Effect of fire and thinning on fine-scale genetic structure and gene flow in fire-suppressed populations of sugar pine (*Pinus lambertiana* Douglas)

Brandon M. Lind[a,b], Malcolm P. North[c], Patricia E. Maloney[d], and Andrew J. Eckert[e]

October 1, 2018

[a]Forest & Conservation Sciences, University of British Columbia, Vancouver, BC V6T1Z4 Canada

[b]Integrative Life Sciences, Virginia Commonwealth University, Richmond, Virginia 23284 USA

[c]USDA Forest Service Pacific Southwest Research Station, Davis, CA 95618 USA

[d]Department of Plant Pathology and Tahoe Environmental Research Center, University of California, Davis, California 95616 USA

[e]Department of Biology, Virginia Commonwealth University, Richmond, Virginia 23284 USA

Running Title: Genetic effects of fire and thinning

Keywords: Sierra Nevada, fire, forest management, sugar pine, gene flow

Corresponding Author:

Brandon M. Lind

3041 - 2424 Main Mall

Forest Science Centre

University of British Columbia

Vancouver, British Columbia V6P 4E9

E-mail: brandon.lind@ubc.ca

Abstract

Historically, frequent, low-severity fires in dry western North American forests were a major driver of ecological patterns and processes, creating resilient ecosystems dominated by widely spaced pine species. However, a century of fire-suppression has caused overcrowding, altering forest composition to shade-tolerant species, while increasing competition and leaving trees stressed and susceptible to pathogens, insects, and high-severity fire. Exacerbating the issue, fire incidence is expected to increase with changing climate, while fire season has been observed to begin earlier and last longer than historic trends. Forest thinning and prescribed fire have been identified as important management tools to mitigate these risks. Yet little is known of how thinning, fire, or their interaction affect contemporary evolutionary processes of constituent pine species that influence fitness and play an important role in the opportunity for selection and population persistence. We assessed the impact of widely used fuel reduction treatments and prescribed fire on fine-scale gene flow on an ecologically important and historically dominant shade-intolerant pine species of the Sierra Nevada, *Pinus lambertiana* Dougl. Treatment prescription (no-thin-no-fire, thin-no-fire, and fire-and-thin) was found to differentially affect both fine-scale spatial and genetic structure as well as effective gene flow in this species. Specifically, the thin-no-fire prescription increases genetic structure (spatial autocorrelation of relatives) between adults and seedlings, while seed and pollen dispersal increase and decrease, respectively, as a function of increasing disturbance intensity. While these results may be specific to the stands at our study site, they indicate how assumptions relating to genetic effects based on spatial structure can be misleading. It is likely that these disequilibrated systems will continue to evolve on unknown evolutionary trajectories. The long-term impacts of management practices on reduced fitness from inbreeding depression should be continually monitored to ensure resilience to increasingly frequent and severe fire, drought, and pest stresses.

Introduction

Many aspects of conifer biology are affected by a tree's surrounding environment as well as the density of hetero- and conspecifics. For instance, outcrossing rates of conifer species are often tied to population density (Farris & Mitton 1984) and surrounding tree heights (O'Connell *et al.* 2003), while removal of proximal individuals can increase pollen and gene flow distances by reducing potential mates and removing once impeding vegetation. Thus, disturbance, *sensu lato*, has the potential to alter contemporary demographic and reproductive dynamics through both direct (population-level) and indirect (ecological-level) impacts (Mouillot *et al.* 2013).

Historically, natural disturbances such as fire were commonplace and equilibrated many ecosystem functions and processes in forests of the western United States (Covington *et al.* 1994). Fire regimes in these regions had return intervals on decadal scales (10-17 years; North et al. 2005), in contrast to wetter climates where fire return intervals were (sub)centennial (50+ years [North *et al.* 2016]). Resultantly, these ecosystems experienced frequent, low-severity burns and were populated by fire-adapted species, creating forests dominated by resilient, widely spaced pine trees. Yet over the past 150 years, anthropogenic influence has resulted in forests that are now fire-suppressed and overgrown by shade-tolerant species, causing increased competition, leaving trees stressed and susceptible to fungal and bark beetle attacks (Bonello *et al.* 2006).

Stand densification has also increased the frequency and probability of contemporary, high-severity fires. Between 2012 and 2014 in California alone, 14,340 fires burned 1.1 million acres and injured or killed nearly 300 individuals (NIFC 2014). Collectively, fires across California, the Great Basin, Southwest, and Rocky Mountain territories have burned a combined 8.8 million acres between 2014 and 2015 (NIFC 2015), while Forest Service scientists predict future fires to reach unprecedented levels, covering over 12-15 million acres annually (USDA Forest Service 2016a) requiring the United States Forest Service (USFS) to budget $2,300,000,000 on wildfire management, suppression, and preparedness for the 2016 fiscal year (USDA Forest Service

71 2016b). Exacerbating the issue, analyses of fire season length and onset have shown that
72 seasons are beginning earlier and lasting longer than historic trends (Westerling 2006) while
73 climate models predict extreme weather favorable to fire to become more frequent, and ignited
74 fires to increase in severity, size, and required suppression efforts (Miller *et al.* 2009).

75 Because of these contemporaneous trends, large-scale forest thinning projects have been
76 implemented to simultaneously restore fire-frequent ecosystems to their pre-settlement resilience
77 as well as to protect urban development and human life, as fuel reduction treatments have been
78 shown to be an effective tool in decreasing fire severity and ignition probability (Agee & Skinner
79 2005; Schwilk *et al.* 2009; Safford *et al.* 2009). For example, the Sierra Nevada Forest Plan
80 Amendment (USDA Forest Service 2004) mandates that 50% of initial thinning treatments take
81 place near urban populations, while the remaining thinning take place in natural wildland stands.
82 To encourage fire resiliency the USFS has implemented fuel reduction treatments across 6.1
83 million acres of western, fire-suppressed forestland in 2014 (USDA Forest Service 2016a).
84 Further, forest and fire scientists are calling for an overhaul of management policy to implement
85 these thinning treatments to a far greater extent (North *et al.* 2015). While congruent with historic
86 forest structure, these actions will orient these already disequilibrated systems on trajectories of
87 unknown evolutionary consequence.

88 Through timber harvests, land use conversion, and fire suppression, forests have
89 undergone systemic shifts in composition, structure, and disturbance regimes that are
90 incongruous to the natural and evolutionary histories of endemic species (Collins *et al.* 2011;
91 Larson & Churchill 2012). Consequentially, anthropogenic forest disturbance has been at the
92 forefront of conservation attention for decades (Ledig 1988; 1992). The extent of human impact
93 on forested land has received particular attention as a result of the empirical expectations
94 developed from population genetic theory. Specifically, because of the reduction in individual tree
95 density overall, and in particular for larger trees that asymmetrically contribute gametes to
96 reproduction (Richardson *et al.* 2014), harvested forests are thought to be specifically subjected

97 to population bottlenecks, potentially altering existing mating systems or available gene pools
98 while decreasing genetic variability within populations and increasing differentiation from native
99 stands (Smouse *et al*. 2001, Cloutier *et al.* 2006; Kramer *et al.* 2008; Lowe *et al.* 2015). These
100 consequences can influence the fitness of affected populations, as drastic changes in gene pool
101 availability or mating system can alter a population's potential to adapt to local conditions where
102 inbreeding depression can have deleterious effects on growth and reproductive potential (e.g.,
103 reproductive capacity or rates of embryo abortion).

104 Past studies investigating the genetic effects of North American forest management show
105 mixed evidence of harvest influence. These studies often sub-sample populations and primarily
106 focus on diversity consequences across a range of molecular markers (often microsatellites).
107 Many management studies of North American conifers compare genotypic diversity indices (e.g.,
108 H_E, H_O, allelic richness, etc.) between treatments to detect management influence (Cheliak *et al.*
109 1988; Gömöry 1992; Buchert *et al.* 1997; Adams *et al.* 1998; Rajora *et al.* 2000; Macdonald *et al.*
110 2001; Perry & Bousquet 2001; Rajora & Pluhar 2003; El-Kassaby *et al.* 2003; Marquardt *et al.*
111 2007; Fageria & Rajora 2013a; b). However, the same diversity values can manifest under
112 completely different scenarios and tests of significance between population values for a small
113 number of markers may therefore be under-informative, particularly for sub-sampled populations,
114 as these differences can result from sampling bias or from evolutionary processes unrelated to
115 management. Additionally, these investigations also often employ F_{ST} analyses to assess
116 statistical significance between treated and untreated stands (Thomas *et al.* 1999; Perry &
117 Bousquet 2001; Marquardt *et al.* 2007; Fageria & Rajora 2013a; b). Though when used in this
118 context, this test is simply signifying whether the allelic frequencies in (sub)populations under
119 study are likely to have been sampled from the same ancestral population (Holsinger & Weir
120 2009). Very often, the treated and untreated stands are physically adjacent (derived of a common
121 ancestral population) and only under extreme perturbation should significance be expected. In
122 cases where significance is detected, and other than to assess relative diversity between stands,

such differentiation does little to inform how management is affecting ongoing evolutionary processes affecting fitness, as such processes may ameliorate bottlenecks due to management. It would therefore be difficult to draw such conclusions without assessing other stand and evolutionary dynamics.

Very seldom in North American studies of forest management are evolutionary processes influencing fitness specifically examined (but see Neale & Adams 1985). Yet when studies are done and nonsignificant findings are found, authors generally caution interpretation (Finkeldey & Ziehe 2004; Namroud *et al.* 2012). Very often the scale of sampling (both in terms of numbers and spatial extent of individuals and the degree of temporal variation), as well as the lack of investigation into evolutionary dynamics have been offered as inadequate, and that further investigation into evolutionary consequences of natural and anthropogenic disturbance could give valuable insight to forest managers and fill a vital knowledge gap in this regard (Namroud *et al.* 2012). Indeed, incongruence between theoretical predictions and empirical results from studies evaluating genetic consequences of forest disturbance has created a paradox within the literature (Kramer *et al.* 2008). Yet as Lowe et al. (2015) point out, we may have been looking in the wrong place. They argue that instead of simply assaying mature cohorts to understand the genetic consequences of disturbance, future attention should include progeny arrays as well as the relative regenerative success across a wide range of influences. Additionally, they contend that the type and magnitude of the genetic response itself may be better understood through the variation in mating and breeding systems of studied species. Of particular importance, Lowe *et al.* (2015) advise scientists that the most fruitful research endeavors will incorporate quantitative approaches to understanding evolutionary mechanisms, specifically those connecting changes in pollination to mating systems and evolutionary fitness, and that these efforts will likely generate critical knowledge regarding the mechanisms driving the dynamics we observe.

