




# Genetic basis of growth reaction to drought stress differs in contrasting high-latitude treeline ecotones of a widespread conifer

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## Abstract

Climate change is increasing the frequency and intensity of drought events in many boreal forests. Trees are sessile organisms with a long generation time, which makes them vulnerable to fast climate change and hinders fast adaptations. Therefore, it is important to know how forests cope with drought stress and to explore the genetic basis of these reactions. We investigated three natural populations of white spruce (*Picea glauca*) in Alaska, located at one drought-limited and two cold-limited treelines with a paired plot design of one forest and one treeline plot. We obtained individual increment cores from 458 trees and climate data to assess dendrophenotypes, in particular the growth reaction to drought stress. To explore the genetic basis of these dendrophenotypes, we genotyped the individual trees at 3000 single nucleotide polymorphisms in candidate genes and performed genotype–phenotype association analysis using linear mixed models and Bayesian sparse linear mixed models. Growth reaction to drought stress differed in contrasting treeline populations. Therefore, the populations are likely to be unevenly affected by climate change. We identified 40 genes associated with dendrophenotypic traits that differed among the treeline populations. Most genes were identified in the drought-limited site, indicating comparatively strong selection pressure of drought-tolerant phenotypes. Contrasting patterns of drought-associated genes among sampled sites and in comparison to Canadian populations in a previous study suggest that drought adaptation acts on a local scale. Our results highlight genes that are associated with wood traits which in turn are critical for the establishment and persistence of future forests under climate change.

## KEYWORDS

Bayesian sparse linear mixed model, dendrophenotype, genotype–phenotype associations, genotyping-by-sequencing, linear mixed model, *Picea glauca*

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## 1 | INTRODUCTION

Under human-induced global warming, drought events are increasing in frequency and intensity (Dai, 2013; IPCC, 2021), affecting boreal forest ecosystems more severely at high latitudes (Collins et al., 2013). Especially in North America, regional warming has led to a decrease in soil moisture and therefore an increase in water deficit (Girardin et al., 2016; Reich et al., 2018). The resulting increased physiological stress is associated with reduced growth and elevated tree mortality rates (Allen et al., 2010; Hynes & Hamann, 2020; van Mantgem et al., 2009). Therefore, it is important to know how trees adapt to increasing drought stress as the speed of adaptation in trees is limited by their long generation time and sessile habit (Shaw & Etterson, 2012). In fact, some tree species already lag behind their potential distribution relative to climate (Aitken et al., 2008). In general, tree populations are characterized by high phenotypic plasticity and adaptive capacity, including high standing genetic variation and a high dispersal ability by pollen, which enables them to cope with environmental changes (Aubin et al., 2016). Extreme events such as droughts exert intense selection pressures on populations and thereby shape genetic variation at adaptive loci (Grant et al., 2017). However, especially in conifers, the high pollen-mediated gene flow keeps populations connected and can introduce pre-adapted alleles (Avanzi et al., 2020; Kremer et al., 2012; Liepelt et al., 2002). Conversely, the introgression of maladapted alleles could counteract local adaptation (O'Connell et al., 2007; Rajora et al., 2005). Within tree populations that have experienced recent selection and are characterized by high gene flow, genes related to local adaptation are expected to interact in a complex way and show small frequency shifts in the process of adaptation (Hornoy et al., 2015). However, it is unclear which specific genes control drought tolerance in trees (Moran et al., 2017).

In many sites, trees will probably have difficulty keeping up with rapid climate change, which makes them more vulnerable to local extinction, particularly in high mountain areas (Dauphin et al., 2021). To explore the molecular mechanisms of stress-tolerant phenotypes in tree populations, recent studies have begun to link genetics with dendroecology (Depardieu et al., 2021; Heer et al., 2018; Housset et al., 2018; Laverdière et al., 2022; Trujillo-Moya et al., 2018). In these studies, genotype-phenotype association analyses are used to identify loci that are associated with dendrophenotypic traits related to drought tolerance. Tree growth dynamics during and after a drought event can reveal the overall drought tolerance of trees (Moran et al., 2017). Using this approach, Depardieu et al. (2020) detected signals of local adaptation to drought among white spruce (*Picea glauca* [Moench] Voss) populations of Eastern Canada planted in a common garden. The follow-up study (Depardieu et al., 2021) then detected 285 genes significantly associated with phenotypic traits or climatic factors, of which 110 were differentially expressed under drought conditions.

Although common garden studies are useful for studying genetic adaptation to the current local site conditions, we cannot investigate the growth reactions of trees in the environment they

are adapted to, using this method (Hoffmann & Sgrò, 2011; Merilä & Hendry, 2014). Further, genotype-by-environment interactions under common garden conditions can make results misleading relative to the natural conditions (Merilä & Hendry, 2014). To our knowledge, genetic association analyses with dendrophenotypes in white spruce have exclusively been done in common garden experiments in Canada. We investigated natural populations of white spruce in contrasting extreme environments in Alaska to determine whether high-confidence genes identified in common garden studies also significantly associate with phenotypic traits related to drought tolerance in natural populations. White spruce is economically and ecologically important in North America and has been found to have an exceptionally high adaptive capacity (Royer-Tardif et al., 2021). To study adaptation to climatic extremes, treeline populations are particularly useful because tree growth is limited and mortality rates are higher because trees experience the limits of their realized niches (Hampe & Jump, 2011; Hampe & Petit, 2005; Restoux et al., 2008). Therefore, we investigated populations of three different sites representing contrasting treeline ecotones to determine genes that control drought tolerance in white spruce. Our study design includes sampling sites in one drought- and two cold-limited ecotones where growth is limited by water or temperature, respectively. The trees in these ecotones experience different climate extremes and, therefore, divergent selection pressures, which should lead to different genetic signatures underlying drought tolerance.

We tested the following hypotheses: (i) the growth reaction to drought stress differs (a) between drought- and cold-limited treelines and (b) between treeline and forest plots, and (ii) the selection pressure of the contrasting treelines leads to divergent signatures in drought-associated genes. To test these hypotheses, we first developed a decision tree to identify growth decline caused by drought stress using dendroecological and climate data because there is no standardized definition (Schwarz et al., 2020; Slette et al., 2019). We then characterized the individual growth reaction to drought stress using tree-ring width. These dendrophenotypic traits are unitless, which makes them more suitable for comparison among natural populations (Opgenoord & Rellstab, 2021). Further, based on a common garden study, moderate narrow-sense heritability values were previously reported for recovery ( $h^2 = 0.34$ ), resilience ( $h^2 = 0.3$ ) and relative resilience ( $h^2 = 0.35$ ) of growth in reaction to drought events (Depardieu et al., 2020). Our genetic data consisted of 3000 single nucleotide polymorphism (SNPs) located in candidate genes that were originally identified in Canadian white spruce populations (Pavy et al., 2017). For the genotype-phenotype association analysis, we used linear mixed-effects models to account for different environments at the sites and Bayesian sparse models to account for interaction and small-effect size SNPs. We compared growth reaction to drought stress and climate sensitivity (standard deviation in growth over the years) between the contrasting ecotones, as well as their underlying genetic basis. Our results provide insights into the genetic architecture underlying drought tolerance of natural populations in contrasting environments.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites

We investigated trees in three sites in nearly monospecific white spruce stands in Alaska under different environmental conditions (Figure 1, Table 1). Each of the three study sites contained two plots, one representing the treeline ecotone and one representing the closed-canopy forest. Two study sites represented the presumably temperature-limited range edge of white spruce. The first study site was located in the Central Brooks Range at the latitudinal treeline on a steep south-exposed slope. The distance between the forest and treeline plot was only 30m, because a steep slope gradient means microenvironmental conditions change quickly over a short

vertical distance. The second study site was situated in the Alaska Range (Denali National Park Preserve) at an elevational treeline on a south-exposed slope. The distance between forest and treeline plot was 1.3 km. A third site was located in Interior Alaska near Fairbanks and belonged to the Bonanza creek experimental forest (Juday & Alik, 2012; Viereck et al., 1986). It was situated at a steep (12–34°) south-exposed bluff of the Tanana River and represented a moisture-limited treeline due to higher water run-off and evapotranspiration rates. Forest and treeline plots were adjacent but had the highest differences in slope angle. The treeline plot was located at the upper edge of the bluff on a steep slope, whereas the forest plot exhibited a shallow slope. For a more detailed description of all study sites see Wilmking et al. (2017) and Trouillier, van der Maaten-Theunissen, Harvey, et al. (2018).



