Phenotypic plasticity and local genetic adaptation in white spruce

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Abstract

Forests influence the climate of our Earth and provide habitat and food for many species and resources for human use. These valuable ecosystems are threatened by fast environmental changes caused by human-induced climte change. Negative growth responses and higher tree mortality rates were associated with increasing physiological stress induced by global warming. Especially boreal forests at high latitudes in the arctic region are threatened, a region predicted to undergo the highest increase in temperature during the next decades. Therefore, it is important to assess the adaptation potential in trees. For this purpose, I studied natural populations of white spruce (*Picea glauca* (Moench) Voss) in Alaska. In this thesis, I present three scientific papers in which my co-authors and I studied the phenotypic plasticity and genetic basis of tree growth, wood anatomy and drought tolerance as well as the genetic structure of white spruce populations in contrasting environments. We established three sites representing two cold-limited treelines and one drought-limited treeline with a paired plot design including one plot located at the treeline and one plot located in a closed-canopy forest, respectively. Additionally, the study design included one forest plot as reference. Within the entire project, in total 3,000 trees were measured, genotyped and dendrochronological data was obtained. I used several approaches to estimate the neutral and adaptive genetic diversity and phenotypic plasticity of white spruce as a model organism to explore the adaptation potential of trees to climate change.

In the first chapter, I combined neutral genetic markers with dendrochronological and climatic data to investigate population structure and individual growth of white spruce. Several individual-based dendrochronological approaches were applied to test the influence of genetic similarity and microenvironment on growth performance. The white spruce populations of the different sites showed high gene flow and high genetic diversity within and low genetic differentiation among populations, rather explained by geographic distance. The individual growth performances showed a high plasticity rather influenced by microenvironment than genetic similarity.

In the second chapter, I investigated the populations of the drought and cold-limited treeline sites to decipher the underlying genetic structure of drought tolerance using different genotype-phenotype association analyses. Based on tree-ring series and climatic data, growth declines caused by drought stress were identified and the individual reaction to the drought stress event was determined. A subset of 458 trees was genotyped, using SNPs in candidate genes and associated with the individual drought response. Most of the associations were revealed by an approach which took into account small-effect size SNPs and their interactions. Populations of the contrasting treelines responded differently to drought stress events. Populations further showed divergent genetic structures associated with drought responsive traits, most of them in the drought-limited site, indicating divergent selection pressure.

In the third chapter, my co-authors and I studied xylem anatomical traits at one of the cold-limited treeline sites to investigate whether genetic or spatial grouping affected the anatomy and growth of white spruce. Annual growth and xylem anatomy were compared between spatial groups and between genetic groups and individuals. Overall, wood traits were rather influenced by spatial than genetic grouping. Genetic effects were only found in earlywood hydraulic diameter and latewood density. Environmental conditions indirectly influenced traits related to water transport.

In conclusion, white spruce showed a high genetic diversity within and a low genetic differentiation among populations influenced by high gene flow rates. Genetic differences

among populations are rather caused by geographical distance and therefore genetic drift. Differing selection pressure at the treeline ecotones presumably lead to divergent genetic structures underlying drought-tolerant phenotypes among the populations. Thus, adaptation to drought most likely acts on a local scale and involves small frequency shifts in several interacting genes. The identified genes with adaptive growth traits can be used to further exlore local adaptation in white spruce. Tree growth and wood anatomical traits are rather influenced by the environment than genetics and showed a high phentoypic plasticity. The high genetic diverstiy and phenotypic plasticity of white spruce may help the species to cope with rapid environmental changes. Still, additional work is needed to further explore adaptation processes to estimate how tree species reacted to rapid climate change. The presented thesis shed some light on the adaptation potential of trees by the example of white spruce using several approaches.

Contents

The conceptual framework of RESPONSE

The ability of organisms to respond to a changing environment increases in importance in an era of human-induced climate change. Species are able to react to environmental change by phenotypic plasticity, genetic adaptation or range shift. Still, the question remains if those response mechanisms are enough to cope with climate change. Especially long-lived and sessile organisms like trees are challenged by the rapidly changing environment and the speed of change could exceed their adaptation ability. Therefore, within the thesis I focused on white spruce (*Picea glauca* (Moench) Voss) populations at Alaskan treelines to explore the adaptation potential of trees. The thesis is part of the research training group "Biological RESPONSEs to novel and changing environments" in the second generation, funded by the German Research Foundation (DFG). The project within the thesis is written, belongs to cluster A which deals with in-situ responses of species, in detail with the phenotypic plasticity and genetic variance of traits in natural populations (Fig. 1). The studies presented in the thesis describe the neutral genetic diversity of white spruce populations, genetic associations with dendrophenotypes and the plasticity of traits like tree growth, wood anatomy and drought tolerance. The results presented help to estimate the adaptation potential of white spruce to better predict the response of trees to climate change. The thesis is written in collaboration with Timo Pampuch from the RESPONSE project B2 who focused on heritability of wood traits and the dispersal capacity of white spruce.

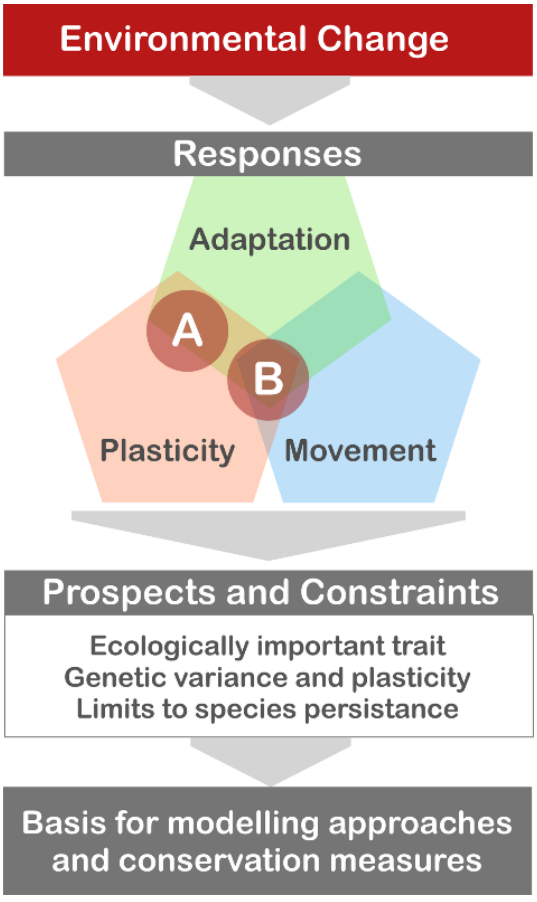

Figure 1: Flowchart concept of the research training group RESPONSE (https://biologie.uni-greifswald.de/forschung/dfg-graduiertenkollegs/research-training-group-2010/)

1. Introduction

1.1. General background

Around 30% of the land surface is covered by forests which contain about 80% of the Earth´s total plant biomass (Pan et al., 2013). Forests influence the climate of our Earth by playing a crucial role in the global carbon cycle (Arneth et al., 2010; Tagesson et al., 2020). Especially boreal forests store more carbon in the soil than other forest biomes. Forests provide habitat and food for many species and resources for human use like timber and non-timber products (Melillo et al., 1993). Further, they fulfill important ecoystem services like air and water filtration, recreation and tourism (Gauthier et al., 2015; Hall et al., 2011; Wells et al., 2020). These precious ecosystems are threatened by human-induced global warming and the accompanying fast changing environmental conditions (Lindner et al., 2010; Yangyang et al., 2018). Extreme weather conditions will increase in frequency and intensity (IPCC, 2021) and the consequently increasing physiological stress is associated with negative growth responses and higher tree mortality rates (Allen et al., 2010; Hynes and Hamann, 2020; van Mantgem et al., 2009). This especially threatens forest ecosystems at high latitudes like boreal forests in North America, where the highest increase in temperature is predicted during the next decades (Collins et al., 2013; IPCC, 2021). Furthermore, forests are affected by human disturbances like habitat fragmentation or overexploration (Allan et al., 2017). Still, questions related to the genomics of local adaptation and the genomic basis of adaptive traits remain unanswered and it is not clear how forests will react to a fast changing environment (Orr, 2005).

The forest ecosystem is influenced by many factors like weather and soil conditions or biotic factors like inter-species competition, mycorrhiza, insects or fungal infections. Studies which aim to answer the question how trees adapt to climate change investigate weather, soil conditions, nutrients, genetic diversity, as well as tree growth, morphology, insect resistance, reproduction and their interactions (Beaulieu et al., 2020; Filipescu and Comeau, 2007; Lamhamedi et al., 2006; Li et al., 1993; Wang and Klinka, 1997). Recent studies more often combine dendroecology with genetics to assess the complex genetic architecture underlying stress response in trees (Depardieu et al., 2021; Heer et al., 2018; Housset et al., 2018; Laverdière et al., 2022; Trujillo-Moya et al., 2018).

The aim of this thesis is to explore adaptation processes in trees by investigating the genetic diversity and phenotypic plasticity of white spruce treeline populations in Alaska. Me, on behalf of my co-authors, combined the analysis of tree growth and wood anatomy together with neutral and adaptive genetic variation to meet the complexity of climate change adaptation in the forest ecosystem. We chose white spruce (*Picea glauca* (Moench) Voss) as model organism and its range margin populations to investigate the effects of microenvironment and genetic similarity on tree growth and wood anatomy, as well as its population structure and genetic basis and plasticity of drought tolerance in contrasting environments. We chose natural populations in Alaska, one of the regions which is warming most in the course of global warming (Handorf et al., 2017), to estimate the adaptation potential. White spruce has a high economic and ecological importance in North America.

1.2. Tree adaptation to climate change

In the face of climate change, forest tree populations will locally adapt to the new environment, migrate to more suitable habitats or go extinct (Aitken et al., 2008). Large ranges will help trees to survive and/or migrate into suitable habitats (Aitken et al., 2008; Mimura and Aitken, 2007), but some species already lag behind their potential distribution range and assisted migration is frequently debated (McLachlan et al., 2007). Recent studies suggest that the long generation times of tree species occuring in high mountain areas hampers the ability to keep up with rapid climate change, which makes them more prone to local extinction (Dauphin et al., 2021).

The adaptation potential of trees is limited by their long generation time and sessile biology, so they can only dispers by seeds (Shaw and Etterson, 2012). Whereas, advantagous traits in regard of adaptation are high disperal capacity as well as adaptive capacity, which includes high phenotypic plasticity, standing genetic variation, mutations and recombination (Aubin et al., 2016; Sultan, 2016). The high fecundity of trees and large populations promote a high genetic diversity because the recombination of different alleles from different loci across the large genome produces a wide range of genotypes (Aitken et al., 2008). This may result in a wide array of phenotypes from which the best locally adapted ones can be selected. However, the distribution of genotypes can be limited by intra- and interspecific competition (Savolainen et al., 2007). In addition, trees have a high phenotypic plasticity, which enables them to cope with climate extremes with relatively fast adaptations (Valladares et al., 2014). Phenotypic plasticity is assumed to play an important role in geographical range shifts or the colonization of new environments (Bonamour et al., 2019). At the same time it can reduce the selection pressure which results in slower genetic adaptation (Botero et al., 2015). Beyond that, phenotypic clines may be further intensified through epigenetic effects induced by local climate on maternal parents (Aitken et al., 2008).

The long-term selection acting on morphological and physiological traits can lead to ecotypic differentiation in contrasting environments, called isolation-by-environment (Gratani, 2014). Additionally, extreme events like droughts set up a strong selection pressure on populations and shape the genetic variation among populations (Grant et al., 2017). These selective pressures among populations act on genetically controlled fitness traits which differ among individuals (Rellstab et al., 2015). Gene flow and the introgression of maladapted alleles into the local gene pool could counteract natural selection and therefore local adaptation (O'Connell et al., 2007; Rajora et al., 2005). Gene flow is especially high in wind-pollinated species and keeps populations connected to maintain the high genetic diversity within populations and low genetic differentiation among populations (Avanzi et al., 2020; Leonarduzzi et al., 2016; Liepelt et al., 2002; Piotti et al., 2009). However, the asynchrony in reproductive phenology of populations in different climates could hinder long-distance gene flow (Aitken et al., 2008).

For natural populations, linkage disequilibrium is low due to large populations and high outcrossing rates mediated by effective gene flow via pollen. Therefore, many genes affecting adaptive traits are inherited largely independently (Aitken et al., 2008). In forest populations characterized by high gene flow and which undergo recent selection, genes related to local adaptation are expected to interact in a complex way and show small frequency shifts (Hornoy et al., 2015). In conifers, traits involved in local adaptation to climate are known to be polygenic and selective sweeps likely affect only a few genes (Aitken et al., 2008; Csilléry et al., 2018; Sork, 2017). In summary, adaptive traits are affected by small effects of many genes enhanced by a high genetic diversity which may promote rapid local adaptation despite high gene flow (Aitken et al., 2008).

1.3. Treeline populations to study adaptation to climate

Treelines are characterized by low tree densities and typically consist of only one tree species (Harsch and Bader, 2011). They are popular in research to investigate abiotic growth-limiting factors (Körner, 2012), because growth at the treeline is mostly limited by a specific environmental factor like low temperature or water availability (Frenne et al., 2013; Lines et al., 2012). Therefore, treelines are suitable to investigate the influence of this one environmental factor on population structure, selection processes and phenotypic plasticity (Cabon et al., 2020; Vitasse et al., 2010). Populations at the treeline are further suitable to study adaptation processes to environmental conditions because trees reach the limits of their realized niches at the treeline, where tree growth and survival are reduced (Case and Taper, 2000; Hampe and Jump, 2011; Restoux et al., 2008).
Alaskan alpine treelines are limited in growth by low temperatures but water limitation has also been shown (Ohse et al., 2012) associated with negative growth responses (Hynes and Hamann, 2020). In contrast, a longer growing season induced by global warming led to an increase in growth and to an advancing treeline (Wilmking et al., 2004; Wilmking et al., 2006). Conifer treeline ecotones are characterized by lower seed production and viability as well as extensive seed immigration and gene flow (Crofts and Brown, 2020; Johnson et al., 2017). In this case, gene flow could introduce preadapted alleles from warmer regions to promote adaptation to a warming climate at high elevations (Aitken et al., 2008; Bontrager and Angert, 2018). On the other hand, gene flow can lead to adaptation lags in climate margin populations (Fréjaville et al., 2019) when maladapted alleles are introduced into the local gene pool and counteract local selection processes (Lenormand, 2002; O'Connell et al., 2007; Rajora et al., 2005). Low rates of allele frequency shifts in high mountain treeline stands are reported, resulting in high genomic vulnerability to rapid climate change and higher vulnerability to local extinction (Dauphin et al., 2021).

1.4. Methodological outline

The traditional way of investigating the influence of environment on phenotype are reciprocal transplant experiments or common garden experiments, which investigate phenotypic and fitness differences among different provenances (Rellstab et al., 2015) (Fig. 2). Common garden studies are suitable to study heritability ex situ and demonstrate genetic adaptation to local site conditions (Merilä and Hendry, 2014; Savolainen et al., 2007). Though, we cannot investigate the speed of adaptation using this set-ups (Hoffmann and Sgrò, 2011). Over the past 20 years, microsatellite markers (simple sequence repeats - SSR) have been widely used for plant genotyping. SSR markers are tandem repeated motifs of 1-6 bp which frequently occur on the genome and can be present in coding and noncoding regions (Kalia et al., 2011). They are codominant and multi-genic markers that are experimentally reproducible and inexpensive (Vieira et al., 2016). SSR markers are useful to investigate gene flow, genetic diversity within and genetic differentiation among populations and to infer the degree of relatedness between individuals (Vieira et al., 2016). Besides that, next-generation sequencing technologies allowed us to screen millions of single-nucleotide polymorphisms (SNPs) across the whole genome to explore the evolutionary and adaptive capacity of more complex genomes like that of conifers (Sork et al., 2013). To increase the efficiency of testing, it is possible to use targeted sequencing for already identified candidate genes. SNPs enable us to identify functional genes and regulatory regions that underly phenotypes. These genotype-phenotype associations correlate

phenotypic measurements with genotypic data to suggest traits involved in local adaptation (Sork et al., 2013). In contrast, genotype-environment association analyses try to identify genetic variants associated with specific environmental conditions to reveal patterns of local adaptation (Rellstab et al., 2015).
When studying local adaptation in trees, wood anatomy traits became more important in the recent years. Heritability of wood anatomical traits is important in regard of climate adaptation because the wood xylem conducts water from roots to the leaves which influences drought stress tolerance (Björklund et al., 2017; Hacke, 2015). Further, the phenotypic plasticity of trees can be investigated by wood anatomical traits, because trees can alter their wood anatomy in response to environmental cues (Fonti et al., 2010). Moreover, tree growth is highly variable in response to environmental conditions. The high inter-individual differences qualify the trait for association analyses. Investigating these phentoypic traits helps to improve the understanding of the interplay of phenotypic plasticity and genetic adaptation in forest trees.

Chapter I mainly deals with population genomics using SSR markers, but also explores if tree growth is rather influenced by the genotype or environment (Fig. 2). Chapter II includes a genome-wide association study (GWAS) using SNPs and dendrochronological data. Whereas chapter III deals with the question if wood anatomical traits are rather influenced by the environment or genotype.

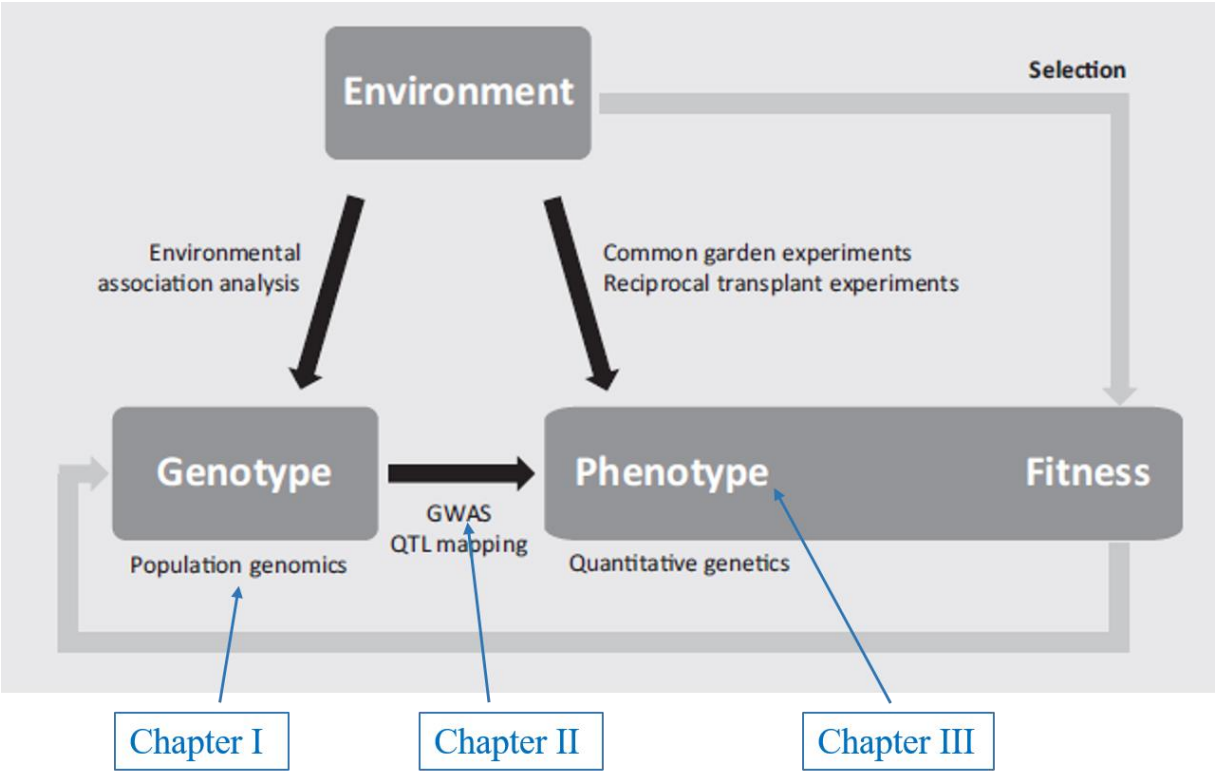

Figure 2: Rellstab et al. 2015: Analyes to detect signs of natural selection and genes involved in local adaptation with the assigned chapters of the thesis.

1.5. White spruce (*Picea glauca* (Moench) Voss)

White spruce has a wide distribution range in North America, reaching from the west coast of Alaska to the east coast of Canada (Burns and Honkala, 1990) and covers a wide range of environmental conditions (OECD, 1999) (Fig. 3). White spruce forms monospecific stands at the latitudinal and elevational treeline but also grows in forest stands together with black spruce (*Picea mariana* Britton, Sterns and Poggenburg), balsam poplar (*Populus balsamifera* L.), quaking aspen (*Populus tremuloides* Michx.) or paper birch birch (*Betula papyrifera* Mashall). In contrast to black spruce, white spruce prefers drainaged and comparatively warm soils without permafrost (Viereck, 1992). White spruce is monoecious and mature at the age of around 30 to 40 years. It is a wind-pollinated species and produces high amounts of seeds, especially in masting years, which are also mainly dispersed by wind (Roland et al., 2014). At the treeline, under harsh environmental conditions, white spruce tends to reproduce vegetatively via layering which leads to groups of clonal trees (Stone and McKittrick, 1976; Wuerth et al., 2018).

Increasing temperatures can favor tree growth and lead to an advancing treeline at the cold-limited sites of white spruce (Wilmking et al., 2004; Wilmking et al., 2006). On the other hand, negative correlations between tree growth and summer temperatures were observed in white spruce in Alaska, probably due to temperature-induced drought stress (Juday and Alix, 2012; Yarie and van Cleve, 2010). White spruce shows different vulnerabilites to moisture-deficits (Hynes and Hamann, 2020) which makes it suitable as model organism to study adaptation processes to different environments. Moreover, raising temperatures further increase the risk of wildfires, frequently occuring in white spruce forests in Interior Alaska (Chapin, 2006). Furthermore, white spruce has a high economic and ecological importance in North America. Therefore, a lot of studies focused on the genetic architecture of wood traits (Beaulieu et al., 2011; Lamara et al., 2016; Lenz et al., 2010) or local adaptation to climate (Hornoy et al., 2015; Namroud et al., 2008). Despite its large genome size (Birol et al., 2013), several genomic resources are already available such as a catalogue of annotated expressed genes (Rigault et al., 2011), genotyping data from SNP arrays (Pavy et al., 2008; Pavy et al., 2013), or high density gene-based linkage maps (Pavy et al., 2012; Pavy et al., 2017).

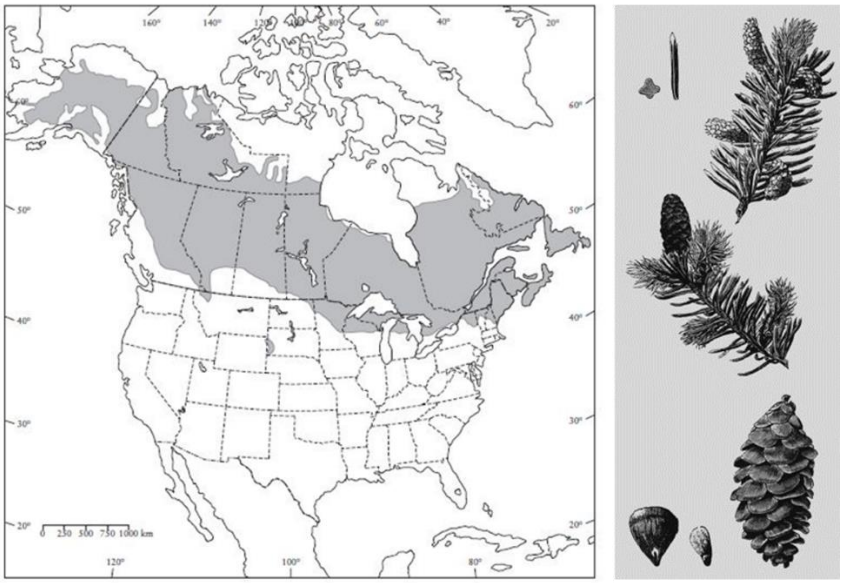

Figure 3: White spruce distribution range (grey) (Earle & Frankis, 2004) in North America (left) and white spruce morphology (Beissner & Fitschen 1930) (right).

1.6. Study sites, field work and sampling

The study design contained three sites in nearly monospecific white spruce stands in Alaska (Fig. 4). Each study site consisted of two plots, one located at the treeline and one plot located in a closed canopy forest. The first study site was located in the Central Brooks Range (67°56'N, 149°44'W) at the latitudinal treeline on a steep south exposed slope. The second study site was located in the Alaska Range (63°43'N, 149°00'W) within the Denali National Park Preserve located at the elevational treeline, also on a south exposed slope. Both sites were established in 2012 by Wilmking et al. (2017). At both sites, tree growth is presumably limited by temperature, therefore they represented cold-limited treelines of white spruce. The third study site was established in 2015 in Interior Alaska near Fairbanks (64°42'N, 148°18'W) located at a south-facing bluff (12-34°) above the Tanana river. Due to higher evapotranspiration rates, tree growth is limited by water availability and the site represented a drought-limited treeline. Additionally, in Interior Alaska one forest plot "Park Loop South" (PLS) complemented the study design, also located near Fairbanks. This plot was situated within a mature and undisturbed closed canopy forest in the center of the white spruce distribution range. The PLS plot as well as the Interior Alaska plots belonged to the Bonanza creek experimental forest (Juday and Alix, 2012; Viereck et al., 1986). In total, approximately 3000 trees were sampled, genotyped, measured (dbh, height) and the location mapped using a differential GPS. About 1000 trees were cored to obtain annual tree-ring data.
For chapter III, genetic material and wood cores of the clonal trees were sampled during field work in 2018 conducted by Timo Pampuch, Andreas Burger, Martin Wilmking and me. For the genetic analyses in chapter II, needles for DNA extraction were sampled from preselected trees during field work in 2019 carried out by Timo Pampuch, Sabine Lichtnau, Andreas Burger and me. Further samples for the genetic data used in chapter I and the tree-ring data analysed in chapter I and chapter III were collected previously by my co-authors and colleagues, described in detail in the corresponding chapters.

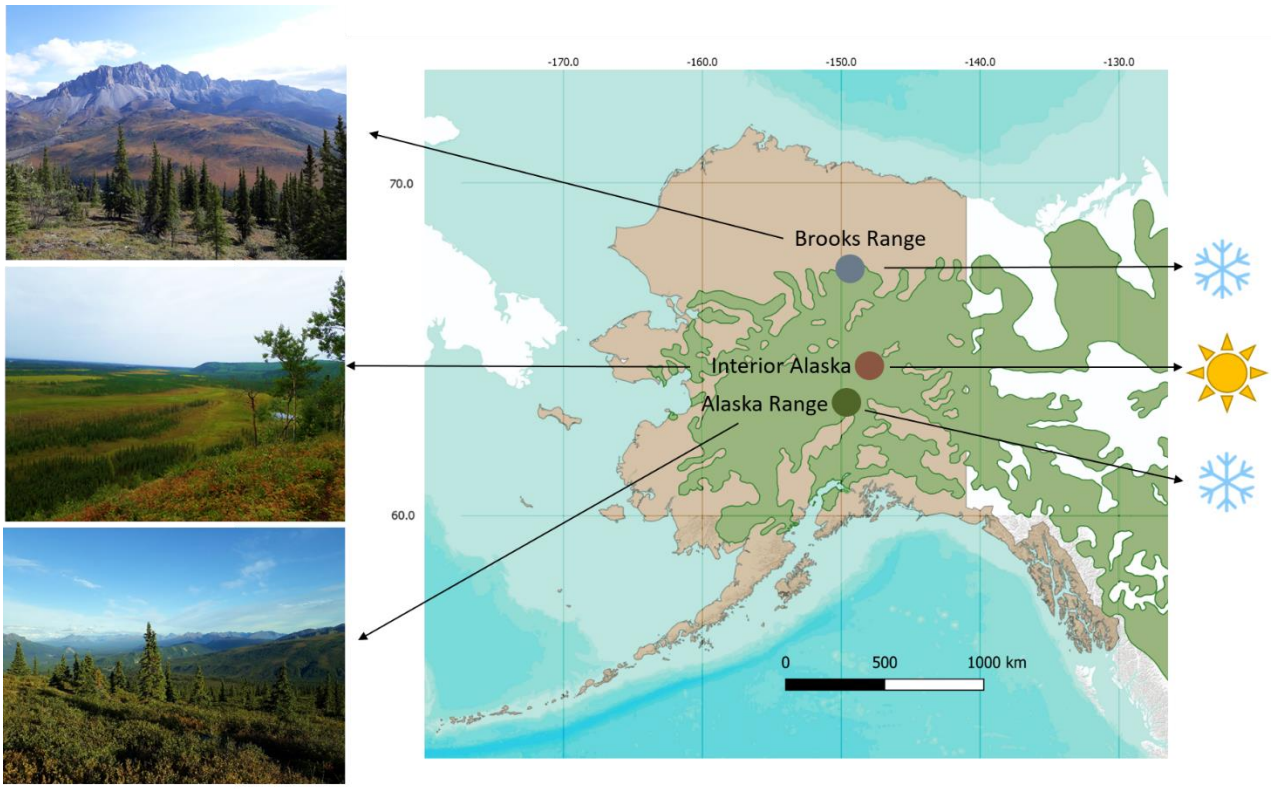

Figure 4: The state of Alaska (light brown) and distribution range (green outline) of white spruce (Picea glauca) in Alaska (Prasad and Iverson 2003). Coloured dots show the location of the three study sites Brooks Range, Interior Alaska and Alaska Range and the corresponding environmental conditions. Photos by me and by Andreas Burger.

2. Author´s contributions to scientific papers in the thesis

Chapter I deals with neutral genetic diverstiy using by microsatellite markers to describe the population structure of the study sites. Morover, it explores if individual tree growth is rather influenced by microenvironmental conditions or genetic similarity. The study of **chapter II** explores the genetic basis of drought tolerance in white spruce by evaluating the individual growth reaction to drought stress associated with single nucleotide polymorphisms. In **chapter III**, the heritability of wood anatomical traits is investigated by using clonal groups of trees at the cold-limited treeline.

Chapter I:

Zacharias, M., Pampuch, T., Heer, K., Avanzi, C., Würth, D.G., Trouillier, M., Bog, M., Wilmking, M., Schnittler, M. (2021): Population structure and the influence of microenvironment and genetic similarity on individual growth at Alaskan white spruce treelines. *Science of the Total Environment* 798 (2021) 149267, doi: 10.1016/j.scitotenv.2021.149267

MW and MS invented the overall study design. DW, MT, MS, MW collected samples with help of others. DW performed genotyping analyses. Conceptualization of statistical analyses was done by KH. R scripts for the growth performance analyses were provided by CA. I evaluated environmental data and conducted all statistical analyses except model adaptation, which was done by TP. I wrote the manuscript with contributions from all authors.

Chapter II:

Zacharias, M., Pampuch, T., Dauphin, B., Opgenoorth, L., Roland, C., M., Schnittler, Wilmking, M., Bog, M., Heer, K. (2022): Genetic basis of growth reaction to drought stress differ in contrasting high-latitude treeline ecotones of a widespread conifer. *Molecular ecology* (submitted)

MW and MS designed the overall study design. CR assisted in realizing the Alaska Range study sites. TP, MW and I collected samples with the help of others. Conceptualization of the GPA analyses was done by KH and LO. I prepared the samples for genotyping. TP performed dendro analyses. I performed population structure and genotype-phenotype association analyses. I wrote the manuscript with contributions from all authors.

Chapter III:

Pampuch, T., Anadon-Rosell, A., **Zacharias, M**., von Arx, G., Wilmking, M. (2020): Xylem anatomical variability in white spruce at treeline is largely driven by spatial clustering. *Frontiers in Plant Science* 11, Article 581378, doi: 10.3389/fpls.2020.581378

TP, MW, and I designed the study and conducted field work and sampling. TP prepared the samples and performed xylem anatomical measurements with help from GA. I performed the genetic analyses. TP performed all statistical analyses with help from AA-R. TP wrote the manuscript with contributions from all authors.

The abstract, introduction, synthesis and conclusion of the thesis is solely written by me.

