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Effects of Wild Forest Fires on Genetic Diversity and Population Structure of a Boreal Conifer, White Spruce (*Picea glauca* (Moench) Voss): Implications for Genetic Resource Management and Adaptive Potential under Climate Change

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Abstract: Climate change is predicted to increase forest fires in boreal forests, which can threaten the sustainability of forest genetic resources. Wildfires can potentially impact genetic diversity and population structure in forest trees by creating population bottlenecks, and influencing demography, effective population size (N_e) and various evolutionary processes. We have investigated this critical issue in a widely-distributed, transcontinental, ecologically and economically important and fire-intolerant boreal conifer, white spruce (*Picea glauca* (Moench) Voss). We tested the hypothesis that in a predominantly outcrossing species with long distance gene flow, such as white spruce, located in primary undisturbed forests, normal forest fires do not adversely affect genetic diversity and population structure. We used 10 nuclear genic and genomic microsatellite loci to examine genetic diversity and population structure of post-fire pristine old-growth (PF-OG) and adjacent post-fire naturally regenerated young (PF-YR) stands. The genetic diversity, inbreeding and genetic differentiation levels, Bayesian population structure, N_e and latent genetic potential were statistically similar between the PF-OG and PF-YR populations. None of the microsatellites showed any signature of selection. Our study demonstrates that normal wild forest fires do not adversely affect genetic diversity, differentiation, and population genetic structure in white spruce. The results should have wide significance for sustainable forest management.

Keywords: natural disturbance; fire intolerant species; genetic biodiversity; population differentiation; effective population size; conifers; microsatellites; sustainable forest management; conservation; forest genetic resources

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1. Introduction

Genetic diversity benefits the functioning and resilience of the ecosystem [1–3] because genetic variation is required for evolutionary processes to condition the adaptation, evolution and survival of populations and species, especially under changed climatic and environmental conditions [4]. There is increasing empirical evidence that genetic diversity is positively associated with population fitness under normal and stressed conditions [5–7]. Forest ecosystems, in which forest trees are normally the keystone species, are extremely important for the environment because they serve as carbon sinks and biodiversity reservoirs and provide many ecological and environmental services, such as nutrient cycling, soil protection, and climate moderation. Therefore, conservation and sustainable management of genetic diversity in forest trees is crucial, especially to buffer the negative effects of climate change and abiotic and biotic stresses.

Natural and anthropogenic disturbances in forests and other ecosystems can impact genetic diversity, differentiation and genetic structure of populations of their constituent

forest trees and other organisms by influencing demography and several evolutionary processes [8–11]. Wild forest fires are the major disturbance in boreal forests, which can influence landscape, ecosystem and forest composition and diversity, energy flow, and biogeochemical cycles [12]. Wild forest fires can potentially impact genetic diversity, differentiation and population structure by creating population bottlenecks and influencing demography, effective population size and various evolutionary processes, such as genetic drift, gene flow and breeding system, that condition genetic diversity in populations. Wild forest fires can also act as a selection force, driving evolutionary changes and adaptive traits, such as cone serotiny in some conifer species, which was evident from the signatures of selection observed on certain genes underlying such traits [13]. The extent of the impact of wild forest fires on genetic diversity and population structure will depend upon the intensity of fires and the life history traits of a species, such as its capability of maintaining canopy and soil seed banks, and the extent of seed and pollen dispersal. Scientific evidence indicates that species which maintain perennial seedbanks in serotinous or semi-serotinous cones in the canopy and in the soil—called seeders, fire resisters and evaders [14,15]—and/or that have long-distance seed and pollen dispersibility, can preserve genetic diversity and population structure under normal fire regimes [16–20].

Although wild forest fires have been an integral part of the boreal forest ecosystems, climate models and scenarios suggest that the boreal fire incidence and intensity as well as the forest area burned will increase significantly as a result of climate change [21–23]. This can threaten the sustainability of forest genetic resources. Furthermore, natural disturbances are being considered as a basis for managing boreal forests [24,25]. Therefore, there is an urgent need to understand the genetic effects of forest fires in boreal forest trees. However, very little is known about the genetic effects of wild or prescribed fires in boreal and other forest trees. Most of the published information on this subject is on fire adaptive/tolerant species that maintain perennial seedbanks in serotinous or semi-serotinous cones in the canopy and in soil. Most of the studies reported have focused on Mediterranean Aleppo pine, *Pinus halepensis* Mill., which is a typical seeder fire-adaptive species. It has been demonstrated that normal fire regimes do not have negative impacts on genetic diversity, genetic differentiation, spatial genetic structure and/or the mating system in this species [18,19,26]. However, the *Pinus halepensis* stands that originated after a high fire intensity were found to have a stronger spatial genetic structure than the stands that originated after a low fire intensity [19]. In addition, evidence for the potential selection of heterozygotes in the post-fire population [18], and high fire-intensity related selection on three SNPs at the regional scale [19] has been reported in *Pinus halepensis*. Among the boreal conifers, information on the genetic effects of forest fires is available for fire adapted black spruce (*Picea mariana* (Mill.) B.S.P.) [16], which retains a perennial seedbank in semi-serotinous cones in the canopy and in the soil and is considered as a fire-evader species [14]. Forest fires did not show any negative impact on genetic diversity, genetic differentiation and inbreeding levels in this species [16].

The information available on the effects of forest fires on genetic diversity and population structure of angiosperm forest trees is also very limited and is mostly restricted to fire adapted species. In *Betula maximowicziana* Regel, a fire-adaptive invader species, genetic diversity was found to be similar between the pre-fire and post-fire stands, but the number of private alleles and the effective population size was higher in the pre-fire stands [27]. Genetic diversity of undisturbed and post-fire naturally regenerated populations of mountain ash (*Eucalyptus regnans* F. Muell.) in Australia were found to be similar [20]. Mountain ash is a fire-dependent seeder species, which has seed stored in serotinous capsules in the canopy [20].

There is very little published information on genetic effects of forest fires in fire sensitive or intolerant species and contrasting results have been reported. In two Tasmanian endemic conifers, *Athrotaxis cupressoides* D. Don and *Diselma archeri* Hook. f., based on the Last Glacial Maximum-modelled distributions, the fossil record and the fire history index of the sampled stands, it was found that the genetic diversity metrics decreased significantly

with an index of fire history [28]. In the fire-sensitive shrub, *Persoonia mollis* ssp. *Nectens* S. Krauss & L.A.S. Johnson, successive fires were not found to result in a decline in genetic diversity or an increase in the spatial genetic structure [17]. The authors suggested that the species' large seed banks with a bet-hedging strategy of staged seed germination, combined with pollen and seed dispersal, provided a good buffer against the negative genetic impacts of frequent fires.

White spruce (*Picea glauca* (Moench) Voss) is a transcontinental, widely distributed and dominant species of the boreal forest in Canada [29] and it has a high ecological and economic importance. It is one of the most important trees for the production of wood pulp and lumber in Canada, and for the stability and sustainability of the North American boreal forest ecosystem. White spruce has high genetic diversity and is predominantly an outcrossing species [9,30–33]. Long-distance pollen and seed dispersal occurs in white spruce [34,35]. There is also evidence for selection against inbreds at an early stage in this species [34]. White spruce does not produce serotinous cones, and it is a fire intolerant species; it is classified as a fire-avoider because it arrives late in succession and becomes dominant where fire-cycle intervals are long [14]. It is imperative to determine genetic effects of forest fires in this ecologically and economically important boreal conifer because wildfire frequencies and intensities are expected to increase under climate change. However, no information is available on the impacts of wildfires on genetic diversity and population structure in white spruce.

The objective of this study was to examine the effects of wild forest fire on genetic diversity, population structure and genetic differentiation by comparing populations of white spruce in adjacent natural old-growth and post-fire naturally regenerated young stands. Since white spruce has high inherent genetic diversity, predominantly outcrossing mating system, long-distance pollen and seed dispersal and selection against inbreds at an early stage, we hypothesized that wild forest fires in a natural primary forest do not have a significant adverse effect on the genetic diversity and population structure of white spruce.

2. Materials and Methods

2.1. Study Sites, Experimental Design and Sampling

Due to post-fire management actions, such as salvage logging, scarification, and seeding or planting by forest management companies or agencies, it is difficult to find optimal locations to study the effects of wildfire. It is also quite expensive and time consuming to conduct controlled forest fire experiments in natural forests. National parks in Canada, where industrial logging, silvicultural activities and other management interventions do not normally occur, provide excellent research sites to examine genetic effects of wild forest fires. Although wildfires are controlled in national parks, actions are generally limited to the use of water and hand-tools to extinguish flames, and there is no subsequent intervention to manipulate post-fire forest regeneration. However, the number of sites where the adjacent populations of old-growth and post-fire natural young regeneration could be studied in Canadian national parks are limited.

