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ORIGINAL ARTICLE

Fine-scale spatial genetic structure of two red oak species, *Quercus rubra* and *Quercus ellipsoidalis*

Jennifer Lind-Riehl · Oliver Gailing

Abstract Peripheral populations located at their range edge, may be at risk due to geographical isolation, environmental changes, human disturbances or catastrophic events such as wildfires. Fine-scale spatial genetic structure (SGS) investigations provide a way to examine the spatial arrangement of genetic variation within populations. SGS can result from restricted seed and pollen dispersal and might be affected by geographic isolation and environmental changes and disturbances even in outcrossing wind-pollinated species like oaks. Studying the SGS of peripheral populations provides information that can be used to develop improved conservation and management plans at the species' range edge. We assessed the level of genetic variation and SGS in twelve range edge populations in northern Wisconsin and the Upper Peninsula of Michigan (USA): eight *Quercus rubra* and four *Quercus ellipsoidalis* populations that were subject to different management regimes and natural disturbances. In contrast to *Q. rubra* populations, the drought tolerant *Q. ellipsoidalis* populations are isolated from the species' main distribution range. These populations are not actively managed but are especially prone to recurring fire events. The four managed and four old growth ("unmanaged") *Q. rubra* populations displayed similar levels of genetic variation. Likewise the *Sp* statistic showed similar SGS levels in managed and unmanaged *Q. rubra* populations ($Sp = 0.005$) comparable to other *Quercus* species (European *Q. robur*: $Sp = 0.003$). *Q. ellipsoidalis* populations showed similar or more pronounced SGS than neighboring *Q. rubra* populations extending up to 83 m in one population. A significant excess of homozygotes across markers in two of the *Q. ellipsoidalis* populations suggests potential inbreeding. In summary, diverse management activities combined with various natural disturbances are likely both influencing SGS patterns. Outcrossing forest trees like oaks hold large amounts of genetic diversity allowing adaptation to environmental changes over their long life spans. Reductions of these genetic stores, through inbreeding for example, can inhibit a species' ability to adapt to changing environmental conditions.

Handling editor: Christoph Oberprieler.

J. Lind-Riehl · O. Gailing (✉)
Michigan Technological University, 1400 Townsend Drive, 49931 Houghton, MI, USA
e-mail: ogailing@mtu.edu

J. Lind-Riehl
e-mail: jflind@mtu.edu

Introduction

Outcrossing forest tree populations are known to harbor high levels of genetic diversity within populations, with relatively little genetic variation among them due to their long life spans, large neighborhood sizes, and extensive gene dispersal (Petit and Hampe 2006; Hamrick et al. 1979). The spatial arrangement of this genetic diversity is expected to vary widely between different forest tree species given their great variety in reproduction, mating, and dispersal systems (Epperson 1992). Fine-scale spatial genetic structure (SGS) can result from, for example, limited seed or pollen dispersal that leads to the clustering

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of genetically similar individuals (McCauley 1997; Trapnell and Hamrick 2004). The incidence and strength of SGS are influenced by dispersal efficiency, mating systems, population density, and life history (Vekemans and Hardy 2004). In turn, distribution of genetic variation in populations reflects patterns of gene flow through pollen and seed dispersal, and microenvironmental selection (Epperson 2000). However, relatively little is known about the distribution of genetic variation at the population level for peripheral or marginal populations (Jump and Peñuelas 2006). Peripheral populations have been shown to be sources of genetic variation (Jimenez et al. 1999; Lorenzo et al. 2009) and the genetic diversity and distinctness in peripheral populations may be the result of local selective pressures or limited gene flow from other populations (Gibson et al. 2009). They may also be particularly vulnerable to climatic changes resulting in increased competition and fire or drought events (Aitken et al. 2008), and/or anthropogenic changes such as fragmentation (Muir et al. 2004; Gibson et al. 2009), both of which may influence the spatial arrangement of genetic variation. Thus, it is imperative to examine SGS in different species with varied life history traits as well as peripheral populations of these species to provide the best information for conservation and management activities of a particular species or population (Epperson 1992).

Studies of fine-scale spatial genetic structure (SGS) in oaks and other wind-pollinated forest tree species have yielded generally low SGS with a few exceptions. Thus, many wind-pollinated species with gravity or animal dispersed seeds showed low, but significant genetic structure over short distances likely as the result of restricted seed dispersal (Berg and Hamrick 1995; Stefenon et al. 2008; Streiff et al. 1998; Troupin et al. 2006). Spatial aggregation of adult trees and seedlings in Abies alba Hill showed reduced SGS, suggesting that wind pollination counterbalanced the effect of restricted seed dispersal (Sagnard et al. 2011). Strong SGS has been observed in studies as the result of clonal reproduction or strong inbreeding in geographic isolation (Berg and Hamrick 1994; Chybicki et al. 2011). For example, strong kinship structure up to 50–100 m was found in highly isolated populations of wind-pollinated Taxus baccata L., whose seeds are dispersed by gravity or birds (Chybicki et al. 2011).

Studies examining range edge populations are rare, but Pandey and Rajora (2012) compared SGS between two peripheral and two core populations of Thuja occidentalis and found relatively higher SGS in peripheral populations. Muir et al. (2004) examined range edge fragmented populations of Quercus petraea in Ireland using both microsatellite and plastid markers and found similar levels of genetic variation to mainland European populations likely maintained by high levels of outcrossing. They caution that

the effects of recent exploitation of the area may not yet be reflected in the current population structure due to limited generations exposed to genetic drift and currently high gene flow levels. In fact, Jump and Peñuelas (2006) have shown that chronic fragmentation of Fagus sylvatica populations for over more than 600 years has had significant impacts on genetic diversity and structure.

