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Arboreal review

At least it is a dry cold: the global distribution of freeze–thaw and drought stress and the traits that may impart poly-tolerance in conifers

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Conifers inhabit some of the most challenging landscapes where multiple abiotic stressors (e.g., aridity, freezing temperatures) often co-occur. Physiological tolerance to multiple stressors (‘poly-tolerance’) is thought to be rare because exposure to one stress generally limits responses to another through functional trade-offs. However, the capacity to exhibit poly-tolerance may be greater when combined abiotic stressors have similar physiological impacts, such as the disruption of hydraulic function imposed by drought or freezing. Here, we reviewed empirical data in light of theoretical expectations for conifer adaptations to drought and freeze–thaw cycles with particular attention to hydraulic traits of the stem and leaf. Additionally, we examined the commonality and spatial distribution of poly-stress along indices of these combined stressors. We found that locations with the highest values of our poly-stress index (PSi) are characterized by moderate drought and moderate freeze–thaw, and most of the global conifer distribution occupies areas of moderate poly-stress. Among traits examined, we found diverse responses to the stressors. Turgor loss point did not correlate with freeze–thaw or drought stress individually, but did with the PSi, albeit inverse to what was hypothesized. Leaf mass per area was more strongly linked with drought stress than the poly-stress and not at all with freeze–thaw stress. In stems, the water potential causing 50% loss of hydraulic conductivity became more negative with increasing drought stress and poly-stress but did not correlate with freeze–thaw stress. For these traits, we identified a striking lack of coverage for substantial portions of species ranges, particularly at the upper boundaries of their respective PSis, demonstrating a critical gap in our understanding of trait prevalence and plasticity along these stress gradients. Future research should investigate traits that confer tolerance to both freeze–thaw and drought stress in a wide range of species across broad geographic scales.

Keywords: abiotic stress, conifers, distributions, drought, freeze-thaw, poly-tolerance.

Introduction

Globally, coniferous forests experience harsh and stressful environmental conditions throughout most of the year. Plants in these ecosystems may be exposed to a range of weather extremes, including freezing temperatures in the winter, high air temperatures in the summer and low water availability seasonally. Transitions between frozen and thawed states (i.e., ‘freeze–thaw’ cycles) and/or periods of drought can impair

physiological functioning in a variety of ways, including photosynthetic decline, hydraulic dysfunction and mortality (Huner et al. 1998, 2003, Allen and Ort 2001, Ivanov et al. 2001, Pittermann and Sperry 2003, 2006, Mayr et al. 2006, Choat et al. 2018, Blackman et al. 2019). Despite recent progress in characterizing stress mitigation and tolerance mechanisms to single stressors (e.g., drought, Choat et al. 2018; freeze–thaw, Guy 2003, Zanne et al. 2014), we currently lack a

comprehensive understanding of the mechanisms by which evergreen conifers mitigate multiple (but not necessarily concurring) physiological stresses (i.e., 'poly-stress'). In the context of a changing climate, it is particularly important that we understand how plants tolerate stressors that currently exhibit strong selective pressure, such as drought and freeze–thaw cycles, in order to improve our predictions of how forest compositions may shift in the future.

Both drought and freeze–thaw cycles are expected to become more frequent in many coniferous forest regions due to rising air temperatures and shifting precipitation regimes associated with global climate change (Henry 2008, IPCC 2019). Global warming, which is greatest at higher latitudes, can lead to mid-winter warming events (Williams et al. 2015), increase the occurrence of rain relative to snow (Knowles et al. 2006), decrease total snow accumulation (Mote et al. 2005) and result in earlier spring snowmelt (Clow 2010). Increased variability in winter temperatures and reduced insulation from temperature fluctuations associated with lower snow cover can increase the frequency of freezing and thawing experienced by plants (Henry 2008). These conditions can also have cascading consequences throughout the growing season as reduced snow cover and, consequently, diminished and less prolonged snowmelt limit available water for plants throughout the growing season (Hu et al. 2010, Williams et al. 2015). When combined with the elevated summer air temperatures and reduced summer precipitation predicted for some of these regions, many trees will likely experience population declines associated with these combined stresses (Mayr et al. 2006, Earles et al. 2018).

Freeze–thaw cycles and drought impose similar impacts on leaves and stems. In leaves, both freezing temperatures and drought can hinder photosynthetic capacity and lead to excess absorbed light energy that causes photoinhibitory damage and leaf death (Demmig-Adams and Adams 2006). In stems, both freeze–thaw cycles and drought disrupt the hydraulic network by inducing or propagating embolisms throughout the xylem (Tyree and Sperry 1989). Although the mechanism of formation differs, embolisms caused by both freeze–thaw events and drought reduce whole plant hydraulic conductance, which can limit plant functioning and growth (Kreyling 2010, Anderegg et al. 2016). Given that these stresses create similar dysfunction in plants, as well as the observations that evergreen conifers often experience freeze–thaw cycles and drought simultaneously in some habitats (Mayr et al. 2006) and that these stresses are likely to have compounding effects (Charrier et al. 2021), the survival and distribution of these taxa likely depends on a tolerance to both stressors (i.e., 'poly-tolerance'). However, some studies have noted a distinct trade-off between drought and cold/freezing tolerance in woody taxa mediated by reduced wood density in frost-tolerant species (e.g., Laanisto and Niinemets 2015, Rueda et al. 2017), suggesting divergent trait coordination that promotes either freeze–thaw or drought tolerance across large geographic scales. Though some

studies have systematically examined organ-level traits that could promote tolerance to multiple abiotic stressors across a wide range of species (e.g., Hallik et al. 2009, Stahl et al. 2013, Rueda et al. 2017), no study has quantified the degree of geographic overlap between freeze–thaw and drought stress to identify key areas where poly-tolerance to these stressors would be most adaptive. Additionally, we do not yet have a comprehensive understanding of what traits confer both freeze–thaw and drought tolerance, how widespread the coordination of these traits is across species or how these traits might influence the survival and distribution of conifers worldwide.

In this review we aim to (i) examine indices of the worldwide spatial distribution of drought, freeze–thaw cycles (hereafter, 'FT cycles') and their combination, and (ii) identify traits that may impart tolerance to drought and FT cycles, and explore to what extent these traits overlap in evergreen conifers. In particular, we focused this review on the physiological, morphological and anatomical traits of conifer leaves and stems. Although roots are also susceptible to freezing and drought stresses, comparatively little research has investigated the topic (but see Ambrose et al. 2020 for a review on frost resistance in crop roots). This review is not intended to comprehensively examine all aspects of winter or drought ecophysiology (but see Chang et al. 2021, Choat et al. 2018, McDowell et al. 2008, Sakai and Larcher 2012). Other recent reviews have covered topics such as extreme winter weather events (Casson et al. 2019); winter climate change impacts on plant species composition, ranges and phenology (Kreyling 2010); and the impacts of severe drought on global forest mortality (Allen et al. 2010, Anderegg et al. 2013, Clark et al. 2016). By identifying the global occurrence of FT and drought, as well as relevant traits that may enhance tolerance to these combined stressors, we aim to identify promising research avenues that will enhance predictions of conifer function, abundance and distributions in a changing climate.