The study sites were located in the northern portion of the Prince Albert National Park of Canada, which is located within the Mid-Boreal Uplands Ecoregion of central Saskatchewan [36]. The forests are dominated by boreal conifers (black spruce, jack pine, *Pinus banksiana* Lamb., and white spruce) and lesser amounts of deciduous species (trembling aspen, *Populus tremuloides* Michx., and balsam poplar, *Populus balsamifera* L.) growing on gray luvisolic soils. The region is a primary forest that has been a protected area since 1928 and has never been subjected to modern industrial forest management activities. The fire regime for the region is characterized by stand-replacing crown fires that initiate conifer regeneration from seeds and deciduous regeneration from root suckers and seeds.

To identify potential sampling sites, the park's forest inventory, fire history maps and aerial photographs were examined to locate old-growth white spruce stands adjacent to recent fire disturbances. Sites with young white spruce adjacent to old growth were rare because fires had burned only a small proportion of the study region between 1945

and 1998 [37]. Two sampling sites (Tibiska Lake and Sanctuary Lake) were identified in 1998 where natural white spruce regeneration (no artificial planting or seeding) occurred after the fire disturbances within recent decades. Adjacent old-growth and post-fire young naturally regenerated stands/populations at each of the Tibiska Lake and Sanctuary Lake sites were sampled (Table 1). The adjacent sampled populations would have been within the same even-aged stands prior to the Tibiska Lake fire in 1964 and the Sanctuary Lake fire in 1986.

Table 1. White spruce populations studied and their geographical coordinates and age at sampling.

Site	Population	Population ID	Longitude W	Latitude N	Age Years
Tibiska Lake (TL)	Post-fire natural old growth (PF-OG)	TL-PF-OG	106°06′41″	54°15′16″	168
	Post-fire natural young regeneration (PF-YR)	TL-PF-YR			34
Sanctuary Lake (SL)	Post-fire natural old growth (PF-OG)	SL-PF-OG	106°33′25″	54°09′50″	128
	Post-fire natural young regeneration (PF-YR)	SL-PF-YR			12

The Tibiska Lake sampling site is located north of the MacLennan River and east of Tibiska Lake. The old-growth white spruce/trembling aspen stand sampled at the Tibiska Lake site originated from a fire in approximately 1830, while the adjacent young regenerating trembling aspen/white spruce stand originated from a fire in 1964 [38]. The 1964 fire burned approximately 50 km^2 within the Prince Albert National Park and an equivalent area in the adjacent Northern Provincial Forest. At the time of sampling in 1998, the old growth was 168 years old, and the post-fire young regeneration was 34 years old (Figure 1).

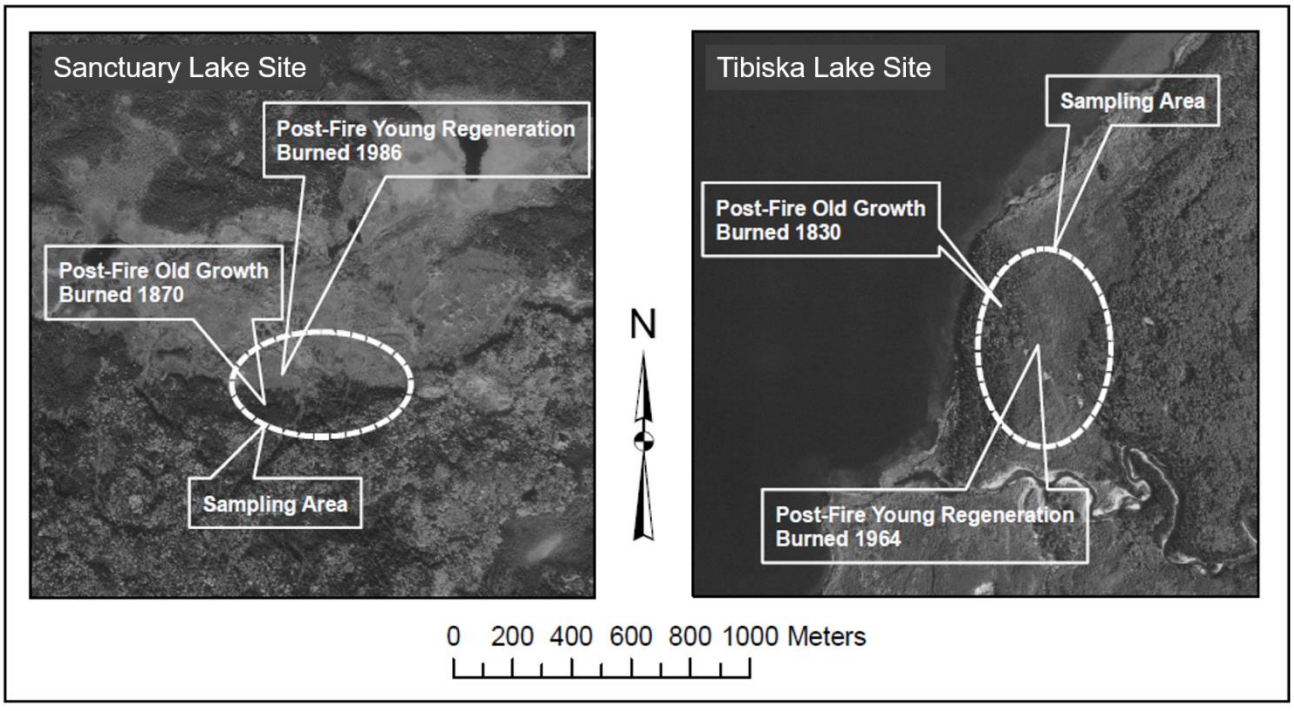

Figure 1. The 1999 aerial photos showing post-fire old growth and post-fire young regeneration at the Sanctuary Lake site (**left**) and Tibiska Lake site (**right**).

The Sanctuary Lake sampling site is located between Sanctuary Lake and Bladebone Lake. The old-growth white spruce/trembling aspen stand sampled at the Sanctuary Lake site originated after a fire in 1870, while the young white spruce/black spruce/trembling aspen regeneration was initiated after a fire in 1986 [38]. The 1986 fire was approximately

1 km^2 in size. At the time of sampling in 1998, the Sanctuary Lake old growth was 128 years old, and the post-fire young natural regeneration was 12 years old (Figure 1).

The field sampling was conducted between June and August 1998. Thirty trees per population were randomly sampled, separated by a minimum distance of 35 m, with a total of 118 individuals from four populations. We could only sample 28 individuals instead of 30 in the post-fire young-regenerated stand of Sanctuary Lake due to logistic reasons. Where necessary, tree climbing spurs were used to access branches on old-growth trees. White spruce needle samples were collected from the most recent year's growth. Needle samples were shaken or brushed off in the field to remove any insects, lichens or other organisms that might contaminate the white spruce DNA. All samples were stored immediately on ice and transported to the lab at the University of Alberta within 48 h, where they were stored at −20 °C until DNA extraction.

2.2. DNA Extraction and Genotyping

Genomic DNA was isolated from needle tissues in 1999 as described in Rajora [9]. DNA samples were stored in a freezer at −20 °C. One hundred and eighteen individuals were genotyped with 10 microsatellite loci (Table 2) as described in Fageria and Rajora [32,33].

Table 2. Microsatellite DNA loci used, and the number and size of alleles detected at each microsatellite DNA locus.

Microsatellite Locus *	Repeat Unit	Total No. of Alleles	Allele Size Range (bp)
	EST-based genic microsatellites *		
RPGSE2	(CTG)3G(CTG)3G(CTG)3	5	170–185
RPGSE5	(GAA)6	2	242–245
RPGSE17	(TCG)6	3	154–160
RPGSE34	(GA)10	16	237–269
RPGSE35	(TA)26	8	151–171
RPGSE44	(TA)9	7	204–218
	Mean no. of alleles per locus	6.83	
	Genomic microsatellites		
SPAG003	(AG)n	15	118–146
PGL14	(AG)22	23	130–182
UAPgGT8	(GT)22	12	192–228
UAPgCA91	(CA)20	28	102–190
	Mean no. of alleles per locus	19.50	
Overall mean no. of alleles per locus		11.20	

* *RPGSE2, RPGSE5, RPGSE17, RPGSE34, RPGSE35* and *RPGSE44* are EST-based genic microsatellites from Rajora and Mann [39], which were developed from white spruce gene coding sequences; *SPAG003* is from Norway spruce, *Picea abies* (L.) H. Karst. (provided by Ivan Scotti); *PGL14* is from Rajora et al. [40]; *UAPgGT8* and *UAPgCA91* are from Hodgetts et al. [41].