Studies comparing different oak species have shown differences in extent of SGS that often correspond to differences in life history characteristics such as efficiency of seed dispersal or mating systems (Berg and Hamrick 1994; Cottrell et al. 2003; Streiff et al. 1998). For instance, more pronounced SGS was detected in Q. petraea than in Q. robur which was attributed to Q. robur's potential for longer range seed dispersal and the ability to grow over a wider range of site conditions (Streiff et al. 1998). Additionally, management of stands may influence the spatial arrangement of trees consequently affecting SGS by influencing mating patterns and gene flow through modification of the spatial distribution of trees (Finkeldey and Ziehe 2004). For example, harvesting and thinning projects could impact genetic diversity of populations and their ability to adapt by selecting for certain desired phenotypic traits (Finkeldey and Ziehe 2004). However, little is known about the effects of management on SGS (Cottrell et al. 2003). Cottrell et al. (2003) found a higher significant SGS in populations of Q. robur and Q. petraea that experienced long-term coppicing and planting as compared to relatively unmanaged populations which they attributed to the influence of artificial regeneration through planting of seeds originating from only a few individuals. Managed (logging and other silvicultural activities) and unmanaged populations of another Fagaceae tree species, Fagus sylvatica L., exhibited similar levels of genetic diversity (Rajendra et al. 2014). However, managed F. sylvatica stands exhibited reduced SGS in comparison to the unmanaged stands potentially as the result of selective removal of trees breaking down family structures (Rajendra et al. 2014; Paffetti et al. 2012).

Here we focus on the two red oak species in northern Wisconsin and the Upper Peninsula of Michigan (USA), Quercus rubra and Q. ellipsoidalis. While Q. rubra is common and has a wide distribution range, Q. ellipsoidalis has a much smaller distribution range with only fragmented populations in the study areas. Both species can co-occur, but generally are more likely to be near one another than sympatric as Q. ellipsoidalis favors dry sandy soils and Q. rubra more mesic conditions (Abrams 1990). Given the dry conditions at these sites, Q. ellipsoidalis stands are prone to fire, and stand replacing fires may occur as frequently as every 30 years (Dickmann and Leefers 2003). While both species maintain distinct adaptations to drought, there is potential for hybridization, and genetic assignment analyses were used to identify Q. rubra and Q. ellipsoidalis

samples (Lind and Gailing 2013). *Quercus rubra* is primarily an outcrossing species with potentially long distance pollen dispersal (Ennos 1994; Moran and Clark 2012). Acorn dispersal by gravity is common and is thought to play a role in limiting gene dispersal since acorns are heavy. Jones et al. (2006) found that *Q. rubra* seedlings showed SGS at small scales (up to 25 m) consistent with limited seed dispersal. However, animals are also important vectors and birds such as blue jays are known to cache acorns and have been implicated in postglacial dispersal of fagaceous trees in eastern North America (Johnson and Webb 1989). Pollen dispersal has been shown to be farther reaching than seed dispersal for *Q. rubra* as would be expected in an outcrossing and wind-pollinated tree species (Moran and Clark 2012). Little is known about gene dispersal in *Q. ellipsoidalis*, but *Q. ellipsoidalis* shows similar life history characteristics to *Q. rubra* and both species are hybridizing; seed dispersal is thought to be carried out by similar animals such as squirrels and blue jays. Additionally seed production seems to be less frequent in *Q. ellipsoidalis* based on field observations in the Ford Research Forest-Baraga Plains area (FRF-BP) since 2009. Little is known about the SGS of *Q. ellipsoidalis* as it is not considered to be a valuable timber species. Aldrich et al. (2005) described SGS in old growth *Q. rubra* populations in Indiana and found significant spatial structure up to 70 m. The stand had maintained high levels of genetic diversity despite the absence of smaller size classes in the core habitat of the stand. This may be indicative of the early stages of a genetic bottleneck likely created by extensive harvesting during European settlement of the area. However, the decline in oak regeneration has become a concern. Many reasons have been cited for this decline including fire suppression, elevated herbivory, and competition with invasive plant species (Huebner 2003; Lorimer 1993).

Quercus rubra is an important component of temperate forests in the Great Lakes region and provides habitat and food for various wildlife (McShea et al. 2007). Economically the species is valuable for many uses including wood production (Aldrich and Cavender-Bares 2011). Considering the importance of *Q. rubra*, both economically and ecologically, further understanding of how management practices affect genetic diversity of *Q. rubra* and the closely related interfertile *Q. ellipsoidalis* would be beneficial to appropriately address issues such as low regeneration rates. Furthermore, since these populations are at the range edge, it will be important to understand current SGS and genetic variation patterns to assess the vulnerability of these populations to climate change and anthropogenic impacts.

Our study aims to characterize fine-scale spatial genetic structure (SGS) in unmanaged and managed peripheral populations of *Q. rubra* and to compare SGS in both *Q. rubra* and *Q. ellipsoidalis* peripheral populations. We want to better understand how differences in the historical management and disturbance regimes affect the level of SGS. We also examine whether there are differences in SGS and levels of inbreeding between the more widely distributed species *Q. rubra* and the more isolated populations of *Q. ellipsoidalis*.

Materials and methods

Sample locations

A total of eight *Q. rubra* and four *Q. ellipsoidalis* populations were sampled from five geographic regions from the northern distribution edge of both species. While both species are at their northern distribution edge, *Q. rubra* remains far more frequent than *Q. ellipsoidalis*. *Q. ellipsoidalis* populations were geographically isolated and separated from the main distribution range of the species (Lind-Riehl et al. 2014). Eight populations (four *Q. rubra* and four *Q. ellipsoidalis*) were subjected to different degrees of management, while four populations (*Q. rubra* only) were mainly unmanaged pre-European settlement forests (Dickmann and Leefers 2003) (Fig. 1; Table 1). All *Q. rubra* populations showed signs of natural regeneration. The managed forests consist of two pairs of *Q. rubra* and *Q. ellipsoidalis* populations in the Western Upper Peninsula of Michigan in the Ford Research Forest-Baraga Plains area (FRF-BP) as well as two pairs of *Q. rubra* and *Q. ellipsoidalis* populations from the Chequamegon-Nicolet National Forest in northern Wisconsin (Fig. 1; Table 1). Like many forests in the temperate regions of the United States both of these areas were heavily logged for pine in the late 1800s and then again for northern hardwoods between the 1920s and 1940s (Dickmann and Leefers 2003; Saetre 1983). Catastrophic wild fires followed the logging of forests in Michigan and Wisconsin. Pine barrens such as in the Baraga Plains and in the Chequamegon-Nicolet National Forest were especially prone to fire and were maintained by fires that could return at intervals of less than 30 years (Dickmann and Leefers 2003; Saetre 1983). Red oaks colonized burnt cutover lands in both Michigan and Wisconsin (Abrams 1992).

