

Drought stress limits the geographic ranges of two tree species via different physiological mechanisms

LEANDER D. L. ANDEREGG and JANNEKE HILLERISLAMBERS

Department of Biology, University of Washington, Box 351800, Seattle, WA 98195, USA

Abstract

Range shifts are among the most ubiquitous ecological responses to anthropogenic climate change and have large consequences for ecosystems. Unfortunately, the ecophysiological forces that constrain range boundaries are poorly understood, making it difficult to mechanistically project range shifts. To explore the physiological mechanisms by which drought stress controls dry range boundaries in trees, we quantified elevational variation in drought tolerance and in drought avoidance-related functional traits of a widespread gymnosperm (ponderosa pine – *Pinus ponderosa*) and angiosperm (trembling aspen – *Populus tremuloides*) tree species in the southwestern USA. Specifically, we quantified tree-to-tree variation in growth, water stress (predawn and midday xylem tension), drought avoidance traits (branch conductivity, leaf/needle size, tree height, leaf area-to-sapwood area ratio), and drought tolerance traits (xylem resistance to embolism, hydraulic safety margin, wood density) at the range margins and range center of each species. Although water stress increased and growth declined strongly at lower range margins of both species, ponderosa pine and aspen showed contrasting patterns of clinal trait variation. Trembling aspen increased its drought tolerance at its dry range edge by growing stronger but more carbon dense branch and leaf tissues, implying an increased cost of growth at its range boundary. By contrast, ponderosa pine showed little elevational variation in drought-related traits but avoided drought stress at low elevations by limiting transpiration through stomatal closure, such that its dry range boundary is associated with limited carbon assimilation even in average climatic conditions. Thus, the same climatic factor (drought) may drive range boundaries through different physiological mechanisms – a result that has important implications for process-based modeling approaches to tree biogeography. Further, we show that comparing intraspecific patterns of trait variation across ranges, something rarely done in a range-limit context, helps elucidate a mechanistic understanding of range constraints.

Keywords: drought avoidance, drought tolerance, ecophysiology, functional trait, intraspecific trait variation, *Pinus ponderosa*, ponderosa pine, *Populus tremuloides*, trembling aspen

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Introduction

Species geographic ranges are ideal ecological study systems because they are a highly visible outcome of the fundamental forces shaping the abundance and distribution of organisms. It is therefore surprising that despite two centuries of study on geographic distributions, the processes controlling range boundaries are still poorly understood (Von Humboldt & Bonpland, 1805; MacArthur, 1972; Gaston, 2009; Sexton *et al.*, 2009). Because evolutionary responses are likely to be too slow to allow species to adapt to rapid anthropogenic climate change in place (especially long-lived species) – (Aitken *et al.*, 2008; Dullinger *et al.*, 2012; Quintero & Wiens, 2013), range shifts are projected to be a major ecological response to climate change over the next century (Parmesan & Yohe, 2003; Root *et al.*,

2003). Unfortunately, ecologists and conservation biologists lack a strong understanding of the fundamental physiological mechanisms that limit species ranges, and therefore have little ability to predict and manage for the ‘emergent risk’ (i.e., complex, multisystem risk that spans local and national boundaries) of climate change-induced range shifts and the resulting potential for species extinction (IPCC, 2014). Indeed, current approaches to predicting range boundary movement are by necessity largely correlational and mechanistic approaches are rare (Handa *et al.*, 2005; Morin, 2009; Sexton *et al.*, 2009; Buckley *et al.*, 2011). Within-species patterns of functional trait variation may provide an underexplored tool for identifying the physiological mechanisms underpinning climate-controlled range boundaries.

Plant functional traits are a fundamental link between the environment and organismal fitness, thus providing a powerful tool for ecological inquiry at multiple spatial, temporal, and taxonomic scales (Violle

Correspondence: Leander D. L. Anderegg, tel. +1 541 790 1096, fax +1 206 543 3041, e-mail: ldla@uw.edu

et al., 2014). Functional traits have therefore become a pillar of many ecological subdisciplines. They serve as a tool for understanding plant community assembly (McGill *et al.*, 2006), drive next-generation vegetation dynamics in land-surface models (Moorcroft *et al.*, 2001; Medvigy *et al.*, 2009; Pavlick *et al.*, 2013; Scheiter *et al.*, 2013) and provide a generalized understanding of plant responses to environmental change (Angert *et al.*, 2011; Buckley & Kingsolver, 2012; Díaz *et al.*, 2013; Mouillot *et al.*, 2013; Soudzilovskaia *et al.*, 2013). In particular, recent global between-species trait comparisons have revealed fundamental constraints on plant physiology (Reich *et al.*, 2003; Reich, 2014) that translate into powerful life history trade-offs (Adler *et al.*, 2014). In addition, intraspecific trait variation, which can be a substantial fraction of between-species trait variation (Albert *et al.*, 2010; Messier *et al.*, 2010), can influence species coexistence (Clark, 2010), predict climate change impacts on plant physiology (Anderegg, 2014), or project future range shifts (Benito-Garzón *et al.*, 2011). However, intraspecific functional trait variation across species ranges is still poorly understood (Martinez-Vilalta *et al.*, 2009; Violle *et al.*, 2014), but could greatly improve our mechanistic understanding of range constraints by suggesting limits to physiological adjustment.

We explore within-species variation in a suite of plant drought stress resistance traits to explore the physiological basis underlying tree range limits along an aridity gradient. Moisture availability controls plant biogeography and productivity across much of the globe (Boisvenue & Running, 2006), and water stress is thought to control the lower elevation range boundaries of many plant species in semi-arid environments (Kelly & Goulden, 2008; Fellows & Goulden, 2012). Moreover, drought is likely to change in spatial and temporal extent and magnitude over the coming century (Dai, 2011; Hartmann, 2011), driving plant range shifts. Thus, we focus on drought resistance traits near the dry range margins of two widely distributed tree species. These traits have classically been divided into 'avoidance traits', 'tolerance traits', and 'recovery traits' (see parallel terms in Larcher *et al.*, 1973 and Levitt, 1980): traits that relate to the ability to avoid experiencing drought stress, the ability to tolerate stress without injury when stress occurs, and the ability to recover when stress injures performance. We explore within-species variation in key plant hydraulic traits (Maherali *et al.*, 2004) and morphological traits (Reich *et al.*, 2003) that together represent multiple aspects of drought avoidance and tolerance (Table 1). We do not focus on recovery traits, belowground traits, or phenological traits (all of which may vary within a species and influence plant drought resistance),

Table 1 Physiological and morphological variables measured on study trees, categorized by whether traits are thought to help trees avoid or tolerate drought stress

Drought avoidance traits	
traits	Physiological implication
Tree height (m)	Decreased height lowers xylem tensions by reducing gravity potential and hydraulic resistance due to path length (Koch & Fredeen, 2005)
Sapwood area-to-leaf area ratio ($A_s:A_L - m^2$ per cm^2)	Decreased leaf area to sapwood area increases hydraulic efficiency and reduces the xylem tensions required to supply evaporative area with water (Martinez-Vilalta <i>et al.</i> , 2009)
Median Leaf Size (cm^2)	Decreased leaf size reduces distances from major leaf veins, decreasing hydraulic resistance from xylem to the leaf evaporative site (Zwieniecki & Boyce, 2004)
Maximum xylem area-specific hydraulic conductivity (K_{max})	Increased K_{max} indicates greater potential xylem hydraulic efficiency (in the absence of embolism), which reduces hydraulic resistance and decreases the xylem tensions needed to move water from root to leaf (Maherali <i>et al.</i> , 2004)
Leaf area-specific native hydraulic conductivity (K_{nat_Leaf})	Increased K_{nat_Leaf} (conductivity with native embolism present) increases hydraulic efficiency and indicates greater hydraulic support of each unit leaf area
Decreased stomatal conductance (g_s)	Stomatal closure prevents the development of large xylem tensions by limiting water loss
Drought Tolerance Traits	
Traits	Physiological Implication
Specific leaf area (SLA)	Decreased SLA (leaf area per unit dry mass) can increase tolerance of leaves to large xylem tensions (Mitchell <i>et al.</i> , 2008)
Xylem vulnerability to cavitation (in branches)	Decreased xylem vulnerability to embolism increases the xylem tensions possible before conductivity is curtailed by drought-induced xylem embolism (Maherali <i>et al.</i> , 2004)
Hydraulic safety margin	Difference between most extreme xylem tension experienced in the field and xylem tension required to cause 50% embolism (Choat <i>et al.</i> 2012)

because these are either poorly understood or difficult to quantify. In general, tree species may avoid increasing water stress at their dry range edge via adjustments to tree height, leaf area-to-sapwood area ratio, leaf size, hydraulic efficiency, and stomatal regulation of water loss. By contrast, species may become more tolerant to water stress at their range edge via changes to specific leaf area, hydraulic vulnerability to cavitation, or hydraulic safety margin (see Table 1).