2.3. Data Analysis

2.3.1. Genetic Diversity, Inbreeding Coefficient and Effective Population Size

MICROCHECKER [42] was used to check the quality of the microsatellite genotype data for null alleles. The commonly used genetic diversity parameters—total number of alleles (A_T), number of alleles per locus (A), effective number of alleles per locus (A_E), number of private alleles (A_P), observed heterozygosity (H_O) and expected heterozygosity (H_E) and inbreeding coefficient (*F*)—for each population were calculated using GENALEX 6.5 [43]. In addition, genotypic diversity for each population was calculated manually as the observed (GA_O) and expected (GA_E) genotype additivity [44] as described in Rajora et al. [10]. Additionally, latent genetic potential (*LGP*) [45] was calculated manually as the difference between the mean number of alleles and the effective number of alleles over the loci. The means of each parameter for old-growth and post-fire young naturally regenerated populations were calculated. The significance of differences in the above genetic and genotypic diversity parameters and inbreeding coefficients between the two population types (old

growth and the post-fire naturally regenerated young) were tested by ANOVA using the SAS 9.1 software package.

We estimated the contemporary effective population size of each of the studied populations using the linkage disequilibrium (LD) method [46] in the NeEstimator2 program [47]. We used the critical allele frequency values of 0.05 and 0.01 because the allele frequencies and sample size affect the N_e estimates from the LD method [46]. The estimates are conservative with high critical values and biased upwards with a low critical value.

2.3.2. Genetic Differentiation and Genetic Structure

The allelic heterogeneity was tested among four populations and between the PF-OG and PF-YR population groups with the Fisher's exact test after 10,000 dememorization steps and a total of 500,000 iterations (100 batches with 5000 iterations/batch) using GENEPOP [48]. The genetic differentiation among all four populations and between the old-growth and post-fire young population groups was determined using Wright's *F*-statistics, Nei's [49] genetic distances and the hierarchical Analysis of Molecular Variance (AMOVA) [50] using GENALEX 6.5 [43]. We constructed an UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster tree based on Nei's [49] genetic distances with 1000 bootstrap iterations using the PHYLIP Software Package [51].

The overall population genetic structure was assessed using the Bayesian clustering approach implemented in the software STRUCTURE 2.3.1 using the admixture and correlated allele frequency models [52,53]. The number of genetic clusters (K) was assumed to range from 2 to 8, and for each value of K, 10 independent runs of the Markov Chain Monte Carlo (MCMC) sampler were performed with 100,000 Markov Chain iterations and 100,000 burn-in iterations. The most likely K-value was selected based on the Evanno et al. [54] method using STRUCTURE HARVESTER [55].

2.3.3. Selection Scan

To ascertain whether any of the microsatellite loci showed signatures of selection, we used BayeScan ver. 2.1 [56], which implements the logistic regression model in a Bayesian framework. The observed values of F_{ST} are partitioned into the effects of populations shared across all loci and the effects of loci shared across all populations using logistic regression [56]. If the effect of a locus shared across all populations deviates significantly from 0 (i.e., FDR q-value < 0.10), the locus is identified under selection. We used the default MCMC settings for BayeScan with 20 pilot runs each a length of 5000 steps, a burn-in of 50,000 steps followed by an additional 50,000 steps thinned every 10 steps. We used the prior odd of 10:1 on a null model to check for convergence as suggested by Foll and Gaggiottii [56] for the type of data that we had.

3. Results

3.1. Genetic Diversity, Fixation Index and Effective Population Size

The allelic diversity, the observed and expected heterozygosity and the genotypic diversity for the genomic microsatellites were several folds higher than that for the genic EST/cDNA-derived microsatellites (allelic diversity in Table 2; the other data is not shown separately for the EST-based and genomic microsatellites). Overall, the allelic patterns were similar across the four studied populations and between the PF-OG and PF-YR populations at each of the two sites (Figure 2). The total number of alleles, number of alleles per locus, effective number of alleles per locus, number of private alleles, observed and expected heterozygosity, observed and expected genotypic diversity, and latent genetic potential were similar among all four populations (Table 3), and these parameters were not significantly different ($p > 0.05$) between the PF-OG and PF-YR population groups. The fixation index (inbreeding coefficient) (*F*) values were high (Table 3) and significantly different from 0. Although the mean *F* value for the PF-YR populations was about 40% higher than that for the PF-OG populations (Table 3), the differences were not statistically significant ($p > 0.05$).

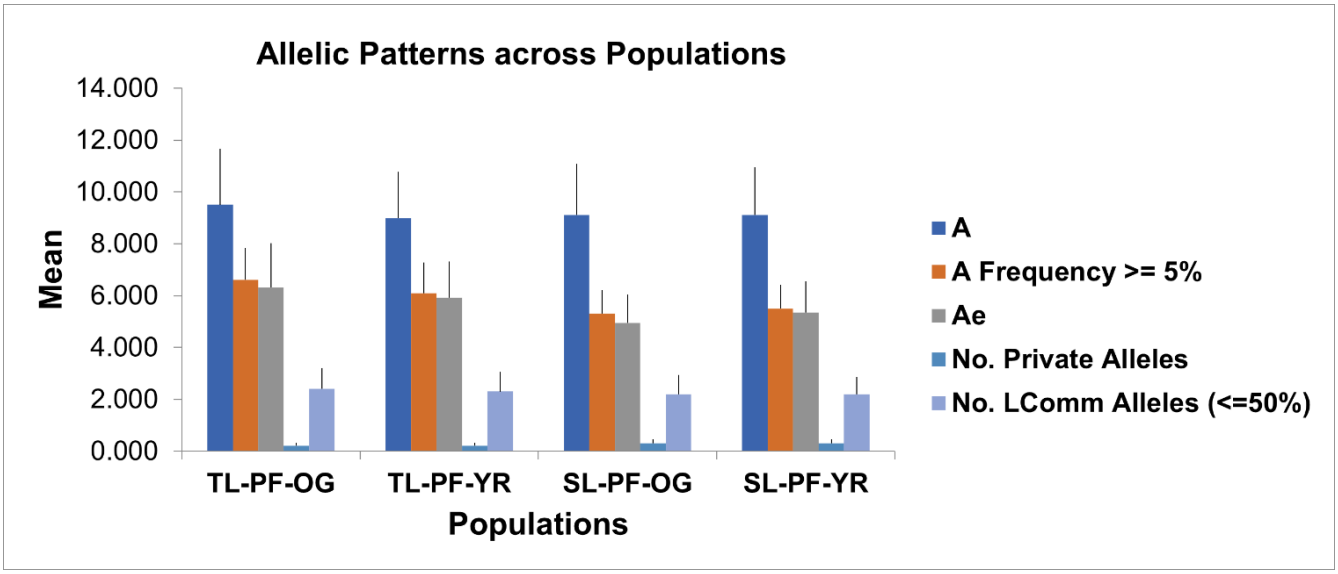

Figure 2. Allele patterns across the white spruce populations. A, number of alleles per locus; A Frequency >= 5%, number of alleles per locus with frequency of ≥0.05 (5%); Ae, effective number of alleles per locus; No. Private Alleles, total number of private alleles (A_P); No. LComm Alleles (<=50%) = number of locally common alleles (Freq. <= 5%) found in 50% or fewer populations.

The estimates of contemporary effective population size ranged from 33 in the Tibiska Lake post-fire young regeneration (TL-PF-YR) to 108 in the Sanctuary Lake post-fire young regeneration (SL-PF-YR), with an average of 64 over the four populations at the critical allele frequency value of 0.05, and from 46 (TL-PF-YR) to 193 (SL-PF-YR) with an overall mean of 97 at the critical allele frequency value of 0.01 (Table 3). As expected, the N_e estimates were higher at the critical allele frequency value of 0.01 than that at 0.05; however, the pattern of the N_e estimates was similar between the 0.01 and 0.05 allele frequency critical values. There was substantial heterogeneity among the populations for their N_e estimates and no pattern between PF-OG and PF-YR was observed. Although, on average, the PF-YR populations had higher N_e than the PF-OG populations (Table 3), the differences were not significant ($p > 0.05$).

3.2. Genetic Differentiation and Population Genetic Structure

Eight loci (*RPGSE2*, *RPGSE34*, *RPGSE35*, *RPGSE44*, *SPAG003*, *PGL14*, *UAPgGT8* and *UAPgCA91*) showed significant ($p < 0.05$) allele frequency heterogeneity among all four populations. Three loci (*RPGSE34*, *PGL14* and *UAPgCA91*) showed significant ($p < 0.05$) allele frequency heterogeneity between the Tibiska Lake PF-OG and PF-YR populations, and seven (*RPGSE34*, *RPGSE35*, *RPGSE44*, *SPAG003*, *UAPgGT8*, *PGL14* and *UAPgCA91*) between the Sanctuary Lake PF-OG and PF-YR populations.

The pairwise among-population F_{ST} ranged from 1.1% to 3.2% (Table 4). The overall F_{ST} among all four populations was 3.5% (Table 5) indicating that most of the genetic variation was among individuals within populations. The genetic differentiation between the two PF-OG populations of 2.2% ($F_{ST} = 0.022$) was similar to that (2.4%) between the two PF-YR populations ($F_{ST} = 0.024$) (Table 5). The hierarchical AMOVA results were similar to the F_{ST} results. The hierarchical AMOVA showed no genetic differentiation between the PF-OG and PF-YR populations, 4% among populations, and 96% variation among individuals (Supplementary Table S1).