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Melanie Zacharias (doctoral student)

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Prof. Dr. Martin Schnittler (supervisor)

3. Publications

Chapter I

Population structure and the influence of microenvironment and genetic similarity on individual growth at Alaskan white spruce treelines

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Population structure and the influence of microenvironment and genetic similarity on individual growth at Alaskan white spruce treelines

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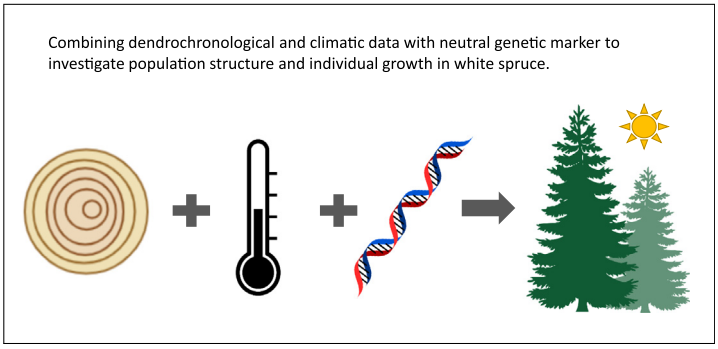
HIGHLIGHTS

- We combined neutral genetic marker with dendrochronological and climatic data to investigate population structure and individual growth of white spruce
- We used individual-based dendrochronological approaches
- White spruce populations showed low differentiation, high genetic diversity and high gene flow
- Growth performances showed high plasticity influenced rather by microenvironmental features than genetic similarity

GRAPHICAL ABSTRACT



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ABSTRACT

Knowledge on the adaptation of trees to rapid environmental changes is essential to preserve forests and their ecosystem services under climate change. Treeline populations are particularly suitable for studying adaptation processes in trees, as environmental stress together with reduced gene flow can enhance local adaptation. We investigated white spruce (*Picea glauca*) populations in Alaska on one moisture-limited and two cold-limited treeline sites with a paired plot design of one forest and one treeline population each, resulting in six plots. Additionally, one forest plot in the middle of the distribution range complements the study design. We combined spatial, climatic and dendrochronological data with neutral genetic marker of 2203 trees to investigate population genetic structure and drivers of tree growth. We used several individual-based approaches including random slope mixed-effects models to test the influence of genetic similarity and microenvironment on growth performance. A high degree of genetic diversity was found within each of the seven plots associated with high rates of gene flow. We discovered a low genetic differentiation between the three sites which was better explained by geographic distances than by environmental differences, indicating genetic drift as the main driver of population differentiation. Our findings indicated that microenvironmental features had an overall larger influence on growth performances than genetic similarity among individuals. The effects of climate on growth differed between sites but were smaller than the effect of tree size. Overall, our results suggest that the high genetic diversity of white spruce may result in a wider range of phenotypes which enhances the efficiency of selection when the

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species is facing rapid climatic changes. In addition, the large intra-individual variability in growth responses may indicate the high phenotypic plasticity of white spruce which can buffer short-term environmental changes and, thus, allow enduring the present changing climate conditions.

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1. Introduction

Boreal forests and woodlands cover around 14.5% of the terrestrial surface, store more carbon in the soil than other forest biomes and provide important ecosystem services like habitat and food for many organisms and resources for human use (Melillo et al., 1993). These valuable ecosystems are threatened by climate change, which is expected to affect boreal forests, most severely at high latitudes (Collins et al., 2013). In this rapidly changing environment, forest tree populations will either go extinct or persist through migration to more suitable habitats or by local adaptation to environmental changes (Aitken et al., 2008). Their dispersal capacity as well as adaptive capacity, which includes high phenotypic plasticity and standing genetic variation, could allow them to cope with local environmental changes (Aubin et al., 2016; Sultan, 2016). However, despite above-average plasticity and genetic variation, trees are sessile organisms with long generation times which limits their speed of adaptation (Shaw and Etterson, 2012). Thus, the question whether tree adaptation will be able to track rapid climatic changes is currently of high relevance. Advantageous traits for climate change adaptation in trees are high fecundity and large populations which promote a high genetic diversity. This may result in a wider range of phenotypes which enhances the efficiency of selection. In addition, large ranges will help to survive and/or migrate into suitable habitats (Aitken et al., 2008; Mimura and Aitken, 2007). Especially conifers show high rates of pollen-mediated gene flow which keeps populations connected and maintain the high genetic diversity within populations (Avanzi et al., 2020; Leonarduzzi et al., 2016; Liepelt et al., 2002; Piotti et al., 2009). Since trees reach the limits of their realized niches at the treeline, where tree growth and survival are reduced, those populations are particularly suitable to study adaptation processes to environmental conditions (Case, 2000; Hampe and Jump, 2011; Hampe and Petit, 2005; Restoux et al., 2008). Conifer treeline ecotones are characterized by extensive seed immigration and gene flow together with lower seed production and viability (Crofts and Brown, 2020; Johnson et al., 2017; Piotti et al., 2009). On one hand, gene flow with preadapted alleles from warmer regions could promote adaptation to a warming climate at high elevations (Aitken et al., 2008; Bontrager and Angert, 2018). On the other hand, the introgression of maladapted alleles into the local gene pool could counteract local selection processes (Lenormand, 2002; O'Connell et al., 2007; Rajora et al., 2005) and lead to adaptation lags in climatic margin populations (Fréjaville et al., 2019).

Besides genetic adaptation, trees show a variability in growth in responses to environmental conditions. In particular, forests at the leading edge are sensitive to increasing temperature, which is apparent in negative growth responses under drought stress (Hynes and Hamann, 2020). On the other hand, growth increase due to a longer growing season ultimately translates into an advancing treeline towards higher latitudes and elevation (Harsch et al., 2009; Wilmking et al., 2004; Wilmking et al., 2006). To study the influence of genetic similarity and environment on tree growth, Avanzi et al. (2019) developed an individual-based approach for Norway spruce (*Picea abies* (L.) Karst.) to identify factors influencing growth performance. In this study, microenvironmental features were more important than genetic similarity in determining similar growth performances. Tree age had a larger effect on individual tree-ring width (TRW) than climate but still a large part of the variance in growth remained unexplained. To disentangle drivers affecting tree growth we applied this individual-based approach to treeline and forest populations of white spruce (*Picea glauca* (Moench) Voss). White spruce forms monospecific stands at the treeline in Alaska (Wilmking and Juday, 2005) and is found all over boreal North America (Burns and Honkala, 1990). Due to its economic and ecological importance in North America, genetic variation in economically important traits as well as candidate genes for local adaptation to climate have been studied, focusing on populations in eastern Canada (Beaulieu et al., 2011; Depardieu et al., 2020; Hornoy et al., 2015; Lamara et al., 2016; Lenz et al., 2010).

In this study we combine neutral genetic marker with spatial, climatic and dendrochronological data to investigate the different processes shaping patterns of genetic diversity and its consequences for growth performance in natural white spruce populations in Alaska. Such information is also highly relevant for the conservation of forest genetic resources, breeding programs and forest management practices. Our study design includes three different sites, one latitudinal and one elevational treeline, both cold-limited, and one moisture-limited treeline. The moisture-limited treeline represents dry and warm conditions predicted for future climate scenarios. Each of the three sites contains a forest and treeline population. Additionally, one forest plot in the middle of the distribution range complements the study design. With these contrasting environments, we investigate genetic differentiation and gene flow among populations and identify drivers of tree growth as well as the influence of genetic similarity and spatial proximity on tree growth. Moreover, we can compare drivers influencing growth between the North American and European spruce species.

Specifically, we investigated (i) the genetic differentiation and (ii) gene flow among forest and treeline populations of white spruce shaped by geographical distance and differing environmental conditions at large scales. Further, we tested (iii) whether genetic similarity and spatial proximity among individuals had an influence on individual growth performance within sites.

Research hypothesis:

1. The population structure of white spruce is shaped by environmental conditions.
2. The individual tree growth of white spruce is influenced by genetic similarity and/or microenvironment.

2. Materials and methods

2.1. Study sites

We established three study sites in nearly monospecific white spruce stands in Alaska (Fig. 1, Table 1). Each study site contained two plots, one at the treeline ecotone and one in a closed canopy forest. The first study site in Central Brooks Range was located at the latitudinal treeline on a steep south exposed slope. The distance between forest and treeline plot was only 30 m, because competition changes fast on a short vertical distance due to the steep slope gradient. The second site in the Alaska Range (Denali National Park Preserve) was located at the elevational treeline, also on a south exposed slope, where forest and treeline plot were 1.3 km apart from each other. These two study sites represented the presumably temperature limited range edge of white spruce. The third site, Interior Alaska, was located near Fairbanks at a steep (12–34°) south exposed bluff of the Tanana river which represents a moisture-limited treeline due to higher evapotranspiration rates. At this site, forest and treeline plot were right next to each other, with the latter plot at the edge of the bluff. For a more detailed description of the study sites see Wilmking et al. (2017), Wuerth et al. (2018) and Trouillier et al. (2018a). Additionally, one forest plot (PLS,

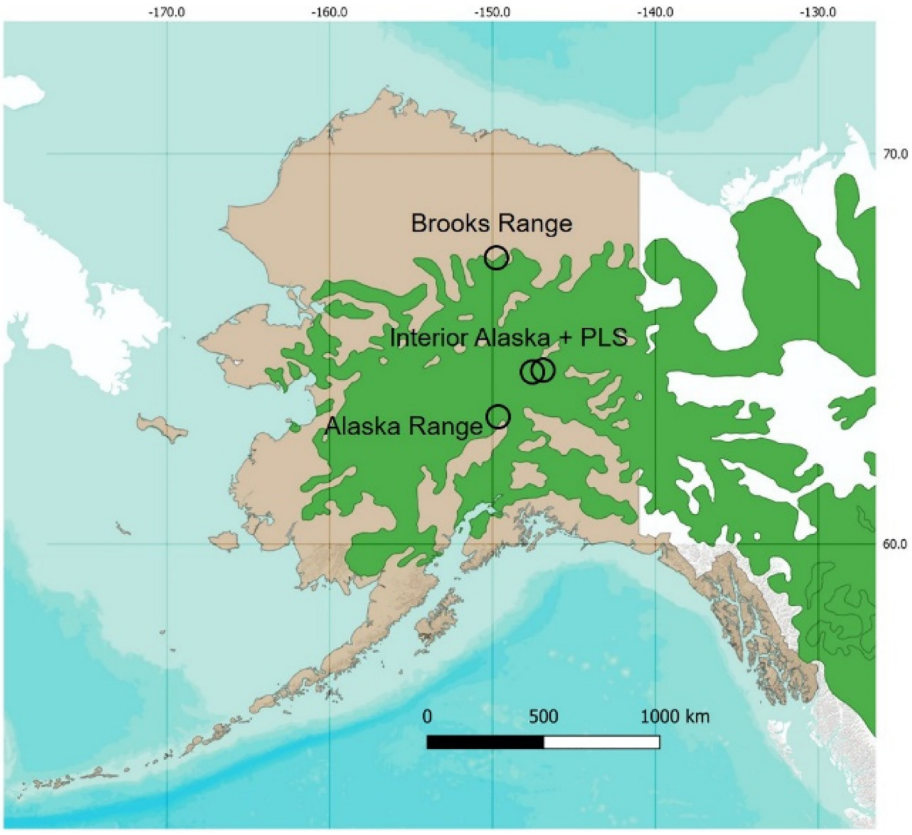

Fig. 1. The state of Alaska (light brown) and distribution range of white spruce (*Picea glauca*) in Alaska (green) (Prasad and Iverson, 2003). Black circles show the location of the three study sites Brooks Range, Interior Alaska + PLS and Alaska Range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ParkS Loop South) in Interior Alaska complemented the study design, representing a mature and undisturbed forest in the middle of the distribution range. The PLS plot and the Interior Alaska bluff plots were 7 km apart and located within the Bonanza creek experimental forest (Juday and Alix, 2012; Viereck et al., 1986).

Each of the seven plots (here referred as populations) contained at least 200 trees and covered an area from 0.5 to 2 ha depending on tree density. Each tree within the plots was sampled, coordinates were recorded with a differential DGPS with a precision of 30 cm and tree height was recorded. Diameter at breast height (dbh) was measured for trees with a height of at least 1.3 m and wood cores were taken from trees with dbh > 5 cm (except for the PLS plot where coring was not permitted). Fresh needles were sampled from each tree and dried on silica gel for genetic analysis. Additionally, we recorded which trees produced cones in 2012 for the plots Brooks Range and Alaska Range as maturity estimator.

2.2. Genetic data

DNA was extracted from dried needles as described in Wuerth et al. (2018). We genotyped 2571 sampled individuals using 11 nuclear microsatellite loci developed by Hodgetts et al. (2001) and Rajora et al. (2001) (Table S1). Microsatellites were combined into three multiplex assays for Brooks Range and Alaska Range (Eusemann et al., 2014), and into two multiplex assays for Interior Alaska and PLS for higher efficiency (Wuerth et al., 2018). PCR conditions and fragment analysis are described in Wuerth et al. (2018). The microsatellite locus PGL 15 was excluded from further analysis because sequences obtained from this locus indicated that the corresponding primers annealed to multiple sites within the genome. Expected and observed heterozygosity as well as proportion of null alleles was calculated with GenAlEx v 6.5. (Peakall and Smouse, 2012) (Table S1). Clonal trees within the plots as well as individuals with more than two markers containing missing

Table 1
Characteristics of the white spruce (*Picea glauca*) research plots. Latitude, longitude and elevation were taken for the center point of each plot, average age was calculated using the oldest ring measured, F = forest plot, T = treeline plot, n.d. = not determined.

Study site	Brooks range		Interior Alaska			Alaska range	
Research plot	BR F	BR T	PLS	Int F	Int T	AR F	AR T
Latitude	67.95	67.95	64.77	64.70	64.70	63.72	63.74
Longitude	149.75	149.74	148.28	148.31	148.30	149.01	149.01
Elevation (m a.s.l.)	876	923	406	180	180	802	1008
Density (trees per ha)	839	232	1128	406	326	507	152
Number of trees sampled for genetic analysis	361	264	489	275	206	338	270
Average dbh ± SD (cm)[a]	8.8 ± 8.7	4.8 ± 6.4	8.5. ± 15.1	12.9 ± 8.6	4.3 ± 8.0	15.5 ± 14.1	5.6 ± 5.9
Number of cored trees	157	67	n.d.	196	92	167	146
Average age ± SD (years) of cored trees	132 ± 54	69 ± 47	n.d.	77 ± 8	64 ± 17	149 ± 46	56 ± 28

[a] Only trees above 1.3 m height were considered.

data were excluded for the analysis, which resulted in a total of 2203 individuals.

Five of 10 markers showed a high degree of null alleles (UAPG_24, UAPG_64, UAPG_87, UAPG_91, PGL_12), as reflected by the divergence between expected and observed heterozygosity (Table S1). Excluding those markers would remove a large part of explanatory power, therefore we tested their influence by running the STRUCTURE analysis (see Section 2.5.1) with only five markers without homozygous excess (UAPG_06, UAPG_08, UAPG_25, UAPG_105, UAPG_144). The results of the two STRUCTURE analyses showed no difference. Thus, we continued using all ten SSR marker. The analysis of NMπ (see Section 2.5.3) and genetic relatedness (see Section 2.5.4) are accounting for missing data as described in detail in the corresponding sections.

2.3. Dendrochronological data

We used a tree-ring dataset containing trees from Brooks Range and Alaska Range originally sampled in 2012 (Eusemann et al., 2016) and updated in 2015 and 2016 (Wilmking et al., 2017), and tree-ring data sampled in Interior Alaska in 2015 (Trouillier et al., 2018a). In total, our dataset contained dated tree-ring series for 779 individuals. Cores were glued onto wooden sample holders and surfaces prepared with a core-microtome. Ring widths were measured from optical scans and crossdating was done visually. For a detailed description of core processing see Wilmking et al. (2017) and (Trouillier et al., 2018a). All series were detrended in "R" v. 4.0.2 (R Core Team, 2015) with the *detrend* function of the package DPLR v. 1.7.1 (Bunn, 2008, 2010; Bunn et al., 2020), using a 30 years' spline. Detrending with a 30 years' spline proved to be a suitable standard method in tree-ring studies conducted within these study sites (Lange et al., 2019; Trouillier et al., 2018b). For each study site a standard and residual chronology was build using the *chron* function with *prewhitening* in DPLR.

2.4. Climatic data

To characterize the different climate conditions among the three study sites, we used monthly climate data (precipitation sum, mean temperature, mean potential evapotranspiration (PET), and mean vapor pressure) downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP) for the period 1950–2015 with a resolution of 2 km^2. From this data the standardized precipitation evapotranspiration index (Vicente-Serrano et al., 2010) was calculated for the summer (Jun–Aug) and the vegetation period (May-Sep) using the R package SPEI v. 1.7 (Beguería and Vicente-Serrano, 2013). For the mixed-effects model SPEI was calculated in a sliding window approach taking into account the previous three (SPEI3) and six months (SPEI6) for each month. Mean vapor pressure was summarized for the summer and the vegetation period. Mean temperature and the sum of precipitation was calculated per year. Additionally, we recorded on-site air temperature with data loggers (EL-USB-1-PRO, Lascar electronics, UK) at 1 h-intervals 2 m above the ground. Loggers were covered with a radiation shield to avoid direct sunlight. On-site temperature measurements were only used as descriptive data because the measurement period was not sufficient to use it for analysis (2016–2019, Alaska Range only 2018–2019). On-site mean annual temperature was higher than database temperatures for all plots (Table S2). A possible reason for this may be the south exposed location of the plots and the climate extremes of the last years. The spatial resolution of the SNAP climatic variables was not high enough to separate forest and treeline plots and on-site air temperature also showed no differences between forest and treeline, thus climate-related analyses were only possible at site level.

To characterize the environmental conditions at the study sites, we identified climate variables which correlate with each other as little as possible ($r^2 < 0.6$) but also separated the study sites (Fig. S2). Therefore, we used a principal component analysis (PCA) and correlation matrices to check for correlation between the initial set of climate variables. The final set of climatic variables consisted of annual temperature, total annual precipitation and mean vapor pressure of the vegetation period. Following the procedure described in Roschanski et al. (2016) the selected variables were used for a PCA (Fig. S2) to explore climatic differences between sites.

2.5. Statistical analyses

2.5.1. Population structure

To assess the genetic structure among sites and plots we used the Bayesian clustering approach implemented in STRUCTURE, v 2.3.4 (Pritchard et al., 2000). K values were tested from 1 to 9 and sample group information was incorporated in the determination of K. Settings were a burn-in period of 5×10^4 iterations and 5×10^4 iterations for the number of membership coefficients with 10 runs. ΔK plots were produced in structure Harvester (Earl and von Holdt, 2012) according to the method of Evanno et al. (2005). Clumpak (Kopelman et al., 2015) was used to visualize the results. Final number of clusters (K) was chosen based on ΔK.

2.5.2. Isolation by distance vs. isolation by environment

To evaluate the influence of isolation by distance vs. isolation by environment on the genetic structure we followed the analysis described in Roschanski et al. (2016). At first a hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN, v 3.5.2.2 (Excoffier and Lischer, 2010) with 10^4 permutations was performed. In case of significant genetic differences among sites we can assume isolation by distance (IBD). In contrast, significant differences between forest and treeline plots could be a sign of isolation by environment (IBE). We used the Mantel and partial Mantel function within the R package ECODIST v 2.0.7 (Goslee and Urban, 2007, 2020) to further evaluate whether IBD or IBE determined the observed genetic differentiation. Genetic distance among sites and plots were determined by calculating G_{ST} values with GENALEX v 6.5. For geographic distances we calculated Euclidean physical distances among sites and plots using longitude, latitude and elevation. As a proxy for environmental distances between sites we used the distances between site scores of the two first principal components (PC1 and PC2) of the PCA calculated on climate variables. As climatic data was only available at the site level, we tested for a pattern of IBE only at site level and for patterns of IBD at plot level.

2.5.3. Influence of gene flow on population differentiation

We assessed gene flow immigration rates within each plot by using the software NMπ v1.1 (Chybicki, 2018). The software implements the neighborhood model, a maximum likelihood approach aimed at reconstructing parent-offspring relationships using individual multilocus genotypes and spatial positions as input. Besides reconstructing seedlings' genealogies, the model estimates seed and pollen immigration rates, parameters of the seed and pollen dispersal kernel, as well as selfing rates. Since NMπ accounts for missing data and genotyping errors, mistyping error rates can be considered as estimable parameters. Individuals were classified either as putative parents or as putative offspring based on tree height which is a better proxy for maturity than age in conifers (Bronson, 2020; Crain and Cregg, 2018). We determined the height at which >30% of the trees produced cones for the plots in Brooks Range and Alaska Range in 2012 and could thus be considered as mature, reproducing trees. Strong external cues like temperature extremes and light conditions can lead to earlier flowering (Bronson, 2020; Crain and Cregg, 2018; Greene et al., 2002; Santos-Del-Blanco et al., 2013), and thus, the height threshold for putative parents was set lower for plots at the distribution edge and low density populations like Alaska Range treeline and Brooks Range forest and treeline (Table S3). Individuals below this height threshold were considered as unlikely to produce seeds, and are thus defined as putative offspring. By setting the height thresholds we removed individuals which could belong to parents as well as to offspring to exclude overlapping generations in the analysis as much as possible.

2.5.4. Influence of genetic similarity and spatial structure on individual growth performances

To test the genetic and spatial influence on growth performance of white spruce populations, we used a two-step analytical framework described in Avanzi et al. (2019). Briefly in a first step, we employed random slope mixed-effects models to quantify the effects of climate and tree size on TRW. We then extracted individual parameters from the models, which characterize the individual growth performances under the consideration of climate and size effects. In a second step, these individual growth performances were tested against genetic and spatial variables and their fine-scale spatial arrangement was assessed through correlograms and kriging. The procedure will be explained in more detail in the following.

For the first step, we used tree-ring chronologies for growth-climate correlations for each study site (see Section 2.3). We calculated the correlation of standard and residual tree-ring chronologies with monthly climate data for each study site using the *dcc* function of the R package TREECLIM v 2.0.3 (Zang and Biondi, 2015). Since there were no striking differences between the correlations of standard and residual chronologies, we proceeded with the standard chronologies. We selected the two to three months which showed the highest and most significant correlation as climatic variables to be included as fixed effect in the model (Table 2). For the Interior Alaska site, we used the sum of precipitation of previous year July, August and September as one climatic driver, since all three months showed similar significant correlations with the tree-ring chronologies. We fitted random slope mixed-effects models for each plot, including the selected climate variables as well as dbh as fixed effects. We used dbh rather than age as fixed-effect variable because tree size, rather than age, alters climate sensitivity in white spruce in Alaska (Trouillier et al., 2018b). To estimate inter-individual variances, we included the ID of individual trees as random factor. We assumed independency of the different random effects. As a response variable we used square root transformed raw TRW data, to fulfill the assumption of normally distributed residuals. The fact that we compare relative (individual slopes) instead of absolute values, allowed us to use transformed data within the model. Model evaluation and reductions of parameters was done in a step-wise manner as described in Avanzi et al. (2019). From these models we extracted individual parameter which characterize individual growth performances.

In the second step, we evaluated whether the individual growth performances that we determined in the random slope mixed-effect models were influenced by genetic similarity and spatial structure using Mantel tests and variance partitioning. For the Mantel tests, we calculated spatial structure as a matrix of pairwise spatial distance and used it as a proxy for microenvironmental heterogeneity, assuming that trees in close proximity are likely to face similar microenvironmental conditions. Pairwise genetic similarity was determined by pairwise relatedness coefficients calculated in POLYRELATEDNESS, v 1.8. (Huang et al., 2016), which takes null allele frequencies into account. We calculated pairwise absolute differences of the extracted individual growth performances from the models to quantify inter-individual differences. For the Mantel test, we used the *mantel()* function implemented in the R package VEGAN v 2.5.6 (Oksanen et al., 2018). Since, Mantel tests suffer from some limitations (Legendre et al., 2015), variance partitioning (Legendre and Legendre, 2012) was used as a second approach to evaluate the effect of genetic similarity and spatial structure on individual growth performances. For this, we estimated the relative contribution of genetic similarity or spatial structure or a combination of both as adjusted R^2 in explaining the variance of individual growth performances (see Avanzi et al., 2019). To determine genetic similarity among individuals, we ran a principal component analysis based on the genetic data with the *dudi.pca* function of the R package ADEGENET v. 2.1.3 (Jombart and Ahmed, 2011) using only components accounting for >50% of the variance. For spatial structure we used a distance-based Moran's eigenvector map as described in Avanzi et al. (2019). Variance partitioning was performed using the *varpart* function of the R package VEGAN. To test for the significance of the variance components we used ANOVA-like permutation tests for redundancy analysis and partial redundancy analysis (RDA) with 10^4 permutations (Legendre and Legendre, 2012). The PLS plot could not be considered for this growth performance analysis since no tree-ring data were available.

Finally, we investigated the fine-scale spatial arrangement of individual growth performances to visualize the spatial clustering of similar growth performances, due to microenvironmental heterogeneity. Therefore, we applied Moran's I correlograms using ten distance classes with even sample size within the R package SPDEP (Bivand and Piras, 2015). To identify the existence and extent of within-plot clusters of trees showing similar individual growth performances, we applied ordinary kriging with an isotropic global neighborhood using the R package GEOR (Ribeiro et al., 2020). Kriging results were displayed only when variograms have a range parameter >3 m and a partial sill >0 (Avanzi et al., 2019).

3. Results

3.1. Genetic diversity

3.1.1. Population structure

STRUCTURE displayed the most pronounced differences for K = 2, which distinguished the study site Brooks Range from the Southern populations (Interior Alaska, PLS and Alaska Range, Fig. 2). When individuals were assigned to three clusters (K = 3), the three study sites

Table 2
REML-estimated parameters (restricted maximum likelihood) which showed statistical significance after model selection procedure with tree-ring width of white spruce (*Picea glauca*) as response variable. Intercept, …, Dbh^2 are parameters of fixed effects (intercept and slopes). Marginal and conditional R^2 reported per model. F – forest, T - treeline.

Study site	Brooks range		Interior Alaska		Alaska range	
Research plot	BR F	BR T	Int F	Int T	AR F	AR T
Intercept (int)	0.94	0.67	0.45	0.22	0.47	0.65
Sum of precipitation previous year July (P-prev_Jul)	1.1×10^{-3}	3.9×10^{-3}	–	–	5.2×10^{-4}	-3.3×10^{-4}
Sum of precipitation of previous year July, Aug, Sept (P-prev_Jul-Sep)	–	–	3.3×10^{-3}	5.8×10^{-3}	–	–
Standardized Precipitation Evaporation Index for July, Aug, Sept in previous September (SPEI3-prev_Sep)	-3.5×10^{-2}	-3.6×10^{-2}	-3.1×10^{-2}	-1.2×10^{-1}	2.2×10^{-2}	–
Mean July temperature previous year (T-prev_Jul)	-3.6×10^{-2}	-2.3×10^{-2}	–	–	–	–
Mean June temperature current year (T-Jun)	-5.8×10^{-2}	1.4×10^{-2}	–	–	1.7×10^{-2}	3.9×10^{-3}
Mean February temperature current year (T-Feb)	–	–	-4.3×10^{-2}	-7.1×10^{-2}	–	–
Dbh	5.0×10^{-2}	6.0×10^{-2}	-3.7×10^{-3}	5.7×10^{-2}	4.1×10^{-2}	6.0×10^{-2}
Dbh^2	2.7×10^{-3}	-3.5×10^{-3}	-3.2×10^{-3}	-3.6×10^{-3}	-1.9×10^{-3}	-5.6×10^{-3}
Marginal R^2	0.2	0.08	0.35	0.26	0.41	0.24
Conditional R^2	0.96	0.91	0.88	0.80	0.99	0.84

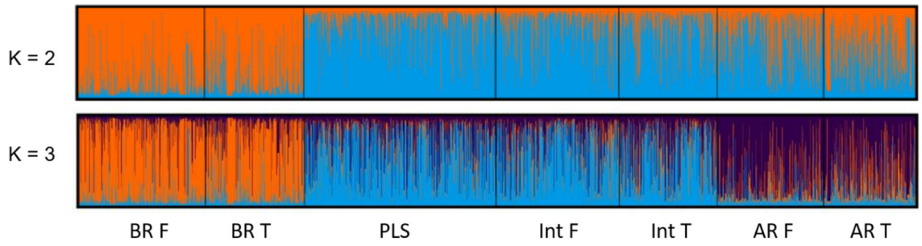

Fig. 2. Population membership coefficient of sampled white spruce (*Picea glauca*) individuals of the three different sites and forest/ treeline plots obtained with STRUCTURE for K = 2 and K = 3 based on 10 SSR loci. BR – Brooks Range, PLS – forest plot (Interior Alaska), Int – Interior Alaska, AR – Alaska Range, F – forest, T - treeline.

were differentiated. Within the sites, forest and treeline plots were not assigned to distinct genetic clusters. Based on ΔK and loglikelihood plots, K = 2 and K = 3 were most representative of the true number of clusters (Fig. S3). Pairwise G_{ST} values showed a low degree of genetic differentiation among sites as well as between plots (mean $G_{ST} = 0.014$, Table S4). The lowest value was found for the pair of Interior forest and Interior treeline ($G_{ST} = 0.001$); the highest between the plots Alaska Range forest and Brooks Range forest ($G_{ST} = 0.026$).

3.1.2. Isolation by distance vs. isolation by environment

The first PC of the PCA on selected climate variables explained 59.1% of the variance and separated Brooks Range and Interior Alaska / PLS due to temperature and vapor pressure. The second PC separated Alaska Range from the other sites due to precipitation differences and explained 37.2% of the variability (Fig. S2).

On site level, the hierarchical AMOVA revealed no significant differentiation (0.28%, $P > 0.05$, $F_{CT} = 0.0028$, Table S5). The partial Mantel test supported the existence of an IBD pattern among sites when accounting for PC1 ($r = 0.961$, $P = 0.04$, Table S6). At the same time, a partial Mantel test considering mean annual temperature while accounting for geographic distance indicated an IBE pattern ($r = 0.865$, $P = 0.043$). On plot level, the AMOVA showed a weak but significant differentiation between the paired plots (forest and treeline) of a study site (0.87%, $P < 0.001$, $F_{SC} = 0.00868$), whereas most of the genetic variance was within the plots (98.85%, $P < 0.001$, $F_{ST} = 0.0115$). The Mantel tests showed that the differentiation among the plots could be explained by geographic distance (IBD) ($r = 0.843$, $P = 0.004$). The differentiation among sites could be rather explained by geographic distance than environmental differences.

3.1.3. Influence of gene flow on population differentiation

Seed and pollen immigration were high in all plots, with mean rates of 62.2% and 34.5%, respectively (Table S3). Seed immigration into treeline plots (72.3%) tended to be higher than into the corresponding forest plots (49.7%). Due to the very high seed immigration rates in the two Interior Alaska plots and Alaska Range treeline plot, the neighborhood model was not able to calculate the remaining parameters due to insufficient data. Test runs with varying thresholds for the group assignments showed a high fluctuation in results except for PLS. Furthermore, the estimated mistyping error rates were high, reflecting the divergence of expected and observed heterozygosity (Table S1).

3.2. Influence of genetic similarity and spatial structure on individual growth performances

3.2.1. Random slope mixed-effects model

In all sites, dbh had a strong influence on TRW (Table 2). The effect of temperature-based climate variables on TRW was overall slightly higher than the effect of precipitation-based climate variables. The selected climate variables differed among sites. For the populations Alaska Range forest and treeline and Brooks Range treeline mean temperature of current June had a positive influence on TRW. TRW of Brooks Range forest was negatively influenced by mean temperature of current June and mean temperature of previous year July. For both Interior Alaska populations, mean temperature of current February had a negative influence on TRW. The drought index SPEI (standardized precipitation evapotranspiration index) calculated for the months July, August and September in previous year September was negatively correlated with TRW in the forest and treeline plots in Brooks Range and Interior Alaska. A positive influence of SPEI3 on TRW was detected for Alaska Range forest and no interaction in Alaska Range treeline. Overall, higher precipitation had a positive influence on TRW. With TreeID as random factor, conditional R^2 values were high in all plots (0.84–0.99), whereas marginal R^2 showed a larger range (0.08–0.41).

3.2.2. Influence of genetic similarity and spatial structure on individual growth performances

Mantel tests between pairwise differences of individual growth performances with spatial proximity revealed significant correlations in six out of 37 cases, with r values ranging from 0.035 to 0.130 (Fig. 3). In contrast, genetic similarity showed a minor correlation with pairwise difference in individual growth performances with only two significant correlations out of 37 comparisons ($r = 0.018$ and 0.026 with dbh^2 and intercept in Interior Alaska forest) (Fig. 4).