In this study, we build on successful across-species trait analyses (e.g., Carnicer *et al.*, 2013; Reich, 2014) to explore the strategies by which individual species deal with water limitation across their ranges. We compare two woody species, a dominant gymnosperm (ponderosa pine – *Pinus ponderosa* Dougl. ex Laws) and a clonal angiosperm (trembling aspen – *Populus tremuloides* Michx.) in the southwestern USA. Annual growth is increasingly sensitive to previous year moisture availability at the low-elevation range boundaries of both species (L.D.L. Anderegg & J. HilleRisLambers in prep.), suggesting that ponderosa pine and aspen's low/dry range boundaries are both constrained by moisture stress at the study site. First, we confirm that performance is constrained at range limits by examining rangewide variations in radial growth and water stress. Second, we quantify trait variation in multiple functional traits to assess the physiological strategies by which each species copes with increasing water stress. Finally, we synthesize growth and trait variation patterns to speculate how and whether the study species differ in the physiological drivers of their dry range limits.

We find that radial growth of both tree species decreased dramatically at the dry range margins, but the species showed contrasting patterns of trait variation. Ponderosa avoided water stress at low elevations by curtailing water loss, whereas aspen maintained transpirational losses but built more drought-tolerant –

yet carbon dense – tissues at its dry range boundary. Thus, ponderosa pine may be limited at its dry range boundary by lack of carbon assimilation, whereas aspen faces the increasing carbon cost of growing drought-tolerant organs.

Materials and methods

Study design

The study was conducted on the west slope of the La Plata Mountains in the San Juan National Forest (37.4825°N, 108.1970°W), Southwest Colorado (USA) in the summer of 2014. Plant communities transition from lowland piñon–juniper woodland to ponderosa pine forest to montane aspen forest to subalpine spruce–fir forest with elevation (Fig. S1), crossing a large temperature and precipitation gradient while maintaining a relatively consistent southwest aspect. Our study sites (Table 2) start at the lower transition from closed canopy forest to scrub/open woodland (at ~2250 m) and nearly reach upper tree line (at ~3550 m). Mean annual temperature ranges from 7.3 °C to 2.6 °C, and mean annual precipitation ranges from 480 mm to 760 mm (Fig. 1). Because precipitation is bimodally distributed throughout the year (~50% falls during the winter and the rest falls as summer monsoons beginning mid- to late-July), these forests usually experience peak water stress in early- to mid-July (Anderegg *et al.*, 2013a).

We investigated the gymnosperm ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) and the clonal angiosperm trembling aspen (*Populus tremuloides* Michx.), both widespread throughout North America and forming monodominant stands across most of the study site. Focal species differ in xylem anatomy (ponderosa have only tracheids, aspen have tracheids and xylem vessels) and leaf lifespan (evergreen vs. winter deciduous). Because aridity strongly increases with decreasing elevation in semi-arid, midlatitude mountains (e.g., Fig. 1), drought stress likely controls the low-elevation limit of most tree species at the study site (Adams & Kolb, 2005; Fellows & Goulden, 2012), including the two study species.

Table 2 Characteristics (mean \pm SD) of study stands (5 stands per elevation). DBH is the mean diameter at breast height (1.3 m) of focal trees (3 per stand), while density and basal area are the mean number of trees per hectare and stand basal area per hectare across stands based on the stand average density and basal area assessed for each focal tree using 15-m (ponderosa pine) or 10-m-diameter (trembling aspen) plots

	Elevation (m)	DBH (cm)	Density (trees ha ⁻¹)	Basal area (m ² ha ⁻¹)	Age (years)
Ponderosa					
Low	2320 \pm 10	44 \pm 11	248 \pm 70	23 \pm 11	100 \pm 8
Mid	2480 \pm 27	47 \pm 7	356 \pm 107	38 \pm 8	102 \pm 8
High	2676 \pm 14	51 \pm 4	281 \pm 84	29 \pm 10	101 \pm 9
Aspen					
Low	2665 \pm 9	25 \pm 4	588 \pm 202	27 \pm 8	101 \pm 5
Mid	2889 \pm 38	40 \pm 9	949 \pm 175	55 \pm 16	98 \pm 26
High	3081 \pm 8	36 \pm 7	1082 \pm 431	57 \pm 11	97 \pm 24

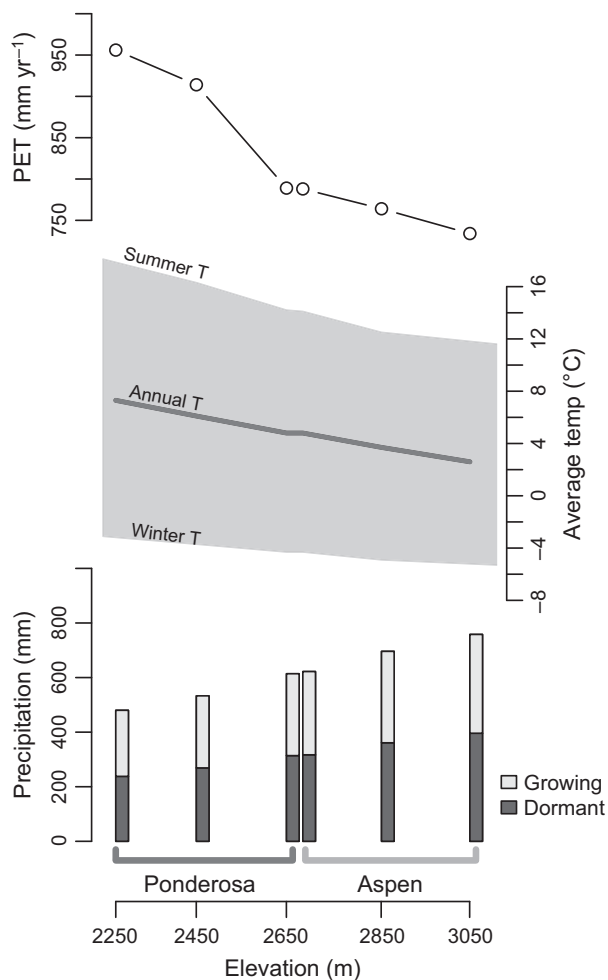


Fig. 1 Climate variation across the study elevation gradient in SW Colorado, USA. Elevation ranges of ponderosa pine and trembling aspen are shown at the bottom of the figure. Mean annual growing season precipitation (light gray bars) and dormant season precipitation (dark gray bars) at the three ponderosa study elevations and three aspen study elevations increase with increasing elevation. Mean annual temperature (solid line), as well as mean summer temperature (upper edge of gray shading) and mean winter temperature (lower edge of shading) decrease with elevation. Annual potential evapotranspiration (PET, points with lines) also decreases with elevation. Data are from 30 years PRISM climate normals (Daly *et al.*, 2002) interpolated using the ClimateWNA algorithm (Wang *et al.*, 2012).

We located five mature stands each at the lower elevation range margin, at the range center, and at the upper range margin of each species (total of 15 stands per species). Stands were >100 m apart on gentle (<8% slope) southwest to west facing aspects and were >30 m from any major topographic or hydrological features such as drainages or hill tops (see Table 2 for stand characteristics). In each stand, we randomly selected three mature, dominant, visually healthy trees for growth and trait measurements (below, see Table 1).

Trait measurements

Growth. To quantify growth, we collected two tree cores from each focal tree at 1.3 m height on opposite sides of the bole perpendicular to the aspect. Cores were sanded and scanned with a high-resolution scanner, and annual growth rings were measured to 0.001 mm using the WINDENDRO software (Version 2008e, Regent Instruments, Quebec City, Quebec, Canada). Cores were visually and statistically cross-dated using the *dplR* package in R (Bunn, 2010, R Development Core Team 2014), and ring widths averaged per tree. We used diameter at breast height (DBH) and bark depth to calculate annual basal area increment (BAI) from annual ring widths, and then calculated mean annual BAI (2003–2012) for each tree. To assess how stand density affects growth, we performed stand surveys and calculated Hegyi's distance-dependent competitive index (Hegyi, 1974) for each tree. Hegyi's index sums the neighbor DBH divided by the focal tree DBH and the distance to the neighbor for all neighboring trees within 15 m (for ponderosa pine) or 10 m (for aspen) of the focal tree. This competition index correlates with BAI in ponderosa forests (Contreras *et al.*, 2011), and was therefore included as a covariate in all statistical tests involving BAI. Because our response variable (BAI) and density covariate (Hegyi's competitive index) were highly variable, we included growth data from 12 to 25 closely co-located trees per elevation (cored in 2013 for a separate project) to increase sample size and statistical power (L.D.L. Anderegg & J. HilleRisLambers, in prep.). Unlike other focal individuals in this study, 2013 trees were selected for high-vs.-low competitive environments; however, other selection criteria were identical to this study (see description with Fig. S2). Competitive environment was estimated for 2013 trees via multiple techniques, which we converted into Hegyi's competitive index (see Fig. S2). When excluding these additional trees, results were qualitatively similar, albeit non-significant for trembling aspen (Fig. S3). The combined dataset resulted in a final sample size of 27–40 trees per elevation (mean = 35 trees) for growth estimates.