Table 3. Genetic diversity parameters (mean (SE)), inbreeding coefficient, *F* (SE), and effective population size (N_e) (95% confidence interval) for the post-fire natural old-growth (PF-OG) and the post-fire young naturally-regenerated (PF-YR) populations of white spruce based on 10 microsatellite loci.

Population	A_T	A	A_P	A_E	H_O	H_E	*F*	LGP	GA_O	GA_E	N_e (0.05)	N_e (0.01)
Tibiska Lake												
PF-OG	95.0	9.50	2	6.32	0.507	0.656	0.224	3.18	131.0	622.0	42	66
		(2.17)		(1.70)	(0.094)	(0.092)	(0.103)	(0.61)	(2.87)	(26.36)	(29–68)	(45–115)
PF-YR	90.0	9.00	3	5.92	0.495	0.658	0.307	3.08	132.0	574.0	33	46
		(1.78)		(1.39)	(0.099)	(0.092)	(0.102)	(1.05)	(2.65)	(20.33)	(23–51)	(32–71)
Sanctuary Lake												
PF-OG	91.0	9.10	2	4.95	0.473	0.644	0.271	4.15	114.0	613.0	74	83
		(1.98)		(1.09)	(0.095)	(0.089)	(0.093)	(0.53)	(2.50)	(16.92)	(37–424)	(51–197)
PF-YR	91.0	9.10	3	5.34	0.404	0.635	0.366	3.76	128.0	589.0	108	193
		(1.85)		(1.20)	(0.082)	(0.095)	(0.093)	(0.71)	(2.55)	(17.23)	(46-infinite)	(78-infinite)
Mean stand types												
PF-OG	93.00	9.30	2	5.64	0.490	0.650	0.247	3.67	122.5	617.5	58	75
PF-YR	90.05	9.05	3	5.63	0.450	0.647	0.336	3.42	130.0	581.5	71	120
Overall mean	91.75	9.18	2.5	5.63	0.470	0.648	0.292	3.52	126.25	599.5	64	97

Note: Details of populations are provided in Table 1. A_T, total number of alleles; A, mean number of alleles per locus; A_E, effective number of alleles per locus; A_P, number of private alleles; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; LGP, mean latent genetic potential [45]; GA_O, genotype additivity observed; GA_E, genotype additivity expected [10]. Values in parenthesis are standard errors. N_e (0.05), effective population size at the critical allele frequency of 0.05, N_e (0.01), effective population size at the critical allele frequency of 0.01.

Table 4. Pairwise F_{ST} estimates (above diagonal) and genetic distances (below diagonal) among the four populations.

	TL-PF-OG	SL-PF-OG	TL-PF-YR	SL-PF-YR
TL-PF-OG		0.022	0.011	0.021
SL-PF-OG	0.064		0.028	0.032
TL-PF-YR	0.015	0.070		0.024
SL-PF-YR	0.068	0.115	0.067	

Note: Details of populations are provided in Table 1.

Table 5. Group-wise mean (SE) *F*-statistics estimates from 10 microsatellites loci.

Population Groups	No. of Populations	F_{IS}	F_{IT}	F_{ST}
All populations	4	0.290 (0.094)	0.314 (0.091)	0.035 (0.002)
PF-OG	2	0.247 (0.095)	0.262 (0.094)	0.022 (0.004)
PF-YR	2	0.336 (0.095)	0.353 (0.093)	0.024 (0.004)

Genetic distances [49] between populations varied from 0.015 (the PF-OG and PF-YR populations of Tibiska Lake) to 0.115 (the PF-OG and PF-YR populations of Sanctuary Lake) (Table 4). The genetic distances between post-fire old-growth and post-fire young populations (0.067) were similar to that between the two post-fire old-growth (0.064) or between the two post-fire naturally regenerated young (0.067) populations. The UPGMA dendrogram, based on Nei's [49] genetic distances grouped all four populations in a single group (Figure 3). The two populations from Tibiska Lake clustered more closely together.

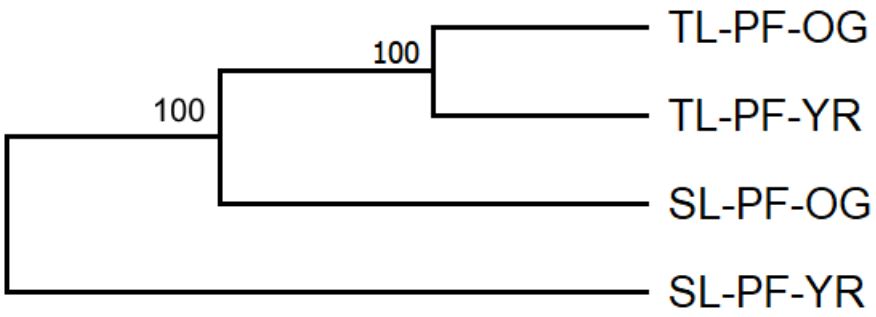

Figure 3. The UPGMA dendrogram, based on genetic distances. The number on the nodes represents the percent bootstrap support from 1000 bootstraps. Details of the populations are provided in Table 1.

The summary bar plot of the estimated membership coefficient (Q) of the studied white spruce individuals from four populations from the STRUCTURE analysis for K = 2–8 is presented in Figure 4. We observed the most prominent peak at Delta$K$ = 4 after Evanno et al. [54] adjustments in STRUCTURE HARVESTER [55] (Figure 5A). Thus, STRUCTURE revealed four genetic groups admixed within and among four populations of white spruce (Figure 5B). The STRUCTURE analysis did not separate the two groups (PF-OG and PF-YR) of populations at K = 2 or any other K values (Figure 4).

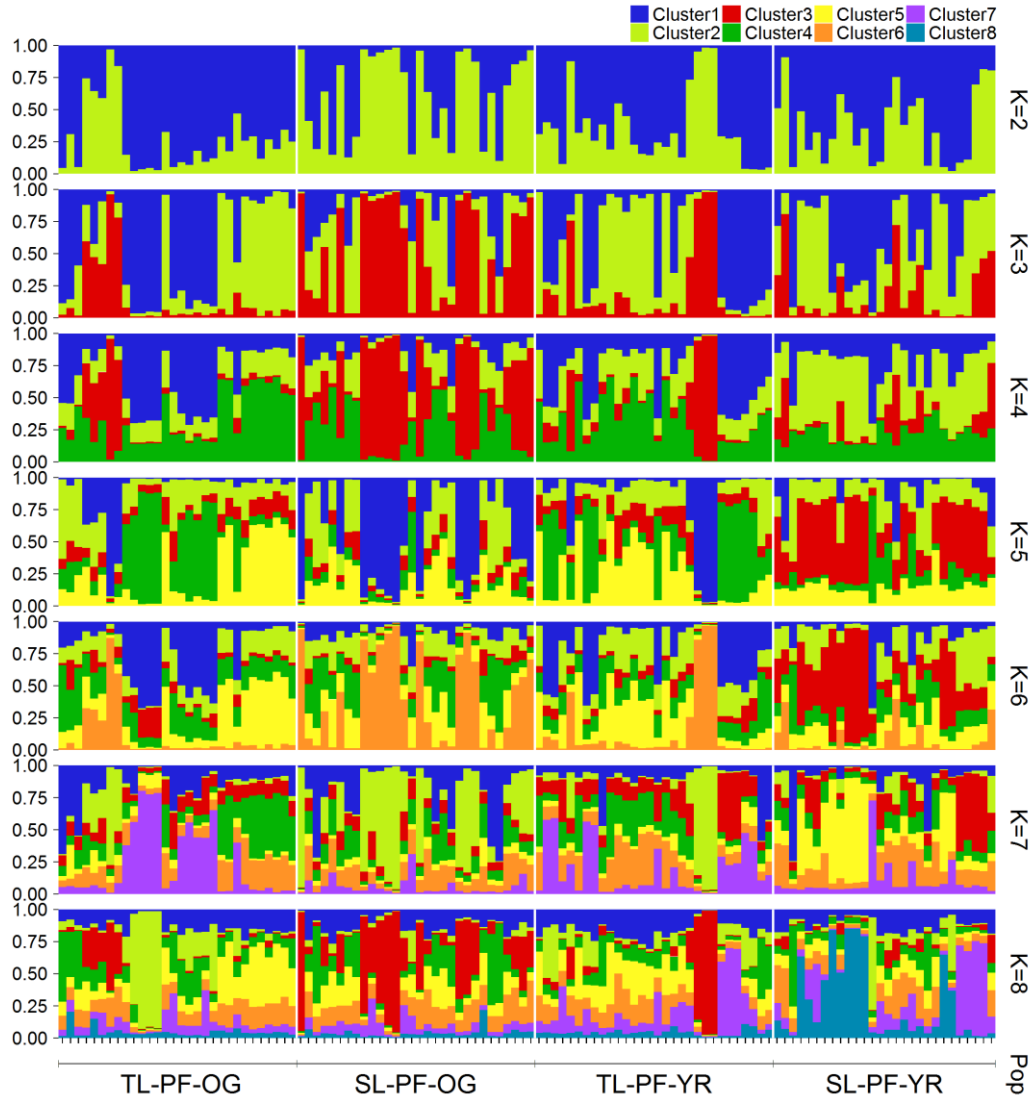

Figure 4. The summary bar plot of the estimated membership coefficient (Q) of the studied white spruce individuals from four populations from the STRUCTURE analysis for K = 2–8. Each individual is represented by a single vertical line while each color represents one cluster/genetic group. TL-PF-OG, Tibiska Lake post-fire natural old-growth; SL-PG-OG, Sanctuary Lake post-fire natural old-growth; TL-PF-YR, Tibiska Lake post-fire natural young regeneration; SL-PF-YR, Sanctuary Lake post-fire natural young regeneration.

