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Research article

Recruitment dynamics and population structure of willows in tundra disturbed by retrogressive thaw slump thermokarst on Alaska's North slope

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ABSTRACT

Retrogressive thaw slumps (RTS) are large (> 1 ha) depressions of exposed mineral soil on hillslopes caused by the thaw and displacement of ice-rich permafrost soils in high-latitude regions; since the 1980s the number of RTS observed on Alaska's North Slope has increased by two-thirds. Some RTS in the Toolik Lake area are filled with tall (≥ 0.5 m) willow thickets, likely within decades after disturbance. Tall shrub thickets are different in structure and function from mixed dwarf tundra communities and may have different long-term impacts on ecosystems and wildlife. Currently it is unknown to what degree seedlings versus clonal recruitment contribute to shrub thickets. We assessed size and distribution of clones (modular stems of individuals) using eight microsatellite (SSR) markers to genotype leaf tissue of 223 willow ramets (stems) at two sites: an RTS aged 11–30 years since disturbance, and nearby undisturbed moist acidic tussock (MAT) tundra. Genotypes of known clones from excavated ramets were used to determine the mutation rate of clones. Spatial arrangement of ramets within clones was assessed in 18 × 18 m sampling grids nested at 2 m ("far clones"), 1 m (intermediate), and 0.25 m ("near clones") between ramets. We identified 121 genotypes including 10 clonal genotypes in the RTS, and 63 genotypes including 11 clonal genotypes in the undisturbed MAT. Percent distinguishable was greater than 76% at both sites. Mean spatial distance between clonal ramets was not different at either site but among far clones, ramets were separated 7–16 m downslope in the RTS. *Salix pulchra* was the dominant willow at both sites; a third of willow genotypes in the RTS were identified as *S. glauca*, a disturbance colonizer. Rarer and hybrid species comprised 4–20% of all genotypes but were more abundant in the RTS. At both sites, within-species expected heterozygosity (H_T) ranged from 0.49 - 0.85. Our results suggest: 1) sexual recruitment and low clonal expansion likely explain genetic diversity of willows in disturbed and late-successional sites; and 2) downslope separation of far clones in the RTS suggests disturbance effects; and 3) species richness was higher in the disturbed site.

1. Introduction

Increased biomass and abundance of woody shrubs over the past century has been documented across the Arctic (Sturm et al., 2001, 2005; Myers-Smith et al., 2011). Concurrent with the observed increase in shrub expansion, degradation of ice-rich permafrost soils leading to soil subsidence (thermokarst) has been documented with increasing frequency in Northern Alaska (Jorgenson et al., 2006; Schuur et al., 2007; Bowden et al., 2008; Gooseff et al., 2009; Abbott and Jones, 2015). Disturbance, including thermokarst, may be important for some deciduous shrub species, such as dwarf birch (*Betula nana*) and willow (*Salix* spp.), because they may rely more on seedling recruitment than on storing seed in soil seedbanks to preserve genetic diversity (Huebner and Bret-Harte, 2019). Currently it is not well known to what extent disturbance affects the genetic structure of plant communities dominated by clonal plants, and particularly, how deciduous shrubs may respond in disturbed tundra versus late-successional tundra. Our study investigated sexual and clonal recruitment of arctic willows at two tundra sites on Alaska's North Slope: one disturbed and the other characterized by late-successional vegetation.

Arctic plants have generally been considered clonal due to limited opportunities for seedling recruitment (Billings, 1987; Hermanutz et al., 1989) but subsequent studies suggest that arctic willow populations may have sufficiently high levels of genetic diversity through frequent seedling recruitment to help them withstand rapid change brought on by climate warming (Steltzer et al., 2008; Douhovnikoff et al., 2010). In permafrost regions, thermokarst forms heterogeneous landscapes (Schuur et al., 2007; Becker et al., 2016) including microsites suitable

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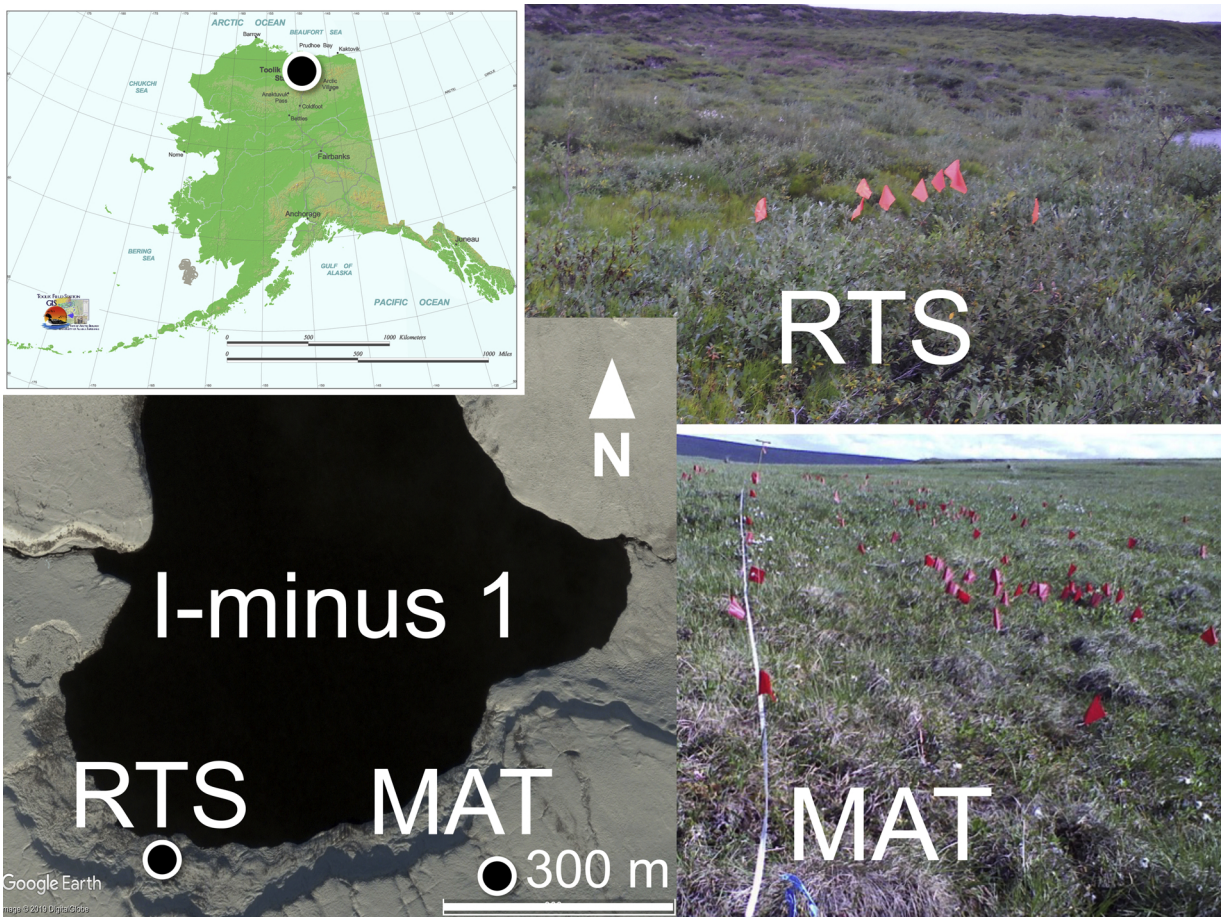

Fig. 1. Sample sites at Lake I-minus 1, North Slope, Alaska (inset maps). A retrogressive thaw slump (RTS) estimated to be middle-aged (11–30 years since disturbance, minimum estimate from woody shrub rings) and moist acidic tussock (MAT) tundra, likely undisturbed for centuries. Tall (≥ 0.5 m) willows (in foreground of top photo) colonize the RTS, compared to low mixed tussock-forb vegetation characteristic of undisturbed MAT (bottom photo). RTS map: Google Earth; Alaska inset map: Toolik Field Station GIS.

for germination and establishment. Microsite conditions in disturbed tundra such as bare soil, increased light and nutrients, and sheltered depressions, have been correlated with increased seedling recruitment of many arctic species (Gough, 2006; Graae et al., 2011; Frost et al., 2013). Increased seedling recruitment in thaw-eroded ground is likely to lead to novel genotypes that could help some populations adapt to rapid change (Petit, 2004) and may especially benefit species that produce short-lived seeds, including willows.

Many willow species also form rooted branches through a process known as layering, as stems become buried in mud or peat or come in contact with moisture (Densmore and Zasada, 1978; Krasny et al., 1988; Jeník, 1994; Collet, 2004) and are thus considered facultatively clonal (Stamati et al., 2007; Douhovnikoff et al., 2010). Studies of arctic plants in disturbed environments should also include a greater understanding of clonal reproduction, as the clonal strategy is likely to affect the success of species and populations coping with rapid change.