Xylem tension measurements. We estimated ψ_{\min} (the largest xylem tension, i.e., most negative plant water potential) for focal trees by measuring branch xylem tension between June 29th and July 9th of 2014. This period captured the driest portion of the growing season (monsoonal rains began on July 10th) and largest xylem tensions of the year. Although 2014 was an average year climatologically (the water year precipitation was 4.7 cm or 6% below the 20-year average, and mean annual temperature was 1.3 °C hotter than the 20-year average at a SNOTEL station ~5 km away), single growing season ψ_{\min} is often used to approximate interannual ψ_{\min} (Sperry, 2000; Choat *et al.*, 2012). Furthermore, our measured xylem tensions were quite similar to previous maximum tensions measured in low-elevation aspen stands in this region (~0.2 MPa less than most extreme tensions measured since 2010; Anderegg *et al.*, 2012, 2013a, 2014).

Xylem tensions were measured twice daily on distal twigs of focal trees: once predawn (03:00–05:30 local time,

generally considered lowest daily tensions) and again at midday (13:00–15:00). Assuming no nighttime transpiration, predawn xylem tensions reflect soil water potential across the rooting depth of the tree and are an indication of soil moisture limitation, whereas midday xylem tensions represent the maximum tensions experienced by the tree (Ritchie & Hinckley, 1975). The assumption of limited nighttime transpiration was supported for both species by preliminary experiments comparing predawn water potentials of juveniles bagged in plastic overnight vs. unbagged juveniles (data not shown). Branches of ~5 cm diameter were collected from the mid-to-upper, sun-exposed, south-facing canopy via shotgun and immediately placed in humid plastic bags. Xylem tension of 1–3 intact twigs from these branches (recut >30 cm from the initial branch break) was measured using a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR) within three minutes of sample collection. Weather was sunny and cloud free when xylem tensions were measured.

Morphological traits. Morphological traits can allow trees to avoid water stress when soil moisture is limiting (Table 1). Large reductions in tree height can limit maximum xylem tensions by reducing gravitational potential and lowering the hydraulic path length between soil and leaf (McDowell *et al.*, 2002; Koch & Fredeen, 2005), while decreased branch leaf area-to-sapwood area ratio ($A_L:A_S$) can increase hydraulic efficiency, thereby decreasing the xylem tensions necessary to deliver water to the leaf (Martinez-Vilalta *et al.*, 2009). In addition, decreased leaf size can increase leaf hydraulic efficiency (decrease hydraulic resistance) by decreasing the distance between leaf evaporative sites and large (low resistance) veins (Zwieniecki & Boyce, 2004; Sack & Holbrook, 2006). We also quantified elevational variation in specific leaf area (SLA = leaf area/leaf dry mass), because decreasing SLA is associated with increased drought tolerance as more structural carbon increases a leaf's ability to withstand high xylem tensions without losing turgor (Mitchell *et al.*, 2008). In addition, water storage and capacitance can increase as SLA decreases (Ishii *et al.*, 2014).

We used various field and laboratory techniques to measure these traits. Tree heights were measured with a digital inclinometer. We used digital photographs and ImageJ image processing software (US National Institute of Health; <http://www.nih.gov/>) to quantify total one-sided leaf area (A_L) and median leaf size (Pérez-Harguindeguy *et al.*, 2013) of one sun-exposed branch 3–15 mm in diameter from the south-facing mid-to-upper canopy of each tree (collected for hydraulic measurements discussed below). We calculated SLA (A_L /leaf dry mass) and calculated $A_L:A_S$ using the sapwood diameter at the basal end of the branch segment. When branch leaf area was very large, leaf area was estimated by calculating the SLA of a subset of leaves/needles and multiplying by the total leaf/needle dry mass of the branch. Following measurement of branch hydraulic conductivity, we measured branch wood density on a 3- to 5-cm section by dividing the green volume (assessed via water displacement on an analytical balance) by sample dry mass.

Hydraulic traits. We measured branch hydraulic efficiency across elevation in both species to quantify drought avoidance-related hydraulic adjustment. We used a shotgun to collect one large (diameter >10 cm), sun-exposed, mid-to-upper canopy branch from the south side of each focal tree at midday during maximum summer water stress (June 29th – July 9th). Because branch severing under tension can cause artificial embolism (Wheeler *et al.*, 2013), an unbranched segment (>12 cm long, bearing no foliage and typically 5–9 mm diameter) was immediately cut from the original branch under water as far away from the initial break as possible (typically >10 cm) to relax xylem tension. This segment was sprayed with water, sealed in a moist plastic bag, and placed in a cooler for transport back to the laboratory. In the laboratory, branch segments were recut underwater using a sharp razor (final length >8 cm). Aspen stems were cut as long as possible (typically >10 cm in length) to accommodate long maximum vessel lengths (between 8 cm and 15 cm, mean vessel length is 1.9 cm; Sperry & Sullivan, 1992; Sperry *et al.*, 1994; Zimmermann & Jeje, 1981). Native or maximum conductivity and branch length were uncorrelated, suggesting no open vessels in shorter aspen branch segments (data not shown). Branch native conductance (k_{nat}) was measured using the standard pressure-flow method (Sperry *et al.*, 1988), stems were flushed of embolisms via vacuum infiltration, and then maximum conductance (k_{max}) was measured. Native conductance values were standardized by the leaf area of the branch and stem segment length to give leaf area-specific conductivity ($K_{\text{nat,Leaf}}$), reflecting how well hydraulically supported each unit of leaf area is. Maximum conductance values were standardized by stem sapwood area and stem length to give maximum sapwood specific conductivity (K_{max}), representing maximum hydraulic efficiency allowed by the branch xylem anatomy. The degree of embolism present in these branches was also quantified as the percentage loss of conductance:

$$\text{PLC} = \frac{(k_{\text{max}} - k_{\text{nat}})}{k_{\text{max}}} \times 100. \quad (1)$$

On a second set of branch segments (collected as above but following the onset of the summer monsoons), we quantified xylem vulnerability to cavitation via a standard vulnerability curve technique. Artificial xylem tensions were induced via air injection, following the protocols of Anderegg *et al.* (2013b) for aspen stems and Maherali & DeLucia (2000) for ponderosa pine stems. Native conductance and maximum conductance were measured for each branch, and then, conductance was measured following air injection-induced xylem tensions of 1, 2, 3, and 4 MPa. This method has previously produced reliable vulnerability curves for both of these species, and results for trembling aspen have been verified against the centrifuge method (Anderegg *et al.*, 2013b). For ponderosa pine, we had difficulty maintaining the slight positive pressure recommended by Maherali & DeLucia (2000) in the six-chamber pressure manifold used to induce xylem tensions. Because of this, we removed data from some branches that appeared to refill considerable cavitation at higher xylem tensions (final $n = 25$ branches). Xylem P50, the xylem tension at which branches reach 50% loss of conductivity, was calculated for

each elevation by fitting an exponential sigmoidal function of the form:

$$PLC = \frac{100}{1 + \exp(a(\psi - b))}, \quad (2)$$

where PLC is the percentage loss of conductance, ψ is the induced xylem tension, a is the shape parameter, and b is the P50 value (i.e., the ψ that causes 50% loss of conductivity) (Pammenter & Vander Willigen, 1998). Parameters a and b and their 95% confidence intervals were estimated for each elevation by nonlinear least squares using the R statistical software (R Core Team 2014) combining data from all branches from that elevation. Vulnerability curve results are reported in terms of percentage loss of conductivity from maximum conductance, but vulnerability curves were also constructed using raw conductance values with qualitatively similar results (see Fig. S4). The hydraulic safety margins (the difference between the xylem tension required to induce 50% embolism – P50 – and the strongest xylem tensions experience in the field— ψ_{\min}) for each elevation were calculated using the P50 estimated from xylem vulnerability curves (above) and the actual midday xylem tensions measured at that elevation.

Finally, we integrated the xylem tension, $A_L:A_S$ and stem conductivity measurements to model stomatal conductance, using the model proposed by Whitehead & Jarvis (1981) to estimate water movement through a plant at steady state. Specifically, canopy gas exchange is modeled as:

$$g_s = c \frac{1}{A_L : A_S} K_s \left(\frac{\Delta\psi}{h} \right) \frac{1}{VPD}, \quad (3)$$

where g_s is stomatal conductance; c is a coefficient representing the specific heat and density of air, the latent heat of vaporization, and the viscosity of water; $A_L:A_S$ is the leaf area-to-sapwood area ratio; K_s is the sapwood area-specific conductivity; $\Delta\psi/h$ is the pressure drop across the plant (midday xylem tension – predawn xylem tension) divided by the total path length (typically approximated by tree height); and VPD is the vapor pressure deficit (Whitehead & Jarvis, 1981). We estimated mean midday VPD (average of measurements at 13:00 hours and 15:00 hours) for each elevation between June 1st and June 18th 2014 using four temperature and relative humidity sensors (Maxim iButtons, DS1923) shielded by white funnels and placed in the canopy of focal trees or nearby conifers (Lundquist & Huggett, 2008), two at ponderosa's low-elevation range margin, one at the ponderosa/aspen transition zone (high ponderosa margin, low aspen margin), and one at aspen's high-elevation margin, and then linearly interpolating VPD at the range center of each species. Using the individual values of branch $A_L:A_S$, branch K_{\max} , $\Delta\psi$ (midday xylem tension minus predawn xylem tension), and h (tree height as a proxy for total path length), we calculated relative g_s for each focal tree. We assume whole tree K and $A_L:A_S$ values to be proportional to branch values and report stomatal conductance values calculated via Eqn (3) as the percentage of mean mid-elevation g_s for each species. Ponderosa $A_L:A_S$ and K_{\max} were corrected for branch diameter based on the branch diameter or log(branch diameter) coefficients from the mixed-effects models for each trait discussed below. As is

heuristically evident by Eqn (3), stomatal conductance (g_s) is intimately tied to whole-plant hydraulics and multiple feedback and feedforward processes relate g_s to leaf xylem tension. Stomatal behavior is often discussed as falling on a spectrum between 'isohydric' (plants that limit conductance to maintain a stable maximum xylem tension) and 'anisohydric' (plants that regulate stomata less strongly in response to either xylem tension or evaporative demand and thus have larger variations in xylem tension)(Klein, 2014), and stomatal behavior is tightly coupled with hydraulic parameters and a plant's general water use strategy (Sperry *et al.*, 2002).