3.3. Selection Scan

The BayeScan analysis did not show any of the 10 SSR loci being identified as outliers (q values ranged from 0.70 to 0.92 at 10:1 prior odds) and showing the signatures of selection.

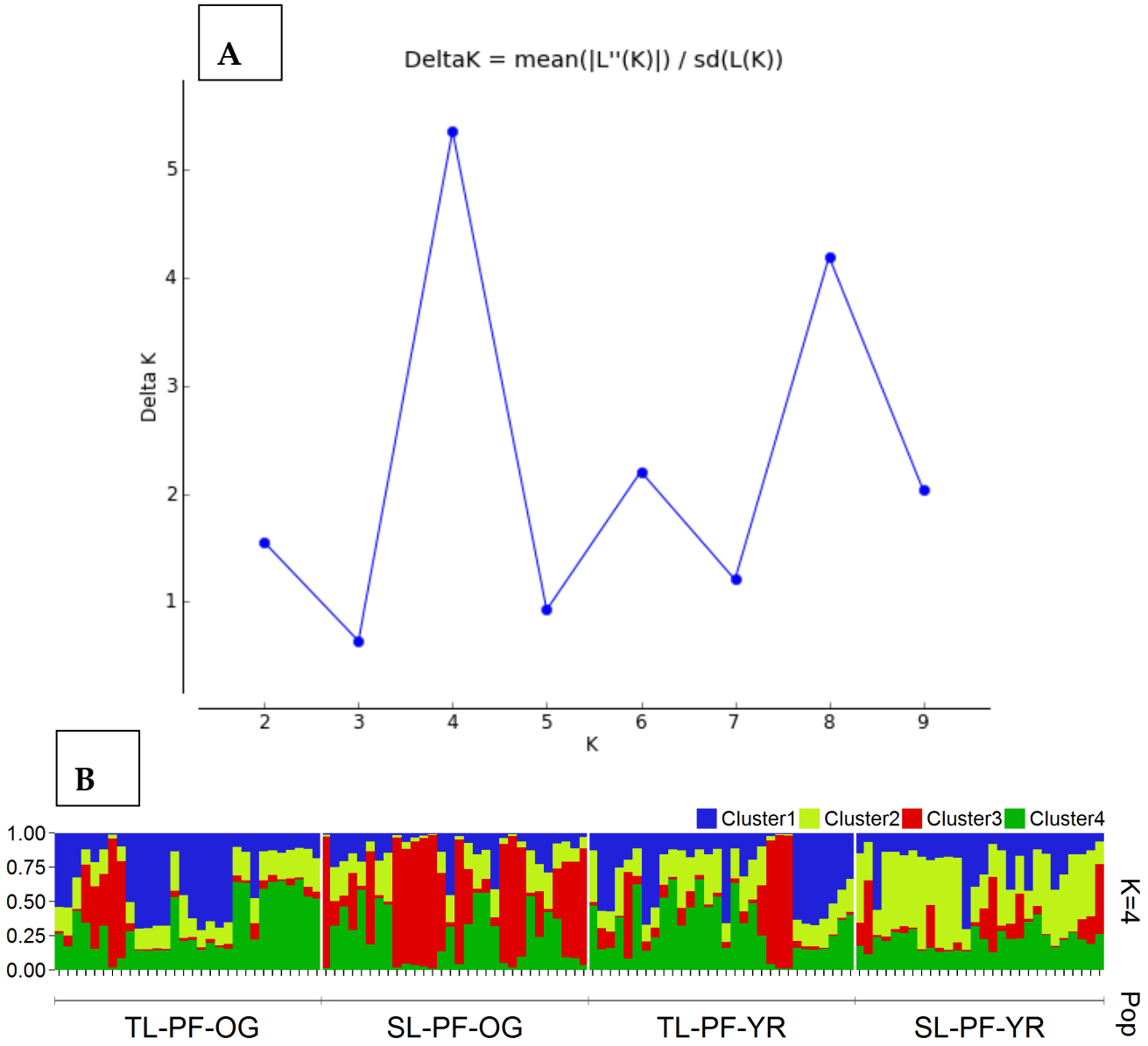

Figure 5. (**A**). A summary scatterplot of DeltaK values for white spruce populations testing K = 2–8 clusters, calculated from the STRUCTURE results using the Evanno et al. [54] method. (**B**). Summary bar plot of estimated membership coefficient (Q) of the studied white spruce individuals from the four populations from the STRUCTURE analysis for K = 4. Each individual is represented by a single vertical line while each color represents one cluster/genetic group. TL-PF-OG, Tibiska Lake post-fire natural old-growth; SL-PG-OG, Sanctuary Lake post-fire natural old-growth; TL-PF-YR, Tibiska Lake post-fire natural young regeneration; SL-PF-YR, Sanctuary Lake post-fire natural young regeneration.

4. Discussion

In this study, we demonstrate that wild forest fires do not adversely affect the genetic diversity, genetic differentiation and genetic structure of white spruce populations. This is most likely the first study of its kind for a boreal conifer species, which is not considered fire adapted/tolerant.

4.1. Genetic Diversity, Inbreeding and Effective Population Size

Considering that wildfires create population bottleneck and fragmentation, and white spruce does not have fire-adaptation traits, such as cone serotiny, wild forest fires may result in the erosion of genetic diversity and increased inbreeding in post-fire populations

and higher genetic differentiation between old growth and post-fire young populations as a result of interacting evolutionary processes, such as genetic drift, selection and gene flow. However, our results demonstrate that wildfires did not adversely affect genetic diversity and increased inbreeding because genetic diversity levels for allelic and genotypic diversity and heterozygosity were similar and not statistically different between post-fire old-growth and post-fire natural young populations (Table 3). Although the inbreeding coefficient (*F*) on average was about 40% higher in the young post-fire regeneration than that in the post-fire old-growth populations, the differences were not statistically significant. This may be due to low statistical power to detect differences resulting from low number of replicates/degrees of freedom. The result of higher *F* values in younger populations is consistent with the notion that heterozygosity is expected to increase with age e.g., [57]. Indeed, the youngest sampled white spruce population from Sanctuary Lake showed the highest inbreeding coefficient (Table 3). Overall, the Tibiska Lake populations showed slightly higher (statistically insignificant) genetic diversity than the Sanctuary Lake populations, suggesting somewhat potential differences in demography and other differences between the sites. Our results also show that the estimates of latent genetic potential, which is the difference between number of alleles per locus and number of effective alleles per locus, are similar between the PF-OG and PF-YR populations of white spruce. Despite the differences in the age of the sampled populations and huge differences in the areas burnt by wildfires (50 km^2 at the Tibiska Lake vs. 1 km^2 at the Sanctuary Lake), the results were consistent between the two sites. This suggests that the age of the populations and the areas of forest burnt by forest fires did not have confounding effects on our results and did not affect the genetic diversity levels of the studied populations. Thus, the results could be considered as consistent between the two study sites.

Our results demonstrating no adverse effects of forest fires on genetic diversity and inbreeding levels are consistent with that reported for sympatric black spruce (*Picea mariana*) [16], and Mediterranean Aleppo pine (*Pinus halepensis*) [18,19,26]. However, somewhat contrasting results were reported in Aleppo pine for the effects of forest fires on spatial genetic structure (SGS). No significant effect of wild forest fires was observed in Aleppo pine on SGS by Gershberg et al. [18]; however, Budde et al. [19] reported a stronger SGS in Aleppo pine populations from the high fire frequency region than from the low fire frequency region. Both black spruce and Aleppo pine are fire-adapted species and retain serotinous cones containing seeds for decades [15,58]. The cones open after fire and release the seeds, which provide a highly diverse gene pool for the next regenerating population. These species are considered as seeders [15,58] because they have both canopy and soil seedbanks, and such traits assist in maintaining population genetic diversity [59].