Interactions between fire and forest thinning management are certain. To ensure forests are resilient to frequent fire and disturbance, and provide habitat for public recreation and native

149 wildlife, the interactive impact of management and fire must be understood in an evolutionary
150 framework. Here, we investigated the evolutionary impact of forest management on fire-
151 suppressed populations of the historically dominant and ecologically important sugar pine (*Pinus*
152 *lambertiana* Dougl.) within Teakettle Experimental Forest (TEF), a USFS site located in the central
153 Sierra Nevada of California. Using microsatellite markers, we employ parentage analysis and
154 assess impact upon various processes known to affect fitness such as mating patterns, effective
155 dispersal distances, and fine-scale (<300m) genetic structure. Our results show that thinning
156 alone increases fine-scale genetic structure of constituent trees (i.e., spatial autocorrelation of
157 relatives), and that the vast majority of pollen and seed dispersal events take place at this same
158 scale. Although the genetic structure of adults is due to an interaction between the evolutionary
159 history of the stand and the applied treatment, mating patterns and seedling ingrowth will
160 determine long-term impacts of management. While effects of such treatments will vary by
161 location, our results show that the degree of thinning and the choice of leave-trees should be
162 tailored to a given stand, and that spatial structure (arrangement of individuals across the
163 landscape) should not be conflated with spatial genetic structure (arrangement of relatives across
164 the landscape). By avoiding treatments that exacerbate genetic structure, managers may be able
165 to decrease seed abortion due to inbreeding and thus increase effective seed rain of species with
166 management importance.

167 Methods

168 Study area, sampling, and focal species

169 Teakettle Experimental Forest (TEF) is a fire-suppressed, old-growth forest watershed in
170 the central Sierra Nevada mountains of California. The 1300-ha forested watershed ranges from
171 1900–2600m in elevation and consists of five conifer species representative of fire-suppressed
172 forests of the Sierra range: white fir (*Abies concolor* [Gordon] Lindley ex Hildebrand), red fir (*A.*
173 *magnifica* A. Murray), incense cedar (*Calocedrus decurrens* [Torr] Florin), Jeffrey pine (*Pinus*

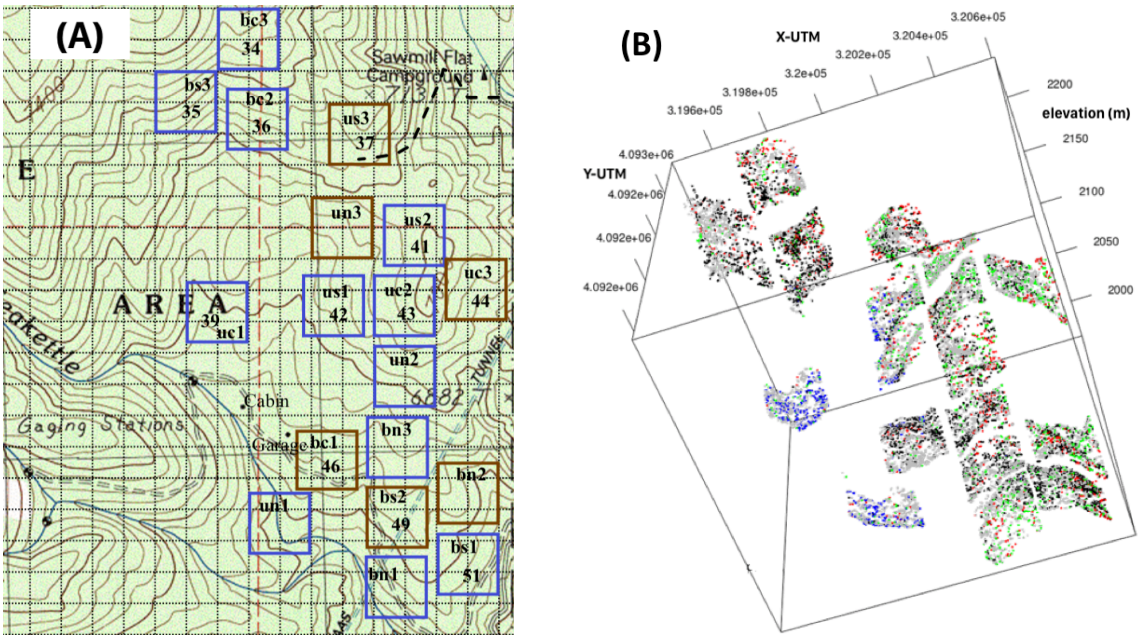

Figure 1 *Teakettle Experimental Forest, California (Latitude: 36.9606, Longitude: -119.0258). (A) Topographic map and spatial arrangement of treatments (BC = burned understory thin; BN = burned no-thin; BS = burned shelterwood thin; UC = unburned understory thin; UN = unburned no-thin; US = unburned shelterwood thin). Replicates for each treatment are numbered one through three from south to north. (B) Mapped coordinates (Universal Transverse Mercator) and elevation (meters) of pre-treatment adults ≥ 5cm diameter at breast height. (green: P. lambertiana, red: P. jeffreyi, gray: A. concolor, blue: A. magnifica, orange: C. decurrens, black: Quercus, Salix, and remaining species.).*

jeffreyi Balf.), and sugar pine (*P. lambertiana*). Historically, fire burned the area every 11-17 years, but has been suppressed for 135 years (North *et al.* 2005) while logging had been completely absent within the watershed (North 2002). Six treatments were applied to neighboring 4-ha plots at TEF (each 200m x 200m, Figure 1a) by crossing two levels of burn (no-fire and fire) with three levels of thinning (no-thinning, overstory-thinning, and understory-thinning). The understory thinning prescription followed guidelines in the California spotted owl (CASPO) report (Verner *et al.* 1992), which is now widely used for fuel management in California (SNFPA 2004). Each treatment was replicated three times for a total of 18 plots covering 72ha. Understory-thinning removed all trees with a diameter at breast height (DBH) ≤76cm and ≤≥25cm, while overstory-thinning removes all trees >25cm DBH except 18-22 of the largest trees per hectare. Treatments were applied to the watershed over 2000 and 2001. Plot inventories of pre-treatment (1999), and post-treatment (2001, 2004, and 2011) conditions mapped individual trees on a 3D coordinate

195 system that was then translated into Universal Transverse Mercator (colored dots, Figure 1b).
196 Only standing boles ≥5cm DBH were included in plot inventories, which recorded species, DBH,
197 spatial coordinates, and decay class and forest health metrics (e.g., presence/absence of insects
198 and pathogens). Post-treatment inventories updated DBH, decay class, forest health metrics, and
199 added individuals to the dataset once they reached 5cm DBH. Here, seedling and saplings are
200 all pine stems <5cm DBH. For these, basal diameter and spatial coordinates were recorded over
201 the summers of 2012 and 2013 by BML while collecting needle tissue samples from the full census
202 of all live *P. lambertiana* (*N* = 3,135). *Pinus lambertiana* is a historically dominant member of
203 mixed-conifer forests of the Sierra Nevada, and continues to play important ecological roles. This
204 species is shade-intolerant and is an important focus of restoration in the Sierra Nevada range.

205 **Analysis of tree spatial structure**

206 Using plot-level *P. lambertiana* individuals, we estimated spatial structure of seedlings and
207 adults across 10-meter distance classes, *r*, separately using univariate inhomogeneous pair
208 correlation functions ($g_{inhom}(r)$) from the `spatstat` library in R (Baddeley *et al.* 2015) with an
209 isotropic edge correction. This statistic was chosen over Ripley's K, or its linearized version (*L*)
210 because of advocacy for $g_{inhom}(r)$ over these statistics (see `spatstat` manual). This analysis
211 tests the null hypothesis that the 2D spatial arrangement of points (adults or seedlings) is not
212 significantly different from complete spatial randomness (CSR; i.e., a Poisson distribution of inter-
213 point distances with inhomogeneous intensities of points), where support for the alternative
214 hypothesis is indicative of ecological factors driving spatial patterning. We calculated null
215 confidence envelopes for each test using 199 null simulations of CSR using the same intensity of
216 the pattern of individuals analyzed (equivalent to an alpha value of 0.01; see `spatstat` manual).
217 For trees that coincide with the null model of CSR $g_{inhom}(r)$ = 1, with spatial aggregation resulting
218 in $g_{inhom}(r)$ > 1, and with spatial inhibition resulting in $g_{inhom}(r)$ < 1 (Baddeley *et al*. 2015);
219 significance was judged using the null confidence envelopes described above. We also repeated
220 this analysis for shade-tolerant individuals (i.e., *A. concolor* and *A. magnifica* grouped together).

221 Further, we extended the univariate inhomogeneous pair correlation function to its bivariate
222 equivalent, $g_{inhom,i,j}(r)$, to test for spatial affinity between two groups *i* and *j*, using similar
223 methods as above for edge correction and null confidence envelopes. We calculated $g_{inhom,i,j}(r)$
224 between unique combinations of *P. lambertiana* adults, *P. lambertiana* seedlings, and shade-
225 tolerant individuals. Hypothesis testing and interpretation of bivariate $g_{inhom,i,j}(r)$ was carried out
226 as with univariate $g_{inhom}(r)$. Results from these analyses will allow us to compare standing
227 spatial structure of trees against spatial genetic autocorrelation (see below) and make inferences
228 about the ecology of these species as well as how treatments at TEF are affecting ongoing
229 evolutionary dynamics.

230 **DNA extraction, microsatellite amplification**

231 Total genomic DNA was extracted according to manufacturer's protocol using the DNeasy
232 96 Plant Kit (Qiagen, Germantown, MD) from finely ground *P. lambertiana* samples within a
233 subset of the factorial treatments at TEF: unburned-no-thin control plots (hereafter UN),
234 understory-thin (CASPO) plots without burn application (hereafter UC), and burned understory-
235 thin plots (hereafter BC) for a total of 1,348 individuals. Herein, we often refer to patterns across
236 these treatments in terms of increasing disturbance intensity (i.e., from UN to UC to BC). For each
237 individual, three chloroplast (paternally inherited, Wofford *et al.* 2014: pt71936, pt87268, pc10)
238 and four nuclear (biparental inheritance, Echt *et al.* 1996: rps50, rps02, rps12, rps39)
239 microsatellite markers were amplified (using fluorescent dyes NED, PET, VIC, and FAM) per the
240 original publications with minor modifications using BIO-RAD iProof high fidelity DNA polymerase
241 (see Supplemental Information). The chloroplast markers were chosen for their primer
242 conservation across *Pinus*, *Trifoliae*, *Parrya*, and *Quinquifolia* subsections of the *Pinus* genus
243 (Wofford *et al.* 2014) while the chosen nuclear markers have been amplified in eastern white pine
244 (*P. strobus* L., Echt *et al.* 1996) and both sets successfully amplified on a subset of individuals at
245 TEF as a proof-of-concept judged by gel electrophoresis. Multiplexed individuals (one fluorescent
246 dye per well) were analyzed using the Applied Biosystems 3730xl fragment analyzer at Cornell

University (http://www.biotech.cornell.edu/brc/genomics-facility) and genotypes were called using GeneMaker v2.6.7 (see Supplemental Info; http://www.softgenetics.com/GeneMarker.php).

Genetic diversity measures

Treatment-specific diversity measures (total number of alleles, A_T; mean number of alleles per locus, A; effective number of alleles per locus, A_e; observed and expected heterozygosity for nuclear markers, respectfully H_o, H_e; average number of private alleles, A_P; and overall means for each category) were calculated for each treatment and averaged across loci in order to compare dynamics at TEF to published studies. For estimates of H_o and H_e, only nuclear markers were used. To quantify variation in these measures we also report standard deviation. We also calculated hierarchical multi-locus F_{ST} according to Weir & Cockerham (1984) for nuclear markers using the `hierfstat` package in R (Goudet & Jombart 2015) and calculated treatment-specific F_{ST} in a similar manner in order to compare fixation indices across treatments. Further, single- and multi-locus exclusion probabilities for parentage analysis (see below) were calculated using python scripts modified from `gstudio` (v1.5.0; Dyer 2016).

