Northern forest tree populations are physiologically maladapted to drought

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Northern forests at the leading edge of their distributions may not show increased primary productivity under climate warming, being limited by climatic extremes such as drought. Looking beyond tree growth to underlying physiological mechanisms is fundamental for accurate predictions of forest responses to climate warming and drought stress. Within a 32-year genetic field trial, we analyze relative contributions of xylem plasticity and inferred stomatal response to drought tolerance in regional populations of a widespread conifer. Genetic adaptation leads to varying responses under drought. Trailing-edge tree populations produce fewer tracheids with thicker cell walls, characteristic of drought-tolerance. Stomatal response explains the moderate drought tolerance of tree populations in central areas of the species range. Growth loss of the northern population is linked to low stomatal responsiveness combined with the production of tracheids with thinner cell walls. Forests of the western boreal may therefore lack physiological adaptations necessary to tolerate drier conditions.
Northern forests play a key role in carbon and water cycles, and influence climate forcings and feedbacks\textsuperscript{1-3}. Northern latitudes will be disproportionately affected by climate change, and reports of boreal forest decline\textsuperscript{4-7} are beginning to replace earlier optimism of benefits due to warming\textsuperscript{8,9}. It is therefore critical to assess the physiological capacity of northern forests to cope with changing climates, including climatic extremes. Drought in particular is anticipated to become more frequent and severe as greater climatic variability coincides with higher evaporative demand\textsuperscript{10-12}. Although trees have high plasticity, there are limitations to trees’ adaptive capacity, meaning drought may cause substantial forest productivity losses and mortality. Severe drought has been linked to vascular damage and disrupted water columns causing hydraulic failure in trees\textsuperscript{13}. Prolonged droughts may also increase forest mortality through slow depletion of tree internal carbon reserves, known as carbon starvation\textsuperscript{14}.

Both wood hydraulic traits and stomatal regulation of water loss affect vascular damage and photosynthesis under moisture limitation\textsuperscript{15-19}. Combined, xylem morphology and stomatal responsiveness contribute to a continuum of tree physiological behaviors for coping with drought. Drought-avoidant behavior involves closure of stomata under drier conditions to maintain xylem water potentials, thereby reducing cavitation risk that may lead to hydraulic failure\textsuperscript{18,20}. Alternatively, trees may produce more cavitation-resistant xylem through the formation of tracheids with thicker cell walls and smaller lumen diameters. This drought-tolerant behavior could enable stomata to remain open under prolonged drought, which may help avoid depletion of carbon reserves. While several studies point to differences in drought coping behaviors among forest tree species\textsuperscript{14,18}, very few studies have examined differences in water-use behaviours of populations within species\textsuperscript{21,22}. More information on the responses within widespread species is needed\textsuperscript{19,23}, both in terms of the amplitude of the response and the physiological mechanisms underlying these responses to drought.

Local adaptations in drought physiology are likely: wide-ranging tree species that occur across a range of climate conditions often consist of populations that have developed unique multi-trait polygenic adaptations to local climates\textsuperscript{24,25}. Provenance trials, also referred to as common garden trials, provide an experimental setting to study differences in local adaptation and effects of seed transfers\textsuperscript{26-28}: by growing multiple seed sources in common gardens, where test environments are homogenous, population-level differences can be revealed. Common garden trials therefore are suitable experiments to test intraspecific variation in water-use strategies\textsuperscript{21}. Large reciprocal transplant experiments additionally act as real-world climate change laboratories since transferring northern seeds southward simulates projected warming, while moving southern seeds northward tests survival under assisted migration. Further combining multi-decade provenance trials with tree-ring methodologies facilitates the observation of a population’s response to extreme climatic events such as drought and cold\textsuperscript{26-29}.