White spruce is not fire adapted [14] and does not have serotinous cones to have a long-term canopy seedbank, and its seeds do not remain viable in soil for long, which essentially equates to having no soil seedbank [60]. Then how does white spruce maintain genetic diversity after wild forest fires? The mating system, seed and pollen dispersal in white spruce, and the forest cover surrounding the burn area could buffer the negative effects of the population bottlenecks and fragmentation caused by wildfires on genetic diversity and inbreeding in the post-fire generation. White spruce is a predominantly outcrossing species (multilocus outcrossing rate ~94%) and most inbred ovules are aborted before the completion of seed formation, resulting in empty seeds [31]. An extensive and long-distance gene flow through pollen and seed dispersal occurs in white spruce. Although most of the seed dispersal occurs within 45–60 m, seeds could disperse for more than 400 m [60]. White spruce pollen could disperse up to 3000 m in a fragmented landscape [34]. The average minimum pollen dispersal distance in outcrossed matings was found to be 619 m [34]. Furthermore, occasionally, crown seeds may be available on unburnt or partially burnt trees [60]. White spruce is generally killed by fires of any intensity and its reestablishment typically occurs from seeds from trees surrounding the burn area and unburnt trees within the burnt area [60]. The burnt areas at both the Tibiska Lake and Sanctuary Lake study sites are surrounded by intact forest with high white spruce

cover. This could have provided a highly diverse seed source for the post-fire regenerated populations. Thus, the availability of high white spruce forest cover surrounding the burn areas, long-distance seed and pollen dispersal, and selection against inbreds at a very early stage before seed formation may have maintained genetic diversity and inbreeding levels in the post-fire regenerated white spruce populations at the study sites. Furthermore, some white spruce trees may have survived post-fire and may have supplied seed onto the burned sites [61,62]. Our results are consistent with those reported for a fire-sensitive shrub *Persoonia mollis* ssp. *Nectens*, where wildfires did not affect genetic diversity in post-fire seedlings after successive fires [17].

Wild forest fires may reduce N_e by reducing the population size and increasing genetic drift and inbreeding. Although the contemporary N_e, as estimated using the linkage disequilibrium method [46], varied substantially among populations and between the two study sites, our study did not find significant differences between the PF-OG and PF-YR groups at both 0.05 and 0.01 allele frequency critical values. Also, there was no pattern in the N_e estimates. The Tibiska Lake PF-YR showed a lower N_e and the Sanctuary Lake PF-YR showed a higher N_e than their PF-OG counterparts. All of this suggests that wild forest fires did not reduce the N_e in the next generation. This may be the first report of the effects of wildfires on the N_e in forest tree species.

The genetic diversity levels observed in the white spruce populations studied were similar to that reported for white spruce populations from other parts of Saskatchewan (A = 10.6, A_E = 6.5, H_O = 0.490 and H_E = 0.637) [33] and from Alberta (A = 11.23, A_E = 6.77, H_O = 0.532 and H_E = 0.655) [32] based on the same 10 microsatellite markers. Also, genetic diversity for the genomic microsatellites was similar to that reported for white spruce from Alberta [30]. Our study suggests a deficiency of heterozygotes relative to the Hardy–Weinberg expectations in the white spruce populations studied. The *F* values observed in this study are very similar to those observed for white spruce populations from other parts of Saskatchewan (F = 0.210) [33] based on the same 10 microsatellite markers, and from Alberta based on genomic microsatellite markers (F_{IS} = 0.226) [30], but somewhat higher than that observed in white spruce populations from Alberta based on the same 10 microsatellite loci (F = 0.175) [32]. Although white spruce has a predominantly outcrossing mating system [31], and selection against inbreds can occur at an early stage [34], heterozygote deficiency appears to be a common phenomenon in this species [30,32,33,63–65]. This may be caused by several factors such as inbreeding, and genetic drift. White spruce has significant self-fertilization (6.2%) and biparental inbreeding (3.2%) [31]. Furthermore, selection against heterozygotes at five allozyme loci was evident in white spruce from Alberta [65]. For the microsatellite loci, the heterozygote deficiency may also result from the presence of null alleles and the non-detection of heterozygotes for null alleles, as well as the non-detection of all heterozygous combinations of genotypes in a sample size of 30 for hypervariable microsatellites, resulting in the artificial inflation of homozygotes. However, we did not find any evidence for the presence of null alleles in the studied microsatellite loci. The heterozygote deficiency is estimated relative to the Hardy–Weinberg equilibrium (HWE) expectations. However, several HWE assumptions are violated in natural forest tree populations. Further studies are needed to examine the cause of the heterozygote deficiency in this species.

4.2. Genetic Differentiation and Genetic Structure

Forest fires can induce genetic differentiation and genetic structure between pre-fire and post-fire gene pools by reducing population size and creating forest fragmentation and isolation of populations, which can result in genetic drift and a curtailed gene flow. However, our results show that reoccurring wild forest fires have not yet significantly affected the genetic constitution and structure of white spruce populations studied and induced genetic differentiation. The results from the F_{ST}, AMOVA, genetic distance and STRUCTURE analyses were consistent and demonstrate no significant differences in genetic differentiation and structure between the post-fire old-growth and post-fire young

populations of white spruce. The F_{ST} and Nei's [49] genetic distances were similar between the two populations each of the PF-OG and PF-YR groups and between the PF-OG and PF-YR population groups (Tables 4 and 5). The AMOVA showed no genetic differentiation between the PF-OG and PF-YR population groups. There was no separation of PF-OG from PF-YR populations from the STRUCTURE results, which showed four genetic groups admixed within and among four white spruce populations, as well as from the genetic distance-based UPGMA or Neighbor-joining (not shown) trees. The homogeneity in the genetic constitution may have been maintained by extensive and long-distance seed and pollen dispersal and the availability of the seed source from the surrounding high white spruce forest cover as discussed above. Although the highest pairwise F_{ST} and genetic distance were observed between the PF-OG and PF-YR in the Sanctuary Lake populations, the trend was opposite for the Tibiska Lake populations. Similarly, the allele frequency heterogeneity did not show a trend that would indicate substantial allele frequency differentiation between the PF-OG and PF-YR populations. The allele frequency heterogeneity may in part be caused by the occurrence of many rare alleles at microsatellite loci.

4.3. The Selection Effects of Fire

Wildfires can be major selective forces in forests, which can result in the evolution of adaptive traits in forest trees, such as cone serotiny. Genetic variation at 11 loci was found to be associated with cone serotiny in lodgepole pine, *Pinus contorta* Douglas [13]. Furthermore, fire-related signatures of selection were detected at three SNPs in *Pinus halepensis* [19]. Although six of the ten microsatellite loci were from functional genes, we did not find any of the loci showing signatures of selection. Microsatellites are assumed to be selectively neutral markers. Therefore, a large number of SNP markers in functional genes are needed to detect any signatures of selection in response to wildfires in white spruce.

4.4. Implications for White Spruce Genetic Resources Management and Adaptive Potential under Climate Change

We have shown that genetic diversity, population genetic structure, latent genetic potential and effective population size were not adversely impacted by wildfires in the white spruce populations studied. Therefore, wildfires of the magnitude studied do not appear to erode the genetic resources and adaptive potential of white spruce. Thus, the white spruce genetic resources could be conserved, and sustainably managed, and their adaptive potential could be maintained in natural boreal white spruce populations provided there is sufficient natural white spruce cover surrounding the burn areas. If prescribed fires mimic the wildfires in our study, they are unlikely to have negative impacts on genetic diversity of boreal white spruce. However, both the severity and occurrence of wildfires are expected to increase under the ongoing climate change in North America [21,22]. One of the effects of climate change is drought. These factors may impact the genetic resources and adaptive potential of white spruce under climate change by reducing post-fire genetic diversity and the N_e as a result of the reduced white spruce abundance through high tree mortality and limited post-fire recruitment. The post-fire establishment of white spruce depends upon seed availability, seedbed condition, fire intensity and soil characteristics, and forest fires typically create suitable seedbeds, such as bare mineral soil, for white spruce establishment [60,66]. However, the climate-change-induced increased severity and frequency of fires may make the soil and bed conditions unfavorable (such as insufficient mineral soil and moisture) for white spruce seed germination, which can adversely affect post-fire white spruce establishment. If this happens, white spruce population size may reduce and consequently its gene pool could be degraded. Although white spruce occurs in all succession stages of boreal forest, it is considered as a mid-to-late successional species that can germinate in relatively cool, mesic microsites under partial shade [60]. Its seeds may germinate under cool and perhaps moist conditions under other plant species. The extent to which this characteristic could buffer the effects of drought and population and seed source reduction under the changed climate conditions remains to be ascertained.

Nevertheless, white spruce genetic resources should be sustainably managed and conserved to maintain their adaptive potential under changing climate conditions.

5. Limitations

While the present study used white spruce as the study species, the results should be broadly applicable to boreal conifers with similar life history traits. The study sites were located in the Prince Albert National Park. National parks in Canada protect natural phenomena, habitats, biodiversity and ecosystems, and forest commercial activities are not allowed. Thus, our results likely present the actual story of the effects of wildfires on genetic diversity and population structure in the natural system without any anthropogenic confounding effects. However, in the post-fire operational management, salvage logging is normally practiced, which can remove some of the seed source left on unburnt trees and crowns. This in turn can negatively affect post-fire white spruce recruitment, demography and genetic diversity.