Statistics

To assess the effect of elevation on individual traits, we constructed mixed-effects models for each species relating raw trait values, or in some cases, power transformed-trait values (see Tables S1 and S2), to elevation, with a random effect of stand to account for the nested data structure. We coded elevation categorically (low, mid, and high), and tested for a significant effect of elevation via a likelihood ratio test (LRT) against a null model (model with only random effects and an intercept). Where elevation proved significant via likelihood ratio testing, we also used the Satterthwaite approximation of marginal fixed effect significance implemented in the 'lmerTest' R package (Kuznetsova *et al.*, 2014) to test post hoc whether trait values at either range margin differed significantly from the range center. We report these significant differences with an asterisk (*) over significantly differing range margins in figures. A subset of ponderosa pine traits showed a relationship with branch diameter, so we also included branch diameter (wood density, $A_L:A_S$) or log(branch diameter) (SLA, $K_{\text{nat_Leaf}}$, K_{max}) as a covariate in these models (Table S1). For mean annual BAI, we performed the same likelihood ratio test to assess the effect of elevation on growth, but included Hegyi's competitive index and diameter at breast height as covariates.

Competitive index was never included in the best model as determined by AIC for any trait other than growth, so we did not include it in analyses of morphological and physiological traits variation. Although tree age did not differ significantly across elevation, we also built trait models including tree age as a covariate to test for maturation-related effects on tree traits. However, with the exception of tree height in ponderosa pine, tree age was never included in the best-fit models and was therefore excluded from the final analysis.

We also fit mixed-effects models with elevation as a continuous linear predictor and a quadratic predictor to assess whether these continuous models better described our results, using AIC to compare categorical vs. continuous models. However, continuous models never showed a ΔAIC of >2 from the null or best categorical models, and results were qualitatively very similar to categorical elevation models, so we report only results from the categorical models.

For xylem tension measurements, we performed model selection on mixed-effects models (with stand and tree as random effects to account for nested data structure) including a null model, elevation as a continuous linear predictor, time of

day, and an elevation*time of day interaction (quadratic elevation*time of day interaction was not included due to the difficulty of interpreting elevation*time of day interactions). We then selected the best-fit model based on AIC and performed a likelihood ratio test against the null model and the next best model. A best-fit model including an interaction effect suggests that predawn and midday xylem tensions of a species did not change similarly across elevation. All models were constructed using the 'lme4' package (Bates *et al.*, 2014) and 'lmerTest' package (Kuznetsova *et al.*, 2014) in the R statistical environment version 3.1.0 (R Core Team 2014). For xylem tension measurements, significance of individual fixed effects was determined using the Satterthwaite approximation implemented in the 'lmerTest' package (Kuznetsova *et al.*, 2014). We verified the normality of all model residuals visually, and either log transformed or power transformed the trait data where necessary (see Tables S1 and S2 for details). Where necessary, extreme outliers were removed (see Tables S1 and S2 for final sample sizes excluding missing data and extreme outliers). Data files and R code for all analyses are provided in the Appendix S1–S6.

Results

Growth analysis

Mean annual basal area increment (BAI) increased with elevation, almost tripling from the low to the high-elevation range margin in ponderosa (from 762 ± 75 to 2179 ± 186 mm² yr⁻¹, mean \pm SE) and almost doubling in aspen (from 696 ± 58 to 1245 ± 97 mm² yr⁻¹ – Fig. 2). After accounting for the effects of DBH and competitive index, BAI of ponderosa pine remained low at the lower range margin and the mid-elevation range center, and significantly increased only at high elevations ($P < 0.0001$, Table S1). Meanwhile, aspen BAI also increased nonlinearly with elevation, increasing from low- to mid-elevation ($P < 0.0001$, Table S2), but then remaining stable from mid- to high elevation.

Xylem tensions across elevation

Predawn measurements of branch xylem tension (a proxy for soil water potential) of both species showed increasingly limited midsummer soil moisture (higher tensions) descending across the elevational range (Fig. 3, effect of elevation on predawn tension from mixed-effects model: ponderosa $P < 0.00001$, aspen $P = 0.008$, see Table S3). For both species, the elev * time of day interaction model was the best mixed-effects model as determined by AIC (likelihood ratio test, ponderosa: $P \ll 0.00001$; aspen: $P = 0.005$). Interestingly, predawn xylem tensions varied considerably more across the range of ponderosa pine (1.44–0.89 MPa from low- to high elevation) than across that

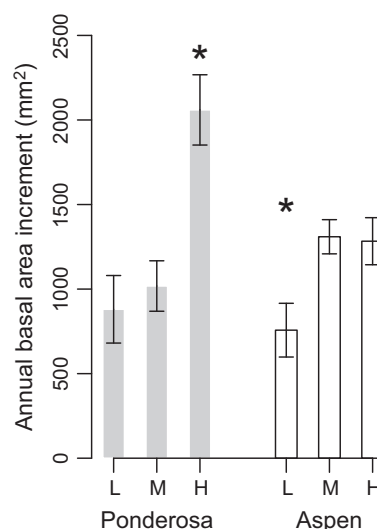


Fig. 2 Mean annual basal area increment (BAI) increased with elevation across the range of ponderosa pine and aspen. Bars show the mean (\pm SE) annual growth (averaged from 2003 to 2012) for a tree of mean DBH and mean competitive environment. Asterisks (*) indicate margins significantly different ($\alpha = 0.05$) from the range center based on mixed-effects models.

of aspen (0.58–0.47 MPa). This variation is more similar to the nonlinear decrease in PET with increasing elevation than the linear increase in precipitation across the study gradient (Fig. 1). Also, even though high-elevation ponderosa stands and low-elevation aspen stands were closely co-located in the ponderosa-aspen transition zone (plot centers of aspen and ponderosa stands sometimes differed by <100 m), aspens showed lower predawn xylem tensions by on average 0.3 MPa. This difference is not explained by their ~ 5 m height difference at this elevation, which accounts for only 0.05 MPa of added gravitational potential.

Midday xylem tensions also were greater at low elevation in both species (Fig. 3). However, in ponderosa pine the increase in midday xylem tensions was significantly less than the increase in predawn xylem tensions (Fig. 3, elev*midday interaction $P = 0.001$), resulting in an average daily change in xylem tension due to daily transpiration ($\Delta\psi$) of 0.73 ± 0.08 MPa (mean \pm SE) at high elevation and only 0.31 ± 0.06 MPa at low elevation. This suggests either a very large increase in hydraulic efficiency or considerable stomatal closure at low elevations. In contrast, approaching the low-elevation margin of aspen midday xylem tensions increased slightly more than did predawn xylem tensions, resulting in a predawn to midday tension difference of 0.94 ± 0.05 MPa at high elevations and 1.12 ± 0.04 MPa at low elevations (Fig. 3, Table S4, elev*midday interaction $P = 0.006$).

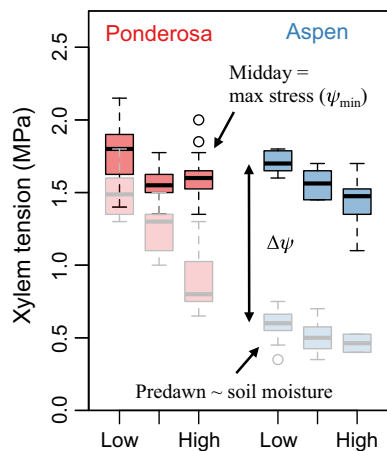


Fig. 3 Predawn (light) and midday (dark) xylem tensions across the elevation range of ponderosa pine (red) and aspen (blue). Xylem tensions were assessed at the peak of growing season water stress. Midday xylem tensions represent the maximum water stress experienced by the sampled trees, while predawn xylem tensions are indicative of soil water potential. The difference between them is the potential drop ($\Delta\psi$) used in Eqn 3.

Morphological traits

The study species showed clinal variation in very different morphological traits. Median leaf size decreased strongly with decreasing elevation in ponderosa (Fig. 4a, Table S1, LRT $P = 0.0018$) but not in aspen (Table S2, LRT $P = 0.14$). The height of adult canopy trees was similar for both tree species, and remained stable across all elevations except at aspen's low elevational range edge (Fig. 4b, ponderosa LRT $P = 0.29$; aspen LRT $P = 0.00003$). In contrast, the ratio of leaf area to sapwood area ($A_L:A_S$) showed no pattern in aspen ($P = 0.73$) but increased significantly at the upper range margin of ponderosa pine (Fig. 4c, LRT $P = 0.025$).

