climate envelope, and physiological traits of non-domesticated grass species. To that end, anatomical traits were measured from greenhouse-grown species of grasses from the subfamily *Pooideae*. These data were also analyzed for relationships between anatomy and climate traits. Based on the previous body of research regarding the correlations between leaf anatomy and climate variables, we predicted that there would be strong relationships between leaf vasculature and climate variables, especially between precipitation and xylem characteristics.

## MATERIALS AND METHODS

### **Plant Material Selection and Germination**

Five species were selected from each of five genera: *Poa*, *Hesperostipa*, *Elymus*, *Festuca*, and *Bromus*. The species selected in this study are all phylogenetically classified as part of the *Pooideae* subfamily; this was done to ensure that all species had a similar evolutionary history. Species were selected to represent sampling of

temperature and precipitation gradients across North America. Plants in this study were grown from seeds provided by the Germplasm Resource Information Network (USDA). Specimens used for gas exchange measurements were grown in summer 2016 in large conetainers, while the remaining specimens were grown in 10-cm conetainers (Ray Leach Cells 3, Stuewe and Sons, Inc), with a 70%/30% mix of potting soil (Promix HP) and fritted clay (Greens' Grade Porous Ceramic Topdressing), respectively. This substrate was fertilized with Osmocote® Plus at a ratio of 10 mL fertilizer/L soil substrate; specimens received no further fertilization. The soil substrate was saturated with water before seeds were planted, and specimens were kept on a misting bench until the full expansion of their 4<sup>th</sup> leaf, then moved to an unmisted bench and watered 3 times per week.

### **Climate Envelope Analysis**

Species distribution data were collected from the Global Biodiversity Information Facility (GBIF) and correlated with climate data from weather stations closest to each specimen GBIF point. Gridded and interpolated climate data were retrieved from WorldClim at 0.1 degree grid points. For each reported occurrence of each species, climate data from the closet grid point to the occurrence were retrieved and added to the data set. If two reported occurrences were closest to the same grid point, that point was only used once in the data set to avoid the geographic bias associated with occurrence data (I need to get you a citation for this). Once this data set was generated for each species, the 5<sup>th</sup>, 50<sup>th</sup>, and 95<sup>th</sup> percentiles of each climate variable (see list of variables in supplement) were calculated and used to define the climate envelope of each species.

## **Anatomical Analysis**

Whole-leaf samples (base of lamina to tip of lamina) were collected from 5 random specimens of each species for the purpose of anatomical examination. The third or fourth leaves were harvested from each plant after full expansion. Samples were stored in a solution of formalin, acetic acid, ethanol, and deionized water (FAA fixative solution). Anatomical samples were collected halfway along the lamina length of each specimen, using a razor to cut a cross-sectional sample ~0.5 mm in thickness. Each cross-sectional sample was stained using safranin-o and fast green. Microscopic images of vein anatomy were taken of half the total lamina width using a ZEISS Axio Scope.A1 in conjunction with ZEN microscope software. Images were measured using both ZEN microscope software and Fiji open-source image analysis software. Total number of veins were counted, and the interveinal distance was measured in micrometers as the distance between the centers of one vein and each pair of neighboring veins. Each vein was classified as either major or minor. Major veins had at least two of the three following characteristics: 1) vein was at least 50% larger than the smallest vein, 2) vein had bundle sheath support structure, 3) vein had at least two xylary vessel elements 100% larger than remaining xylary vessel elements. The anatomical traits of up to five major veins per replicate were measured, and the traits of one minor vein was measured for each major vein measured. Vein density was calculated as number of veins number/leaf width (veins/ $\mu\text{m}$ ). Bundle sheath diameter ( $\mu\text{m}$ ) was measured in micrometers as the distance from the outer edge of the bundle



sheath to the opposite outer edge. Vein diameter ( $\mu\text{m}$ ) was measured from the innermost edge of the bundle sheath to the opposite inner edge. Bundle sheath cell size was estimated by calculating the difference between bundle sheath diameter and vein diameter. The diameters of vessel elements were measured as the distance in micrometers between the inner edge of the vessel element cell wall to the opposite inner edge. Vessel element wall thickness was measured as the distance in micrometers between one edge of the vessel element cell wall to the other.

### **Statistical Analysis**

Multiple regression analyses were conducted using the 'lm' function in R to investigate relationships between anatomical traits and climate (R Core Team, 2018). Multivariate analysis were conducted using the 'bestglm' package (A.I. McLeod and Changjiang Xu) in R to identify principle components of anatomical variables before regression analyses were conducted between the principle climate variables accounting for at least 40 percent of the variation in anatomical variables.

## **RESULTS**

### **Comparisons Among Genera**

Figure 1 shows that vein density (VD) was highest in *Hesperostipa*, lowest in *Bromus*, and intermediate for all other genera ( $p = <0.05$ ). For the diameter of the bundle sheath cells of major veins ( $D_{\text{MBSC}}$ ), *Bromus* had the largest diameter, *Hesperostipa* had the lowest diameter, and all other genera were of intermediate diameter (Fig. 1) ( $p = <0.05$ ). For the lumen diameter of major veins ( $D_{\text{Mlumen}}$ ), *Bromus*

had the largest lumen, *Poa* had the smallest lumen, and the other genera were intermediate (Fig 1) ( $p = < 0.05$ ). For the ratio of conduit wall thickness over conduit diameter in major veins ( $t/b_{maj}$ ), no genus was significantly different from any other ( $p = > 0.05$ ).

### **Anatomical Relationships**

Multiple anatomical traits scaled with vein density (VD). Lumen breadth of major veins ( $D_{Mlumen}$ ) decreased as vein density increased (Fig. 2,  $p = < 0.001$ ). The wall thickness in the lumen of major veins ( $t_M$ ) decreased as vein density increased (Fig. 2,  $p = < 0.01$ ). The ratio of major vein vessel wall thickness to lumen breadth ( $t/b_{maj}$ ) increased with vein density ( $p = < 0.05$ ).  $D_{MBSC}$  was correlated positively with increasing interveinal distance (IVD) ( $p = < 0.001$ ) (Fig 4). The ratio of bundle sheath diameter to mesophyll thickness in minor veins ( $\%_{mBSD:mimeso}$ ) was positively correlated to IVD ( $p = < 0.05$ ), however, species in the *Festuca* genus had a significant negative correlation with IVD ( $p = < 0.05$ ) (Fig 6), an inverse relationship from all other species in the study. When species in the genus *Festuca* were removed, the relationship between  $\%_{mBSD:mimeso}$  improved considerably (Fig. 5,  $p = < 0.001$ ).

### **Climate Relationships to Anatomical Variables**

Multiple regression analysis of anatomical variables to climate variables showed that a significant proportion of variability in VD was explained by three climatic variables; the 5<sup>th</sup> quintile of the minimum temperature of the coldest month ( $T_{mincoldm5th}$ ), the 95<sup>th</sup> quintile of the mean temperature of the warmest quarter ( $T_{meanwarmq95th}$ ), and the 5<sup>th</sup> quintile of the precipitation in the wettest quarter ( $P_{wetq5th}$ ) (Table 1). The variability in the diameter of bundle sheath cells of major veins ( $D_{MBSC}$ ) was explained by the 95<sup>th</sup> quintile

of the mean temperature during the driest quarter ( $T_{\max\text{warm}95\text{th}}$ ), the 5<sup>th</sup> quintile of precipitation seasonality ( $P_{\text{seasonality}5\text{th}}$ ), and the 50<sup>th</sup> quintile of the precipitation during the warmest quarter ( $P_{\text{warm}q50\text{th}}$ ) (Table 1). The variability in the ratio of vessel element thickness by vessel element diameter in major veins ( $t/b_{\text{maj}}$ ) was explained in significant proportion by the 5<sup>th</sup> quintile of the minimum temperature during the coldest month ( $T_{\text{mincold}m5\text{th}}$ ), the 5<sup>th</sup> quintile of the mean temperature during the warmest quarter ( $T_{\text{meanwarm}q5\text{th}}$ ), and the 50<sup>th</sup> quintile of the mean temperature during the driest quarter ( $T_{\text{dry}q50\text{th}}$ ). Mean annual precipitation (MAP) was also correlated with the ratio of major to minor veins (M:m) ( $r^2 = 0.31$ ,  $p = < 0.05$ ) (Figure 3).