Our study was based on the paired comparison at only two sites from the same geographical area; however, the burn size and age of the pre- and post-fire populations were not the same at the two sites. Thus, the sites were not truly replicated. It is not easy to find such study sites where adjacent forest tree populations of successive fire regimes of over 100 years apart exist in natural systems unaffected by anthropogenic activities. Fires are managed in the national parks. Indeed, the helicopter that we used for transportation to the study sites was deployed for monitoring and controlling wildfires. Additionally, it is almost impossible to find the exact replicate of fire severity and a burn area in the case of wildfires. Nevertheless, despite the differences in the burn size and population age, our results were consistent between the two study sites and additional paired comparisons both in the protected and commercially managed forest areas could help to confirm the results on a wider scale and perhaps improve their precision.

6. Conclusions

Our study demonstrates that genetic diversity, genetic differentiation and population genetic structure, inbreeding levels and effective population size are similar between post-fire old-growth and post-fire young populations of white spruce in the Prince Albert National Park of Canada. Therefore, subsequent wildfire regimes of over 100 years apart have not yet adversely affected genetic diversity, N_e, population structure and the adaptive potential of white spruce. However, the climate-change-induced increased severity and frequency of fires may have negative impacts on the genetic diversity, N_e and adaptive potential of white spruce; thus, white spruce genetic resources need to be carefully managed and conserved for future generations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14010157/s1 Table S1. Proportion of genetic variation between post-fire natural old-growth (PF-OG) and post-fire natural young regeneration (PF-YR) population groups, and among and within populations of white spruce from hierarchical AMOVA.

Author Contributions: Overall direction of the study and funding, O.P.R.; Study conception, experimental design and sampling, O.P.R. and M.F.; DNA extraction: M.F.; Microsatellite genotyping: M.S.F.; Data analysis, M.S.F. and O.P.R.; Manuscript writing: O.P.R., M.S.F. and M.F.; Manuscript re-writing and revision, O.P.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data is available from the corresponding author upon request.

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References

1. Reusch, T.B.H.; Ehlers, A.; Hammerli, A.; Worm, B. Ecosystem recovery after climate extremes enhanced by genotypic diversity. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2826–2831. [CrossRef]
2. Roger, F.; Godhe, A.; Gamfeldt, L. Genetic diversity and ecosystem functioning in the face of multiple stressors. *PLoS ONE* **2012**, *7*, e45007. [CrossRef]
3. Salo, T.; Gustafsson, C. The effect of genetic diversity on ecosystem functioning in vegetated coastal ecosystems. *Ecosystems* **2016**, *19*, 1429–1444. [CrossRef]
4. Rajora, O.P.; Zinck, J.W.R. Genetic diversity, structure and effective population size of old-growth versus second-growth populations of keystone and long-lived conifer, eastern white pine (*Pinus strobus*): Conservation value and climate adaptation potential. *Front. Genet.* **2021**, *12*, 650299. [CrossRef]
5. Vandewoestijne, S.; Schtickzelle, N.; Baguette, M. Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. *BMC Biol.* **2008**, *6*, 46. [CrossRef] [PubMed]
6. Markert, J.A.; Champlin, D.M.; Gutjahr-Gobell, R.; Grear, J.S.; Anne Kuhn, A.; McGreevy, T.J., Jr.; Roth, A.; Mark, J.; Bagley, M.J.; Nacci, D.E. Population genetic diversity and fitness in multiple environments. *BMC Evol. Biol.* **2010**, *10*, 205. [CrossRef] [PubMed]
7. Takahashi, Y.; Tanaka, R.; Yamamoto, D.; Noriyuki, S.; Kawata, M. Balanced genetic diversity improves population fitness. *Proc. R. Soc. B* **2018**, *285*, 2017–2045. [CrossRef] [PubMed]
8. Buchert, G.P.; Rajora, O.P.; Hood, J.V.; Dancik, B.P. Effects of harvesting on genetic diversity in old-growth eastern white pine (*Pinus strobus* L.) in Ontario, Canada. *Conserv. Biol.* **1997**, *11*, 747–758. [CrossRef]
9. Rajora, O.P. Genetic biodiversity impacts of silvicultural practices, phenotypic selection in white spruce. *Theor. Appl. Genet.* **1999**, *99*, 954–961. [CrossRef]
10. Rajora, O.P.; Rahman, M.H.; Buchert, G.P.; Dancik, B.P. Microsatellite analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. *Mol. Ecol.* **2000**, *9*, 339–348. [CrossRef]
11. Banks, S.C.; Gary, G.J.; Smith, A.L.; Davies, I.D.; Driscoll, D.A.; Gill, A.M.; Lindenmayer, D.B.; Peakall, R. How does ecological disturbance influence genetic diversity? *Trends Ecol. Evol.* **2013**, *28*, 670–679. [CrossRef]
12. Stocks, B.J.; Wotton, B.M.; Flannigan, M.D.; Fosbert, M.A.; Cahoon, D.R.; Goldammer, J.G. Boreal forest fire regimes and climate change. In *Remote Sensing and Climate Modeling: Synergies and Limitations*; Beniston, M., Verstraete, M.M., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 2001; pp. 233–246.
13. Parchman, T.L.; Gompert, Z.; Mudge, J.; Schilkey, F.D.; Benkman, C.W.; Buerkle, C.A. Genome-wide association genetics of an adaptive trait in lodgepole pine. *Mol. Ecol.* **2012**, *21*, 2991–3005. [CrossRef] [PubMed]
14. Rowe, S. Concepts of fire effects on plant individuals and species. In *The Role of Fire in Northern Circumpolar Ecosystems*; Wein, R.W., MacLean, D.A., Eds.; John Wiley & Sons Ltd.: Toronto, ON, Canada, 1983; pp. 135–1554.
15. Ne'eman, G.; Goubitz, S.; Nathan, R. Reproductive traits of *Pinus halepensis* in the light of fire—A critical review. *Plant Ecol.* **2004**, *171*, 69–79. [CrossRef]
16. Rajora, O.P.; Pluhar, S.A. Genetic diversity impacts of forest fires, forest harvesting, alternative reforestation practices in black spruce (*Picea mariana*). *Theor. Appl. Genet.* **2003**, *106*, 1213–1224. [CrossRef]
17. Ayre, D.J.; Ottewell, K.M.; Krauss, S.L.; Whelan, R.J. Genetic structure of seedling cohorts following repeated wildfires in the fire-sensitive shrub *Persoonia mollis* ssp. *nectens*. *J. Ecol.* **2009**, *97*, 752–760. [CrossRef]
18. Gershberg, A.; Neeman, G.; Ben-Shlomo, R. Genetic structure of a naturally regenerating post-fire seedling population: *Pinus halepensis* as a case study. *Front. Plant Sci.* **2016**, *7*, 549. [CrossRef] [PubMed]
19. Budde, K.B.; Gonzalez-Martınez, S.C.; Navascue´s, M.; Burgarella, C.; Mosca, E.; Lorenzo, Z.; Zabal-Aguirre, M.; Vendramin, G.G.; Verdu, M.; Pausas, J.G.; et al. Increased fire frequency promotes stronger spatial genetic structure and natural selection at regional and local scales in *Pinus halepensis* Mill. *Ann. Bot.* **2017**, *119*, 1061–1072. [CrossRef]
20. von Takach Dukai, B.; Peakall, R.; Lindenmayer, D.B.; Banks, S.C. The influence of fire and silvicultural practices on the landscape-scale genetic structure of an Australian foundation tree species. *Conserv. Genet.* **2020**, *21*, 231–246. [CrossRef]
21. Flaniggan, M.D.; Van Wagner, C.E. Climate change and wild fires in Canada. *Can. J. For. Res.* **1991**, *21*, 66–72. [CrossRef]
22. Flaniggan, M.D.; Stocks, B.J.; Wotton, B.M. Climate change and forest fires. *Sci. Total Environ.* **2000**, *262*, 221–229. [CrossRef]
23. Amiro, B.D.; Flannigan, M.D.; Stocks, B.J.; Todd, J.B.; Wotton, M.B. Boreal forest fires: An increasing issue in a changing climate. In Proceedings of the XII World Forestry Congress, Quebec City, QC, Canada, 21–28 September 2003; Available online: http://www.fao.org/3/xii/0207-b3.htm (accessed on 12 April 2022).