Assessing the worldwide prevalence of drought stress, FT stress and their combination

Drought and freezing temperatures profoundly influence the global distribution of plant groups (Stuart et al. 2007, Engelbrecht et al. 2007, Normand et al. 2009, Stahl et al. 2014). The distribution and trait evolution of evergreen woody angiosperms can be sharply defined by exposure to freezing temperatures (Zanne et al. 2018), while maximum temperature and precipitation are more strongly associated with tolerance traits and distributions in conifers (Rueda et al. 2017). This suggests a role for overlapping stressors as a primary driver of conifer occurrence as opposed to temperature or precipitation singly. Furthermore, the impacts of minimum annual temperatures on conifer traits and distributions are likely small compared with the impacts of the FT cycles that occur in cooler climates. Freezing temperatures alone can reduce photosynthetic capacity

(Huner et al. 1998, 2003, Ivanov et al. 2001) and increase the hydraulic resistance in soil (Tranquillini 1976), while FT cycles can cause severe hydraulic dysfunction in xylem via embolisms (Hammel 1967, Sucoff 1969). Cold hardiness, studied extensively in conifers, often varies by genotype and is correlated with local minimum temperatures (e.g., Sebastian-Azcona et al. 2018), although foliage is commonly hardened to temperatures far beyond those minimums (Sakai 1960, Strimbeck et al. 2007, Wisniewski et al. 2018). However, there is no broad-scale relationship between minimum temperature and FT events (Figure S1 available as Supplementary data at *Tree Physiology* Online). As such, common ranking systems for cold tolerance or cold hardiness do not show any clear relationship with FT events (Figure 1 available as Supplementary data at *Tree Physiology* Online), thus providing an opportunity to investigate the spatial distribution and impact of FT cycles on plant function and ecology.

To visualize the global patterns of drought, FT cycles and their combination, we used available global climate data to develop spatial indices of drought stress (Di, based on soil water content and vapor pressure deficit (VPD); comparison with other water availability metrics shown in Figure S2 available as Supplementary data at *Tree Physiology* Online), FT stress (FTi, based on daily maximum and minimum temperatures) and poly-stress index (PSi, based on the product of the individual stresses; see Supplemental Materials for detailed methods). Our PSi shows bimodal latitudinal peaks at ca. 34.8°S and 32.8°N (Figure 1, and Figure S3 available as Supplementary data at *Tree Physiology* Online), and the distribution of conifers overlaps considerably with areas of land that experience moderate levels of FT and drought stresses (i.e., index values of 30–40; Figure 2). Regions of moderate poly-stress that have high conifer abundance and diversity include the western USA, southern Argentina and Chile, northern Morocco, the Iberian Peninsula and the northern extents of central Asia (Sundaram et al. 2019). Locations that have more extreme values of the PSi (i.e., high index values), including Iran and southeastern Australia, are rare globally (Figure S4 available as Supplementary data at *Tree Physiology* Online) and are dominated by angiosperms, which are cold deciduous in Iran but evergreen in Australia. The PSi does not indicate high levels of poly-stress in the majority of the vast boreal forests of the northern hemisphere (Figure 1C), which are instead characterized by a high number of FT days but low drought stress (Figure 1A and B). It is worth noting, though, that some regions of the boreal forest do experience some drought stress, particularly in British Columbia, Canada, which has recently experienced severe heatwaves and droughts that have exacerbated widespread wildfires. For the majority of the boreal forests that experience little water stress, the main climatic driver of this biome type is likely associated with the stresses of repeated freezing and thawing, along with the extreme cold. At extreme degrees of drought, conifers are generally replaced by plants (often angiosperms)

that exhibit succulence and CAM photosynthesis, or the rapid growth of desert annuals (however, see Larter et al. 2015, 2017), while at extreme FT stress, conifers are replaced by deciduous angiosperms. In locations with relatively extreme poly-stress, the vegetation type tends to be desert and xeric shrubland without conifers (Figure 2L).

Importantly, the data used to quantify the distributions of drought, FT and poly-stress (e.g., Figure 1) are from the recent past (see Supplemental Materials for details). As the global climate changes, shifting precipitation patterns will reduce soil moisture availability in some areas (Dai et al. 2018) and higher temperatures will increase the VPD broadly (Yuan et al. 2019). Furthermore, variations in the extent and location of polar vortices, along with milder winter temperatures in the arctic, could increase the number of FT cycles in regions of the northern hemisphere (J. Zhang et al. 2016, Matsumura et al. 2021). Thus, the spatial area experiencing multiple stresses should increase in the future, as should the intensities of poly-stress.

Traits and stress tolerance

The apparent biome shifts away from conifers at the extreme abiotic stress levels in Figure 2 likely represent the physiological limits of conifers. However, which specific traits limit individual species within this broader distribution remains unclear. Figure 3 shows the occurrence records of multiple species from five genera along the axes of the FT and drought stress indices, illustrating that these taxa vary considerably in their distribution within our poly-stress space. Some species are absent in areas of drought stress but are present in areas of high FT stress (e.g., *Podocarpus nubigenus*, Figure 3W), while others show the reciprocal pattern (e.g., *Pinus herrerae*, Figure 3R), suggesting a trade-off. Indeed, we found a significant negative relationship between species' median positions on the drought and FT stress index ($R^2 = 0.19$, $P < 0.001$; data not shown). However, some species occur in areas subject to both stressors (e.g., *Pinus halepensis*, Figure 3Q).

In the next sections, we discuss specific traits that could impart tolerance to drought, FT cycles and their combination in conifer leaves and stems, thus influencing their geographical distribution and position on the Di, FTi and PSi. Comparative studies on interspecific hydraulic traits have shown significant relationships between these traits and either drought or FT cycles (Maherali et al. 2004, Pittermann and Sperry 2006, Bartlett et al. 2012, Choat et al. 2012, Larter et al. 2017, Rosas et al. 2019, Sanchez-Martinez et al. 2020, Rosas et al. 2021), which may provide insight in to mechanisms facilitating the tolerance of particular conifer species to both stresses. We test how these traits relate to drought and FT stresses using data compiled from databases and our own literature search (see Supplemental Materials). Relationships were analyzed using linear regressions. We checked the appropriateness of a linear model with a Shapiro normality test. All models that included leaf