Table 1. Table of multiple regression analysis results for vein density (VD), ratio of lumen wall thickness to lumen diameter in major veins ( $t/b_{\text{major}}$ ), and diameter of bundle sheath cells in major veins ( $D_{\text{MBSC}}$ ) against climatic distribution traits: 5<sup>th</sup> quintile of the minimum temperature of the coldest month ( $T_{\text{mincold}m5\text{th}}$ ), 95<sup>th</sup> quintile of the mean temperature of the warmest quarter ( $T_{\text{meanwarm}q95\text{th}}$ ), 5<sup>th</sup> quintile of the precipitation in the wettest quarter ( $P_{\text{wet}q5\text{th}}$ ), 5<sup>th</sup> quintile of the precipitation seasonality ( $P_{\text{seasonality}5\text{th}}$ ), 5<sup>th</sup> quintile of the mean temperature of the warmest quarter ( $T_{\text{meanwarm}q5\text{th}}$ ), 50<sup>th</sup> quintile of the mean temperature of the driest quarter ( $T_{\text{dry}q50\text{th}}$ ), 95<sup>th</sup> quintile of the mean temperature of the driest quarter ( $T_{\text{dry}q95\text{th}}$ ), 50<sup>th</sup> quintile of the precipitation of the warmest quarter ( $P_{\text{warm}q50\text{th}}$ ).

		Independent Variables (p-values)			$r^2$
<b>Dependent Variables</b>	<b>VD</b>	<b>T<sub>mincoldm5th</sub></b>	<b>T<sub>meanwarmq95th</sub></b>	<b>P<sub>wetq5th</sub></b>	
		0.012	0.030	0.003	0.412
	<b>t/b<sub>major</sub></b>	<b>T<sub>mincoldm5th</sub></b>	<b>T<sub>meanwarmq5th</sub></b>	<b>T<sub>dryq50th</sub></b>	
		0.001	0.007	0.013	0.482
	<b>D<sub>MBSC</sub></b>	<b>T<sub>dryq95th</sub></b>	<b>P<sub>seasonality5th</sub></b>	<b>P<sub>warmq50th</sub></b>	
		0.007	0.002	0.006	0.493

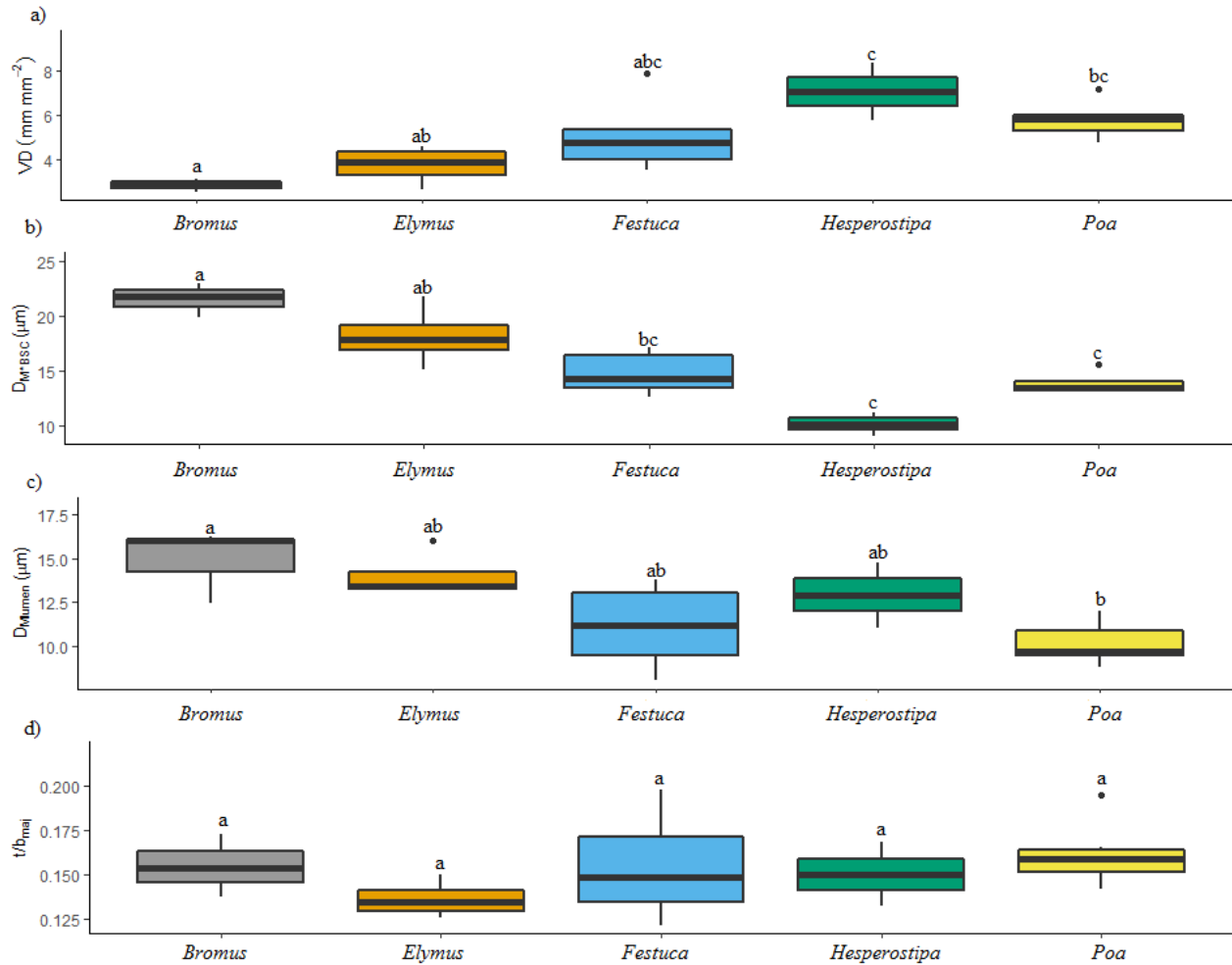


Figure 1. Box plots anatomical traits across 5 genera of grasses: a) vein density (VD), b) major vein bundle sheath cell diameter ( $D_{MBS}$ ), c) diameter of lumen in major veins ( $D_{lumen}$ ), and d) the ratio of vessel element thickness by vessel element diameter in major veins ( $t/b_{maj}$ ). Means with a letter in common are not different based on analysis of variance with Tukey's honest significant comparisons of means ( $p = <0.05$ ).