Finally, SLA and wood density showed an increase in the carbon cost of tissues at aspen's low-elevation range boundary, but showed no elevational trends for ponderosa pine. SLA of low-elevation aspen trees was significantly lower (i.e., more carbon per unit leaf area) than SLA at the range center or upper range margin (Fig. 5a, LRT $P = 0.012$, low elevation differed from mid $P = 0.016$) whereas ponderosa pine showed no significant change in SLA. Branch wood density also showed no significant relationship with elevation in ponderosa pine (Fig. 5b), while wood density decreased strongly across the elevational range of aspen (LRT $P = 0.0008$).

Hydraulic traits

At the height of midsummer water stress, neither native leaf area-specific hydraulic conductivity ($K_{\text{nat_Leaf}}$:

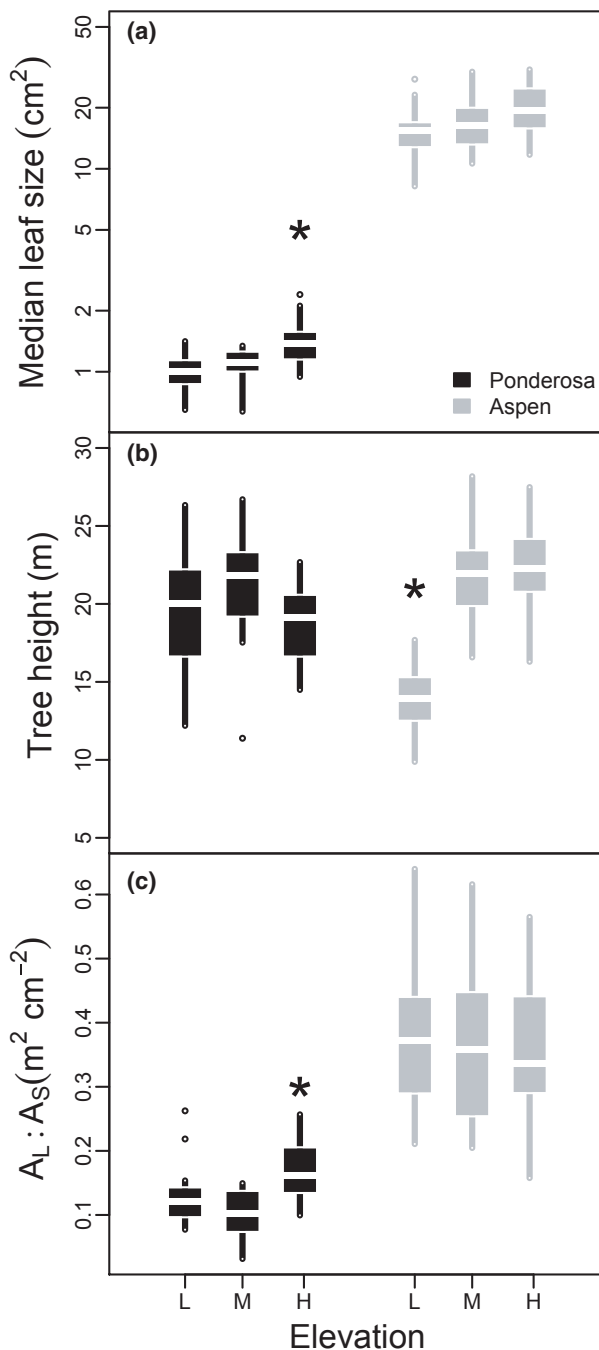


Fig. 4 Morphological adjustments across the elevation range of ponderosa pine (black) and trembling aspen (gray). (a) Median leaf/needle size (note log y axis), (b) tree height, (c) leaf area-to-sapwood area ratio ($A_L:A_S$). Asterisks (*) denote a significant difference ($\alpha = 0.05$) of the range margin from the range center. Boxplots show the median (bar), interquartile range (box), range (lines), and outliers (points).

a measure of how well hydraulically supported a unit leaf area is) nor sapwood area-specific maximum conductivity (K_{max} : conductivity with embolism removed,

a measure of maximum xylem hydraulic efficiency) showed any trend with elevation in aspen (Fig. 6). In ponderosa, once $\log(\text{stem diameter})$ was included as a covariate in the mixed-effects models, $K_{\text{nat_Leaf}}$ also showed no relationship with elevation (Fig. 6a). Meanwhile, ponderosa K_{max} decreased significantly from mid- to low elevation (Fig. 6b, LRT $P = 0.001$, low differs from mid-elevation $P = 0.02$).

Xylem vulnerability to cavitation increased significantly with elevation in aspen, but showed no significant clinal variation in ponderosa (Fig. 7). The vulnerability curve of low-elevation aspen was considerably more resistant than that of mid- and high-elevation aspen (Fig. 7b), resulting in a higher P50 value (xylem tension required to cause 50% cavitation) at low elevation (Fig. 7b, 95% confidence interval 2.4–2.8 MPa at low elevation vs. 1.6–1.9 MPa at mid-elevation and 1.4–1.9 MPa at high elevation). We found a slight but non-significant decrease in the P50 with increasing elevation in ponderosa (Fig. 7a, all 95% confidence intervals overlap), although there was considerably more uncertainty in our estimates of ponderosa P50 than aspen P50.

By subtracting the midday xylem tensions measured in the field from P50 values estimated for each elevation from the xylem vulnerability curves above, we calculated the ‘hydraulic safety margin’ for each elevation. Even though aspen midday xylem tensions were elevated at lower elevations, the hydraulic safety margin was much larger in low-elevation trees than mid- or high-elevation trees (e.g., low-elevation safety margin of 0.96 ± 0.02 MPa compared to mid-elevation margin of 0.21 ± 0.03 MPa, LRT $P \ll 0.001$ Fig. 8a). This suggests that aspen do not just grow stronger xylem at low

elevations, but are actually more conservative in their xylem anatomy at low elevations. Hydraulic safety margin also decreased significantly with elevation in ponderosa ($P < 0.001$, Fig. 8a, Table S2). However, these calculations do not incorporate the relatively large uncertainty in ponderosa P50 values (Fig. 7a) because safety margins for each tree were calculated using the elevation mean P50 value. Because the documented change in safety margin is small (0.25 MPa from low- to mid-elevation) compared both to the uncertainty in P50 value (mean P50 confidence interval range was 1.1 MPa) as well as to the safety margin differences observed in aspen (0.75 MPa from low- to mid-elevation), the observed differences in hydraulic safety margin for ponderosa pine are not necessarily biologically significant.

Further corroborating a biologically relevant increase in hydraulic safety margin in low-elevation aspens, native embolism (measured as percentage loss of conductivity or PLC) increased with increasing elevation in this species (Fig. 8b, LRT $P = 0.001$, low differs from mid $P = 0.004$), despite decreasing midday xylem tensions. Meanwhile, ponderosa pine branch PLC at mid-summer was consistently quite low at all elevations (Fig. 8b), suggesting that the significant decrease in hydraulic safety margin in this species may be either a statistical artifact or not biologically significant.

Finally, estimates of steady state, midsummer stomatal conductance (g_s) appeared stable across the elevational range of aspen (Fig. 8c), but decreased precipitously across the elevation range of ponderosa pine (Fig. 8c, LRT $P = 0.002$). In ponderosa, large differences in g_s despite relatively small differences in

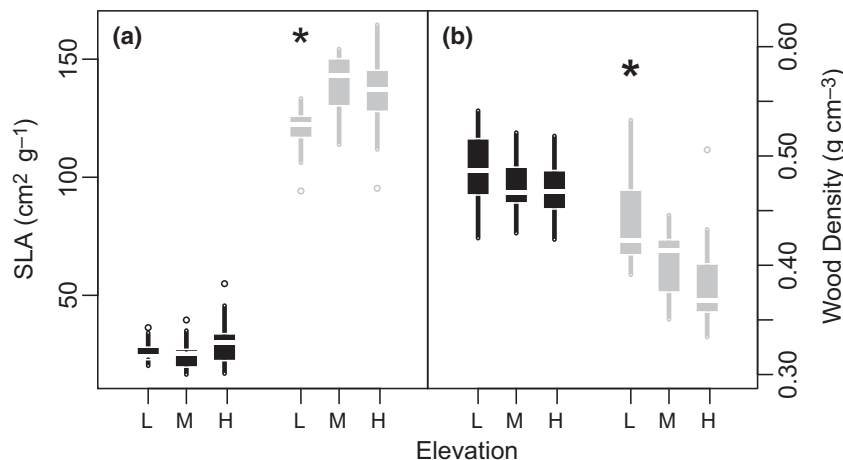


Fig. 5 Variation in specific leaf area (a) and branch wood density (b) with elevation in ponderosa pine (black) and aspen (gray). Specific leaf area (SLA = leaf area/dry mass) decreased and branch wood density increased near the lower (dry) range boundary of trembling aspen but showed no significant variation with elevation in ponderosa pine. Asterisks (*) denote a significant difference ($\alpha = 0.05$) of the range margin from the range center. Boxplots show the median (bar), interquartile range (box), range (lines), and outliers (points).