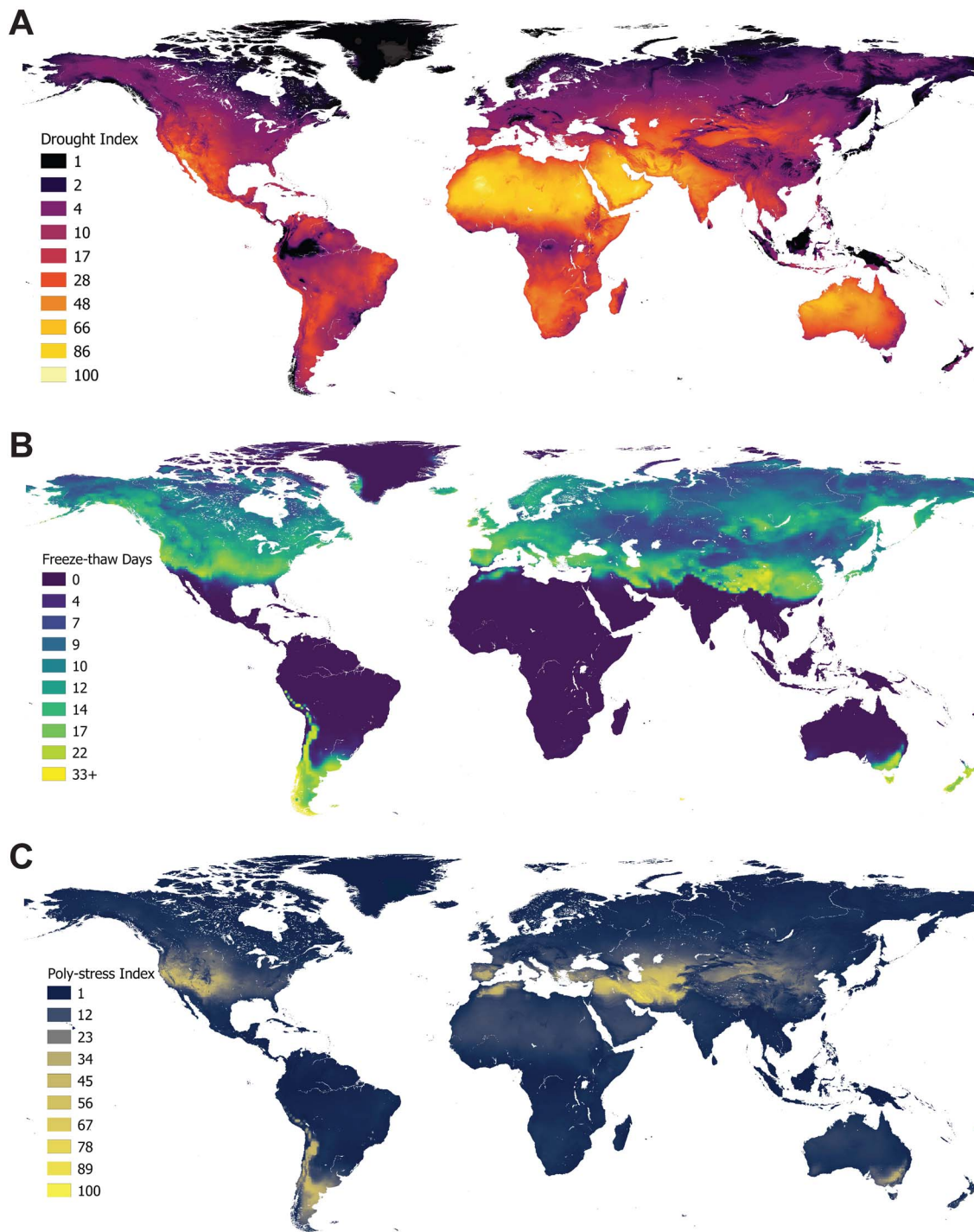


Figure 1. Spatial distribution of (A) annual average Di, (B) number of freeze–thaw days and (C) a PSI based on their spatial overlap. Indices (A, C) range from 1 to 100 in order of increasing stress. Freeze–thaw days are shown in (B) for interpretability of common units (days), see Supplemental Methods for details about the conversion to the freeze–thaw index (FTi) used in other figures. Details of both indices (A, C) are described in the methods. Color thresholds were determined to maximize contrast.

mass per area (LMA) failed to meet the proper assumptions and data were log-transformed.

Traits that impart tolerance in leaves

Among the most well-established characteristics associated with tolerating drought stress are those that maintain turgor pressure

in living cells. Positive turgor is crucial for proper metabolic functioning within cells. Turgor can be maintained under water deficits by lowering the turgor loss point (TLP), which can occur in multiple ways. First, the TLP can be lowered through a reduction in the cell's osmotic potential, which limits water loss. Lower TLPs associated with reductions in osmotic potentials have