Analysis of spatial genetic structure

To quantify spatial genetic autocorrelation at a distance class (lag) h (hereafter r_g^h), we used multi-locus genetic distances (Smouse & Peakall 1999) and Euclidean geographic distances among spatial coordinates of individuals to calculate r_g^h across distances classes h corresponding to approximately 10-meter bins for *P. lambertiana* seedlings, *P. lambertiana* adults, as well as a bivariate approximation for the clustering of *P. lambertiana* adult genotypes to those of seedlings. For a given distance class, h, spatial patterning of multi-locus genotypes are unrelated to (i.e., random relative to) the spatial patterns of individuals if $r_g^h = 0$, aggregated if $r_g^h > 0$, and dispersed if $r_g^h < 0$. We estimated null confidence intervals by taking the 2.5th and 97.5th quantiles of M = 1000 estimates of $r_{g,m}^h$, where 999 of these estimates were computed by randomly permuting individual genotypes across empirical spatial coordinates, with the Mth permutation being the

empirical estimate of r_g^h itself (Smouse & Peakall 1999). Using the `PopGenReport` package in R (Adamack & Gruber 2014) we created correlograms for nuclear and chloroplast markers both in isolation and in combination, but present only those using full genotypes as correlograms by marker type showed similar patterns as full genotypes. We used these correlograms to quantify spatial aggregation of genotypes so that conclusions based on treatment effects could be compared and contextualized with ongoing evolutionary dynamics at TEF such as that of fine-scale gene flow.

Parentage analysis

To quantify fine-scale gene flow at TEF, we conducted parentage analysis using our genetic markers and spatial coordinates of individuals. Joint estimation of parentage and dispersal parameters (i.e., mean dispersal distances of seed and pollen) were achieved by expanding methods of Moran & Clark (2011). This method simultaneously estimates parentage and dispersal kernel parameters for seed and pollen within a Bayesian framework, taking into account genotyping error and variation in individual fecundity while treating dispersal processes inside and outside of the mapped areas in a coherent manner, which is critical if the dispersal kernel is to reflect both long- and short-distance movement. Here, all sampled adults are characterized by a multi-locus genotype and a mapped coordinate. Additionally, there exists a sample of seedlings, each of which has not only a genotype and location, but an estimated pedigree as well, which can consider any adult as either mother or father, or of a selfing event (though we excluded possible selfing events from analyses). The probability of the pedigree considering two in-plot parents, before incorporating information regarding genotype, is estimated from the probability of pollen to mother movement over the given distance and of seed movement over the distance between mother and seedling, as well as the parental prior distribution for fecundity and pollen production. For the study here, pollen production was considered proportional to fecundity (as in Moran & Clark 2011) and was estimated by fitting a 2nd-order power polynomial regression to data from Figure 6 in Fowells & Schubert (1956) where Cone Count = $0.0098(\text{dbh}^2) - 0.4811(\text{dbh}) + 10.651$.

After calculating cone counts using this regression, we set fecundity for all adults <25cm DBH to zero given observed cone counts from Fowells & Schubert (1956). For dispersal priors, we set the seed dispersal kernel shape parameter, u_{s}, to 253.31, corresponding to a mean dispersal distance of 25m (Millar *et al*. 1992; Fowells & Schubert 1956) while the pollen dispersal kernel shape parameter prior, u_{p}, was set to 2279.72, corresponding to a mean pollen distance of 75m (Wright 1976; Neale 1983; Millar *et al*. 1992). For priors to the standard deviation of mean dispersal we set seed (pollen) to 1013.21 (9118.90) corresponding to standard deviations of 50m (75m).

Given that either parent could have produced the offspring, the likelihood that this pair is the true parents relative to all other possible parent pairs depends on the dispersal kernel priors for seed and pollen, and the seed and pollen production of all trees both inside and outside of the plot (the fraction of all possibilities; Moran & Clark 2011). To evaluate the probability of an offspring having one parent in the plot and the other outside of the plot, a set of potential out-of-plot parent-densities, $dp_1,\ldots,dp_{20}$, each 10m progressively outside of the plot is considered (see supplemental figure S3.1 in Moran & Clark 2011). Pollen and seed movement into the plot is approximated by assuming first that all seed/pollen produced within each quarter-polygon, v, originates from a tree located dp_v meters from the midpoint of each side outside of the plot. The expected out-of-plot pollen (seeds) reaching an in-plot mother (a seedling's location) from each quarter-polygon outside of the plot is calculated based on the average density and average fecundities of trees outside of the plot and then multiplied by the probability of dispersal to the point within the plot. Summing over each distance class over each side gives the total expected out-of-plot pollen/seed dispersal to points inside of the plot. However, to calculate the probability of an in-plot versus an out-of-plot father, the expected pollen arriving at an out-of-plot mother from another out-of-plot father must first be calculated using the concentric polygons around the sampled plot and the distance classes described above. The fraction of rings falling outside the

plot determines the fraction of pollen received from each distance class, dp_v, expected to come from outside trees. Once error rates (e_1) and dropout rates (e_2) of genotyping are calculated through regenotyping individuals (see Supplemental Information), the probability of a pedigree, seed and dispersal parameters given the offspring genotype, distances, error rates, and pollen/seed production can be estimated (Moran & Clark 2011). Very rarely have previous studies investigating effects of forest management (or using parentage analysis towards such goals) incorporated error and dropout rates into subsequent inferences.

For the current study, out-of-plot densities were extrapolated for each side of the nine plots used at Teakettle from densities and DBH distributions (our proxy for fecundity) revealed in pre-treatment surveys (North 2002). Due to the proximity of the treated plots, all adult trees and seedlings across UC, BC, and UN treatments were considered simultaneously for parentage assignment. Our methods therefore extend Moran & Clark (2011) from a single plot of sampled individuals to multiple plots across the landscape. Additionally, instead of considering any given pedigree as symmetrical (i.e., with no consideration for which tree was the pollen or seed donor) we utilize genotyped markers separately to consider whether a given pedigree is for a mother-father pair, or for a father-mother pair (i.e., we only considered nuclear markers for a potential mother, and all markers for a potential father). The most probable pedigree for each seedling was identified by assessing the proportion of the proposed pedigree across chains in the Gibbs sampler (as in Moran & Clark 2011), in which we used 500,000 steps and a burn-in of 30,000. This method was further modified to improve computational efficiency by multiprocessing appropriate elements of the script by utilizing custom python scripts run on the VCU Center for High Performance Computing cluster (CHiP) and the `SNOW` library (v0.4-2; Tierney *et al*. 2016) in R (v3.3.3; R Core Team 2017). We replicated each run three times, and judged convergence within and between runs in R.

Using parentage analysis to further quantify fine-scale gene flow

In addition to estimates of the mean seed and pollen dispersal from dispersal kernels estimated during parentage analysis (see above), we used these parentage assignments to further classify fine-scale gene flow at TEF. Using the full set of most probable pedigrees identified from parentage analysis, we first quantified the number of in-plot vs. out-of-plot dispersal events averaged across each replicate for a given treatment. Then, using the most probable parentage assignment for each offspring, we quantified mean dispersal distances from sampled mothers to seedlings, as well as between sampled fathers to sampled mothers. To better account for uncertainty in parentage assignment (i.e., to account for fractional parentage assignment), we also calculated mean dispersal distance by treatment by considering all pedigrees with known individuals weighted by the probability of assignment. Specifically, for mean seed dispersal, for each seedling we calculated the weighted average of mother-offspring distances across pedigrees of non-zero probability that included known mothers in the dataset. Here, each weight was the probability of assignment, $p_{seed,pedigree}$, divided by the probability of assignment of this seedling to a known mother (1 - U_M) where U_M is the sum of the probabilities across all non-zero pedigrees that included an unsampled mother. Treatment-level averages were then calculated across these weighted distances. For pollen dispersal, for each seedling we considered only pedigrees of non-zero probability where both the mother and father were known, weighting each distance by the probability of assignment, $p_{seed,pedigree}$, divided by the probability of assignment to known parents (1-$U_{seed,pedigree}$) where $U_{seed,pedigree}$ is the sum of the probabilities across all non-zero pedigrees that included at least one unsampled parent. Treatment-level averages were then calculated from these weighted distances and significance was determined using a Kruskal-Wallis test with an alpha value of 0.05.

Scripts used in analyses described above can be found in IPython notebook format (Pérez & Granger 2007) at https://github.com/brandonlind/teakettle.

Results

Analysis of tree spatial structure

Univariate Analysis

Across treatments, *P. lambertiana* adults generally exhibited spatial aggregation at distance classes less than 20 meters, where this signal decreased with increasing disturbance intensity with UN plots showing the greater magnitudes of $g_{\mathrm{inhom}}(r)$ than UC or BC plots at these small distance classes (Figure 2 first row). For adult shade-tolerant species (*A. magnifica* and *A. concolor* combined), the extent of spatial aggregation at large distance classes decayed with increasing disturbance intensity (Figure 2 second row) where UC treatments generally exhibited greater magnitudes of $g_{\mathrm{inhom}}(r)$ than BC treatments in small distance classes. For *P. lambertiana* seedlings the spatial structure of individuals was also similar across treatments, though UN treatments generally had significant aggregation and much larger magnitudes of $g_{\mathrm{inhom}}(r)$ at larger distance classes than other treatments, while seedlings in BC treatments exhibited greater magnitudes of $g_{\mathrm{inhom}}(r)$ across small distance classes than either UC or UN plots (Figure 2 third row).