**FIGURE 1** Studied locations and distribution range (green) of white spruce (*Picea glauca*) in Alaska (Prasad & Iverson, 2003). The state of Alaska is coloured in light brown. Circles show the location of the three study sites Brooks Range, Interior Alaska and Alaska Range.

TABLE 1 Characteristics of the sampled locations

Study site	Brooks Range		Interior Alaska		Alaska Range	
	BR F <sup>a</sup>	BR T <sup>b</sup>	Int F <sup>a</sup>	Int T <sup>b</sup>	AR F <sup>a</sup>	AR T <sup>b</sup>
Research plot	BR F <sup>a</sup>	BR T <sup>b</sup>	Int F <sup>a</sup>	Int T <sup>b</sup>	AR F <sup>a</sup>	AR T <sup>b</sup>
Latitude (°N)	67.95	67.95	64.70	64.70	63.72	63.74
Longitude (°W)	149.75	149.74	148.31	148.30	149.01	149.01
Elevation (m.a.s.l.)	876	923	181	180	802	1008
Mean annual temperature (°C)	-8.18		-1.89		-3.58	
Total annual precipitation (mm)	314		305		511	
Density (trees per ha)	839	232	406	326	507	152
Number of analysed trees	94	44	105	49	106	60
Average dbh ± SD (cm)	15.4 ± 5.3	13.9 ± 4.7	16.3 ± 5.2	17.6 ± 5.6	20.2 ± 8.8	12.9 ± 4.2
Average height ± SD (m)	8.5 ± 2.3	6.9 ± 2.1	13.1 ± 3.8	11.4 ± 2.4	10.2 ± 3.3	5.3 ± 1.2
Average age ± SD (years)	136 ± 39	102 ± 49	76 ± 8	68 ± 9	133 ± 45	75 ± 26

Note: Latitude, longitude and elevation values were taken from the centroid of each plot. Temperature and precipitation data were downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP) for the 1950–2015 reference period. Average age was calculated using the oldest tree ring measured.

<sup>a</sup>Forest plot.

<sup>b</sup>Treeline plot.

Each plot contained at least 200 trees and covered an area from 0.5 to 2 ha depending on tree density (Table 1). For each tree within the plots, tree height was recorded and diameter at breast height (dbh) was measured for trees with a height of at least 1.3 m. Within the plots we selected trees with a dbh between 10 and 40 cm, a height of 4–20 m, and a minimum age of 50 years, from which wood cores were taken for further analysis. We calculated the maximum likelihood estimates of relatedness between individuals using microsatellite markers described in Zacharias et al. (2021) and the software ML-RELATE (Kalinowski et al., 2006). We excluded trees that were closely related ( $r > .5$ ). In the case of clonal groups, only the oldest individual was selected for further analysis. For genetic analyses, fresh needles were sampled from selected trees and dried with silica gel.

## 2.2 | Drought year identification and individual-level response parameters

We used a tree ring data set that contained trees sampled in Interior Alaska in 2015 (Trouillier, van der Maaten-Theunissen, Harvey, et al., 2018) as well as trees from the Brooks Range and the Alaska Range initially sampled in 2012 (Eusemann et al., 2016) and complemented in 2015 and 2016 (Wilmking et al., 2017). In brief, cores were glued onto wooden sample holders and surfaces prepared with a core-microtome. Ring widths were measured from optical scans and cross-dating was done visually. For a detailed description of core processing see Wilmking et al. (2017) and Trouillier, van der Maaten-Theunissen, Harvey, et al. (2018).

For the genotype–phenotype association (GPA) analysis, we derived measures of the individual growth reaction to drought stress as phenotypic data. We first identified years with a growth decline caused by drought stress for each site. As there is no standardized method to identify growth decline associated with drought in

dendroecology (Schwarz et al., 2020), we combined tree ring and climatic data to make a standardized decision for each of the three study sites (Figure S1). Following Slette et al. (2019), we provided standardized climatic index values and a quantitative definition of what we consider as drought conditions.

As a first step, we used R version 4.0.2 (R Core Team, 2015) with the package POINTRES version 1.1.3 (van der Maaten-Theunissen et al., 2015) to identify years of growth decline in our tree ring data set. Event years are defined as years in which the individual showed a substantial reduction in growth. We identified years in which at least 50% of the trees showed such an event year, which we defined as a pointer year (Schweingruber et al., 1990). As suggested by Schwarz et al. (2020), we used raw radial growth data and series detrended with the *detrend* function of the R package DPLR version 1.7.1 (Bunn, 2008, 2010; Bunn et al., 2020), using a 30-year spline. We performed a sensitivity analysis to check whether the choice of the thresholds influenced the outcome. We applied a moving window approach, initially proposed by Cropper (1979), using different settings of 3, 5 or 7 years with the common thresholds 0.9, 1.0, and 1.1 for standard deviation in each combination. All combinations were calculated using raw and detrended data. We only considered a pointer year for further analyses when it was identified by at least half of the applied combinations (Table S1).

As a second step, to identify whether the growth decline in the pointer year was caused by drought, we checked the climatic conditions from 2 years before until 2 years after the pointer year. Due to the memory effect, trees can show a delayed growth reaction to drought stress (Hackett-Pain et al., 2015). Because of the low accuracy of climatic data in Alaska before 1950, we only considered years after 1950 in the analysis. To characterize the climatic conditions, we used three different drought-related indices. The first is the standardized precipitation evapotranspiration index (SPEI6), which accounts for precipitation and potential evapotranspiration (PET) in a moving

window approach taking into account a period of 6 months (Vicente-Serrano et al., 2010). Second, we used the climate moisture index (CMI6), which includes precipitation and PET. SPEI and CMI were calculated using the Thornthwaite method (Thornthwaite, 1948) for the growing season (May–September). We also calculated the CMI by Hogg for the period of 1 year, including the sum of monthly CMI values from the first of the preceding August until the July 31 of the current year. In previous studies about drought impacts on growth in white spruce, this index had shown strong growth–climate relationships (Hogg et al., 2017; Hogg & Wein, 2005). Monthly climate data (precipitation sum, mean temperature, mean PET) were downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP) for the period 1950–2015 with a resolution of 2 km<sup>2</sup>. We defined drought as a periodic lack of water compared to normal conditions at the site, characterized by SPEI6 and CMI values with a negative standard deviation >1.25 from the 5-year mean.