The number of retrogressive thaw slump thermokarst (RTS) features observed on Alaska's North Slope is concurrent with climate warming in the Arctic and represents an increase of about two-thirds since the 1980's (Bowden et al., 2008). RTS are often large (> 1 ha; Lantz et al., 2009) depressions of bare soil on hill slopes caused by permafrost thaw and mass soil wasting. Over time, several RTS in this area have become colonized by tall (≥ 0.5 m) deciduous shrubs, primarily willows and dwarf birch (Pizano et al., 2014; Huebner and Bret-Harte, 2019). These shrub thickets are much taller and more homogeneous in plant functional type than undisturbed tundra and likely represent a strong vegetation response to RTS disturbance. The goal of this study is to quantify the relative contribution of sexual and clonal recruitment in the formation of shrub thickets in RTS relative to the surrounding landscape.

Trade-offs between sexual and clonal growth have been observed in vascular plant species in heterogeneous environments (Hutchings and Wijesinghe, 2008; Macek and Lepš, 2008; Li et al., 2018). Clonal expansion may be more likely where conditions are favorable to plant growth (Liu et al., 2009; Schulze et al., 2012) or persistence (Callaghan and Emanuelsson, 1985), and less likely where disturbance frequency and severity limits re-sprouting in favor of seedlings (Klimešová and Klimeš, 2003). It is also possible that conditions in RTS favoring increased seedling recruitment could favor increased clonal growth, which could benefit clonal species and populations, especially if it increases their likelihood of becoming dominant (Dormann and Brooker, 2002). In the Toolik Lake area, deciduous shrubs have shown a strong growth response to nutrients by increasing their biomass and becoming the dominant vegetation in fertilized plots of moist acidic tussock (MAT) tundra (Shaver et al., 2001; Bret-Harte et al., 2004). Similarly, increased clonal expansion following RTS could particularly benefit fast-growing woody species like dwarf birch and willows, leading to changes in MAT communities that may have long-term effects on ecosystem services and plant-animal interactions (Becker et al., 2016; Tape et al., 2010, 2018).

We used genetic markers to compare the genotypic structure of willows in a given area of disturbed and undisturbed tundra to test the hypothesis that willow recruitment will be higher in RTS microsites, which are more heterogeneous and often feature more open space,

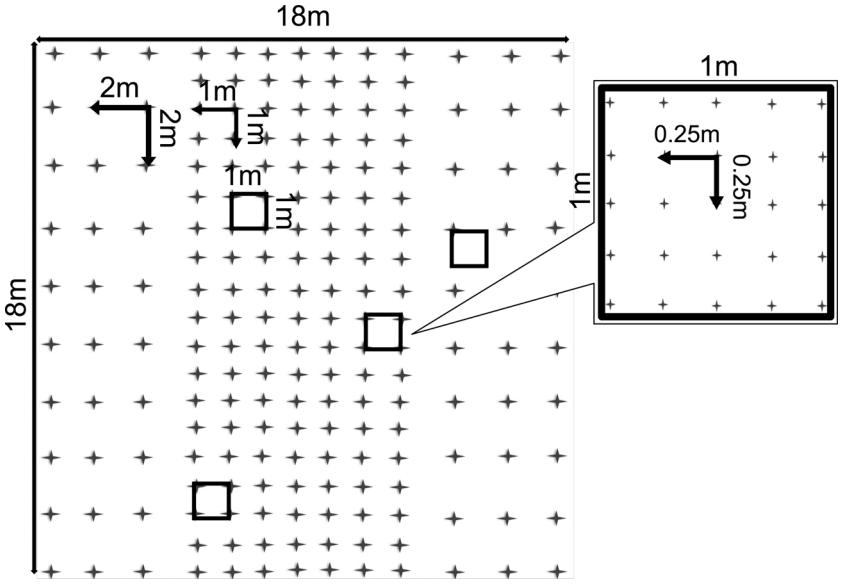

Fig. 2. Nested sampling grid (18 × 18 m) used to sample willow leaves from ramets. Grids were composed of pin flags placed at three spatial scales: 2 m = broad; 1 m = intermediate; 0.25 m = fine. Inset shows fine-scale flag placement in 0.25 m increments. Fine-scale grids were randomly placed inside the sample area. Sample points (stars) are ordered from upper left to lower right in all grids.

light, and available nutrients than undisturbed MAT. If so, we can expect: (1) More willow genotypes and higher levels of genetic diversity in a given area of RTS than MAT (greater sexual recruitment); alternatively, better conditions in RTS will result in greater asexual (clonal) recruitment: fewer genotypes and lower genetic diversity than in MAT; (2) Willow clones will be larger, expanding through more area than in MAT; alternatively, clones in RTS will be more irregularly spaced in RTS than in MAT due to resource patchiness or physical disturbance of clonal bud banks; (3) Willow species richness and abundance will be higher in RTS than in MAT.

2. Material and methods

Study Site

This study was performed near Toolik Field Station (68° 37′ 39″ N, 149° 35′ 51″ W), on Alaska's North Slope (Fig. 1). The climate is typical of the Low Arctic, with cold temperatures (-10 °C year^{-1}) and low precipitation (300 mm year^{-1}), half of which falls as snow (Huebner and Bret-Harte, 2019). Sampling was done at two sample areas near Lake I-minus 1 (Pizano et al., 2014): an area of moist acidic tussock tundra (MAT) likely undisturbed for > 300 years, based on radiocarbon dates of peat (Pizano et al., 2014) and an RTS estimated from shrub ring counts to be 11–30 years since disturbance (Huebner and Bret-Harte, 2019). This is likely a minimum estimate; aerial photos from 1949 show a developed RTS headwall around the south shore of the lake (U.S. Geological Survey, 2019), although a previous study of vegetation and soils indicates the site comprises a chronosequence of older and more recent RTS activity (Pizano et al., 2014). The RTS is located on the south shore of the lake (68° 33′ 11.57″ N, 149° 34′ 16.64″ W, elevation: 817 m, slope: 3, aspect: north) and is characterized by tall (≥ 0.5 m) willow thickets (Fig. 1); the MAT sample area occurs on a level plain above the lake (68° 33′ 11.11″ N, 149° 33′ 34.92″ W, elevation: 838.5 m, slope: unknown, aspect: north) (Bowden et al., 2008) (Fig. 1). The two sample areas represent a subsample of > 2000 leaves collected from eight sample areas at two RTS chronosequence sites (Huebner and Bret-Harte, 2019). We chose the I-minus 1 site because willow species distributions in the area were similar and because the undisturbed control location was representative of MAT communities. Because willow species composition was different at the other chronosequence site, including the undisturbed control location, we could not make direct comparisons across sites and thus replication of disturbance categories was precluded.

MAT is the most widespread tundra type in the area (Bliss and Matveyeva, 1992) and is characterized by tussock-forming sedge *Eriophorum vaginatum* (cottongrass) as well as non-tussock forming sedges in the genus *Carex*, and a heterogeneous mix of plant functional types including herbaceous forbs, evergreen shrubs, and dwarf deciduous shrubs, mostly *Betula nana,* and roughly 10% *Salix,* of which *S. pulchra*, an erect layering species, is the most common (Huebner and Bret-Harte, 2019). Soils are thin and peat-rich and underlain by continuous permafrost (> 200 m depth) with a shallow active layer that thaws to < 0.5 m sub-surface depth in summer (Romanovsky et al., 2002). Compared to MAT, more than half of the plant cover in the RTS sample area was composed of erect willows, mostly *S. pulchra* and *S. glauca,* with < 5% of *S. hastata*, *S. lanata, S. alaxensis* and others, and < 2% of the prostrate species *S. arctica, S. reticulata* and *S. chamissonis*. Less than 25% of cover in the RTS was composed of other species including *B. nana,* horsetails (*Equisetum arvense*), forbs, graminoids, and mosses, with the remainder comprising < 10% of litter and bare soil (Huebner and Bret-Harte, 2019).

Sampling design

In July of 2014 and 2015, we collected fresh fully-expanded leaves from a total of 226 willow ramets (stems) focusing our study on dominant erect species, mainly *S. pulchra* and *S. glauca*. To quantify the frequency of genotyping errors, we randomly selected 10 willows as known clonal controls, determined by partially excavating the shrubs to confirm ramets were produced by the same individual (Douhovnikoff and Dodd, 2003). Samples consisted of 127 individual ramets plus 17 ramets of six known clones (2–5 ramets per known clone) from the RTS, and 71 individual ramets plus 11 ramets of four known clones from the MAT. Samples were taken within one 18 × 18 m nested sampling grid at each sample location. Grids were composed of pin flags nested at broad- to fine-scale distances: 2 m (broad, to identify "far clones"), 1 m (intermediate), and 0.25 m (fine, to identify "near clones") (Fig. 2). 18 × 18 m grids were placed along one side of our original cover transects along hillslopes (Huebner and Bret-Harte, 2019). Replicate 1 × 1 m fine-scale grids subdivided into 0.25 m increments were placed inside the larger sample area using a random numbers generator (3 fine-scale grids inside the MAT area and 4 grids in the RTS). This nested design refines the clone size sampling scheme of Douhovnikoff et al. (2010) by capturing a range of distances between ramets from 2 m to 0.25 m. Where a ramet touched a flag, 2–5 leaves per ramet were collected, identified to species using dichotomous keys (Hultén, 1968;

Table 1
Eight microsatellite loci from primer pairs (developed by [a]Stamati et al., 2003 and [b]Lauron-Moreau et al., 2013) used to genotype 223 willows at a retrogressive thaw slump (RTS, aged ≤ 3 decades since disturbance) and a site of undisturbed (> 300 years) moist acidic tussock (MAT) tundra near Toolik Lake, Alaska. Observed product size is in base pairs (bp). H_O measures observed heterozygosity per locus.