24. Bergeron, Y.; Leduc, A.; Harvey, B.D.; Gauthier, S. Natural fire regime: A guide for sustainable management of the Canadian boreal forest. *Silva Fenn.* **2002**, *36*, 81–95. [CrossRef]
25. Kuuluvainen, T.; Angelstam, P.; Frelich, L.; Jõgiste, K.; Koivula, M.; Kubota, Y.; Lafleur, B.; Macdonald, E. Natural disturbance-based forest management: Moving beyond retention and continuous-cover forestry. *Front. For. Glob. Change* **2021**, *4*, 629020. [CrossRef]
26. Schiller, G.; Ne'eman, G.; Korol, L. Post-fire vegetation dynamics in a native *Pinus halepensis* Mill. forest on Mt. Carmel, Israel. *Isr. J. Plant Sci.* **1997**, *45*, 297–308. [CrossRef]
27. Uchiyamaa, K.; Gotob, S.; Tsudaac, Y.; Takahashib, Y.; Yuji, I. Genetic diversity and genetic structure of adult and buried seed populations of *Betula maximowicziana* in mixed and post-fire stands. *For. Ecol. Manag.* **2006**, *237*, 119–126. [CrossRef]
28. Worth, J.R.P.; Jordon, G.J.; Marthick, J.R.; Sakaguchi, S.; Colhoun, E.A.; Williamson, G.J.; Ito, M.; Bowman, M.J.S. Fire is a major driver of patterns of genetic diversity in two co-occurring Tasmanian paleoendemic confers. *J. Biogeogr.* **2017**, *44*, 1254–1267. [CrossRef]
29. Hosie, R.C. *Native Trees of Canada*; Fitzhenry and Whiteside Ltd.: Don Mills, ON, Canada, 1979.
30. Rajora, O.P.; Mann, I.K.; Shi, Y.Z. Genetic diversity, population structure of boreal white spruce (*Picea glauca*) in pristine conifer-dominated, mixed-wood forest stands. *Can. J. Bot.* **2005**, *83*, 1096–1105. [CrossRef]
31. O'Connell, L.M.; Mosseler, A.; Rajora, O.P. Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (*Picea glauca*) at the landscape level. *Heredity* **2006**, *97*, 418–426. [CrossRef]
32. Fageria, M.S.; Rajora, O.P. Effects of harvesting of increasing intensities on genetic diversity and population structure of white spruce. *Evol. Appl.* **2013**, *6*, 778–794. [CrossRef]
33. Fageria, M.S.; Rajora, O.P. Effects of silvicultural practices on genetic diversity and population structure of white spruce in Saskatchewan. *Tree Genet. Genomes* **2014**, *10*, 287–296. [CrossRef]
34. O'Connell, L.M.; Mosseler, A.; Rajora, O.P. Extensive long-distance pollen dispersal in a fragmented landscape maintains genetic diversity in white spruce. *J. Hered.* **2007**, *98*, 640–645. [CrossRef]
35. Stewart, J.D.; Hogg, E.H.; Hurdle, P.A.; Stadt, K.J.; Tollestrup, P.; Lieffers, V.J. Dispersal of white spruce seed in mature aspen stands. *Can. J. Bot.* **1998**, *76*, 181–188.
36. Padbury, G.A.; Acton, D.F.; Stushnoff, C.T. *The Ecoregions of Saskatchewan*; University of Regina Press: Regina, SK, Canada, 1998.
37. Weir, J.M.H.; Johnson, E.A.; Miyanishi, K. Fire frequency and the age mosaic of the mixed-wood boreal forest in western Canada. *Ecol. Appl.* **2000**, *10*, 1162–1177. [CrossRef]
38. Weir, J.M.H. The Fire Frequency and Age Mosaic of a Mixed-Wood Boreal Forest. Master's Thesis, University of Calgary: Calgary, AB, Canada, 1996.
39. Rajora, O.P.; Mann, I.K. Development and characterization of Novel EST-based single-copy genic microsatellite DNA markers in white spruce and black spruce. *Mol. Biol. Rep.* **2021**, *48*, 2963–2971. [CrossRef]
40. Rajora, O.P.; Rahman, M.H.; Dayanandan, S.; Mosseler, A. Isolation, characterization, inheritance and linkage of microsatellite DNA markers in white spruce (*Picea glauca*) and their usefulness in other spruce species. *Mol. Genet. Genom.* **2001**, *264*, 871–882. [CrossRef]
41. Hodgetts, R.B.; Aleksiuk, M.A.; Brown, A.; Clark, C.; Macdonald, E.; Nadeem, S.; Khasa, D. Development of microsatellite markers for white spruce (*Picea glauca*) and related species. *Theor. Appl. Genet.* **2001**, *102*, 1252–1258. [CrossRef]
42. van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.M.; Shipley, P. Micro-checker, software for identifying, correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **2004**, *4*, 535–538. [CrossRef]
43. Peakall, R.; Smouse, P.E. GENALEX6, genetic analysis in Excel. Population genetic software for teaching, research. *Mol. Ecol. Notes* **2006**, *6*, 288–295. [CrossRef]
44. Rajora, O.P. Effects of forestry practices on genetic diversity: Implications for sustainable forest management and gene conservation. In *Proceedings of the IUFRO Conference, Diversity and Adaptation in Forest Ecosystems in a Changing World, University of British Columbia, Vancouver, BC, Canada, 5–10 August 1996*; Kluwer Academic Publishers: Amsterdam, The Netherlands, 1996.
45. Bergmann, F.; Gregorius, H.R.; Larsen, J.B. Levels of genetic variation in European silver fir (*Abies alba*). *Genetica* **1990**, *82*, 1–10. [CrossRef]
46. Waples, R.S.; Do, C. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. *Evol. Appl.* **2010**, *3*, 244–262. [CrossRef] [PubMed]
47. Do, C.; Waples, R.S.; Peel, D.; Macbeth, G.M.; Tillett, B.J.; Ovenden, J.R. NeEstimator V2: Reimplementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol. Ecol. Resour.* **2014**, *14*, 209–214. [CrossRef] [PubMed]
48. Raymond, M.; Rousset, F. GENEPOP version 1.2: Population genetics software for the exact tests and ecumenicism. *J. Heredity* **1995**, *86*, 248–249. [CrossRef]
49. Nei, M. Estimation of average heterozygosity, genetic distance from a small number of individuals. *Genetics* **1978**, *89*, 583–590. [CrossRef] [PubMed]
50. Excoffier, L.; Smouse, P.E.; Quattro, J.M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* **1992**, *131*, 479–491. [CrossRef]
51. Felsenstein, J. *PHYLIP (Phylogeny Inference Package) version 3.6*; Distributed by the author; Department of Genome Sciences, University of Washington: Seattle, DC, USA, 2005.

52. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
53. Falush, D.; Stephens, M.; Pritchard, J.K. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **2003**, *164*, 1567–1587. [CrossRef] [PubMed]
54. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
55. Earl, D.A.; von Holdt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
56. Foll, M.; Gaggiotti, O. A genome-scan method to identify selected loci appropriate for both dominant and codominant marker: A Bayesian perspective. *Genetics* **2008**, *180*, 977–993. [CrossRef]
57. Ledig, T.F.; Guries, R.P.; Bonefeld, B.A. The relation of growth to heterozygosity in pitch pine. *Evolution* **1983**, *37*, 1227–1238. [CrossRef] [PubMed]
58. Fryer, J. Picea mariana. In *Fire Effects Information System*; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory: Fort Collins, CO, USA, 2014. Available online: https://www.fs.usda.gov/database/feis/plants/tree/picmar/all.html (accessed on 7 April 2020).
59. Templeton, A.R.; Levin, D.A. Evolutionary consequences of seed pools. *Am. Nat.* **1979**, *114*, 232–249. [CrossRef]
60. Abrahamson, I. *Picea glauca*, white spruce. In *Fire Effects Information System*; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory: Fort Collins, CO, USA, 2015. Available online: https://www.fs.fed.us/database/feis/plants/tree/picgla/all.html (accessed on 7 April 2020).
61. Greene, D.F.; Zasada, J.C.; Sirois, L.; Kneeshaw, D.; Morin, H.; Charron, I.; Simard, M.J. A review of the regeneration dynamics of North American boreal forest tree species. *Can. J. For. Res.* **1999**, *29*, 824–839. [CrossRef]
62. Bergeron, Y. Species and stand dynamics in the mixed woods of Quebec'ssouthern boreal forest. *Ecology* **2000**, *81*, 1500–1516. [CrossRef]
63. Alden, J.; Loopstra, C. Genetic diversity and population structure of *Picea glauca* on an altitudinal gradient in interior Alaska. *Can. J. For. Res.* **1987**, *17*, 1519–1526. [CrossRef]
64. Tremblay, N.; Simon, J.P. Genetic structure of marginal populations of white spruce as its northern limit of distribution in Nouveau-Quebec. *Can. J. For. Res.* **1989**, *19*, 1371–1379. [CrossRef]
65. Rajora, O.P.; Dancik, B.P. Population genetic variation, structure, and evolution in Engelmann spruce, white spruce, and their natural hybrid complex in Alberta. *Can. J. Bot.* **2000**, *78*, 768–780.
66. Gartner, F.M.; Lieffers, V.J.; Macdonald, S.E. Ecology and management of natural regeneration of white spruce in the boreal forest. *Environ. Rev.* **2011**, *19*, 461–478. [CrossRef]