Bivariate Analysis

The spatial affinity of *P. lambertiana* seedlings to *P. lambertiana* adults, $g_{inhom,seedling,adult}(r)$, generally ranged from randomness ($g_{inhom,seedling,adult}(r)$ = 1) to spatial inhibition ($g_{inhom,seedling,adult}(r)$ < 1) with decreasing intensity of disturbance (i.e., from undisturbed UN plots, to thin-only UC plots, to thinned-and-burned BC plots). UN plots tended to show consistent inhibition across distance classes greater than about 15m, whereas observed $g_{inhom,seedling,adult}(r)$ for UC plots tended to align with the lower extent of the confidence interval with fewer instances of significant inhibition between adult and seedlings (Figure). A similar trend for increasing spatial inhibition between *P. lambertiana* seedlings and shade-tolerant adults ($g_{inhom,seedling,adult}(r)$), as well as for *P. lambertiana* adults and shade-tolerant adults

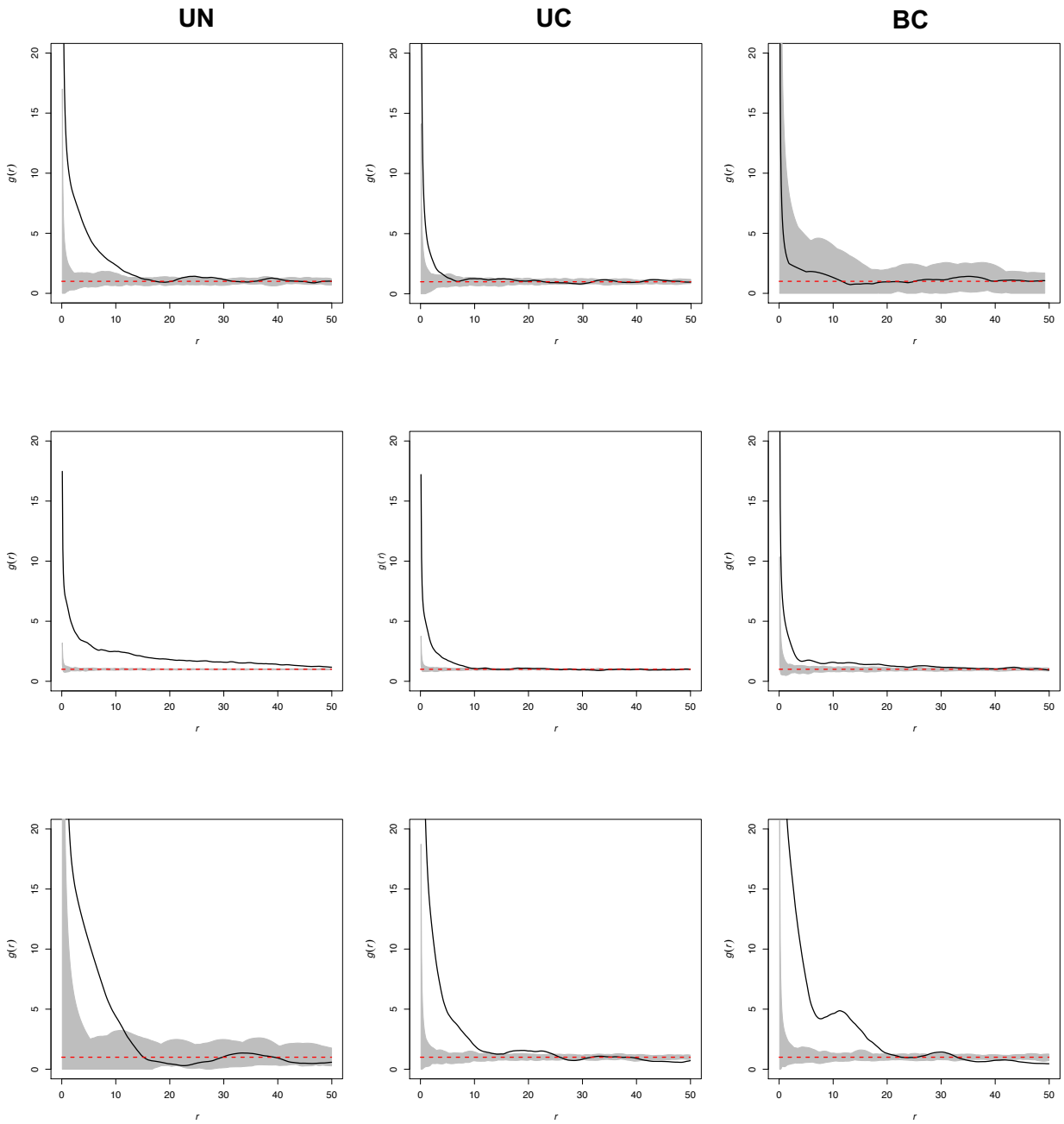

Figure 2 *Representative figures of univariate analysis of spatial structure, $g_{inhom(r)}$, by treatment replicate for each distance class, r. First row: adult P. lambertiana (PiLa); second row: adult shade tolerant (A. magnifica and A. concolor = ShadeTol); third row: P. lambertiana (PiLa) seedlings. Disturbance intensity increases by column from left to right. These figures show that with increasing disturbance intensity there is a diminution of the degree of spatial structure within classes. Gray : null confidence envelope; Solid black line : observed $g_{inhom}(r)$. Red dashed line : null expectation of complete spatial randomness, $g_{inhom}(r)$ = 1. Individuals are aggregated if $g_{inhom}(r)$ > 1, inhibited if $g_{inhom}(r)$ < 1. See Supplemental Figures S1-S3 for all plots.*

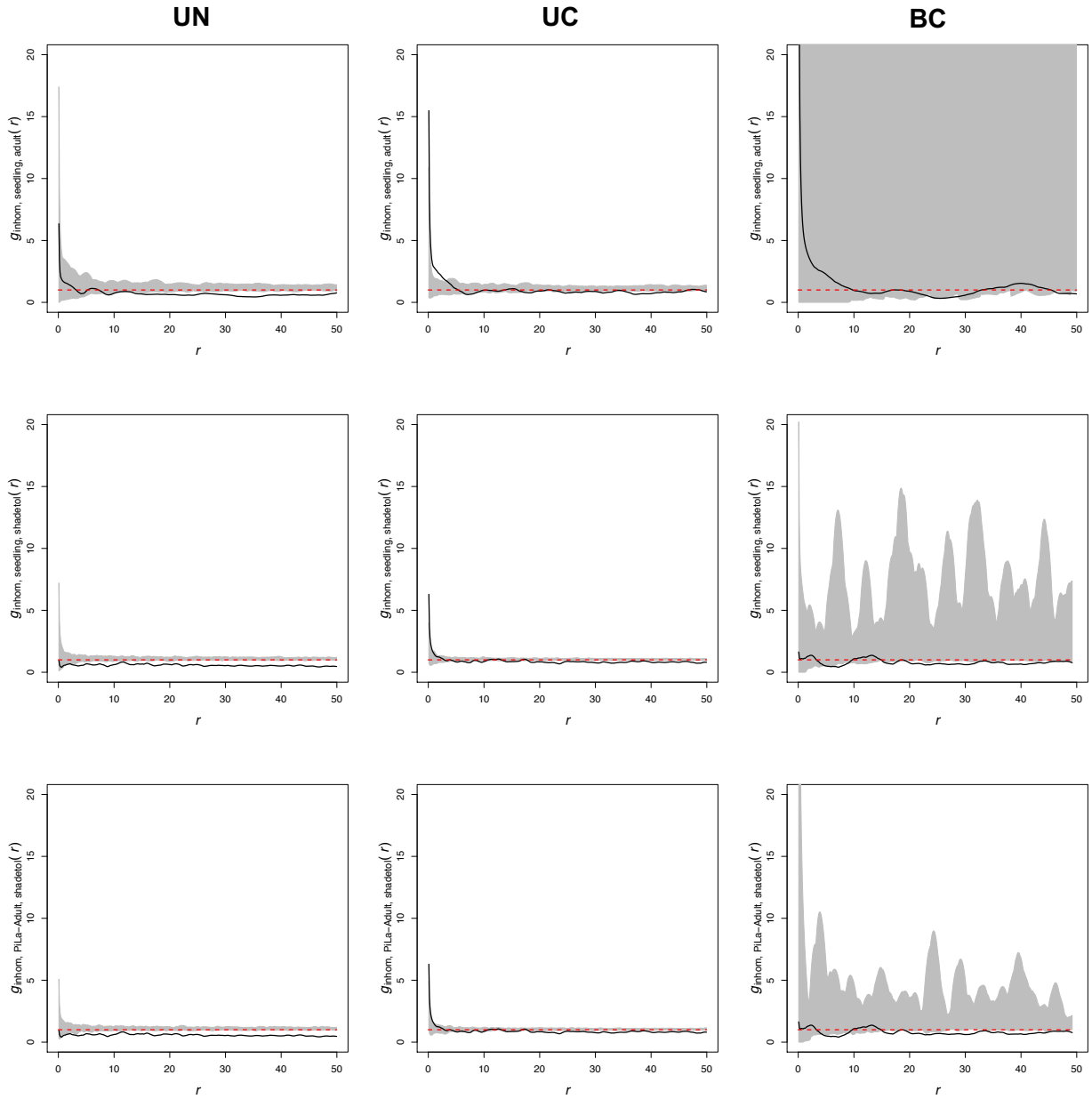

Figure 3 *Representative figures of bivariate analysis of spatial structure, $g_{inhom,i,j}(r)$, between: first row: P. lambertiana seedlings (seed) and P. lambertiana adults; second row: P. lambertiana seedlings and shade tolerant adults; third row: P. lambertiana adults to shade tolerant adults. Disturbance intensity increases by column from left to right. These figures show that the two classes compared are generally inhibited spatially by the presence of the other, and that with increasing disturbance there is a diminution of the degree of spatial inhibition between classes. Gray : null confidence envelope; Solid black line : observed $g_{inhom,i,j}(r)$. Red dashed line : null expectation of complete spatial random-ness, $g_{inhom,i,j}(r)$ = 1. Individuals are aggregated if $g_{inhom,i,j}(r)$ > 1, inhibited if $g_{inhom,i,j}(r)$ < 1. The gray shading in the third column of the first row indicates the null confidence envelope extended beyond the limit of the y-axis, where the pattern of the confidence envelope seen in the third column of the second and third rows is caused by sample size varying among distance classes. It should be noted that the observed values for all comparisons generally fall below the y = 1 expectation except for some short distance classes. See Supplemental Figures S4-S6 for all plots.*

423 ($g_{inhom,PiLa-adult,shadetol}(r)$), was also observed (Figure 3) where UN treatments generally had a
424 greater inhibition than UC or BC plots, though BC plots also exhibited some evidence of spatial
425 inhibition between groups. The results from the uni- and bivariate analyses of spatial patterns at
426 TEF suggest that pines are generally clustered with other pines, shade-tolerant individuals are
427 clustered with other shade-tolerant individuals, but spatial patterns of shade-tolerant adults
428 generally show spatial inhibition with pine individuals of both classes. Additionally, *P. lambertiana*
429 seedlings showed similar clustering across all treatments, suggesting a similar pattern of
430 response to the environment by individual trees. Further, together with the univariate spatial
431 clustering of *P. lambertiana* seedlings at small distance classes, these bivariate results suggest
432 there may be ecological drivers influencing realized patterns of seedlings across
433 microenvironments at TEF (e.g., perhaps sites with decreased competition for [or optimal levels
434 of] water, nutrients, or light).

435 **Diversity measures**

436 To compare our results with measures often used across the literature to investigate
437 genetic effects of forest management we calculated various genetic diversity measures (see
438 Methods). Genetic diversity measures (Table 1) seemed to be most influenced by census size
439 across the various measures we estimated here. For instance, census size increased from BC (n
440 = 109 individuals) to UN (n = 557 individuals) to UC (n = 682 individuals) where related diversity
441 measures of A_{T}, A, A_e, and A_P followed this trend. Observed heterozygosity was greatest for UN
442 plots, followed by BC and UC plots, while expected heterozygosity decreased from UC to BC to
443 UN (Table 1). Thus, no trend was observed between diversity measures and increasing
444 disturbance treatment at TEF.