The results of variance partitioning and partial RDA analysis supported the Mantel test results (Fig. 5). Genetic similarity explained a minor part of the variance (adjusted R^2 up to 0.17) and showed four significant cases out of 37 cases in partial RDA analysis. In contrast, spatial structure explained up to 26% of the variance in individual growth performances (adjusted R^2 up to 0.26) in eleven out of 37 cases. The joint effect of genetic and spatial structure together resulted in an adjusted R^2 of up to 0.1. Most of the variance in individual growth performances was not explained as shown by the high values of the residuals (Fig. 5).

Spatial autocorrelograms revealed the existence of non-random spatial arrangements for individual growth performances for mean June temperature and dbh for Brooks Range forest, as well as dbh in Brooks Range treeline (Fig. S4). Interior Alaska treeline showed a spatial clumping in individual growth performances for intercept. A clear non-random spatial arrangement was also shown in Alaska Range forest and precipitation of previous year July and the clearest pattern in Alaska Range treeline for dbh. These non-random spatial arrangements of individual growth performances were confirmed by the results of the kriging analysis except for precipitation of previous July in Alaska Range forest (Fig. S5). Additionally, a signal of spatial clumping was found for the intercepts of all plots except Interior Alaska forest, the mean June temperature in Brooks Range treeline and SPEI in September for both Brooks Range plots as well as temperature of previous July in Brooks Range forest.

4. Discussion

4.1. Genetic differentiation

Similar to other conifer species, the investigated white spruce populations showed low but significant genetic differentiation together with a high degree of genetic diversity within a population (Hamrick and Godt, 1996; O'Connell et al., 2007; Rajora et al., 2005; Roschanski et al., 2016). In our case, geographic distance explained more of the

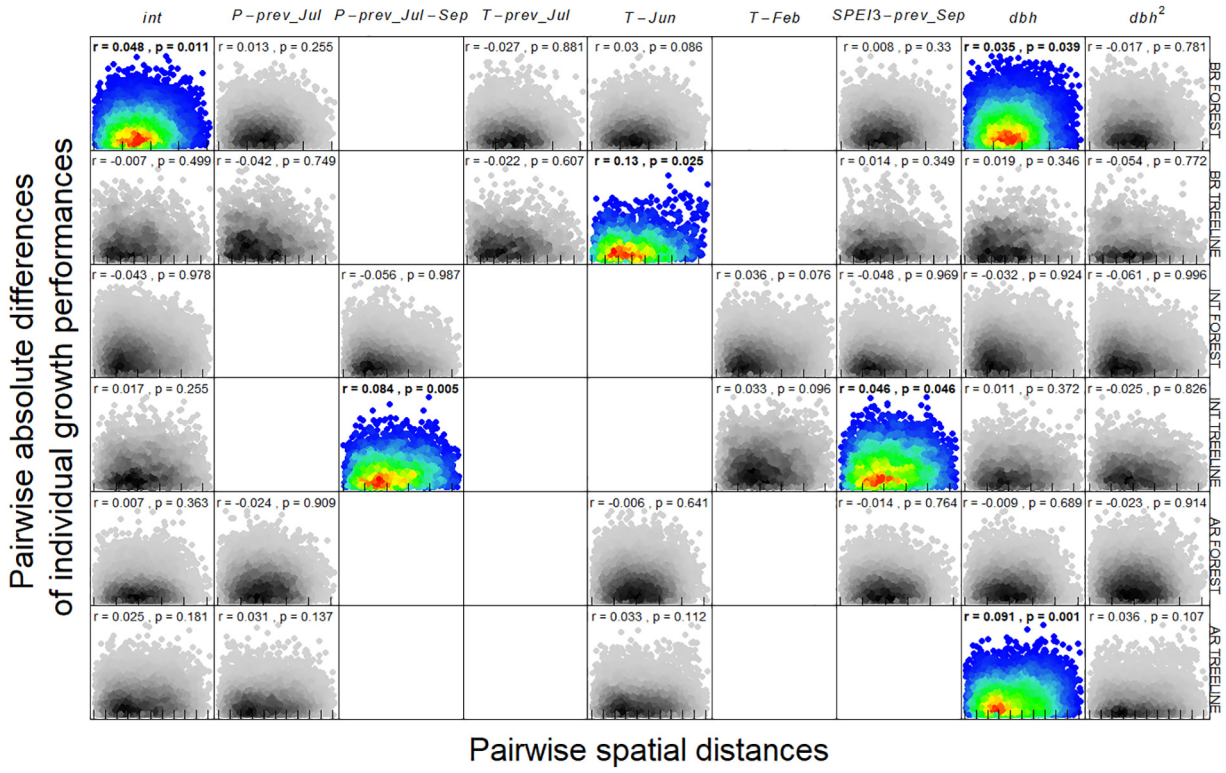

Fig. 3. Scatterplots of pairwise spatial distances (x axis) vs. pairwise absolute differences of individual growth performances (y axis) of white spruce (*Picea glauca*). Each tick on x axis corresponds to 20 m of linear distance. Mantel test results (i.e. correlation coefficients and *P*-values) are reported for each combination of plot × individual parameters of the model depending on plot location (BR, Int, AR). The graph is coloured only when the Mantel test was significant, otherwise it is in greyscale. The R function densCols (GRDEVICES v3.6.2) was used to colour points accordingly to their local densities in each area of the scatterplot, ranging from black/red (high density) to light grey/blue (low density). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

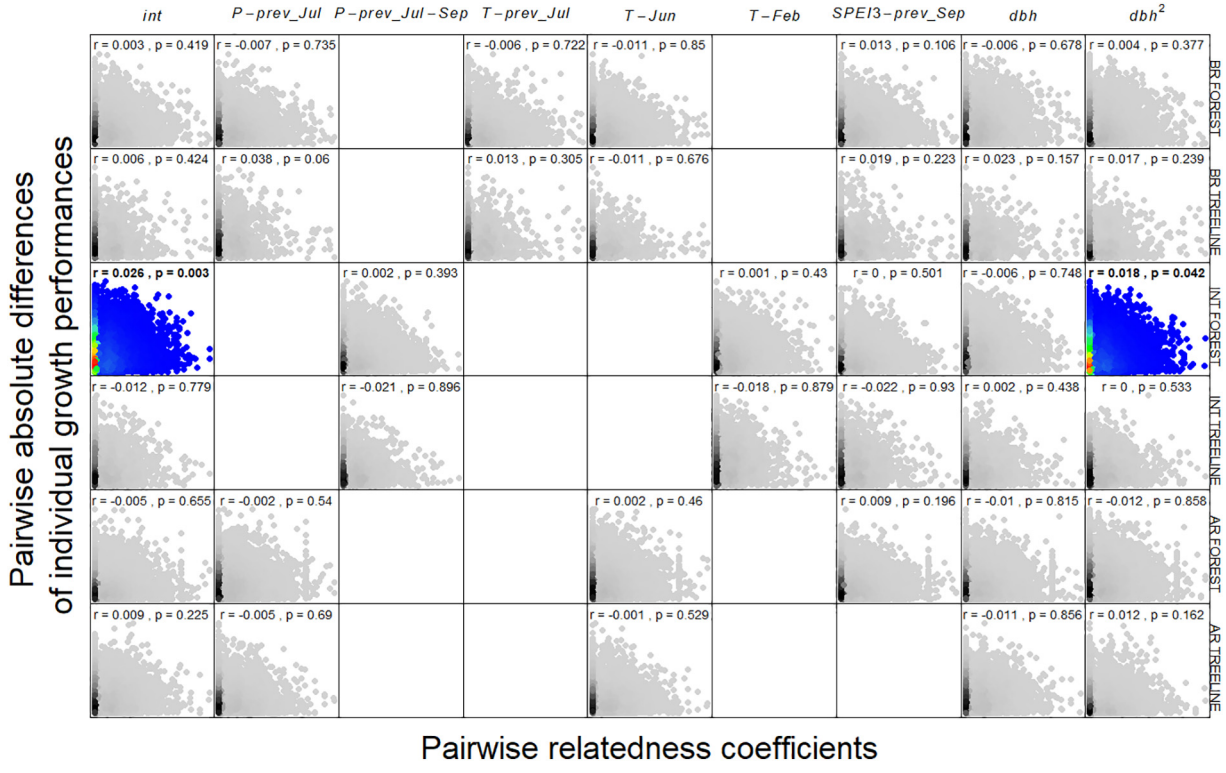

Fig. 4. Scatterplot of pairwise relatedness coefficients (x axis, scaled in a 0–1 range) vs. pairwise absolute differences of individual growth performances (y axis) of white spruce (*Picea glauca*). X axis values range from 0 to 1. Mantel test results (i.e. correlation coefficients and P-values) are reported. Colours are used as in Fig. 3.

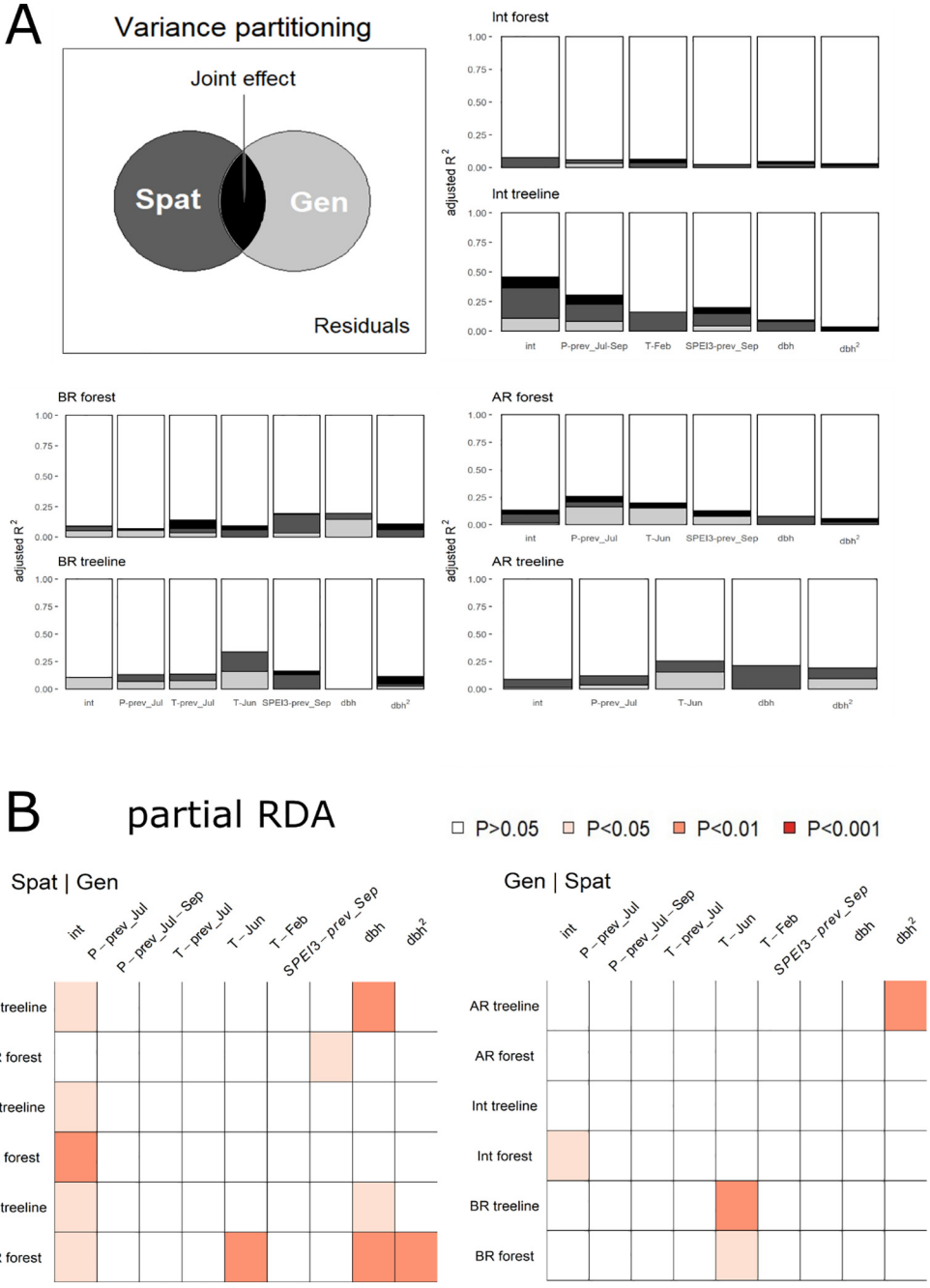

Fig. 5. The upper figures (A) represent the results of variance partitioning on individual growth performances of white spruce (*Picea glauca*) depicted by Venn diagram as conceptual representation (top left) and plot-by-plot results grouped by study sites (BR – Brooks Range, Int – Interior Alaska, AR – Alaska Range). In the bar plots dark grey, light grey and black represent the portions of variance (adjusted R^2) uniquely explained by spatial structure (Spat) and genetic similarity (Gen), and their joint effect, respectively. The two bottom figures (B) represent the effect of spatial structure on individual growth performances of white spruce (*Picea glauca*) while controlling for genetic similarity (left) and the effect of genetic similarity while controlling for spatial structure (right). Statistical significance of the variance components, assessed through ANOVA like permutation tests for partial redundancy analysis (pRDA), is reported in the heat maps.

differentiation among populations than environmental differences. Only the mean annual temperature explained a minor part of the differentiation, indicating some selective influence by the environment. A weak genetic differentiation between forest and treeline plots was only visible within the AMOVA, attributed to geographic distance. The missing significant differentiation among sites within the AMOVA can be explained by the low number of sites included in our analysis. In accordance with our results, several studies have shown that isolation by distance has a stronger effect on genetic similarity than isolation by environment in conifers (Mimura and Aitken, 2007; Mosca et al., 2014) and other plant species (Sexton et al., 2014). Moreover, Anderson et al. (2011) named genetic drift as a main driver in structuring Alaskan white spruce populations. In contrast, Roschanski et al. (2016) reported differentiation between high and low elevation plots due to environmental differences, using SNP markers in *Abies alba*. This could be to the fact that the SNPs were located in candidate genes which represent a part of the adaptive genetic diversity compared to SSR markers which mainly represent neutral genetic variation. Further, the study design contains greater horizontal and elevational distances between high and low elevation plots than our study design. Still, most of the genetic variance remains within the plots (Roschanski et al., 2016).

The low genetic differentiation but high genetic diversity can mainly be explained by extensive long-distance pollen dispersal in white spruce (O'Connell et al., 2007). Several studies have shown that pollen-mediated gene flow plays a major role in connecting tree populations to maintain the high genetic diversity within populations (Burczyk et al., 2004; Hamrick, 2004; Liepelt et al., 2002). Especially the wind-pollination and light seeds of white spruce favor the exchange of genetic material (Kling and Ackerly, 2021; Nienstaedt and Zasada, 1990) and counteract selection processes which can lead to local adaptation (Lenormand, 2002). On the other hand, a high genetic diversity in treeline populations can facilitate the evolution of new climatic niche limits (Aguilée et al., 2016). The high rates of gene flow between plots were reflected in the high seed and pollen immigration rates in the NMπ results. Similar high seed and pollen immigration rates were reported for Norway spruce (Avanzi et al., 2020; Piotti et al., 2009) and other conifers in treeline ecotones (Johnson et al., 2017; Leonarduzzi et al., 2016). A study investigating white spruce populations in Canada reported that 85.1% of the seeds were sired by pollen of trees from at least 250 to 3000 m away (O'Connell et al., 2007). At this point it is important to mention that parentage assignment methods have a limited application to treeline plots because treeline populations are young and are mainly colonized from seed sources outside the plots. Thus, only few offspring can be assigned to adults within the same plot and the runs were aborted (I. Chybicki, personal communication). Moreover, homozygous excess and the high amount of null alleles in our SSR data might have hampered the correct assignment of progeny to putative parents. To account for these problems in other analyses, we calculated relatedness using a software which considers for null alleles. In addition, Carlsson (2008) argued that null alleles influence the power to correctly assign individuals but probably do not change the overall outcome of the assignment. Furthermore, Anderson et al. (2011) described significantly fewer alleles in the microsatellites of Alaskan white spruce populations relative to outside Alaska, which could result in a lower variation in microsatellites and therefore assignment problems.

4.2. Drivers of tree growth

When studying growth dynamics, the high discrepancy between individual-level and plot-level growth-climate correlations underlines the importance of individual-based modelling approaches (Carrer, 2011). In this regard, random slope mixed-effect models offer great advantages for analyzing individual growth performances (Avanzi et al., 2019). In this study, we modified the random slope mixed-effects model developed by Avanzi et al. (2019) to better assess the drivers of tree growth. Specifically, we included dbh instead of tree age and more precise climate variables than mean temperature and precipitation. We used individual parameters obtained from the model to test if microenvironment or genetic similarity had a larger impact on individual growth performances in natural Alaskan white spruce populations. The similar study design of Avanzi et al. (2019) allowed us to compare drivers affecting TRW among an American and European spruce species.

In the random slope mixed-effects models, we found higher conditional R^2 values for white spruce than for Norway spruce. This might be related to the application of a detrending technique using a 30 years' spline on the tree-ring data. Further, the explained variance in TRW was higher for white spruce than Norway spruce, which could be due to the fact that we used dbh instead of age as a proxy for size. For both species, tree size had a larger effect on TRW than most of the climate variables within the models. Also, the importance of climate variables differed between the spruce species. In Norway spruce, temperature had a larger effect than precipitation on TRW, which was explained by high water availability at the sampling sites (Avanzi et al., 2019). In our study, the investigated white spruce populations differed in their reaction to climate. At the Interior Alaska study site, which represents a moisture-limited treeline, precipitation during the summer had a high influence on growth compared to Brooks Range and Alaska Range. In contrast, temperature showed a larger effect size on TRW in Brooks Range and Alaska Range, which represent edge populations at the cold-limited treeline. The effect was slightly larger in Brooks Range, the study site at the northern distribution treeline of the species in Alaska. These results indicate that tree growth in Brooks Range and Alaska Range is limited by cold temperatures in contrast to the moisture-limited Interior Alaska site. Further, the negative growth response in the Brooks Range plots to temperature in July of the previous year and the negative growth response in the plots of Interior Alaska and Brooks Range to SPEI3 in previous year September could be related to drought stress (Ohse et al., 2012; Wilmking et al., 2004). Forest and treeline plots showed similar effect sizes for a site, which can be explained by similar climatic conditions at treeline and forest plots per site.

The extracted individual growth performances showed a large part of unexplained variance in variance partitioning. This was consistent with the large part of inter-individual variation in TRW within the models associated with the intercepts in all plots. The intercepts reflect how constant each tree grows influenced by factors which were not explicitly defined in the model, like nutrient availability, mycorrhiza, water holding capacity of the soil, light conditions and other environmental factors. This heterogeneity seemed to have the largest influence on tree growth by creating microhabitats which are more or less beneficial (Carrer et al., 2013). The results of the Mantel tests and partial RDA using the individual growth performances confirmed that spatial structure had a larger effect on individual growth than genetic similarity. Forest plots did not differ from treeline plots. Moreover, model intercepts were spatially structured in almost all white spruce plots and in the Italian Norway spruce plots located on a steep and rocky sandstone slope (Avanzi et al., 2019). This indicates that microenvironmental features have an even larger effect on growth under extreme growth conditions.

The negligible effect of genetic similarity on individual growth performances compared to spatial structure was also found in the Norway spruce populations as well as in other conifer species (Avanzi et al., 2019; King et al., 2013; Rozas et al., 2020). Additionally, spatial structure also had a larger effect on wood anatomical traits of white spruce than genetic similarity (Pampuch et al., 2020). In contrast, a greater genetic diversity could positively be associated with growth in juvenile trees of alpine treeline populations (González-Díaz et al., 2020). However, all these studies were based on neutral markers like microsatellites which only represent a small and presumably neutral fraction of the large conifer genome and thus, might have limited explanatory power for growth traits.

In summary, in both species microenvironmental conditions seemed to have a larger effect on individual growth performances than genetic similarity. Tree size had a larger effect on TRW than climate in white spruce as well as in Norway spruce. Overall, white and Norway spruce showed similar results when explaining TRW with tree size and climate, which was probably due to the close relatedness and similar physiology of both *Picea* species even though they occur in different parts of the world.

4.3. Conclusions and outlook

The overall genetic diversity of the investigated white spruce populations was high due to high gene flow favored by high seed and pollen immigration rates. Gene flow was higher into the treeline than core plots due to the leading edge position within the distribution range and lower reproductive success at the treeline. The high gene flow rates are also reflected in the low degree of genetic differentiation among sites. The observed population structure was better explained by geographic distance than environmental distance. This leads to the assumption that genetic drift together with decreasing gene flow with

increasing distance play an important role in structuring white spruce populations in Alaska. Further, in Alaskan white spruce, tree size has a larger effect on TRW than climatic conditions. Climate variables which drive TRW differ depending on the growth limiting factor at the site, with temperature at the cold-limited treeline and precipitation at the moisture-limited treeline. At the local scale, microenvironment was likely to be more relevant for tree growth than genetic similarity.

In future studies it is worth testing if sequence-based information from both, coding and non-coding regions, would have a greater explanatory power for growth traits compared to our SSR based analysis. A further step in model development may be the inclusion of competition among trees to explain a higher proportion of the variance in TRW.

The high plastic growth response of white spruce could be advantageous to buffer short-term environmental changes. In regard to long-term adaptation, the high gene flow among Alaskan populations could support adaptation to a warming climate by the possible introgression of preadapted alleles from warmer regions into the gene pool of northern populations.

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CRediT authorship contribution statement

MW and MS invented the overall study design. DW, MT, MS, MW collected samples with help of others. DW performed genotyping analysis. Conceptualization of statistical analysis was done by KH. R scripts for the growth performance analysis were provided by CA. MZ evaluated environmental data and conducted all statistical analysis except model adaptation, which was done by TP. MZ wrote the manuscript with contributions from KH, TP, MB and MS. All authors revised and refined the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Information

Table S1: SSR loci used to analyze population structure and differentiation and genetic similarity of 2,571 individuals of white spruce (Picea glauca) with mistyping error rate (ER) of NMπ runs, expected (He) and observed heterozygosity (Ho) and proportion of null alleles (null) per plot calculated in GENALEX.; AR – Alaska Range, Int – Interior Alaska, BR – Brooks Range, F – forest plot, T – treeline plot.

	loci	**UAPG_06**	**UAPG_08**	**UAPG_24**	**UAPG_25**	**UAPG_64**	**UAPG_87**	**UAPG_91**	**UAPG_105**	**UAPG_144**	**PGL_12**
BR forest	error rate	0.16	0.20	0.21	0.13	0.20	0.21	0.24	0.14	0.07	0.21
	Ho	0.77	0.65	0.30	0.09	0.46	0.54	0.44	0.73	0.68	0.54
	He	0.79	0.88	0.91	0.43	0.87	0.91	0.89	0.77	0.71	0.83
	null	0.00	0.01	0.15	0.00	0.04	0.04	0.00	0.00	0.00	0.05
BR tree-line	error rate	0.10	0.06	0.06	0.04	0.08	0.06	0.06	0.02	0.07	0.05
	Ho	0.81	0.66	0.33	0.18	0.46	0.53	0.53	0.75	0.62	0.42
	He	0.77	0.88	0.92	0.17	0.86	0.91	0.92	0.80	0.66	0.79
	null	0.00	0.01	0.13	0.01	0.10	0.02	0.00	0.00	0.00	0.06
PLS	error rate	0.03	0.04	0.22	0.05	0.15	0.19	0.22	0.01	0.09	0.10
	Ho	0.72	0.71	0.33	0.09	0.38	0.69	0.51	0.82	0.62	0.46
	He	0.71	0.87	0.90	0.90	0.87	0.94	0.87	0.81	0.81	0.84
	null	0.03	0.01	0.23	0.00	0.14	0.02	0.01	0.00	0.00	0.14
Int forest	error rate	0.24	0.22	0.35	0.07	0.40	0.42	0.24	0.11	0.22	0.01
	Ho	0.76	0.67	0.26	0.14	0.45	0.72	0.42	0.83	0.61	0.39
	He	0.75	0.87	0.87	0.19	0.83	0.92	0.90	0.81	0.64	0.78
	null	0.00	0.00	0.26	0.02	0.07	0.01	0.03	0.00	0.00	0.12
Int tree-line	error rate	0.19	0.16	0.46	0.07	0.35	0.20	0.37	0.20	0.11	0.29
	Ho	0.75	0.66	0.31	0.17	0.39	0.70	0.41	0.83	0.55	0.35
	He	0.76	0.88	0.91	0.26	0.84	0.92	0.89	0.82	0.61	0.82

	null	0.00	0.01	0.23	0.02	0.04	0.01	0.02	0.00	0.00	0.10
AR forest	error rate	0.09	0.14	0.19	0.01	0.17	0.13	0.17	0.04	0.06	0.25
	Ho	0.71	0.79	0.51	0.10	0.41	0.49	0.50	0.78	0.67	0.37
	He	0.69	0.85	0.88	0.11	0.83	0.92	0.79	0.74	0.69	0.82
	null	0.00	0.00	0.11	0.00	0.06	0.08	0.00	0.00	0.00	0.05
AR tree-line	error rate	0.25	0.13	0.40	0.09	0.19	0.24	0.20	0.06	0.09	0.25
	Ho	0.75	0.78	0.39	0.10	0.39	0.66	0.45	0.71	0.68	0.48
	He	0.76	0.90	0.91	0.10	0.86	0.94	0.86	0.76	0.71	0.87
	null	0.00	0.00	0.11	0.00	0.06	0.05	0.01	0.00	0.00	0.09

Table S2: Mean annual temperature in °C downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP) for each site and measured on-site temperature at the white spruce (Picea glauca) plots for various time periods used as climatic data.

	Temperature SNAP database	**Temperature measurements**	
		Forest plot	Treeline plot
Brooks Range	-8.18	-2.55	-2.26
PLS	-1.89	1.2	n.d.
Interior Alaska	-1.89	0.66	1.24
Alaska Range	-3.58	1.32	1.56
Time period	1950 – 2015	09/2016 – 08/2019 09/2018 – 08/2019 for Alaska Range	

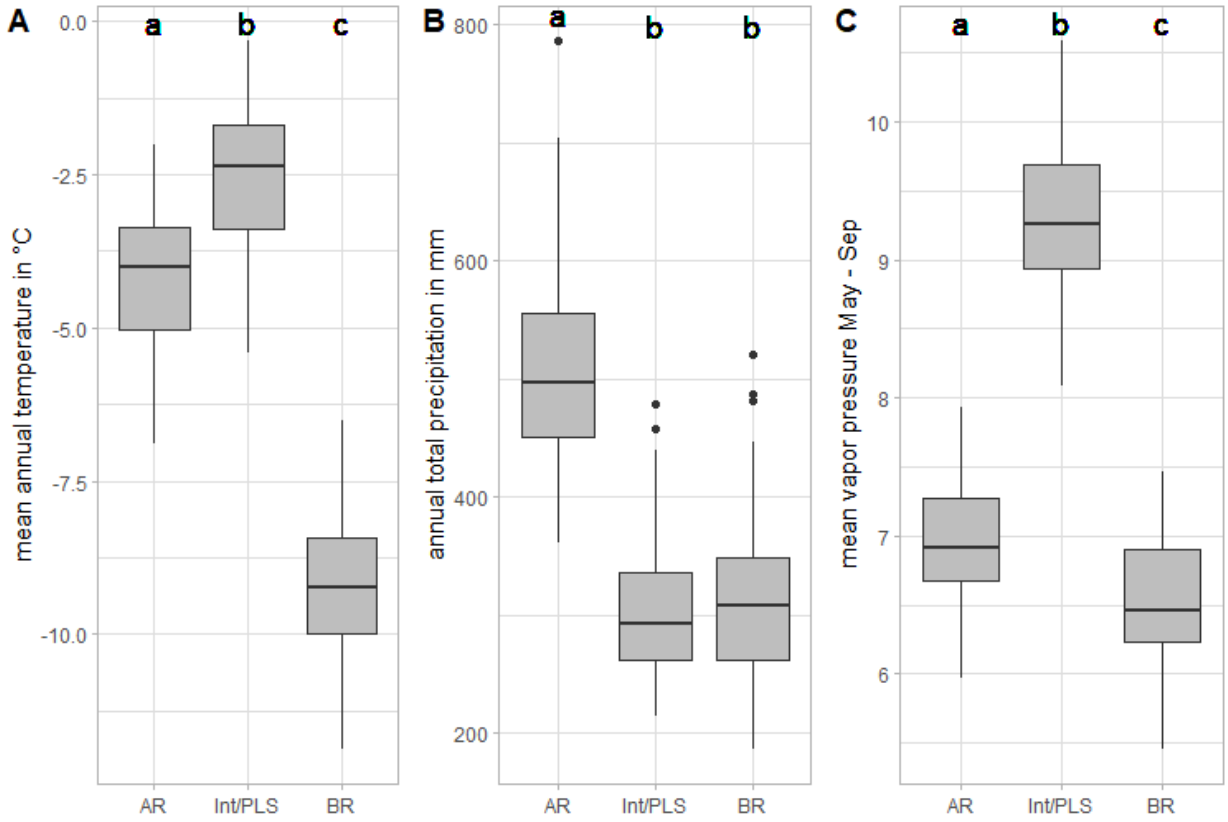

Figure S1: Interannual variation in the selected climate variables mean annual temperature (A), annual total precipitation (B) and mean vapor pressure (C) for the white spruce (Picea glauca) sites Alaska Range (AR), Interior Alaska / PLS (Int/PLS) and Brooks Range (BR) for the period 1950 – 2015 downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP). Letters (a, b, c) indicate significant differences between sites.

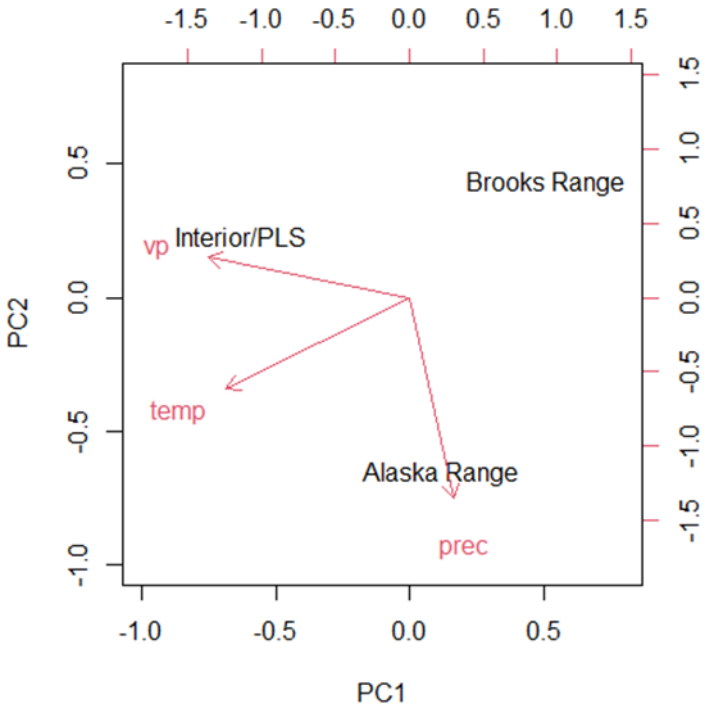

Figure S2: PC1 versus PC2 of a PCA of the climate variables mean annual temperature (temp), total annual precipitation (prec) and vapor pressure of May until September (vp) downloaded from the Scenarios Network for Alaska and Arctic Planning (SNAP) for analysis of isolation by environment; differentiating the sites white spruce (Picea glauca) Brooks Range, Interior Alaska / PLS and Alaska Range

Table S3: Parameters and results of the gene flow analysis of white spruce (Picea glauca) plots with NMπ (Chybicki 2018). The top rows indicate the size thresholds (tree height in m) for assigning trees as putative parents and putative offspring, the central rows indicate the number of individuals assigned as parents and offspring, and the bottom rows represent the proportion of offspring resulting from seed immigration, selfing and pollen immigration and offspring with both parents assigned.

Threshold group assignment (height in m)							
	BR F	BR T	PLS	Int F	Int T	AR F	AR T
Putative parents	> 5	> 5	> X*	> 6	> 6	> 6	> 5
Putative offspring	< 2.5	< 2.5	< 4	< 3	< 3	< 3	< 3
Number of trees							
Putative parents	158	43	146	184	64	185	59
Putative offspring	162	191	327	71	111	102	141
Results (%)							
Seed immigration	38.8	40.2	69.4	57.9	86.5	52.5	90.2
Selfing	27.0	5.3	1.5	n.d.	n.d.	11.9	5
Pollen immigration	28.2	56.8	25.4	n.d.	n.d.	27.7	n.d.
Both parents assigned	6	0	3.7	n.d.	n.d.	7.9	n.d.