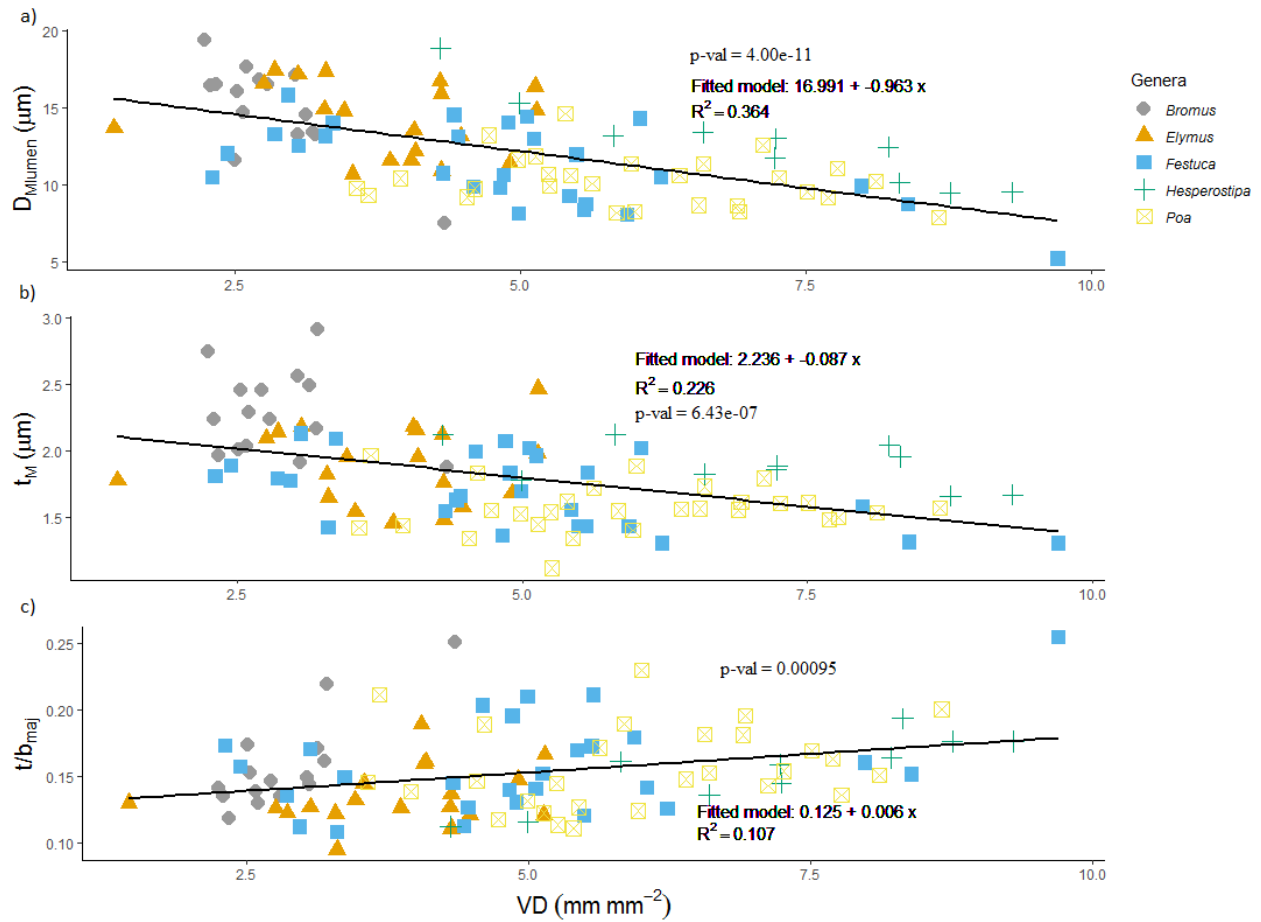


Figure 2. a) Plot of major vein vessel element diameter ( $D_{\text{Mlumen}}$ ) against vein density (VD). b) Plot of major vein vessel element wall thickness ( $t_{\text{M}}$ ) against vein density. c) Plot of major vein t/b ratio ( $t/b_{\text{maj}}$ ) against vein density.

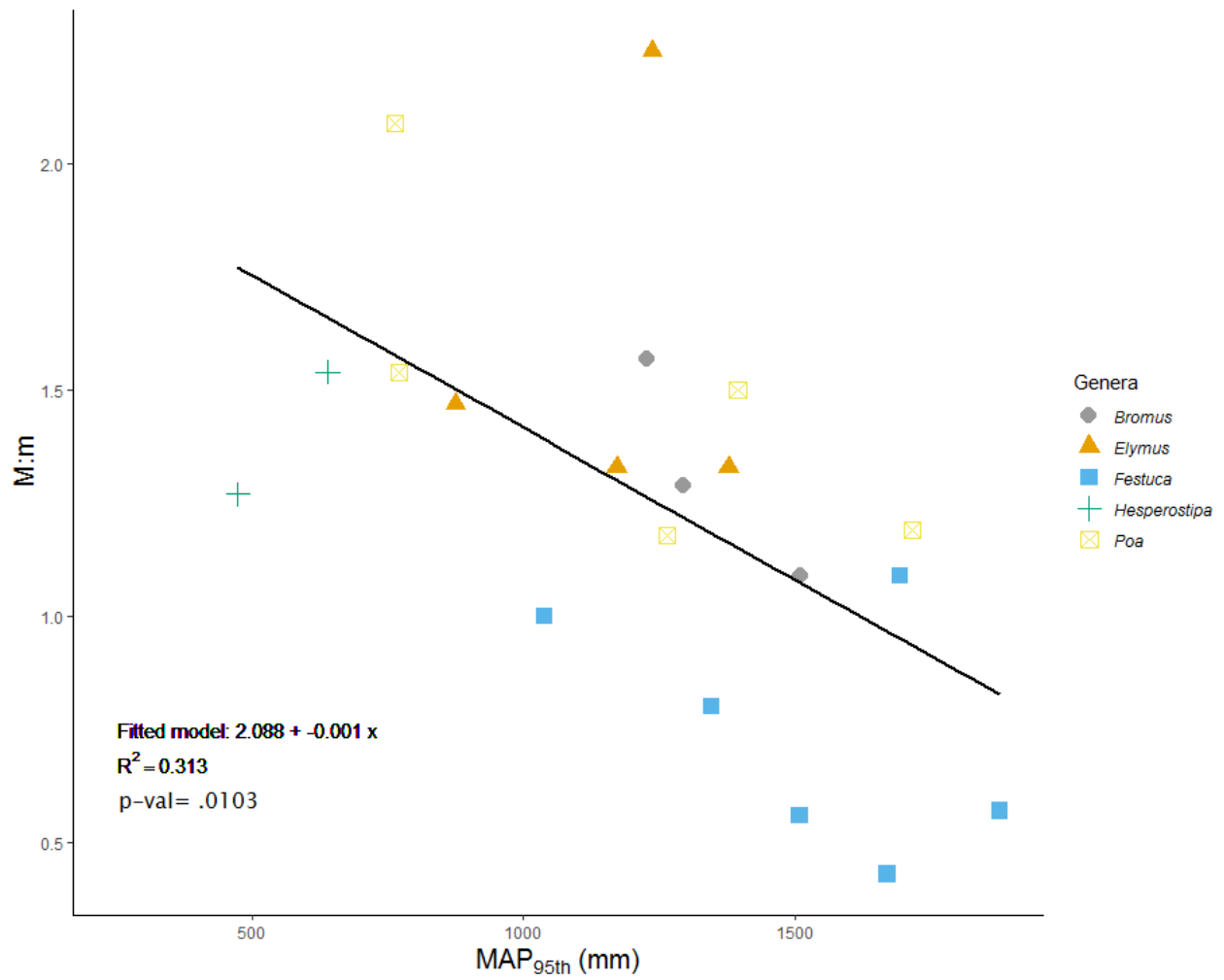


Figure 3. Plot of the relationship between mean annual temperature (MAP) and the ratio of major to minor veins (M:m).