Population-level differences in physiological response to drought could additionally have important implications for risk trade-offs in forest management. Reforestation with local seed sources is associated with risks because climate change is causing a mismatch of local plant populations to emerging climates. The rate of decoupling is expected to exceed the ability of long-lived forest trees to acclimate, migrate through dispersal or gene flow, or adapt through natural selection\textsuperscript{30-32}. While trailing-edge populations are expected to accumulate extinction debts\textsuperscript{33}, decreased forest health and productivity can be expected throughout a species’ range due to locally adapted populations being genetically maladapted to new climate conditions\textsuperscript{34}.

Designed to overcome these issues, assisted migration is an adaptation strategy involving planting a percentage of pre-adapted seed sources from warmer, drier populations\textsuperscript{35,36}. While this approach may help realign tree populations with their historic climatic optima, a careful assessment of the nature of adaptation is critical to effectively evaluate potential risks with longer-distance seed transfers\textsuperscript{37,38}.

Recent work quantifying genetic differences in drought tolerance in terms of annual growth loss suggested northern boreal pine forests are most at-risk to drought under additional warming\textsuperscript{39}. Meanwhile, central populations were moderately drought tolerant. However, explaining the underlying physiological mechanisms behind these responses is key for assessing vulnerability of forests to drought. This is required for vegetation modeling and accurate growth predictions as new climate assemblages appear and for assessing the efficacy of assisted migration\textsuperscript{40}.

An excellent tree species for studying the effects of climate change, climatic extremes and assisted gene flow is lodgepole pine (\textit{Pinus contorta} Dougl. ex Loud.). Being one of the most common tree species in its native range of western North America, it has an extensive distribution covering ~1.3 million km\textsuperscript{2} in diverse climates. Due to its ecological and economic value, an extensive provenance trial was established in western Canada as a reciprocal transplant experiment in 1974\textsuperscript{39}. Given the age of the trial, it can now be combined with tree-ring analyses to study genetic differences to annual events, including extremes such as drought and cold events.

Here, we test the physiological ability of different lodgepole pine populations to acclimate to warmer and drier conditions under climate change. We evaluate long-term growth, as well as annual losses in productivity following a severe spring drought that occurred in western North America in 2002. Growth responses were assessed by measurements of total tree height, annual height increments and tree-ring widths. The physiological mechanisms underlying the growth response to drought are assessed by comparing relative contributions of xylem adaptations and stomatal regulation. These are inferred by combining functional wood anatomical and dual-isotope analyses focusing on a 10-year window capturing the 2002 drought. Samples are collected from lodgepole pine trees grown in three common garden experiments in British Columbia’s southern interior. The 20 provenances investigated here are sourced from across a 4000 km range and grouped into four populations designed to represent major climate regions. By combining population genetic research with retrospective insights into growth, physiological and wood anatomical responses to drought, this study contributes an analysis of complex drought adaptations in a wide-ranging tree species under realistic field conditions. Northern trees produce more vulnerable hydraulic systems combined with lower stomatal responsiveness. This maladaptive response to drought means that western boreal forests may not cope well with droughts expected to increase in frequency with climate change.

**Results**

**Differences in stable isotopes and xylem properties.** Physiological adaptations to drought varied greatly among the four regional populations of lodgepole pine. These pronounced differences in growth and physiological parameters were also maintained over the duration of the study (Fig. 1a). Seed sources from the central part of the lodgepole pine distribution showed consistently higher growth rates, hydraulic diameters of the xylem conduits as well as higher $\delta^{13}$C and $\delta^{18}$O values (Fig. 1a). In contrast, seed sources from northern areas of the lodgepole pine range showed consistently lower growth, hydraulic diameter of...
the xylem, and δ¹³C and δ¹⁸O values (Fig. 1a). Far southern provenances grew slower than central interior and southern interior populations (Fig. 1a). They also had moderate values in all physiological traits relative to central populations, except they had relatively thicker cell walls (Fig. 1a).