445 Hierarchical *F*-statistics were calculated with nuclear markers to compare the extent of
446 fixation within and across treatment types, with individuals nested in replicates, replicates nested
447 in treatments, and treatments nested within TEF. The overall multilocus F_{ST} ($F_{rep,TEF}$) at TEF was
448 0.075, consistent with estimates of many *Pinus* species across various spatial scales (Howe et

Table 1 *Genetic diversity measures (standard deviation) by treatment. N : census number of individuals [adults, seedlings];* A_T *: total number of alleles;* A *: mean number of alleles per locus;* A_e *: effective number of alleles (harmonic mean across loci);* H_o*,* H_e *: respectively the observed and expected heterozygosity for nuclear markers;* A_P *: average number of private alleles. For* A*,* A_e*,* H_o*, and* H_e*, values indicate averages across loci, where values for each locus were calculated across all three treatment replicates simultaneously.* H_o *and* H_e *used only nuclear markers, whereas other genetic diversity columns considered all loci.*

Treatment	N	A_{T}	A	A_{e}	H_{o}	H_{e}	A_{P}
UN	557 [236,321]	180	25.71 (6.50)	3.23 (1.58)	0.87 (0.06)	0.77 (0.06)	46
UC	682 [307,375]	210	30.00 (7.76)	6.20 (3.07)	0.57 (0.30)	0.84 (0.10)	73
BC	109 [42,67]	107	15.29 (6.80)	4.80 (2.46)	0.82 (0.08)	0.82 (0.07)	5
Mean	449 [195,254]	165.67	23.67	4.74	0.75	0.81	41.3

al. 2003), suggesting that the majority of genetic variation was partitioned more so within plots than between plots. The $F_{rep,TEF}$ for individual markers varied: rps02 ($F_{rep,TEF}$ = 0.019), rps12 ($F_{rep,TEF}$ = 0.037), rps39 ($F_{rep,TEF}$ = 0.148), rps50 ($F_{rep,TEF}$ = 0.103). Considering only genotypes across replicates of a given treatment, treatment-level estimates of $F_{\mathrm{rep,tx}}$ also varied ($F_{\mathrm{rep,UN}}$ = 0.011, $F_{\mathrm{rep,UC}}$ = 0.109, $F_{\mathrm{rep,BC}}$ = 0.035) but showed no pattern with increasing disturbance intensity. Pairwise $F_{\mathrm{rep,tx}}$ comparisons between treatments were calculated by considering genotypes across two treatments simultaneously and were used to compare the extent of fixation across disturbance intensity. Here, the three comparisons ranged from 0.050 (UC and UN) to 0.055 (UC and BC) to 0.075 (BC and UN) indicative of increasing relative fixation with increasing disparity for the intensity of disturbance for a given comparison.

Analysis of spatial genetic structure

Analysis of spatial genetic autocorrelation (sensu Smouse & Peakall 1999) was carried out at TEF to better understand how treatment affects standing genetic structure (*P. lambertiana* adults x *P. lambertiana* adults), how this standing genetic structure relates to the genetic structure of seedlings (*P. lambertiana* seedlings x *P. lambertiana* seedlings), and the tendency of alike genotypes to be aggregated or inhibited across the treatments as the stands continue to develop

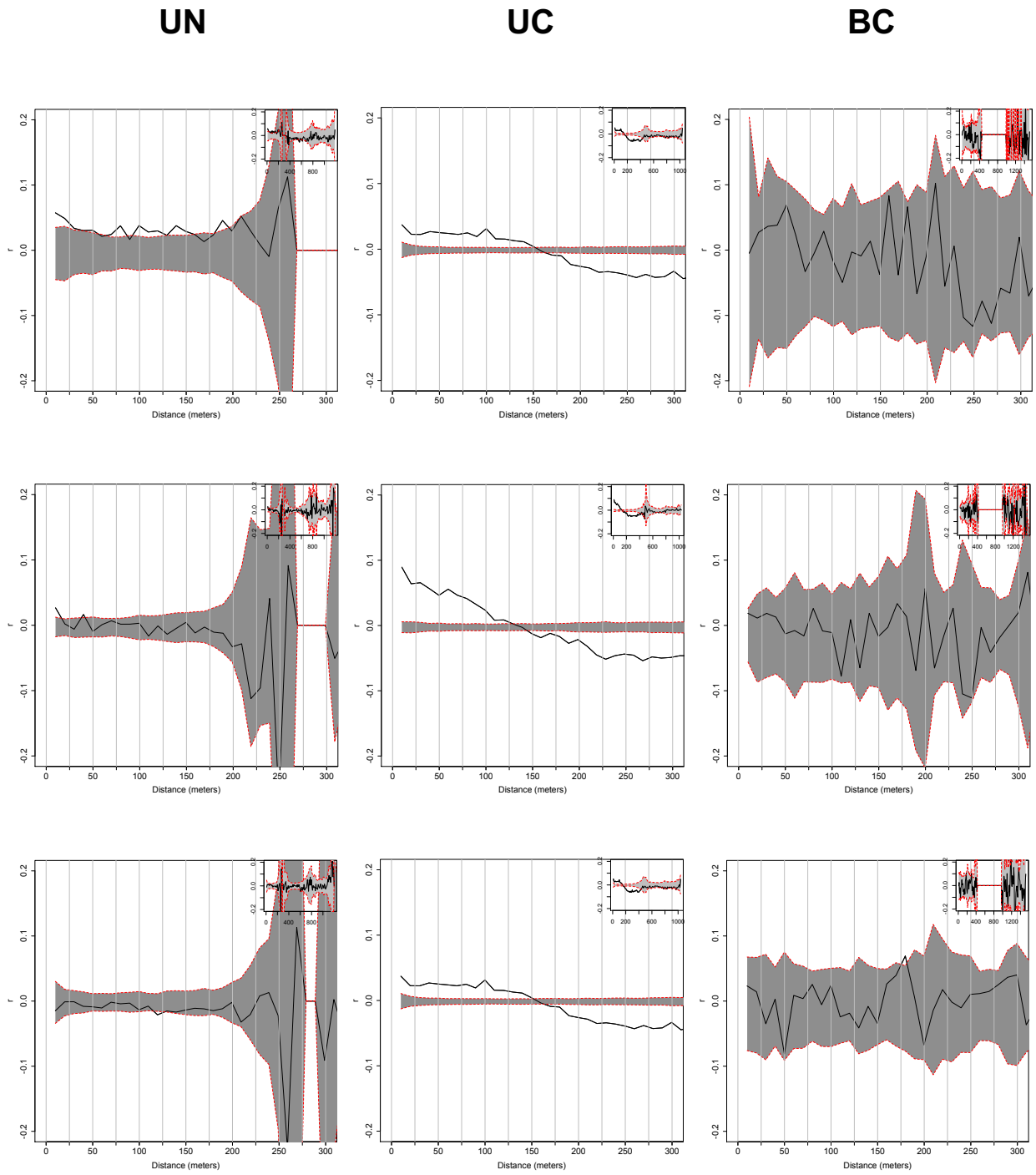

Figure 4 *Analysis of spatial genetic structure (sensu Smouse & Peakall 1999) between P. lambertiana adults (first row), P. lambertiana seedlings (second row), and between P. lambertiana adults and seedlings (third row) by treatment (columns) across distance classes within plots (main panel) or across TEF (insets). Values of* r_g^h *= 0 indicate random spatial patterns of genotypes,* r_g^h *> 0 indicate clustering of alike genotypes, and* r_g^h *< 0 indicate spatial inhibition of alike genotypes.*

484 after treatment (*P. lambertiana* adults x *P. lambertiana* seedlings). In all comparisons, spatial
485 genetic structure in BC treatments did not differ significantly from a random spatial distribution of
486 genotypes (last column Figure), perhaps due to the relatively small census sizes (Table 1) in
487 distance-class bins. However, there seems to be an effect of treatment on the spatial patterning
488 of genotypes of adults in the UC and UN stands (first row Figure). While the natural fire-
489 suppressed stands (UN) exhibited small but significant spatial genetic structure for most distance
490 classes up to 200m, UC stands resulted in significant aggregation of adult genotypes at a greater
491 degree than UN up to 150m, where genotypes became spatially inhibited up to the maximum
492 distances in stands ($200(\sqrt{2})$m; Figure). These patterns resulted in spatial distributions of
493 seedling genotypes that were randomly distributed except for very short distance classes in UN
494 treatments, and for UC seedlings, resulted in the general pattern observed for UC adults albeit to
495 a higher degree of both aggregation and inhibition (second row Figure). As a result, alike
496 genotypes between adults and seedlings were aggregated up to 150m in UC plots, whereas this
497 relationship in UN treatments resulted in negative values of $r^{h}_{g,adult,seed}$ that bordered the
498 confidence envelope for spatial inhibition but were not significantly different from a random spatial
499 distribution of genotypes (third row of Figure 4). While the genetic structure of adults is due to the
500 interaction of the effect of treatment on pretreatment conditions, the long-term dynamics of these
501 stands will be influenced by seedling ingrowth. These results suggest that UC treatments may, in
502 the long term, increase the relatedness of individuals across short spatial scales less than 150m
503 relative to either BC or UN treatments. This will be particularly exacerbated if gene flow occurs at
504 similarly fine spatial scales (see below).

505 **Quantifying fine-scale gene flow**

506 *In-plot vs. out-of-plot dispersal events*

507 To understand how gene flow across plots is influenced by treatment, we quantified the
508 number of in-plot and out of plot dispersal events from pedigrees identified as