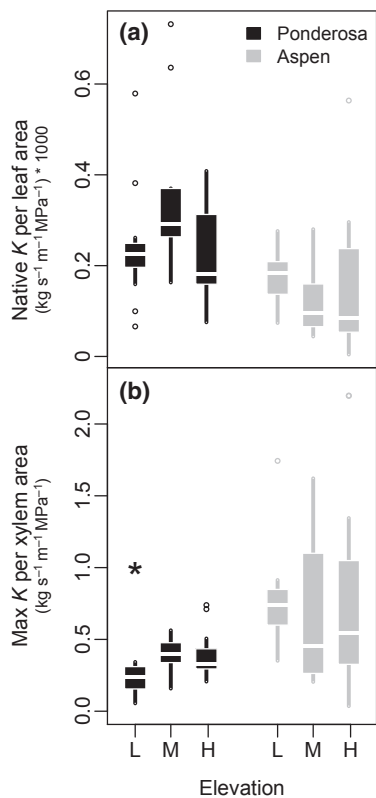


Fig. 6 Ponderosa show decreased hydraulic efficiency at low elevation while aspen show no clinal hydraulic trait variation. (a) Branch native conductivity per unit leaf area ($K_{\text{nat_Leaf}}$) shows no elevational variation in either species (b) maximum hydraulic conductivity (post embolism removal) per unit xylem area (K_{max}) is higher at mid- and high elevations than at low elevation for ponderosa pine (*Pinus ponderosa*). Boxplots show the median (bar), interquartile range (box), range (lines) and outliers (circles). Asterisks (*) indicate a margin significantly different ($\alpha = 0.05$) from the range center based on mixed-effects models.

maximum xylem tensions (Fig. 3) suggest a relatively isohydric strategy compared to aspens, which experience larger geographic variation in xylem tensions with no decrease in g_s (although such a delineation can be somewhat difficult, Klein, 2014; Franks *et al.*, 2007). By integrating values of morphological (Fig. 4c) and hydraulic traits (Fig. 6b) with xylem tensions measured in the field (Fig. 3) and changes in evaporative demand (VPD, not shown), our model estimates suggest that mid- and low-elevation ponderosas have 41% and 22% (respectively) of the stomatal conductance of ponderosa at the high-elevation range margin, implying drastically curtailed transpiration near the dry range boundary of this species. Meanwhile, the model suggests that, decreases in height in low-elevation aspen sufficiently offset increases in VPD to maintain g_s equal to or greater than mid-elevation g_s .

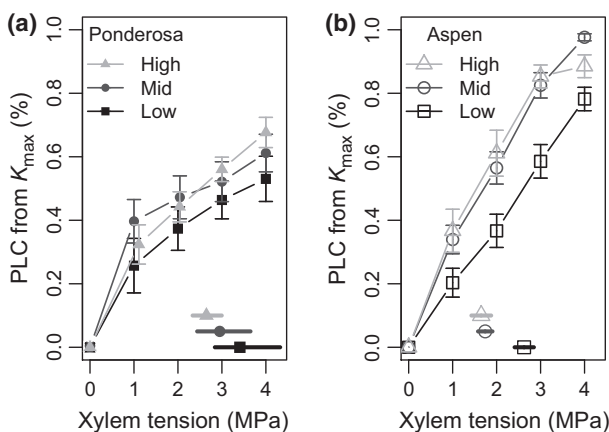


Fig. 7 Xylem vulnerability curves for low-, mid-, and high-elevation trees of (a) ponderosa pine, and (b) trembling aspen. Points in curve show means \pm SE of PLC values (percentage loss of conductivity from K_{max}) at xylem tensions induced via air seeding. P50 values, or the xylem tension at which 50% PLC is reached, are displayed as points along the bottom of the figure (solid horizontal lines indicate 95% confidence intervals for P50 estimates). Xylem vulnerability to cavitation did not differ significantly across elevation in ponderosa, while low-elevation aspen showed significantly more resistant xylem than mid- and high-elevation aspen.

Discussion

Our results suggest that two major North American tree species occurring along different portions of an aridity gradient employ drastically different strategies for coping with increased water limitation at their dry range boundary. Ponderosa pine showed little variation in key morphological and hydraulic traits influencing drought avoidance and drought tolerance, and thus appeared to minimize water stress primarily by strongly limiting transpiration in drier habitats. In contrast, trembling aspen showed a considerable decrease in the vulnerability of its hydraulic system to drought-induced cavitation at its dry range edge, suggesting a strategy of increased drought tolerance in response to aridity. Despite these adjustments, growth of both species was constrained at their low-elevation range boundary, potentially indicative of a limit to ponderosa's drought avoidance capacity and aspen's drought tolerance capacity at higher levels of aridity. Below we discuss each species in turn.

Trembling aspen

Aspen trees tolerate water limitation near their dry range edge by protecting their hydraulic system against xylem cavitation during chronic stress, rather

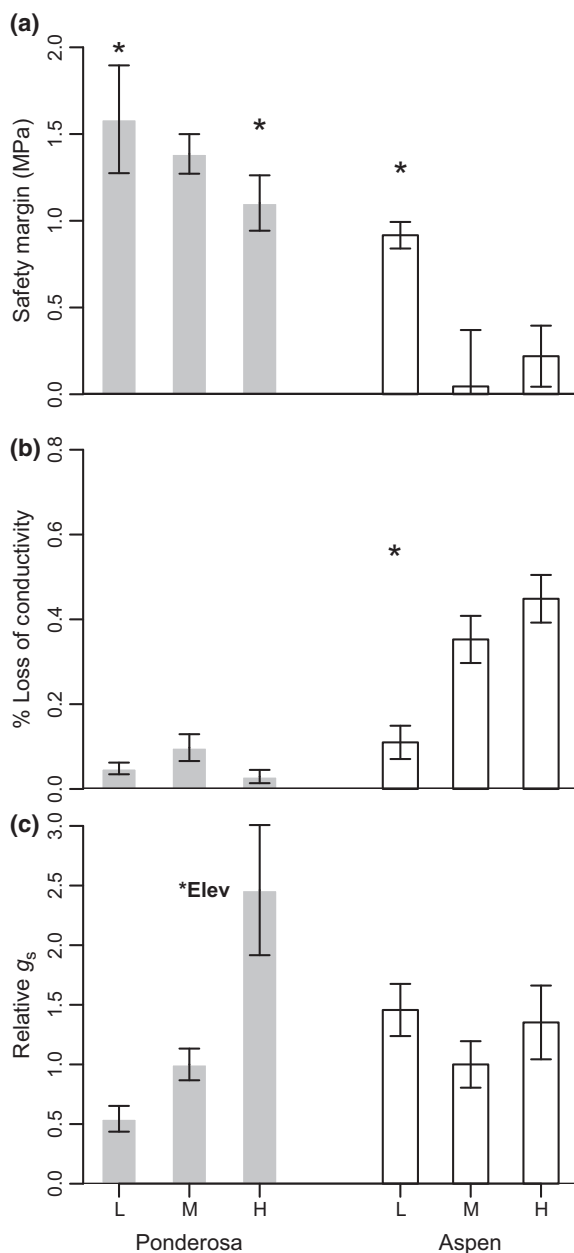


Fig. 8 At low elevations, aspen increase hydraulic safety while ponderosa pine curtail stomatal conductance. (a) Hydraulic safety margin (\pm SE) calculated from xylem vulnerability curves and midday water potentials, (b) percentage loss of conductivity (PLC) due to extant embolism in branches, and (c) modeled stomatal conductance (g_s) of ponderosa pine (gray) and trembling aspen (white). g_s is standardized so that mid-elevation g_s equals one for each species. Trembling aspen exhibits an increased hydraulic safety margin and decreased PLC at low elevation, while ponderosa shows greatly decreased g_s at mid and low elevations. Asterisks (*) show range margins that significantly differ ($\alpha = 0.05$) from the range center based on linear mixed-effects models, and '*Elev' indicates a significant effect of elevation but no post hoc significant differences between range margins and range center.

than by avoiding water stress through increased hydraulic efficiency or decreased water use. Pre-dawn xylem tension measurements suggested that soil moisture becomes only slightly more limited approaching aspen's low-elevation boundary. However, maximum water stress (i.e., midday xylem tensions) increased significantly approaching aspen's low-elevation margin (Fig. 3) presumably due to increased evaporative demand. Aspens respond by growing shorter trees with denser wood, bearing smaller, lower SLA (i.e., more carbon dense) leaves, and by growing stronger xylem. Previous research suggests that the main mechanism of drought-induced mortality in aspen is the deterioration of hydraulic function caused by cavitation during drought and subsequent xylem 'cavitation fatigue' (Anderegg *et al.*, 2012, 2013b). Given the possibly fatal consequences of cavitation, evolutionary forces may drive aspen to become increasingly hydraulically conservative where drought is most prevalent (Fig. 8). This intraspecific pattern contrasts with the findings of Choat *et al.* (2012), who found little relationship between climate dryness and angiosperm hydraulic safety margins across species.