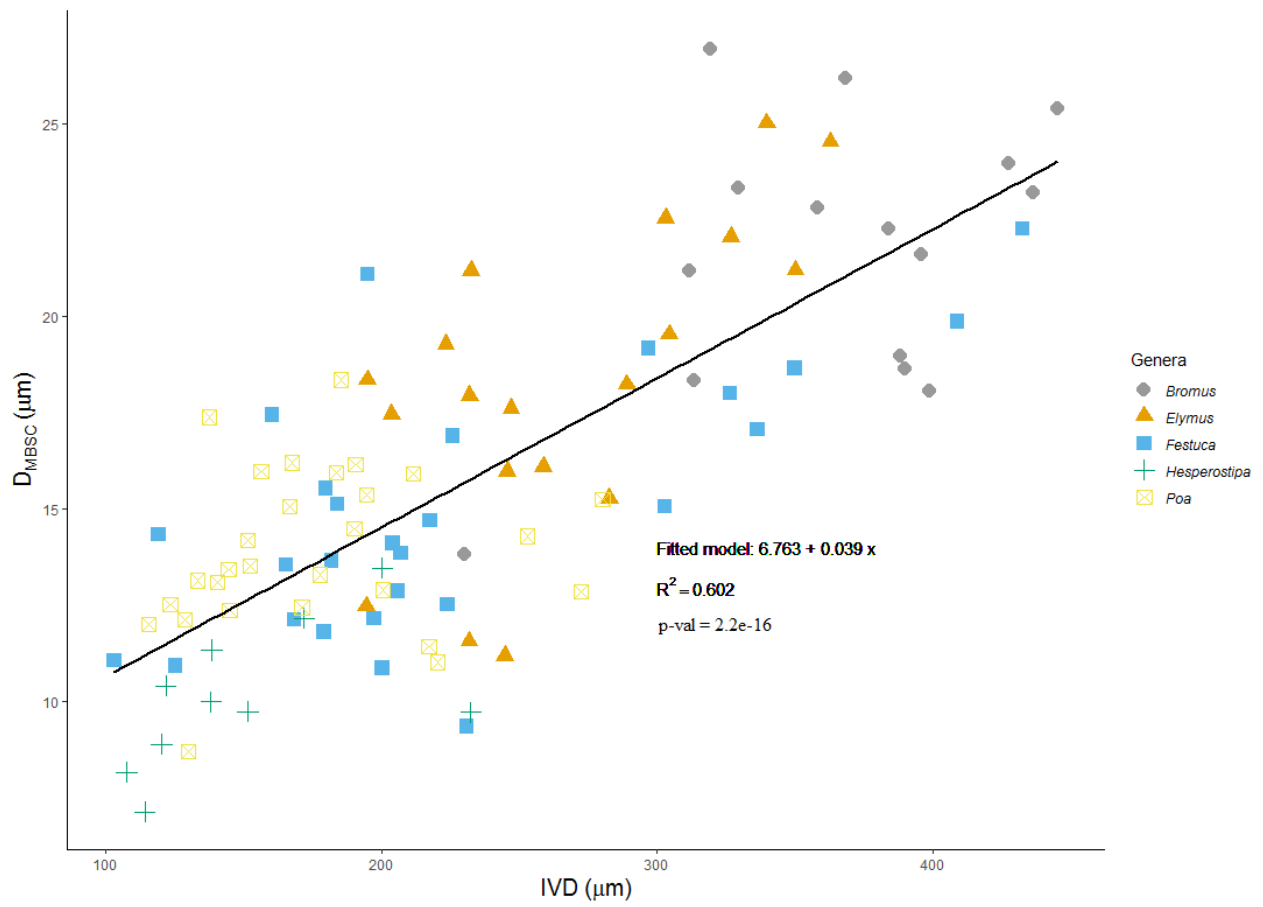


Figure 4. Plot of the relationship between interveinal distance IVD and diameter of major vein bundle sheath cells ( $D_{MBSC}$ ).

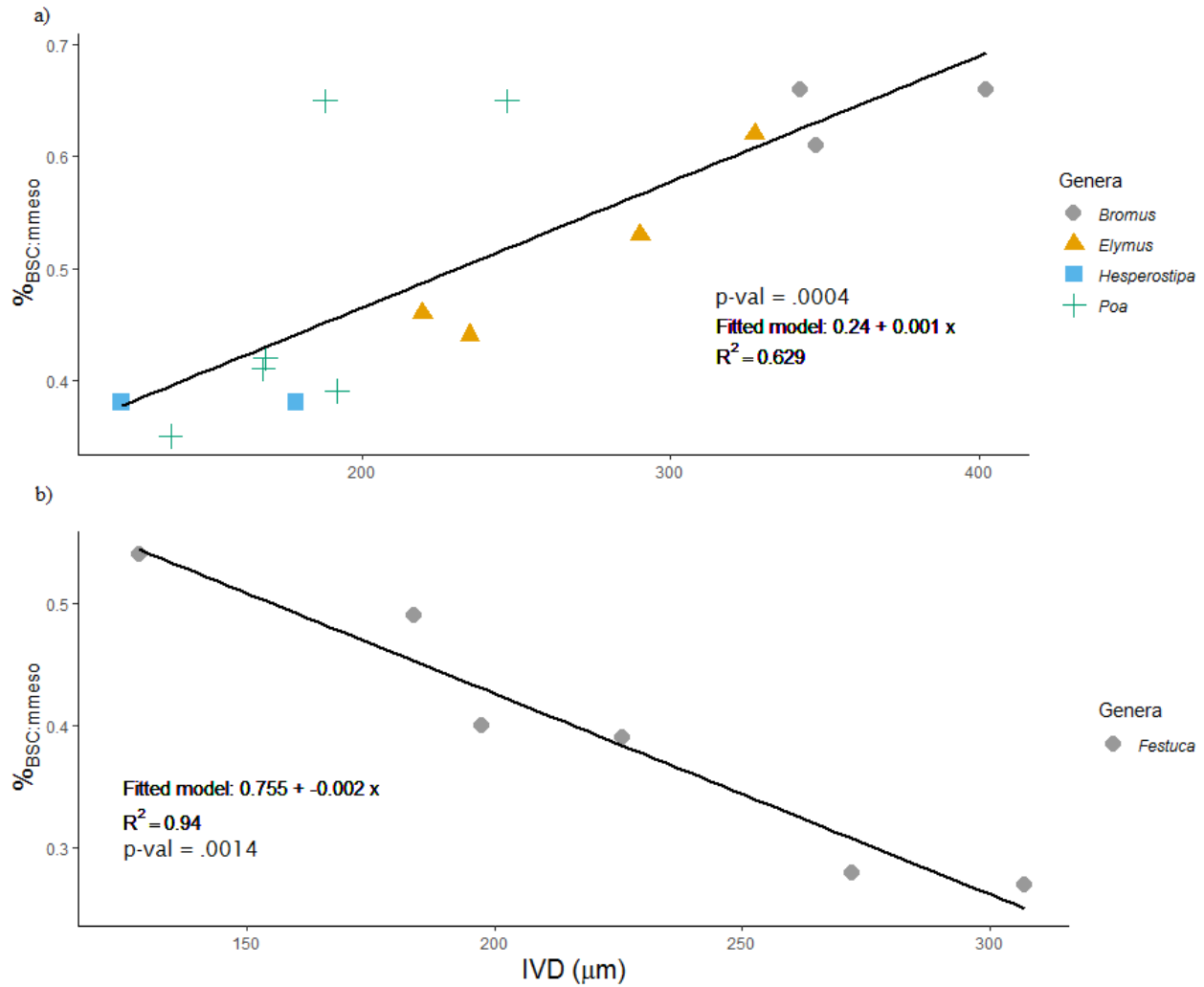


Figure 5. a) Relationship between ratio of bundle sheath to mesophyll in minor veins (%BSC:mmeso) and interveinal distance (IVD) with *Festuca* species removed. b) Relationship between ratio of bundle sheath to mesophyll in minor veins and IVD in *Festuca* species alone.