Primer name	Observed product size (bp)	Observed no. of alleles	RTS H_O	MAT H_O
gSIMCT011[a]	298-420	11	0.688	0.768
gSIMCT024[a]	300-342	10	0.475	0.268
gSIMCT035[a]	150-320	13	0.234	0.366
gSIMCT041[a]	173-243	11	0.128	0.049
gSIMCT052[a]	244-378	27	0.489	0.695
PMGC223[b]	166-250	7	0.567	0.634
WPMS15[b]	136-208	11	0.191	0.146
WPMS16[b]	118-248	7	0.496	0.366

Viereck and Little, 1972), and air-dried for processing.

DNA extraction and amplification

Genomic DNA was extracted from 0.2 g leaf fragments using a modified CTAB method of Doyle and Doyle (1987) (Cortes-Palomec and McCauley, 2009). Extracted DNA was quantified on an Epoch Microplate spectrophotometer (BioTek Instruments, Inc.) to assess quantity and quality (ng/ul). Eight microsatellite (SSR) primer pairs were selected out of twelve markers tested to amplify DNA fragments using methods modified from Stamati et al. (2003, 2007) and Lauron-Moreau et al. (2013) (Table 1). PCR amplicons were screened on 2% agarose gels via gel electrophoresis for the presence of bands (Aaij and Borst, 1972). The markers were chosen because they consistently amplified for the majority of our samples.

A protocol modified from Schuelke (2000) was used to amplify microsatellite regions in such a way that only 4 fluorescently-labeled primers were needed, with one for each dye color. This method uses a specific forward primer with a M13 tail at its 5′ end, a specific reverse primer, and a fluorescently-labeled M13 primer (5′-AGGGTTTTCCCA GTCACGACGTT-3′). PCR reactions were carried out using the KAPA 3 G Plant PCR Kit (Kapa Biosystems, Inc.) in 10 µL total volume reactions, each containing approximately 50 ng of template DNA, 5.0 u L Kapa 3 G Plant PCR buffer (2x, containing MgCl2 and dNTPs), 0.10 µM each of forward and reverse primers, 0.10 µM of fluorescent-labeled M13 primer (Integrated DNA Technologies), and 2.5 U/µL KAPA 3 G Plant DNA Polymerase (Kapa Biosystems, Inc.). A Bio-Rad PTC 240 thermal cycler (Bio-Rad Laboratories, Inc.) was used to amplify DNA fragments under the following conditions for all primers: initial denaturation at 95 °C for 3 min followed by 35 cycles of 20 s at 95 °C; 15 s at 54 °C, 30 s at 72 °C followed by a final extension at 72 °C for 5 min. Amplified fragments were visualized on agarose gel (2%). Fragment sizes were quantified on an ABI 3730XL Genetic Analyzer (Applied Biosystems) in 10 u L of formamide and 0.5 u L of GeneScan 600 LIZ dye size standard (Applied Biosystems).

Genetic analysis

Alleles were called using a combination of Peak Studio (McCafferty et al., 2012); the R package MsatAllele (Alberto, 2009) (R Core Team, 2019); and STRand (Toonen and Hughes, 2001). Three individuals produced no data for ≥ 5 loci and were omitted from analysis, on the assumption that DNA was of low quality. Because hybridization among different species is common in *Salix* (Argus, 1997), the willows we studied were likely polyploid, and to some extent allopolyploid, however, the majority of samples produced 1–2 peaks in capillary analysis. Amplification of alleles from a single parent species is known to occur in allopolyploid willows (King et al., 2010), thus we analyzed our samples as though they were diploids (single peaks were scored as homozygous for that locus). 3% of samples produced more than two peaks, but because these individuals also produced peaks common to the majority of samples, extra peaks were excluded from analysis.

Genotypes were further analyzed as follows: (1) a ramet-based model, in which all known clonal ramets (repeat genotypes) were included (Douhovnikoff and Leventhal, 2016) to determine number and spatial arrangement of clones and clonal diversity within each population using GENODIVE 2.0b23 (Meirmans and Van Tienderen, 2004); and (2) a genet-based model, in which clonal genotypes were represented only once (Douhovnikoff and Leventhal, 2016), used in GENODIVE to compare clonality estimates obtained in (1), and to assign each ramet to a species and determine levels of admixture using STRUCTURE 2.3.4 (Pritchard et al., 2000), as species identification based on morphology is notoriously difficult in willows.

In this study, we defined a clone as two or more ramets sharing an identical multilocus genotype, and clonality as the number or proportion of genotypes shared between ramets. Tests of clonality were conducted using GENODIVE's Assign Clones algorithm, which makes pairwise comparisons across increasing thresholds of clonality (allelic differences allowed between ramets assigned to the same clone) from 0 (no differences allowed) until all ramets are assigned to the same clone. We used the stepwise mutation option appropriate for SSRs (Meirmans and Van Tienderen, 2004). In order to determine an appropriate threshold of clonality, we first examined the distribution of the number of ramet pairs assigned to the same clone under increasing threshold of clonality. For both RTS and MAT the first peak in the distribution is at 0 (Figure S1). According to Meirmans and Van Tienderen (2004), the first peak is likely to represent distances between ramets of the same clone, whereas later peaks represent distances between ramets that are not clones of each other. We then compared clonal assignments at threshold values of 0 and 1 because they are similar to the 0.983 threshold value calculated for willows by Douhovnikoff and Dodd (2003). Although our known-clone controls showed no evidence of somatic mutations at either site, we chose the 1 threshold value for final analysis to correct for possible scoring errors or somatic mutations in some samples (Arnaud-Haond et al., 2007). We repeated the analysis using the genet-based approach (each clonal control genotype was represented once) and found the clonal assignments did not change. For comparisons between sites the Clonal Diversity algorithm in GENODIVE was used with bootstrapping to detect significance at $P < 0.05$ in 999 permutations, with the "subsampling to match population size" option used, and Nei's (1987) G_{st} diversity values corrected for sample size. With clonal reproduction, clonal diversity will be reduced compared to sexual reproduction. GENODIVE tests the null hypothesis that all reproduction is sexual, by comparing observed genotype frequencies to those expected under random mating (Meirmans and Van Tienderen, 2004). Nei's (1987) diversity index (expected heterozygosity) measures the amount of genetic variation based on allele frequencies, with values between 1 (high diversity) to 0 (no diversity). For spatial relationships of clonal ramets in sampling grids, the Assign Clones algorithm in GENODIVE was used for each site using the same methods as above (Meirmans and Van Tienderen, 2004).

Species assignment and determinations of species admixture were carried out using the admixture model in STRUCTURE 2.3.4 (Pritchard et al., 2000). The dataset was reduced to represent only sexually produced genotypes thus each clone was included once. Because there is no information in the literature specifying otherwise, all loci were assumed to be unlinked (Stamati et al., 2003, 2007; Lauron-Moreau et al., 2013). Allele frequencies were not assumed to be correlated. PCR reactions with no peaks were assumed to be homozygous for null alleles, since we repeated the PCR reactions twice. Morphological species assignments were used as prior information to assist in clustering, as is suggested for weakly informative datasets. Different clusters (K) were allowed to have different alpha values (POPALPHAS = 1), allowing for asymmetric admixture, following recommendations by Wang (2017). The

burn-in for the Markov Chain was set to 100,000 steps, and we collected data from 100,000 subsequent steps. To identify the K with the highest probability of explaining the data, we compared the mean likelihood of models with K = 1–10, repeated 5 times each. The best value of K was chosen as the smallest value of K where the mean log likelihood of the model plateaued, as suggested by the STRUCTURE manual and Wang (2017) (Figure S2). Individuals with ≥ 30% membership in more than one cluster were designated as hybrids. Species composition maps at each site were created from STRUCTURE results using PhyloGeoViz (Tsai, 2011) and DISTRUCT (Rosenberg, 2004).

To quantify species diversity at each site we used two standard ecological indices for each population: the Shannon Diversity Index and Simpson's Index (Magurran, 1988). The Shannon Diversity Index (H) is expressed as the proportion of each species multiplied by the natural logarithm of each proportion ($H' = - p_i \Sigma \ln p_i$) with higher values indicating greater species richness and evenness. Simpson's Index (D) is expressed as the probability that any two individuals drawn from a community belong to the same species ($D = \Sigma \ln p^2_i$) with higher values indicating fewer, more dominant species (Magurran, 1988).