Basal area growth decreased strongly in low-elevation aspen, even as leaf and stem tissue showed increased carbon investment. Although wood density arises through many aspects of xylem anatomy, reduced xylem vulnerability to cavitation via increased vessel wall thickness likely plays some role in increasing branch wood density in low-elevation aspens (Lens *et al.*, 2010) while making each unit of conducting area more energy intensive to grow. Likewise, SLA varies in response to multiple environmental cues and anatomical differences, but low SLA aspen leaves likely increase leaf drought tolerance, possibly by decreasing leaf turgor loss point, (Merchant *et al.*, 2007; Bartlett *et al.*, 2012) and/or increasing leaf capacitance (Ishii *et al.*, 2014). This increased drought tolerance appears to come at the cost of more structural carbon for every unit of leaf area. We lack data on whole-plant carbon balance, but a significant positive relationship between SLA and mean BAI (LRT = 0.046) and a negative but non-significant relationship between wood density and BAI (LRT = 0.289) at the individual level supports this idea (Fig. S5). Even though available photosynthate and nonstructural carbon stores do not directly regulate growth (Körner, 2015), this coordination between increased cost of growth and decreased amount of growth may indicate a limit to aspen's drought tolerance with increasing aridity mediated by the carbon cost of tolerance traits.

Ponderosa pine

Low-elevation ponderosa pines considerably curtail their transpiration, and most likely carbon uptake, during some if not most of the growing season. This reduction in water use is associated with vastly reduced growth at the low-elevation range margin and range center compared to the high-elevation margin (Fig. 2). In contrast to aspen, ponderosa pine showed remarkably few trait adjustments toward either increased drought tolerance or drought avoidance at the lower range margin, despite increasing soil moisture limitation and evaporative demand (Figs 1 and 3). For example, ponderosa pine exhibited no elevational variation in height and wood density, and only slight clinal variation in leaf morphology (Fig. 4). Surprisingly, we found only subtle, although statistically significant, variation in branch-level leaf area:sapwood area ratio – $A_L:A_S$ (Fig. 4, $0.151 \pm 0.027 \text{ m}^2 \text{ cm}^{-2}$ at low elevation vs. $0.199 \pm 0.023 \text{ m}^2 \text{ cm}^{-2}$ at high elevation), a trait that has been implicated as a main mediator for hydraulic adjustment across space in other pine species (e.g., branch level: Martinez-Vilalta *et al.*, 2009; whole tree level: Mencuccini & Bonosi, 2001) and in previous studies of montane vs. desert ponderosa pine populations (Maherali & DeLucia, 2000; Maherali *et al.*, 2002). Variation in $A_L:A_S$ appeared to be primarily driven by changes in leaf area, rather than branch scale adjustments – as changes in leaf size and $A_L:A_S$ were similar in magnitude from high to low elevation (median leaf area –28.2% and $A_L:A_S$ –23.7% decrease). Indeed, when trees were relativized to percentage change from the high-elevation average, the slope of the total least squares regression between $\% \Delta A_L:A_S$ and $\% \Delta$ median leaf area was near one (Fig. S5). A different study of water relations in ponderosa pine in a contiguous riparian and hill-slope population did not find ecotypic variation in branch $A_L:A_S$ (Stout & Sala, 2002), which suggests that intraspecific variation in $A_L:A_S$ may only be detectable across larger geographic gradients, possibly because specific hydraulic adjustments are tailored to the moisture release curves of the soils on which the trees grow (Barnard *et al.*, 2011). In addition, our study trees had limited variation in age, density, and tree size (Table 2), which further constrains potential variation compared to previous studies. Future study is required to determine how changes in branch $A_L:A_S$ relate to whole tree $A_L:A_S$, as whole tree characteristics can be modified by canopy structure (Berninger *et al.*, 1995) and stand development/tree size (Mencuccini & Bonosi, 2001; McDowell *et al.*, 2002; Martinez-Vilalta *et al.*, 2009) and represent an additional scale of hydraulic adjustment not measured here.

Ponderosa pine also exhibited little hydraulic adjustments to tolerate or avoid high xylem tensions in drier habitats in our study. Specifically, we found no clinal variation in branch vulnerability to cavitation, consistent with previous work on ponderosa populations (Maherali & DeLucia, 2000; Stout & Sala, 2002) and more geographically extensive work in other pine species (Martinez-Vilalta *et al.*, 2009; Lamy *et al.*, 2013; Sáenz-Romero *et al.*, 2013). We found a small increase in hydraulic safety margins in low-elevation ponderosa; however, this did not translate into increased cavitation in trees with smaller margins. We also found slight elevational differences in xylem area-specific maximum conductivity (K_{max}), suggesting *less* hydraulic efficiency at low elevation (Fig. 6) contrary to expectations and to some previous findings in ponderosa pine (Maherali *et al.*, 2002; Barnard *et al.*, 2011 but see Stout & Sala, 2002). Xylem capacitance (the amount of water stored in xylem) is a final mechanism that ponderosa may use to buffer their hydraulic system against extreme xylem tensions without curtailing transpiration (Domec & Gartner, 2003; Barnard *et al.*, 2011). However, capacitance has previously been found to correlate strongly with xylem P50 and less strongly with K_{max} in trunk xylem of ponderosa pine (Domec & Gartner, 2003; Barnard *et al.*, 2011), suggesting minimal elevational differences in xylem capacitance in our system.

Our estimates of relative stomatal conductance (g_s) suggest that study ponderosa pines strongly regulate water loss via stomatal closure at low elevations (Fig. 8c), rather than avoiding or tolerating drought through hydraulic or morphological adjustment. This 78% decrease in g_s is likely associated with a smaller but considerable decrease in assimilation. Maintaining assimilation at such reduced conductance rates would require water use efficiency (WUE) to more than double, which is considerably beyond the ~40% increase in WUE to be expected at low values of g_s based on ponderosa assimilation curves (e.g., Cregg, 1994). Nor is such a large WUE decrease consistent with plastic or genetic differences in WUE documented in ponderosa pine provenance trials (e.g., Cregg & Olivas-García, 2000), drought experiments (e.g., Cregg, 1994; Zhang *et al.*, 1997), or observations across elevation (McDowell *et al.*, 2010). Thus, the greatly reduced g_s per leaf area suggested by our measurements and decreased branch $A_L:A_S$ likely results in decreased whole-tree carbon assimilation.

Alternatively, high transpiration and assimilation could theoretically be maintained at low elevation despite small potential differences between predawn and midday xylem tensions ($\Delta\psi$) (Fig. 3) via drastic increases in hydraulic efficiency or capacitance. How-

ever, our hydraulic results show decreasing efficiency (K_{\max}) at low elevations (Fig. 6). While mid- and low-elevation branch $A_L:A_S$ did show on average a ~25% and ~27% decrease (respectively) from high elevation $A_L:A_S$ (Fig. 4), the Whitehead & Jarvis (1981) model suggests that a decrease of 76% and 86% would be necessary to offset the decreased $\Delta\psi$ and increased VPD at mid- and low elevation and maintain transpiration. Martinez-Vilalta *et al.* (2009) found $A_L:A_S$ changes approaching this magnitude across the entire geographic range of Scots pine (*Pinus sylvestris* L.) in Europe, but this trait appears more constrained either in ponderosa pine as a species, in the study population in the absence of local adaptation, or in stands of very similar structure.

Other factors influencing drought responses

Although this study suggests that trembling aspen is drought tolerating and ponderosa pine is drought avoiding at their dry range edges, additional drought avoidance and tolerance mechanisms may also be important. In particular, belowground traits related to rooting depth, morphology, hydraulics, and allometry (not measured in this study) could influence the elevational trends in xylem tensions that we documented. Lower predawn xylem tensions of low-elevation aspen compared to co-occurring high-elevation ponderosa pine (some aspen and ponderosa stands were located <50 m apart) suggests that rooting depth may play a role in the two species' drought resistance strategies (Fig. 3). However, aspens at the study site have extremely shallow functional rooting depths even when the shallow soil is dry (i.e., during natural or experimental drought, Anderegg *et al.*, 2013a). This suggests that lower predawn xylem tensions in aspen result from either slightly wetter micro-sites or a more extensive rooting area with less local soil-dry down, not from a deeper rooting depth than ponderosa. In addition, elevational differences in recovery potential following drought stress (a third class of drought resistance strategy) could be important. Assessment of recovery potential in the field is difficult because easily measured functional traits have rarely been linked to recovery ability, although some evidence suggests that embolism refilling may be correlated with wood density and P50 in angiosperms (Ogasa *et al.*, 2013). This relationship suggests that low-elevation aspens may be both more drought tolerant and better at postdrought recovery if it holds within species as well as between species. Finally, phenology of leaf/needle expansion and senescence compared to xylem growth may vary across elevation, and could alter plant hydraulics over the growing season by shifting leaf area:sapwood area

ratios on relatively short time scales. Our study provides only a midsummer (peak water stress) snapshot of these traits, and additional temporally resolved studies are warranted.