After the drought event identification, we calculated the individual growth reaction to drought stress of each tree using detrended and raw tree-ring data. Because there were no striking differences, we continued with the detrended data. We obtained the resistance, recovery, resilience and relative resilience indices after Lloret et al. (2011) for each tree and pointer year using the R package POINTRES version 1.1.3 (van der Maaten-Theunissen et al., 2015). The number of years considered for pre- and post-disturbance periods when we determined the growth reaction components was set to 2 years because of the short periods between growth declines. Resistance describes the ratio between growth during and before the pointer year, recovery the ratio between growth after and during the pointer year, resilience the ratio between growth after and before the pointer year, and relative resilience the resilience weighted by the growth decrease during the pointer year (van der Maaten-Theunissen et al., 2015).

In addition to these named indices, we estimated climate sensitivity for 1970–2012 by calculating the standard deviation in growth for each individual tree. Trees with a high deviation from the mean were characterized as highly sensitive with a high variability in growth depending on climate conditions, which has been associated with higher mortality (Cailleret et al., 2017). The described measures of individual reaction to drought stress and climate sensitivity were used as phenotypic data (dendrophenotypes) within the GPA analysis. A normal distribution of the phenotypic data was checked statistically by performing the *shapiro.test* function (Shapiro–Wilk normality test) and visually by applying the *qqnorm* function in the R package STATS version 4.1.0. In the case of non-normally distributed data, we transformed the data with the R function *boxcoxTransform* of ENVSTATS version 2.4.0 (Millard, 2013) using the lambda value calculated with *transformTukey* of the R package RCOMPANION version 2.4.1 (Mangiafico, 2017). Furthermore, we plotted the correlation between climate sensitivity and tree height using the R function *ggscatter* in GGPUBR version 0.4.0 (Kassambara & Kassambara, 2020). In addition, outlier phenotypes which disturbed the normal distribution of the data were excluded to avoid spurious associations in linear mixed models (Interior Alaska—four individuals for resistance

2010, one individual for resilience 2010, three individuals for recovery 2010; Brooks Range—one individual for relative resilience 1993).

## 2.3 | SNP genotyping and filtering

The sampled needles were sent to LGC Genomics for DNA extraction and targeted genotyping by sequencing (SeqSNP, LGC April 11, 2019). All trees were genotyped using the Illumina NextSeq 550 platform, targeting SNPs located in coding regions mapped in a high-resolution reference genetic map of white spruce (Pavy et al., 2017). The first step was selecting a subset of 7511 SNPs genotyped in white spruce in Pavy et al. (2017) in eastern Canada populations, which were distributed across the 12 chromosomes of white spruce. These SNPs showed good quality and minor allele frequency (MAF) of at least 6% in previous white spruce studies (Pavy et al., 2017). All selected SNPs were located in coding regions that have a higher probability of sequence conservation and were mapped to the reference maps of Pavy et al. (2017) and Galalova et al. (2022). The sequences surrounding the SNPs (at least 75 bp) were blasted against the transcriptome of white spruce (Birol et al., 2013) and only sequences of SNPs with a full hit in the genome were retained. The corresponding oligo probes for SNP detection were designed on the transcriptome and validated by running test sequencing. Based on this information, we selected 3000 SNPs whose oligo probes had only one hit in the genome (Table S6). Each SNP was located in a single gene, resulting in 3000 different genes. In addition to the 478 samples, we sequenced 12 negative controls and 15 duplicates to control the sequencing quality. We compared the sequences of the duplicated individuals using the function *dupGenotypes* implemented in the R package STRATAG version 2.0.2 (Archer et al., 2017). This function calculates the proportion of shared loci between the duplicates. Duplicated individuals with more than 5% of missing data were excluded, which was the case for four individuals (Table S3). Note that 98.7%–99.8% of all loci were shared between duplicate samples, demonstrating the reliability of the genotyping approach and the SNP detection method used.

In the first filtering steps, performed by LGC Genomics, SNPs were filtered for a minimum coverage of eight reads per sample and locus. Then, we subsequently removed SNPs with more than 80% missing data, individuals with more than 10% missing data and finally SNPs with more than 10% missing data. Only biallelic SNPs were kept in the data set. SNPs with an MAF >2.5% were retained, resulting in a data set of 458 individuals and 2744 SNPs. Furthermore, to exclude linked loci, we tested all SNPs for pairwise population-based linkage disequilibrium (LD) using the function *gl.report.ld* implemented in the R package DARTR version 1.8.3 (Gruber et al., 2018). In the case of tightly linked SNPs ( $r > .9$ ), the first SNP of the pair was removed. For SNPs located on the same contig, this threshold was set to  $r > .5$ . With this step, we excluded 120 SNPs. Furthermore, if paralogues are targeted, it might negatively affect the outcome of the analysis. Since paralogues are expected to show a greater proportion of heterozygotes than singleton loci (McKinney, 2016),

we calculated the expected and observed heterozygosity as well as the deviation from Hardy–Weinberg-equilibrium (HWE) per locus and population ( $\alpha = .05$ ) using DARTR. Loci that showed heterozygous excess and deviation from HWE in more than one population can probably be assigned to oligo probes that are binding on multiple sites within the white spruce genome and were therefore excluded from the analysis, which was the case for a further 161 loci. As a result, the final data set consisted of 2463 SNPs from 458 individuals.

## 2.4 | Population genetic structure

In GPA studies, population structure can cause spurious associations (Sul et al., 2018). Therefore, we investigated genetic structure within and among plots using two approaches. First, we conducted a principal component analysis (PCA) as implemented in the R package ADEGENET version 2.1.3 (Jombart, 2016). Second, we used a variational Bayesian framework implemented in FASTSTRUCTURE (Raj et al., 2014) to infer the levels of admixture within populations and individuals. We defined the optimal number of genetic clusters ( $K$ ) using the script *chooseK.py* in PYTHON 2, which parsed through the output of the runs to provide an appropriate number of clusters for the model complexity of our data (van Rossum & Drake Jr, 1995). We summarized and visualized the results of the 15 independent runs for each  $K$  value using the R package POPHELPER version 2.3.1 (Francis, 2017). In addition, we checked the overall population genetic structure using five neutral microsatellite markers described in Zacharias et al. (2021) in STRUCTURE version 2.3.4 (Pritchard et al., 2000) in comparison with the SNP data. Pairwise population genetic differentiation values ( $F_{ST}$ ) were calculated using the SNP data in the R package DARTR version 1.8.3 (Gruber et al., 2018).