** no height data available for adult trees, dbh > 10 cm corresponds to 7.5 m height*

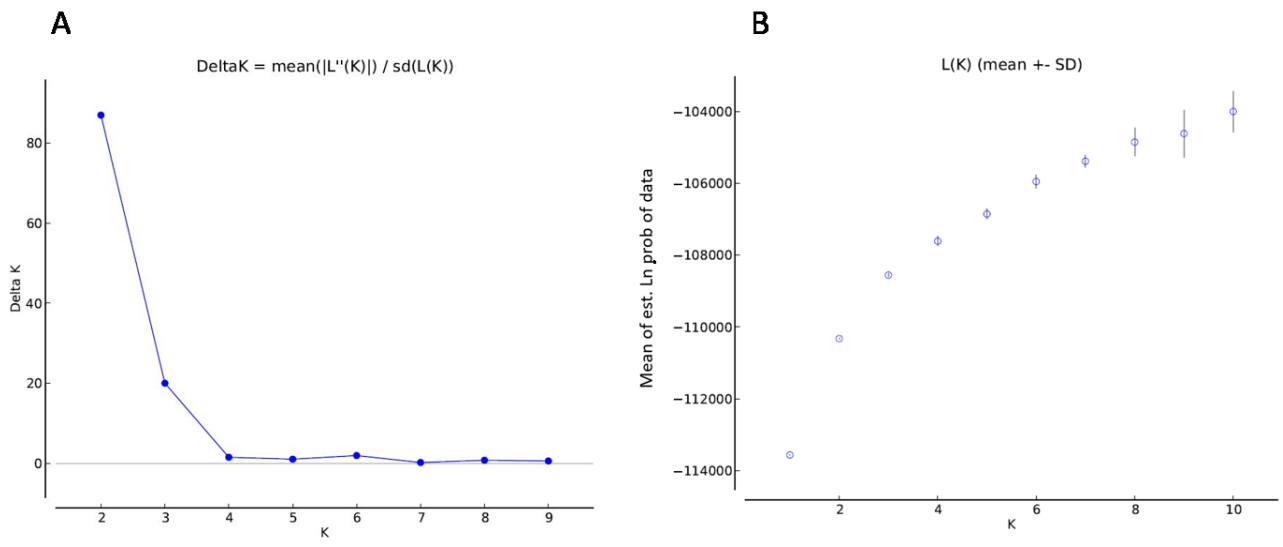

Figure S3: A - Evaluation of the optimum number of clusters (K) from the STRUCTURE analysis with the deltaK method (Evanno et al. 2005) for white spruce (Picea glauca). B - Evaluation of loglikelihood of clusters (K) from the STRUCTURE analysis with the deltaK method (Evanno et al. 2005).

Table S4: Population differentiation described by G_{ST} values between the white spruce (Picea glauca) plots (BR, Int, AR stands for Brooks Range, Interior, Alaska Range sites; F and T for forest and treeline plot) obtained from GENALEX.

	BR F	**BR T**	**PLS**	**Int F**	**Int T**	**AR F**
BR F						
BR T	0.007					
PLS	0.024	0.021				
Int F	0.021	0.019	0.005			
Int T	0.019	0.017	0.005	0.001		
AR F	0.026	0.022	0.012	0.014	0.013	
AR T	0.016	0.013	0.011	0.011	0.008	0.009

Table S5: Results of hierarchical AMOVA analysis to test for isolation by distance for all white spruce (Picea glauca) sites using microsatellite data.

Source of variation	**Sum of Squares**	**Variance components**	**Percentage variation**	**P**	**Fixation index**
Among sites	13359.32	1.67	0.28	> 0.05	F_{CT} = 0.0028
Among plots within sites	16071.58	5.10	0.87	< 0.001	F_{SC} = 0.0087
Within plots	2738353.03	582.21	98.85	< 0.001	F_{ST} = 0.0115

Table S6: Mantel test and partial Mantel test for isolation by distance (on plot and site level) and isolation by environment (on site level) for all white spruce (Picea glauca) sites using microsatellite data.; significant results are in bold type.

		Mantel test		**Partial Mantel test**		
		r	**p-value**	**Accounting for**	**r**	**p-value**
Isolation by distance	Site level	0.906	0.083	PC 1	**0.961**	**0.040**
				PC 2	0.906	0.209
	Plot level	**0.843**	**0.004**	-	-	-
Isolation by environment (site level)	PC1	0.562	0.083	Geographic distance	0.841	0.087
	PC2	0.398	0.665		-0.397	0.628
	Mean annual temperature	0.976	0.086		**0.865**	**0.043**
	Total annual precipitation	-0.180	0.999		-0.360	0.622
	Mean vapor pressure (May-Sep)	0.528	0.080		0.836	0.083

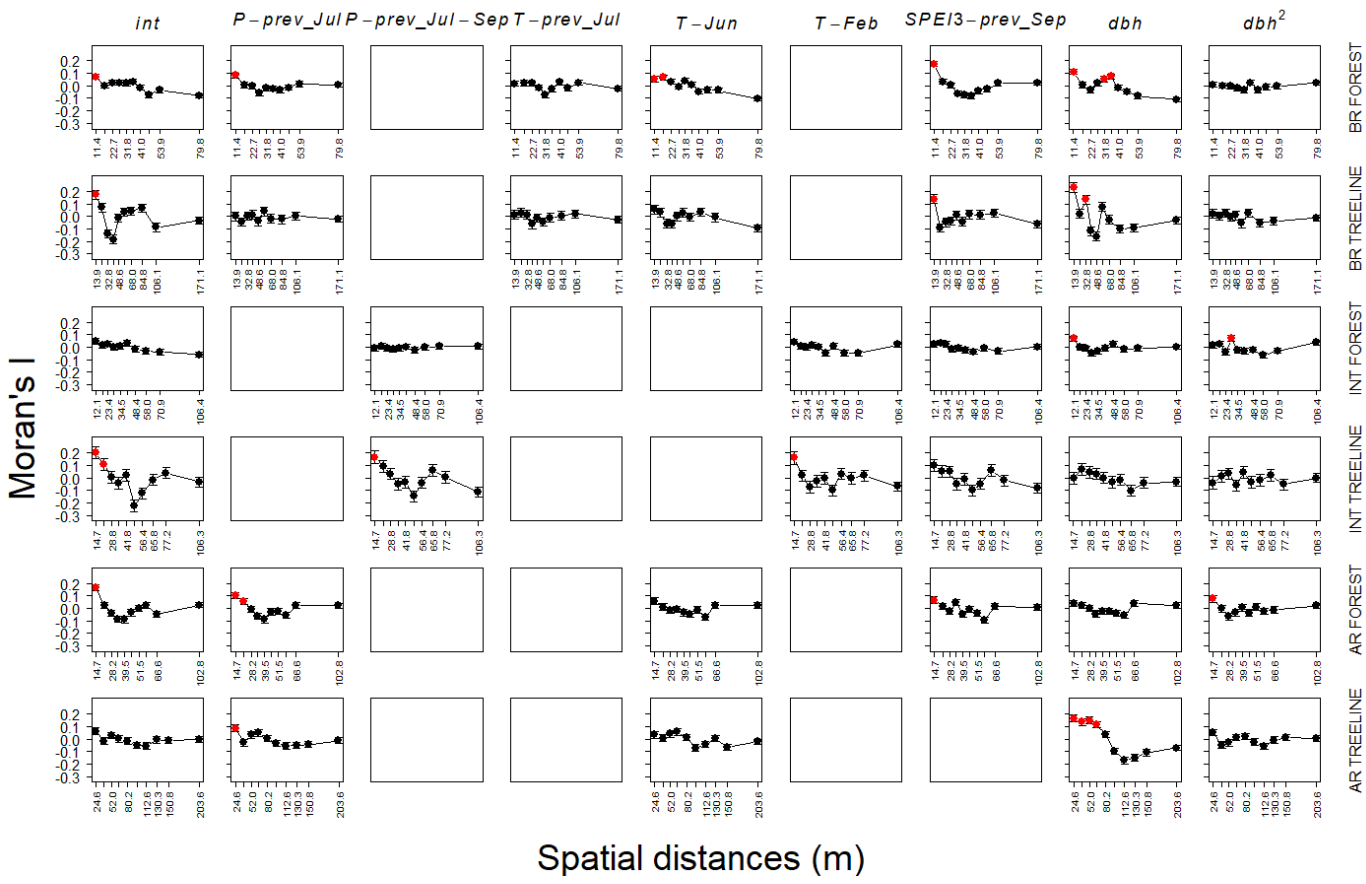

Figure S4: Moran´s I spatial correlograms on individual growth performances for each white spruce (Picea glauca) plot to test for spatial clumping of individual growth performances.

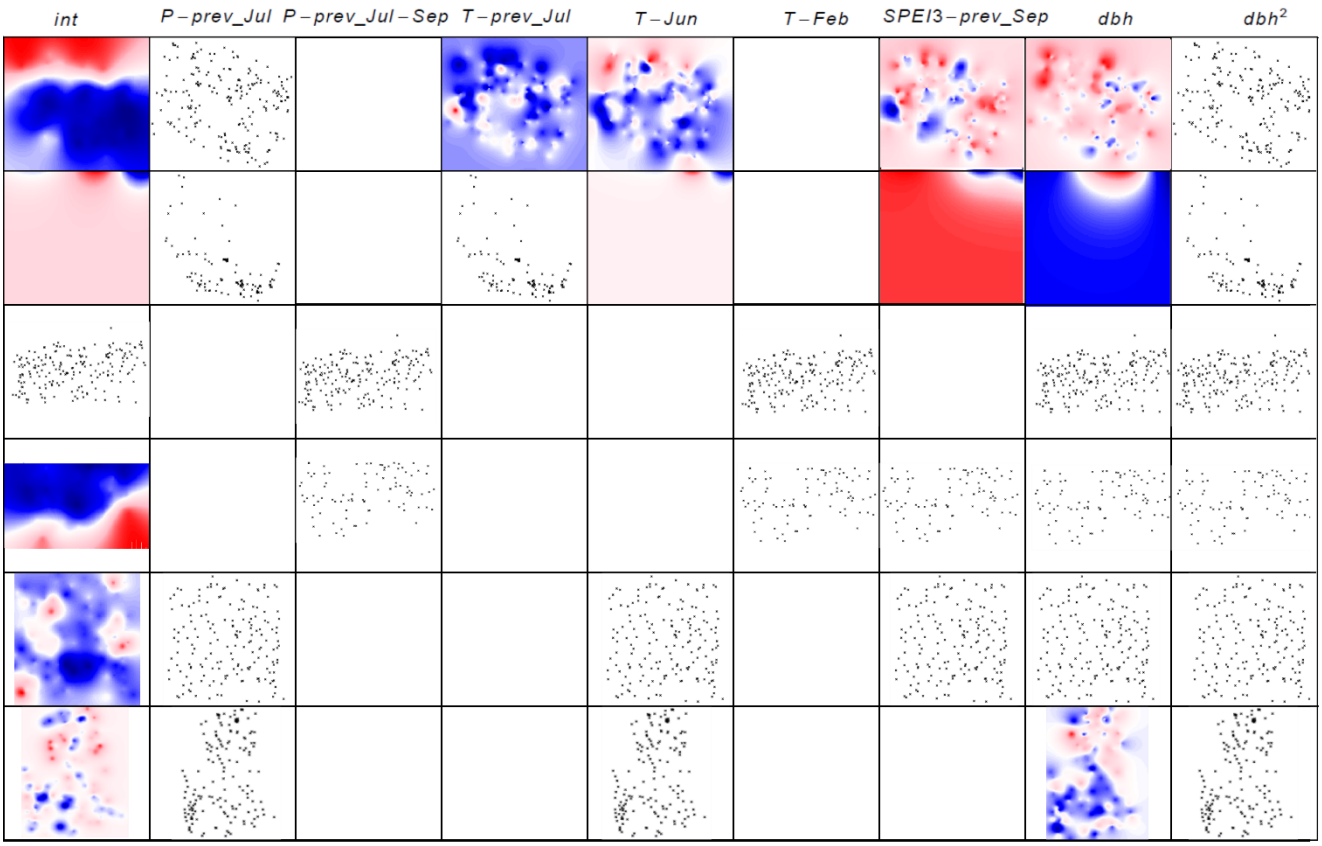

Figure S5: Distribution maps from spatial interpolation (kriging) of individual parameters for each white spruce (Picea glauca) plot. Colors range from the highest values of within-plot parameters (red) to the lowest ones (blue)

Chapter II

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

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

Running title: Genotype-phenotype associations *Picea glauca*

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Abstract

Climate change will increase the frequency and intensity of drought events in many boreal forests. As tree species are sessile and have a long generation time, it is essential to know how forests will cope with such extreme environmental conditions and what is the genetic basis of fitness-related phenotypic traits that enable drought tolerance. We therefore 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 drought stress-induced growth declines. To explore the genetic basis of these dendrophenotypes, we genotyped the individual trees at 3,000 SNPs in candidate genes and performed genotype-phenotype association analysis using linear mixed models and Bayesian sparse linear mixed models. Growth responses to drought stress differed in contrasting treeline populations and are likely to be affected unevenly by climate change. We identified 40 genes associated with dendrophenotypic traits, which differed between the treeline populations. Most genes were identified in the drought-limited site, indicating covariance of alleles with drought-tolerant phenotypes. The genetic basis of drought tolerance contrasted between the sampled sites as well as in comparison with Canadian populations, suggesting that drought adaptation acts on a local scale. Our results highlight a set of genes that genetically determines wood traits critical for the establishment and persistence of tomorrow´s forests under climate change.

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

1. Introduction

Under human-induced global warming, drought events increase in frequency and intensity (IPCC 2021; Dai 2013), affecting boreal forest ecosystems more severely at high than low latitudes (Collins *et al.* 2013). Especially in North America, regional warming leads to a decrease in soil moisture and therefore

53 increase in water deficit (Reich *et al.* 2018; Girardin *et al.* 2016). The resulting increased physiological
54 stress is associated with negative growth responses and elevated tree mortality rates (Allen *et al.* 2010;
55 van Mantgem *et al.* 2009; Hynes & Hamann 2020). Therefore, it is important to know how trees adapt
56 to increases in drought events as the speed of adaptation in trees is limited by their long generation time
57 and sessile biology (Shaw & Etterson 2012). In fact, some tree species already lag behind their potential
58 distribution range (Aitken *et al.* 2008). In general, tree populations are characterized by high phenotypic
59 plasticity and adaptive capacity, including high standing genetic variation and a high dispersal ability
60 by pollen, which enables them to cope with environmental changes (Aubin *et al.* 2016). Extreme events
61 like droughts exert intense selection pressure on populations and thereby shape genetic variation at
62 adaptive loci (Grant *et al.* 2017). However, especially in conifers, the high pollen-mediated gene flow
63 keeps populations connected (Liepelt *et al.* 2002; Avanzi *et al.* 2020) and the introgression of
64 maladapted alleles could counteract local adaptation (Rajora *et al.* 2005; O'Connell *et al.* 2007). Within
65 tree populations that experienced recent selection and that are characterized by high gene flow, genes
66 related to local adaptation are expected to interact in a complex way and show small frequency shifts
67 (Hornoy *et al.* 2015). However, it is unclear which genes do control drought tolerance in trees (Moran
68 *et al.* 2017).

69 Due to their long generation times, trees will probably have difficulties to keep up with rapid
70 climate change, especially in high mountain areas, what makes them more vulnerable to local extinction
71 (Dauphin *et al.* 2021). Therefore, recent studies started to link genetics with dendroecology to explore
72 the molecular mechanisms of stress-tolerant phenotypes in tree populations (Heer *et al.* 2018; Housset
73 *et al.* 2018; Trujillo-Moya *et al.* 2018; Depardieu *et al.* 2021; Laverdière *et al.* 2022). To achieve this
74 goal, genotype-phenotype association analysis is commonly used to identify loci that are associated to
75 phenotypic traits related to drought tolerance. Tree growth during and following a drought event informs
76 about the overall drought tolerance of trees (Moran *et al.* 2017). Using this approach, Depardieu *et al.*
77 (2020) detected signals of local adaptation to drought among white spruce (*Picea glauca* (Moench)
78 Voss) populations of Eastern Canada planted in a common garden. The follow-up study (Depardieu *et*
79 *al.* 2021) then combined genetic association analysis using dendrophenotypes and climate data with

gene expression data to detect genes related to drought adaptation in white spruce. Depardieu *et al.* (2021) detected 285 genes significantly associated with phenotypic traits or climatic factors, of which 110 were differentially regulated under drought conditions. The study identified eight high-confidence genes, associated with phenotypic traits as well as climatic factors, including four drought-responsive genes related to white spruce adaptation to drought (Depardieu *et al.* 2021).

Although common garden studies are suitable to study genetic adaptation to local site conditions, we cannot investigate the speed of adaptation using this set-ups (Merilä & Hendry 2014; Hoffmann & Sgrò 2011). Further, genotype-by-environment interactions can make results misleading in regard to the natural situation (Merilä and Hendry 2014). To our knowledge, genetic association analyses with dendrophenotypes in white spruce are exclusively done in common garden experiments in Canada. We investigated natural populations of white spruce in contrasting extreme environments in Alaska to check whether high confidence genes identified in common garden studies also show to be significantly associated with phenotypic traits related to drought tolerance in natural populations. White spruce has a high economic and ecological importance in North America and is described to have an exceptional high adaptive capacity (Royer-Tardif *et al.* 2021). To study adaptation to climatic extremes, treeline populations are particularly suitable because tree growth and survival are limited and 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 infer 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 populations of this ecotones experience different climate extremes and, therefore, divergent selection pressures, which is estimated to lead to different genetic signatures underlying drought tolerance.

With our data, we wanted to test the following two hypotheses: (1) the individual reaction to drought stress differs i) between drought and cold-limited treelines and ii) between treeline and forest plots, and (2) the selection pressure of the contrasting treelines lead to divergent signatures in drought-associated genes. To test these hypotheses, we first developed an evidence-based approach 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). Second, we calculated the individual reaction to drought events using tree-ring data as phenotypic data. Because resilience traits are unitless, they can be compared between natural populations (Opgenoorth & Rellstab 2021). As genetic data, we used 3,000 SNPs located in candidate genes, 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, as a second approach, Bayesian sparse model to account for interaction and small-effect size SNPs. We compared growth reaction to drought stress as well as climate sensitivity and the underlying genetic basis among the contrasting ecotones. 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 Central Brooks Range at the latitudinal treeline on a steep south exposed slope. The distance between the forest and treeline plot was only 30 m, because due to a steep slope gradient microenvironmental conditions changed fast on 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 & Alix 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 plot were right next to each other, but had the highest differences in inclination. 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 *et al.* (2018a).

Each plot contained at least 200 trees and covered an area from 0.5 to 2 ha depending on tree density. 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. Wood cores were taken from trees with dbh > 5 cm. Within the plots we selected trees with a dbh between 10 – 40 cm, a height of 4 – 20 m and a minimum age of 50 years for further analyses. We calculated the maximum likelihood estimates of relatedness (r) 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 > 0.5). In the case of clonal groups, we selected the oldest individual to be included in the analysis. For genetic analyses, fresh needles were sampled from selected trees and dried on silica gel.

2.2. Drought year identification and individual-level response parameters

We used a tree ring dataset that contained trees sampled in Interior Alaska in 2015 (Trouillier *et al.* 2018a) as well as trees from Brooks Range and 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 crossdating was done visually. For a detailed description of core processing see Wilmking *et al.* (2017) and Trouillier *et al.* (2018a).

For the genotype-phenotype association (GPA) analysis, we derived measures of the individual growth reaction to drought stress as phenotypic data. For this purpose, 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 and evidence-based decision for each of the three study sites (Figure 2). According to the recommendation for drought studies of 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 v. 4.0.2 (R Core Team 2015) with the package POINTRES v. 1.1.3 (van der Maaten-Theunissen *et al.* 2015) to identify extreme events in our tree ring data set. We calculated event years, defined as years in which the individual tree shows a substantial reduction in growth. Based on these individual event years, we identified years in which at least 50% of the trees showed such an event year which was then defined as 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 v. 1.7.1 (Bunn 2008; Bunn 2010; Bunn *et al.* 2020), using a 30 year spline. We did a sensitivity analysis to check if the choice of the thresholds influences 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 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.

As a second step, to identify whether the growth decline in the pointer year was caused by drought, we checked the climatic conditions in a time span of two years before until two years after the pointer year. 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 one 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 six months (Vicente-Serrano *et al.* 2010). Second, we used the climate moisture index (CMI6), which includes precipitation and PET. Both climate values were calculated for the growing season (May-September). We also calculated the CMI by Hogg for the period of one year, including the sum of monthly CMI values from 1st of preceding August till the 31st of current year July. This index already showed strong growth-climate relationships and was applied in previous studies about drought impacts on growth in white spruce (Hogg & Wein 2005; Hogg *et al.* 2017). Monthly climate data (precipitation sum, mean temperature, mean PET) was downloaded from the Scenarios

187 Network for Alaska and Arctic Planning (SNAP) for the period 1950-2015 with a resolution of 2 km^2.
188 We defined drought as a periodic lack of water compared to normal conditions at the site, characterized
189 by SPEI6 and CMI values with a negative standard deviation > 1.25 from the 5-year mean. We also
190 accounted for the climate conditions in the years before the pointer year, due to the memory effect, trees
191 can show a later response to drought stress (Hacket-Pain *et al.* 2015).

192 After the drought event identification, we calculated the individual response to drought stress of
193 each tree using detrended and raw tree-ring data. Because there were no striking differences, we
194 continued with the detrended data. We obtained the resistance, recovery, resilience and relative
195 resilience indices after Lloret *et al.* (2011) for each tree and pointer year using the R package POINTRES
196 v.1.1.3 (van der Maaten-Theunissen *et al.* 2015). The number of years considered for pre- and post-
197 disturbance periods in calculating the resilience components was set to two years because of the short
198 periods between growth declines. Resistance describes the ratio between growth during and before the
199 pointer year, recovery the ratio between growth after and during the pointer year, resilience the ratio
200 between growth after and before the pointer year and relative resilience the resilience weighted by the
201 growth decrease during the pointer year (van der Maaten-Theunissen *et al.* 2015).

202 Additional to the named indices, we estimated the climate sensitivity of each individual for
203 1970-2015 by calculating the standard deviation in growth. Trees with a high deviation from the mean
204 were characterized as highly sensitive with a high variability in growth depending on climate conditions,
205 which is associated with higher mortality (Cailleret *et al.* 2017). The described growth indices were used
206 as phenotypic data within the GPA analysis. Normal distribution of the phenotypic data was checked by
207 performing the *shapiro.test* function (Shapiro-Wilk normality test) and visually by applying the *qqnorm*
208 function in the R package STATS v.4.1.0. In case of not normal distributed data, we transformed the
209 data with the R function *boxcoxTransform* of ENVSTATS v.2.4.0 using the lambda value calculated with
210 *transformTukey* of the R package RCOMPANION v2.4.1. In addition, outlier phenotypes which disturbed
211 the normal distribution of the data were excluded to avoid spurious associations in linear mixed models
212 (Interior Alaska - four individuals for resistance 2010, one individual for resilience 2010, three
213 individuals for recovery 2010; Brooks Range - one individual for relative resilience 1993).

214

215 **2.3. Genotyping**

216 The sampled needles were sent to LGC Genomics GmbH (Berlin, Germany) for DNA extraction and
217 targeted genotyping by sequencing (SeqSNP, LGC April 11, 2019). All trees were genotyped using the
218 Illumina NextSeq 550 platform, targeting SNPs located in coding regions mapped in a high-resolution
219 reference genetic map of white spruce (Pavy *et al.* 2017). In a first step, we selected a subset of 7,511
220 SNPs genotyped in white spruce in Pavy *et al.* (2017) in East Canadian populations which were
221 distributed across the 12 chromosomes of white spruce. These SNPs showed good quality and minor
222 allele frequency (MAF) of at least 6% in previous white spruce studies (Pavy *et al.* 2017). All selected
223 SNPs were located in coding regions which have a higher probability of sequence conservation and were
224 mapped to the reference maps of Pavy *et al.* (2017) and (Gagalova et al. *in review*). The sequences
225 surrounding the SNPs (at least 75 bp) were blasted against the transcriptome of white spruce (Birol *et*
226 *al.* 2013) and only sequences of SNPs with a full hit in the genome were retained. The corresponding
227 oligo probes for SNP detection were designed on the transcriptome and validated by running a test
228 sequencing. Based on this information, we selected 3,000 SNPs whose oligo probes had only one hit in
229 the genome (Table S5). Each SNP was located in a single gene, resulting in 3,000 different genes.
230 Additional to the 478 samples, we included 12 negative controls and 15 duplicates into the sequenced
231 samples to control the sequencing quality. We compared the sequences of the duplicated individuals
232 using the function *dupGenotypes* implemented in the R package STRATAG v. 2.0.2 (Archer *et al.* 2017).
233 This function calculates the proportion of shared loci between the duplicates. Duplicated individuals
234 with more than 5% of missing data were excluded.

235 In the first filtering steps, performed by LGC, SNPs were filtered for a minimum coverage of 8
236 reads per sample and locus. We removed SNPs with more than 80% missing data and individuals with
237 more than 10% missing data. Only biallelic SNPs were kept in the dataset. SNPs with a minor allele
238 frequency > 2.5% were retained, resulting in a dataset of 458 individuals and 2,744 SNPs. Further, to
239 exclude linked loci, we tested all SNPs for pairwise population-based linkage disequilibrium (LD) using
240 the function *gl.report.ld* implemented in the R package DARTR v.1.8.3 (Gruber *et al.* 2018). In the case

of tightly linked SNPs (r > 0.9), the first SNP of the pair was removed. For SNPs located on the same contig this threshold was set to r > 0.5. With this step, we excluded 120 SNPs. Further, when paralogs are targeted, this might negatively affect the outcome of the analysis. Since paralogs are expected to show a greater proportion of heterozygotes than singleton loci (McKinney 2017), we calculated the expected and observed heterozygosity, as well as the deviation from Hardy-Weinberg-equilibrium (HWE) per locus and population using DARTR. Loci which showed heterozygous excess and deviation from HWE in more than one population can probably be assigned to oligo probes which are binding on multiple sites within the white spruce genome and were therefore excluded from the analysis, which was the case for further 161 loci. As a result, the final dataset consisted of 2,463 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 v. 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*) by 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 v. 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 v. 2.3.4 (Pritchard *et al.* 2000) in comparison with the SNP data. Pairwise population genetic differentiation values (F_{ST}) were calculated in the R package DARTR v. 1.8.3 (Gruber *et al.* 2018).

2.4. Genetic association analysis

2.4.1. Genotype-phenotype association analysis

We tested the association between each dendrophenotype (Lloret index) and each SNP to characterize the underlying genetic variation of drought stress tolerance. For this purpose, we used linear mixed models (LMM) implemented in the R package GENESIS v.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 mean alternate allele count by using the allele frequency. 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 v.1.27.0 (Zheng *et al.* 2012). As a next step, we conducted a PC-AiR using unrelated individuals which are maximally informative about all ancestries in our sampled populations. We ran a Principal Component Analysis (PCA) with the unrelated individuals and then projected the relatives onto the PCs with a kinship threshold of degree 3 (unrelated is less than first cousin) using the *pcair* function (GENESIS). Second, to account for genetic relatedness we used the first 2 PCs to compute kinship estimates with the *pcrelate* function (GENESIS). We obtained a genetic relatedness matrix as covariance matrix for the null model using the function *pcrelateToMatrix* (GENESIS). As a next step, we 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 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 size (dbh) as a covariate since the latter influences climate sensitivity in white spruce in Alaska (Trouillier *et al.* 2018b). In case of 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. Further, we used the function *assocTestSingle* (GENESIS) to test each SNP with each quantitative trait in conjunction with the output of the null model fit. At last, we controlled for multiple testing using the *qvalue* function with a false discovery rate of 0.05 using the R package QVALUE v.2.25.0 (Storey *et al.* 2021).

To account for small effect size SNPs as well as for interaction effects, we applied a Bayesian sparse linear mixed model (BSLMM) using Markov chain Monte Carlo (MCMC) as implemented in GEMMA v.0.98.5 (Zhou *et al.* 2013). This polygenic model accounts for single larger effect size SNPs and multiple SNPs with small effects at the same time while correcting for population genetic structure by calculating a centered relatedness matrix. GEMMA excludes individuals with missing values. Therefore, we imputed missing genotypes using the function *na.roughfix* implemented in the R package RANDOMFOREST v.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 runs was assessed using Gelman-Rubin diagnostics implemented in the R package CODA (Plummer *et al.* 2006). The harmonic mean of the posterior inclusion probabilities (PIP) were calculated across the three runs. The PIP is the sum of all posterior probabilities of all regressions including the specific variable and thus a ranking measure to assess the extent to which the data favor 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; Pfeifer *et al.* 2018; Depardieu *et al.* 2021). 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% confidence interval (Depardieu *et al.* 2021).

2.4.2 Gene annotation

The GCAT3.3 white spruce gene catalogue (Rigault *et al.* 2011) was used for structural annotation of SNPs. Sequence description for the associated genes was obtained using BLAST2GO (Götz *et al.* 2008), described in Gagalova et al. (in review). BLAST2GO was also used to obtain Gene Ontology (GO)

annotations. GO biological process, molecular function and cellular component terms were gained for each individual transcript.

3. Results

3.1. Identified drought years

The trees of Interior Alaska showed the highest climate sensitivity, whereas the trees of the Alaska Range forest plot were the least sensitive. Within each study site, the treeline plots had consistently a higher sensitivity than the corresponding forest plots, which was significant for Alaska Range and Brooks Range (Figure 3A). The climate sensitivity also differed significantly between sites.

We identified several pointer years which can be associated with drought stress for each study site: For the Brooks Range we identified 1993 as a pointer year associated with a low CMI6 in 1991. In the Alaska Range, trees also showed a growth reduction in 1993 likely 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 also preceded by low values of CMI6 and SPEI6 in 1997. The trees of Interior Alaska showed a second pointer year in 2010 in the wake of low CMI6 in 2009.

The reaction of trees showed significant site-specific differences to the same pointer year. For example, in 1998 Interior Alaska showed a higher relative resilience and recovery but Alaska Range a higher resistance (Figure 3E). In 1993, Alaska Range had a higher resistance, resilience, relative resilience and recovery than Brooks Range (Figure 3D). Further, trees also showed a significantly different reaction to different pointer years within the same site, like 1993 and 1998 in Alaska Range or 1998 and 2010 in Interior Alaska for half of all parameters (Figure 3B, C). The individual-tree reaction during a pointer year differed between the treeline and the forest plot within one site. In Alaska Range for 1998, the forest plot showed a higher resilience, relative resilience and recovery compared to the treeline plot (Figure 3B). This pattern was also shown in Interior Alaska for 1998 and 2010 (Figure 3C). No significant differences between forest and treeline could be detected in Brooks Range and Alaska

Range for 1993 (Figure 3B – D). Recovery showed to have the highest inter-individual variation in all sites (Fig 3 B – E).

3.2. Population genetic structure

Distinct genetic clusters appeared between study sites based on PCA, with a clear separation of the Brooks Range study site from the Southern populations (Interior Alaska and Alaska Range; Figure 4A). No separation between forest and treeline plots was visible, except for two groups of individuals of the Alaska Range forest plot which separated from the remaining individuals of their study site. The two first axes (PC1 and PC2) together explained 3.94 % of the total genotypic variation. Note that 98.7 – 99.8 % of all loci were shared between duplicate samples (Table S2), demonstrating the reliability of the genotyping approach and the SNP detection method used.

This pattern of population genetic structure was supported by the results of Bayesian clustering analysis (Figure 4B). When individuals were assigned to two genetic clusters (K = 2), the study site Alaska Range was mainly distinguished from Brooks Range and Interior Alaska. At K = 3 the three study sites were mainly differentiated and a difference between Alaska Range forest and treeline became visible. Interior Alaska and Alaska Range were admixed from several genetic clusters. Furthermore, the pairwise F_{ST} values revealed a lower differentiation (F_{ST} = 0.014 – 0.017) between the Alaska Range treeline plot and the plots of the Brooks Range and Interior Alaska sites compared to the Alaska Range forest plot (F_{ST} = 0.023 – 0.025; Table S1).

3.3. Genotype-phenotype association analysis

3.3.1 Among-site associations

We conducted genotype-phenotype association (GPA) analysis of genotypes with dendrophenotypes using LMM. When we integrated Interior Alaska and Alaska Range in a single analysis for the pointer year 1998, we detected 12 SNPs associated with resilience in 1998, after correcting for individual relatedness (Table S4). No significant associations were detected for climate sensitivity.