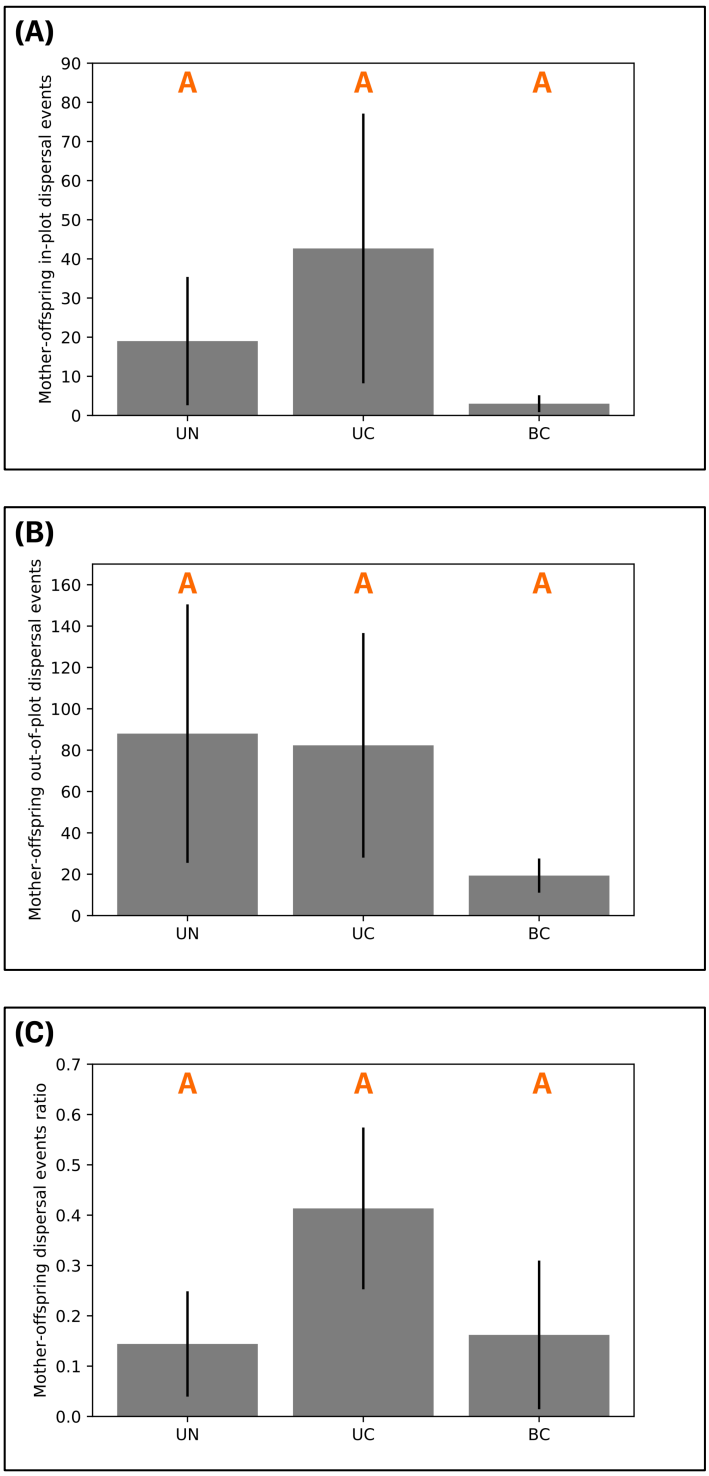

Figure 5 *Mother-offspring dispersal events by treatment for (A) dispersal between in-plot individuals, (B) dispersal into plot from an out-of-plot mother, and (C) the ratio of these values. There were no events in which a known mother dispersed seed to another plot, therefore B is utilizing information from parentage analysis that indicated the mother of a given seedling was not sampled. Orange letters within each plot show significant differences between medians, as inferred from separate Kruskal-Wallis tests (see main text of Results). Vertical lines indicate standard deviations.*

518 most probable from our parentage analysis. To account for sample size differences, we also
519 calculated the ratio of these values. The number of in-plot and out-of-plot dispersal events
520 between mother and offspring differed by treatment (Figure A-B) but not significantly so (p >
521 0.4297). The ratio of these values differed by treatment as well (Figure C), with UC having the
522 greatest proportion of in-plot dispersal events but overall there were no significant differences
523 among treatments (p = 0.1926).

524 We next quantified the number of in-plot and out-of-plot dispersal events of pollen from
525 the most probable pedigrees identified from parentage analysis. In these cases, out-of-plot pollen
526 dispersal events were tallied as an in-plot mother receiving pollen from an unsampled or out-of-
527 plot father. As with mother-offspring dispersal events we also calculated a ratio of these values.
528 The UC treatment exhibited the most in-plot pollen dispersal events, followed by UN and BC
529 (Figure A), though these comparisons were not significant using a Kruskal-Wallis test (p =
530 0.5073). UN and UC treatments exhibited similar levels of out-of-plot dispersal events (Figure B),
531 which differed (though not significantly, p = 0.1376) from BC out-of-plot events. The ratio of in-
532 plot vs. out-of-plot dispersal events increased with increasing disturbance (Figure C) but did not
533 differ significantly (p = 0.1030).

534 *Median dispersal distances by treatment*

535 Considering the most probable parentage from our model, we calculated the median seed
536 dispersal distances between offspring and known mothers, as well as between the median pollen
537 dispersal between known mothers and fathers (see Methods). Median seed dispersal varied by
538 treatment, being greatest for UN and decreasing with increasing disturbance intensity (Figure A).
539 Results from a Kruskal-Wallis test indicated significant differences between groups (p = 0.0480),
540 with *post hoc* tests indicating significant differences between UN and BC (H = 4.34, p = 0.0372)
541 but not between UN and UC (H = 2.77, p = 0.0959) or between UC and BC (H = 2.75, p = 0.0970;
542 Figure A). Median pollen dispersal also varied by treatment, being greatest for BC treatments,
543 followed by UN and UC treatments, which did not differ significantly (p = 0.1381; Figure B).

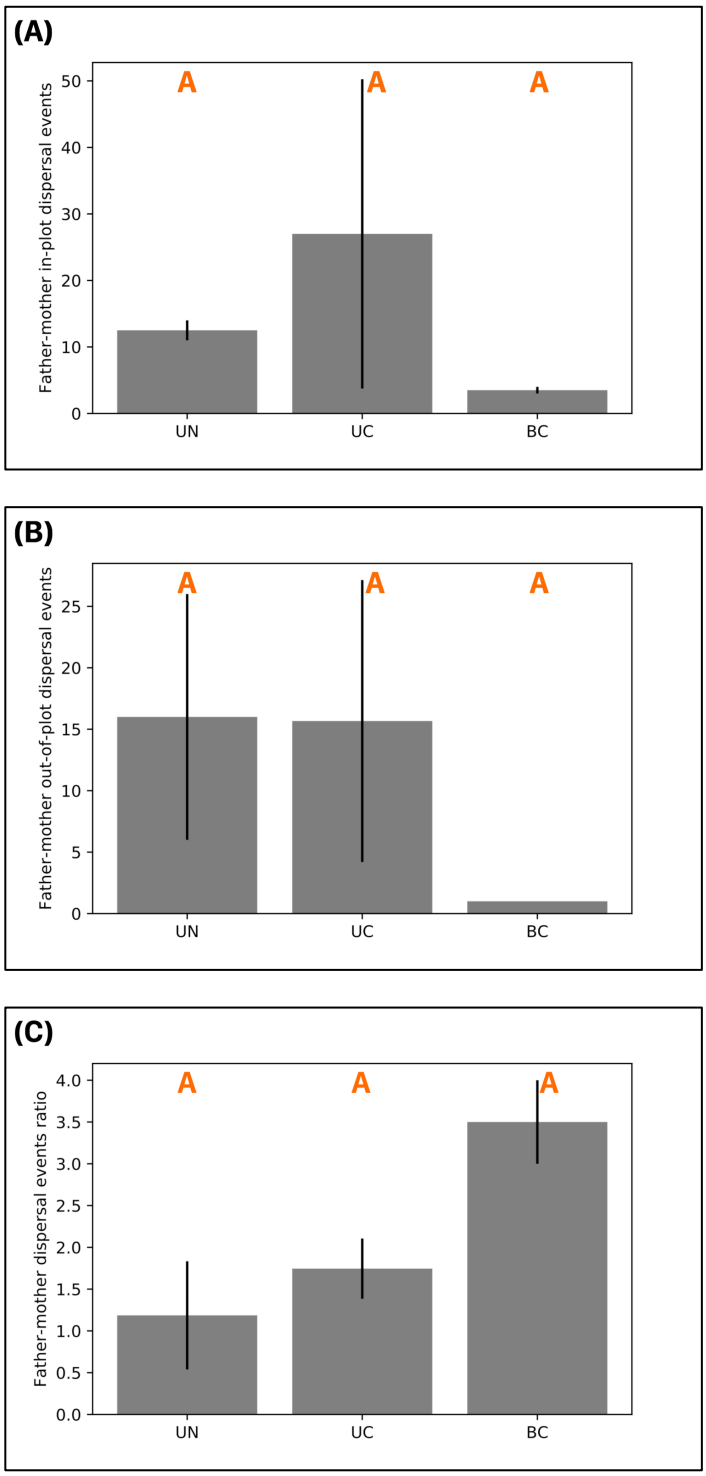

Figure 6 *Father-mother dispersal events by treatment for (A) dispersal between in-plot individuals, (B) dispersal into plot from an out-of-plot mother, and (C) the ratio of these values. Plot-level tallies were those of in-plot mothers receiving pollen from either an in-plot father (A) or an out-of-plot (sampled or unsampled) father (B). Orange letters within each plot show significant differences between medians, as inferred from Kruskal-Wallis tests (see main text of Results). Vertical lines indicate standard deviations.*

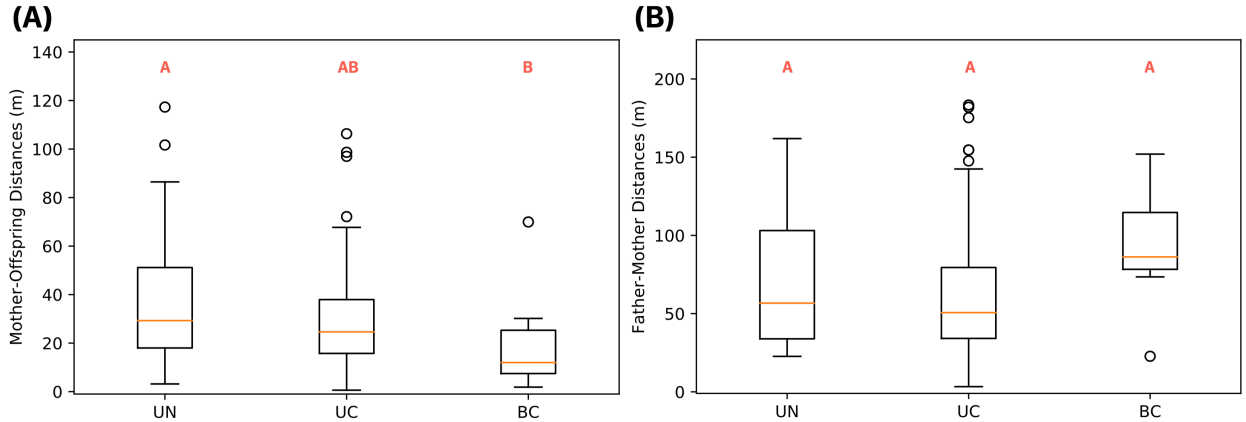

Figure 7 *Dispersal distances for seed (A) and pollen (B) calculated from the most probable pedigree from parentage analysis, considering only pedigrees with known mothers (A) or known parents (B). Orange letters within each plot show significant differences between medians, as inferred from Kruskal-Wallis tests for mother-offspring and father-mother dispersal distances (see main text of Results).*

These realized distances were roughly in line with, but smaller than, mean dispersal distances estimated from dispersal kernel shape parameters in the parentage analysis: mean seed dispersal = 65m (95% credible interval: 57-75); mean pollen dispersal = 170m (95% CI: 150-190; Figure).