## 2.5 | Genetic association analysis

### 2.5.1 | Genotype–phenotype association analysis

We tested the association between each dendrophenotype (climate sensitivity and growth reaction parameters) and each SNP to characterize the underlying genetic variation of drought tolerance. For this, we used linear mixed models (LMMs) implemented in the R package GENESIS version 2.23.3 (Gogarten et al., 2019), which takes into account population structure using the PC-AiR method (Conomos et al., 2015), and genetic relatedness using the PC-Relate method (Conomos et al., 2016) to control for false-positive associations. For missing values in the genotype data, GENESIS imputed the most frequent allele from all analysed individuals. First, to adjust for population structure in the mixed models, we estimated the kinship among individuals using the *snpgdsIBDKING* function of the R package SNPRELATE version 1.27.0 (Zheng et al., 2012). Next we used the estimated kinship to conduct a PC-AiR using unrelated individuals, which are maximally informative about all ancestries in our sampled populations. We ran a PCA with the unrelated individuals and then

projected the relatives onto the PCs with a kinship threshold of degree 3 (unrelated means less than a first cousin) using the *pcAir* function (GENESIS). Second, to account for genetic relatedness, we used the first two PCs to compute kinship estimates with the *pcRelate* function (GENESIS). We obtained a genetic relatedness matrix including all individuals as the covariance matrix for the null model using the function *pcRelateToMatrix* (GENESIS). We then created a household matrix to account for different environmental conditions among the study sites or treeline/forest plots. Within this binary code matrix, 0 represents two individuals from the same and 1 represents two individuals sampled from different study sites or plots. The first step in association testing was to fit the null model with the hypothesis that each SNP has no effect. We fit different null models depending on the tested study sites using the *fitNullModel* function (GENESIS). For each study site, we fit a null model with the first PC and tree height as fixed effect covariates and genetic relatedness matrix and household matrix accounting for treeline and forest plot as random effect covariates based on the Gaussian distribution. We included tree height rather than tree age as a covariate, because tree size influences climate sensitivity in white spruce in Alaska (Trouillier, van der Maaten-Theunissen, Scharnweber, et al., 2018). In cases with overlapping pointer years among sites, we fit the null models for multiple study sites with the first PC and tree height as fixed effect covariates, and genetic relatedness matrix and household matrix accounting for different study sites as random effect covariates based on the Gaussian distribution. Furthermore, we used the function *assocTestSingle* (GENESIS) to test each SNP with each quantitative trait in conjunction with the output of the null model fit. Finally, we controlled for multiple testing using the *qvalue* function with a false discovery rate of 0.05 using the R package QVALUE version 2.25.0 (Storey et al., 2021).

To account for small-effect size SNPs and interaction effects, we applied a Bayesian sparse linear mixed model (BSLMM) using Markov chain Monte Carlo (MCMC) as implemented in GEMMA version 0.98.5 (Zhou et al., 2013). This polygenic model accounts for single large-effect size SNPs and multiple SNPs with small effects at the same time while correcting for population genetic structure by calculating a centred relatedness matrix. GEMMA excludes individuals with missing values. Therefore, we imputed missing genotypes using the function *na.roughfix* implemented in the R package RANDOMFOREST version 4.6–14 (Liaw & Wiener, 2002). Missing genotypes were filled with the mean genotype of the SNP, which was the case for 0.31% of the SNPs. We then tested all SNPs for association with each phenotypic trait by performing 5,000,000 iterations and a burn-in of 1,000,000, running three independent chains for each trait. The convergence across the independent chains was assessed using Gelman–Rubin diagnostics implemented in the R package CODA (Plummer et al., 2006). The harmonic means of the posterior inclusion probabilities (PIP) were calculated across the three chains. The PIP is the sum of all posterior probabilities of all regressions including the specific variable and are therefore a ranking measure to assess the extent to which the data favour the inclusion of a variable in the regression. We filtered SNPs with  $PIP > 0.1$  to identify SNPs with the strongest

evidence of association (Chaves et al., 2016; Depardieu et al., 2021; Pfeifer et al., 2018). We summarized the hyperparameters for each trait using the R package CODA by calculating the mean, standard deviation and upper and lower bound of the 97.5% credible interval (Depardieu et al., 2021).

### 2.5.2 | Gene annotation

The GCAT3.3 white spruce gene catalogue (Rigault et al., 2011) was used for structural annotation of SNPs. Sequence descriptions for the associated genes were obtained by submitting the sequences to BLAST2GO (Götz et al., 2008). BLAST2GO was also used to obtain Gene Ontology (GO) annotations. GO biological process, molecular function and cellular component terms were acquired for each individual transcript.

## 3 | RESULTS

### 3.1 | Pointer years and site-specific dendrophenotypic variation

The climate sensitivity differed significantly among sites. Specifically, the trees of Interior Alaska showed the highest climate sensitivity, whereas the Alaska Range forest plot trees were the least sensitive. Within each study site, the treeline plots consistently had higher sensitivity than the corresponding forest plots, which was significant for both our Alaska Range and Brooks Range sites (Figure 2a). On average, shorter trees were more climate-sensitive than taller individuals (Figure S2).

We identified several pointer years associated with drought stress for each study site. For the Brooks Range, 1993 was a pointer year associated with a low CMI6 in 1991. In the Alaska Range, trees also showed a growth reduction in 1993, probably in response to low CMI6 in 1991. Additionally, the year 1998 was identified as a pointer year in the Alaska Range with low values of SPEI6, CMI6 as well as CMI by Hogg in the previous year (1997). The same pointer year (1998) occurred in Interior Alaska and was also preceded by low values of CMI6 and SPEI6 in 1997. The trees of Interior Alaska showed a second pointer year in 2010 following the low CMI6 in 2009.

The reaction of trees showed significant site-specific differences to the same pointer year. For example, in 1998 Interior Alaska trees showed higher relative resilience and recovery while the Alaska Range had higher resistance (Figure 2e). In 1993, the Alaska Range trees had higher resistance, resilience, relative resilience and recovery than trees in the Brooks Range (Figure 2d). Furthermore, study trees also showed a significantly different reaction to different pointer years within the same site, such as 1993 and 1998 in the Alaska Range or 1998 and 2010 in Interior Alaska for half of all parameters (Figure 2b,c). The individual-tree reaction during a pointer year differed between the treeline and the forest plot within one site. In the Alaska Range in 1998, trees in the forest plot showed

higher resilience, relative resilience and recovery compared to trees in the treeline plot (Figure 2b). This pattern was also found in Interior Alaska in 1998 and 2010 (Figure 2c). No significant differences between forest and treeline plot trees could be detected in the Brooks Range and the Alaska Range for 1993 (Figure 2b–d). Within each site, recovery of trees had the highest interindividual variation when comparing the Lloret indices (Figure 2b–e).