374

375 **3.3.2 Within-site associations**

376 The association analysis of genotypes with the dendrophenotypes using LMM revealed no significant
377 associations when testing pointer years at individual sites separately. Our second approach, the
378 polygenic BSLMM and testing sites separately, revealed strong associations involving 30 SNPs,
379 representing 30 different genes (Table S4). Three of these were associated with two traits. Of the 30
380 SNPs, 13 were associated with climate sensitivity, including 11 in Alaska Range and one for Brooks
381 Range and Interior Alaska, respectively. The remaining 17 SNPs showed strong associations with
382 drought parameters. We revealed the majority of the associations in the drought-limited Interior Alaska
383 site (11 SNPs) with two of the SNPs being associated with two different traits. In addition, we found the
384 highest PIP values for resistance to drought in 2010 (PIP = 0.74 & 0.44). No overlap between strongly
385 associated SNPs with individual response parameters could be detected among the sites. When
386 comparing the amount of associated SNPs between the phenotypic traits, resistance was most frequently
387 associated (8 SNPs) followed by relative resilience (5 SNPs), resilience (4 SNPs) and recovery (3 SNPs;
388 Figure S5). Of all traits, climate sensitivity encompassed the highest amount of strong associations
389 biased by the SNPs of Alaska Range (11 of 13 SNPs). This is also reflected by the proportion of
390 phenotypic variance explained in BSLMM as well as in LMM (Figure 5, Figure S3). For Interior Alaska
391 in BSLMM, genetic variance explained the largest proportion of phenotypic variance for the traits
392 resilience, relative resilience and recovery in 1998 (53%, 70% and 62%; Figure 5, Table S3). The
393 phenotypic variance explained of the remaining parameters had a range from 12 – 45%. In Brooks Range
394 15 – 33% of the phenotypic variation was explained by genetic variation and in Alaska Range 17 – 77%.
395 The proportion of phenotypic variance which could be explained by large-effect size SNPs was the
396 highest for Interior Alaska for the traits resistance (59%) and relative resilience (42%) in 2010 (Table
397 S3, Figure S4). In Brooks Range large effect size SNPs explained a higher proportion of the phenotypic
398 variance (38 – 43%) compared to Alaska Range (30 – 37%; Table S3, Figure S4). The credible interval
399 of the hyperparameters showed a wide distribution which is depending on the sample size (pers. comm.
400 Zhou; Table S3).

Both 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 & ss524300164 on chromosome 9) were identified with both methods independently, associated with resilience in 1998 in Interior Alaska (Table S4). In total, 40 unique SNPs could be associated with the individual response parameters to drought or climate sensitivity.

3.4. Annotation of candidate SNPs

All associated SNPs were mapped and represent different genes (Table S4). Genes containing SNPs with strong associations differed in their location on the genome between the sites. In Interior Alaska, the majority of the genes were located on chromosome 1 and 10 (3 genes each), whereas in Brooks Range chromosome 3 (2 genes) and in Alaska Range chromosome 7 (4 genes) and chromosome 4 (3 genes) contained the majority of the associations. GO annotation was possible for 24 of the 40 associated genes (Table S4). Eight of them were related to the cellular component membrane and six genes related to transferase and / or hydrolase activity. One gene (GQ03312_O11) could be related to lignin biosynthetic process. Further, for six of the SNPs we could derive the information if the mutation was synonymous (4 SNPs) or non-synonymous (2 SNPs).

4. Discussion

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

When investigating the population genetic structure with a Bayesian clustering analysis, Alaska Range could clearly be distinguished from Interior Alaska and Brooks Range, whereas in the PCA, Brooks Range was more separated from the other two sites. A further STRUCTURE analysis with five microsatellite markers also differentiated Brooks Range from Interior Alaska and Alaska Range for *K*

427 = *2* (Figure S1). This supports the results of the PCA and indicates a pattern of isolation by distance
428 (Zacharias *et al.* 2021). Further, the separating group of individuals in the Alaska Range forest plot was
429 weakly supported by the available data and was also not shown with the microsatellite markers. It is
430 unlikely that it is the result of human interference.
431
432 **4.1. Climate sensitivity**
433 All treeline plots showed a higher climate sensitivity than the corresponding forest plots due to the more
434 extreme climate conditions at the treeline compared to the more protected closed-canopy forest
435 environment. In fact, the treeline populations represent an environment where the species experiences
436 its physiological limits within the realized niche. Growth is limited by low water availability (Interior
437 Alaska) or low temperatures (Brooks Range, Alaska Range), resulting in a stronger climate signal
438 (Hampe & Jump 2011). Consequently, treeline populations are preferably sampled in dendroecology to
439 study the influence of these environmental variables (Fritts 1976; Cook & Kairiukstis 1990).
440 Furthermore, in Brooks Range an elevated climate sensitivity is reported for small and young white
441 spruce trees (Trouillier *et al.* 2018b), like those found in the treeline plots. By far the most significant
442 associated genes with climate sensitivity were found in the Alaska Range population (11 genes)
443 compared to only one gene for Interior Alaska and Brooks Range, respectively. Therefore, for the trait
444 climate sensitivity, the phenotypic variance explained by genetic variants was highest in Alaska Range
445 (77%), intermediate in Interior Alaska (38%) and lowest in Brooks Range (15%). In Alaska Range,
446 forest and treeline plot have the highest distance to each other and the largest difference in elevation.
447 Therefore, environmental conditions and consequently climate sensitivity differ the most between the
448 plots within Alaska Range compared to the other two sites. These phenotypic differences among the
449 individuals together with the separating cluster of Alaska Range forest within the population genetic
450 structure analysis probably led to the high amount of significant associations. Five of the associated
451 SNPs in Alaska Range could be annotated and related to gene functions such as hydrolase activity or
452 cell wall organization, among others. Further, two of the associated SNPs with climate sensitivity in
453 Alaska Range are non-synonymous mutations, which change the amino acid sequence of a protein and

454 are therefore subjected to natural selection. This indicates a genetic basis of climate sensitivity. Climate
455 sensitivity exhibited a higher variability among the sites than the drought related traits. In contrast, for
456 white spruce populations planted in a common garden, climate sensitivity traits had the lowest
457 phenotypic variance explained (11 – 18.5%) (Depardieu *et al.* 2021).
458
459 **4.2. Growth reaction to drought stress**
460 In contrast to climate sensitivity, there was no consistent pattern in the growth reaction to drought events
461 when comparing forest and treeline. In general, in Alaska Range and Interior Alaska, trees within the
462 forest plots seemed to recover better from a drought event. For the drought-limited site (Interior Alaska)
463 the growth reaction differed significantly between forest and treeline trees for most of the traits, even
464 though the plots were positioned right next to each other. This suggests that the site´s location at a steep
465 south-exposed bluff results in a strong microenvironmental gradient at short geographical distance,
466 which seems to gain influence during a drought event with stronger effects on the bluff site that is more
467 exposed to radiation (Nicklen *et al.* 2018). In the cold-limited sites (Brooks Range, Alaska Range),
468 forest and treeline plots exhibited only minor differences in growth reaction probably due to similar
469 environmental conditions. Trees of different sites reacted significantly to a drought event in the same
470 year, probably due to distinct growth conditions among sites. For the growth reaction in 1998, the
471 drought-limited site (Interior Alaska) showed a significant higher recovery compared to the cold-limited
472 site (Alaska Range), which in return showed a significant higher resistance. A similar pattern was
473 observed in maritime pine with high resistance in Atlantic and high recovery in Mediterranean
474 provenances planted in a common garden (Zas *et al.* 2020). Thus, populations experiencing contrasting
475 environmental conditions seem to use various strategies to cope with drought stress. This supports our
476 hypothesis that the individual reaction to drought stress differs between drought and cold-limited
477 treelines as well as between forest and treeline plots. Nevertheless, we could not identify a common
478 pattern within the growth reaction except for climate sensitivity. For gymnosperms, a reduced recovery
479 is related to high drought-related mortality risk (Desoto *et al.* 2020).

480 Even though we developed an evidence-based approach to identify years of growth decline
481 caused by drought, we acknowledge that factors other than drought stress may had affected growth
482 reduction. Furthermore, drought induces masting in white spruce (Ascoli *et al.* 2020) which could also
483 be the reason for growth reduction at the population level (Hacket-Pain *et al.* 2015; Nicklen *et al.* 2018).
484 Mast seeding events were recorded for the Alaska Range site in 1998 and the Interior Alaska site in
485 2010 overlapping with the analyzed pointer years (Roland *et al.* 2014).
486
487 **4.3. Contrasting genetic basis underlying drought tolerant phenotypes**
488 There was no overlap in drought-associated genes among sites, which supports our hypothesis that the
489 selection pressure at the contrasting treeline ecotones led to divergent genetic signatures underlying
490 drought tolerance. Even the two cold-limited sites (Brooks Range, Alaska Range) showed different
491 genetic signatures associated with drought tolerance. However, it is important to mention that the two
492 cold-limited sites differ in precipitation as well as in temperature. At these treelines frost tolerance may
493 be a strong selective driver in addition to drought. Thus, signatures of selection are population-specific
494 and led to different alleles associated with drought-tolerant phenotypes, such as reported in populations
495 of *Arabidopsis halleri* in heterogenous alpine environments (Rellstab *et al.* 2017). Moreover, the
496 location of the associated genes on the genome varied widely with genes on different chromosomes
497 associated for different sites. Even though we analyzed two drought years for the Alaska Range site
498 compared to only one for the Brooks Range site, the Alaska Range site showed the lowest number of
499 GPAs with growth reaction, possibly explained by the comparably high precipitation rates at the Alaska
500 Range site. We identified most of the significantly associated genes with drought tolerance in the
501 drought-limited site (Interior Alaska), as well as the highest proportion of phenotypic variance explained
502 by genetic variance (70%). This indicates comparatively strong selection of drought-tolerant phenotypes
503 within the drought-limited site. Populations experience the strongest selection pressure under extreme
504 events like droughts which shape the genetic variation among populations (Grant *et al.* 2017). This high
505 selection pressure leads from small to moderate shifts in allele frequencies (Depardieu *et al.* 2021). A
506 high resilience to extreme drought events was also found in white spruce populations from dry regions

planted in a common garden, which leads to the assumption that genetic variation among populations plays a significant role in growth resilience in response to drought (Depardieu *et al.* 2020). Further, significant 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 is high, as demonstrated by the high seed- and pollen-migration rates and the low genetic differentiation among the investigated sites (Zacharias *et al.* 2021). The linear mixed model revealed only significant SNPs when taking together Interior Alaska and Alaska Range, the two sites which genetically differentiated within the Bayesian clustering analysis for $K = 2$. Therefore, there could be a covariance between the genetic differences between the sites and site-specific growth responses to drought, resulting in spurious associations. Nevertheless, two of the associated SNPs could also be identified in the Bayesian sparse linear mixed model, independently.

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 linear mixed models, pointing towards a complex genetic architecture of drought tolerance in white spruce. When analyzing complex traits such as growth related traits, multi-locus 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 rather driven by interacting small-effect size alleles instead of 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 tolerance. The two SNPs with the highest posterior inclusion probabilities were found for this trait in

the Interior Alaska site. This could be a hint towards selective sweeps like reported for *Sequoia sempervirens* and *Sequoiadendron giganteum* in relation to local adaptation (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 are higher than the ones reported for white spruce populations in a common garden (11 – 33.6%) or natural *Pinus albicaulis* populations (14.4 – 37.6%) (Depardieu *et al.* 2021; Lind *et al.* 2017). Most of the associated genes were found in climate sensitivity at the Alaska Range site. For the traits related to drought reaction, resistance had the most associated genes suggesting a strong genetic basis for this trait.

4.5. Significant associated genes 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 S4) and two other genes (GQ03701_H09: auxin response factor 6, GQ03417_G17: uridine-cytidine kinase C) were associated with resilience by both association approaches independently. These genes were associated with various biological processes, molecular functions and cellular components like transferase or hydrolase activity suggesting their relevance in relation to drought tolerance. Sixteen of the genes 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 greenhouse experiment (Depardieu *et al.* 2021). Furthermore, eight of the 40 associated genes were already associated with wood anatomy traits like wood density in white spruce provenances sampled in Québec (Lamara *et al.* 2016). Wood density is known to influence drought tolerance in conifers (Martinez-Meier *et al.* 2008). In a common garden experiment of *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 investigated sites xylem anatomical traits are rather influenced by microhabitat, but latewood density and earlywood hydraulic diameter showed a 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 which are heritable (Depardieu *et al.* 2020). 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 are therefore related to drought tolerance. One gene (GQ03617_M21: *21 kDa protein-like*) associated to resilience in Interior Alaska in our study, was also identified as 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 reason for the limited number of overlapping genes between our study and study of Depardieu *et al.* (2021) could be that we investigated natural populations with trees of different sizes and ages and varying environmental conditions in contrast to their controlled common garden setting. Still, we tried to account for the differing environments by using the household matrix (a binary encoded matrix indicating if individuals are from the same or different study sites) within the linear mixed models when testing multiple sites together or testing the sites separately in BSLMM. Further, the Alaskan and Canadian study sites are located at the western and eastern edge of the white spruce distribution range, which not only reduces gene flow but also represents populations of different glacial refugia (Anderson *et al.* 2011). Adaptation to drought can also occur in independent routes like 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 supports the important role of these genes.

4.6. Conclusions

Treeline plots showed a higher sensitivity in their growth response to climate than the corresponding forest plots because of the more extreme environmental conditions. Climate sensitivity showed a high phenotypic plasticity but our results indicated a minor genetic basis. Tree populations growing in different environments responded differently to drought stress, thus supporting that populations were

differentially affected by drought events induced by climate change. In addition, populations from different environments had divergent genetic signatures underlying drought stress tolerance with most genes found in populations more exposed to drought. As a consequence, our results support the hypothesis that selection pressure in populations in different environments resulted in differing strategies to cope with drought stress and, thus, adaptation is a local process in populations with restricted gene flow. Further, the high amount of small-effect size SNPs demonstrated the polygenic and complex architecture of drought tolerance in trees. Genes that were identified by several analyses or which were associated with wood traits or expressed under drought conditions in other studies can be considered as potential targets for further gene expression and landscape genomic studies focusing on drought response. These are critical resources to help inform assisted migration programs in the context of more severe and recurrent extreme climatic events.

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Data accessibility statement

Raw and filtered genotypic data, tree ring data and R scripts containing the filtering steps and analysis are deposited on Zenodo (DOI: 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.

Author contributions

MW and MS designed the overall study design. CR assisted in realizing the Alaska Range study sites. MZ, TP and MW collected samples with the help of others. Conceptualization of the GPA analysis was done by KH and LO. MZ prepared samples for genotyping. TP performed dendro analysis. MZ performed population structure and GPA analysis. MZ wrote the manuscript with contributions from all authors. All authors revised and refined the final manuscript.

Tables and Figures

Table 1: Characteristics of the sampled locations. 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.

Study site	**Brooks Range**		**Interior Alaska**		**Alaska Range**	
Research plot	**BR F†**	**BR T‡**	**Int F†**	**Int T‡**	**AR F†**	**AR T‡**
Latitude	67.95	67.95	64.70	64.70	63.72	63.74
Longitude	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 in °C	-8.18		-1.89		-3.58	
Total annual precipitation in mm	314		305		511	
Density (trees per ha)	839	232	406	326	507	152
Number of analyzed 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

† forest plot
‡ treeline plot

Supplementary tables

Table S 1: Population differentiation described by pairwise F_{ST} values between the white spruce (Picea glauca) plots calculated in R package DARTR.

	Brooks Range Forest	**Brooks Range Treeline**	**Interior Alaska Forest**	**Interior Alaska Treeline**	**Alaska Range Forest**
Brooks Range Forest	-	-	-	-	-
Brooks Range Treeline	0.005	-	-	-	-

Interior Alaska Forest	0.024	0.023	-	-	-
Interior Alaska Treeline	0.025	0.024	0.001	-	-
Alaska Range Forest	0.025	0.024	0.023	0.024	-
Alaska Range Treeline	0.017	0.016	0.014	0.015	0.007

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902 *Table S 2: Proportion of shared loci between duplicated samples of 2931 SNP loci. Mismatches are due to missing data.*

Tree ID	Sample 1	Sample 2	Shared loci in %	Loci with missing data sample 1	Loci with missing data sample 2
45508	P02_G06_45508	P02_G12_45508	99.8	9	7
DF143	P05_A12_DF143	P05_E02_DF143	99.7	15	14
BF070	P02_B05_BF070	P03_G11_BF070	99.6	14	13
BF373	P03_E01_BF373	P03_F11_BF373	99.6	14	14
45205	P06_D07_45205	P06_D10_45205	99.5	21	22
45486	P04_C02_45486	P05_B12_45486	99.5	27	16
DF266	P04_H10_DF266	P05_A02_DF266	99.4	8	18
DF205	P05_E01_DF205	P06_F10_DF205	99.4	9	22
45248	P01_F11_45248	P06_E10_45248	99.3	11	25
45473	P04_A12_45473	P04_F01_45473	98.8	12	35
45263	P01_C12_45263	P01_D11_45263	98.7	39	15

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905 *Table S 3: Hyperparameters of the Bayesian Sparse Linear Mixed Mmodels (BSLMM) testing dendrophenotypes.*

Site	Phenotypic trait	Hyperparameters	Mean	Standard deviation	2.5% Confidence interval	97.5% Confidence interval
Brooks Range	Resistance 1993	PVE†	0.33	0.20	0.02	0.75
		PGE‡	0.43	0.30	0	0.97
		LE SNPs§	61	77	0	270
	Resilience 1993	PVE†	0.24	0.17	0.01	0.63
		PGE‡	0.38	0.3	0	0.96
		LE SNPs§	55	73	0	263
	Rel. resilience 1993	PVE†	0.25	0.17	0.01	0.64
		PGE‡	0.38	0.3	0	0.96
		LE SNPs§	59	77	0	268
	Recovery 1993	PVE†	0.23	0.17	0.01	0.63
		PGE‡	0.41	0.3	0	0.96
		LE SNPs§	59	74	0	261
	Climate sensitivity	PVE†	0.15	0.12	0	0.46
		PGE‡	0.42	0.31	0	0.97
		LE SNPs§	48	71	0	261
	Resistance 1998	PVE†	0.4	0.22	0.04	0.89

Interior Alaska		PGE‡	0.33	0.28	0	0.94
		LE SNPs§	64	77	0	270
	Resilience 1998	PVE†	0.53	0.23	0.1	0.98
		PGE‡	0.33	0.28	0	0.94
		LE SNPs§	67	79	0	273
	Rel. resilience 1998	PVE†	0.70	0.19	0.29	0.99
		PGE‡	0.24	0.24	0	0.87
		LE SNPs§	74	83	0	278
	Recovery 1998	PVE†	0.62	0.21	0.20	0.99
		PGE‡	0.28	0.26	0	0.91
		LE SNPs§	79	83	0	275
	Resistance 2010	PVE†	0.45	0.20	0.12	0.92
		PGE‡	0.59	0.27	0.02	0.98
		LE SNPs§	28	43	1	166
	Resilience 2010	PVE†	0.21	0.15	0.01	0.56
		PGE‡	0.38	0.30	0	0.96
		LE SNPs§	61	76	0	265
	Rel. resilience 2010	PVE†	0.16	0.12	0.01	0.45
		PGE‡	0.42	0.3	0	0.97
		LE SNPs§	37	62	0	240
	Recovery 2010	PVE†	0.12	0.11	0	0.39
		PGE‡	0.38	0.3	0	0.96
		LE SNPs§	49	72	0	261
	Climate sensitivity	PVE†	0.38	0.23	0.03	0.91
		PGE‡	0.34	0.29	0	0.95
		LE SNPs§	63	78	0	269
Alaska Range	Resistance 1993	PVE†	0.42	0.26	0.02	0.96
		PGE‡	0.35	0.28	0	0.94
		LE SNPs§	57	73	0	264
	Resilience 1993	PVE†	0.44	0.26	0.02	0.97
		PGE‡	0.33	0.28	0	0.94
		LE SNPs§	63	80	0	271
	Rel. resilience 1993	PVE†	0.25	0.2	0.01	0.75
		PGE‡	0.34	0.29	0	0.94
		LE SNPs§	55	74	0	269
	Recovery 1993	PVE†	0.22	0.19	0.01	0.70
		PGE‡	0.36	0.30	0	0.95
		LE SNPs§	55	76	0	269
	Resistance 1998	PVE†	0.33	0.23	0.01	0.89
		PGE‡	0.35	0.3	0	0.95
		LE SNPs§	63	77	0	270
	Resilience 1998	PVE†	0.26	0.20	0.01	0.74
		PGE‡	0.35	0.29	0	0.95
		LE SNPs§	69	82	0	275
	Rel. resilience 1998	PVE†	0.17	0.13	0.01	0.5
		PGE‡	0.37	0.3	0	0.96
		LE SNPs§	59	76	0	269
	Recovery 1998	PVE†	0.19	0.14	0.01	0.53
		PGE‡	0.36	0.3	0	0.95
		LE SNPs§	60	78	0	272
		PVE†	0.77	0.20	0.30	0.99

	Climate sensitivity	PGE‡	0.30	0.26	0	0.91
		LE SNPs§	86	88	0	282

† proportion phenotypic variance explained
‡ proportion of PVE explained by large effect size SNPs
§ large effect size SNPs

Table S4: Significantly associated SNPs identified with linear mixed models (LMM) and SNPs with posterior inclusion probabiity (PIP) > 0.1 identified by Bayesian sparse linear mixed models (BSLMM) with dendrophenotypes in Alaska Range (AR), Interior Alaska (Int) and Brooks Range (BR). SNPs are mapped on genes according to the GCATv3.3 gene catalog and sequence description and GO terms are obtained from blast2go runs. Literature review of genes known to be regulated under drought (Depardieu et al. 2021) and associated with wood traits (Lamara et al. 2016) in white spruce.

Table S5: All initially selected SNPs for genotyping and filtering steps.

Figure 1: Studied locations and distribution range (green) of white spruce (Picea glauca) in Alaska (Prasad and 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.

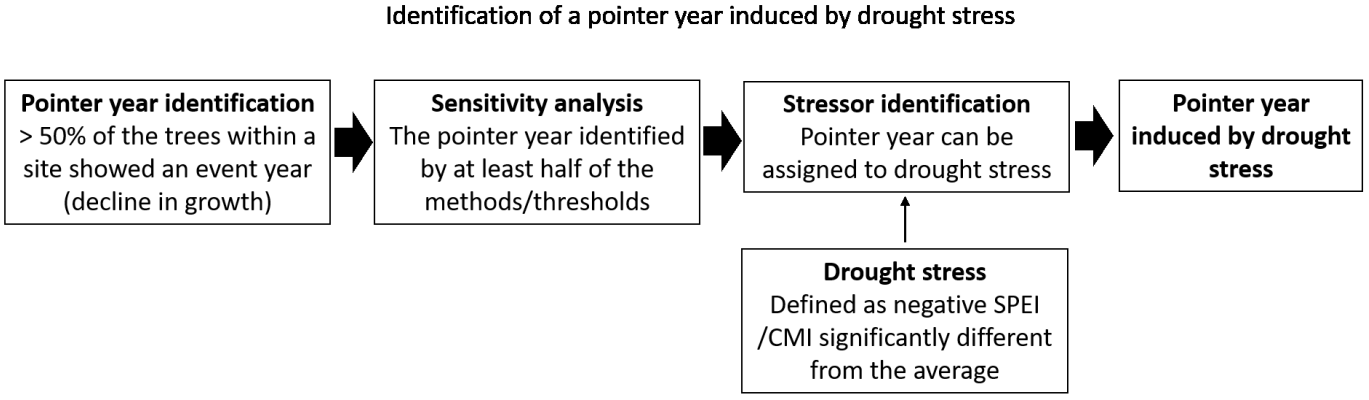

Figure 2: Decision tree for pointer year identification induced by drought stress.

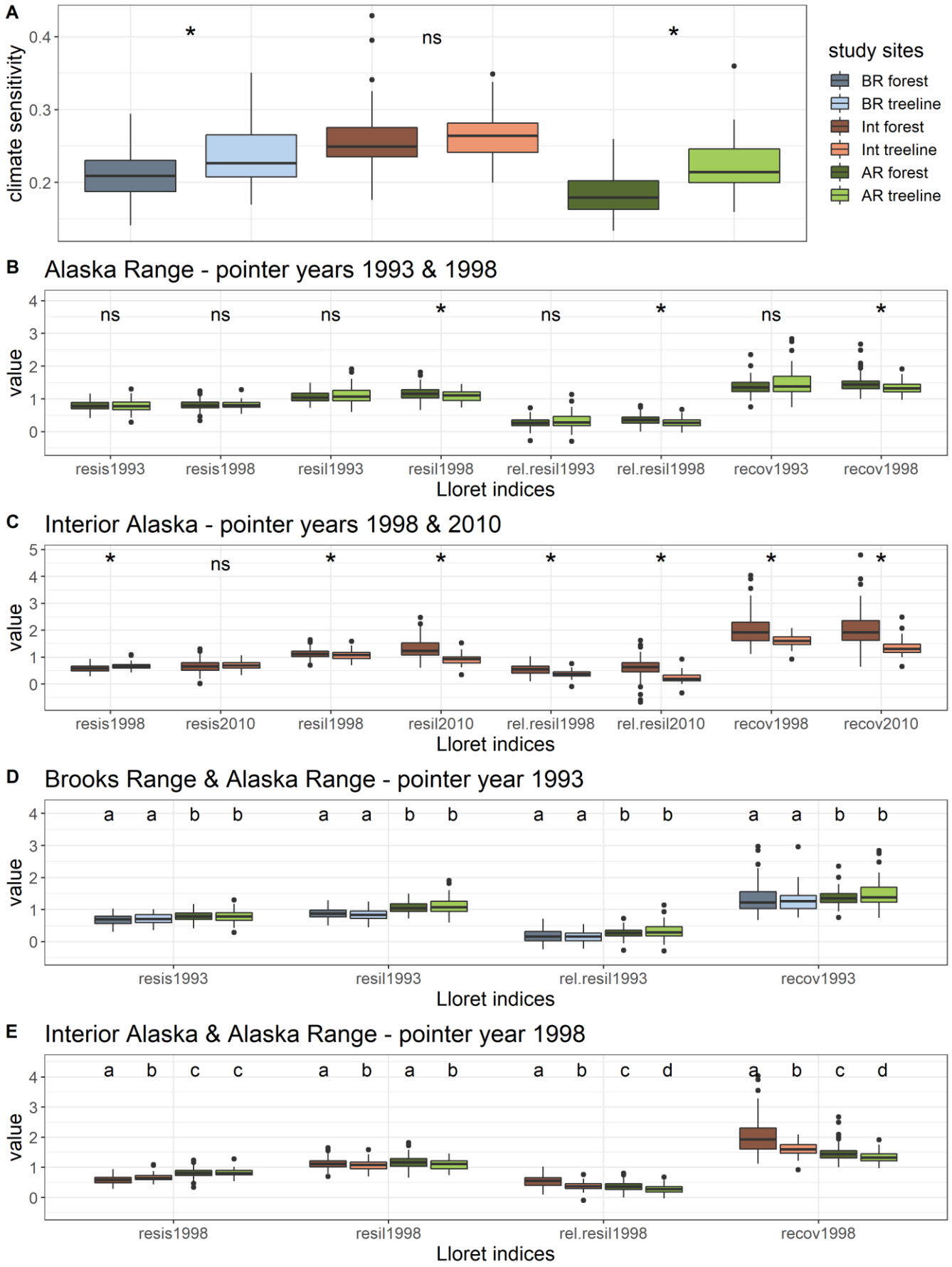
A
climate sensitivity
0.4
0.3
0.2
*
ns
*
study sites
BR forest
BR treeline
Int forest
Int treeline
AR forest
AR treeline
B
Alaska Range - pointer years 1993 & 1998
value
ns ns ns * ns * ns *
resis1993 resis1998 resil1993 resil1998 rel.resil1993 rel.resil1998 recov1993 recov1998
Lloret indices
C
Interior Alaska - pointer years 1998 & 2010
value
* ns * * * * * *
resis1998 resis2010 resil1998 resil2010 rel.resil1998 rel.resil2010 recov1998 recov2010
Lloret indices
D
Brooks Range & Alaska Range - pointer year 1993
value
a a b b a a b b a a b b a a b b
resis1993 resil1993 rel.resil1993 recov1993
Lloret indices
E
Interior Alaska & Alaska Range - pointer year 1998
value
a b c c a b a b a b c d a b c d
resis1998 resil1998 rel.resil1998 recov1998
Lloret indices

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*Figure 3: Comparison of 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 tested with Wilcoxon test. * means significant (p-value < 0.05), ns means not significant. Letters indicate significant different groups.*

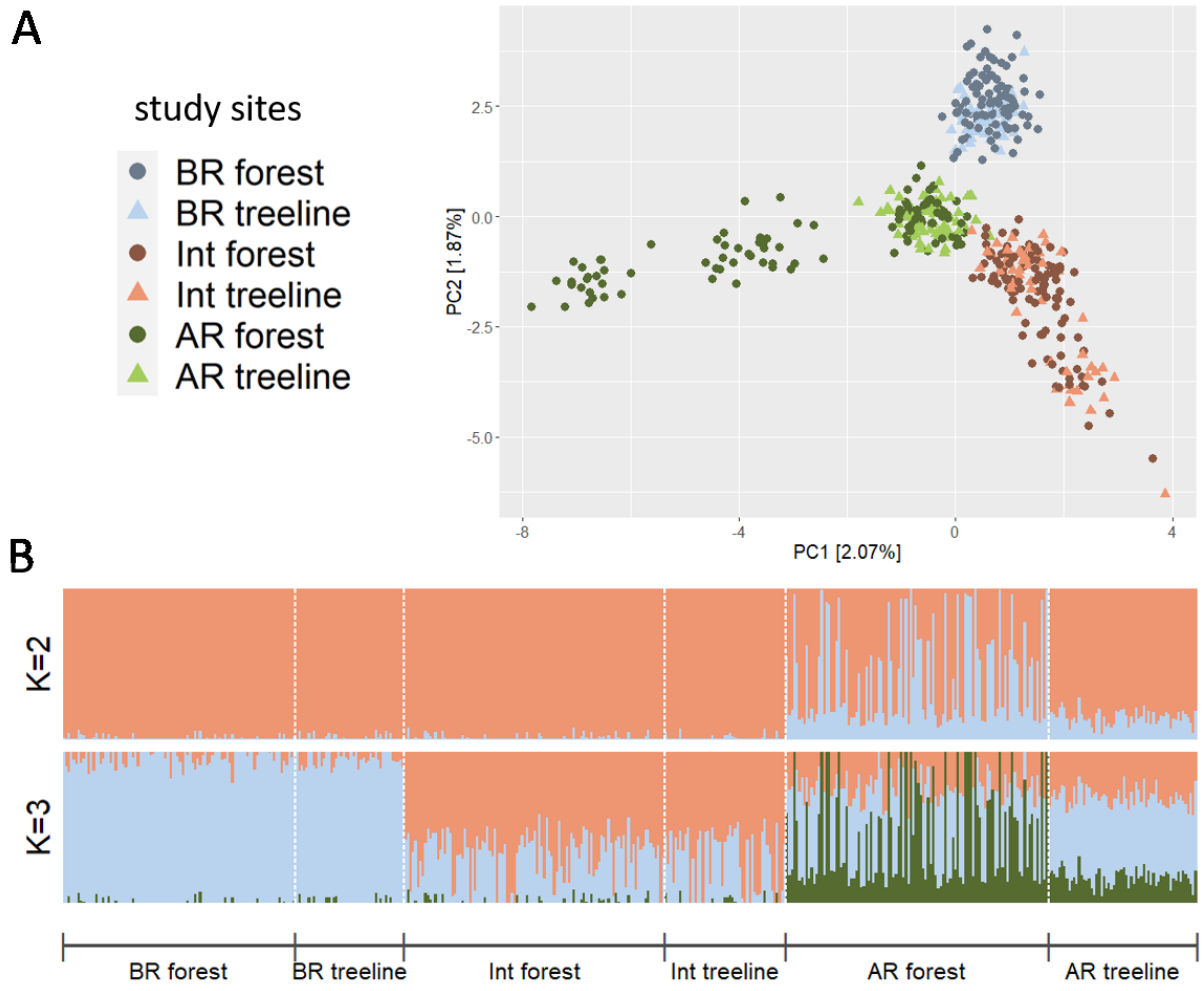

Figure 4: Principal Component Analysis (A) and Bayesian clustering analysis (B) for K = 2 and K = 3 based on 2,463 SNP loci genotyped in white spruce (Picea glauca) individuals sampled from three different sites and forest/treeline plots. BR – Brooks Range, Int – Interior Alaska, AR – Alaska Range.