To consider uncertainty in parentage assignment, we calculated weighted average dispersal distances for seed and pollen dispersal (see Methods). Assignments to mothers of out-of-plot adults were less common than for assignments to in-plot fathers, as can be seen from the blocks (replicates) within treatment of Figure . Using this set of fractional parentage, we calculated weighted average distances for each seed and nested these distances within treatments (see Methods). We first considered mother-offspring and father-mother dispersals from fractional parentage where the identified adults could originate in any treatment at TEF. Distances differed significantly by treatment (Figure A; H = 7.91, p = 0.0191) where UN and UC were significantly different (H = 8.11, p = 0.0044) but not between any other comparison (H range = [0.0042,0.6755], p > 0.4111). Father-mother distances (Figure B) also differed by treatment (H = 41.16, p = 1.15E-9), with median dispersal distance decreasing from BC to UN to

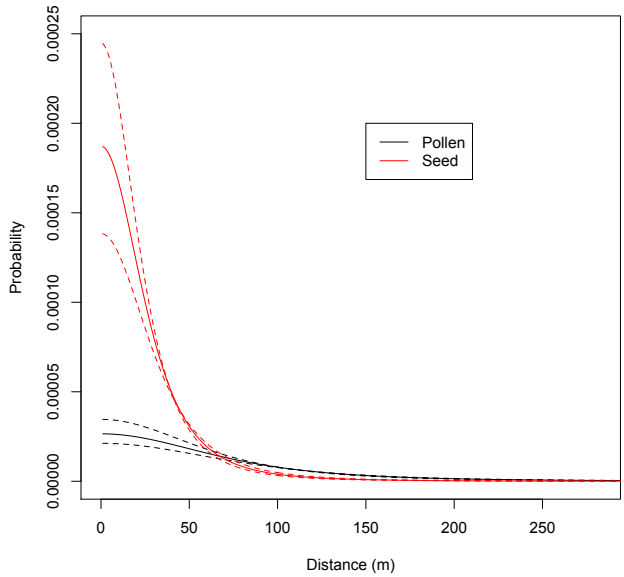

Figure 8 *Fitted 2D-t dispersal kernel for seed (red) and pollen (black) using shape parameters inferred from parentage analysis (sensu Moran & Clark 2011). Dashed lines show the 95% credible interval. This figure is truncated at the maximum distance within plots (200√2m) to focus on differences at short distances.*

UC, where all pairwise considerations were also significant (*H* range = [5.21,27.18], *p* range = [1.85E-07, 0.0224]).

Because the proximity of the treatment replicates at TEF may interact with dispersal distance estimates, we also considered dispersal distances within plot tallied within treatments using weighted distances as described above. Median values of mother-offspring in-plot distances decreased with increasing disturbance intensity (Figure C) and differed by treatment (*H* = 47.10, *p* = 5.91E-11), but only between UN and UC (*H* = *4.29*, *p* = 0.0382) and between UN and BC (*H* = 5.83, *p* = 0.0253) and not between UC and BC treatments (*H* = 0.95, *p* = 0.3291). In-plot father-mother distances (Figure D) were significantly different across treatments (*H* = 13.89, *p* = 0.0010), with BC having greater distances that either UN (*H* = 5.83, *p* = 0.0157) or UC (*H* = 5.07, *p* = 0.0242), and UC exhibiting greater distances than UN (*H* = 5.00, *p* = 0.0253).

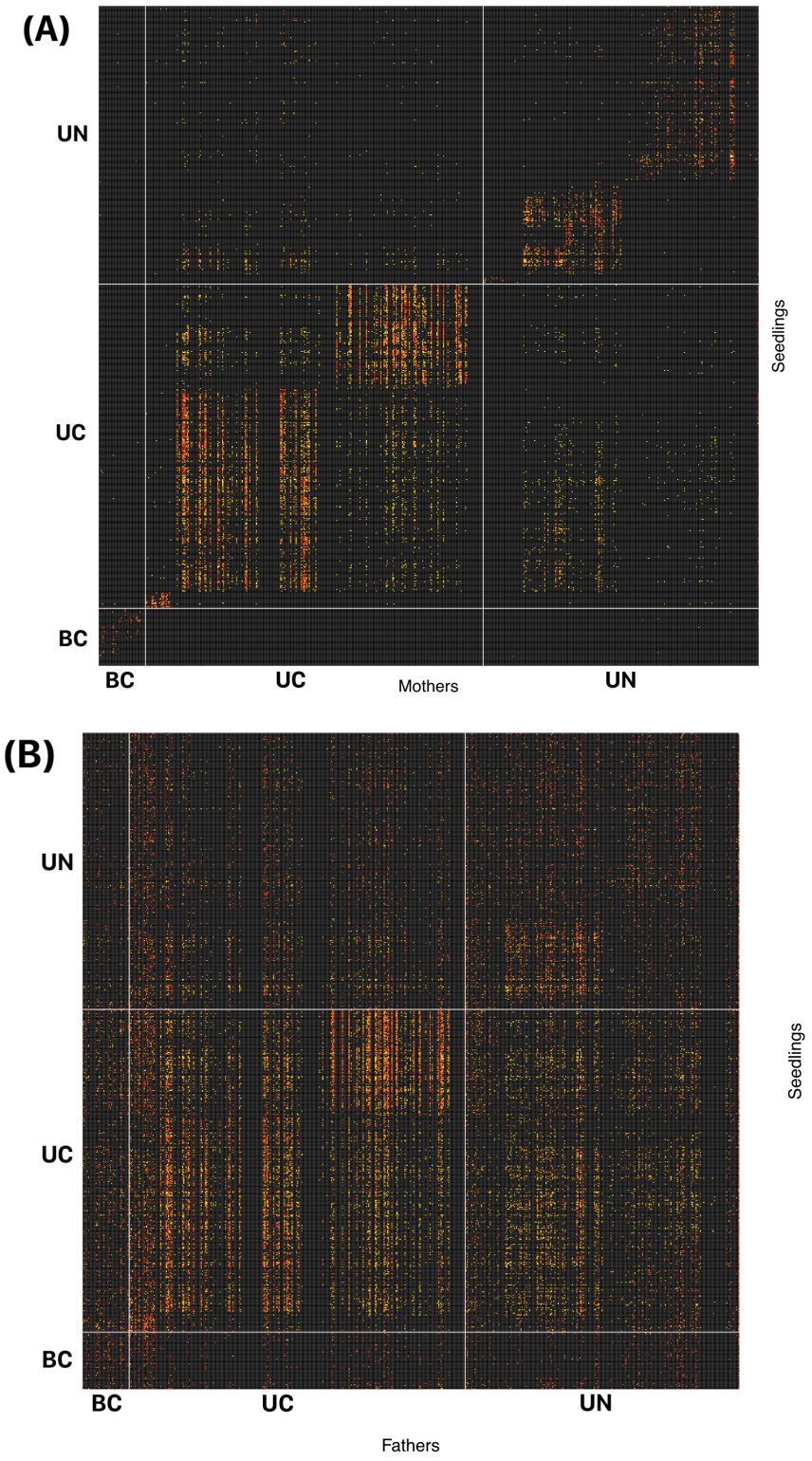

Figure 9 *Fractional parentage across parentage analysis cycles for (A) maternal assignment and (B) paternal assignment (see Methods) with adult individuals along x-axes and seedling individuals along y-axes. Each cell represents the fraction of the cycles a particular seedling was assigned to a given adult (black ~ 0 to red to orange to yellow to white ~1).*

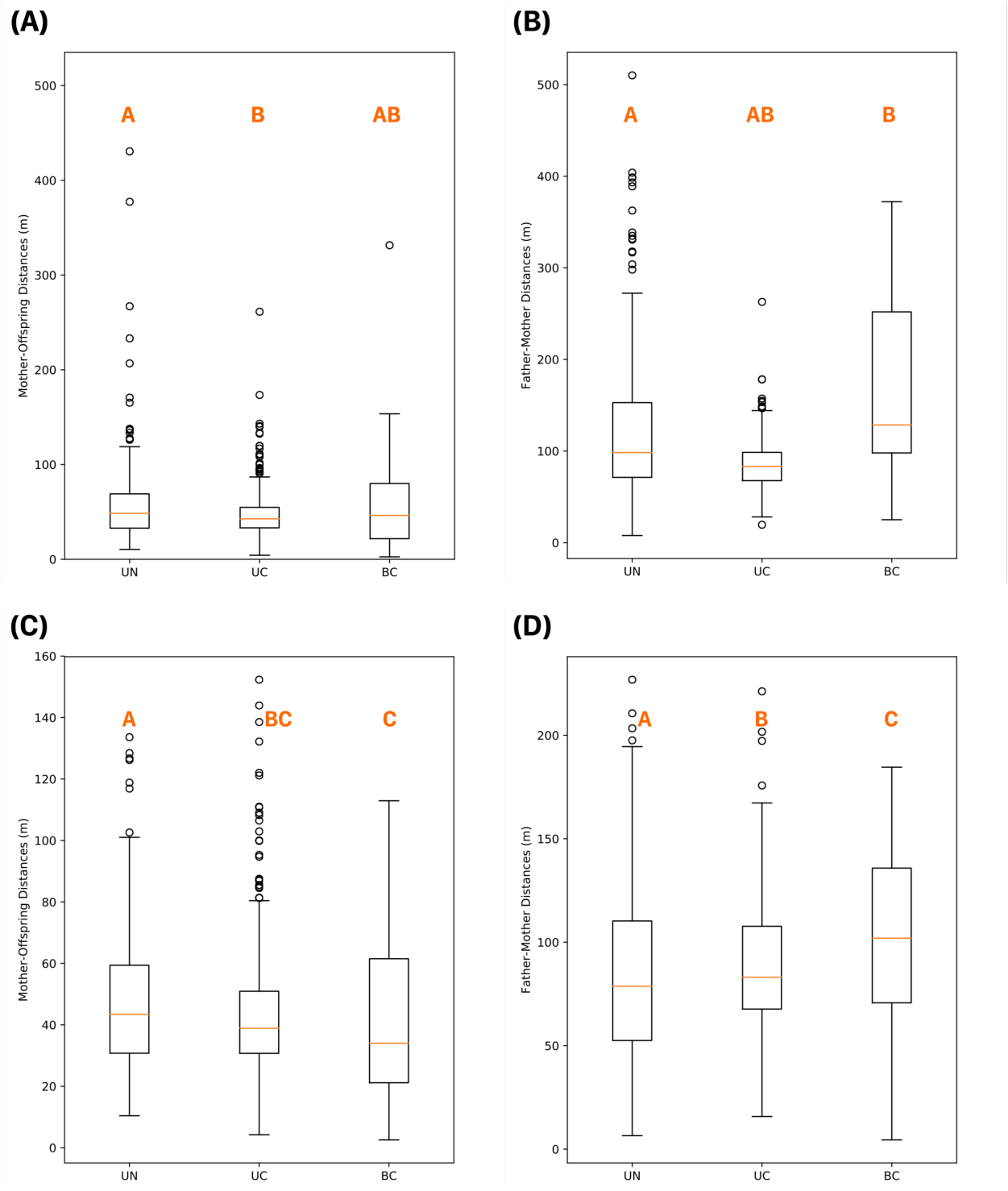

Figure 10 *Dispersal distances between mothers and offspring (first column) and between fathers and mothers (second column) using assigned adults from any location (A-B) and for only in-plot individuals (C-D). Orange letters within each plot show significant differences between medians, as inferred from separate Kruskal-Wallis tests (see main text of Results).*

601 **Discussion**

602 Frequent fires were commonplace in historic forests of the Sierra Nevada, where forests
603 exhibited relatively lower tree densities and a higher proportion of pine species (North *et al*. 2005;
604 Knapp *et al*. 2013). Yet post-settlement fire suppression has led to forest densification that has
605 caused instability in these systems and has increased the chances of uncharacteristic high-
606 severity wildfire. As a result, thinning prescriptions are used to increase the resilience of
607 constituent stands (SNFPA 2004; Agee & Skinner 2005; Schwilk *et al.* 2009; Safford *et al.* 2009).
608 While these prescriptions can mimic the density-reducing effects of fire, and reduce fire severity,
609 it is currently unknown how thinning, in isolation or through its interaction with managed fire, will
610 alter evolutionary dynamics of ecologically important species such as *P. lambertiana* (SNEP
611 1996). We characterized spatial and genetic structure of a fire-suppressed forest treated with a
612 common thinning prescription in the Sierra Nevada and compared this with its interaction with
613 prescribed fire and with a no-thin-no-fire control treatment. Our results suggest that spatial
614 structure of constituent species is a result of the interaction between treatment and ecology where
615 pines are often clustered with other pines, shade-tolerant trees are often clustered with other
616 shade-tolerant trees, and pine seedlings often are inhibited by both adult pine and shade-tolerant
617 individuals. While genetic diversity statistics are informative of stand-level diversity, they are less
618 informative regarding ongoing evolutionary dynamics as a result of treatment as they do little to
619 predict inbreeding of future generations nor the scale at which mating events are to occur. Used
620 in isolation, diversity indices leave researchers to speculate about ongoing processes and future
621 outcomes, while monitoring of processes that affect fitness provides more meaningful inferences
622 which can be directly used by land managers.