### 3.2 | Population genetic structure

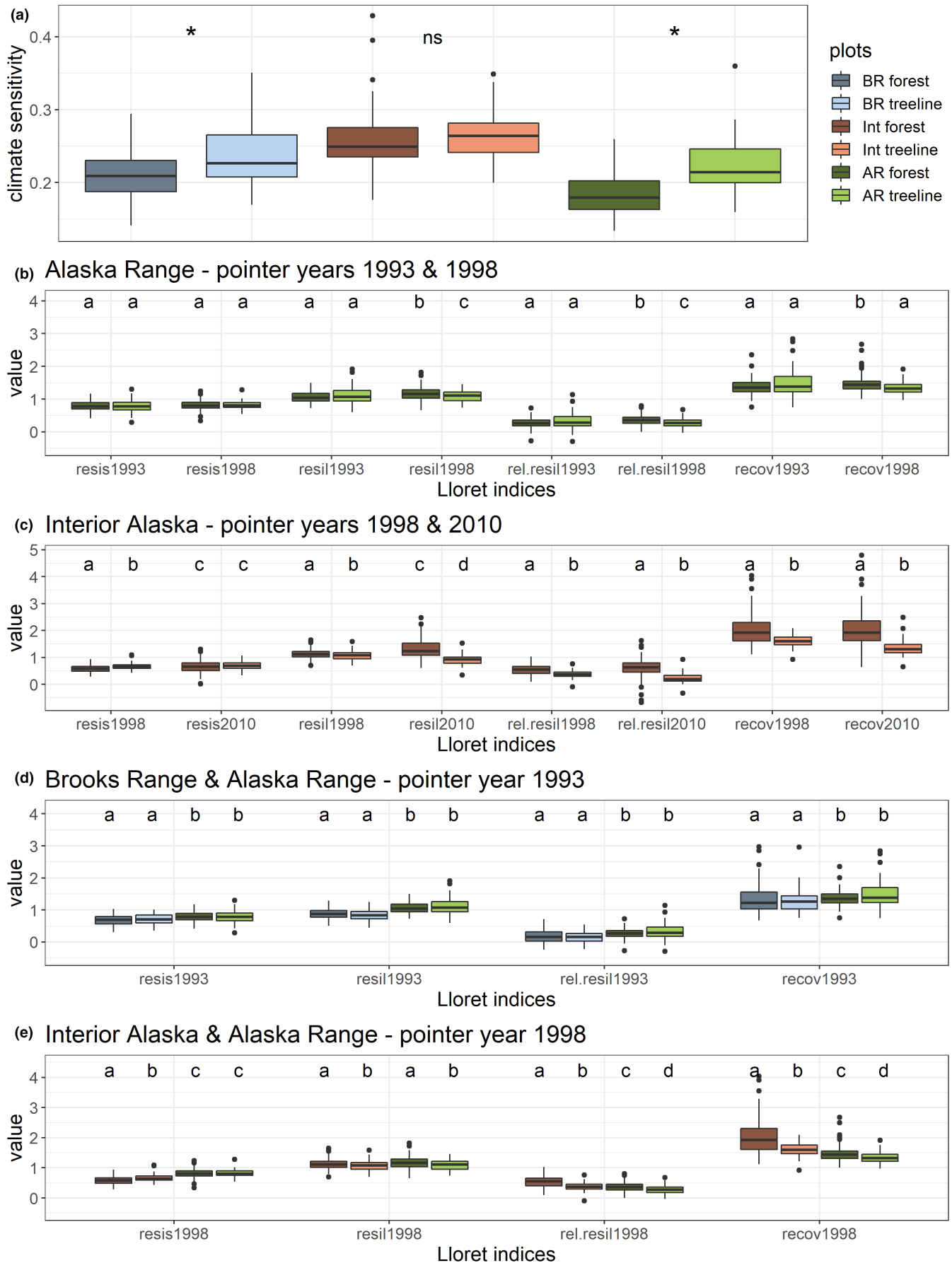
We identified distinct genetic clusters in our study sites based on PCA, with a clear separation of all three study sites (Figure 3a). We observed no separation between trees in respective forest and treeline plots, except for two groups of individuals of the Alaska Range forest plot that separated from the remaining individuals of this study site. The two first axes (PC1 and PC2) together explained 3.94% of the total genotypic variation.

This pattern of population genetic structure was supported by the results of Bayesian clustering analysis (Figure 3b), wherein individuals were assigned to one of two genetic clusters ( $K = 2$ ). Trees in the Alaska Range were mainly distinguished from those in the Brooks Range and in Interior Alaska. At  $K = 3$ , all three study sites were differentiated and a difference between the Alaska Range forest and treeline trees was revealed. Interior Alaska and the Alaska Range were admixed from several genetic clusters. Furthermore, the Brooks Range and Interior Alaska trees showed less differentiation from the Alaska Range treeline plot ( $F_{ST} = 0.014–0.017$ ) than from the Alaska Range forest plot ( $F_{ST} = 0.023–0.025$ ; Table S2). The STRUCTURE analysis with five microsatellite markers differentiated the Brooks Range from Interior Alaska and the Alaska Range for  $K = 2$  (Figure S3). This supports the results of the PCA and indicates a pattern of isolation by distance (Zacharias et al., 2021). Furthermore, the separating group of individuals in the Alaska Range forest plot was not shown with the microsatellite markers. It is unlikely that this was the result of human interference, such as plantings or cuttings.

### 3.3 | Genotype–phenotype association analysis

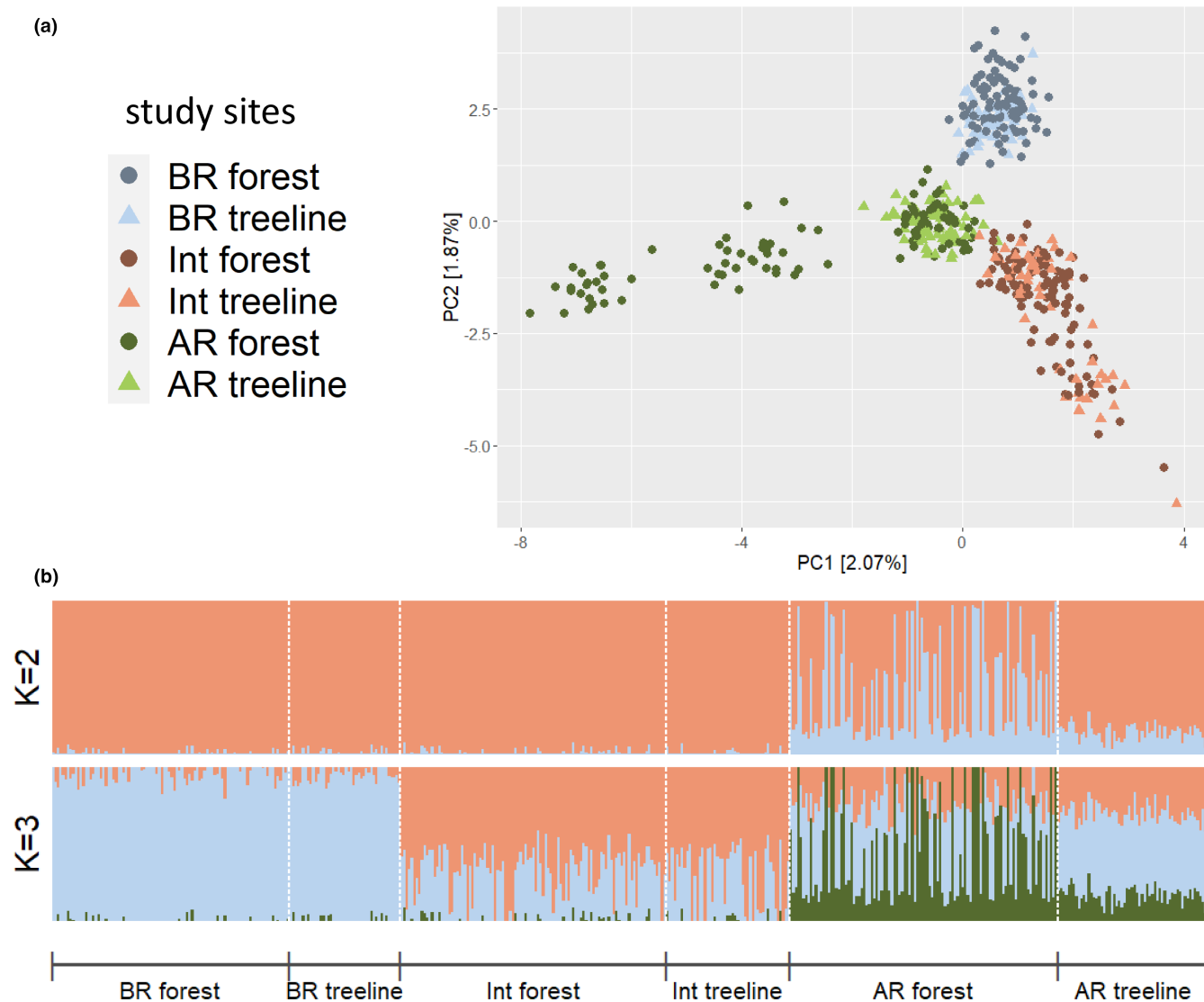
We conducted GPA analysis of genotypes with dendrophenotypes using LMM. When we integrated Interior Alaska and the Alaska Range in a single analysis for the pointer year 1998, we detected 12 SNPs associated with resilience in 1998 after correcting for genetic relatedness and population genetic structure (Table 2; Table S5). No significant associations were detected for climate sensitivity.