Statistical significance of mean spatial distance between ramets within clones at each site was determined using the Welch Two Sample t-test of unequal variance. We chose this test due to the difference in the number of clonal ramets at each site. To avoid bias, clonal controls were excluded from spatial distance estimates because their ramets were sampled non-systematically.

3. Results

Clonal assignments in the RTS stayed the same at the 0 and 1 threshold, but in MAT assignments increased from 7 clonal groups at the 0 threshold to 11 groups at the 1 threshold (Figure S1). Known-clone controls did not change and are excluded from these counts. We identified 121 genotypes in the RTS including 10 clones, versus 63 genotypes in MAT including 11 clones. Percent distinguishable (PD), the number of genotypes over the total number of samples, was ostensibly higher in the RTS than MAT, likely due to 23 more alleles found in RTS willows compared to MAT (Table 2), but PD was greater than 76% at both sites and not statistically different (Two Sample z-test Comparing Two Proportions: χ -squared = 0.231, df = 1, P = 0.128; Table 2). Clonal genetic diversity at the two locations was similar and not different from levels of genetic diversity due to random mating (bootstrapped confidence intervals P = 0.570; Table 2). There was no statistical difference between sites in the total number of ramets assigned to clones or in the number of clonal groups (Welch Two Sample t-test of the number of clonal groups: t = -0.7361, df = 18.998, P = 0.722; Table 2). Within spatial categories, the MAT had more near clones than the RTS, but other categories were similar (Fig. 3). Likewise, mean spatial distance between ramets within clones was not different between sites: 4.5 (± 1.9 SEM) and 1.8 (± 0.7 SEM) m for RTS and MAT, respectively (Welch Two Sample t-test: t = -1.10, df = 16.265, P = 0.289). The larger standard of error around the mean

Table 2
Comparison of genotypes derived from 8 microsatellite loci of willows sampled at two sites: a retrogressive thaw slump (RTS) aged ≤ 3 decades since disturbance, and nearby moist acidic tussock (MAT) tundra likely undisturbed for centuries. PD is percent distinguishable (n genotypes /n samples), total alleles is the sum of alleles. Clonal diversity is based on Nei's (1987) G_{st} index of diversity. Numbers of genotypes and clones (using the Welch Two Sample t-test), PD (using the Two Sample z-test Comparing Two Proportions) and clonal diversity (using bootstrapped confidence intervals, n = 999 permutations) were not statistically different between populations (each at $P > 0.05$).

Site	Samples	Genotypes	Clones	PD (%)	Total alleles	Clonal diversity
RTS	141	121	10	85.8	93	0.997
MAT	82	63	11	76.8	70	0.993

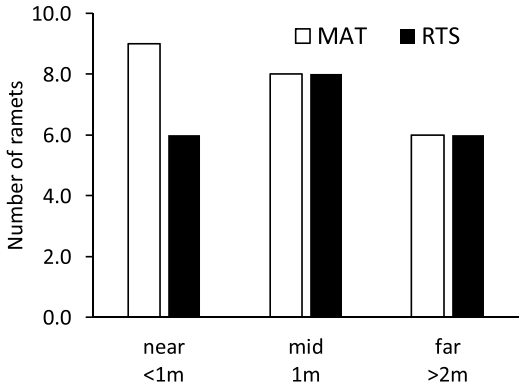

Fig. 3. Total number of clonal ramets by spatial category (in meters) in undisturbed moist acidic tussock (MAT) tundra and retrogressive thaw slump (RTS) aged ≤ 3 decades since disturbance.

spatial distance was likely due to greater spatial variation between ramets within clones in the RTS: 0.5–16 m (Fig. 4) versus 0.5–9 m in MAT (Fig. 5). Ramets within far clones were separated 7 – 16 m downslope in the RTS (Fig. 4); in the MAT, ramets within far clones were separated 2.5–9 m apart at same elevation on comparatively level ground (Fig. 5).

STRUCTURE analysis of microsatellite results suggests the dataset is best explained by 2–5 species (K clusters). Evanno et al.'s (2005) method to estimate the number of clusters suggests K = 2, as this shows the greatest change in the log likelihood of the models, however, K = 5 has the maximum likelihood overall (Figure S2). Although species assignments were somewhat less reliable for the rare clusters identified when K = 5, morphology strongly suggests that there were ~ 5 willow species present in our sample. *Salix pulchra* was the most common willow species in the area, consisting of 51% and 84% of genotypes at the RTS and MAT, respectively (Fig. 6). *Salix glauca* was the next most common species, comprising 28% at the RTS, but only 6% at the MAT (Fig. 6). Species composition of clones, including clonal controls, reflected overall species composition. In RTS, half the ramets were identified as *S. pulchra* and 40% as *S. glauca* or *S. glauca* hybrids; by contrast, all clonal ramets in MAT were identifed as *S. pulchra*. Species assignment by STRUCTURE for *S. pulchra* and *S. glauca* was generally consistent with morphological species assignment. Three other clusters, which we designate as *Salix* spp. 3–5, are likely *S. hastata*, *S. lanata* L. subsp. *richardsonii* (Hook.), or others, based on hirsuteness of twigs and other morphological characters. Less common species and hybrids comprised 4–20% of all genotypes across sites but were more abundant in the RTS (Fig. 6; Table 3). However, there is not a strong correspondence between STRUCTURE assignments and morphological species assignments for these clusters.

Expected heterozygosity of individuals in each cluster (H_T) ranged from 0.49 for common species to 0.85 for less common species (Table 3). The Shannon Diversity Index estimate of species diversity in the RTS was roughly twice that of MAT, while Simpson's Index showed the opposite trend, indicating fewer, more dominant species in MAT (Table 3). These results are corroborated by the area distributions of less common and hybrid genotypes at the two sites (Figure S3).

4. Discussion

Prediction (1): If RTS microsites are more productive and heterogeneous than in undisturbed MAT, we can expect to find more willow genotypes and higher levels of genetic diversity in a given area of RTS than MAT (greater sexual recruitment); alternatively, better conditions in RTS will result in greater asexual (clonal) recruitment: fewer genotypes and lower genetic diversity than in MAT.

Response of clonal plants to disturbance is expected to vary

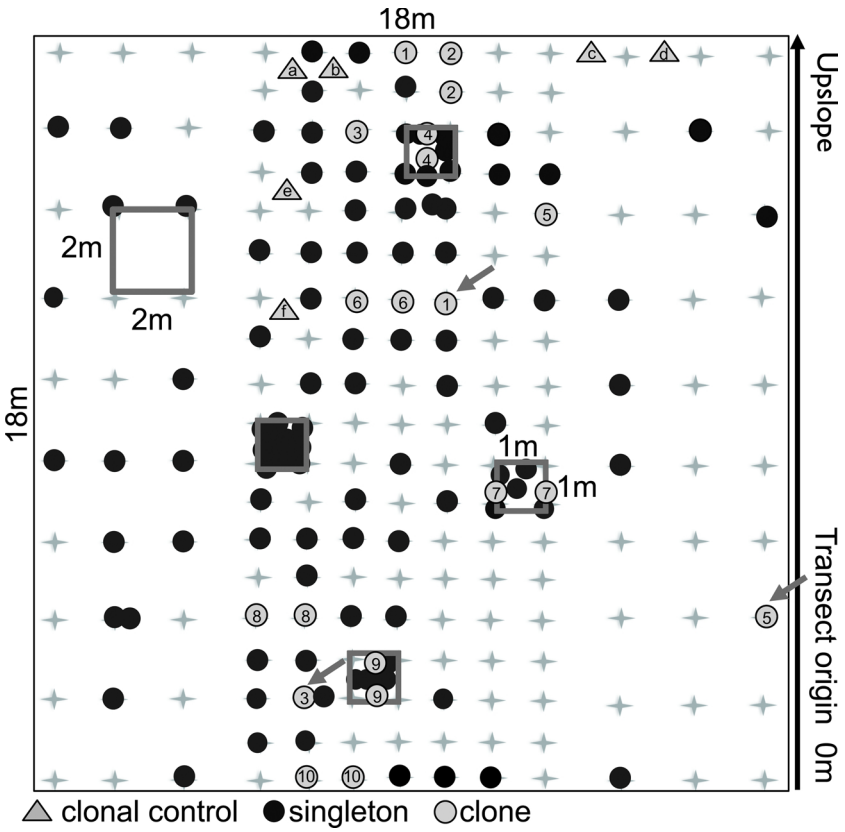

Fig. 4. Spatial relationships of willow clones (matching clonal genotypes are grouped by number) and singletons (black circles) genotyped with 8 microsatellite loci in a retrogressive thaw slump (RTS) aged ≤ 3 decades since disturbance. Triangles show location of 2–4 excavated ramets of known clonal controls (known clonal genotypes are grouped by letter). Arrows indicate position of clonal ramets separated downslope.