623 From the analysis of spatial genetic structure (*sensu* Smouse & Peakall 1999), and despite
624 spatial inhibition between adults and seedlings across treatments, our results suggest that
625 unburned thinned stands (UC treatments) result in the increase of fine-scale similarity of adult to
626 seedling genotypes relative to control (UN treatments) or thinned-and-burned stands (BC

627 treatments). Parentage analysis offered additional quantification of fine-scale gene flow and
628 suggested that effective seed and pollen dispersal within plots generally decreased and
629 increased, respectively, with the increasing intensity of disturbance, perhaps due to an increase
630 in microsite suitability for *P. lambertiana*, or for adults, the availability of potential mates. Our
631 results were measured from individuals remaining or regenerating 13 years post-treatment, very
632 near the historical fire return interval for this area. Thus, ongoing dynamics should be monitored,
633 and will likely change through time, as stands with different treatments continue to develop and
634 respond to subsequent disturbances such as fire.
635
636 *The genetic effects of forest management*
637 With some exceptions, studies investigating the genetic consequences of forest
638 management have centered around the impact on genetic diversity indices (see Table 1 in
639 Ratnam *et al*. 2014). This focus is likely due to the fact that highly outcrossing tree species often
640 suffer from elevated inbreeding depression, where survival and reproduction of subsequent
641 generations may be impacted. In such cases, genetic diversity has been used as an index for
642 evolutionary potential, likely attributable to the consequences of the relative contribution of
643 additive genetic variance to phenotypic variance (i.e., narrow-sense heritability) in the breeder's
644 equation (Lynch & Walsh 1998), but the use of heritability itself as a measure of evolvability comes
645 with important caveats (e.g., see Hansen *et al*. 2011). Further, such diversity indices have been
646 used to assess the relative reduction of alleles due to harvest intensity, where the removal of
647 individuals from stands will likely reduce the diversity of alleles present. Here, management
648 resulting in population bottlenecks is of concern. While these premises are important to
649 investigate, the use of genetic diversity indices as the sole method for inference of management
650 impact are limiting with regard to evolutionary outcomes. If the focus is to be on management
651 impact on evolutionary potential, processes that influence evolutionary fitness should be
652 investigated instead (e.g., mating systems, effective dispersal, fecundity, spatial genetic structure,

653 pollen pool heterogeneity, juvenile survival; Lowe *et al*. 2015). Many traits with fitness
654 consequences in trees are of a polygenic basis (Lind *et al*. 2018), where any given underlying
655 positive-effect locus has minimal influence on the trait. In such cases, fixation (as measured by a
656 handful of putatively neutral markers) at some of the underlying causative loci can be ameliorated
657 by selection for combinations of alleles at other loci. Therefore, while alleles with little to no effect
658 on fitness are informative for demographic processes, these should not be conflated with loci
659 under selection, particularly loci under strong negative selection with important implications for
660 inbreeding depression. Such neutral markers could be better utilized in assessing consequences
661 *within* processes that directly affect fitness. However, in cases where spatial genetic relatedness
662 is increased as a result of management, or individuals become increasingly sparse, wasted
663 reproductive effort (e.g., embryo abortion, or high juvenile mortality) due to increased instances
664 of consanguineous or self-mating events may play an important role in ongoing population
665 dynamics (Kärkkäinen *et al*. 1999; Sorensen 2001), particularly when seed rain of heterospecifics
666 exceeds effective reproductive output of historical or ecologically important species (e.g., as for
667 *P. lambertiana* at TEF, Zald *et al*. 2008).
668
669 *Dispersal dynamics of tree species*
670 The analysis of spatial genetic structure and gene flow within and across populations of
671 trees can elucidate ongoing evolutionary dynamics, as this spatial structure is a result of selective
672 and neutral processes acting across temporal and spatial scales (Hardy & Vekemans 1999;
673 Oddou-Muratorio *et al*. 2004; Robledo-Arnuncio *et al*. 2004; Oddou-Muratorio *et al*. 2011). Thus,
674 quantifying dispersal and mating system is an important component in understanding such
675 patterns. There are multiple biological and ecological factors that shape dispersal dynamics and
676 resulting mating systems, such as population density, degree of fragmentation, manner of
677 pollination (e.g., anemophily, entomophily, or zoophily), relative reproductive output, phenotype
678 (such as crown shape or height), interannual climatic variation, as well as stochastic variables

679 such as wind direction and strength (Burczyk *et al*. 1996; Dow & Ashley 1998; Robledo-Arnuncio
680 *et al*. 2004, Burczyk *et al*. 2004; O'Connell *et al*. 2004). Compared with herbaceous and annual
681 plants, trees have more extensive gene flow (Hamrick *et al*. 1992), though such distances are
682 idiosyncratic to a given population, species, and system. For instance, estimates of pollen
683 dispersal for *Pinus sylvestris* varied from between 17-29m based on paternity assignment
684 (Robledo-Arnuncio *et al*. 2004) to 136m (Robledo-Arnuncio & Gil 2005) using the TwoGener
685 method (Smouse *et al*. 2001) where 4.3% of mating events came from pollen dispersed over
686 30km (Petit & Hampe 2006; Savolainen *et al*. 2007). Seed dispersal distances can also vary
687 idiosyncratically, particularly for winged seeds or those that are also dispersed by animals, such
688 as with *P. lambertiana*.

689 Spatial genetic structure will be a function of these dispersal consequences as well as
690 their ecological interaction with the environment. While much of the quantification of such
691 structure in trees has been carried out at regional or continental scales, examples exist for
692 investigations at fine spatial scales below a few hundred meters. For instance, Marquardt *et al*.
693 (2007) assessed spatial genetic structure of eastern white pine (*Pinus strobus* L.) as a function of
694 management influence at Menominee Indian Reservation in northeastern Wisconsin. While
695 spatial genetic structure within 100m differed by population, the strongest autocorrelation
696 occurred at the least disturbed site (Marquardt *et al*. 2007). However, while they sampled both
697 adults and natural regeneration they did not distinguish these two groups when inferring spatial
698 genetic structure. Conversely, in Norway spruce (*Picea abies* L. Karst.) populations of northern
699 Italy, Scotti *et al*. (2008) assessed spatial genetic structure of mitochondrial (maternally inherited)
700 and chloroplast (paternally inherited) loci across both adults and saplings. While chloroplast
701 haplotypes were uncorrelated across most distance classes up to 90m for both classes, the
702 maternally inherited mitochondrial markers showed strong affinity below 30m, where this affinity
703 was greater for saplings than for adults. This pattern was seen for *P. lambertiana* individuals at
704 TEF as well, where both adults and seedlings were genetically structured at small distance

classes in UC treatments, though seedling genotypes were clustered to a higher degree than adults (Figure). To our knowledge however, few instances in the literature compare both spatial structure of trees with spatial genetic structure of tree genotypes. At TEF, seedlings were clustered at fine spatial scales across all treatments likely due to microsite suitability (as most cached seeds will likely persist only in suitable sites), but were only clustered genetically in UC treatments. As such, without genotypic data, investigators may be lead to spurious conclusions where it may be assumed that clustering of individuals also indicates clustering of genotypes. Further, ingrowth of *P. lambertiana* in UC treatments will likely be more related to nearby individuals, which may cause inbreeding and embryo abortion to a greater degree in subsequent generations than in other stands at TEF.

Management implications

Our results suggest that management is affecting dispersal through the availability of suitable microsites for seedling establishment, as well as through the availability of mates. As disturbance intensity increased at TEF, mean effective seed dispersal generally decreased while effective pollen dispersal generally increased (Figure A-B), likely due to the proximity of suitable (e.g., unshaded) microsites and the availability of potential mates, respectfully. Using the inferred dispersal kernels (Figure), the vast majority of dispersal occurs across small distance classes, with the estimated probability of dispersal of pollen below 150m accounting for more than 90.2% of pollen dispersal events, while dispersal of seed below 50m (150m) accounts for 87.3% (99.2%) of dispersal events across TEF. Such a dispersal tendency will drive spatial genetic structure and will interact with environment (including management) to ultimately determine the patterns we observe across the landscape. Because UC treatments generally resulted in an increased spatial affinity of alike genotypes between adults and seedlings (Figure), short-term dynamics (decadal scales) may be dominated by mating events between related individuals. However, long-term dynamics will likely affect this structure as well. The strong levels of spatial genetic structure

observed in seedlings have been shown to decrease in adult stages because of self-thinning processes in other tree species (Hamrick *et al*. 1993; Epperson & Alvarez-Buylla 1997; Chung *et al*. 2003; Oddou-Muratorio *et al*. 2004), and may well occur at TEF as well. Even so, such consequences are dependent upon initial structure that may vary to differing degrees in undisturbed stands, or across the landscape. Long-term dynamics should be monitored as these stands continue to develop and respond to contemporaneous ecological pressures.

Conclusion

Understanding how thinning and fire prescriptions intended to decrease fire severity and restore ecosystem resilience influence evolutionary dynamics of historically dominant and ecologically important pine species is of paramount significance. We found that treatment of fire-suppressed populations of *P. lambertiana* differentially affects fine-scale spatial and genetic structure, and that seed and pollen dispersal increase and decrease, respectively, with disturbance intensity. Such dynamics are likely to remain unequilibrated in the short term, and therefore management would benefit from further monitoring of evolutionary dynamics that affect fitness in these forests (e.g., reproductive output, survival of seedlings). Further monitoring across broader spatial scales would also inform how these management prescriptions affect dynamics across a greater extent of environmental heterogeneity and how these evolutionary dynamics vary by locality. Such information will allow management to prescribe treatments in a regionally- and site-specific manner.

Acknowledgments

The authors wish to thank Britta Austin, Casey Harless, Erin Hobson, Nate Stearrett, and Alexandrea Stylianou for help with field and lab work (and for being great people), VCU's Center for High Performance Computing, and to VCU for start-up funds awarded to AJE.

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