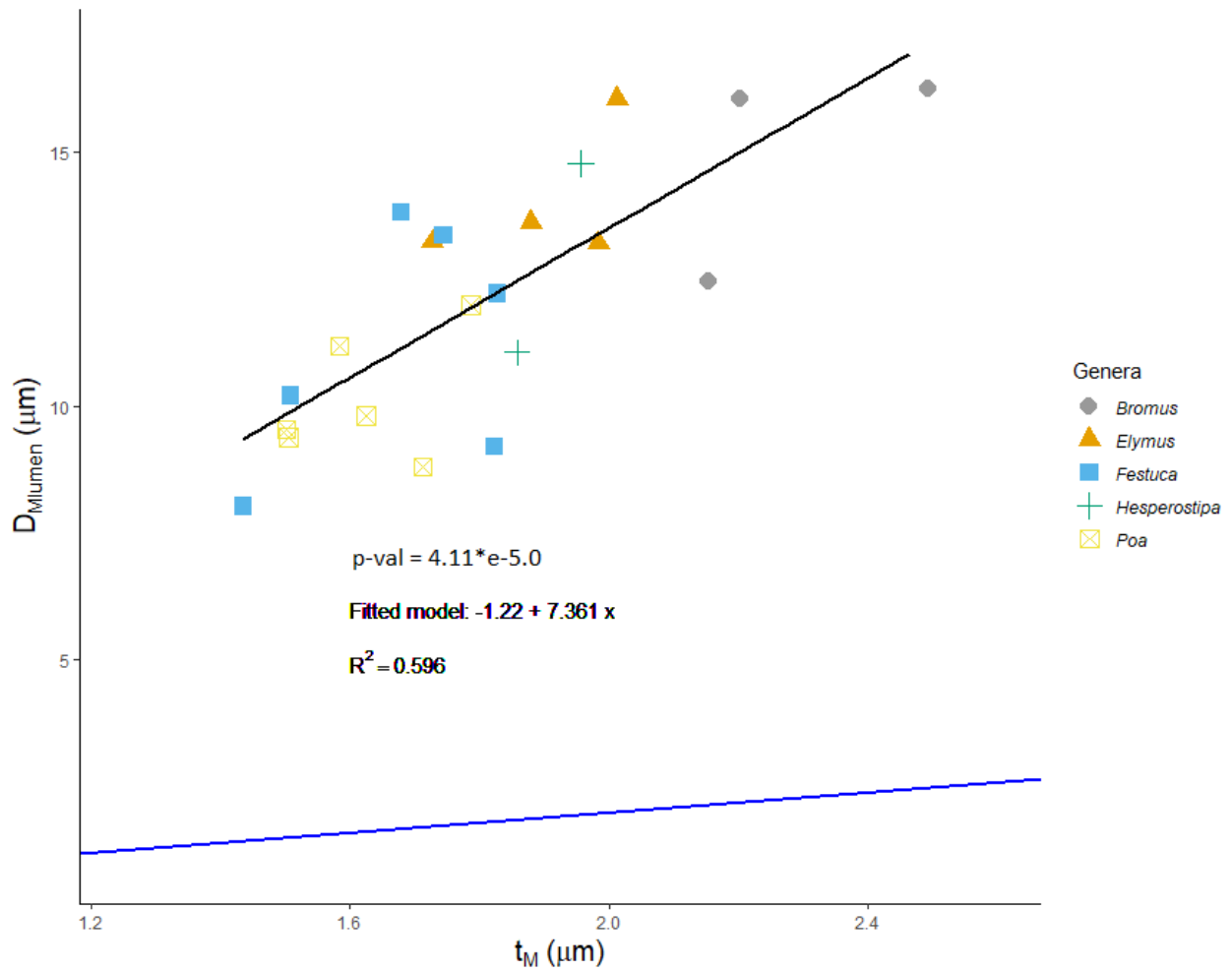


Figure 6. Relationship between the diameter of major vein vessels ( $D_{\text{Mlumen}}$ ) and the vessel wall thickness of major veins ( $t_M$ ). The solid black line indicates the trend of the relationship, while the solid blue line represents the trend if  $D_{\text{Mlumen}}$  and  $t_M$  were increasing at the same rate.

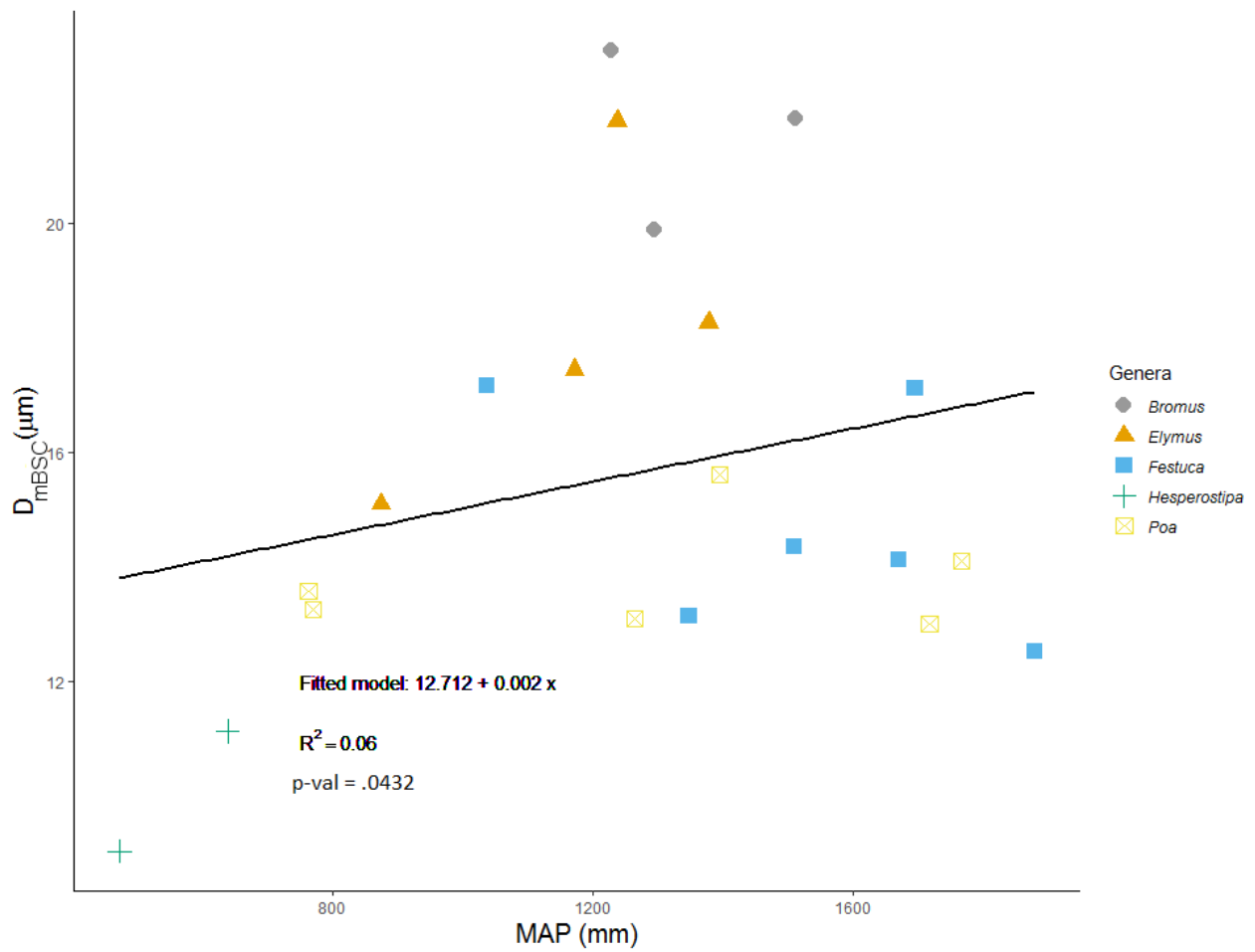


Figure 7. Relationship between the diameter of minor vein bundle sheath cells ( $D_{mBSC}$ ) and the 95<sup>th</sup> percentile of mean annual precipitation (MAP).