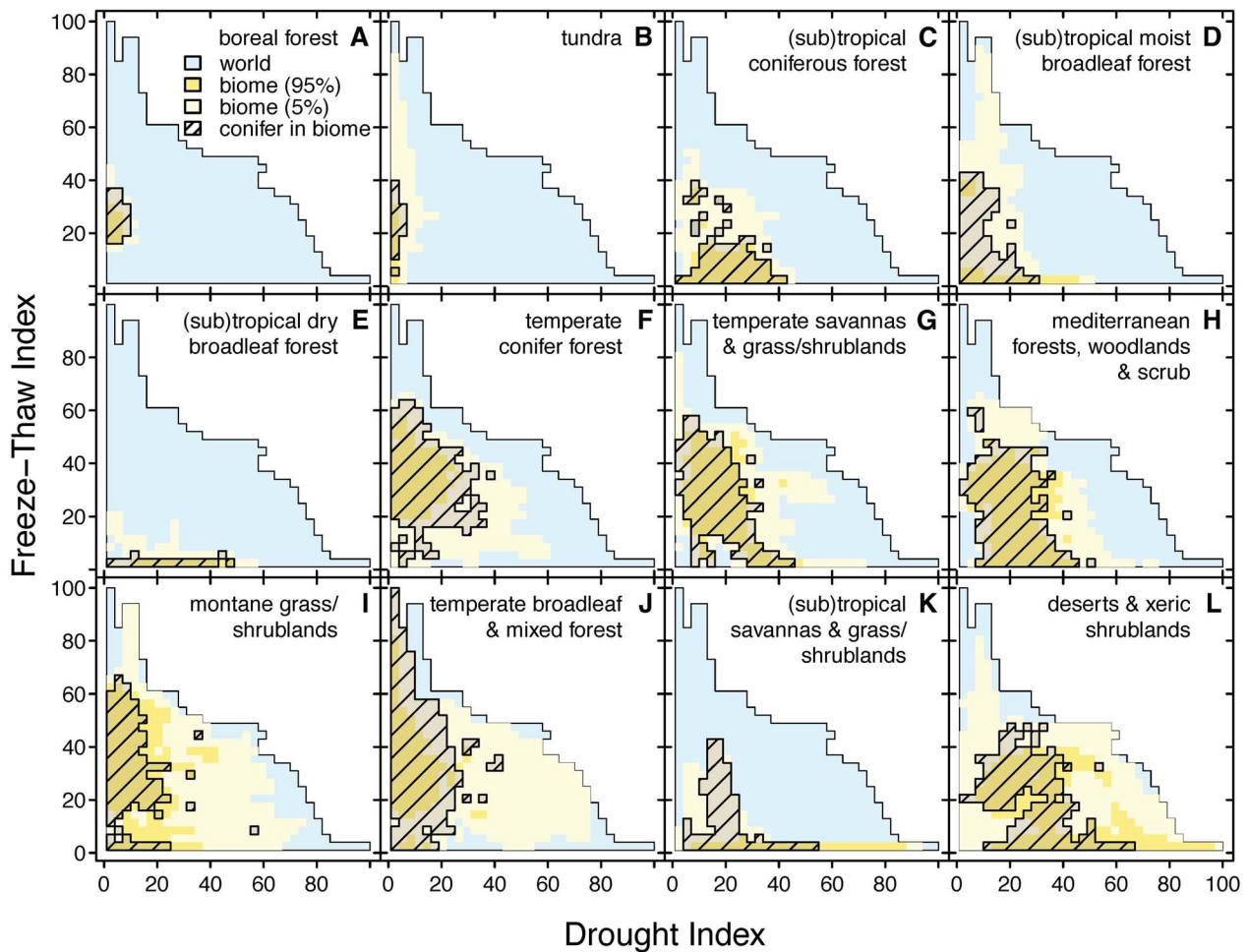


Figure 2. The distribution of 12 major biomes in FTi–Di space, relative to the occurrence of all global landmass (excluding Antarctica - blue; “world”) are shown in shades of yellow (“biome”). The darker shade indicates the bulk of common FTi–Di combinations (“biome 95%”), while the pale shade shows the remaining, rare combinations (“biome 5%”). Hatched regions indicate the reported occurrences of conifer species within each biome. Drought and freeze–thaw indices range from 1 to 100 in order of increasing stress. Each pixel is 3 × 3 index units. For every conifer species, we included it in a biome only if at least 5% or 10 observations were recorded in that biome.

been studied extensively, and there is considerable evidence that across biomes, more arid-adapted plants exhibit lower TLP values due to higher concentrations of osmotically active solutes (Bartlett et al. 2012), although the majority of data come from angiosperms. When we compared the TLP of conifers reported in the literature (see Supplemental Methods) with the species’ median Di, we found no relationship ($P = 0.19$; Figure 4A). The lack of a relationship is surprising, given previous research on the importance of TLP. However, TLP in conifers is not as well studied as in angiosperms, and our dataset lacks measurements of species living in the more severe end of the Di range that conifers occupy. Second, TLP can be lowered by decreasing the elastic modulus of cell walls, which allows the cell wall to collapse slightly as water is lost (Saito and Terashima 2004). Meinzer et al. (2014) found evidence of rapid adjustment of elastic modulus when *Juniperus monosperma* leaves were rehydrated, which resulted in a far milder TLP than the trees

achieved in situ. Conversely, no adjustment was observed in the elastic modulus of *Pinus edulis* leaves in the same study, nor did Bartlett et al. (2012) find evidence of elastic modulus adjustments in response to drought across a broad range of mostly angiosperm species. This discrepancy suggests not all species adjust TLP using the elastic modulus. Third, plants can reduce TLP by increasing the water content within the apoplast relative to that in the symplast (called the ‘apoplastic water fraction’, Bartlett et al. 2012). This concentrates the solutes within the cell, reduces the osmotic potential and consequently lowers the TLP (assuming the other components of cellular water relations remain constant). Regardless of how TLP is reduced or maintained, lower TLP typically results in a greater tolerance of water stress. The TLP has also been shown to be tightly coordinated with a large number of other traits that impart drought tolerance, such as the ability to maintain leaf hydraulic conductance (Brodribb and Holbrook 2006,