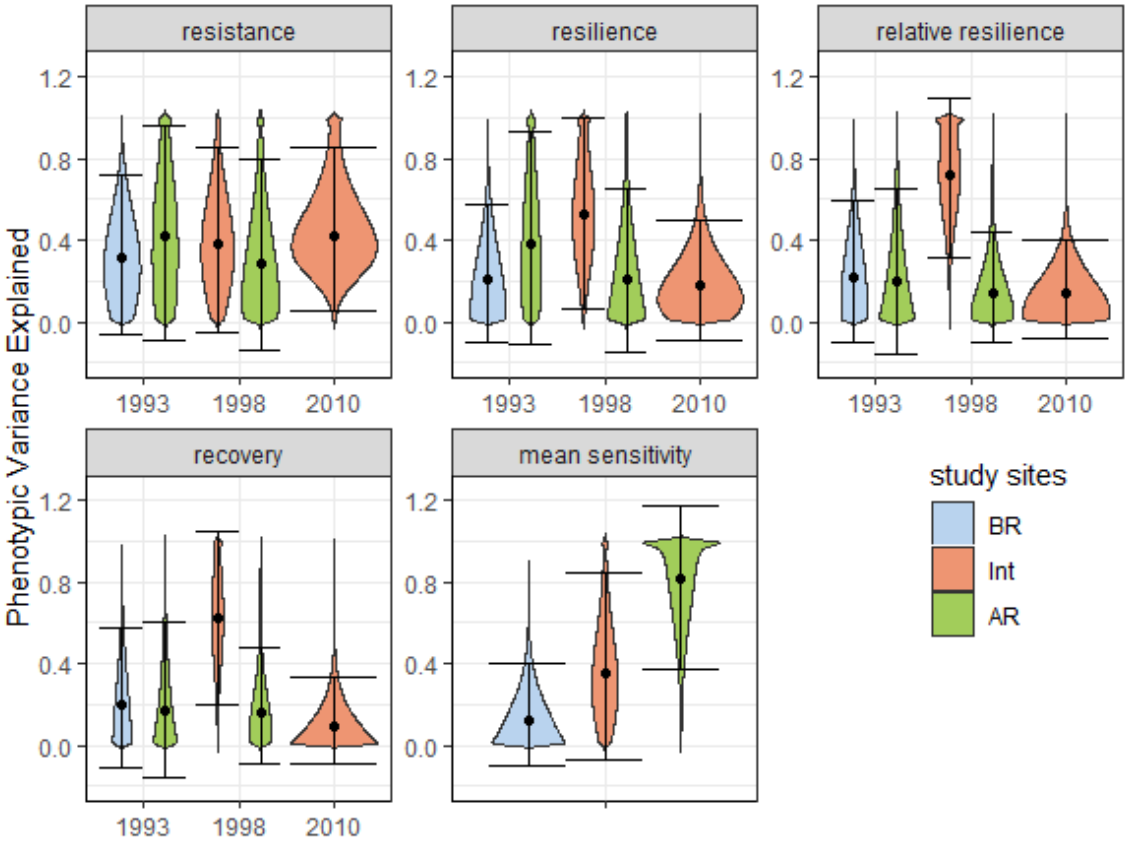

Figure 5: Results of the BSLMM analysis. Violin plots represent the posterior distributions of the proportion of the phenotypic variance explained by genetic variance (PVE). The median (black circle) and standard deviation (error bars) for each phenotypic trait is shown for Brooks Range (BR), Interior Alaska (Int) and Alaska Range (AR).

Supplementary figures

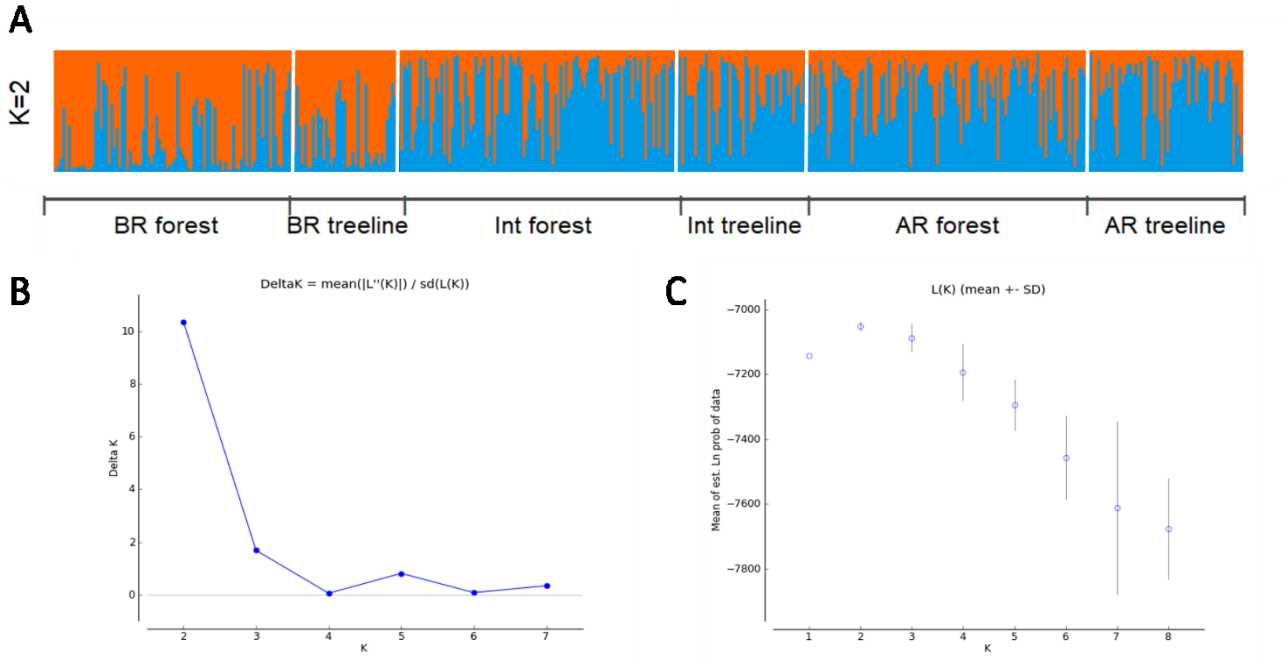

Figure S 1: Population membership coefficient (A) of sampled white spruce (Picea glauca) individuals of the three different sites and forest/treeline plots obtained with treeline plots obtained with STRUCTURE for K = 2 based on ΔK (B) and loglikelohood distribution (C) based on five SSR loci described in (Zacharias et al. 2021). F – forest, T – treeline.

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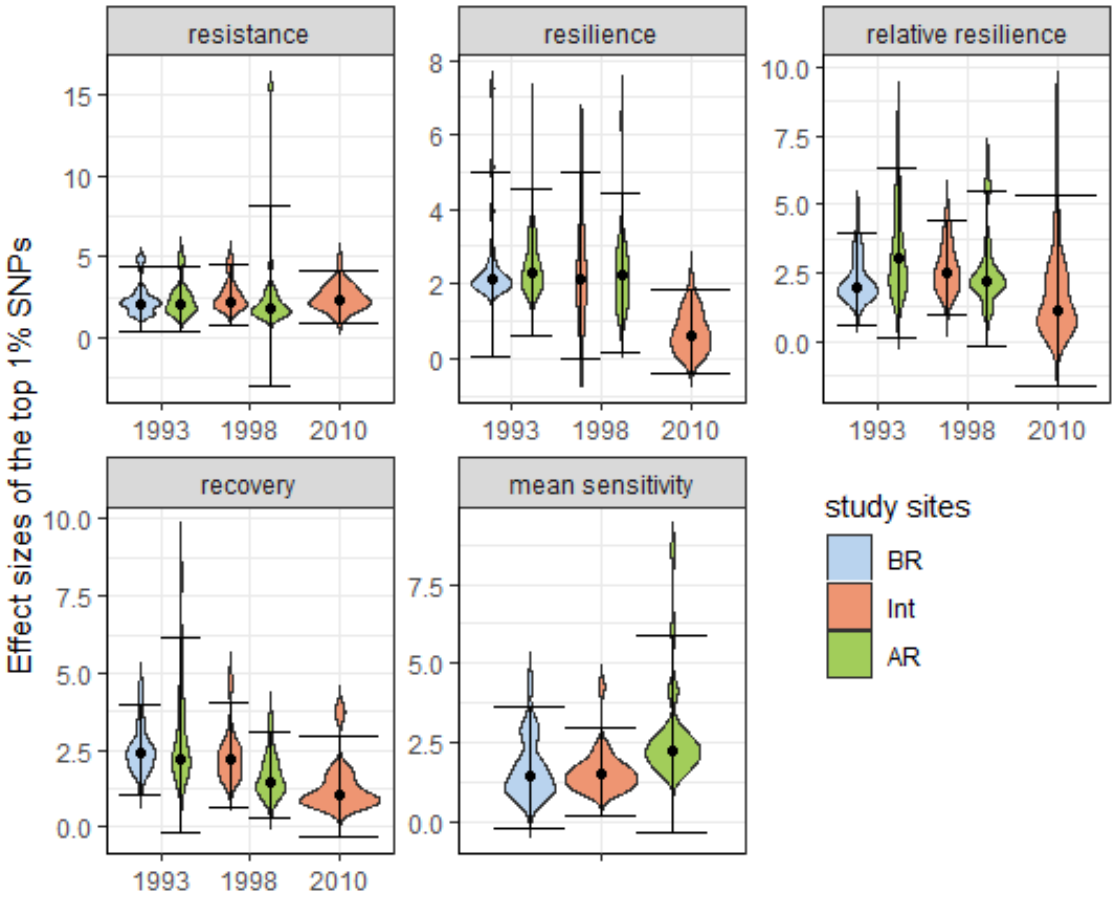

962
963 *Figure S 2: Results of the LMM analysis. Violin plots represent the absolute values of the effect sizes of the top one percent*
964 *SNPs. The median (black circle) and standard deviation (error bars) for each phenotypic trait is shown for Brooks Range (BR),*
965 *Interior Alaska (Int) and Alaska Range (AR).*

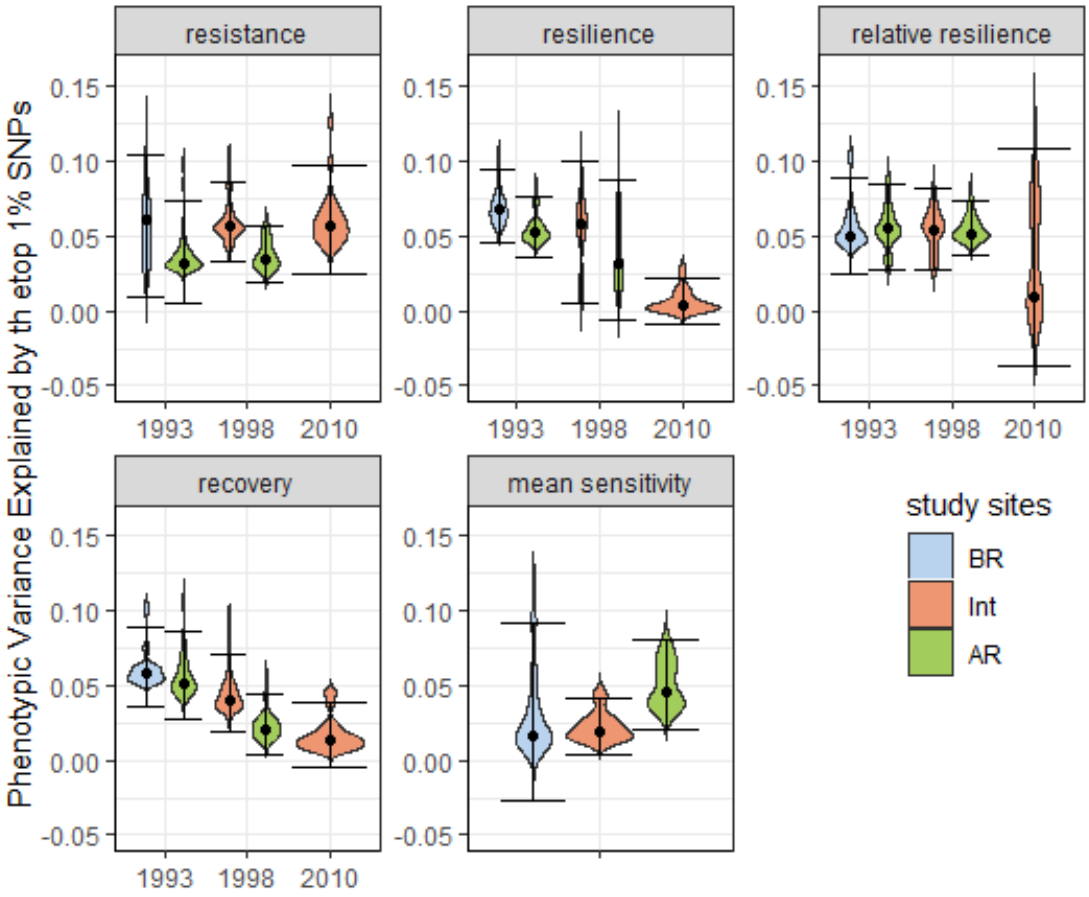

Figure S 3: Results of the LMM analysis. Violin plots represent the phenotypic variance explained by the top one percent SNPs. The median (black circle) and standard deviation (error bars) for each phenotypic trait is shown for Brooks Range (BR), Interior Alaska (Int) and Alaska Range (AR).

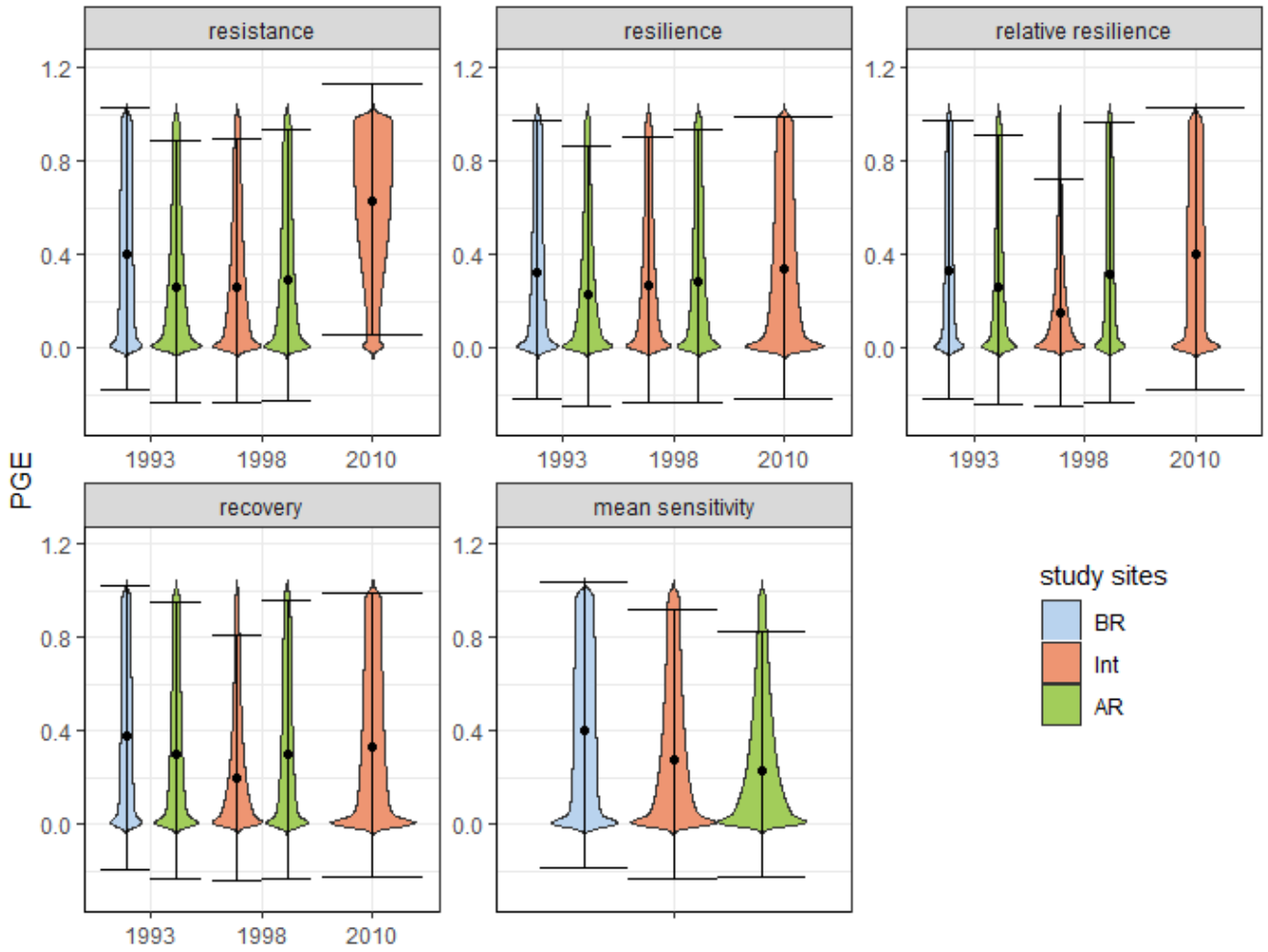

Figure S 4: Results of the BSLMM analysis. Violin plots represent the posterior distributions of the proportion of PVE explained by large effect size SNPs (PGE). The median (black circle) and standard deviation (error bars) for each phenotypic trait is shown for Brooks Range (BR), Interior Alaska (Int) and Alaska Range (AR).

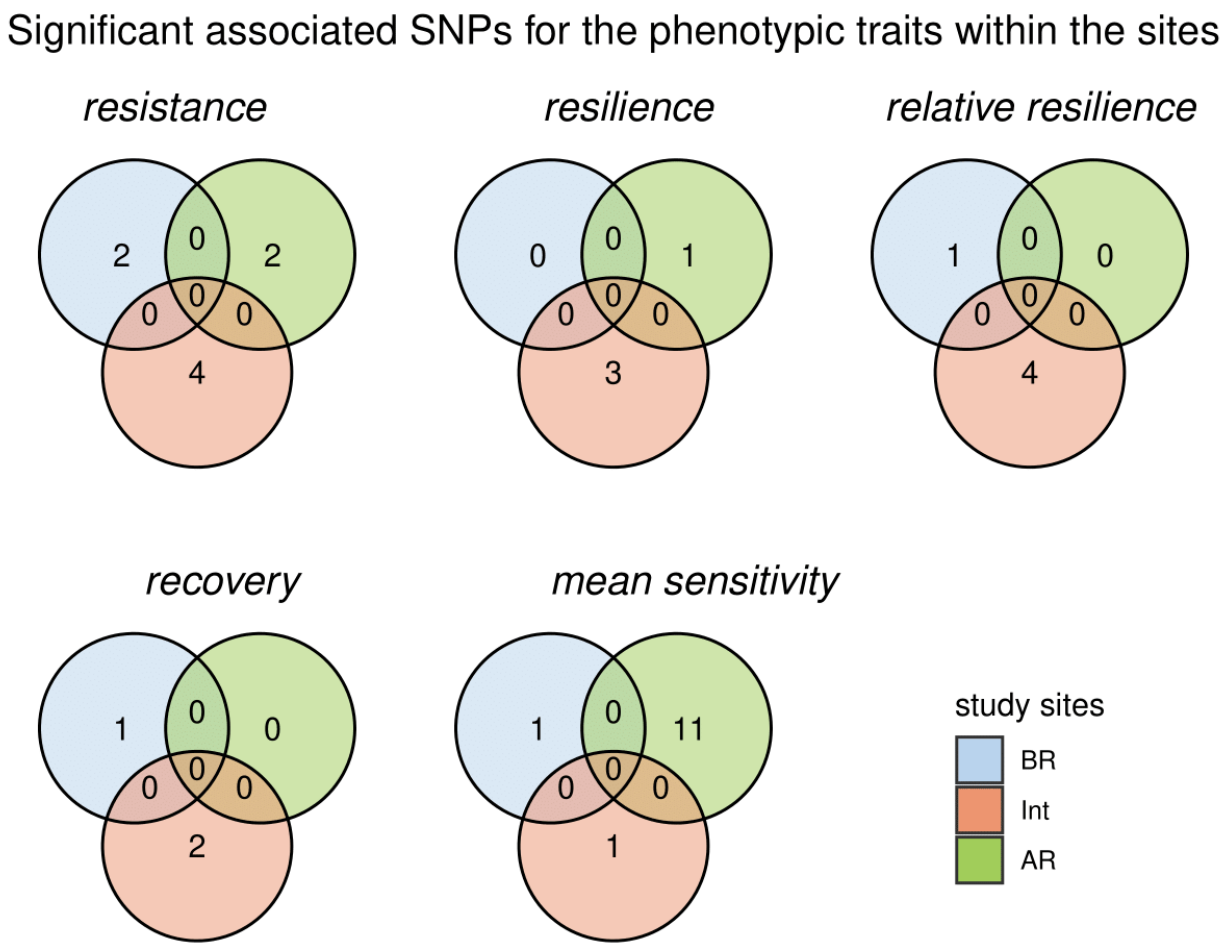

Figure S 5: Venn diagrams of significantly associated SNPs in BSLMM analysis for each phenotypic trait derived in sites. One SNP represents one gene. BR – Brooks Range, Int – Interior Alaska, AR - Alaska Range.

Table S 4: Significantly associated SNPs identified with linear mixed models (LMM) and SNPs with posterior inclusion probabiity (PIP) > 0.1 identified by Bayesian sparse linear mixed models (BSLMM) with dendrophenotypes in Alaska Range (AR), Interior Alaska (Int) and Brooks Range (BR). SNPs are mapped on genes according to the GCATv3.3 gene catalog and sequence description and GO terms are obtained from blast2go runs. Literature review of genes known to be regulated under drought (Depardieu et al. 2021) and associated with wood traits (Lamara et al. 2016) in white spruce.

Gene	SNP ID	chrom	BSLMM			LMM				Sequence description	GO terms[§]	Regulated under drought	Wood traits[¶]	Syn[#]
			site	trait[†]	PIP	site	trait[†]	effect size	PVE[‡]					
GQ03701_H09	ss524300164	9	Int	Rl98	0.15	Int & AR	Rl98	0.05	0.04	auxin response factor 6	P:regulation of transcription, DNA-templated; P:response to hormone; P:auxin-activated signaling pathway; F:DNA binding; C:nucleus			
GQ03417_G17	ss538950708	1		Rl98, Rr98	0.25, 0.11			-0.05	0.04	uridine-cytidine kinase C	P:UMP biosynthetic process; P:phosphorylation; F:uridine kinase activity; F:ATP binding; C:integral component of membrane			
GQ04005_G08	ss538952487	1						-0.09	0.03	senescence/dehydration-associated protein At4g35985, chloroplastic-like	C:plasma membrane			
GQ0014_K23	ss538945558	10						-0.05	0.03	unnamed product	C:membrane; C:integral component of membrane	Down		
GQ0045_F02	ss518105220	5						-0.07	0.03	zinc finger protein CONSTANS-LIKE 9-like isoform X2		Down	MFA/ MOE	
GQ03207_K07	ss538948979	7						-0.06	0.04	PREDICTED: uncharacterized protein LOC107018276 isoform X1	P:cell redox homeostasis; P:obsolete oxidation-reduction process; P:cellular oxidant detoxification; F:antioxidant activity; F:oxidoreductase activity; C:obsolete cell			
GQ0043_O18	ss538945672	7						0.07	0.03	F-box/kelch-repeat protein At1g22040		Down	MOE/ RW	

GQ04007_P09	ss538952592	3						-0.11	0.03	28S ribosomal protein S33, mitochondrial				
GQ03706_G11	ss538944095	1						-0.09	0.04	phospholipase D beta 2-like	F:phospholipase D activity; F:N-acylphosphatidylethanolamine-specific phospholipase D activity	up	MOE	s
GQ03113_N18	ss538948383	10						0.05	0.03	mannosylglycoprotein endo-beta-mannosidase isoform X3	F:hydrolase activity, hydrolyzing O-glycosyl compounds			
GQ04006_C07	ss538952518	12						0.17	0.06	U2 small nuclear ribonucleoprotein B" isoform X2	F:nucleic acid binding	up		
GQ03001_M17	ss538947781	1						0.09	0.04	tRNA (cytosine(34)-C(5))-methyltransferase-like isoform X2				
GQ03814_E07	ss524905003	3	BR	Rc93, Rr93	0.13, 0.12					O-fucosyltransferase 23-like	P:carbohydrate metabolic process; F:transferase activity			
GQ04105_B09	ss511222837	8		Rs93	0.25					transcription factor SRM1-like isoform X1		up	RW/ WD	
GQ04104_L11	ss538952912	10		Rs93	0.11					beta-1,6-galactosyltransferase GALT29A	F:glycosyltransferase activity; C:membrane			
GQ02829_O21	ss538947535	3		CS	0.12					vacuolar fusion protein CCZ1 homolog B-like	P:vesicle-mediated transport; C:Mon1-Ccz1 complex	up		
WS00110_K01	ss538953160	4	Int	Rc98, Rs10	0.11, 0.44					probable inactive leucine-rich repeat receptor-like protein kinase At3g03770	P:protein phosphorylation; F:kinase activity; C:membrane			
GQ02809_D01	ss538946877	1		Rc98	0.11					LRR receptor-like serine/threonine-protein kinase GSO2		down		
GQ03617_M21	ss538954933	10		Rs98	0.10					21 kDa protein-like	P:negative regulation of catalytic activity; F:enzyme inhibitor activity; F:hydrolase activity; F:pectinesterase activity; C:membrane;	down	MOE/ WD	

											C:integral component of membrane			
GQ03308_B05	ss538950030	10		Rl98	0.11					AT-hook motif nuclear-localized protein 16	F:minor groove of adenine-thymine-rich DNA binding; F:DNA-binding transcription factor activity; C:nucleus; C:membrane	up		
GQ03105_E13	ss538948127	3		Rs10	0.12					ras-related protein Rab5-like	F:GTPase activity; F:GTP binding		RW	
GQ03318_K18	ss538950306	6		Rs10	0.74									
GQ03123_B21	ss524904113	12		Rr98	0.11					CTD small phosphatase-like protein 2 isoform X1				
GQ03234_J18	ss538949723	11		Rr98	0.13					protein SGT1 homolog B-like	F:transferase activity			
GQ03312_O11	ss538943411	12		Rr10	0.17					probable cinnamyl alcohol dehydrogenase	P:lignin biosynthetic process; P:obsolete oxidation-reduction process; F:zinc ion binding; F:cinnamyl-alcohol dehydrogenase activity; F:sinapyl alcohol dehydrogenase activity			s
GQ03001_D10	ss538941983	10		CS	0.16					tubulin beta chain		down		s
GQ03204_D08	ss538948868	4		Rs93	0.25					protein phosphatase 2C 29-like	P:protein dephosphorylation; F:protein serine/threonine phosphatase activity	Down		
GQ04106_C04	ss524903752	7		Rs98	0.11					protein GPR107-like	C:integral component of membrane			
GQ04101_B06	ss524905087	8	AR	Rl93	0.16					F-box protein At4g35930		Up		
GQ03006_D16	ss538942046	1		CS	0.12					subtilisin-like protease SBT1 7	F:peptidase activity		WD/ RW	s
GQ03916_G13	ss538952280	4		CS	0.16					uracil phosphoribosyltransferase isoform X1				
GQ02812_L03	ss538940375	4		CS	0.17					protein DETOXIFICATION 29-like			WD	ns

GQ03711_F07	ss524904949	7		CS	0.26					farnesylcysteine lyase	P:prenylated protein catabolic process; P:prenylcysteine catabolic process; F:prenylcysteine oxidase activity			
GQ02801_A19	ss511222916	7		CS	0.12					probable glucan 1,3-alpha-glucosidase		up		
GQ0035_E21	ss538945615	7		CS	0.12					GDSL esterase/lipase At1g71691	F:hydrolase activity, acting on ester bonds			
GQ03218_J10	ss538949337	9		CS	0.14					BUD13 homolog	C:nucleus	up		
WS00725_O13	ss538953241	9		CS	0.11							up		
GQ02828_D02	ss538947479	10		CS	0.10					pentatricopeptide repeat-containing protein At1g07590, mitochondrial isoform X1				
GQ03808_P10	ss538944519	11		CS	0.20					probable polygalacturonase	P:carbohydrate metabolic process; P:cell wall organization; F:polygalacturonase activity; C:extracellular region	up	RW	ns
GQ03221_I23	ss538949416	12		CS	0.11									

Abbreviations: † CS: climate sensitivity, Rc: recovery, Rl: resilience, Rr: relative resilience, Rs: resistance
‡ phenotypic variance explained
§ P: biological process; C: cellular component, F: molecular function
¶ WD: wood density, MOE: modulus of elasticity, RW: ring width, MFA: microfibril angle
# s: synonymous, ns: non-synonymous SNP

Chapter III

Xylem anatomical variability in white spruce at treeline is largely driven by spatial clustering

frontiers in Plant Science

ORIGINAL RESEARCH
published: 21 October 2020
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Xylem Anatomical Variability in White Spruce at Treeline Is Largely Driven by Spatial Clustering

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The ecological function of boreal forests is challenged by drastically changing climate conditions. Although an increasing number of studies are investigating how climate change is influencing growth and distribution of boreal tree species, there is a lack of studies examining the potential of these species to genetically adapt or phenotypically adjust. Here, we sampled clonally and non-clonally growing white spruce trees (*Picea glauca* [Moench] Voss) to investigate spatial and genetic effects on tree ring width and on six xylem anatomical traits representing growth, water transport, mechanical support, and wood density. We compared different methods for estimating broad sense heritability (H^2) of each trait and we evaluated the effects of spatial grouping and genetic grouping on the xylem anatomical traits with linear models. We found that the three different methods used to estimate H^2 were quite robust, showing overall consistent patterns, while our analyses were unsuccessful at fully separating genetic from spatial effects. By evaluating the effect size, we found a significant effect of genetic grouping in latewood density and earlywood hydraulic diameter. However, evaluating model performances showed that spatial grouping was a better predictor than genetic grouping for variance in earlywood density, earlywood hydraulic diameter and growth. For cell wall thickness neither spatial nor genetic grouping was significant. Our findings imply that (1) the variance in the investigated xylem anatomical traits and growth is mainly influenced by spatial clustering (most probably caused by microhabitat conditions), which (2) makes it rather difficult to estimate the heritability of these traits in naturally grown trees *in situ*. Yet, (3) latewood density and earlywood hydraulic diameter qualified for further analysis on the genetic background of xylem traits and (4) cell wall thickness seems a useful trait to investigate large-scale climatic effects, decoupled from microclimatic, edaphic and genetic influences.

Keywords: boreal forest, broad-sense heritability, clonal trees, spatial clustering, treeline, white spruce, xylem anatomy

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INTRODUCTION

Boreal forests are ecologically and commercially valuable ecosystems that make up almost a third of the global forest cover (Apps et al., 1993; Hansen et al., 2003). They act as a sink for global atmospheric carbon dioxide (Arneth et al., 2010; Tagesson et al., 2020) but are heavily influenced by human-induced and natural cover loss (Hansen et al., 2010). According to climate projections, boreal forests will face exceptional changes in climatic conditions within the 21st century (Soja et al., 2007; IPCC, 2013; Charney et al., 2016), threatening ecosystem functions (Gauthier et al., 2015). To preserve their functionality, it is of outmost importance to understand how these ecosystems work and how boreal forest tree species adjust to environmental changes.

A great majority of tree species are able to cope with a range of environmental conditions (Reich et al., 2016). To this end, they adjust phenotypically, adapt genetically and/or disperse into new habitats to track their niche of suitable conditions (Lenoir et al., 2008; Yeaman et al., 2016). In sessile and long-lived organisms like trees, the ability to adjust to changing conditions is essential (Schlichting, 1986). However, in the long term it is also necessary for a population to adapt genetically. Adaptation can occur when phenotypes of traits improving fitness are heritable. A common way to quantify heritability of a trait is to estimate the amount of phenotypic variance of the trait that occurs due to genetic variance. Heritability can be estimated in a "narrow sense" (h^2, based on additive genetic variance) and a "broad sense" (H^2, based on total genetic variance; Visscher et al., 2008; Wray and Visscher, 2008). Estimating heritability of traits in trees can thus help to inform species distribution models to create more precise predictions of future development of forests, and can also guide projects aiming at maintaining the functionality of boreal forests (e.g., assisted migration; Gauthier et al., 2015; Correia et al., 2018).