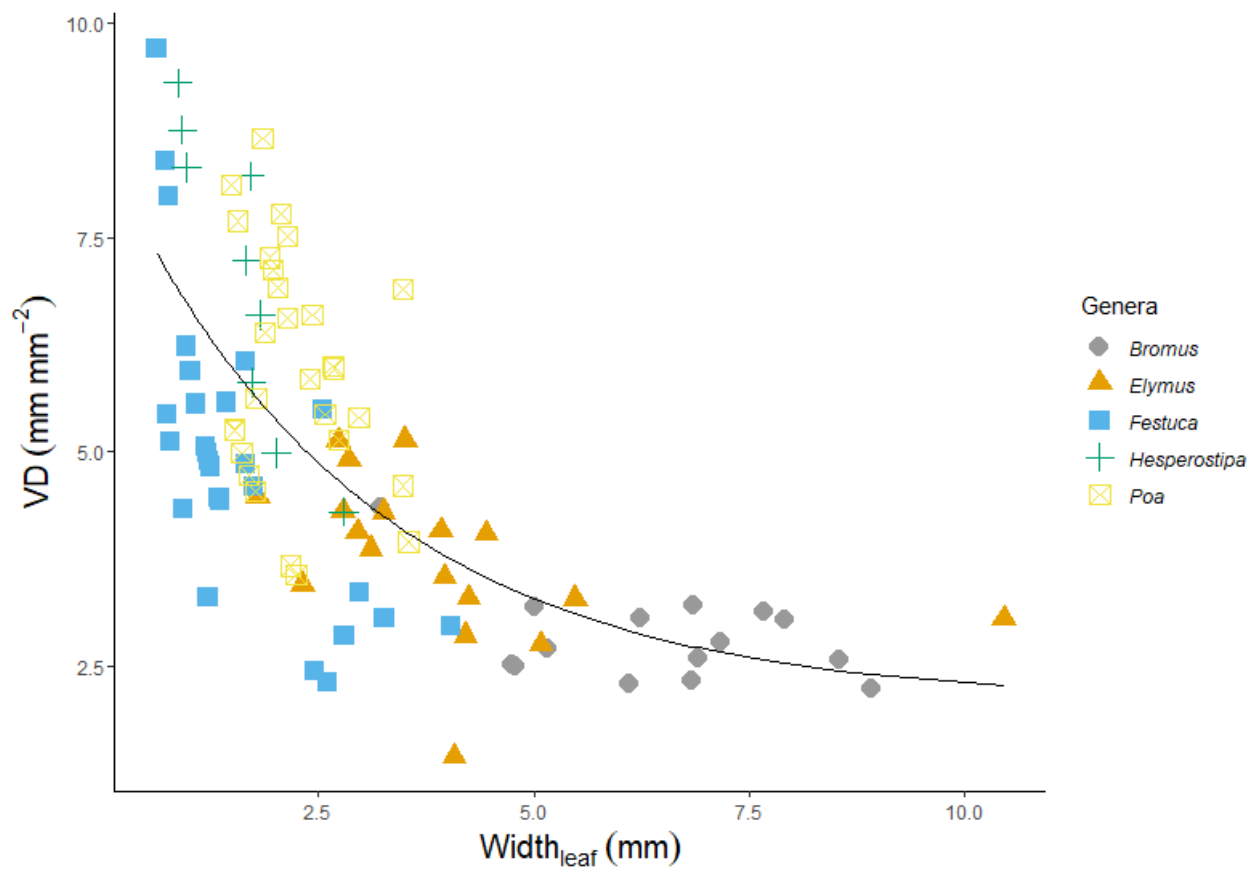


Figure 8. Relationship between vein density (VD) and leaf width (Width<sub>leaf</sub>).

## DISCUSSION

Anatomical characteristics of plant leaves can provide valuable insight into the function of plants (Ocheltree *et al.*, 2012; Sack and Holbrook, 2006), including their tolerance of abiotic conditions and resulting distribution on the landscape (Volaire *et al.*, 2018; Ivanova *et al.*, 2018). Studies among closely related C3 grass species are rare, but these species occupy regions that span vastly different climate patterns, from alpine environments to deserts. To begin understanding the anatomical constraints to C3 grass distributions, we investigated the relationship among anatomical characteristics of grass leaves within a single subfamily to investigate the coupling between plant structure and the climates plants can occupy. In general, we found strong scaling relationships between many of the anatomical variables we measured, suggesting coordination among different cell types in leaves. We only found a few correlations between anatomy and climate, however, suggesting some decoupling of anatomy from climate among the species we studied.

### **Scaling of Anatomical Vein Features**

One of the more striking findings of this study is the strong scaling relationships of vein anatomical traits with one another across wide climatic distribution of the species used in this study. Of note are the relationships these vein traits have with vein density (VD), illustrating that cells involved in vein architecture have adapted to become smaller as veins move closer together. For example, conduit diameter of major veins along with the thickness of conduit walls decreases as vein density increases (see Fig. 2). This suggests that, rather than these traits being regulated independently and thus capable

of being adjusted singly, the coordination of these anatomical traits provides some advantage or is a response to some constraint and is related in some way to vein density. Reduced vessel diameter could mean an overall decrease in per-vein conducting capacity, a relationship found in previous studies on woody plant species (Zweiniecki, Melcher and Holbrook 2001; Zaehle 2005) and supported by the Hagen-Poiseuille equation, which predicts that any reduction in conduit size also decreases the total conductive capacity of the conduit by a factor of four. Blackman *et al* (2010) found that an increasing  $t/b$  ratio (the ratio of the thickness of the vessel conduit wall over width of vessel conduits) led to smaller conduit diameters in woody angiosperms and thus reduced conductance capacity, but also found that this reduction in conduit size conferred a greater resistance to cavitation and xylem embolism, and also found no relationship between  $t/b$  and vein density, unlike our study (Fig. 2). A later study by Blackman *et al.* (2018) found that this increase in  $t/b$  was driven primarily by an increase in conduit wall thickness relative to conduit diameter, a pattern also observed in our data (Fig. 6). Blackman *et al.*'s study linked this conduit reinforcement to greater embolism resistance in the plants of that study.

It is possible that these scaling relationships in anatomical traits are a result of advantageous selection; Brodribb *et al.* (2013) found a negative correlation between xylem lumen diameter and vein density among tree species. They suggested that these relationships may be part of a system of linked anatomical traits that remain constant and allow plants a level of hydraulic security and baseline water transport around which other water transport traits may adapt. Scoffoni *et al.* (2011) described a similar correlation between vein density, available moisture, and xylem diameter, which they

associated with conferring increased protection against hydraulic failure. The idea that anatomy may operate as a sort of functional bedrock around hydraulic safety or efficiency has some supporting evidence. Gleason *et al.* (2018) examined scaling of conduit widths and conduit numbers in leaves of 36 tree and shrub dicots and found that they did not differ significantly from those predicted by Murray's law, which represents the most hydraulically efficient network configuration that will distribute resources to all distant points of a symmetric network, for a given expenditure of energy or material, like carbon. They concluded that for the dicots studied, conduit systems that maximized water transport per unit of material and energy had been strongly selected for.

### **Correlations Between Anatomy and Climatic Distribution Variables**

Previous studies on trees, have found relationships of varying strength between anatomical traits and climate traits, like precipitation and temperature. Larger diameter vessels and tracheids are generally found in plants growing in mesic climates (Rita *et al.* 2015, Castagneri *et al.* 2018) or regions with high summer temperatures (Pandey *et al.* 2018). While the reasons for these correlations are subject to speculation, all three of these studies concluded that the anatomical traits of trees were associated with climate variables. Multiple regression analyses (see Table 1) in our study found a relationship between vein density, coldest temperatures in the coldest month, the warmest three-month period, and precipitation in the warmest three-month period. Vessel element diameter of major veins was related to the highest temperatures during the warmest month, precipitation seasonality and precipitation of the driest month. The t/b ratio was related to the coldest temperatures in the coldest month, and the mean temperatures of the warmest three-month period and driest quarter. The diameter of major vein bundle

sheath cells was related to the mean temperature of the driest three-month quarter, precipitation seasonality, and precipitation of the warmest three-month period. These analyses indicate that relationships do exist between grass leaf anatomical traits and climate variables but suggests that they are responding to the interaction of multiple climate variables related to both precipitation and temperature, rather than just a single factor or broad average annual factors such as mean annual temperature and mean annual precipitation.