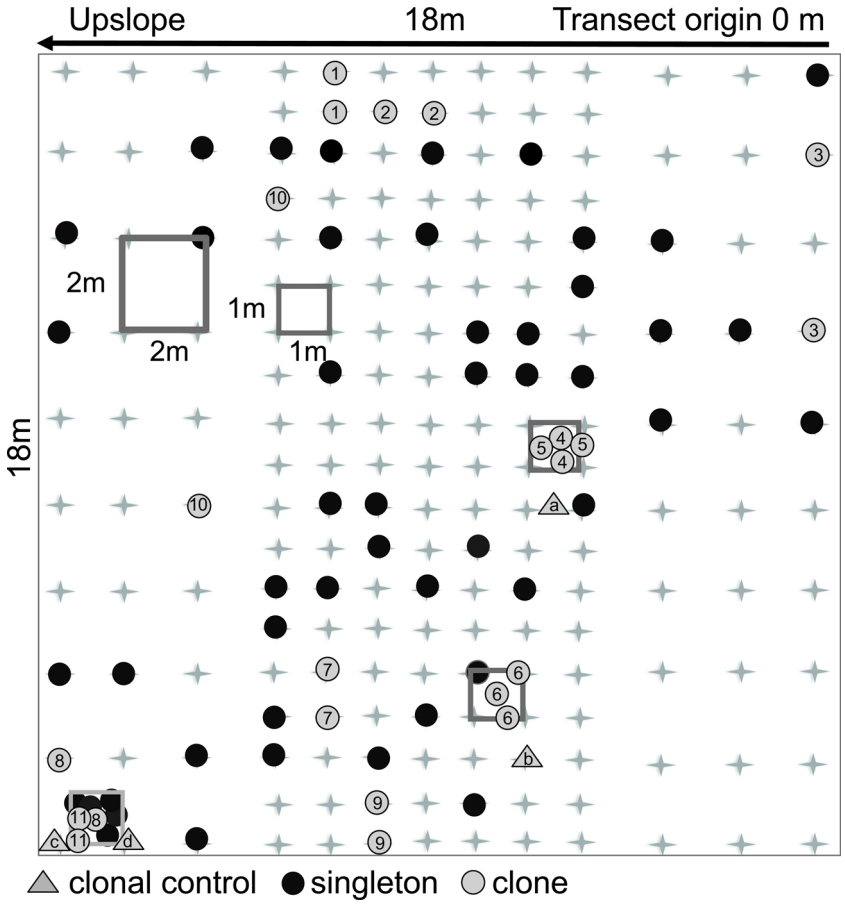

Fig. 5. Spatial relationships of willow clones and singletons (genotyped with 8 microsatellite loci) in moist acidic tussock (MAT) tundra undisturbed by RTS erosion. Symbols and numbers follow the convention used in Fig. 4.

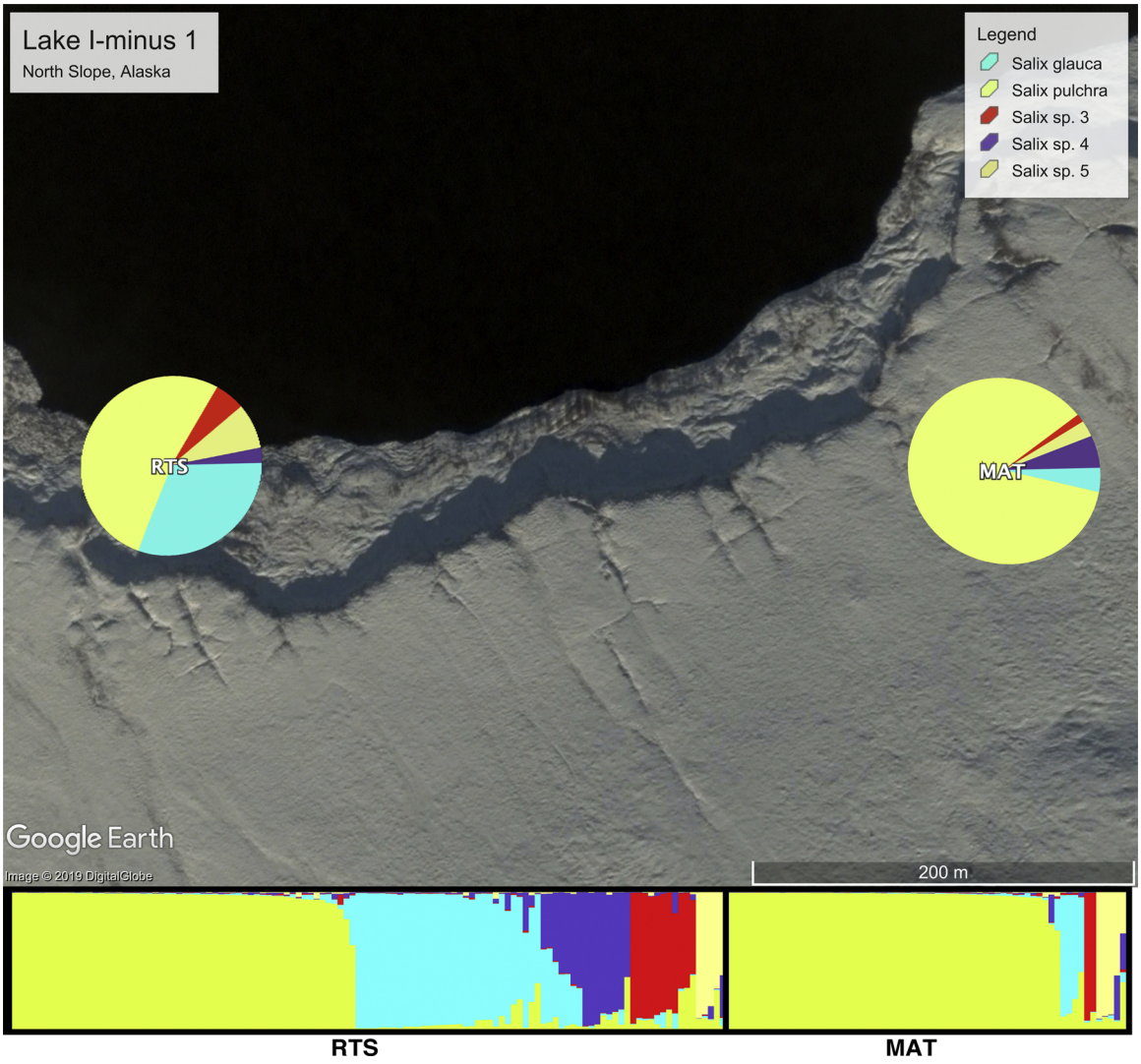

Fig. 6. Species composition of willows at Lake I-minus 1 on Alaska's North Slope genotyped from ramets in 18 × 18 m nested grids in retrogressive thaw slump (RTS) aged ≤ 3 decades since disturbance, and moist acidic tussock (MAT) tundra undisturbed by RTS erosion. Pie charts show species composition by population, line plot (below map) shows same information by individual (vertical lines). *Salix* spp. 3, 4 and 5 are likely *S. hastata*, *S. lanata* L. subsp. *richardsonii* (Hook.), or others. Map: Google Earth.

Table 3
Numbers of individuals assigned to each species of *Salix* and genetic diversity (expected heterozyosity H_T) among individuals of each species derived from STRUCTURE analysis (K = 5 clusters) of willow SSR genotypes sampled at two sites: a retrogressive thaw slump (RTS) aged ≤ 3 decades since disturbance, and nearby moist acidic tussock (MAT) tundra likely undisturbed by RTS for centuries. Putative hybrids are individuals with ≥ 30% membership in more than one cluster.

	Site			
	RTS		MAT	
Taxon	*n*	H_T	*n*	H_T
Salix pulchra	61	0.541	56	0.490
Salix glauca	34	0.632	4	0.846
Salix sp. 3	9	0.765	2	0.848
Salix sp. 4	12	0.735	1	0.848
Salix sp. 5	4	0.814	4	0.846
Putative hybrids	14	–	3	–
Shannon's Index (*H*)	1.239		0.654	
Simpson's Index (*D*)	0.355		0.707	

depending upon the species, disturbance type, and severity (Klimešová and Klimeš, 2003; Bret-Harte et al., 2013). The high percent distinguishability and low clonality we found at both sample locations agrees with other studies of genetic diversity of willow populations in the Arctic and other regions (Steltzer et al., 2008; Alsos et al., 2009; Stamati et al., 2007; Douhovnikoff et al., 2010; Sochor et al., 2013) and suggests that clonal spread of willows in this area is somewhat limited. In our previous study, we found greater seedling recruitment in the field and in greenhouse germination trials of RTS seedbanks from this site (Huebner and Bret-Harte, 2019), suggesting that sexual recruitment plays an important role in the revegetation of RTS-disturbed tundra. Our genotyping results further suggests that recruitment also has long-lasting effects on the genetic diversity of willow populations in late-successional tundra.