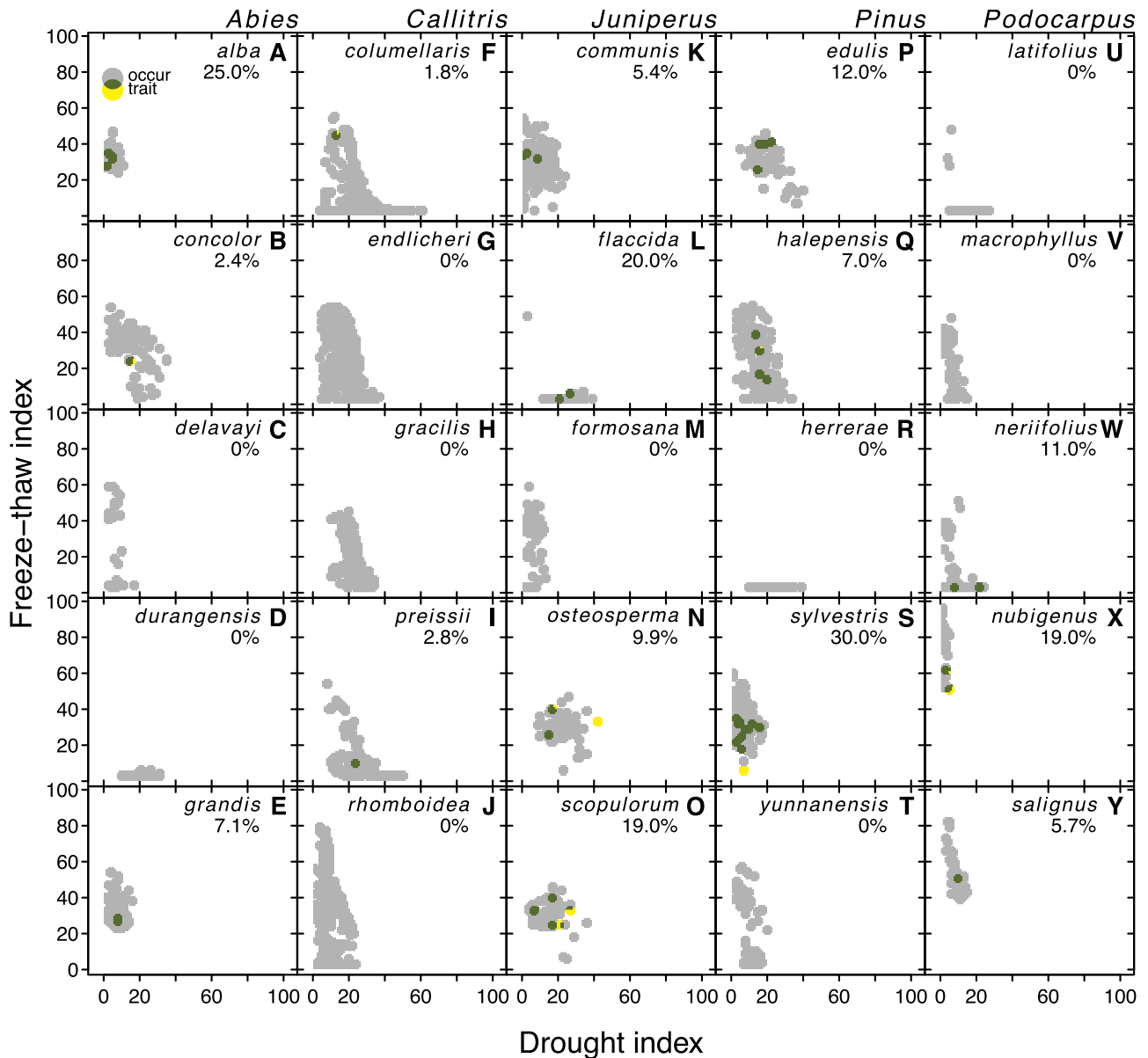


Figure 3. The availability of occurrence data (grey; “occur”), trait data (yellow; “trait”) and their overlap (dark green) in FTi-Di space for five representative species in five representative genera. Each data point was rounded to the nearest FT and drought index and then buffered by a radius of two index units. The approximate completeness of trait data that is available relative to the environmental space that each species occupies was calculated as the area of yellow and dark green pixels divided by the area of yellow, dark green and grey pixels, and expressed as a percentage in the top right of each panel.

Nardini et al. 2012, Nardini and Luglio 2014, Johnson et al. 2018, Yao et al. 2021), which is known to be an indicator of drought tolerance in conifers (Brodrribb and Cochard 2009), and stomatal conductance (Brodrribb and Holbrook 2003, Li et al. 2018) at low water potentials.

Extracellular ice formation is one of the most damaging aspects of FT events. The freezing point of water within the apoplast is higher than that of the cytoplasm, and consequently, intercellular water typically freezes first. Freezing lowers the water potential of the apoplast and draws water out from the living cells, which may lead to cellular dehydration and damage

to the structure and function of the plasma membrane (Guy 1990, 2003). The amount of cellular dehydration that occurs with freezing is temperature-dependent because water potential declines with the temperature of the ice, which in turn reduces the water potential of the cytoplasm (Xin and Browse 2000). Thus, plants that are able to reduce the osmotic potential of living cells might experience less damage due to extracellular ice formation (e.g., *Tsuga canadensis*; Tyree et al. 1978). Indeed, substantial evidence shows that plants acclimated to cold temperatures have lower leaf osmotic potentials and also experience less freezing damage than plants not acclimated

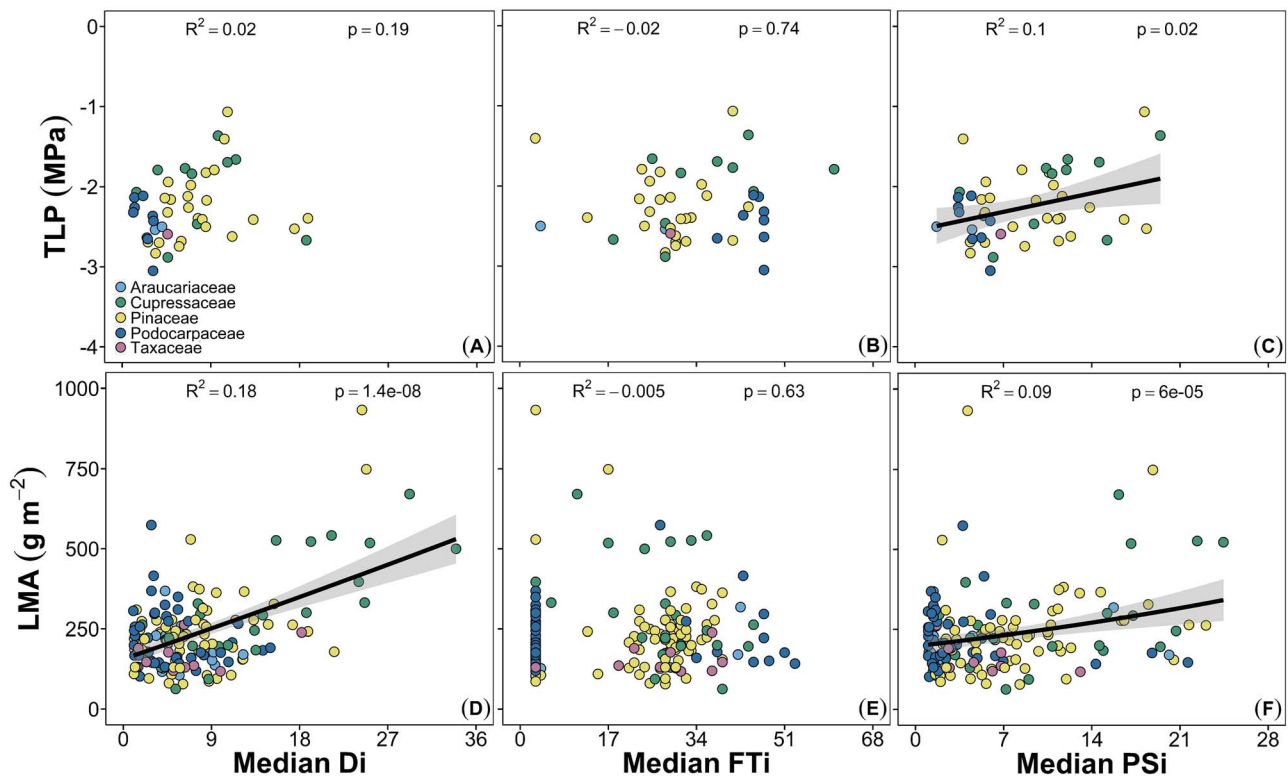


Figure 4. Linear regression of mean species' turgor loss point (TLP; $n = 52$) and LMA ($n = 202$) on median species' occurrence on the drought (Di, A, D), freeze–thaw (FTi, B, E) and poly-stress (PSi, C, F) indices. Regression lines and 95% confidence intervals are presented where significant. The LMA data were not normally distributed and were log transformed. The regressions present in these panels are logarithmic. Each dot represents one species in the families indicated.

to cold temperatures prior to freezing (Kasuga et al. 2007, Charrier et al. 2013, Ting et al. 2014, Arias et al. 2015, 2017). Although a lower osmotic potential is generally associated with lower TLP, we found no significant correlation between FTi and TLP in our dataset ($P = 0.74$; Figure 4B). One explanation for the lack of a relationship is that a lower TLP does not impart greater tolerance to higher FT stress. This may be true if a threshold exists below which reducing the TLP does not increase tolerance to FT cycles. Another explanation is that the TLP values we compiled report values that were only collected in the summer. Conifer TLP values have been shown to adjust throughout the growing season in response to drought (Meinzer et al. 2014, Johnson et al. 2018), and in response to winter (Ritchie and Shula 1984, Gross and Koch 1991, Grossnickle 1992). When apoplastic water freezes and draws water from the symplast, this concentrates the solutes and reduces the osmotic potential and likely the TLP (Mayr and Améglio 2016, Lintunen et al. 2018). The adjustment of pressure–volume curve parameters in the winter deserves far greater attention.