Single linear regression analyses revealed only a relationship between the 95<sup>th</sup> quintile of mean annual precipitation (MAP) and the ratio of major to minor veins (M:m) ( $p < 0.02$ ), as well as a weak ( $p < 0.05$ ,  $r^2 = 0.06$ ) relationship between bundle sheath cell thickness and mean annual precipitation. No other significant relationships ( $p < 0.05$ ) between anatomy traits and MAP or MAT were found. If this lack of strong relationships is valid, it means that the leaf anatomical traits in the species examined do not interact strongly with broad-scale annual climate variables. This suggests that xylem and whole-vein anatomy characteristics are unimportant to plant-level interactions with the climate, and do not contribute meaningfully to a species' ability to exist within a given precipitation or temperature regime. Leaf anatomical traits might instead have more significant relationships to other abiotic factors, such as soil type, soil nutrients, or light levels. There has been at least one study that established a strong correlation between leaf anatomical traits in grasses and soil type (Kuzmanovic *et al.* 2011).

The way we used the climate data might be responsible for the lack of correlations we observed; the data were mean temperature and precipitation over the course of the average year for that area, and for the hottest, coldest, wettest and driest

three-month periods, but the time of year for those periods was not available, and the climate data were pulled from weather stations based on the simple presence or absence of the species on the landscape. Because this methodology does not take into account species density or distribution on the landscape in a given climate envelope, it is impossible to say if that species is well-represented there or if it is dependent on microsite variations in precipitation or temperature, which would be obscured with simple consideration of broad, landscape-scale climate variables (Nippert *et al.* 2011). Additionally, intra-annual variation in precipitation has been shown to cause shifts in species composition on the landscape even while total yearly precipitation remains the same, but this intra-annual climate variation is obscured in broad, averaged climate data, and may result in a misrepresentation of a species preferred climate conditions (Knapp *et al.*, 2002; Li *et al.*, 2019; Mulhouse *et al.*, 2017). Phenotypic plasticity might also have a role, as grass species do not all share the same phenotypes for growth strategies, responses to environmental factors, or other phenological traits. Even perennial grass species that share the same photosynthetic pathway and genus, such as *Poa secunda* and *Poa bulbosa*, have significantly different phenological traits, and these traits respond differently to the same water availability, including time and timing of flowering events and aerial productivity potential, and this variation might be acting to obscure relationships between anatomical traits and the climate traits that were collected for this study (Balachowski, Bristiel and Volaire 2016). Multiple regression relationships between anatomical traits and suites of climate variables highlight the possibility that species-level phenological traits might be responding to the interaction of several climatic factors, instead of single specific variables. A relationship between



grass leaf morphological and physiological traits with time of season has been found in a previous study, but this study was conducted examining annual grass species (Ma *et al.* 2011). Other studies have been conducted on woody species regarding the anatomical response to climate-related variables such as varying soil moisture levels (Tng *et al.* 2018), and seasonal variation in physiological and morphological traits have been studied in wood and herbaceous plants (Miyashita *et al.* 2012; Reich *et al.* 1991; Volkova *et al.* 2011), but relationships between leaf anatomy and seasonal climate data have not been explored. All of these explanations contrast with the fact that numerous studies on woody plant species, both angiosperms and gymnosperms, have found relationships between anatomy and climate, whether these links are to conduit size, mesophyll area, stomatal density, or vein density (Rita *et al.*, 2015; Castagneri *et al.* 2018; Pandey *et al.* 2018; Carlson *et al.* 2015; Kivimaenpaa *et al.* 2014; Liang *et al.* 2019; Locosselli and Ceccantini 2012; Schollert *et al.* 2016).

Future studies of anatomy-climate relationships in grasses might address these potential problems through several methodological considerations. First, the use of multi-year averaged climate data for a landscape might not be suitable for the purposes of establishing whether anatomy-climate connections exist, because landscape-level variation in moisture and temperature is lost. Multi-year averaged climate data also obscures potentially important temporal climate variation, which has been demonstrated to have significant effects on herbaceous monocots (Knapp *et al.*, 2002; Li *et al.*, 2019; Mulhouse *et al.*, 2017). For capturing the climate conditions that are most relevant for a species, collecting daily or weekly climate data for a given year would be more relevant and useful, as any climate variation is captured, and data can be compared on a daily,

weekly, or monthly scale for the year. Lembrechts *et al.* (2019) provide several methods to obtain this finer scale, microclimate data that can more accurately reflect the actual conditions grass species experience in their climate ranges, such as employing remotely-sensed surface temperature data (especially relevant to the generally low-growing morphology of *Poaceae*). To improve the utility of finer-scale climate data, it is also important to collect more detailed data about phenotypic plasticity, and the plasticity of phenological traits more specifically. Previous research has demonstrated that variability in precipitation and temperature can influence the phenology of grassland species and impact species composition of the landscape (Cook *et al.*, 2012; Wolkovich *et al.*, 2013; Cebrino *et al.*, 2017; Cleland *et al.*, 2007). Therefore, it is important to collect phenological data for any herbaceous monocot as part of any research study investigating anatomy-climate relationships. Even phenological data as simple as time of leaf green-up and time of leaf senescence would improve our ability to directly associate leaf anatomy traits to the relevant climate variables. Because phenology is coupled with climate, these phenological data would have to be gathered from specimens that exist in the climate of interest, or high-resolution climate data must be available alongside detailed phenological data on the species in question. In addressing these methodological concerns, future research might establish conclusively the existence and/or significance of anatomy-climate links in members of the *Poaceae* family.

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## CONCLUSION

The results of this study indicate that many anatomical variables across a subfamily of species belonging to *Poaceae* closely correlate and scale with each other, but the significant relationships between anatomical traits and climate traits are a result of interactions between climate variables, rather than bivariate relationships. Some of the more interesting scaling relationships in anatomical traits were the negative correlation of conduit diameter and wall thickness with vein density, and that vein density acts as a coarse predictor of the size of anatomical traits, with higher vein density being associated with smaller anatomy. The coordination of these traits suggests that some advantage is being conferred, or some constraint is being addressed, by the relationship between xylem anatomy and vein density. Our results suggest that increasing t/b ratio was driven by increasing vessel diameter, unlike the results found in a study of a different plant functional group. It is possible that the species in this study have anatomical traits specifically designed for increased hydraulic efficiency or safety, as other research has indicated for other plant functional groups, but the differences in venation between grasses and other angiosperms make it difficult to draw conclusions about the impact of scaling relationships without additional evidence.

The ratio of major to minor veins and bundle sheath thickness had some correlation with precipitation variables, but overall this study did not unearth any major connections between anatomy and climate. It is possible, because of the climate traits we analyzed, that important resolution on microsite variations in precipitation and



temperature were lost, and averaged climate data obscures the importance of climatic variability, especially in precipitation. Additionally, phenologic plasticity was not taken into account, which could have been responsible for significant differences in plant responses, even if the plants were operating in the same conditions.

Previous research into the links between anatomy traits and climate variables have found connections of various strength, but variability in species studied, study type and treatment type, anatomical traits affected, phenology, morphology, and functional group has made it difficult to apply broad assumptions to climate-anatomy relationships across species, creating a need for more research to test our expectations of these relationships in different functional groups, such as herbaceous monocots. Our research has focused exclusively on this functional group, and identified the existence of some of these anatomy-climate relationships among grass species in the subfamily *Pooideae*. We have also reinforced the idea that these relationships are complex and require specific focus, especially on the resolution of climate data and the phenology of the species being studied, in order to make these relationships clear.