Local adaptation and climate correlations. Responses of the four populations were associated with the climate conditions at the origin of the seed sources (Fig. 1b, Supplementary Tables 1, 3), indicative of local adaptations. Responses were also linked to interannual variation of climate at the planting sites (Fig. 1c, Supplementary Tables 2, 4), suggesting plastic responses. Growth, tracheid wall thickness and δ¹⁸O showed stronger latitudinal clines of adaptation linked to temperature and growing season conditions (Fig. 1b). In contrast, the δ¹³C and intrinsic water-use efficiency (iWUE) values were found to be more linked to site climate conditions than could be explained by climate of seed origin (Figs. 1b, 1c, Supplementary Tables 3, 4). We find a positive correlation between δ¹⁸O and δ¹³C among all populations studied here but note that the northern population has less variation in δ¹³C (Supplementary Figure 1). The positive relationships generally indicate that δ¹³C is governed more by stomatal conductance than photosynthesis given the importance of relative humidity in our study (Fig. 1c).

Total growth and drought tolerance. Total height and diameter growth, representing average metrics of overall success, were compared to drought-tolerance indicators and 10-year averages of
Fig. 2 Genetic adaptations revealed from growth, drought tolerance and physiology. Each dot represents the average response in units of standard deviation to show relative rankings among the four regional populations \((n = 1170): 4 \text{ populations} \times 5 \text{ provenances} \times 1 \text{ tree} \times 3 \text{ sites} \times 2 \text{ blocks} \times 10 \text{ years}, \text{ minus 3 trees}. \) Regional populations are coloured as such: blue represents the leading edge (northern) population; green represents the central interior population; yellow represents the southern interior population; and orange represents the trailing-edge (far southern) population. Response is tested across three planting sites in British Columbia’s southern interior. Therefore a positive climate transfer distance exists for the northern population (tests climate warming) while a negative climate transfer distance exists for the southern population (tests assisted migration scenarios). Bars are standard errors of the mean.

### Table 1 Multiple comparisons of growth, drought indicators and physiological traits in interior lodgepole pine populations

| Test | BAI | HI | Resis. | Reco. | Resil. | Rel. Resil. | Hydr. Diam. | Wall. Thick. | δ18O | δ13C | iWUE |
|------|-----|----|--------|-------|-------|-------------|-------------|-------------|------|------|------|
| LE-Cl = 0 | < 0.001 | < 0.001 | > 0.999 | 0.130 | 0.145 | 0.033 | 0.011 | 0.002 | 0.025 | 0.001 | 0.001 |
| SI-Cl = 0 | 0.481 | 0.999 | 0.968 | 0.999 | 0.995 | > 0.999 | 0.927 | 0.985 | 0.570 | 0.674 | 0.678 |
| TE-Cl = 0 | 0.108 | 0.104 | 0.016 | 0.004 | > 0.999 | 0.025 | 0.023 | 0.811 | 0.999 | 0.018 | 0.018 |
| SI-LE = 0 | < 0.001 | < 0.001 | > 0.983 | 0.176 | 0.087 | 0.046 | < 0.001 | 0.005 | 0.001 | 0.038 | 0.036 |
| TE-LE = 0 | 0.144 | 0.013 | 0.012 | < 0.001 | 0.156 | < 0.001 | 0.999 | 0.033 | 0.035 | 0.882 | 0.876 |
| TE-SI = 0 | 0.001 | 0.082 | 0.004 | 0.003 | 0.955 | 0.019 | 0.002 | 0.948 | 0.522 | 0.241 | 0.239 |