Many Alaskan willow species in high-stress environments exhibit a creeping habit (Viereck and Little, 1972). We expected that as seedling recruitment decreases over time under limiting conditions in MAT, we would see more clonal willow ramets in undisturbed MAT relative to RTS, but we found no evidence of a shift from primarily sexual to primarily asexual strategies at either site. The number of clones and levels of genetic diversity were relatively similar in both RTS and MAT, suggesting both populations are sexually derived. Our previous study of the

area found greater seedling recruitment of RTS seedbanks with no difference in seed rain between disturbed and undisturbed tundra (Huebner and Bret-Harte, 2019). We propose that if seed dispersal is more or less equal, sexual recruitment may have declined at undisturbed MAT due to a decrease in available space, light and nutrients. Genetic diversity is more likely to decrease over time because of clonal spread and decreased opportunities to replace genotypes that die off. Thus, we expected to see more clonal growth and fewer genotypes in the MAT.

Alternatively, we considered the possibility that conditions in RTS 2–3 decades after disturbance might promote greater clonal expansion of willows than the more limiting conditions of undisturbed MAT, but we found no difference in overall clonality in a given area of RTS versus MAT. These results are initially somewhat surprising, given that greater allocation to clonal growth rather than to sexual organs has been demonstrated in some clonal species when nutrient levels are adequate (Liu et al., 2009). In our previous study of I-minus 1 we found that compared to undisturbed MAT, available soil N was up to six times higher in the RTS used in this study, and shrubs were twice as tall and nearly four times as wide (Huebner and Bret-Harte, 2019). How could two seemingly different physical environments have resulted in such similar genetic outcomes? If clonality is a response to conditions that reduce seedling recruitment, our results show, on one hand, that willows in RTS can be *at least* as clonal as those in mature MAT, and 2 or 3 decades since disturbance might be enough time for arctic willows to transition from sexual to clonal reproduction as microsite conditions change. On the other hand, current conditions under climate warming may lead to tundra disturbance that favors sexual recruitment over clonal growth. The similarities we found at the MAT site could be the result of a different type of recruitment scenario characterized by continuous dieback of clonal ramets during extreme historical conditions at a time when sexual recruitment was likely more sporadic and clonal growth was the more common strategy.

In comparison to prostrate willows, erect species may be somewhat less efficient at forming clonal ramets because branches may require sufficient burial to stimulate rooting. If burial is an important stimulus to clonal growth of erect willows, however, we would expect to find many more clones than we did, especially in the RTS. Viereck (1966) states that MAT develops once deep moss layers accumulate, allowing lateral expansion of shrubs such as dwarf birch and blueberry, but not willows, which are early-successional species of disturbed ground. This is supported by Argus (2006) who states that riparian willows have brittle stems that are easily dispersed by seasonal flooding and beaver activity, whereas layering species are known to form distinctive clonal patches because opportunities for layering may be more limited. Our results agree with this in part, in that most ramets within clones at our sites were spaced at relatively short distances of 1 m or nearer, suggesting processes that stimulate layering in tundra environments may be more spatially and temporally variable than in seasonally-disturbed habitat.

Prediction (2): If RTS microsites are more productive and heterogeneous than in undisturbed MAT, willow clones will be larger, expanding through more area than in MAT; alternatively, clones in RTS will be more irregularly spaced in RTS than in MAT due to resource patchiness or physical disturbance of clonal bud banks.

Although it is likely that the greater vertical and lateral growth of RTS shrubs in our previous study can be attributed in part to adequate nutrient supply and other microsite conditions, such as shelter from wind provided by RTS depressions (Huebner and Bret-Harte, 2019), we did not find that greater shrub growth in RTS resulted in larger clone sizes or more clones in a given area of RTS. We did find a greater range in spatial separation across ramet categories in the RTS, and among far clones, we found a somewhat unusual physical separation between ramets within clones that occurred on a slope gradient in the RTS but not in the MAT. This latter result suggests that physical disturbance was responsible for the separation of these ramets in the RTS.

Accurate estimation of clone size involves many parameters, including the appropriate sampling distance between ramets, the number of genetic markers to use, and the level of sampling replication. Previous studies of arctic and alpine willows using roughly the same number of microsatellite markers (3 to 4) produced different assessments of clonality that appeared to be based largely on the chosen spatial scale. With minimum sampling distances $\geq$ 2 m between ramets, more singleton genotypes were found (Douhovnikoff et al., 2010); whereas more clonal genotypes were found sampling 0.25 m between ramets (Reisch et al., 2007). Using a subset of three microsatellite markers used in this study, Reisch et al. (2007) found a PD of 18%, suggesting the population was composed mainly of clones. By sampling within a single 3 × 3 m plot it is unknown whether clonality was accurately estimated or overestimated. By comparison, ramets sampled at our sites at 0.25 m distances were replicated 3 to 4 times each in 1 × 1 m plots (although we did not replicate our 18 × 18 m plots), and our PD values, using eight markers, ranged from 25 to 100%. As with optimization of spatial distance to account for clones versus singletons, the number of genetic markers also appears to be critical. The eight markers we chose consistently performed well for the majority of our samples, including known clonal control ramets in repeated tests. The low levels of clonality and high levels of genetic diversity we found in this study, using a threshold of clonality calculated from known genotypes of true clones and siblings (Douhovnikoff and Dodd, 2003), suggest that these willow genotypes mainly originated from seedling recruitment.

It was surprising that ramets within far clones were separated downslope in the RTS and along relatively level ground at the same elevation in the MAT. Previous research has found disturbance may alter the spatial arrangement and species composition of vegetative propagules, or bud banks, of clonal plants, leading to plant community changes in some areas (Du et al., 2013) but not in others (VanderWeide and Hartnett, 2015). Other research suggests the effect of disturbance to limit the ability of clonal propagules to re-sprout may be overestimated (Klimešová and Klimeš, 2003). In our previous study of RTS-disturbed tundra we did not account for the role of non-sexual propagules, but here we find evidence, in support of other research (Du et al., 2013), of disturbance effects upon the existing bud bank. Given the destructive nature of RTS, we believe that mass soil wasting likely caused the downslope translocation of viable clonal fragments, which we inferred because we ran our sampling grids along the same slopes as our original cover transects (Huebner and Bret-Harte, 2019), resulting in downslope separation of identical genotypes in the RTS but not in the MAT. The fact that all of the far ramets within clones in the RTS were located downslope appears to support downslope translocation, which may be a unique feature of RTS to alter the spatial arrangement of clones and redistribute extant genotypes across the landscape.

Prediction (3): If RTS microsites are more productive and heterogeneous than in undisturbed MAT, willow species richness and abundance will be higher in RTS than in MAT.

Without the ability to replicate willow population structure in multiple RTS features and controls of similar age, type, and vegetation composition, our assessment of clonal growth response in disturbed and undisturbed tundra is somewhat limited. However, the key difference we found was in the abundance of genotypes identified as *S. glauca,* which is regarded as a pioneer species (Viereck and Little, 1972), and which was nine times more abundant in the RTS than in MAT. The spatial arrangement of ramets illustrates not only patterns of clonal spread and fragmentation, but the underlying patterns of recruitment that lead to differences in species composition in tundra succession (Figure S3). It may be unsurprising that dominant species, colonizing species, less common species and hybrids were all more abundant in the RTS than in the MAT site. Their spatial arrangement suggests the patchy nature of recruitment as seed rain interacts with microsite (Eriksson and Fröborg, 1996). In comparison to the RTS arrangement formed within decades, the predominance of mostly unhybridized *S. pulchra*

ramets at the MAT site implies population persistence, and to some extent, distinctive clonal groups formed through layering (Argus, 2006). It is unknown whether RTS-disturbed tundra represents a shift to tall shrub tundra or a mid-successional stage of MAT with its characteristic stands of low-growing *S. pulchra*. RTS are patchy disturbances that appear to be confined to hillslope tundra on Alaska's North Slope and similarly hilly areas in the Canadian Arctic and elsewhere. If RTS become more frequent in the future, MAT communities may feature patchworks of RTS scars characterized by tall willow clumps of different species that persist for decades.

Species analysis suggests that *S. pulchra* is the dominant willow species in disturbed and undisturbed tundra, likely because it is more persistent than other willow species. The earliest fossil records of modern *Salix* are from North America in the early Eocene (Collinson, 1992), and pollen records from the Kobuk Valley in the Alaskan Arctic indicate that willows were dominant in that area during the middle and late Wisconsin glaciation (Hamilton et al., 1993). This evidence, and radiocarbon analysis of the I-minus 1 sites (Pizano et al., 2014), suggests that these plant communities have persisted for centuries.