At the FRF-BP site, a stand replacing fire occurred sometime around 1910 that burned through the Baraga Plains reaching almost to Alberta, which encompasses the areas occupied by all four sampled stands (J. Schmierer, personal communication). Fire scars are still visible on some of the older surviving trees in the FC-A stand (field observation). No major fires have occurred in the area since then. In 1954, the Ford Motor Company donated 1,700
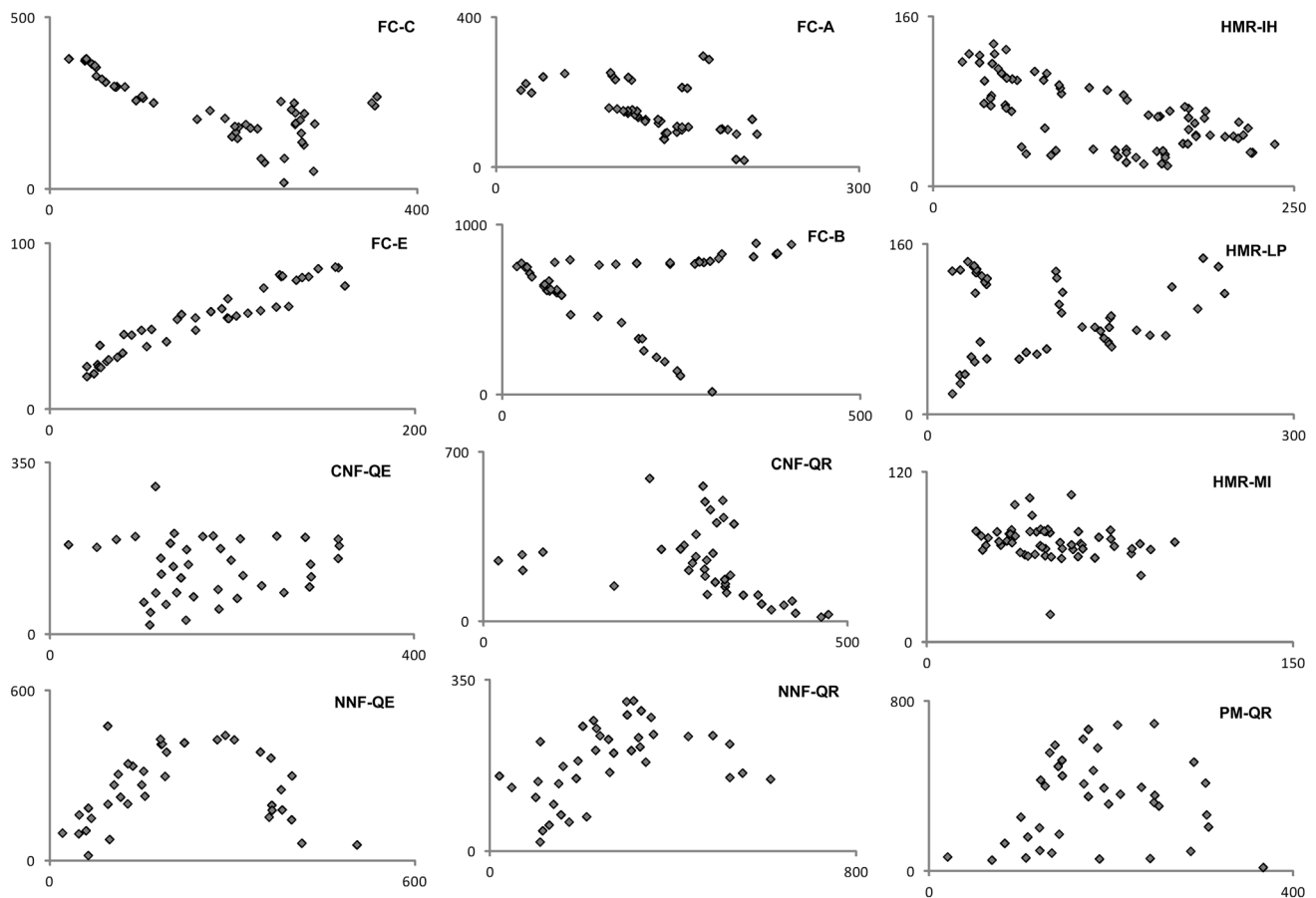
Fig. 1 Spatial distribution of sampled populations in northern Wisconsin and the Upper Peninsula of Michigan (USA) (the population abbreviations follow those in Table 1)

acres of land including the current Ford Research Forest. Since then, management for hardwoods, including *Q. rubra*, has been based on a selection system silviculture. In the Baraga Plains jack pine has been managed using even-aged methods and various regeneration techniques including scarification or spot fires, with incidental management of *Q. ellipsoidalis* populations (J. Schmierer, personal communication). The *Q. ellipsoidalis* stands, FC-C and FC-E, are both located in pine barrens known as the Baraga Plains with jack pine surrounding them. The *Q. rubra* stand FC-A is in a mixed mesic deciduous forest with major sugar maple and pine components, while FC-B is in a mixed deciduous hardwood forest with maple and hemlock (field observation).

Starting in the late 1920s Wisconsin began acquiring land for the creation of national forests. By 1933, the Chequamegon and Nicolet National Forests (CNF and NNF) were officially named and now consist of more than 1.5 million acres of land across northern Wisconsin. Prior to 1930, fire was common in northern Wisconsin with about 2,500 fires burning half a million acres a year. Since that time fires have decreased, replanting increased and

more sustainable management practices have been adopted (Saetre 1983). Jack pine that surrounded the *Q. ellipsoidalis* stand in the NNF has been periodically clear cut (last cut in 1992). The area around the CNF *Q. ellipsoidalis* stand, which is partially on private land, has also been periodically clear cut (last cut in 1994) and is now mostly covered with jack pine. The two *Q. rubra* stands, one in CNF and one in NNF, have been managed through thinning and shelter wood cutting, most recently between 2000 and 2010, allowing for the establishment of even-aged stands. *Q. rubra* is the dominant species in the NNF stand and the CNF stand contains mostly *Q. rubra* with sugar maple and some aspen and spruce [D. Veen (USFS) and A. Sullivan, personal communication]. In general, *Q. rubra* has been directly managed in both the FRF-BP, CNF, and NNF populations, while the *Q. ellipsoidalis* populations in these areas experienced only incidental management.