The association analysis of genotypes with the dendrophenotypes using LMM revealed no significant associations when testing pointer years at individual sites separately. Our second approach, which involved the polygenic BSLMM and testing sites separately, revealed strong associations with 30 SNPs (Table 2; Table S5). Three of these were associated with two traits. Of the 30 SNPs, 13 were associated with climate sensitivity, including 11 in the Alaska Range



**FIGURE 2** Comparison of measures of individual reaction to drought stress (Lloret indices) between the Brooks Range, Interior Alaska and Alaska Range study sites for different pointer years calculated in the R package POINTRES and visualized with GGPLOT. Pairwise significance was tested with a Wilcoxon test. \*Significant ( $p < .05$ ); ns, not significant. Letters indicate significantly different groups.





**FIGURE 3** Principal component analysis (a) and Bayesian clustering analysis (b) for  $K = 2$  and  $K = 3$  based on 2463 SNP loci genotyped in white spruce (*Picea glauca*) individuals sampled from three different sites and forest/treeline plots. BR, Brooks Range; Int, Interior Alaska; AR, Alaska Range.

and one for the Brooks Range and Interior Alaska, respectively. The remaining 17 SNPs showed strong associations with measures of individual reaction to drought stress. We observed the majority of the associations in the drought-limited Interior Alaska site trees (11 SNPs), with two of the SNPs being associated with two different traits. In addition, we found the highest PIP values for resistance to drought in 2010 (PIP = 0.74 and 0.44). No overlap among strongly associated SNPs with measures of individual reaction to drought stress could be detected among the sites. When comparing the number of associated SNPs among the phenotypic traits, resistance was most frequently observed (eight SNPs), followed by relative resilience (five SNPs), resilience (four SNPs) and finally recovery (three SNPs; Figure S7). Of all traits, climate sensitivity encompassed the highest number of strong associations, biased towards the SNPs of the Alaska Range (11 of 13 SNPs). This is also reflected in the proportion of phenotypic variance explained in BSLMM as

well as in LMM (Figure 4; Figure S5). For Interior Alaska in BSLMM, genetic variance explained the largest proportion of phenotypic variance for the traits resilience, relative resilience and recovery in 1998 (53%, 70% and 62% respectively; Figure 4; Table S4). The phenotypic variance explained by the remaining parameters ranged from 12% to 45% (Table S4). In the Brooks Range, 15%–33% of the phenotypic variation was explained by genetic variation and in the Alaska Range it was 17%–77%. The proportion of phenotypic variance explained (PVE) by large-effect size SNPs was the highest for Interior Alaska for the traits resistance (59%) and relative resilience (42%) in 2010 (Table S4, Figure S6). In the Brooks Range trees, large-effect size SNPs explained a higher proportion of the phenotypic variance (38%–43%) compared to the Alaska Range trees (30%–37%; Table S4, Figure S6). The hyperparameters had large credible intervals which depended on the sample size (X. Zhou, pers. comm.; Table S4).

Site	Trait	Number of associated SNPs	Number of analysed individuals
BSLMM			
Brooks Range	Resistance 1993	2	138
	Resilience 1993	0	138
	Rel. resilience 1993	1	137
	Recovery 1993	1	138
	Climate sensitivity	1	138
Interior Alaska	Resistance 1998	1	154
	Resilience 1998	3	154
	Rel. resilience 1998	3	154
	Recovery 1998	2	154
	Resistance 2010	3	150
	Resilience 2010	0	153
	Rel. resilience 2010	1	154
	Recovery 2010	0	151
Alaska Range	Climate sensitivity	1	154
	Resistance 1993	1	166
	Resilience 1993	1	166
	Rel. resilience 1993	0	166
	Recovery 1993	0	166
	Resistance 1998	1	166
	Resilience 1998	0	166
	Rel. resilience 1998	0	166
	Recovery 1998	0	166
Climate sensitivity	11	166	
LMM			
Interior Alaska & Alaska Range	Resilience 1998	12	320

TABLE 2 Associated SNPs and analysed individuals for each phenotypic trait tested in BSLMM and LMM for each site

Linear mixed model and BSLMM analyses revealed the highest number of significantly and strongly associated SNPs with drought-related parameters in the drought-limited site Interior Alaska. Genomic regions associated with drought tolerance differed between sites. Two SNPs (ss538950708 on chromosome 1 and ss524300164 on chromosome 9) were associated with resilience in 1998 in Interior Alaska by both methods independently (Table S5). In total, 40 unique SNPs could be associated with the measures of individual reaction to drought stress or climate sensitivity.

### 3.4 | Annotation of candidate SNPs

Genes containing SNPs with strong associations differed in their location on the genome among the sites (Table S5). In Interior Alaska, the majority of the genes were located on chromosomes 1 and 10 (three genes each), whereas in the Brooks Range most were located on chromosome 3 (two genes), and in the Alaska Range, chromosome 7 (four genes) and chromosome 4 (three genes) contained the majority of the associations. GO annotation was possible for 24 of

the 40 associated genes (Table S5). Eight of them were related to the cellular component membrane and six genes were related to transferase and/or hydrolase activity. One gene (GQ03312\_O11) could be related to lignin biosynthetic process. Furthermore, for six of the SNPs, we could determine whether the mutation was synonymous (four SNPs) or nonsynonymous (two SNPs).

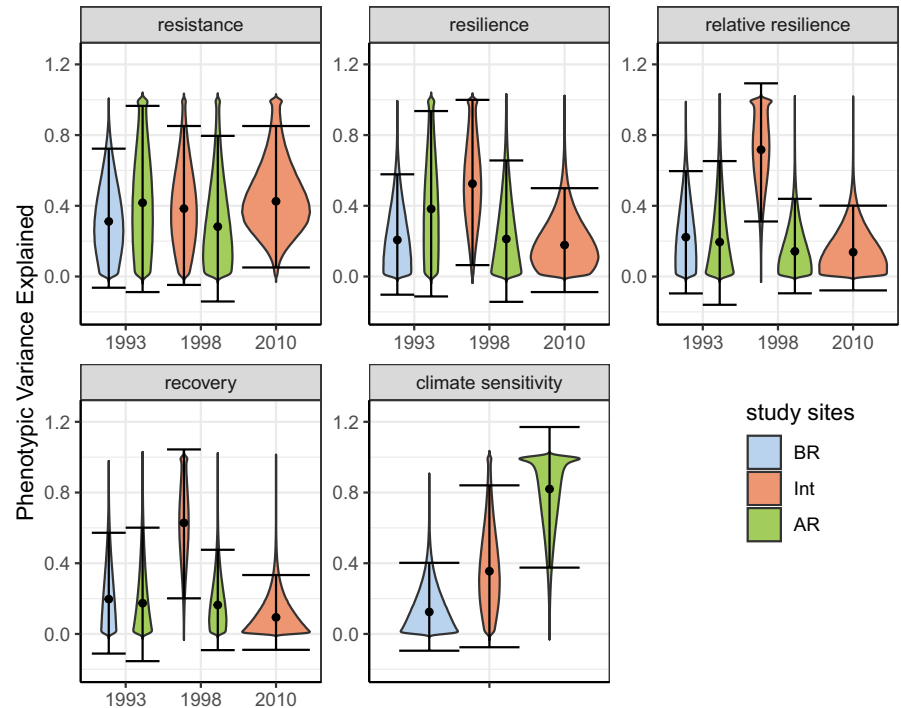
## 4 | DISCUSSION

We investigated three populations of white spruce representing contrasting treeline ecotones at high latitudes, and identified 40 genes associated with dendrophenotypes that informed us about the drought tolerance and climate sensitivity of the trees.