5. Conclusions

Our work suggests that genetic diversity conferred by seedling recruitment may have long-lasting effects within clonal plant populations, particularly in environments where recruitment and growth are limited. It also suggests that clonality of willows in recently-disturbed and late-successional tundra is not vastly different, likely because tundra willows may be exposed to conditions that stimulate root layering less frequently than riparian species. Interestingly, the downslope movement of clonal propagules across wide spatial distances in RTS was suggested in our results. Analysis of additional sites may be necessary to more thoroughly investigate the dynamics of recruitment within these expanding physical features.

Although we focused on a small subset of thermokarst lakes on Alaska's North Slope, our study provides results that address the same concerns raised by more comprehensive studies, namely: how do plants respond to rapid anthropogenic change, and how do their responses affect global biodiversity and future ecosystem health? The human footprint in Alaska is still relatively small compared to other regions, but the rise of large and severe disturbances such as RTS and tundra wildfires in the Alaskan Arctic (Bret-Harte et al., 2013), the latter disturbance similar in size and severity to the recent devastating wildfires in California (Nauslar et al., 2018), urgently illustrate the importance of the continued study of vegetation responses to disturbance associated with global warming. An expanded understanding of seed bank and bud bank responses to disturbance is critical in being able to predict whether susceptible landscapes have sufficient genetic resources to recover, or whether they will undergo radical shifts in species composition, structure and function, and genetic diversity. Our study reveals useful insights about arctic shrub response to what is likely to become a more frequent type of tundra disturbance.

Author contributions

DCH, VD, MSBH and DEW conceived of the ideas and designed the methodology; DCH conducted field sampling and laboratory work with laboratory assistance from DEW; DCH and DEW analyzed the data, with assistance from VD, MSBH, DW, and others. All authors contributed critically to the drafts and gave their final approval.

Declaration of Competing Interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

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References

Aaij, C., Borst, P., 1972. The gel electrophoresis of DNA. Biochim. Biophys. Acta 269, 192–200.
Abbott, B., Jones, J., 2015. Permafrost collapse alters soil carbon stocks, respiration, CH4, and N2O in upland tundra. Glob. Change Biol. Bioenergy 21, 4570–4587. https://doi.org/10.1111/gcb.13069.
Alberto, F., 2009. MsatAllele: 1.0: an R package to visualize the binning of microsatellite alleles. J. Hered. 100, 394–397. https://doi.org/10.1093/jhered/esn110.
Alsos, I.G., Alm, T., Normand, S., Brochmann, C., 2009. Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. Glob. Ecol. Biogeogr. 18, 223–229. https://doi.org/10.1111/j.1466-8238.2008.00439.x.
Argus, G., 1997. Infrageneric classification of *Salix* (Salicaceae) in the new world. Syst. Bot. Monogr. 52, 1–121. https://doi.org/10.2307/25096638.
Argus, G., 2006. Guide to *Salix* (Willow) in the canadian maritime provinces (New Brunswick, Nova Scotia, and prince Edward island). Canadian Museum of Nature, Ottawa, Canada 49.
Arnaud-Haond, S., Duarte, C.M., Alberto, F., Serrao, E.A., 2007. Standardizing methods to address clonality in population studies. Mol. Ecol. 16, 5115–5139.
Becker, M.S., Davies, T.J., Pollard, W.H., 2016. Ground ice melt in the high Arctic leads to greater ecological heterogeneity. J. Ecol. 104, 114–124.
Billings, W.D., 1987. Constraints to plant growth, reproduction and establishment in arctic environments. Arc. Alp. Res. 19 357-357.
Bliss, L.C., Matveyeva N.V., 1992. Circumpolar arctic vegetation, F.S. ChapinIII, Jefferies, R.L., Reynolds, J.F., Shaver, G.R., Svoboda, J. Arctic ecosystems in a changing climate: an ecophysiological perspective. Academic Press, New York, pp. 59–89.
Bowden, W.B., Gooseff, M.N., Balser, A., Green, A., Peterson, B.J., Bradford, J., 2008. Sediment and nutrient delivery from thermokarst features in the foothills of the North Slope, Alaska: potential impacts on headwater stream ecosystems. J. Geophys. Res. 113, G02026. https://doi.org/10.1029/2007JG000470.
Bret-Harte, M.S., García, E.A., Scaré, V.M., Whorley, J.R., Wagern, J.L., Lipper, S.C., Chapin, F.S., 2004. Plant and soil responses to neighbor removal and fertilization in Alaskan tussock tundra. J. Ecol. 92, 635–647.
Bret-Harte, M.S., Mack, M.C., Shaver, G.R., Huebner, D.C., Johnston, M., Mojica, C.A., Reiskind, J.A., 2013. The response of Arctic vegetation and soils following an unusually severe tundra fire. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 368, 20120490. https://doi.org/10.1098/rstb.2012.0490.
Callaghan, T., Emanuelsson, U., 1985. Population structure and processes of tundra plants and vegetation. In: White, J. (Ed.), The Population Structure of Vegetation. Springer, Dordrecht, pp. 399–439.
Collet, D.M., 2004. Willows of Interior Alaska. U.S. Fish and Wildlife Service, Yukon Flats National Wildlife Refuge, Fairbanks.
Collinson, M., 1992. The early fossil history of Salicaceae: a brief review. Proc. R. Soc. Edinb. B. Biol. Sci. 98, 155–167. https://doi.org/10.1017/S0269727000007521.
Cortes-Palomec, A., McCauley, R., 2009. CTAB/Chloroform-Isoamyl Alcohol DNA Extraction Protocol. (accessed 31 July 2019). http://faculty.fortlewis.edu/mccauley_r/ecol_mol/ctab_dna_extraction.doc.
Densmore, R., Zasada, J.C., 1978. Rooting potential of Alaskan willow cuttings. Can. J. For. Res. 8, 477–479. https://doi.org/10.1139/x78-070.
Dormann, C.F., Brooker, R.W., 2002. Facilitation and competition in the High Arctic: the importance of the experimental approach. Acta Oecol. Montrouge (Montrouge) 23, 297–301. https://doi.org/10.1016/S1146-609X(02)01158-X.
Douhovnikoff, V., Dodd, R.S., 2003. Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theor. Appl. Genet. 106, 1307–1315.
Douhovnikoff, V., Goldsmith, G.R., Tape, K.D., Huang, C., Sur, N., Bret-Harte, M.S., 2010. Clonal diversity in an expanding community of arctic Salix spp. And a model for recruitment modes of arctic plants. Arct. Antarct. Alp. Res. 42, 406–411. https://doi.