Additionally, four unmanaged *Q. rubra* populations were collected in the Western Upper Peninsula of Michigan (Fig. 1; Table 1). Compared to those described above, these populations have not been actively managed. Three of them are located in the Huron Mountain Reserve

Table 1 Sampled population characteristics

Population	Region	Species	Sample Size (N)	Latitude (S)	Longitude (W)	Altitude (m)	Sampled area (m²)	Mean DBH (cm)
Managed Stands								
CNF-QE	Chequamegon National Forest (CNF)	*Q. ellipsoidalis*	40	46°44′43″N	91°04′20″W	384	33,076	19.1
FC-C	Ford Research Forest-Baraga Plains (FRF-BP)	*Q. ellipsoidalis*	50	46°39′14.454″N	88°35′25.616″W	394	41,354	19.2
FC-E	Ford Research Forest-Baraga Plains (FRF-BP)	*Q. ellipsoidalis*	47	46°39′55.879″N	88°33′19.775″W	398	2,575	12.3
NNF-QE	Nicolet National Forest (NNF)	*Q. ellipsoidalis*	39	45°19′19″N	88°19′ 53″W	301	38,755	18.7
CNF-QR	Chequamegon National Forest (CNF)	*Q. rubra*	40	46°42′54″N	91°02′8″W	323	69,969	25.5
FC-A	Ford Research Forest-Baraga Plains (FRF-BP)	*Q. rubra*	48	46°39′9.407″N	88°30′6.962″W	297	29,743	35.3
FC-B	Ford Research Forest-Baraga Plains (FRF-BP)	*Q. rubra*	48	46°40′27.937″N	88°31′27.397″W	423	87,754	32.0
NNF-QR	Nicolet National Forest (NNF)	*Q. rubra*	40	45°20′53″N	88°23′17″W	354	36,596	NA
Unmanaged Stands								
HMR-IH	Huron Mountain Reserve (HMR)	*Q. rubra*	76	46°51′12.884″N	87°50′42.824″W	257	11,696	20.5
HMR-LP	Huron Mountain Reserve (HMR)	*Q. rubra*	52	46°50′59.813″N	87°49′48.806″W	246	8,570	21.4
HMR-MI	Huron Mountain Reserve (HMR)	*Q. rubra*	60	46°51′20.783″N	87°51′24.026″W	307	2,359	31.8
PM-QR	Porcupine Mountains Wilderness State Park (PMWSP)	*Q. rubra*	40	46 44′23.070″N	89°46′23.460″W	NA	100,931	44.8

NA information not available, *DBH* diameter at breast height

(HMR), which was established as the privately owned Huron Mountain Shooting and Fishing Club in 1889. It contains one of the few remaining extensive tracts of intact hardwood-hemlock-pine forests in the Upper Great Lakes (Dickmann and Leefers 2003). In the 1930s the Club approached Aldo Leopold for advice on how to best manage their lands and his 1938 report focused on preserving natural habitat for native wildlife and as large a sample as possible of uncut timber. In the 1940s, the Club teamed up with the US Forest Service to implement many of Leopold's recommendations which have guided their management strategies to this day (Flaspohler and Meine 2006). Only about 20 % of the Club's perimeter lands were selectively logged for white pine in the late 1800s and about 6 % of the Club's land consists of oak-pine communities (Davis 1996). None of the sampled populations were located in the selectively logged lands. The fourth population is located in the Porcupine Mountains Wilderness State Park (PM). The PM was formed in 1945 to protect large stands of old growth northern hardwood forest that are the largest west of the Adirondacks, with 35,000

acres of unlogged forest. The forest is primarily composed of sugar maple, American basswood, eastern hemlock and yellow birch and may have been lightly logged for white pine in the late 1800s (Davis 1996). The area is open to the public for recreation as a state park with trail management activity, but timber production is not a focus of the management plan for this forest.

Sample collection

Leaf material was collected and GPS coordinates were recorded for each tree in all sampled populations. Exhaustive sampling was undertaken in the HMR, FRF-BP populations resulting in a large number of comparisons in small distance classes (Online Resource 1). In the CNF, NNF, and PM populations the smaller distance classes were less well represented (Fig. 1; Online Resource 1). While we will not be able to detect SGS at a very small scale due to a lower representation in the small distance classes for the latter populations, we can compare the presence and extent of SGS for populations with similar sampling design. The

leaf material was stored at $-20\ ^\circ\text{C}$ until DNA extraction. Total genomic DNA (~ 10–20 ng) was extracted using the Qiagen DNeasy96 Plant Kit (Qiagen, Valencia, CA) following the manufacturer's instructions.

Microsatellite genotyping

All populations were characterized at 15 microsatellite markers including eight nuclear simple sequence repeats (nSSRs: 1P10, 2P24, 3A05, 3D15, QpZAG15, quru-GA-0C11, quru-GA-0E09, quru-GA-1F07) and seven expressed sequence tag-SSRs (EST-SSRs: FIR004, FIR048, GOT004, GOT009, GOT021, PIE040, PIE099) described in an earlier study (Lind and Gailing 2013). According to Cavers et al. (2005), 10 microsatellite loci are sufficient to characterize fine-scale SGS. Nuclear and EST-SSRs developed in *Q. robur* were adapted for use in *Q. rubra* and *Q. ellipsoidalis* and tested for the presence of null alleles (Lind and Gailing 2013; Lind-Riehl et al. 2014, Sullivan et al. 2013). PCR amplification and electrophoretic separation were performed according to Lind and Gailing (2013), but the 10 μL PCR reaction was scaled to a 15 μL reaction.