Multiple comparisons among tree populations representing the full north-south range of interior lodgepole pine \( (Pinus contorta ssp. latifolia) \). Populations are represented by five provenances grouped into four climatic regions, LE, CI, SI and TE: LE stands for the Leading Edge, i.e. the northern population occupying the area expected to be the leading edge of tree species migrations under climate warming; CI is Central Interior population, located in the central areas of the lodgepole pine range; SI is the Southern Interior population covering the southern range of the central areas of the lodgepole pine range; TE stands for the Trailing Edge, which represents seed sources from the far south of the lodgepole pine range, which is expected to see increased forest maladaptation under climate warming. BAI is basal area increment, HI is height increment, Resis. is drought resistance, Reco. is drought recovery, Rel. Resil. is drought relative resilience, Hydr. Diam. is mean hydraulic diameter, Wall. Thick. is mean tracheid wall thickness, and iWUE is intrinsic water-use efficiency derived from tree-ring δ13C. Significance \((p < 0.005)\) is indicated in bold and p-values were adjusted with the Benjamini & Hochberg false discovery rate method.

### Physiological plasticity under drought

The plasticity of responses under contrasting climate conditions also varied among the four lodgepole pine populations studied here (Fig. 3). Relative to leading and trailing-edge populations, dual-isotope signatures of central interior and southern interior populations indicated a high degree of plasticity. These populations showed the greatest differences in values between dry and non-dry years (Fig. 3a). Under drought, the ratio of carbon-13 to carbon-12 increased while oxygen-18 became enriched relative to oxygen-16. Under drier conditions, however, these populations did not modify their xylem structure to the same degree as the trailing-edge (far southern) population (Fig. 3b). The far southern, trailing-edge population showed a moderate range in stable isotope values under contrasting conditions, but showed the most flexibility in adjusting their hydraulic properties (Fig. 3a, b). Under drought, far southern seed sources produced fewer tracheids, but those tracheids had smaller lumen diameters and thicker cell walls (Fig. 3b). In contrast, the northern population showed the lowest ability to modulate its response in terms of isotopic signatures or physiological parameters in Fig. 2. Higher productivity and moderate drought tolerance of central and southern interior populations were linked to higher δ13C and δ18O values, larger hydraulic diameter, and thicker cell walls (Fig. 2). Values for these traits were almost always significantly different from the northern, leading-edge population (Fig. 2, Table 1, Supplementary Tables 6-9). The far southern, trailing-edge population showed the highest drought tolerance of all four populations, as measured by recovery and relatively resilience following the 2002 drought (Fig. 2). Relative to the most productive central and southern interior populations, they also showed moderate productivity linked with significantly lower tracheid hydraulic diameter (Fig. 2, Table 1 Supplementary Tables 6, 9). The trailing-edge population had lower average iWUE as well as lower δ13C and δ18O values compared to central populations (Fig. 2, Table 1, Supplementary Table 8). In contrast, the northern, leading-edge population showed the lowest values for average growth, δ13C and δ18O, and for xylem hydraulic properties—notably, significantly thinner tracheid walls on average (Fig. 2, Table 1, Supplementary Table 9).
of carbon reserves being depleted under extended drought\textsuperscript{14}. A recent experiment on saplings, however, found that drought-induced mortality in an isohydric tree species was not linked to carbon starvation over a period of 3 years\textsuperscript{42}. Nevertheless, carbon starvation could eventually lead to mortality, but more likely weakens the tree, making it susceptible to pest and pathogens as other mortality agents\textsuperscript{43}. Therefore, decline of central and southern interior forests in the future remains a risk depending on the rate and magnitude of warming and drying, as well as interactions with other biotic and abiotic factors. Judicious northward seed transfer to central interior and south–central regions of the species range may therefore be warranted.