org/10.1657/1938-4246-42.4.406.
Douhovnikoff, V., Leventhal, M., 2016. The use of Hardy-Weinberg equilibrium in clonal plant systems. Ecol. Evol. 6, 1173–1180. https://doi.org/10.1002/ece3.1946.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.
Du, H.D., Jiao, J.Y., Kou, M., Wang, N., 2013. Seasonal dynamics and vertical distribution pattern of bud bank in different erosion environments on hilly-gully Loess Plateau of Northwest China. Ying yong sheng tai xue bao = J. Appl. Ecol. 24, 1269–1276.
Eriksson, O., Fröborg, H., 1996. "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in Vaccinium shrubs. Can. J. Bot. 74, 1369–1374.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.
Frost, G.V., Epstein, H.E., Walker, D.A., Matyshak, G., Ermokhina, K., 2013. Patterned-ground facilitates shrub expansion in Low Arctic tundra. Environ. Res. Lett. 8, 15035. https://doi.org/10.1088/1748-9326/8/1/015035.
Gooseff, M.N., Balser, A., Bowden, W.B., Jones, J.B., 2009. Effects of hillslope thermokarst in northern Alaska. Eos Trans. Amer. Geophys. Union 90, 29–30. https://doi.org/10.1029/2009EO040001.
Gough, L., 2006. Neighbor effects on germination, survival, and growth in two arctic tundra plant communities. Ecography 29, 44–56. https://doi.org/10.1111/j.2005.0906-7590.04096.x.
Graae, B.J., Ejrnæs, R., Lang, S.I., Meineri, E., Ibarra, P.T., Bruun, H.H., 2011. Strong microsite control of seedling recruitment in tundra. Oecologia 166, 565–576.
Hamilton, T.D., Ashley, G.M., Reed, K.M., Schweger, C.E., 1993. Late pleistocene vertebrates and other fossils from Epiguruk, Northwestern Alaska. Quaternary Res. 39, 381–389. https://doi.org/10.1006/qres.1993.1045.
Hermanutz, L.A., Innes, D.J., Weis, I.M., 1989. Structure of arctic dwarf birch (*Betula glandulosa*) at its Northern Limit. Amer. J. Bot. 76, 755–771. https://doi.org/10.1002/j.1537-2197.1989.tb11370.x.
Huebner, D.C., Bret-Harte, M.S., 2019. Microsite conditions in retrogressive thaw slumps may facilitate increased seedling recruitment in the Alaskan Low Arctic. Ecol. Evol. 9, 1880–1897. https://doi.org/10.1002/ece3.4882.
Hultén, E., 1968. Flora of Alaska and Neighboring Territories. Stanford University Press, Stanford.
Hutchings, M.J., Wijesinghe, D.K., 2008. Performance of a clonal species in patchy environments. Evol. Ecol. 22, 313–324. https://doi.org/10.1007/s10682-007-9178-4.
Jeník, J., 1994. Clonal growth in woody plants: a review. Folia Geobot. 29, 291–306.
Jorgenson, M.T., Shur, Y.L., Pullman, E.R., 2006. Abrupt increase in permafrost degradation in Arctic Alaska. Geophys. Res. Lett. 33. https://doi.org/10.1029/2005GL024960.
King, R.A., Harris, S.L., Karp, A., Barker, J.H.A., 2010. Characterisation and inheritance of nuclear microsatellite loci for use in population studies of the allotetraploid *Salix alba-Salix fragilis* complex. Tree Genet. Genomes 6, 247–258.
Klimešová, J., Klimeš, L., 2003. Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham-Sparrow's model? Oikos 103, 225–229. https://doi.org/10.1034/j.1600-0706.2003.12725.x.
Krasny, M.E., Zasada, J.C., Vogt, K.A., 1988. Adventitious rooting of four Salicaceae species in response to a flooding event. Can. J. Bot. 66, 2597–2598. https://doi.org/10.1139/b88-352.
Lantz, T.C., Kokelj, S.V., Gergel, S.E., Henry, G.H.R., 2009. Relative impacts of disturbance and temperature: persistent changes in microenvironment and vegetation in retrogressive thaw slumps. Glob. Change Biol. Bioenergy 15, 1664–1675. https://doi.org/10.1111/j.1365-2486.2009.01917.x.
Lauron-Moreau, A., Pitre, F.E., Brouillet, L., Labreque, M., 2013. Microsatellite markers of willow species and characterization of 11 polymorphic microsatellites for *Salix eriocephala* (Salicaceae), a potential native species for biomass production in Canada. Plants 2, 203–210. https://doi.org/10.3390/plants2020203.
Li, L., Lan, Z., Chen, J., Song, Z., 2018. Allocation to clonal and sexual reproduction and its plasticity in *Vallisneria spinulosa* along a water-depth gradient. Ecosphere 9, e02070. https://doi.org/10.1002/ecs2.2070.
Liu, F., Chen, J.M., Wang, Q.F., 2009. Trade-offs between sexual and asexual reproduction in a monoecious species *Sagittaria pygmaea* (Alismataceae): the effect of different nutrient levels. Plant Syst. Evol. 277, 61–65.
Macek, P., Lepš, J., 2008. Environmental correlates of growth traits of the stoloniferous plant Potentilla palustris. Evol. Ecol. 22, 419–435.
Magurran, A.E., 1988. Ecological Diversity and Its Measurement. Princeton University Press, Princeton.
McCafferty, J., Reid, R., Spencer, M., Hamp, T., Fodor, A., 2012. Peak Studio: a tool for the visualization and analysis of fragment analysis files. Env. Microbiol. Rep. 4, 556–561. https://doi.org/10.1111/j.1758-2229.2012.00368.x.
Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x.
Myers-Smith, I.H., Forbes, B.C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., Hik, D.S., 2011. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environ. Res. Lett. 6, 045509.
Nauslar, N.J., Abatzoglou, J.T., Marsh, P.T., 2018. The 2017 North Bay and Southern California fires: a case study. Fire 1, 18. https://doi.org/10.3390/fire1010018.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
Petit, R.J., 2004. Biological invasions at the gene level. Divers. Distrib. 10, 159–165. https://doi.org/10.1111/j.1366-9516.2004.00084.x.
Pizano, C., Barón, A.F., Schuur, E.A.G., Crummer, K.G., Mack, M.C., 2014. Effects of thermo-erosional disturbance on surface soil carbon and nitrogen dynamics in upland arctic tundra. Environ. Res. Lett. 9, 75006.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using mutilocus genotype data. Genetics 155, 945–959.
R Core Team, 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, AT (accessed 31 July 2019). https://www.R-project.org.
Reisch, C., Schurm, S., Poschlod, P., 2007. Spatial genetic structure and clonal diversity in an alpine population of *Salix herbacea* (Salicaceae). Ann. Bot. 99, 647–651. https://doi.org/10.1093/aob/mcl290.
Romanovsky, V., Burgess, M., Smith, S., Yoshikawa, K., Brown, J., 2002. Permafrost temperature records: indicators of climate change. Eos Trans. Am. Geophys. Union 83, 589–594. https://doi.org/10.1029/2002EO000402.
Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4, 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x.
Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234.
Schulze, J., Rufener, R., Ernhardt, A., Stoll, P., 2012. The relative importance of sexual and clonal reproduction for population growth in the perennial herb *Fragaria vesca*. Pop. Ecol. 54, 369–380. https://doi.org/10.1007/s10144-012-0321-x.
Schuur, E.A.G., Crummer, K.G., Vogel, J.G., Mack, M.C., 2007. Plant species composition and productivity following permafrost thaw and thermokarst in Alaskan tundra. Ecosystems 10, 280–292.
Shaver, G.R., Bret-Harte, M.S., Jones, M.H., Johnstone, J., Gough, L., Laundre, J., Chapin, F.S.I.I.I., 2001. Species composition interacts with fertilizer to control long-term change in tundra productivity. Ecology 82, 3163–3181. https://doi.org/10.1890/0012-9658(2001)082[3163:SCIWFT]2.0.CO;2.
Sochor, M., Vasut, R.J., Bartova, E., Majesky, L., Mracek, J., 2013. Can gene flow among populations counteract the habitat loss of extremely fragile biotopes? An example from the population genetic structure in Salix daphnoides. Tree Genet. Genomes 9, 1193–1205.
Stamati, K., Blackie, S., Brown, J.W.S., Russell, J., 2003. A set of polymorphic SSR loci for subarctic willow (*Salix lanata*, *S. Lapponum* and *S. herbacea*). Mol. Ecol. Notes 3, 280–282. https://doi.org/10.1046/j.1471-8286.2003.00426.x.
Stamati, K., Hollingsworth, P.M., Russell, J., 2007. Patterns of clonal diversity in three species of sub-arctic willow (*Salix lanata*, *S. Lapponum* and *S. herbacea*). Plant Syst. Evol. 269, 75–88. https://doi.org/10.1007/s00606-007-0578-2.
Steltzer, H., Hufbauer, R.A., Welker, J.M., Casalis, M., Sullivan, P.F., Chimner, R., 2008. Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: implications for a terrestrial feedback to climate change in the High Arctic. J. Geophys. Res. 113, G03S10. https://doi.org/10.1029/2007JG000503.
Sturm, M., Racine, C., Tape, K., 2001. Climate change: increasing shrub abundance in the Arctic. Nature 411, 546–547. https://doi.org/10.1038/35079180.
Sturm, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauser, S.F., Liston, G.E., … Romanovsky, V.E., 2005. Winter biological processes could help convert arctic tundra to shrubland. BioScience 55, 17–26. https://doi.org/10.1641/0006-3568(2005)055[0017:WBPCHC]2.0.CO;2.
Tape, K.D., Lord, R., Marshall, H.P., Ruess, R.W., 2010. Snow-mediated ptarmigan browsing and shrub expansion in Arctic Alaska. Ecoscience 17, 186–193. https://doi.org/10.2980/17-2-3323.
Tape, K.D., Jones, B.M., Arp, C.D., Nitze, I., Grosse, G., 2018. Tundra be dammed: beaver colonization of the Arctic. Glob. Change Biol. Bioenergy. https://doi.org/10.1111/gcb.14332.
Toonen, R.J., Hughes, S., 2001. Increased throughput for fragment analysis on ABI Prism 377 Automated Sequencer using a membrane comb and STRand software. BioTechniques 31, 1320–1324.
Tsai, Y.H.E., 2011. PhyloGeoViz: a web-based program that visualizes genetic data on maps. Mol. Ecol. Res 11, 557–561. https://doi.org/10.1111/j.1755-0998.2010.02964.x.
U.S. Geological Survey, 2019. USGS EarthExplorer Website. (accessed 31 July 2019). https://earthexplorer.usgs.gov/.
VanderWeide, B.L., Hartnett, D.C., 2015. Belowground bud bank response to grazing under severe, short-term drought. Oecologia 178, 395–806. https://doi.org/10.1007/s00442-015-3249-y.
Viereck, L.A., 1966. Plant succession and soil development on gravel outwash of the Muldrow Glacier. Alaska. Ecol. Monogr. 36, 181–199. https://doi.org/10.2307/1942416.
Viereck, L.A., Little Jr, E.L., 1972. Alaska Trees and Shrubs. U.S. Department of Agriculture. Agricultural Handbook No. 410. pp. 1–265.
Wang, J., 2017. The computer program STRUCTURE for assigning individuals to populations: easy to use but easier to misuse. Mol. Ecol. Res. 17, 981–990. https://doi.org/10.1111/1755-0998.12650.