Genetic variation analyses

GeneAlEx 6.5 (Peakall and Smouse 2006) was used to calculate most genetic diversity parameters including average number of alleles per locus (N_A), number of rare alleles (N_{RARE}, frequency $<5\ \%$), effective number of alleles ($N_E = 1/(1 - H_S)$), observed heterozygosity (H_O), expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}) for all populations as well as for both managed and unmanaged populations and for each species. Fisher's exact tests to determine significance of F_{IS} values were calculated in GenePop 4.2 (Raymond and Rousset 1995). FSTAT (Goudet 1995) was used to calculate the measures of genetic diversity allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity within groups (H_E), and genetic differentiation (F_{ST}) for all three groups (*Q. ellipsoidalis* and managed and unmanaged *Q. rubra*). A rarefaction index as suggested by Petit et al. (1998) was used to correct for unequal sample sizes before calculating A_R. Means over all populations were estimated and 5,000 permutations were used to test differences between the means in FSTAT (Goudet 1995). This was also done to test for differences of diversity estimates between species and for both managed and unmanaged populations. MICRO-CHECKER (Van Oosterhout et al. 2004) was used to check for null alleles in each population and markers showed generally low null allele frequencies (see also Lind and Gailing 2013; Lind-Riehl et al. 2014).

Spatial genetic structure analyses

Fine-scale spatial genetic structure, SGS, was analyzed through spatial autocorrelation analysis in SPAGeDi version 1.4 (Hardy and Vekemans 2002). Spatial autocorrelation analyzes the degree of dependency among observations, genetic variation in this case, in a geographic space. Specifically, allelic information is correlated between pairs of individuals in the same distance class. To to assess the extent of SGS, the relationship between genetic similarity and geographic distance between individuals in each population was assessed through Moran's I statistics and regression analysis of kinship coefficients (Loiselle et al. 1995) on geographical distances using a jackknife method to estimate standard errors. The results provide insight as to whether genetic structure exists and at what scale (Sokal and Oden 1978). To determine the significance of the coefficients averaged over all loci, they were tested against the null hypothesis of no spatial genetic structure through the creation of a null distribution of randomly permuting individuals among distance classes 10,000 times (95 % confidence interval). Additionally, the Sp statistic (Vekemans and Hardy 2004) was calculated to allow quantitative comparisons of the extent of spatial genetic structure among species and/or populations. This statistic is not affected by the sampling scheme as heavily as the kinship coefficient as it is primarily dependent on the rate of decrease of pairwise kinship coefficients with the logarithm of the distance between individuals. The Sp statistic is calculated based on the regression slope of the kinship coefficient, such that $Sp = -b_f/1 - F_1$, where F_1 is the mean kinship coefficient over all loci between individuals belonging to the first distance class and b_f is the regression slope of F_1. Statistical significance of F_1 and b_f was determined under a 95 % confidence interval of F_{ij} created by 10,000 permutations of individuals among distance classes.

The size of the distance classes was determined by SPAGeDi to ensure an equal number of comparisons within each distance class. Initial analyses using various numbers of distance classes were run to determine the optimal number of distance classes to ensure at least 50 comparisons per distance class for all populations and to best capture fine-scale spatial genetic structure. The use of 15 distance classes was determined to be optimal. Runs were performed for each population with only individuals with a diameter at breast height (DBH) of ≥ 10 cm for *Q. rubra* samples and ≥ 7 cm for *Q. ellipsoidalis* which has a more shrubby growth (Online Resources 2–4).

The area was estimated by taking the area of a polygon created around the sampled area in ArcMap (ESRI 2011) for each population to provide a clearer view of the scale

and sampling in each population and to calculate the effective census density according to Stefenon et al. (2008). Effective census density was used to indirectly estimate the gene dispersal rate using SPAGeDi assuming equilibrium of isolation by distance in the fine-scale spatial genetic structure as described by Vekemans and Hardy (2004).

Additionally, to determine the relative diversity of diameter classes, the Shannon Wiener diversity index was determined for each population. The equation is often used to calculate species diversity, but can also be applied to age or diameter diversity within a single species (McPherson and Rowntree 1989). Populations with individuals evenly distributed among all diameter or age classes will show high values, indicative of high diameter or age class diversity. To calculate the Shannon Wiener diversity index (H), the equation below was used, where p_i is the proportion of the total sample represented by diameter class i:

$$H = -\sum_i [p_i \cdot \ln(p_i)]$$

Results

Managed vs. unmanaged *Quercus rubra* stands

No significant differences between managed and unmanaged stands were detected for most genetic variation parameters (Table 2). However, managed stands had slightly, but significantly more genetic diversity (H_E) within populations than unmanaged stands (Online Resource 5). Level and extent of SGS were similar in most managed (0–36 m) and unmanaged stands (0–28 m) with unmanaged stand PM-QR showing weak SGS up to 60 m (Fig. 2l; Table 3, Online Resource 1). No significant SGS was found in the managed stands CNF-QR and NNF-QR in the first distance class (Fig. 2e, h). Also the unmanaged stand, HMR-MI, with the highest census density showed no significant SGS and low values for the Sp statistic (0.002) and Moran's I (0.011) (Table 3). The census densities of the other two unmanaged stands from the same geographic region, HMR-IH and HMR-LP, are lower than in HMR-MI but still much higher than in the managed *Q. rubra* stands (Table 3). Overall there was no significant relationship between Moran's I ($R^2 = 0.0194$) or Sp ($R^2 = 0.0053$) and census density. Finally, *Q. rubra* Sp values were similar to other *Quercus* species, such as the European *Q. robur* (Table 4). A linear regression of Moran's I and Sp against the Shannon Wiener diversity index (SWI) of diameter classes revealed significant negative associations across all populations ($R^2 = 0.47$, $p < 0.001$; $R^2 = 0.17$, $p < 0.05$). However, our analysis did not include the managed NNF-QR population due to missing diameter data. The inclusion of this even-aged population with non-significant SGS and

Table 2 Genetic variation at 15 microsatellites loci in 12 managed and unmanaged *Quercus rubra* and *Q. ellipsoidalis* populations (the population abbreviations follow those in Table 1)