The drought-tolerant behaviour exhibited by trees from the southern, trailing-edge population was linked to different physiological behaviours compared to central populations. Far southern provenances showed some ability to adjust their hydraulic diameter, xylem cell lumen diameter and cell wall thickness in response to drier conditions. Stable isotope signatures suggest that while their stomata do respond to drought (Fig. 3a), they also, on average, leave their stomata open longer than central and southern interior populations (Fig. 2). Assuming respiration rates are not disproportionately greater\textsuperscript{44}, this behavior may be better suited to cope with prolonged drought. By continuing to assimilate carbon, carbon starvation can be avoided, while cavitation risk is reduced through adjustments of the xylem. The investment in such safety mechanisms further explains the classic growth versus drought tolerance trade-off exhibited by the far southern provenances\textsuperscript{27}. Placing a proportion of such southern seed sources in the south–central and central interior areas of the species distribution could therefore increase drought tolerance by introducing a different drought behaviour to those forests. While complementary physiological strategies could increase the adaptive portfolio of southern interior forests to drought, shorter transfer distances are recommended since maladaptation to cold could otherwise limit forest productivity\textsuperscript{29}.

Most importantly, the northern population appears to be unsuitably adapted to cope with drought. The dual-isotope signatures suggest that they do not modify stomatal behaviour while, at the

Discussion

Diverse physiological mechanisms underlie the differences observed in drought tolerances of divergent lodgepole pine populations. Wood anatomical responses and isotopic signatures revealed different relative contributions of xylem and stomatal control, suggesting varied strategies to cope with drought. The higher productivity of central populations can be linked to larger tracheid lumens, which form an efficient hydraulic pathway to the photosynthesizing crown. This facilitates growth when moisture is available, giving the trees a competitive advantage in photosynthesis and growth potential under optimal conditions\textsuperscript{37}. At the same time, cavitation risk appears to be reduced through stomatal closure under drought, inferred through stable isotope signatures (Figs. 1–3). Central populations showed the greatest differences in dual-isotope signatures in response to changing climatic conditions but did not modify their xylem structure to the same degree as the trailing-edge (far southern) population (Fig. 3). The genetic history of the provenances may help explain the higher iWUE exhibited by the central interior group. This population shows introgression between the coastal sub-species (\textit{P. contorta} ssp. \textit{contorta})\textsuperscript{38}. For lodgepole pine, higher moisture at the seed origin has been linked to higher iWUE and growth potential\textsuperscript{39,40}.

The drought-avoidant behavior exhibited by central and southern interior populations could explain their moderate resilience to drought\textsuperscript{27}. This may provide the impression that forests in central and southern interior areas are not as urgently in need of assisted migration interventions relative to northern areas. However, their physiological plasticity may be limited, and the mechanisms may not continue to be successful under prolonged drought conditions because larger tracheids are more prone to cavitation. In a European pine species, for example, individuals that had produced larger tracheids after drought showed symptoms of dieback\textsuperscript{41}. A common assumption is that stomatal sensitivity to drier conditions may be associated with increased risk
same time, their xylem cell walls become thinner under drought—a maladaptive response due to higher cavitation risk. Under drought, the combination of sustained stomatal conductance and low cell wall thickness unsuited to compensate for increasing xylem tension would likely cause the northern population to be more cavitation prone. This is consistent with their low drought tolerance; these trees did not regain pre-drought performance. Interestingly, these data also show no evidence of an expected dual adaptation to cold and drought tolerance, which may rely on similar mechanical properties. The smaller lumen diameters could be an avoidance strategy against embolisms caused by drought or freeze–thaw events. However, the relatively thin cell walls of northern populations observed in this study would have an opposite effect: low drought resilience for northern populations of lodgepole pine.

Trade-offs affecting phenology, wood formation and growth response to drought may also exist for each of the four populations. Trees that flush and begin the process of xylogenesis early in the growing season may risk late spring frosts, but, at the same time, may have a competitive advantage by capturing early season moisture. As annual growth dynamics vary among populations, the intra-seasonal pattern may also vary accordingly. Hence, the drought in 2002 occurred early in the growing season. Therefore, all provenances can be assumed to have been affected during wood formation, which would be reflected in isotopic signatures as well. Although all trees studied here were grown in common environments, genotypes that begin growing earlier may acquire slightly different oxygen isotope signatures from melting snow. When northern provenances are grown in warmer planting environments, they appear to meet their heat-sum requirements early, which can lead to cold damage in spring under climate change. However, by comparing the responses of the four populations in a drought and non-drought year (Fig. 3), it is evident that the northern population shows the most limited plasticity in response, which appears to be less likely due to phenological differences.