White spruce (*Picea glauca* [Moench] Voss) is one of the most common tree species of the North American boreal forests (Little and Viereck, 1971). Due to its ability to grow at the latitudinal and altitudinal treeline, it is widely used as a model organism to study plasticity and adaptation patterns (Lloyd and Fastie, 2002; Wilmking and Juday, 2005; Sherriff et al., 2017). Most studies on this species focus on general tree growth, often exclusively investigating annual (radial) growth increments. While tree rings provide valuable information on the integrated response to environmental conditions during the vegetation period, investigating the xylem anatomical structure may reveal crucial information on the tree functionality (Hacke et al., 2015; Amoroso et al., 2017).

Studies investigating xylem anatomical traits that are directly related to tree functioning such as tracheid lumen diameter or cell wall thickness (Wiedenhoeft, 2012) have become increasingly available for boreal tree species (Lange et al., 2019; Mvolo et al., 2019). Yet, little is known about the genetic background of xylem anatomical trait variation in white spruce (Lenz et al., 2010; Hassegawa et al., 2019). White spruce is able to vegetatively reproduce by layering (Stone and McKittrick, 1976; Würth et al., 2018) and thus it is able to grow genetically identical individuals (i.e., clones). Clones offer the unique opportunity to study genetic effects (i.e., broad sense heritability) on growth, hydraulic and structural traits in natural populations (Nyquist and Baker, 1991).

In this study, we identified and sampled naturally growing clones of white spruce at the latitudinal treeline in Alaska. We aimed at estimating broad sense heritability (H^2) of growth and xylem anatomical traits of three trait groups (water transport, mechanical support, and wood density) by comparing three different methods: (1) using raw data, (2) using data predicted with a linear mixed effects model and (3) using estimated variance extracted from a linear mixed effects model. Since vegetative reproduction in trees leads to an unavoidable spatial clustering of individuals, we additionally focused on the spatial patterns. We evaluated the results of our H^2 estimations by using models to identify whether spatial grouping is the main driver for similarities in growth and xylem anatomical traits, or if genetics also influence these patterns. The advantage of this novel approach is that it combines spatial analyses with genetic analyses at an anatomical level. This informs about which xylem anatomical traits qualify for studying genetic patterns potentially leading to genetic adaptation and which qualify better for studying spatial patterns driven by the influence of microenvironment or by climatic effects.

MATERIALS AND METHODS

Study Species and Site

White spruce grows under a variety of climatic conditions and its distributional range covers most of the boreal area in Canada and Alaska, and parts of the northernmost United States mainland (Little and Viereck, 1971). It is often the dominant tree species at the elevational and latitudinal treeline in the north-western parts of its distributional range (Abrahamson, 2015), and is of large economic importance (Attree et al., 1991).

The study site is located at the latitudinal treeline on a south-facing slope of Nutirwik Creek valley, in the central Brooks Range of Alaska (67°56′N, 149°44′W). The study site is a nearly monospecific white spruce stand of approximately two hectares, ranging in elevation from 860 to 940 m a.s.l. The mean annual temperature is around −7.9°C with a mean temperature of around −23.8°C in January (coldest month) and 11.1°C in July (warmest month). The annual precipitation is around 289 mm, 96 mm of which fall in July and August. The information about precipitation and temperature is taken from Lange et al. (2019) and based on modeled data averaged across the 1901–2013 data period provided by the Natural Resources Canada, Canadian Forest Service (NRCAN[1]; McKenney et al., 2011).

Sampling Design and Data Acquisition

In 2018, we sampled all the spatially clustered groups of white spruce that were scattered throughout the study area ranging from the forest line to the treeline and appearing to be the result of vegetative reproduction (see Wilmking et al., 2017;

[1]http://cfs.nrcan.gc.ca/projects/3/1

Würth et al., 2018) (**Figure 1**). We sampled all trees present within each spatially clustered group (**Supplementary Figure S1** and **Supplementary Table S1**). We took one bark-to-bark increment core through the pith from 47 trees (thus resulting in 94 radii) that grew in eleven spatially clustered groups with a 4.3 mm increment borer (Haglöf, Sweden) for growth and xylem anatomical measurements. Additionally, we collected the most recently grown needles from North-facing branches of each tree for genetic analyses.

All cores were air dried and glued onto wooden sample holders. The surface was polished with progressively finer sandpaper (up to 800 grit) and scanned with a flatbed scanner (Epson Perfection V700 Photo; Seiko Epson Corporation, Japan) with 3200 dpi. Ring widths (TRW) were subsequently measured using CooRecorder (version 9.3.1; Cybis Elektronik and Data AB, Sweden) and all radii were cross-dated using CDendro (version 9.3.1; Cybis Elektronik and Data AB, Sweden). We used the cross-dated tree ring chronologies solely to correctly date xylem anatomical measurements. For the analysis, we used TRW measurements obtained from the anatomical sections together with the other anatomical traits.

For the xylem anatomical measurements we cut 12 μm-thick cross-sections from one radius of each tree using a rotary microtome (Leica RM 2245; Leica Camera AG, Germany). The cross-sections were stained with 1:1 safranin and astra blue solution, rinsed with ethanol solutions of increasing concentration (50%, 70%, 96%), mounted on microscope slides with Euparal and dried at 60°C for 48 h. We scanned the slides with a slide scanner (Zeiss Axio Scan.Z1; Carl Zeiss AG, Germany) at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. We used the scans to quantify TRW and several xylem anatomical traits (**Supplementary Table S2**) with the image analysis tool ROXAS v3.0.326 (von Arx and Carrer, 2014; Prendin et al., 2017). Due to the large amount of anatomical data to process in long cores, we selected the growth years 2007–2017 for the measurements of xylem anatomical traits. This allowed us to maximize the amount of data while still maintaining a number of samples feasible to process with high quality. Measurements on lumen diameter and cell wall thickness were used to distinguish between early- and latewood using Mork's index (Denne, 1989). We calculated the total mean, the mean for earlywood and the mean for latewood per year of each trait using R v3.6.1 (R Core Team, 2019).

Earlywood ring width (EWW) and latewood ring width (LWW) were detrended for each tree to minimize the influence of low frequency growth trends without losing too much information on the variance among the trees. For this, we compared two linear models with raw ring width (EWW and LWW separately) as a response variable and year as an explanatory variable. One model was fitted with a linear term of the explanatory variable while the other model was fitted with a linear and a quadratic term of the explanatory variable. We chose the better performing model based on the corrected Akaike Information Criterion (AICc; Hurvich and Tsai, 1991) and calculated the detrended EWW and LWW by adding the model residuals to the mean annual growth of the investigated time period. Mean hydraulic diameter (DH) was calculated for earlywood (DH.ew) and latewood (DH.lw) of each ring based on lumen area (LA) according to Kolb and Sperry (1999). We estimated wood density (DEN) as the proportion of the estimated cell wall area (CWA) to the total cell area [i.e., the sum of CWA and lumen area (LA); Eq. 1] according to Björklund et al. (2017).

$$DEN = \frac{CWA}{CWA + LA} \quad (1)$$

DNA Isolation and SSR Genotyping

To identify clones, we genotyped all sampled trees. We ground 20 mg of silica-gel dried needle tissue in a Retsch ball mill MM301 (Retsch, Germany). For DNA extraction we used the Mag-Bind plant DNA DS Kit (Omega, United States) in combination with the KingFisher^{TM} Flex 96-well plate robot system (ThermoFisher Scientific, United States) following the manufacturer protocols. For genotyping we combined 11 microsatellite loci developed by Hodgetts et al. (2001) and Rajora et al. (2001) in two multiplex reactions according to Eusemann et al. (2014) and Würth et al. (2018). We performed PCR on Eppendorf Mastercyclers (Eppendorf, Germany) using the Qiagen Multiplex PCR Plus Kit (Qiagen, Netherlands) and a modified protocol with a total volume of 10 μl and PCR conditions as described in Würth et al. (2018) with initially 5 min/95°C, 30 cycles 30 s/95°C, 90 s/58°C, 90 s/72°C, final extension 10 min/68°C. For fragment analysis we used a 3130xl Genetic Analyzer (Life Technologies, United States) using 1 μl undiluted PCR product, 0.15 μl 500 GeneScan LIZ® size standard (Life Technologies) and 12 μl HiDi Formamide (Life Technologies).

We performed fragment size determination and binning with the GeneMapper® Software 5.0 (Life Technologies). To account for genotyping errors, we used the algorithm programmed by Schnittler and Eusemann (2010). Since genotyping errors are much more likely to split than to merge clones, we set the threshold for members of a clone to maximum two deviating loci. For the analysis, we considered only trees with a maximum of two null allele-containing loci. These settings are consistent with Würth et al. (2018).

Of the 47 sampled trees in 11 spatially clustered groups, we found that 35 trees belonged to nine clonal groups, while 12 trees did not belong to any clonal group. Five spatially clustered groups consisted of clonal trees only. In four groups, clones grew spatially clustered with non-clonal individuals. Two groups consisted of non-clonal individuals only (**Figure 1**).

Statistical Analysis

To reduce the number of study parameters, we explored the relationship between the measured anatomical traits with a principal component analysis (PCA) using the R function prcomp (Grey et al., 1981) (**Supplementary Figure S2**). We classified the traits in four groups according to the PCA: growth, water transport, mechanical support, and wood density. We chose one representative trait of each group for which we carried out the analyses for earlywood and latewood separately (**Table 1**).

The first principle component (PC1) of the PCA explained 41.3% of variance, the second principle component (PC2) explained 28.7% and the third principle

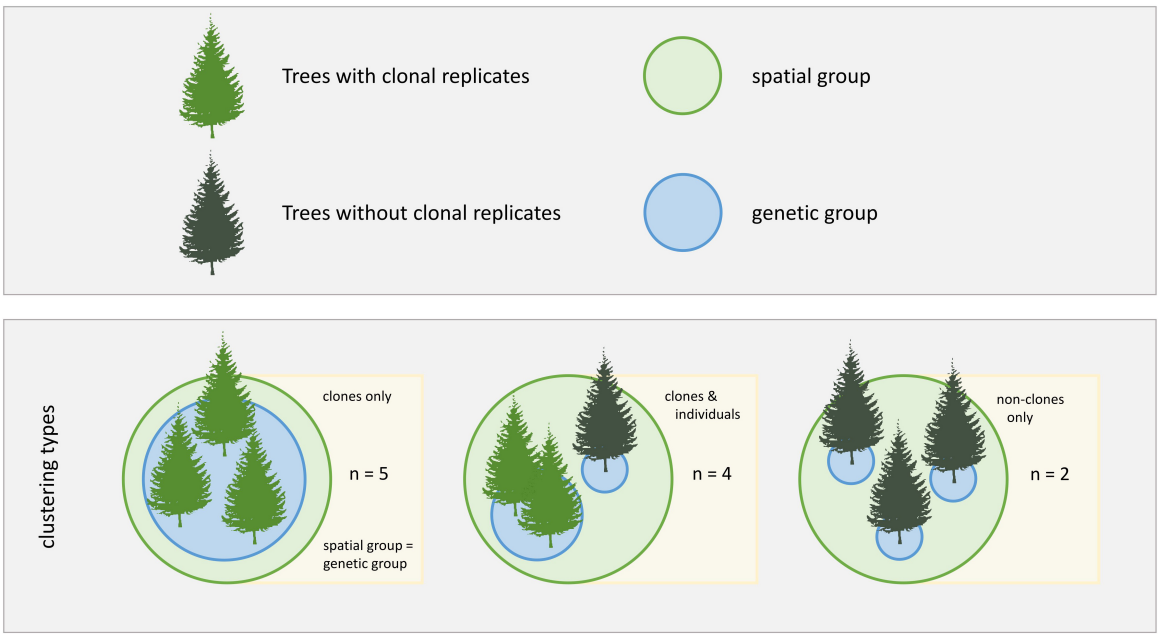

FIGURE 1 | Study design. Different clonal contexts and grouping types are indicated by differently colored trees and circles, respectively. Three clustering types were analyzed in this study, with *n* being the number of study cases for each clustering type.

TABLE 1 | Explanation of growth and xylem anatomical traits selected for analysis and their ecological function.

Group	Selected traits	Unit	Explanation
Growth	EWW, LWW	µm	Earlywood width, latewood width
Mechanical support	CWT.ew, CWT.lw	µm	Mean overall cell wall thickness (earlywood, latewood)
Wood density	DEN.ew, DEN.lw	Proportion	Mean relative anatomical wood density (earlywood, latewood)
Water transport	DH.ew, DH.lw	µm	Mean hydraulic diameter (earlywood, latewood)

component (PC3) explained an additional 14.7% of variance (**Supplementary Figure S2**). The PCA showed a strong relationship among the traits within each group. Traits associated with mechanical support were mostly explained by PC1 while growth and water transport related traits were mainly explained by PC2. Wood density traits were explained by both PC1 and PC2 to a similar extent. Some latewood traits (e.g., latewood density (DEN.lw) and LWW) were mainly explained by PC3. In the following analyses, we used EWW and LWW as proxies for growth, DH.ew and DH.lw for water transport, CWT.ew and CWT.lw for mechanical support and earlywood density (DEN.ew) and DEN.lw for wood density (**Table 1**).

For the H^2-estimations we only used data from trees that were growing in groups of genetically identical individuals (i.e., a subset of 35 trees in nine groups). In order to explore the potential of using estimated data compared to raw data, we used three different methods to estimate H^2. First (1), we estimated H^2 using raw data of all selected xylem anatomical traits (H^2_{raw}; **Table 1**; Eq. 2; For additional information see Eq. S1 – Eq. S5; Klug et al., 2006). Second (2), we fitted a linear mixed effects model for each trait using the nlme package (Pinheiro et al., 2020), where the investigated trait was included as the response variable, clonal group and year were included as fixed effects, cumulative stem diameter at breast height (cDBH) was included as a covariate and tree ID as a random effect. To correct for autocorrelation in time between multiple measurements in each individual, a first-order autoregressive correlation structure was included in the model using the constructor corAR1 of the nlme R package. The Constant Variance Function (varIdent) of the same package was used to account for the non-homoscedastic distribution of residuals between the clonal groups. Since tree height has a strong influence on xylem anatomical traits (Carrer et al., 2015), we used the model to predict trait values on a new set of data where the cDBH (as a proxy for height) was standardized to represent the average growth of the sampled trees in the investigated time period (i.e., an increase of DBH from 11 to 12 cm during the 10-year period resulted in an average annual growth of 0.1 cm). Heritability was then estimated on the predicted values (H^2_{pred}; Eq. 2; Eq. S1 – Eq. S5). Finally (3), we fitted a linear mixed effects model, where the investigated trait was the response variable, year was included as a fixed effect, cDBH as a covariate and clonal group as a random effect. We used the VarCorr function of the nlme R package to extract estimated genetic variance (σ^2_G) and the residual variance (σ^2_R) from the model. The modeled variance was then used to estimate H^2 according to Eq. 3 (H^2_{mod}). All models were fitted using restricted

maximum likelihood estimation (REML).

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2} \quad (2)$$

where σ_G^2 is the genetic variance and σ_P^2 the total phenotypic variance.

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_R^2} \quad (3)$$

where σ_R^2 is the residual variance extracted from the models.

The coefficient of variation (CV) was calculated as an error measurement for H^2 estimations (Eq. 4; Everitt, 1999).

$$CV = \frac{\sqrt{\sigma_G^2}}{\bar{x}} \quad (4)$$

where $\sigma_G^2$is the genetic variance and $\bar{x}$ the trait total mean.

To evaluate whether the calculated H^2 values truly represent genetic effects or rather a spatial pattern caused by the spatial grouping of the clonal trees we used the full dataset of 47 sampled trees in 11 spatially clustered groups, including clonal and non-clonal individuals. We created two categorical groups, genetic group and spatial group, and each tree was assigned a level in each. For the genetic group, each tree was assigned either the clonal group ID or, in non-clonal trees, the individual tree ID; for the spatial group, all trees growing spatially clustered, with a maximum distance of 3 m, were assigned the same code, regardless of the genetic background (**Figure 1**). This method did not allow us to isolate genetic grouping from the spatial grouping, but allowed us to test whether spatial clustering had a large effect on the variability of our study traits. To avoid computational errors related to this issue we did not compare spatial and genetic grouping in one model, but we compared three different models for each trait: (i) a null model, (ii) a genetic model and (iii) a spatial model. The (i) null model was fitted using the selected trait as the response variable, year as a fixed effect, cDBH as a covariate and tree ID as a random effect. The (ii) genetic model was fitted with the genetic group and year as fixed effects, cDBH as a covariate and tree ID as a random effect. The (iii) spatial model was fitted with the spatial group and year as fixed effects, cDBH as a covariate and tree ID as a random effect. The corAR1 constructor was used in all three models to account for autocorrelation in time within tree individuals. The varIdent function was used in the spatial and genetic models to account for non-homoscedastic distribution of the residuals between groups. All models were fitted using REML.

For the comparison of the model performance we calculated the corrected Akaike Information Criterion (AICc) for each model and selected the model that performed best when the AICc was lowest with more than four units difference. Since AICc is only slightly penalizing small differences in the number of parameters, we considered the null model best in case of equal values, following the principle of parsimony (Burnham and Anderson, 2002, 2004).

To evaluate the effect of spatial and genetic grouping and to find potential significant effects of spatial or genetic clustering that are independent of model performance we conducted an analysis of variance (ANOVA; Chambers and Hastie, 1992). We used R v 3.6.1 (R Core Team, 2019) for all statistical analyses.

RESULTS

H^2 Estimates of Growth and Xylem Anatomical Traits

The three different methods used to estimate H^2 showed overall similar results (**Figure 2**). In general, traits associated with growth (EWW and LWW) showed the highest H^2 values and traits associated with mechanical support (CWT.ew and CWT.lw) the lowest. Traits associated with wood density and water transport (DEN.ew, DEN.lw, DH.ew, and DH.lw) showed low to intermediate values (**Figure 2**).

In traits related to growth, H^2_{pred} and H^2_{mod} showed a pattern of similar values, while H^2_{raw} was slightly higher for EWW and lower for LWW. For traits related to mechanical support, wood density, and water transport, H^2_{pred} showed the highest values, while H^2_{raw} and H^2_{mod} showed similar values both for earlywood and latewood.

Comparing earlywood and latewood, H^2 values were similar for growth and mechanical support across methods, while earlywood H^2 values were generally slightly higher for wood density and notably higher for water transport.

The CV values for H^2_{raw} in both EWW and LWW were notably high (0.507 and 0.435), but for all other estimates and traits they were relatively low (0.001–0.104, mean = 0.052; **Figure 2**).

Comparing Genetic and Spatial Grouping

In general, spatial models outperformed all other models. In one case the null model performed better and in two cases genetic and spatial models performed similarly but better than the null model. In no case did the genetic model outperform the other model types.

For both growth traits (EWW, LWW) and all earlywood traits (CWT.ew, DEN.ew, and DH.ew) the spatial model performed better than the other models. For latewood density and latewood CWT there was no difference between the genetic and the spatial model, but in both cases the grouped models performed better than the null model. For latewood DH the null model was considered to show the best performance, as it had an AICc equal to the spatial model and lower than the genetic model (**Figure 3**).

The analysis of the spatial and genetic models using ANOVA showed that the spatial grouping was significant (p-value < 0.05) for EWW and LWW, early- and latewood density and earlywood DH. Genetic grouping was significant for EWW and LWW, latewood density and earlywood DH. No significant grouping effect was found in early- and latewood CWT and latewood DH.

DISCUSSION

The rapidly changing climate conditions threaten the functioning of boreal forests. White spruce is one of the most important

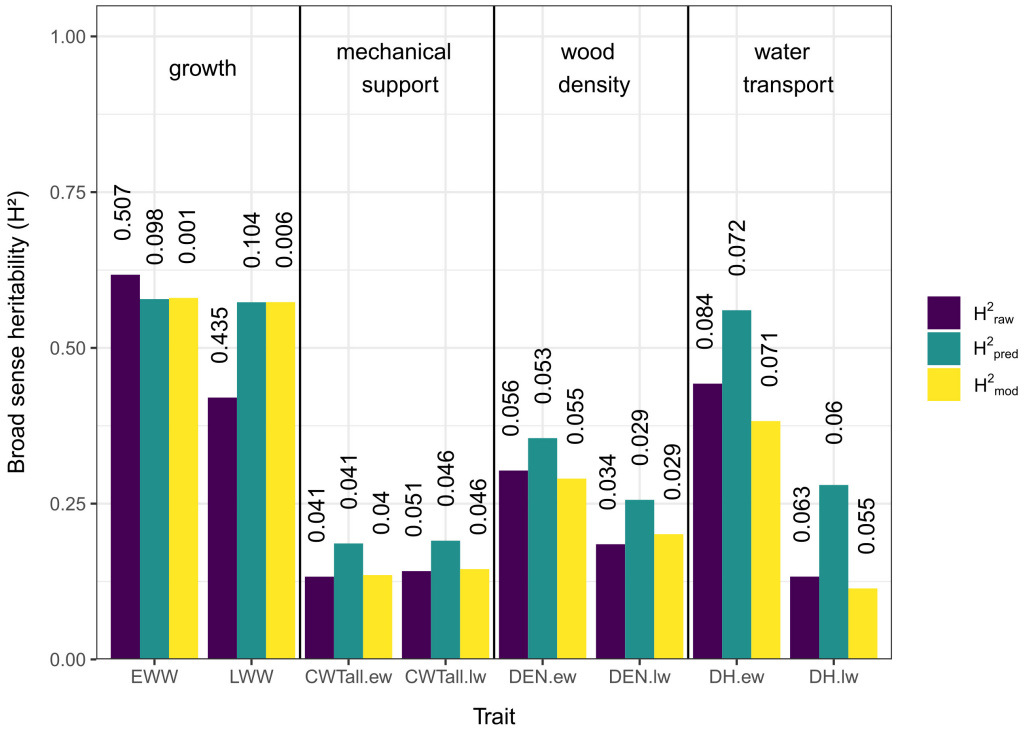

FIGURE 2 | Broad sense heritability (H^2) estimates for growth and xylem anatomical traits. Different colors indicate the method used (purple = heritability based on raw measurements, green = heritability based on predicted values, yellow = heritability based on mixed-effect model), see "Materials and Methods" section for details. Vertical numbers above the columns are the coefficients of variation (CV).

and abundant boreal forest tree species of North America. In order to learn more about its potential to adapt to changing environments, we explored its genetic background of xylem anatomical trait variation. The goal of our study was to investigate the broad sense heritability (H^2) of growth and xylem anatomical traits. We compared three methods to estimate H^2, which overall showed consistent patterns in the resulting values. However, further analyses revealed a large influence of spatial clustering on xylem anatomy, which seemed to overlay any genetic patterns. Nonetheless, we found some evidence for a genetic influence on early- and latewood growth (ring widths), latewood density and earlywood hydraulic diameter.

H^2 Estimates of Growth and Xylem Anatomical Traits

The three methods of estimating H^2 showed overall consistent results, with the only inconsistency found in the estimates based on raw data (H^2_{raw}) of early- and latewood ring width. This inconsistency was likely caused by a high dispersion in the raw data, shown by high CVs (**Figure 2**). The H^2 estimates for EWW and LWW based on the raw data are therefore likely not accurate (Everitt, 1999). All other H^2 estimates showed much lower CVs and more stable patterns. The H^2 estimates based on predicted data were higher in most cases. This pattern was likely introduced by the method of using predictions from a linear mixed effects model. By predicting new values under the assumption of equal diameters in all trees we avoided size-related effects, which are known to largely influence xylem anatomical traits (Carrer et al., 2015). The higher values of H^2_{pred} could suggest an underestimation of H^2_{raw} and H^2_{mod} as a result of this size effect.

In general, most of our calculated heritability estimates are in line with other studies focusing on narrow sense heritability (h^2) of xylem anatomical traits in white spruce. Though narrow sense heritability is based on additive genetic variance instead of total genetic variance, h^2 is comparable to H^2 because over 50% of the total genetic variance is usually additive (Hill et al., 2008; Wang et al., 2013). Similar to our study, Lenz et al. (2010) reported a high h^2 in earlywood radial cell diameter (a trait that strongly correlates with mean hydraulic diameter; Kolb and Sperry, 1999) and a lower h^2 in the latewood radial cell diameter in white spruce on a provenance trial in East Canada. They also found a low h^2 in latewood density and latewood cell wall thickness. In contrast to our estimates, Lenz et al. (2010, 2011) reported a high h^2 in the earlywood cell wall thickness and earlywood density. These opposite results could be explained by their populations being less climatically constrained than our populations. The studies of Lenz et al. (2010, 2011) were conducted on sites in Eastern Canada (Quebec), where climatic conditions are milder and wetter than at our treeline site in northern Alaska. It is possible that the lower heritability estimates in our study are a result of a larger climate control on earlywood parameters at the treeline.

Regarding growth-related traits like ring width, estimates of heritability in white spruce are quite scarce in other studies and

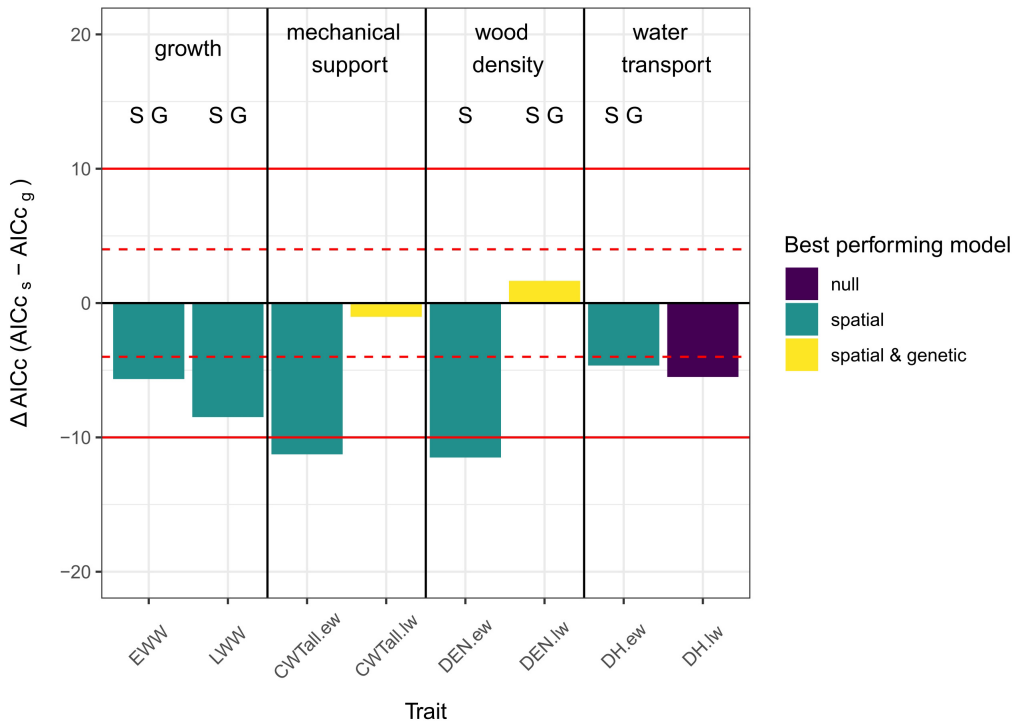

FIGURE 3 | Column plot of the differences in AICc values between spatial and genetic models. A negative ΔAICc means that the AICc of the spatial model is lower than the AICc of the genetic model and vice versa. The color of the columns indicates, which model showed the best performance [purple = null model performed either best, equal to one or equal to both of the other two models; green = spatial model performed best; yellow = grouped models (i.e., genetic and spatial models did not differ significantly between each other but both performed better than the null-model)]. The letters over the columns indicate which model had a significant group effect (S, spatial grouping sign.; G, genetic grouping sign.; significance threshold: $p < 0.05$ - based on ANOVA). Red lines show the thresholds for evaluating differences between the models: $|\Delta AICc| < 4$: no substantial differences (dashed lines); $4 < |\Delta AICc| < 10$: considerable differences (solid lines); $|\Delta AICc| > 10$ - essential differences (Burnham and Anderson, 2002).

considerably differ from our results. While Ying and Morgenstern (1979) and Lenz et al. (2010) reported low, or insignificant levels of h^2 in DBH (0.04–0.10) and ring width (reported as insignificant), respectively, Corriveau et al. (1991) and Merrill and Mohn (1985) estimated intermediate values for ring width (0.32) and DBH (0.35), respectively. This inconsistency in results might occur due to the complex nature of secondary growth itself (Rathgeber et al., 2016) and the strong influence of climatic parameters (Hughes et al., 2011).

In general, we cannot accurately say how representative our H^2 estimates are. Per definition, heritability can only be calculated for a specific population in a specific environment (Stoltenberg, 1997). However, the analysis of spatial grouping showed that we have strong spatial effects in our data, which implies that the assumption of common environmental conditions for heritability estimations was violated and thus makes our estimates uncertain.

Spatial Grouping Has the Strongest Effect on Trait Variability

Comparing models with genetic and spatial grouping showed that in all earlywood traits the spatial model performed better than the genetic model. In the latewood traits, the spatial model was only better for latewood width, but the genetic model did not outperform the spatial model for any trait.

Earlywood is formed at the beginning of the vegetation period. During this time, trees ideally allocate most of the available resources to grow in circumference and height, without risking losing structural integrity or suffering from drought-induced cavitation and other potential effects caused by resource limitation (Willson and Jackson, 2006; Rathgeber et al., 2016; Cartenì et al., 2018). Thus, a high plasticity in the earlywood could promote efficient growth. This high plasticity is evidenced in our results in the form of spatially structured patterns in earlywood anatomical traits. These spatial patterns are probably caused by small-scaled differences in resource availability (i.e., microclimatic and edaphic differences, which are potentially caused by topographic characteristics of the area) between clonal groups, leading to trait variability independent of the genetic background.

At the end of the vegetation period, when latewood is formed, height growth also declines. It becomes more important for the tree to use available resources to produce cells with thicker cell walls, which are responsible for mechanical support for the tree body (Cartenì et al., 2018), while building new tissue for water transport only plays a minor role (Domec, 2002; Tyree and Zimmermann, 2002). Accordingly, latewood anatomy features

were less variable in our study. Small-scale differences in resource availability are more likely to cause differences in wood growth and wood density than in hydraulic diameter at the end of the growing season. Consequently, our results showed that for latewood ring width the spatial model was performing best. For the latewood density, genetics might also have an influence, since both models were performing equally well.

Investigating the significance of spatial and genetic grouping independent from the performance of the models revealed a significant effect of genetic grouping on early- and latewood width, latewood density and earlywood DH. Despite the high heritability estimates for early- and latewood width, the effect of genetic grouping was rather unexpected. As mentioned before, secondary growth is strongly influenced by climatic parameters, and several studies suggested that small-scale environmental conditions rather than genetics affect growth (King et al., 2013; Wilmking et al., 2017; Avanzi et al., 2018). Since our study was performed *in situ* we were not able to truly decouple genetic from spatial effects. Therefore, a combination of the strong impact of micro-environmental conditions (i.e., spatial grouping; Avanzi et al., 2018; Montpellier et al., 2018), combined with individual growth characteristics (Carrer, 2011) could have led to the false assumption of a significant genetic effect. We cannot exclude that this also affected the traits latewood density and earlywood DH. However, previous studies already indicated that variability in lumen size and wood density were linked to adaptation to local conditions (Carlquist, 1980; Aroca, 2012; Hacke et al., 2015; Klisz et al., 2019). Thus, it is likely that a genetic effect on latewood density and earlywood DH actually exists.

Both early- and latewood CWT were not significantly influenced by spatial or genetic grouping, indicating that in our study species at our site CWT is not strongly determined by neither small-scale environmental nor genetic effects. We did not test correlations with climatic parameters since our time series (2007–2017) are very short. However, other studies found significant correlations between CWT and climatic parameters in white spruce (Lange et al., 2019) and also in black spruce (Puchi et al., 2020). Placing our results in the context of these studies suggests that CWT qualifies as a proxy for climatic conditions at larger scales (i.e., range-wide differences and past climatic variability), decoupled from strong small-scale environmental and genetic influences. This view is also supported by a study with Scots pine showing that the thickness of the radial cell walls in the latewood registers a stronger temperature signal than any other tree-ring proxy including the commonly used maximum latewood density (Björklund et al., 2020).