Population	N	N_A	N_E	N_{RARE}	A_R[b]	H_O	H_E[a]	F_{IS}
Quercus rubra								
Unmanaged								
HMR-IH	76	13	6	5	12	0.698	0.778	0.080
HMR-LP	52	13	6	5	13	0.666	0.780	0.120
HMR-MI	60	12	6	6	12	0.685	0.776	0.092
PM-QR[c]	36	11	5	6	12	0.635	0.723	0.105
Mean	56	12	6	6	12	0.671	0.757	0.099
Managed								
CNF-QR	40	13	7	6	13	0.748	0.797	0.033
FC-A	48	12	6	6	11	0.669	0.805	0.144
FC-B	48	12	6	6	12	0.652	0.787	0.150
NNF-QR	40	13	7	7	13	0.713	0.798	0.084
Mean	44	12	7	6	13	0.696	0.797	0.103
Quercus ellipsoidalis								
CNF-QE	40	12	6	6	12	0.692	0.777	0.091
FC-C	50	11	6	6	11	0.690	0.767	0.083
FC-E	47	10	5	5	10	0.590	0.749	0.214
NNF-QE	39	14	7	6	14	0.711	0.801	0.139
Mean	44	12	6	6	13	0.671	0.773	0.132

N sample size, N_A number of alleles averaged over all loci, N_E number of effective alleles, N_{RARE} number of different alleles with a frequency of ≥ 5 %, A_R allelic richness, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient

[a] Unbiased expected heterozygosity (Peakall and Smouse 2006)

[b] Corrected for unequal sample sizes using the rarefaction index suggested by Petit et al. (1998)

[c] Only analyzed at 14 markers

expected low SWI would have resulted in a non-significant association between SGS and SWI.

Species differences

Genetic variation parameters for both species were similar, but managed *Q. rubra* displayed significantly higher genetic diversity (H_E) within populations than *Q. ellipsoidalis* and unmanaged *Q. rubra* (Table 2, Online Resource 5). The unmanaged PM-QR population showed the lowest genetic diversity of all populations. Additionally, most of the *Q. ellipsoidalis* populations also displayed higher F_{IS} values across most markers than *Q. rubra* populations, particularly for FC-E and NNF-QE indicating potential inbreeding (Table 2, Online Resource 6). The extent of SGS was similar for species pairs of populations in the FRF-BP region (Fig. 2b, g; Table 3: *Q. ellipsoidalis*: up to 26 m and *Q. rubra*: up to 36 m). In the *Q. ellipsoidalis* populations SGS extended to 37 m and 83 m in the CNF and NNF regions, respectively, while no significant SGS was
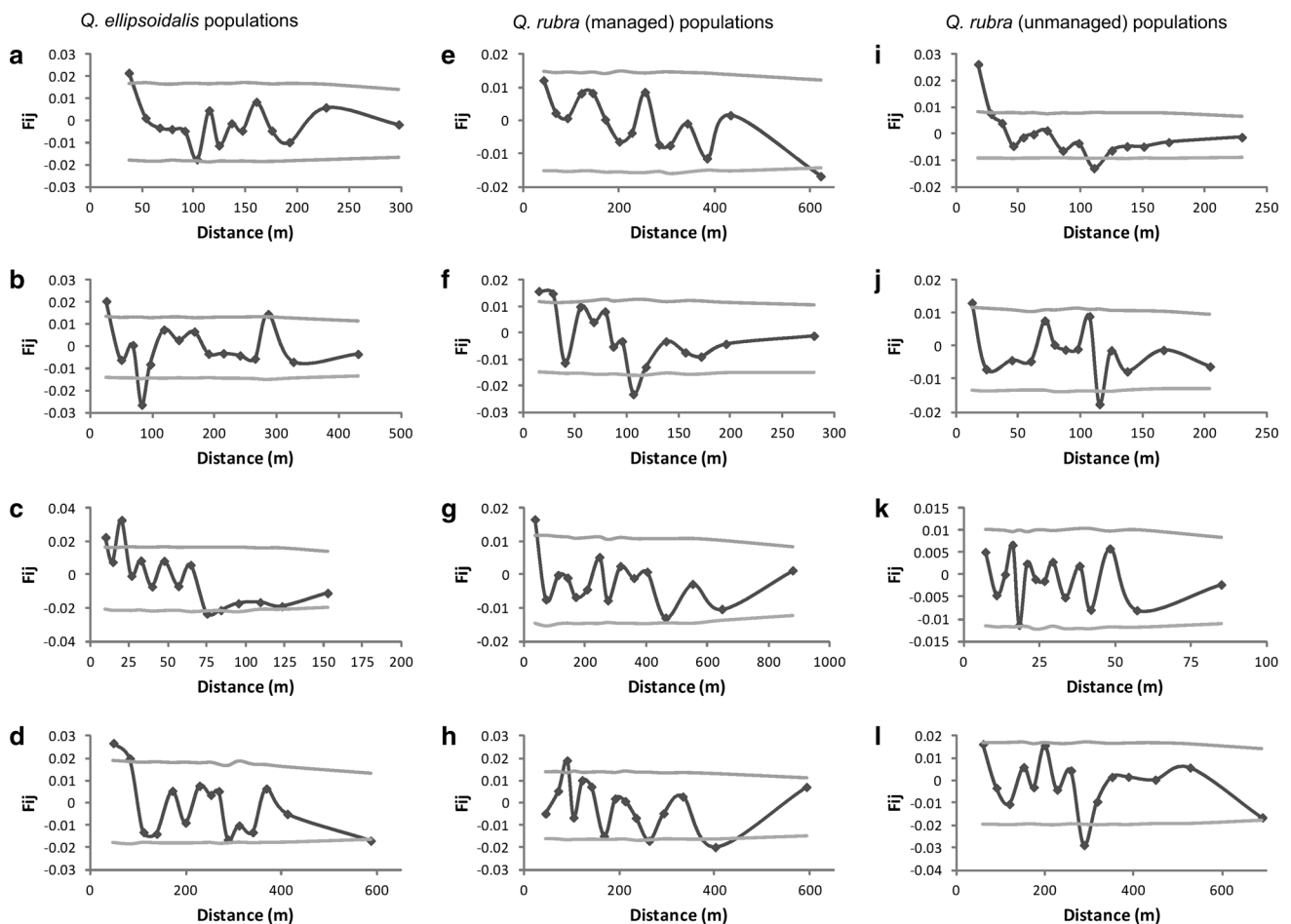
Fig. 2 Correlograms showing fine-scale spatial genetic structure (SGS) of studied populations (the population abbreviations follow those in Table 1): **a** CNF-QE, **b** FC-C, **c** FC-E, **d** NNF-QE, **e** CNF-QR, **f** FC-A, **g** FC-B, **h** NNF-QR, **i** HMR-IH, **j** HMR-LP, **k** HMR-MI, **l** PM-QR