### 4.1 | Climate sensitivity

Spruce trees in our treeline plots showed a higher climate sensitivity than trees in the nearby corresponding forest plots, which may

**FIGURE 4** Results of the BSLMM analysis. Violin plots represent the posterior distributions of the proportion of the phenotypic variance explained by genetic variance (PVE). The median (black circle) and standard deviation (error bars) for each phenotypic trait are shown for Brooks Range (BR), Interior Alaska (Int) and Alaska Range (AR).



be due to the more stressful climate conditions experienced at the treeline as compared to the more protected, closed-canopy forest environment. In fact, the treeline populations were located in an environment where the species experiences its physiological limits within the realized niche. Growth is limited by low water availability (Interior Alaska) or low temperatures (Brooks Range, Alaska Range), resulting in a stronger climate signal (Hampe & Jump, 2011). Consequently, treeline populations are frequently prioritized in dendroecological studies to study the influence of these environmental variables (Cook & Kairiukstis, 1990; Fritts, 1976). Furthermore, we found that shorter trees showed a higher climate sensitivity similar to those found in the treeline plots (Table 1; Figure S2). By far the highest number of genes associated with climate sensitivity were found in the Alaska Range population (11 genes), while only one gene was found for Interior Alaska and the Brooks Range, respectively. Therefore, for the trait climate sensitivity, the phenotypic variance explained by genetic variance was highest in the Alaska Range (77%), intermediate in Interior Alaska (38%) and lowest in the Brooks Range (15%). Our Alaska Range forest and treeline plots were separated both by greater distance and more elevation than was the case in our other sites. Therefore, environmental conditions and consequently climate sensitivity may have differed the most between the plots in the Alaska Range as compared to the other sites. The phenotypic differences among the individuals together with the separating cluster of the Alaska Range forest within the population genetic structure analysis probably led to the high percentage of phenotypic variance explained by genetic variance and we could not exclude the possibility that this was also related to false positives. Five of the associated SNPs in the Alaska Range could be annotated and related to gene functions such as hydrolase activity or cell wall organization. Furthermore, two of the associated SNPs with climate sensitivity in

the Alaska Range were nonsynonymous mutations, which change the amino acid sequence of a protein and are therefore subjected to natural selection. This indicated a genetic basis of climate sensitivity. Climate sensitivity exhibited a higher variability among the sites than the drought-related traits.

## 4.2 | Growth reaction to drought stress

In contrast to climate sensitivity, there was no consistent pattern in the growth reaction to drought stress when comparing trees in forest vs. treeline within our sites. In general, in the Alaska Range and Interior Alaska, trees within the forest plots seemed to recover better from a drought event. For the drought-limited site (Interior Alaska), the growth reaction differed significantly between forest and treeline trees for most of the traits, even though the plots were positioned adjacent to each other. This suggested that the sites' location at a steep south-exposed bluff resulted in a strong microenvironmental gradient at short distance, which seemed to have a strong influence during drought events with stronger effects on the bluff site that was more exposed to radiation (Nicklen et al., 2018). In the cold-limited sites (Brooks Range, Alaska Range), forest and treeline plots exhibited only minor differences in growth reaction, probably due to greater similarity in environmental conditions. Trees in different sites had significantly different reactions to a drought event in the same year, probably due to distinct growth and drought conditions among sites. For the growth reaction in 1998, the drought-limited site (Interior Alaska) showed a significantly higher recovery as compared to the cold-limited site (Alaska Range), which in turn showed a significantly higher resistance. A similar pattern was observed in maritime pine (*Pinus pinaster*), with high resistance in Atlantic and

high recovery in Mediterranean provenances planted in a common garden (Zas et al., 2020). Thus, populations experiencing contrasting environmental conditions seem to adopt various strategies to cope with drought stress. This supports our hypothesis that the individual reaction to drought stress differs between drought and cold-limited treelines as well as between forest and treeline plots. Nevertheless, we could not identify a common pattern within the growth reaction except for climate sensitivity. For gymnosperms, reduced recovery is related to high drought-related mortality risk (DeSoto et al., 2020).

Even though we identified years of growth decline caused by drought based on the above described decision tree, we acknowledged that factors other than drought stress may have affected the reductions in growth that we observed. Furthermore, drought induces masting in white spruce (Ascoli et al., 2020), which could also be the reason for growth reduction at the population level (Hackett-Pain et al., 2015; Nicklen et al., 2018). Mast seeding events were recorded for the Alaska Range site in 1998 and the Interior Alaska site in 2010, overlapping with the analysed pointer years (Roland et al., 2014).

### 4.3 | Contrasting genetic basis underlying drought-tolerant phenotypes

There was no overlap in drought-associated genes among sites, which supports our hypothesis that the selection pressure at the contrasting treeline ecotones has led to divergent genetic signatures underlying drought tolerance. Even in the two cold-limited sites (Brooks Range, Alaska Range) spruce trees showed different genetic signatures associated with drought tolerance. However, it is important to mention that the two cold-limited sites differed in precipitation as well as in temperature. At these treelines, frost tolerance may also have been a strong selection driver in addition to drought. Thus, signatures of selection were population-specific and have led to different alleles being associated with drought-tolerant phenotypes, similar to the finding reported for populations of *Arabidopsis halleri* in heterogeneous alpine environments (Rellstab et al., 2017). Moreover, the location of the associated genes on the genome varied widely, with genes on different chromosomes being identified for different sites. Even though we analysed two drought years for the Alaska Range site compared to only one for the Brooks Range site, the Alaska Range site showed the lowest number of GPAs with growth reaction, possibly explained by the comparatively high precipitation sums that occur at the Alaska Range site. The drought-limited site, Interior Alaska, had the highest number of significantly associated genes with growth reaction to drought stress and the highest proportion of phenotypic variance explained by genetic variance (70%). This indicated comparatively strong selection of drought-tolerant phenotypes within the drought-limited site. Populations experience the strongest selection pressure under extreme events such as droughts, which shape the genetic variation among populations (Grant et al., 2017). This high selection pressure causes small to moderate shifts in allele frequencies (Depardieu et al., 2021). A high

resilience to extreme drought events was also found in white spruce populations from dry regions planted in a common garden, which led to the assumption that genetic variation among populations plays a significant role in growth resilience in response to drought (Depardieu et al., 2020). Heritability estimates for drought response traits indicated significant natural genetic variation among polycross families of white spruce (Laverdière et al., 2022). Furthermore, the adaptive genetic variation and phenotypic correlations between drought response and wood traits differed among provenances of *Picea abies*, indicating different selection intensities (Trujillo-Moya et al., 2018). Still, gene flow among sites was high, as demonstrated by the high seed- and pollen-immigration rates and the low genetic differentiation among the investigated sites (Zacharias et al., 2021). The LMM only revealed significant SNPs when simultaneously analysing Interior Alaska and the Alaska Range, the two sites which genetically differentiated within the Bayesian clustering analysis for  $K = 2$ . Therefore, there could have been a covariance between the genetic differences between the sites and site-specific growth reaction to drought stress, resulting in spurious associations. Nevertheless, two of the associated SNPs were identified independently in the BSLMM.