Adjustment of the elastic modulus may also reduce damage associated with extracellular ice formation, as more rigid cell walls can prevent cell collapse (Scholz et al. 2012, Le Gall et al. 2015, Y.J. Zhang et al. 2016). Finally, the ability to adjust the apoplastic water fraction while tissues are experiencing freezing

temperatures may help plants avoid ice formation and tolerate lower temperatures (Goldstein et al. 1985, Arias et al. 2015, 2017). The extent to which this pattern holds in conifers or imparts tolerance to FT cycles (and not simply low temperatures) remains unclear (Grossnickle 1992). However, the most poly-tolerant conifers would likely exhibit the greatest ability to adjust osmotic, elastic and apoplastic properties because doing so is associated with tolerance and/or avoidance to both drought and freezing. For example, the ability to seasonally adjust the apoplastic water fraction would allow summer increases during drought stress and nongrowing season decreases to tolerate frequent FT cycles. However, there are currently no data available to test if conifers adjust in this way, as the available data are heavily biased toward summertime measurements.

Given the theoretical importance of TLP in conifer leaf functioning under drought and FT, as well as empirical support showing that TLP is a key drought tolerance trait in angiosperms (e.g., Bartlett et al. 2012), we predicted conifers would also exhibit strong correlations between TLP and PSi. As discussed above for the individual stresses, the data we compiled indicated no relationships between TLP and Di or FTi (Figure 4). However, our results indicate that the PSi weakly correlated with TLP values ($R^2 = 0.1$; $P = 0.02$; Figure 4C), but in the reverse direction to what we expected, with more negative

TLP associated with species inhabiting low stress regions. This relationship should be explored further, and there are several things that should be considered. First, the range of TLP values is fairly limited. It would be useful to measure species expected to exhibit very negative TLP values. Second, there is evidence that some conifers are able to adjust their TLP values very rapidly and that this shift can indicate artifactually high TLP values when not accounted for (Meinzer et al. 2014). Although this has not been explored extensively, the artifactually high TLP values have been observed in *J. monosperma*, and another study found large temporal variation in TLP values over a growing season in *Juniperus asheii*, suggesting this ability may be common in the Cupressaceae. Finally, we predict that plants adapted to high P_{Si} values would be able to adjust their TLP values under stressful conditions. If the reported TLP values were measured during favorable seasonal conditions, they could be independent of the growing conditions reflected in the P_{Si} value. It remains unclear what is driving the patterns between the stress indices and TLP, and further investigation into this important trait is warranted.

A morphological trait of leaves that may be associated with poly-tolerance to both drought and FT cycles is LMA. Although LMA is not explicitly a hydraulic trait, changes in this trait are associated with changes in leaf hydraulic conductance expressed on a per mass basis (Nardini et al. 2012, Simonin et al. 2012). In habitats with lower water availability, leaves tend to become thicker and/or heavier for a given area (i.e., LMA increases). Mechanistically, this change is caused by more proximal responses to drought stress, such as increases in cell wall thicknesses to withstand more negative water potentials and the need for thicker, longer-lived leaves in resource-limiting environments (Givnish 2002, Hodgson et al. 2011, Simonin et al. 2012). Although LMA generally increases across species in more arid environments, the relationship is fairly weak (Reich 2014), likely because considerable variation exists among species and clades that have evolved to fill different niches within a community in any given habitat (Bruehlheide et al. 2018, Treurnicht et al. 2020). As with plants that experience drought stress, plants adapted to colder annual temperatures also tend to produce leaves with higher LMA (Niinemets 2016, Jankowski et al. 2017). However, whether higher LMA is also associated with a greater number of FT cycles or how this trait may vary intraspecifically across the FT and drought poly-stress gradient is unknown. When we compared species' mean LMA with Di, FTi and P_{Si}, we found that LMA increased with increasing drought ($R^2 = 0.27$; $P < 0.001$; Figure 4D), exhibited no relationship with FTi ($R^2 = -0.005$; $P = 0.63$; Figure 4E) and increased with increasing poly-stress ($R^2 = 0.12$, $P < 0.001$; Figure 4F). This indicates that the response to poly-stress is likely being driven by the strong relationship between LMA and the Di. However, LMA may have stronger relationships with other aspects of FT cycles that our index does not incorporate. For example, our FTi is based on the typical number of FT cycles per year, but it does not

account for temperatures prior to or after FT events. Evidence indicates that freezing events can be more damaging (and thus a stronger driver of trait evolution) if they exceed the rate of cold acclimation and/or occur during the deacclimation phase (Sakai 1960, Stirmbeck et al. 2007, 2015). It also remains unclear whether relatively minor morphological adjustments result in greater protection to more frequent FT cycles compared with increases in drought stress.

Traits that impart tolerance in stems

Research on traits that impart tolerance to drought and FT stress in stems has largely focused on maintaining xylem function. Loss of xylem function due to drought stress is caused by the propagation of air bubbles (embolisms) throughout the conduit network. The propagation of an embolism from one tracheid to a neighboring, functional tracheid occurs through the bordered pits on the walls between two tracheids. Theory and empirical evidence support that more drought resistant wood in conifers has a greater overlap between the impermeable portion of the pit membrane, the torus and the pit aperture (Domec et al. 2006, Hacke and Jansen 2009, Delzon et al. 2010, Pittermann et al. 2010, Bouche et al. 2014, Song et al. 2022). As this overlap increases, the water in the functional tracheid must experience increasingly negative pressure to break the pit membrane seal and release a bubble (Cochard et al. 2009). Thus, embolisms propagate throughout the xylem network at less negative pressures in wood containing pits with less overlap.

A commonly used metric to compare the capacity of species to transport water under increasingly negative water potentials is the xylem pressure inducing a 50% loss of hydraulic conductivity in branches (P_{50}). Previous work across species has shown that P_{50} values generally become more negative in more arid environments, but that there is considerable variation within a given habitat (Maherali et al. 2004, Choat et al. 2012, McCulloh et al. 2019). When we compared P_{50} values reported in the literature (see Supplemental Methods), we found a relationship consistent with previous results: P_{50} became more negative with increasing Di values ($R^2 = 0.41$, $P < 0.001$; Figure 5A). However, the overall relationship is largely driven by data from the Cupressaceae and Taxaceae. The remaining families did not exhibit significant relationships between P_{50} and Di. This distinction highlights the importance of evaluating commonly used metrics such as P_{50} within the context of a species' physiology. Many Pinaceae species tend to close their stomata at relatively mild water potential values even when living in arid habitats (e.g., *P. edulis*). This conservative approach to maintain favorable water potential values eliminates the need to construct xylem that can continue transporting water under extremely water-stressed conditions.