observed in the *Q. rubra* populations in the first distance class (Fig. 2a, d, e, h). In population NNF-QR significant SGS was found in only one distance class from 90 to 103 m (Fig. 2h). The *Sp* statistic showed the most pronounced SGS for *Q. ellipsoidalis* populations FC-E and NNF-QE (Fig. 2c, d) exceeding the values for neighboring *Q. rubra* (FC-A, FC-B, NNF-QR) (Fig. 2f–h) and *Q. ellipsoidalis* populations (FC-C) (Fig. 2b; Table 3). In general, *Q. ellipsoidalis* populations also show higher kinship coefficients than *Q. rubra* populations even though the extent of SGS is similar for both species (Table 3). Finally, even though gene dispersal estimates did not reach convergence for most populations tested, estimated distances were larger for the *Q. rubra* populations (FC-B: 259 m, CNF-QR: 269 m) than for the *Q. ellipsoidalis* populations (FC-E: 32.6 m, NNF-QE: 158 m). Similar differences in SGS were observed by others for the interfertile sympatric oak species *Q. petraea* and *Q. robur* (Vekemans and Hardy 2004), where the Sp value was larger for *Q. petraea* which has a more limited seed dispersal range than *Q. robur* (Table 4).

Discussion

We find that genetic diversity is slightly but significantly higher in managed than in unmanaged *Q. rubra* stands. Although there are not very many studies comparing managed to unmanaged stands, those that do have also generally shown low to no differences in diversity between managed and unmanaged populations of outcrossing temperate forest trees. Thus, investigations of the impact of management on fine-scale spatial genetic structure (SGS) in two European oak species (*Q. robur* and *Q. petraea*) found that only the heavily managed stand had slightly lower genetic diversity than the unmanaged stand (Cottrell et al. 2003). Likewise, several studies in another Fagaceae species, *F. sylvatica*, have shown no significant differences in levels of genetic variation as the result of a range of management practices including shelterwood, plantation, and semi-natural regeneration (Buiteveld et al. 2007; Rajendra et al. 2014), but one study noted that some rare alleles were lost in fragmented populations (Paffetti et al.

Table 3 Estimation of the fine-scale genetic structure at 15 microsatellite markers in managed and unmanaged *Quercus rubra* and *Q. ellipsoidalis* populations for sampled trees with a DBH ≥10 cm for *Quercus rubra* and a DBH ≥7 cm for *Q. ellipsoidalis*

Population	CD	F_1^a	b_f	Sp	Moran's I^a	Extent of SGS (m)	SWI
Quercus rubra							
Unmanaged							
HMR-IH	75	0.026***	−0.009	0.009	0.049***	0–28	0.29
HMR-LP	81	0.013*	−0.002	0.002	0.026*	0–13	1.17
HMR-MI	284	0.005	−0.002	0.002	0.011	ns	1.92
PM-QR[b]	4	0.016*	−0.006	0.006	0.032*	0–60	1.80
Managed							
CNF-QR	6	0.012	−0.006	0.006	0.025	ns	1.23
FC-A	16	0.016**	−0.007	0.007	0.030**	0–28	1.81
FC-B	6	0.017**	−0.004	0.004	0.032**	0–36	1.92
NNF-QR	11	−0.005	−0.004	0.004	−0.006	ns	–
Quercus ellipsoidalis							
CNF-QE	12	0.022**	−0.006	0.006	0.042**	0–37	0.94
FC-C	14	0.020**	−0.005	0.005	0.039**	0–26	1.47
FC-E	194	0.023*	−0.014	0.014	0.042*	0–10 and 20–25	0.86
NNF-QE	10	0.027**	−0.017	0.017	0.039**	0–83	1.34

CD census density (individuals per hectare), F_{IS} inbreeding coefficient calculated in SPAGeDi, N_A number of alleles averaged over all loci, F_1 multilocus kinship coefficient between individuals of the first distance class (Loiselle et al. 1995), b_f regression slope of F on natural log distance, *Sp* quantification of the SGS, *SWI* Shannon Weiner Index (using diameter class)

15 distance classes with a minimum of 50 pairs per distance class

[a] Level of significance after 10,000 permutations (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

[b] Only analyzed at 14 markers

Table 4 Quantification of the fine-scale genetic structure (SP values) for selected Fagaceae species

Species	Sp	Reference
Quercus rubra	0.005	–
Quercus ellipsoidalis	0.011	–
Quercus petraea	0.008	Vekemans and Hardy (2004)
Quercus robur	0.003	Vekemans and Hardy (2004)
Fagus sylvatica	0.011	Rajendra et al. (2014)

2012). *Fagus sylvatica* shares many life history traits with *Quercus* species such as wind pollination, outcrossing nature, and abundant seed and pollen production. The similar levels of genetic variation within both natural and managed populations may be explained by the life history traits of these forest tree species. Hamrick et al. (1979) analyzed relationships between 12 life history traits and various ecological variables and their impact on the levels of genetic variation within populations of 113 different plant taxa. They found that species with the highest genetic diversity tend to be those with large ranges, high fecundities, an outcrossing mode of reproduction, wind pollination, long generation times, and habitats representing later stages of succession. *Quercus rubra*, like many other

outcrossing temperate tree species, possesses most of these traits and may be able to maintain high levels of genetic diversity despite natural and anthropogenic interferences. This may also hold true for peripheral populations. Accordingly, similarly high within population genetic diversity was evidenced in peripheral *Q. petraea* populations as in the central distribution range of the species (Muir et al. 2004). Another study that examined the long-term impact of fragmentation on the population genetic structure of 14 *Q. macrocarpa* populations found a lack of large-scale population genetic structure with most diversity existing within populations suggesting that wind-pollinated trees with large distribution ranges may be resilient to human impacts like fragmentation as well (Craft and Ashley 2007). Most studies examine the impact of fragmentation on genetic diversity of forest tree populations that has occurred within the last 200 years. However, Jump and Peñuelas (2006) found significant effects of long-term (>600 years) fragmentation on the genetic diversity and population structure of *F. sylvatica*. Thus, chronic fragmentation may reduce resilience in the long run and should not be ignored.