### 4.4 | The polygenic basis of drought tolerance

The association approach, which took into account multiple SNPs and their interactions together with small-effect size SNPs (BSLMM), resulted in a much higher number of significant associations than the LMMs, pointing towards a complex genetic architecture of drought tolerance in white spruce. When analysing complex traits such as those relating to growth, multilocus approaches commonly outperform single-locus approaches (Moser et al., 2015). In conifers, traits involved in local adaptation to climate are known to be polygenic (Csilléry et al., 2018; Sork, 2017), and adaptation is driven by interacting small-effect size alleles rather than a few large-effect alleles (Hornoy et al., 2015; Le Corre & Kremer, 2012). Especially in populations with high gene flow and recent selection events, local adaptation involves small allele frequency changes that interact in complex pathways (Hornoy et al., 2015). Nevertheless, for resistance within the Interior Alaska site, the phenotypic variance explained by large-effect size SNPs was highest, indicating the influence of a few genes with larger effects on drought resistance. The two SNPs with the highest PIP were found for this trait in the Interior Alaska site. This could suggest selective sweeps such as those reported for *Sequoia sempervirens* and *Sequoiadendron giganteum* in relation to local adaptation (De La Torre et al., 2022). When testing our phenotypic traits, the main and polygenic SNPs within the BSLMM analysis explained 12%–77% of the phenotypic variance. These values were higher than those reported for drought-responsive growth traits in white spruce populations in a common garden (11%–33.6%) or fitness-related traits in natural *Pinus albicaulis* populations (14.4%–37.6%) (Depardieu et al., 2021; Lind et al., 2017), probably due to the higher number of individuals and populations in the cited studies.

Most of the associated genes were found in climate sensitivity at the Alaska Range site. For the drought-related traits, the highest number of associations was found for resistance, thereby suggesting a strong polygenic basis for spruce trees' ability to resist the effects of drought.

#### 4.5 | Genes significantly associated with drought tolerance

Three of the identified genes (GQ03814\_E07: O-fucosyltransferase 23-like, WS00110\_K01: probable inactive leucine-rich repeat receptor-like protein kinase At3g03770, GQ03417\_G17: uridine-cytidine kinase C) were associated with multiple traits (Table S5), and two other genes (GQ03701\_H09: auxin response factor 6, GQ03417\_G17: uridine-cytidine kinase C) were independently associated with resilience by both GPA analyses. These genes were associated with various biological processes, molecular functions, and cellular components such as transferase or hydrolase activity, suggesting their relevance to drought tolerance. Sixteen of the genes that associated with drought-relevant phenotypic traits in our analysis were also represented among the 110 differentially expressed genes in white spruce in response to drought in a glasshouse experiment (Depardieu et al., 2021). Furthermore, eight of the 40 associated genes have been associated with wood anatomy traits such as wood density in Canadian white spruce provenances (Lamara et al., 2016). Wood density influences drought tolerance in conifers (Martinez-Meier et al., 2008). Indeed, in a common garden experiment using *Pseudotsuga menziesii*, all trees that survived a strong drought had a higher stem wood density, ring density and latewood density than the individuals that died (Martinez-Meier et al., 2008). Nevertheless, within the study sites, xylem anatomical traits were influenced by microhabitat, but latewood density and earlywood hydraulic diameter showed moderate heritability (Pampuch et al., 2020). Moreover, eight of the associated genes were related to the cellular component membrane and one to lignin biosynthetic process, which may alter the wood anatomy and drought tolerance in trees. Still, selection can only act on traits that are heritable. Five of the associated genes were both differentially regulated under drought and associated with wood traits (Depardieu et al., 2021; Lamara et al., 2016) and were therefore related to drought tolerance. One gene (GQ03617\_M21: 21kDa protein-like) was associated with resilience for trees in Interior Alaska in our study and it was also identified as a high-confidence gene in correlation with phenotypic and environmental data in white spruce in a common garden setting in eastern Canada (Depardieu et al., 2021). The fact that these genes were repeatedly related to drought stress supports their status as candidate genes for drought tolerance-related growth traits. The reason for the limited number of overlapping genes between our study and the study of Depardieu et al. (2021) could be that we investigated natural populations with trees of different sizes and ages and inhabiting locations with varying environmental conditions in contrast to their controlled common garden settings. Still, we tried to account

for the differing environments by using the household matrix (i.e., a binary encoded matrix indicating if individuals are from the same or different study sites) within the LMMs when testing multiple sites together or testing the sites separately in BSLMM. Furthermore, the Alaskan and Canadian study sites were located at the western and eastern edge of the white spruce distribution range, which not only reduced gene flow but also represented populations from different glacial refugia (Anderson et al., 2011) and broadly different climatic norms. Adaptation to drought can also occur via independent routes such as those described for two populations of *Brassica rapa* which shared parallel shifts in allele frequency in only a few genes (Franks et al., 2016). Many genes related to climate adaptation are known to be involved in transferase and hydrolase activities in white spruce (Depardieu et al., 2021; Hornoy et al., 2015) and Norway spruce (Azaiez et al., 2018). In our study, six of our 24 successfully annotated genes could be associated with hydrolase and/or transferase activities, which supported the important roles of these genes.

## 5 | CONCLUSIONS

White spruce trees growing in treeline plots showed a higher sensitivity in growth response to climate than trees in nearby, paired forest plots due to more extreme environmental conditions experienced in the treeline. Still, our results indicated a minor genetic basis for climate sensitivity. Our tree populations growing in different environments responded differently to drought stress, suggesting that future drought events induced by climate change will have contrasting effects on white spruce across the landscape of Interior Alaska. In addition, populations from different environments had divergent genetic signatures underlying drought tolerance, with most genes found in populations that are exposed to more frequent and intense drought stress. As a consequence, our results supported the hypothesis that different combinations of genes respond to selection pressure in populations in different environments, and thus adaptation to drought is a local process. Furthermore, the large number of small-effect size SNPs demonstrated the polygenic and complex architecture of drought tolerance in trees. Genes that were associated with wood traits in our study and also differentially expressed under drought conditions in other studies are very probably involved in drought response of white spruce. Thus, these genes are important resources that help us understand possible trajectories of future change in boreal forests, and can inform assisted migration programmes in the context of more severe and recurrent extreme climatic events.

### AUTHOR CONTRIBUTIONS

M.W. and M.S. designed the overall study design. C.R. assisted in realizing the Alaska Range study sites. M.Z., T.P. and M.W. collected samples with the help of field work assistants. Conceptualization of the GPA analysis was done by K.H. and L.O. M.Z. prepared samples for genotyping. T.P. performed dendro analysis. M.Z. performed the population structure and GPA analysis with support

from B.D. and M.B. M.Z. wrote the manuscript with contributions from all authors. All authors revised and refined the final manuscript.

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## CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

Raw and filtered genotypic data, tree ring data, and R scripts containing the filtering steps and analysis are deposited on Zenodo (Doi: <https://doi.org/10.5281/zenodo.6104140>).

## BENEFIT-SHARING STATEMENT

We developed a research collaboration with scientists from the University of Alaska Fairbanks and Denali National Park Preserve to be enabled for sampling. Collaborators were included as co-authors. We share the results with the provider communities. The research addresses a priority concern, in this case the conservation of white spruce.

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## SUPPORTING INFORMATION

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