APPENDIX

Appended Table 1. Bivariate relationship table for microanatomical traits, indicating both correlation coefficients and p-val ranges (a = < .05, b = < .01, c = < .001).

	Width Mmesophyll	Width mmesophyll	D <sub>M</sub> *BSC/ Width Mmeso	D <sub>m</sub> *BSC/ Width mmeso	M:m	VD	Width leaf	VD M	IVD	D Mbundle
Width Mmesophyll	1									
Width mmesophyll	0.93 <sup>c</sup>	1								
D <sub>M</sub> *BSC/ Width Mmeso	-0.75 <sup>c</sup>	-0.66 <sup>b</sup>	1							
D <sub>m</sub> *BSC/ Width mmeso	-0.68 <sup>c</sup>	-0.69 <sup>c</sup>	0.91 <sup>c</sup>	1						
M:m	-0.05	-0.12	0.15	0.14	1					
VD	-0.27	-0.29	-0.32	-0.35	-0.16	1				
Width leaf	-0.01	-0.02	0.49 <sup>a</sup>	0.54 <sup>a</sup>	0.43	-0.76	1			
VD <sub>M</sub>	-0.30	-0.29	-0.17	-0.18	-0.6 <sup>b</sup>	0.83 <sup>c</sup>	-0.78 <sup>c</sup>	1		
IVD	0.20	0.24	0.43	0.45 <sup>a</sup>	0.17	-0.94 <sup>c</sup>	-0.94 <sup>c</sup>	-0.79 <sup>c</sup>	1	
D Mbundle	0.38	0.36	0.26	0.25	0.21	-0.88 <sup>c</sup>	0.77 <sup>c</sup>	-0.74 <sup>c</sup>	0.90 <sup>c</sup>	1
D M*BSC	0.11	0.15	0.48 <sup>a</sup>	0.50 <sup>a</sup>	0.28	-0.89 <sup>c</sup>	0.87 <sup>c</sup>	-0.74 <sup>c</sup>	0.92 <sup>c</sup>	0.88 <sup>c</sup>
D <sub>M</sub>	0.45 <sup>a</sup>	0.41	0.18	0.16	0.18	-0.84 <sup>c</sup>	0.71 <sup>c</sup>	-0.71 <sup>c</sup>	0.86 <sup>c</sup>	0.99 <sup>c</sup>
D <sub>M</sub> lumen	0.32	0.22	0.23	0.25	0.33	-0.74 <sup>c</sup>	0.71 <sup>c</sup>	-0.73 <sup>c</sup>	0.77 <sup>c</sup>	0.90 <sup>c</sup>
t <sub>M</sub>	0.12	0.05	0.36	0.35	0.33	-0.64 <sup>c</sup>	0.80 <sup>c</sup>	-0.66 <sup>b</sup>	0.73 <sup>c</sup>	0.79 <sup>c</sup>
D <sub>mbundle</sub>	0.32	0.39	0.30	0.35	0.088	-0.89 <sup>c</sup>	0.78 <sup>c</sup>	-0.69 <sup>c</sup>	0.93 <sup>c</sup>	0.89 <sup>c</sup>
D <sub>m</sub> *BSC	0.11	0.16	0.34	0.47 <sup>a</sup>	0.089	-0.77 <sup>c</sup>	0.70 <sup>c</sup>	-0.55 <sup>a</sup>	0.76 <sup>c</sup>	0.67 <sup>c</sup>
D <sub>m</sub>	0.38	0.44 <sup>a</sup>	0.26	0.28	0.082	-0.87 <sup>c</sup>	0.76 <sup>c</sup>	-0.69 <sup>c</sup>	0.92 <sup>c</sup>	0.91 <sup>c</sup>
D <sub>m</sub> lumen	0.40	0.49 <sup>a</sup>	0.15	0.10	-0.079	-0.68 <sup>c</sup>	0.53 <sup>a</sup>	-0.56 <sup>b</sup>	0.74 <sup>c</sup>	0.76 <sup>c</sup>
t <sub>m</sub>	0.19	0.26	0.23	0.22	0.23	-0.60 <sup>b</sup>	0.56 <sup>a</sup>	-0.59 <sup>b</sup>	0.57 <sup>b</sup>	0.63 <sup>b</sup>
t/b <sub>M</sub>	-0.38	-0.29	0.03	-0.02	-0.17	0.47 <sup>a</sup>	-0.25	0.46 <sup>a</sup>	-0.41	-0.56 <sup>b</sup>
t/b <sub>m</sub>	-0.40	-0.44 <sup>a</sup>	-0.13	-0.09	0.20	0.67 <sup>c</sup>	-0.43	0.51 <sup>a</sup>	-0.71 <sup>c</sup>	-0.73 <sup>c</sup>

Appended Table 1 cont'. Bivariate relationship table for microanatomical traits, indicating both correlation coefficients and p-val ranges (a = < .05, b = < .01, c = < .001).

	D M*BSC	D <sub>M</sub>	t <sub>M</sub>	D Mlumen	D mbundle	D m*BSC	D mvein	D mlumen	t <sub>m</sub>	t/b <sub>M</sub>
D M*BSC	1									
D <sub>M</sub>	0.81 <sup>c</sup>	1								
D Mlumen	0.71 <sup>c</sup>	0.91 <sup>c</sup>	1							
t <sub>maj</sub>	0.68 <sup>c</sup>	0.79 <sup>c</sup>	0.77 <sup>c</sup>	1						
D mbundle	0.91 <sup>c</sup>	0.85 <sup>c</sup>	0.73 <sup>c</sup>	0.67 <sup>c</sup>	1					
D m*BSC	0.86 <sup>c</sup>	0.59 <sup>b</sup>	0.45 <sup>a</sup>	0.41	0.86 <sup>c</sup>	1				
D <sub>m</sub>	0.86 <sup>c</sup>	0.88 <sup>c</sup>	0.78 <sup>c</sup>	0.72 <sup>c</sup>	0.98 <sup>c</sup>	0.74 <sup>c</sup>	1			
D mlumen	0.60 <sup>b</sup>	0.77 <sup>c</sup>	0.72 <sup>c</sup>	0.56 <sup>b</sup>	0.78 <sup>c</sup>	0.48 <sup>a</sup>	0.83 <sup>c</sup>	1		
t <sub>min</sub>	0.58 <sup>b</sup>	0.62 <sup>b</sup>	0.62 <sup>b</sup>	0.65 <sup>b</sup>	0.69 <sup>c</sup>	0.49 <sup>a</sup>	0.71 <sup>c</sup>	0.72 <sup>c</sup>	1	
t/b <sub>maj</sub>	-0.38	-0.59 <sup>b</sup>	-0.73 <sup>c</sup>	-0.14	-0.42	-0.27	-0.44 <sup>a</sup>	-0.53 <sup>a</sup>	-0.25	1
t/b <sub>min</sub>	-0.53 <sup>a</sup>	-0.75 <sup>c</sup>	-0.72 <sup>c</sup>	-0.50 <sup>a</sup>	-0.70 <sup>c</sup>	-0.40	-0.76 <sup>c</sup>	-0.94 <sup>c</sup>	-0.52 <sup>a</sup>	0.62 <sup>b</sup>

#### Appendix I

Variables collected from weather stations were mean diurnal temperatures, isothermality, temperature seasonality, the maximum temperature for the warmest month, the minimum temperature for the coldest month, the annual temperature range, the mean temperature for the wettest quarter, the mean temperature for the driest quarter, the mean temperature for the warmest quarter, the mean temperature for the coldest quarter, annual precipitation, precipitation for the driest and wettest months, precipitation seasonality, precipitation for the wettest and driest quarter, and precipitation for the coldest and warmest quarters.