Finding such differences among tree populations further highlights the value of combining genetic field experiments with tree-ring approaches to evaluate intraspecific variation of physiological responses to drought. Assessing annual responses of tree populations grown in multi-decade provenance trials provides an informative approach to determining adaptive capacity to extreme events that are expected to become more prevalent in the future. The reconstruction of annual growth, height increment, wood anatomical and ecophysiological behavior in combination with genetic provenance testing provides insight into the nature and geographic distribution of drought adaptation. This method could be applied to other long-term reciprocal transplant experiments that have been established around the world to infer limits to adaptive capacity within forest species.

Forest health issues, productivity losses and carbon-cycle feedbacks are unlikely to be uniform across tree species distributions due to physiological adaptations at the population level. Countreintuitively, our study shows that northern pine forests will experience the consequences of forest maladaptation first, due to an inability to cope with drought. This suggests that the potential benefit of increased growth due to warming may be counterbalanced by physiological maladaptation to drought, contributing to negative predictions for the western boreal. Intervention in northern forests therefore appears to be more urgent than elsewhere in the species range. In northern forests, therefore, introducing pre-adapted alleles to the leading edge may be a low-risk solution targeting forest maladaptation under climate change.

Methods

Experimental design. All trees used in this study were grown in the Illingworth provenance trial, a genetic field experiment established in 1974 for lodgepole pine. Many planting sites incurred high mortality in 2006 due to the mountain pine beetle epidemic. We were therefore permitted to fell dead trees within this valuable multi-decade experiment in 2013 and 2014, which allowed to accurately measure growth parameters and collect stem disks. The family structure in the Illingworth trial was not maintained; provenances (seed sources) represent stand samples as opposed to single progenies. Although several sub-species exist within the lodgepole pine range, we sampled provenances originating from the drier interior region of the range represented by the economically important timber subspecies P. contorta ssp. contorta. Provenances were grouped into four regional populations: (1) the boreal North representing the cold leading edge (LE) of the species range; (2) the cool central interior (CI) area of the species range; (3) warm, lower-elevation southern interior (SI) areas of the species range; and (4) the trailing-edge (TE) group representing the most southern samples of the provenance trial in the United States, with the warmest and driest climatic conditions. Including the leading and trailing-edge populations was necessary to inform our understanding of how these important populations will respond to climate change and to evaluate assisted migration scenarios from a drought perspective.

Each of the four regional populations is represented by five provenances, grown on three planting sites in British Columbia’s southern interior and replicated on two blocks per site in a randomized block design (Fig. 1d, Supplementary Figure 2, Supplementary Tables 1 and 2). Growth and drought-tolerance data from a previous study relied on 4 trees per provenance per block. Due to the high financial and labour costs involved in stable isotope and functional wood anatomical analyses, however, this work relies on a sub-sample of 117 trees. This sub-sample is represented by the tree of median height per provenance (20) per block (2) per site (3). Three samples were not available.

Field and tree-ring measurements. After felling and de-limbing each tree, we used an Eslon tape to measure height and annual height increment, indicated by the distance between branch whorls. We then cut stem disks for further growth and physiological analyses. In the field, we confirmed the accuracy of annual height increment counts by comparing them to the number of tree rings from stem disks at a given height. Although we were limited to sampling trees killed by mountain pine beetle we were able to capture future work to drought adaptation studies; we could quantify leaf-to-sapwood area ratios in living trees in provenance trials. After all data was obtained, the accuracy of height increments was again verified against the same tree’s tree-ring widths. All tree-ring, wood anatomical and isotopic analyses presented here are based on stem disks taken from diameter at breast height (1.3 m). After sanding and scanning these disks, we measured annual radial growth on four radii per stem disk using Windendo (version 2016) to a precision of 0.01 mm and derived basal area increment (BAI). Stem disks were then cut into two adjacent sections of ~1 cm along a radius to the pith: one each for functional wood anatomical and stable isotope analyses.