We also note that the clinal trait variation we observed in trembling aspen may be somewhat larger than that observed elsewhere in aspen's range, owing to a massive drought-induced mortality event in the early 2000s (affecting ~20% of the aspen in the study area – Huang & Anderegg, 2011), principally at low elevations. This mortality event may have selected for ramets with extreme trait values at low elevations, although some of the traits showing clinal variation have previously shown considerable temporal plasticity in the study area (e.g., P50: Anderegg *et al.*, 2013b; leaf size: Anderegg *et al.*, 2014). However, the P50 differences within aspen documented herein are in the opposite direction of the xylem fatigue documented by Anderegg *et al.* (2013b) in low-elevation aspen following the mortality inducing drought, suggesting this trait may actually show *larger* clinal trait variation in other parts of aspen's geographic range.

In addition, the morphological and physiological adjustments to geographic variations in water availability documented here are distinct from but still relevant to the short-term physiological responses of plants to acute drought, including those leading to mortality. Drought-induced tree mortality is an area of active research (Hartmann *et al.*, 2015), centering around the interlinked roles of the hydraulic and carbon economies in trees (McDowell *et al.*, 2011; Anderegg *et al.*, 2012). It has become clear that mortality is a complex set of many interacting processes and mechanisms, many of which will be strongly influenced by the traits explored here. In particular, widespread mortality of aspen in the region has been linked to the gradual deterioration of plant hydraulic transport (Anderegg *et al.*, 2014, 2015), fitting our observation of increased drought tolerance with apparently little stomatal closure in chronically dry, low-elevation aspens (suggesting a relatively anisohydric stomatal strategy). This may maximize tree performance in dry habitats during most years but make them susceptible to hydraulic damage during drought. In addition, drought-induced mortality in ponderosa pine has been associated with increased growth sensitivity to climate and chronically constrained gas exchange (McDowell *et al.*, 2010), which aligns well with the responses to chronic water limitation documented here.

Determining whether the functional trait variation documented in this study is driven by phenotypic plasticity or local genetic adaptation is critical for understanding future range boundary dynamics of these two species. We believe phenotypic plasticity

likely plays a predominant role in this system. For ponderosa pine, gene flow is large and population differentiation is low across much larger geographic distances than studied here (Maherali *et al.*, 2002), suggesting that elevational trait differences in this species are principally plastic. For aspen, much trait variation likely resulted from phenotypic plasticity as well. We were able to partially examine plasticity for this clonal species by comparing trait variation within stand (representing within-individual variation) and between stands (representing variation between individuals) for all traits except P50 (not measured per individual). Within-stand (likely plastic) variation was considerably larger than between-stand variation for leaf size, SLA, $A_L:A_S$, PLC, and wood density (Table S3). Meanwhile, height, $K_{\text{nat_Leaf}}$ and g_s showed equal or greater between-stand variation than within-stand variation, which may possibly indicate a genetic or micro-site signal.

Implications for range shifts

Both focal species showed very large decreases in basal area growth at their low-elevation range boundary likely related to general fitness decreases – implying that the differences in the physiological strategies employed by the two species to cope with drought stress will matter during climate change-induced range shifts. It is also possible that these growth decreases may represent adaptive responses to water stress (particularly if carbon is invested below ground instead). More study is clearly warranted, but we think fitness differences are more likely. For one, reproductive output is typically a function of tree size that then compounds over a tree's life time (such that growth and fecundity are correlated if mortality rates are constant). In addition, at least within a stand, growth and mortality rate tend to be inversely correlated (slow growing trees die more often, Wyckoff & Clark, 2000, 2002). While a trade-off between growth and survival (i.e. a positive correlation between growth and mortality or 'demographic compensation') has been documented across the range of alpine perennials (Doak & Morris, 2010), we found no evidence for such a trade-off, at least in aspen (tree age did not differ between high and low elevation in aspen, see Fig. S7).

The diametrically opposite drought tolerance and drought avoidance strategies and (therefore) contrasting physiological range constraints for ponderosa and aspen may imply differing range boundary dynamics over the coming century. Drought avoidance via stomatal closure is a rapid response to water stress (timescale of hours to days), likely helping ponderosa pine avoid extreme spikes in water deficit such as single-year droughts. However, heavy reliance on stomatal

closure rather than longer term adjustments may decrease ponderosa pine's ability to reach maturity and/or maintain significant reproductive output under sustained drought or long-term drying trends if low-elevation trees are carbon limited. Long-term decreases in assimilation in low-elevation ponderosas may also increase their susceptibility to bark beetles, both through decreased resin duct formation and decreased resin pressures (Kane & Kolb, 2010). While ponderosa's drought-avoidant physiology would suggest that range shifts driven by increasing drought and long-term aridification should be gradual, the synergistic potential between drought and insect attack could still lead to very rapid range contractions via mass die-off. Indeed, one such die-off-induced range contraction has been documented in ponderosa pine, which occurred in conjunction with an extreme drought as well as a bark beetle outbreak (Allen & Breshears, 1998).

In contrast, trembling aspen's strategy of increasing drought tolerance by building more tolerant organs is a much slower response than the stomatal closure of ponderosa pine (timescale of months to decades). This could allow aspens to acclimate to long-term drying trends but leaves them vulnerable to short-term drought extremes. In the absence of short-term avoidance measures such as stomatal closure, aspen experience catastrophic embolism that can lead to rapid mortality (Anderegg *et al.*, 2014), suggesting that aspen range dynamics will be dominated by episodic contractions initiated by short but severe droughts. In addition, aspen and ponderosa pine may be sensitive to different changes in seasonal precipitation. Because it sustains midsummer transpiration, aspen may be sensitive primarily to extreme midsummer moisture stress tied to summer precipitation, growing season length, and temperature-driven evaporative demand. Indeed, a massive aspen die-off across much of the western United States was precipitated in 2002 by the most extreme single summer evaporative and soil moisture deficit of the past century (Anderegg *et al.*, 2013a), which caused fatal hydraulic failure in affected aspens (Anderegg *et al.*, 2012). Ponderosa pine, on the other hand, can perform 50–70% of its carbon assimilation outside of the growing season (Law *et al.*, 2000). Thus, ponderosa pine may respond most strongly to precipitation changes in the fall, winter, and spring that curtail assimilation during the productive 'shoulder seasons'.

These inferences assume that low-elevation trees are carbon limited. Emerging evidence suggests that this may not necessarily be the case for all trees (Körner, 2003; Sala *et al.*, 2012). However, the range dynamic implications of a drought-avoidant vs. drought-tolerant strategy are supported by the recent landscape level die-off event at the study site following the extreme

2002 drought mentioned above, which affected aspen but not ponderosa (Worrall *et al.*, 2008). The hot 2002 drought predominantly affected stands at aspen's low-elevation range margin (Worrall *et al.*, 2008, 2010) indicating probable range contractions. Meanwhile ponderosa pine trees at low elevations showed little or no growth during the drought (L.D.L. Anderegg, unpublished data). However, ponderosa pine experienced little mortality at our site and showed elevated mortality elsewhere in the southwestern USA only where beetle outbreaks occurred (Negrón *et al.*, 2009). Given the projected drying of the southwestern USA over the next century (Diffenbaugh *et al.*, 2008), ponderosa's dry range boundary may slowly contract in response to long-term drying trends that chronically depress assimilation (in the absence of pest outbreak), while aspen may be more prone to rapid and episodic range contractions in response to extreme events.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Species relative abundance across elevation at the study site in the La Plata Mountains, (San Juan National Forest, Colorado, USA).

Figure S2. Description of methods for combining Basal Area Increment datasets and Relationship between Hegyi's competitive index (2014 dataset) and number of live trees within 5m (2013 dataset).

Figure S3. Mean annual Basal Area Increment for 2014 data only and combined 2013 & 2014 data.

Figure S4. Xylem vulnerability curves showing raw xylem area specific conductivity (K) as a function of induced xylem tension.

Figure S5. Mean annual Basal Area Increment (BAI) for individual trembling aspen trees as a function of (a) SLA and (b) branch wood density.

Figure S6. Percentage difference from high elevation average $A_L:A_S$ and average median leaf area for individual ponderosa pine trees.

Figure S7. Aspen tree age across elevation from all trees in the full dataset used to calculate BAI for which ages could be estimated from tree cores ($n = 86$).

Table S1. *Pinus Ponderosa* – Details for linear mixed-effects models of functional trait data.

Table S2. *Populus tremuloides* – Details for linear mixed-effects models of functional trait data.

Table S3. Details from the best linear mixed effects models explaining branch xylem tensions for ponderosa pine and trembling aspen.

Appendix S1–S5. Comma separated value data files for Basal Area Increment analysis (LDLA_JHRL_BasalAreaIncrement_Full-dataset_102115.csv), hydraulic and morphological trait analysis (LDLA_JHRL_Traits_all_final_10_25_15.csv), and xylem vulnerability curves (ponderosa pine: LDLA_JHRL_PIPO_VcurveLong_FinalData_102215.csv, trembling aspen: LDLA_JHRL_POTR_VcurveLong_FinalData_102215.csv). Also includes metadata file with column descriptions for all four data files (LDLA_JHRL_metadata.csv).

Appendix S6. R code (file: AndereggAndHilleRisLambers2015_Code.R) used for analysis, including mixed effects models and figure generation.