CONCLUSION

The vegetative reproduction of white spruce at the latitudinal treeline offered the opportunity to gather data on genetically identical trees *in situ*. The comparison of three methods to estimate broad sense heritability (H^2) resulted in mostly consistent patterns. This suggests that in general the estimates are quite robust, independent from the method used for their calculation. However, due to spatial clustering of the trees we had to evaluate our heritability measures by testing the strength of the grouping effect. The analyses showed that spatial clustering had a strong influence on the xylem anatomy, especially in the earlywood. We assume that this strong spatial effect is related to differences in micro-environmental conditions, which implies that it is rather difficult to estimate the magnitude of genetic effects in a naturally grown population.

Nonetheless, we found some evidence for genetic effects in early- and latewood ring width, latewood density and earlywood hydraulic diameter. Based on previous studies we assume that results on early- and latewood width might rather be reflecting environmental conditions and individual growth patterns than actual genetics. Latewood density and earlywood hydraulic diameter, however, show a plausible significant genetic component, suggesting they are suitable traits for assessing potential local adaptation. Cell wall thickness, on the other hand, seems neither to be influenced by small-scale spatial (i.e., differences that occur within one study site) nor genetic patterns, potentially qualifying as a proxy for climatic conditions on a larger scale (e.g., range-wide differences).

Exploring the interacting effects of phenotypic plasticity and genetic adaptation in xylem anatomical traits related to wood density and tree hydraulics will lead to a more comprehensive understanding of the adaptation potential of tree species to global change in general, and of white spruce in particular. Yet, it is challenging to balance the reliability of experimental set-ups, which may be far from real-life conditions, with real-world studies, which may have high error potential. We believe that real-world studies dealing with clonal trees are valuable, but highlight the necessity to carefully evaluate any potential spatial effects, as they can drastically influence the growth of trees and obscure any potential genetic signal.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

TP, MW, and MZ designed the study and conducted field work and sampling. TP prepared the samples and performed xylem anatomical measurements with help from GA. MZ performed the genetic analyses. TP performed all statistical analysis with help from AA-R. TP wrote the manuscript with contributions from all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.581378/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

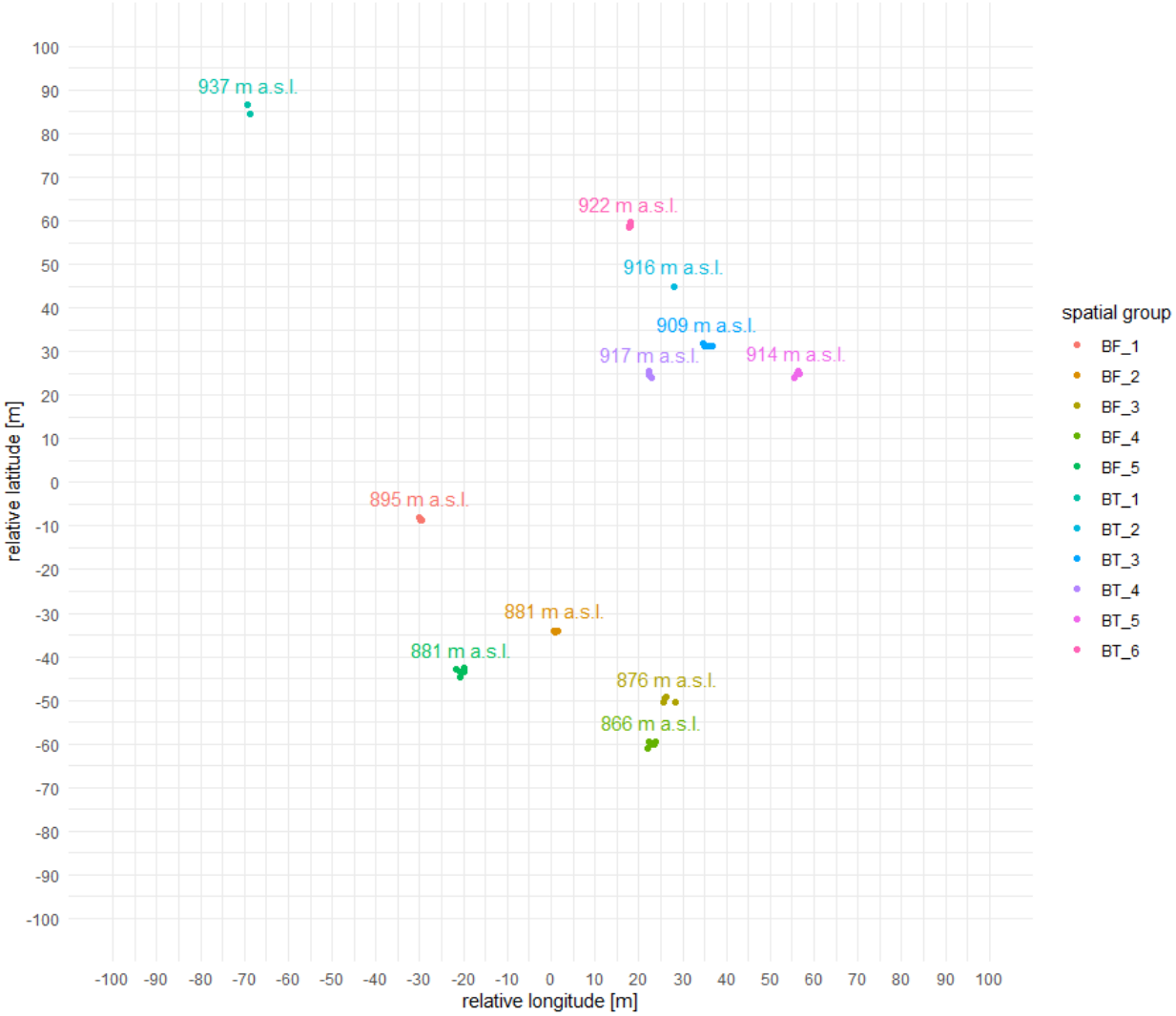

Figure S1: Map of sampled trees. Dots represent the sampled trees on a relative coordinate system in metric units. Distances were measured using a Vertex Laser Geo (Haglöf, Sweden). Colors indicate the spatial group. For some groups not all trees can be represented, since the distance between the trees was smaller than the measuring error (ultra-sound accuracy: 1%). Numbers above the groups show the group mean elevation in meters above sea level, derived from GPS coordinates measured with the Vertex Laser Geo (GPS accuracy: 2.5m in open terrain).

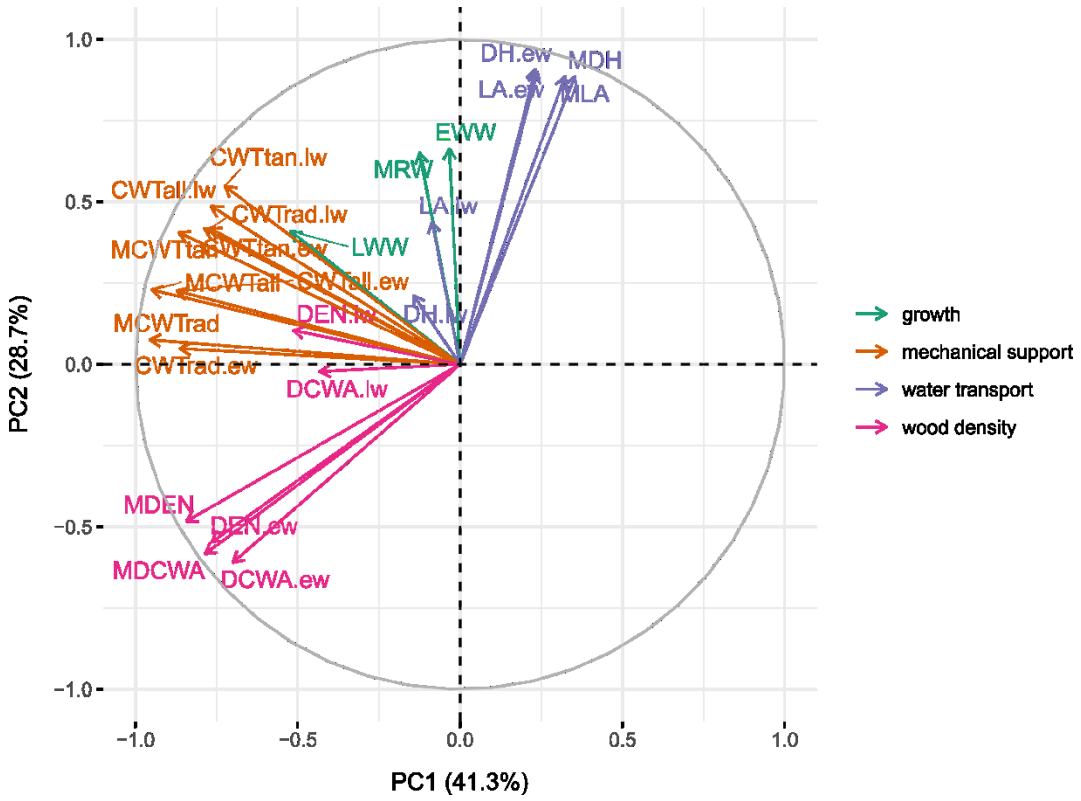

Figure S2: Principal component analysis (PCA) of all measured growth and xylem anatomical traits. Colors indicate different trait groups (green = growth, orange = mechanical support, purple = water transport, pink = wood density).

Table S1: Metadata on sampled trees

ID	CORING DIRECTION	CORING HEIGHT (CM)	DBH (CM)	HEIGHT (M)	CLONE_GROUP	SPATIAL_GROUP	RELATIVE LONGITUDE (M)	RELATIVE LATITUDE (M)	ALTITUDE (M A.S.L.)
101B	N_S	130	18	11.2	BT_F	BT_4	22.3	25.7	917
102B	E_W	140	13	8.40	BT_F	BT_4	22.4	24.6	917
103.1B	NE_SW	110	11	7.70	BT_F	BT_4	22.9	23.9	917
103.2B	N_S	115	9	4.55	BT_F	BT_4	22.9	23.9	917
112B	E_W	80	6.5	3.60	BT_B	BT_5	55.3	24.1	914
114B	N_S	110	14.5	6.20	BT_B	BT_5	56.4	25.6	914
115B	E_W	100	11.5	5.58	BT_B	BT_5	56.1	24.8	914
116B	N_S	120	8	3.70	BT_B	BT_5	56.6	24.9	914
126B	NE_SW	110	8	4.12	BT_D	BT_6	17.9	58.8	922
128B	NW_SE	110	9	4.55	BT_D	BT_6	17.6	58.5	922
131B	N_S	145	13	7.50	BT_D	BT_6	17.9	59.1	922
47B	N_S	85	12	7.50	BT_D	BT_6	17.9	59.6	922
150B	N_S	100	8	5.00	BF_C	BF_1	-29.6	-8.7	895
151B	N_S	95	12	5.50	BF_C	BF_1	-29.8	-8.7	895
152B	E_W	75	10	6.80	152b	BF_1	-30.3	-8.0	895
216B	N_S	150	16	7.33	216b	BF_2	0.6	-34.1	881
217B	S_N	120	14	6.57	217b	BF_2	1.0	-34.4	881
218B	W_E	135	16.5	7.52	218b	BF_2	1.5	-34.1	881
279B	NW_SE	150	15.5	7.14	BF_A	BF_3	28.4	-50.4	876
281B	N_S	160	13.5	6.37	BF_A	BF_3	26.1	-49.3	876
284B	NW_SE	115	15	6.95	BF_A	BF_3	25.5	-50.3	876
285B	N_S	115	11.5	5.58	BF_A	BF_3	26.0	-49.5	876
292B	NE_SW	80	11.5	5.58	BF_D	BF_4	23.7	-59.6	866
293B	NW_SE	120	10.5	5.17	BF_D	BF_4	23.4	-60.2	866
294B	N_S	135	10.5	5.17	BF_D	BF_4	23.3	-60.0	866
295B	NE_SW	110	13.5	6.37	BF_D	BF_4	22.4	-59.6	866
296B	W_E	115	12	5.78	BF_D	BF_4	21.9	-60.9	866
32.1A	N_S	110	14	5.20	BT_E	BT_1	-69.2	86.7	937
32.2A	N_S	110	11.5	5.58	32.2a	BT_1	-69.2	86.7	937
32.3B	E_W	135	8.5	4.34	BT_E	BT_1	-69.2	86.7	937
32.4B	NW_SE	120	10.5	5.17	BT_E	BT_1	-69.2	86.7	937
33B	S_N	75	11	8.00	33b	BT_1	-68.8	84.5	937
355B	NW_SE	115	15	6.95	355b	BF_5	-20.0	-42.7	881
356B	N_S	100	9	4.55	356b	BF_5	-19.8	-43.4	881
357B	NW_SE	95	13	6.17	357b	BF_5	-20.8	-43.5	881
358A	N_S	50	24	10.27	358a	BF_5	-20.7	-44.6	881
361B	W_E	130	15	6.95	361b	BF_5	-21.6	-42.9	881
81.1B	W_E	125	18	8.00	BT_C	BT_2	28.1	44.8	916
81.2B	N_S	120	18.5	8.27	BT_C	BT_2	28.1	44.8	916
81.3A	S_N	100	18.5	8.27	BT_C	BT_2	28.1	44.8	916
81.4B	SE_NW	135	10.5	5.17	BT_C	BT_2	28.1	44.8	916

81.5B	SE_NW	130	9.5	4.76	BT_C	BT_2	28.1	44.8	916
95B	S_N	120	10.5	4.80	95b	BT_3	36.6	31.4	909
96A	N_S	135	11.5	5.80	BT_A	BT_3	35.7	31.3	909
97B	N_S	115	8.5	3.80	BT_A	BT_3	35.6	31.4	909
98B	NE_SW	115	11.5	5.80	BT_A	BT_3	34.8	31.2	909
99B	E_W	125	11.5	5.40	BT_A	BT_3	34.6	32.0	909

Table S2: List of all measured traits.

Abbreviation	Trait	Group
TRW	Tree ring width	Growth
EWW	Earlywood width	Growth
LWW	Latewood width	Growth
MCWT	Mean cell wall thickness	Mechanical support
CWT.ew	Earlywood cell wall thickness	Mechanical support
CWT.lw	Latewood cell wall thickness	Mechanical support
MCWTrad	Mean radial cell wall thickness	Mechanical support
CWTrad.ew	Earlywood radial cell wall thickness	Mechanical support
CWTrad.lw	Latewood radial cell wall thickness	Mechanical support
MCWTtan	Mean tangential cell wall thickness	Mechanical support
CWTtan.ew	Earlywood tangential cell wall thickness	Mechanical support
CWTtan.lw	Latewood tangential cell wall thickness	Mechanical support
MDEN	Mean wood density based on cwt	Wood density
DEN.ew	Earlywood density based on cwt	Wood density
DEN.lw	Latewood density based on cwt	Wood density
MDCWA	Mean wood density based on cell wall area	Wood density
DCWA.ew	Earlywood density based on cell wall area	Wood density
DCWA.lw	Latewood density based on cell wall area	Wood density
MLA	Mean lumen area	Water transport
LA.ew	Earlywood lumen area	Water transport
LA.lw	Latewood lumen area	Water transport
MDH	Mean hydraulic diameter	Water transport
DH.ew	Earlywood hydraulic diameter	Water transport
DH.lw	Latewood hydraulic diameter	Water transport

Equation S1 $\sigma_P^2 = \sigma_G^2 + \bar{\sigma}_p^2$

Where $\bar{\sigma}_p^2$ is the mean clonal group trait variance, calculated as:

Equation S2 $\bar{\sigma}_p^2 = \frac{\sum_{j=1}^{N} \sigma_j^2}{N}$

Where σ_j^2 is the trait variance in group *j* and N is the number of clonal groups:

Equation S3 $\sigma_j^2 = \frac{\sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{n_j - 1}$

Where x_{ij} is the mean of the investigated trait across the study years 2007-2017 in individual *i* of clonal group *j*, $\bar{x}_j$ is the mean of the investigated trait in clonal group *j* and n is the number of individuals in group *j*:

Equation S4 $\bar{x}_j = \frac{\sum_{i=1}^{n_j} x_{ij}}{n_j}$

Equation S5 $\sigma_G^2 = \frac{\sum_{j=1}^{N} (\bar{x}_j - \bar{x})^2}{N - 1}$

Where $\bar{x}$ is the trait total mean.

4. Synthesis

Within this thesis I investigated the adaptive potential of white spruce in the context of climate change. The adaptive potential of trees is proportional to their standing genetic variation, their degree phenotypic plasticity, as well as to their dispersal capacity which allows to cope with novel local conditions and track their preferred habitats (Aubin et al., 2016; Sultan, 2016). To assess the adaptive potential of white spruce, my co-authors and I studied the rate of gene flow and neutral genetic diversity using SSR markers, the adaptive genetic diversity using SNPs and the phenotypic plasticity of tree growth and wood traits of natural populations in Alaska. This region is strongly affected by global warming (Collins et al., 2013; Girardin et al., 2016; Reich et al., 2018) and thus, perfect to study adaptation to rapid climate change. Further, we chose treeline populations because they are assumed to react directly and strongly to climate change (Case and Taper, 2000; Hampe and Jump, 2011; Hampe and Petit, 2005; Restoux et al., 2008). Here, I studied adaptation processes in natural populations by investigating local adaptation and phenotypic plasticity of white spruce to better estimate adaptation of trees to climate change to support our forest ecosystems.

4.1. Genetic diversity

Neutral genetic diversity

I investigated the population structure and gene flow of three natural white spruce populations of one drought and two cold-limited treeline ecotones in Alaska using SSR markers. SSR markers are mainly located in non-coding regions which only enables the investigation of neutral genetic variation (Vieira et al., 2016). In addition, white spruce has a large genome with highly repetitive sequences and to the present day, the genome of white spruce is not fully sequenced (Birol et al., 2013). This hampers the development and applicability of SSR markers, which I experienced by the high amount of null alleles and homozygous excess in the SSR data set. Therefore, I needed to account for these problems by using software which considers for null alleles (Huang et al., 2016). Nevertheless, the relatedness between individuals was most likely underestimated but probably did not change the overall outcome of the study (Carlsson, 2008). Further, significantly fewer alleles in the microsatellites of Alaskan white spruce populations are described relative to outside Alaska, highlighting the limited applicability of genetic markers developed on local populations (Anderson et al., 2011).

The genetic differentiation among populations was low, even though the studied populations were distinct by large geographical distances and environmental differences. At the same time, there was a high genetic diversity within the populations. The high genetic diversity within and the low genetic differentiation among populations can be explained by the extensive long-distance pollen dispersal of white spruce (O'Connell et al., 2007). This hypothesis is also supported by the high observed pollen and seed immigration rates into the plots. The high pollen-mediated gene flow in wind-pollinated and wind-dispersed conifers keeps populations connected (Avanzi et al., 2020; Leonarduzzi et al., 2016; Liepelt et al., 2002; Piotti et al., 2009), resulting in a high genetic diversity within and low genetic differentiation among populations. This was found for several conifer species (Hamrick and Godt, 1996; O'Connell et al., 2007; Rajora et al., 2005; Roschanski et al., 2016). The particularly high pollen and seed immigration rates into the treeline populations, pointing towards a colonization of the treeline ecotones from seed sources outside the plots, like reported for other coniferous treeline ecotones (Johnson et al., 2017; Leonarduzzi et al., 2016). This effect is even more pronounced due to lower seed

production and variability in treeline ecotones (Crofts and Brown, 2020; Johnson et al., 2017; Piotti et al., 2009). Especially at advancing treelines on cold-limited sites, high gene flow can be an advantage when preadapted alleles from warmer regions are introduced into the local population to promote adaptation to a warming climate (Aitken et al., 2008; Bontrager and Angert, 2018). One the other hand, gene flow can also counteract local selection processes by intogression of maladapted alleles into the local gene pool, called outbreeding depression (Lenormand, 2002; O'Connell et al., 2007; Rajora et al., 2005), leading to adaptation lags in climate margin populations (Fréjaville et al., 2019). High gene flow and the resulting high genetic diversity within populations could provide a broad set of phenotypes, potentially increasing the evolvability of the species (Houle, 1992). Moreover, white spruce is a wind-dispersed species with light seeds, resulting in a high dispersal capacity which helps colonize new suitable habitats (Aitken et al., 2008; Mimura and Aitken, 2007; Nienstaedt and Zasada, 1990).

The analyses suggested that the low genetic differentiation among populations was rather caused by isolation by distance than isolation by environment, like reported for other conifers (Mimura and Aitken, 2007; Mosca et al., 2014). Although, the investigation of only three populations in regard of isolation by distance vs. isolation by environment analyses limits the validation. Results indicate genetic drift as the main driver of population differentiation (Anderson et al., 2011). The mean annual temperature explained only a minor part of the differentiation, pointing towards a selective influence by the environment.

Adaptive genetic diversity

As neutral markers, SSR motifs respresent only a minor part of the present genetic variation and the estimation of natural selection by environmental conditions is limited. Therefore, I focused on SNP markers in my second study. I investigated the genetic basis of drought tolerance in the three contrasting treeline populations by using SNPs. I used growth declines caused by drought stress as phenotypic data because drought events increase in frequency and intensity under global warming (IPCC, 2021). In dendroecology there is no standardized method to identify growth decline caused by drought stress (Schwarz et al., 2020). Therefore, my co-authors and I developed a standardized and evidence-based decision tree to identify growth decline associated with drought stress. To explore the genetic basis of drought-tolerant phenotypes, I applied two different approaches of genotype-phenotype association analyses. I used SNPs in candidate genes which already showed some sort of association with climate or phenotypic traits in previous studies (Pavy et al., 2017).

The genetic basis of drought tolerance contrasted between the treeline ecotones. Most genes were identified in the drought-limited site, as well as the SNPs with the strongest associations, indicating a comparatively strong selection of drought-tolerant phenotypes within the site. The differences in adaptive genetic variation among populations were probably shaped by drought events which exert intense selection pressure on populations and thereby shape genetic variation at adaptive loci (Grant et al., 2017). However, high gene flow, as highlighted by the SSR data, keeps populations connected and could counteract local adaptation (O'Connell et al., 2007; Rajora et al., 2005). The divergent genetic structures underlying drought tolerance indicate differing selection pressure of the contrasting treelines which led to a covariance of alleles with drought-tolerant phenotypes. Even the two cold-limited treeline ecotones differed in their genetic structures underlying drought tolerance. In addition to drought, frost tolerance may be a strong selective driver. To my knowledge, genotype-phenotype associations using drought

indices in white spruce were so far exclusively investigated in common garden settings in Eastern Canada (Depardieu et al., 2020; Depardieu et al., 2021; Laverdière et al., 2022). Here, I provide insights into the genetic structures underlying drought-tolerance in natural populations, which also enables us to compare drought-tolerance associated genes from different geographical parts of the white spruce distribution range. Drought-related genes in Alaskan populations differed from the genes identified in Canadian populations, suggesting that drought adaptation acts on a local scale and differs in populations with restricted gene flow (Rellstab et al., 2017). Divergent genetic structures underlying drought tolerance and therefore signatures of selection are population-specific and led to different alleles associated with drought-tolerant phenotypes, like reported for *Picea abies* (Trujillo-Moya et al., 2018). Furthermore, genetic variation among populations plays a significant role in growth resilience in response to drought (Depardieu et al., 2020).

Moreover, the association approach which took into account multiple SNPs and their interactions together with small effect-size SNPs, identified a much higher number of associations than the second approach. This points towards a polygenic architecture of drought tolerance in white spruce, like it is reported for traits involved in local adaptation to climate in conifers (Csilléry et al., 2018; Sork, 2017). Adaptation is rather driven by interacting small-effect size alleles instead of a few large-effect alleles, especially in populations with high gene flow and recent selection events (Hornoy et al., 2015; Le Corre and Kremer, 2012).

I identified 40 genes associated with dendrophenotypic traits, some of them already associated with wood traits or regulated under drought in other studies (Depardieu et al., 2021; Lamara et al., 2016). In contrast to the used SSR markers, the SNPs could be annotated to genes and for some genes, even molecular functions were identified. Still, the selected SNPs represent only a small portion of the white spruce genome. I tried to specify the analysis by using SNPs in candidate genes which were developed for analyses of wood traits or adaptation to climate. Further, the costs of next generation sequencing methods limit the number of trees which can be analysed. Therefore, I needed to select a subset of the available trees with about the same age and size. Moreover, a second drought-limited treeline site would have been helpful to validate the results. Nevertheless, the identified genes demonstrated their relevance in capturing signals of local adaptation and qualified for further analyses.

4.2. Phenotypic plasticity

To further explore the adaptation potential, my co-authors and I investigated the phenotypic plasticity of tree growth and wood anatomy in white spruce. Therefore, the study combined neutral genetic markers (SSR) with dendrochronological and climatic data to investigate the individual growth. The individual-based dendrochronological approaches demonstrated a high phenotypic plasticity of growth performance rather influenced by microenvironmental features than genetic similarity. The effects of climate on growth differed between sites and were smaller than the effect of tree size. There was a large inter-individual variability in growth responses indicating the high phenotypic plasticity of white spruce.

Second, the individual growth response to drought stress using the dendrochronological and climatic data of the three populations was investigated. Climate sensitivity and the growth responses to drought stress differed in the contrasting treeline populations. Trees at the treeline reacted more sensitive to the climate than trees in the forest, due to the more extreme environment. The differing drought stress responses in growth showed a high variability among individuals and among sites.

Additionally, my co-authors and I investigated xylem anatomical traits representing growth, water transport, mechanical support and wood density on clonally and non-clonally growing white spruce trees at one of the cold-limited treelines. Using these data, broad sense heritability of each trait and the effects of spatial and genetic grouping could be estimated. The xylem anatomical traits were mainly influenced by spatial clustering and therefore most probably microenvironmental conditions, similar to the analyses of tree growth. Although, in latewood density and earlywood hydraulic diameter, a significant effect of genetic grouping could be detected.

Tree growth is known to show a high variation affected by several factors like competition or masting events, but especially climate (Hacket-Pain et al., 2015; Trouillier et al., 2018; Wilmking et al., 2020). Within the thesis, several analyses showed a high inter-individual variability in tree growth, suggesting a high phenotypic plasticity. Even the same individuals responded differently to different drought events. The higher climate sensitivity of the trees at the treeline further shows the high phenotypic response to extreme environmental conditions. Within this environment the species experiences its physiological limits because growth is limited by water availability or low temperatures (Hampe and Jump, 2011). The resulting stronger climate signal led to the preference of treeline populations in dendrochronological studies when investigating the influence of climate variables (Cook and Kairiukstis, 1990; Fritts, 1976).
The high phenotypic plasticity of trees helps to cope with short-term environmental changes (Valladares et al., 2014). Whereby, the high phenotypic plasticity of white spruce probably led to the wide distribution range of the species which covers a wide range of environmental conditions (OECD, 1999). Further, phenotypic plasticity was positively correlated with gene flow, which is high among the investigated populations according to the results of the SSR analyses (Lind et al., 2011). Still, with the available data I cannot estimate to which extent the phenotypic plasticity is genetically determined and to which extent selection for phenotypic plasticity is present.
The results of tree growth and xylem anatomical analyses were coherent in the sense that they are mostly influenced by environmental conditions, which was also found in other conifer species (Avanzi et al., 2019; King et al., 2013; Rozas et al., 2020). Nevertheless, there was a moderate heritability of the wood anatomical traits and I identified genes associated with growth responses to drought, indicating some genetic basis of the investigated traits. This is coherent with the literature, which reports moderate heritabilities and genes associated with wood traits and drought-tolerant phenotypes in white spruce (Beaulieu et al., 2011; Depardieu et al., 2020; Depardieu et al., 2021; Lamara et al., 2016; Laverdière et al., 2022), Norway spruce (Baison et al., 2018; Trujillo-Moya et al., 2018) and other conifer species (Dillon et al., 2010; Heer et al., 2018). Even for the highly variable trait climate sensitivity, I could identify some genetic basis. These traits are suitable to assess potential local adaptation, because natural selection can only act on traits which are heritable (Depardieu et al., 2020). Still, we need to consider anatomical influences like tree size when investigating tree growth and wood traits (Trouillier et al., 2018).

5. Conclusion and outlook

The high phenotypic plasticity of white spruce could buffer short-term environmental changes but could also reduce the selection pressure, resulting in slower genetic adaptation. More research is needed to explore the genetic basis of and selection for phentoypic plasticity in white spruce and other tree species. The high genetic diversity within white spruce populations may provide a wider range of phenotypes which enhances the efficiency of selection when the species is facing rapid climatic changes. This can be seen as a phenomenon counterbalancing tree longevity: in the investigated region, environmental conditions did considerably change over the life time of most adult trees. The high genetic diversity is favored by the high pollen-mediated gene flow rates of white spruce. On one hand, the high gene flow into the local gene pool can counteract adaptation by the introduction of maladapted alleles (outbreeding depression). On the other hand, the introduction of alleles preadapted to a warmer climate could accelerate adaptation, especially at the cold-limited treelines of white spruce. Genetic drift seems to be the main driver of the low population differentiation in neutral genetic diversity. Nevertheless, environmental differences probably led to different selection pressure shaping divergent adaptive genetic diversity among populations. Moreover, adaptation to drought involves small frequency shifts in several interacting genes and seems rather to act on a local scale. My results highlight a set of genes that genetically determines wood traits critical for the establishment and persistence of tomorrow´s forests under climate change. These genes can be further used to study the genetic basis of drought tolerance in trees, especially conifers. Moreover, the developed method to identify growth decline caused by drought stress can be applied in future studies to investigate the genetic basis of drought-tolerant phenotypes.

The costs of genotyping are continuously decreasing, which will enable us to conduct analyses including a higher amount of trees to validate the results. This knowledge can be used to support trees in climate adaptation by assisted migration or by the development of marker-based breeding of drought-tolerant phenotypes to maintain the necessary resilience of forest ecosystems and their ecosystem services. Still, more research is needed to further explore the adaptation processes in white spruce and other tree species.

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Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.
Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Greifswald den _____, _____
Melanie Zacharias

Curriculum Vitae

Work experience

04/2018 – present	University Greifswald (Germany), Insitute of Botany and Landscape Ecology *PhD position in the research training group RESPONSE*
01/2017 – 09/2017	University of Auckland (New Zealand) *Internship*
06/2014-12/2016	Technical University Dresden (Germany), Institute for Forest Botany *Student assistant*

Education

2014 – 2018	Technical University Dresden (Germany) *Master of Science in Forestry*
2011 – 2014	Technical University Dresden (Germany) *Bachelor of Science in Forestry*
2011	Bernhard-von-Cotta-Gymnasium, Brand-Erbisdorf (Germany) *Abitur*

Memberships

2013 – 2018	International Forestry Students‘ Association (IFSA) *Member*
04/2015 – 11/2016	International Forestry Students‘ Association (IFSA) *Representative of the local committee Tharandt*

Skills

Languages	German	Native
	English	Fluent
	French	Basics
Computing skills	R	Advanced skills in statistical analyses
	Python	Basic skills
	MS Office	Advanced skills in MS Word and MS Excel

Teaching experience

2019	supervision of Master thesis of Seema Naupane

Conference contributions

January 2020	**Poster** *"Spatial genetic differentiation in Picea glauca stands in Alaska"*, Gentree conference - Genetics to the rescue, Avignon Université (France)
May 2021	**Poster** *"Population structure and the influence of microenvironment and genetic similarity on individual growth at Alaskan white spruce treelines"*, 2021 Forest Genetics Student Symposium (virtual event)
September 2021	**Presentation** *"Genetic signatures of drought stress tolerance in contrasting treeline ecotones of a widespread conifer in Alaska"*, EvolTree Conference 2021 – Genomics and Adaptation in Forest Ecosystems, WSL Birmensdorf (Switzerland)

Publications

2020	**Xylem anatomical variability in white spruce at treeline is largely driven by spatial clustering** Authors: Timo Pampuch, Alba Anadon-Rosell, Melanie Zacharias, Georg von Arx & Martin Wilmking *Journal: Frontiers in Plant Science* *URL: https://doi.org/10.3389/fpls.2020.581378*
2021	**Population structure and the influence of microenvironment and genetic similarity on individual growth at Alaskan white spruce treelines** Authors: Melanie Zacharias, Timo Pampuch, Katrin Heer, Camilla Avanzi, David G. Würth, Mario Trouillier, Manuela Bog, Martin Wilmking, Martin Schnittler *Journal: Science of the Total Environment* *URL: https://doi.org/10.1016/j.scitotenv.2021.149267*
2022	**Genetic basis of growth reaction to drought stress differ in contrasting high-latitude treeline ecotones of a widespread conifer** Authors: Melanie Zacharias, Timo Pampuch, Benjamin Dauphin, Lars Opgenoorth, Carl Roland, Martin Schnittler, Martin Wilmking, Manuela Bog, Katrin Heer *Journal: Molecular ecology (submitted)*

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