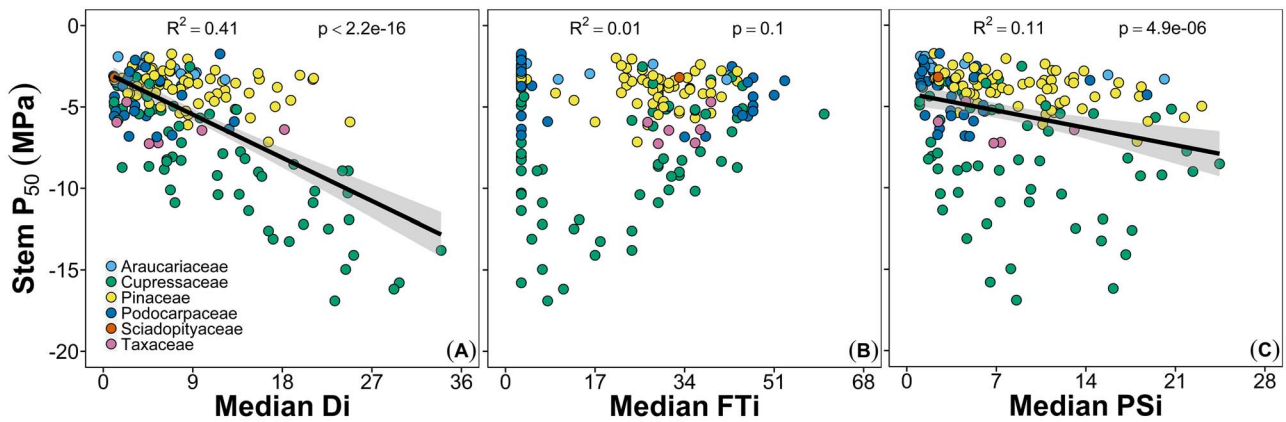


Figure 5. Linear regression of mean species' stem hydraulic vulnerability (stem P_{50}) versus median species' occurrence on the drought (Di, A), freeze-thaw (FTi, B) and poly-stress (PSi, C) indices. Regression lines and 95% confidence intervals are presented where significant. Each dot represents one species ($n = 210$) in the families indicated.

One of the costs of having stems that can maintain hydraulic function at low water potentials is a necessary increase in wood density. In conifers that function at more negative water potentials, the walls surrounding the tracheids are thicker to prevent collapse during periods of extreme negative pressure and this thickening increases the wood density (Hacke et al. 2001, Hacke and Jansen 2009). Species with high wood density and low capacitance tend to exhibit suites of traits related to drought tolerance more than drought avoidance (McCulloh et al. 2019).

The loss of hydraulic function due to FT-induced embolisms is well documented in conifers (Sperry and Sullivan 1992, Sparks and Black 2000, Feild and Brodrigg 2001, Sparks et al. 2001, Mayr et al. 2002, 2003a, 2003b, 2007, Pittermann and Sperry 2003, 2006, McCulloh et al. 2011). In contrast to drought stress, interspecific tolerances to FT events are much more directly dependent on tracheid dimensions. During FT cycles, gases that are dissolved in the xylem water will form bubbles due to their insolubility in ice (Hammel 1967, Sucoff 1969). Upon thawing, a bubble has two potential fates: it can dissolve back into the water or it can expand and form an embolism within the xylem (Pittermann and Sperry 2006). The probability of the gas dissolving into the water depends on the size of the bubble, which in turn depends on the diameter of the conduit (and the xylem pressure potential; Pittermann and Sperry 2006). Wider diameter conduits will produce wider diameter bubbles, which will expand at less negative xylem pressures (as described by La Place's law). Thus, narrower conduits are more resistant to embolism caused by FT cycles, a trend that has been observed across conifers (Pittermann and Sperry 2003, 2006, Willson and Jackson 2006). Freeze-thaw tolerance may also be related to pit characteristics. Theory suggests that pits with greater porosity facilitate the movement of larger particles that allow ice to nucleate at warmer temperatures (Lintunen et al. 2013). Thus, conifers characterized by pits with lower porosity would likely exhibit greater tolerance to FT cycles. Despite the

mechanistic link between stem P_{50} and pit dimensions, we found no relationship between P_{50} values and FTi values ($R^2 = 0.01$, $P = 0.1$; Figure 5B).

The impact of wood density and capacitance on tolerating FT cycles has not been explored. Theoretically, the high specific heat of water suggests that high water storage in stems may prevent the stem from rapidly freezing or thawing during short periods of low or high temperatures. However, capacitive water that is stored apoplastically would freeze at higher temperatures (compared with symplastically stored water) and could dehydrate the stem symplast, just as the apoplastic water fraction does in leaf tissue (Ball et al. 2002). If so, high density wood with a lower total volume of apoplastically stored water and smaller individual storage compartments may experience less FT damage than low density wood during FT cycles. While wood density has been shown to be negatively correlated with frost tolerance in the woody taxa of North America (Rueda et al. 2017), data are not currently available to determine if these patterns extend to tolerance of FT cycling.

Where the stresses of drought and FT stress overlap, highly poly-tolerant populations would likely have high wood density, narrow conduits and large torus-aperture overlap. Despite some evidence that embolisms induced by FT cycles increase when combined with winter drought, very few studies have measured both FT- and drought-induced embolism on the same species. Loss of hydraulic function in the winter and resistance to drought stress have been examined in four species in the Pacific Northwest of the USA, and no differences were found among the species in either trait (McCulloh et al. 2011, McCulloh et al. 2014). In contrast, found that *Picea abies* lost more hydraulic function than that of neighboring *P. cembra* in the winter but was more resistant to loss of function caused by drought alone. The key difference among these alpine species seems to be the cuticular conductance, which was higher in *P. abies* and caused lower water potential values over the winter than *P. cembra*

experienced. If xylem pressure decreases during FT cycles, the probability increases that a gas bubble will expand instead of redissolving, even in narrower conduits (Pittermann and Sperry 2006). Low cuticular and/or minimum stomatal conductance has long been identified as a drought tolerance trait in crops (Sinclair 2000) and has been linked to desiccation tolerance in angiosperms more generally (Gleason et al. 2014, Blackman et al. 2016). A recent review found that 15 *Pinales* species had relatively low values of minimum stomatal conductance compared with angiosperms (Duursma et al. 2019), but it remains unclear the extent to which this trait changes across species ranges in response to greater stress from drought, FT and/or their combination.

Our results also suggest that a more negative P_{50} value imparts some advantage in habitats with higher PSi values ($R^2 = 0.11$, $P < 0.001$; Figure 5C). Although this correlation was weak, it was highly significant, and, like the relationship between P_{50} and Di , was driven by trends in the Cupressaceae and Taxaceae. The remaining families did not have significant relationships, although the correlation for the Podocarpaceae suggested a trend ($P = 0.09$). The similarity in the relationships between P_{50} versus Di and PSi suggests that drought tolerance is driving the observed relationship between P_{50} and PSi .