Drought indicators and period of study. A study period of 10 years from 1996 to 2005 was chosen as it captured a spring and summer drought event occurring in 2002. Compared to normal, this year was a drop of one standard deviation in mean annual precipitation, while standard deviations from mean precipitation in June, July, August, and September were 0.61, 0.62, 0.90, and 0.75, respectively. Designation of these years as pre-drought, drought and post-drought are important for the super-epoch analysis involved in calculating the four drought indicators, as coined by Lloret et al. Resistance, refers to the drop in growth in the drought year relative to the three preceding years; recovery is the speed with which growth resumes; resilience is the ability to return to pre-drought growth; and relative resilience is the ability to return to pre-drought growth relative to the severity of the growth drop during the drought year. These values were defined with respect to BAI to be consistent with a previous study where 1999–2001 are considered pre-drought years, 2002 is the drought year and 2003–2005 are post-drought years. Although 2003 was also considered warm, it was not quite as dry in the early part of the growing season when growth is critical. Since BAI had already begun to recover in 2003, by definition, it was considered a post-drought year.

Functional wood anatomy. After applying a corn-starch solution to the wood sections, we cut micro sections of 10–20 µm thickness using a GSI-1 microtome. The samples were washed, stained with aniline blue, rinsed and bleached. The samples were then soaked in a stain made of equal portions of Safranin and Astra blue for 5 min, rinsed with distilled water, and washed with increasing concentrations of ethanol. After applying Canada balsam, the sections were covered with glass coverslips and placed in an oven at 65 °C for 8 h to solidify. Micrographs were taken at ×200 magnification at a resolution of 5 megapixels and 12-bit colour depth using a Nikon Eclipse Ni-E upright microscope and automatically stitched together.
Relative comparisons among populations are possible since differences in xylem anatomy due to shade stress are largely accounted for by using the provenance trial setting, where trees were grown in a common age structure.

**Pilot: confirm methodology for stable isotope analyses.** Regulation of water loss through reduced stomatal aperture also affects the stable isotope ratios (i.e. δ13C or δ18O) in photosynthesized xylem. Water-stressed conifers have a reduced ability to discriminate against the disfavored heavy carbon-13 isotope relative to the more abundant light carbon-12 isotope, and leaf water becomes increasingly enriched in the heavy oxygen-18 isotope. A positive correlation between δ13C or δ18O points to stomatal closure rather than changing rates of photosynthesis55,58. With an awareness of other complexities affecting isotope composition of tree rings53,57, a dual-isotope approach can be used to retrospectively infer stomatal responsiveness to drought.

We conducted a pilot study to confirm that our methodology for annually-resolved isotopic analysis on our lodgepole pine samples. It has long been recognized that wood components vary isotopically9,39. The ratio of lignin to cellulose in a tree ring may therefore alter the stable isotope ratios59. Cellulose is often used for stable isotope analysis due to its stability and immobility40,42,43. However, cellulose extraction is time-consuming, and whole wood is preferred when there is a consistent offset. For lodgepole pine, Guy and Holowachuk44 previously reported a strong relationship between carbon isotope signatures in cellulose and whole sapwood. Here, however, it was important to test the reliability of this signal over time in tree rings, the key component in our study, from trees that had been dead-standing for 8 years for both oxygen, as well as carbon isotope ratios. We also wanted to exclude the influence of blue-stain fungus affecting many of our lodgepole pine samples on the isotope ratios, as reported for other conifer species64. This pilot additionally compared latwood to the entire ring to test if earlywood cells were formed with stored photosynthate, possibly leading to different climatic correlations65,66.