Discussion and future directions

Our compiled dataset indicates that conifers occupy a large percentage of the worldwide FT and drought poly-stress space (Figure 2), and that individual species differ substantially within this space (Figure 3). Though some species occupy regions with moderate drought and FT stress, no species occupy space with both severe drought and FT stress (Figure 3). Along with the negative relationship of all species' occurrence along the drought and FT stress indices ($R^2 = 0.17$; $P < 0.001$; data not shown), this distribution suggests a potential functional trade-off in tolerance to these stresses (Figure 3). Although the observed species' ranges may be limited by other stressors and/or geographic barriers, the lack of data on traits across the FTi, Di and PSi prevents us from fully quantifying the impact of these stresses on distribution patterns. Further analysis of traits across species' poly-stress ranges may answer whether trait combinations conferring poly-stress tolerance are equivalent for all conifers or if different trait combinations lead to the same tolerance across species.

Our analyses indicate a tendency for individual conifer species to occupy a larger range of FTi than of Di (except when FT tolerance is very low; Figure 3). This tendency remains across all 603 species studied. As a whole, conifers generally occur in habitats with more FT stress than drought stress (Figure 2). These findings may suggest that conifers are better at tolerating FT stress and/or that one unit of the FTi is inherently less stressful than one unit of the Di . This observation drives

several questions as follows. In species that occupy a broad range of FT space (e.g., *Callitris rhomboidea* in Figure 3) do traits shift linearly with increasing FT days or does a threshold exist beyond which additional FT days do not correspond to further trait changes? Such a threshold may indicate a diminishing impact of additional FT days. Additionally, while much focus goes toward understanding what allows tolerance to greater stresses, what limits some species occupying locations with lower stress is unknown. Do the traits that provide FT-tolerance have trade-offs that prevent FT tolerant species from competing successfully in low FT areas? Is there a growth penalty for maintaining FT-tolerating traits when FT events are less frequent? Another important point that Figure 3 highlights is the lack of trait data available for many species. Even well-studied species, such as *Pinus sylvestris*, have trait data for locations representing less than half of their distribution along these axes. This lack of data is not unique to these species. Table S1 available as Supplementary data at *Tree Physiology Online* lists all species with their percent trait coverage. Only five species (*Araucaria laubenfelsii*, *Pinus daltensis*, *Pinus krempfii*, *Pinus koraiensis* and *Retrophyllum minus*) have trait coverage exceeding 50% but these have low occurrences. Many species (76%) have no trait coverage and the median among species with any coverage is 10%. Without a more complete picture of how and if traits vary across species' ranges, our ability to determine which specific traits are limiting is hindered.

Conifers have a worldwide presence, collectively experiencing a wide range of FT and drought stresses, yet the trait data do not yet exist to fully determine how exactly conifers cope with this poly-stress. For example, the numerous studies that have measured hydraulic vulnerability to drought in a wide range of species contrasts with the comparatively fewer studies that have characterized hydraulic vulnerability to FT cycles. Studies that examine the interaction between low xylem water potential and FT-induced embolism (i.e., the combination of drought and FT cycles) in conifers are even rarer still (Mayr et al. 2006, Pittermann and Sperry 2006, Mayr and Sperry 2010). To help make progress in the study of poly-tolerance in conifers, contrasting high versus low poly-stress ecoregions with abundant conifer species (e.g., Western Turkey sclerophyllous and mixed forests vs Sinaloan dry forests of Mexico) may provide the easiest route to understanding traits conferring FT–drought poly-tolerance.

Finally, it is worth reflecting on the limitation of our analyses regarding future conifer distributions. Rising air temperatures that increase VPD values, unstable atmospheric circulation that drive polar vortices and increased precipitation variability will combine to increase drought, FT and poly-stress globally. The vast boreal forests provide an excellent example of the need for more information on which traits are needed for tolerance to each stress and their combination and what the limits are to those tolerances. Specifically, our FTi, Di and PSi showed

that boreal forests currently experience high levels of FT stress but low drought and, consequently, low levels of poly-stress. However, these regions may become drier in the future as altered precipitation patterns result in long, dry periods between summer rain events (Wang et al. 2014). This potential decrease in soil water content will be exacerbated by increases in VPD, which would intensify the drought stress (Yuan et al. 2019, López et al. 2021). These changes will increase the occurrence and intensity of poly-stress, and thus, a tolerance to both FT and drought will be critical for the survival and growth of coniferous species at these latitudes. Consequently, we need a more fundamental understanding of the traits that permit tolerance of these stresses for a broader range of species across their distributions to predict how these forests will respond to FT and drought stress in the future.

Conclusion

In this review, we present four key points: (i) the global peak co-occurrence of drought and FT stresses is the combination of moderate drought and moderate FT; (ii) the distribution of conifers overlaps considerably with moderate levels of FT and drought stress; (iii) the distribution of individual species along the two indices varies, with some species exhibiting broad tolerance to both stresses and others exhibiting tolerance to only one (typically FT); and (iv) while several hydraulic traits may confer tolerance to both FT cycles and drought in stems and leaves, the lack of trait data across species ranges limits our understanding of poly-tolerance in conifers globally. Given that global climate change will likely increase the frequency and severity of both FT cycles and drought, addressing this knowledge gap will be of critical importance for predicting how coniferous forests will function in the future.

The emergent science of poly-tolerance is primarily limited by the scarcity of comprehensive intra- and interspecific datasets across broad geographical scales. Many hydraulic traits that may be involved in poly-tolerance—such as conduit and pit dimensions or TLP—are typically measured in a few species at small spatial scales due to the time-intensive nature of these measurements. Recent efforts to expedite these measurements (e.g., measuring TLP by osmometry; Bartlett et al. 2012) have been successful and will facilitate the broad-scale survey of these traits across ecological gradients, especially with increasing utilization of global field experiment networks such as DroughtNet (Knapp et al. 2015) and the Center for Forest Global Earth Observatory ‘ForestGeo’ (Davies et al. 2021). Furthermore, the observed distribution of species across these indices suggests that different species may have varying levels of tolerance to each stress, indicating the response of conifers to increasing poly-stress will likely be species-specific. Coniferous forests dominated by species that have low poly-tolerance to these stressors may consequently experience range shifts or

extirpation in the future. Drastic changes in the distribution of conifer species will have far-reaching industrial, agricultural and cultural implications, underscoring the need to strengthen poly-tolerance concepts.

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Authors' contributions

K.A.M. led the writing, to which all authors contributed substantially; S.P.A., D.D.S., A.G., and C.P.K. led the compilation of data, data analysis and created figures. After K.A.M., authors are listed alphabetically.

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