The pilot study was based on tree rings covering 7 years (1999–2006, inclusive), representing four trees from one provenance grown on one planting site block. Tree rings were separated with a scalpel under a stereo microscope (Leica Wild M3B, Wetzlar, Germany). Whole-wood samples were homogenized with a ultra-centrifugal mill (ZM 2000, Retsch GmbH, Germany). This setting, where trees were grown in a common garden setting, where trees were grown in a common garden setting.

Climate data. All climate data were derived from the ClimateWNA (version 3.4) software interface. We extracted annual climate variables for planting sites. For the provenance climates (climate of seed origin), we aim to approximate the climates to which the tree populations are adapted. We derived climate variables for the 1961–1990 long-term average. This climate normal is the earliest period with a high density of climate data and precedes the most recent anthropomorphic warming trend.

Statistical analyses. All statistical analyses were conducted in the R statistical programming environment, and graphical implementation was implemented with various functions from the ggplot2 package53. Correlations to climate at the planting site were conducted at the provenance level and were calculated with the rcorr function from the Hmisc package24 to obtain p-values. Due to the slightly imbalanced design, provenance values for the correlations relied on least square means calculated using lmtestT and ImerTest7, where provenance was specified as the fixed effect, and block and a unique tree identity were specified as random effects. For testing differences among the four regional populations, the fixed effect in each model was region. Model selection was verified by confirming lower values for Akaike's Information Criteria.

For models based on functional wood anatomy, the distance from the apex was included as a random effect because it affects conduit size28. It is necessary to include distance to the top of the tree since conduit size is partially determined by growth patterns over time. As the tree grows higher, the new growth at the base of the tree requires large conduits28. This is therefore reflected in functional wood anatomy in a gradient from the top to the stem base of the core76. Correlations between growth and annual climate conditions at the site were based on height increments and BAI. The altitude of the average climate of seed origin (the long-term mean from 1961–1990) to all growth, drought, anatomical and isotopic traits are designed to show adaptation to climate. For these correlations, we used total height and diameter at breast height after 32 years of growth in real-world conditions (year 2005). All correlations were also adjusted using the Benjamini & Hochberg false discovery rate method77 for reporting significance in tables (Table 1, Supplementary Tables 6-9).

**Data availability**

All relevant data is available from the authors.
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79. Keeler, K. T. & Feldesman. Project funding: NSERC (STPGP-430183), DFG (SP 437/18-1) and SNF (200021_175888).

Acknowledgements
We gratefully acknowledge the British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development (FLNRORD) for establishing and permitting sampling in their provenance trial. We thank R. Guy, U. Hacke, V. Liefers, B. Roskilly and A. Sala for comments. Special thanks to A. Bueno and L. Schneider for laboratory assistance. We further acknowledge administrative, laboratory and field assistance from: W. Abdi, F. Baab, V. Berger, J. Braun-Wimmer, S. Giese, U. Graf, J. Grossmann, U. Hacke, M. Harrhy, K. Klasing, C. Koch, E. Koertels, P. Middlestead, J. R. Babenschlag, N. Ukrainetz, A. Vorländer, P. Wickham and A. Wiegelm. Project funding: NSERC CGS-D, Alberta Innovates (AITP), Feodor Lynen Research Fellowship.

Author contributions
M.I.-R. and D.M. developed the experimental design with input from K.T. and A.H. Field logistic planning, data collection and tree measurements were conducted equally by M.I.-R. and D.M., with contributions by H.S. in the field. The isotope laboratory work was conducted by M.I.-R. with assistance from K.T. and P.C. The wood anatomical laboratory work and measurements were conducted by D.M. M.I.-R. conducted statistical analyses and prepared the manuscript with input from D.M. and K.T. as well as contributions from all co-authors.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-07701-0.

Competing interests: The authors declare no competing interests.

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