While there was no clear difference in SGS between managed and unmanaged *Q. rubra* stands, non-significant SGS was found for the two populations that were subject to

even-aged management. Among *Q. rubra* populations, the FRF-BP stands have been managed primarily using a selection system silviculture method (uneven-aged management), while the CNF and NNF populations were largely managed through shelterwood cutting (even-aged management) where up to 1/3 to 1/2 of the trees are left after harvest (Dickmann and Leefers 2003). Accordingly, the CNF population shows clumps of similar sized trees near each other, while the FC-A and FC-B populations show a more mixed spatial structure of different sized trees (Online Resources 7–9). Additionally, FC-A and FC-B show a higher variation in diameter classes as shown by a higher SWI value than the CNF population (Fig. 3, no data on NNF). The even-aged management in the CNF and NNF populations mimics large-scale disturbances such as stand replacing fires, while the uneven-aged management in the FRF-BP populations is similar to natural small scale disturbances. The absence of significant SGS in the CNF and NNF populations (Fig. 2e, h) could be a result of management, but the lower representation in small distance classes in the CNF and NNF populations may also have prevented the detection of fine-scale SGS at smaller distances (see "Materials and methods"). Uneven-aged management allows reproduction to occur in overlapping generations and may promote family structures (Finkeldey and Ziehe 2004), which is displayed by the presence of significant SGS up to 36 m in the FRF-BP populations. Similar to the managed stands, the magnitude and extent of SGS in unmanaged stands were highly variable ranging from no SGS in the population with the highest density (HMR-MI, 284 individuals/ha) to extended SGS in the stand with the lowest density (PM-QR). The absence of SGS in HMR-MI might have been caused by overlapping seed shadows. However, across all populations no clear association of SGS was found with either census density or SWI. Since detailed records on management activities and natural disturbances are missing, it is difficult to disentangle the effects of management and natural disturbances on SGS. Long-term silvicultural trials including managed and unmanaged populations might provide a resource to better assess the effect of disturbance regimes on genetic variation and SGS. In the limited number of studies comparing SGS in managed and unmanaged stands, management has been found to both decrease (Paffetti et al. 2012; Rajendra et al. 2014) and increase SGS (Cottrell et al. 2003). Specifically, Cottrell et al. (2003) found a managed stand to exhibit the highest level of SGS and attributed this to human planting of acorns using seeds from a small number of mother trees. Additionally, there was evidence for pronounced SGS in a clumping of trees within the managed site that represented 50 % of the total number of mature trees paired with young trees (DBH <30 cm) less than 50 m apart.
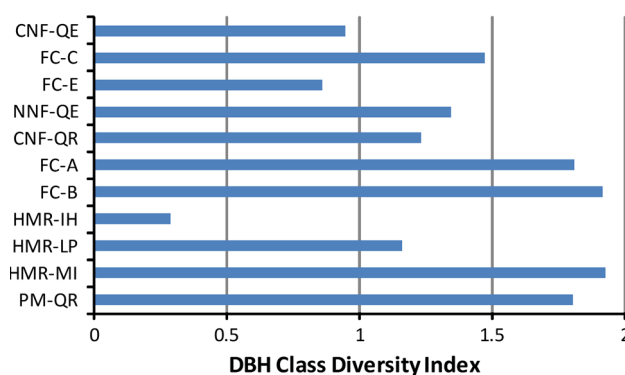

Fig. 3 Diameter class diversity for all sampled populations of *Quercus rubra* and *Q. ellipsoidalis* (the population abbreviations follow those in Table 1). *DBH* diameter at breast height

Studies that have compared SGS between species have generally found that life history characteristics impact the extent and significance of SGS (Berg and Hamrick 1994; Cottrell et al. 2003; Streiff et al. 1998). For example, the more pronounced SGS in *Q. petraea* as compared to *Q. robur* was attributed to *Q. robur*'s potential for longer range seed dispersal and the ability to grow over a wider range of site conditions (Streiff et al. 1998). In our study, differences in level and extent of SGS of *Q. rubra* and *Q. ellipsoidalis* stands suggest that disturbance history (natural and human impact) and species abundance strongly affect SGS. Thus, more pronounced SGS was found in *Q. ellipsoidalis* stands as compared to neighboring *Q. rubra* stands. Oaks show a synchronous reproduction system referred to as masting where at variable intervals (usually every few years) a larger than usual seed crop is produced. If natural regeneration is established in a mast year and that coincides with recent management activity, the new seedling generation could be produced from a limited number of trees and extended family structures might develop. The number of reproducing trees seems to be lower and the intervals between mast years were longer in *Q. ellipsoidalis* than in *Q. rubra* populations. Thus in *Q. ellipsoidalis* stands FC-C and FC-E, fewer reproducing trees were present (multiple year field observations) as compared to neighboring *Q. rubra* stands. While seed production was observed every year since 2009 in *Q. rubra*, only a small group of *Q. ellipsoidalis* trees produced seeds in a single year at the FRF-BP site. Lower numbers of reproducing adults can also lead to inbreeding, which was observed in two of the *Q. ellipsoidalis* populations. This may be characteristic for *Q. ellipsoidalis* which is often found in pine barrens and deals with more harsh conditions such as drought. Fires are also more frequent in pine barren habitats and while the sampled stands have not had extensive fires in the past 100 years, the *Q. ellipsoidalis* stands have been subject to incidental clear cutting due to their proximity to *P. bansksiana* stands that are managed through this

method mimicking the historical fire regime of this type of habitat. It is possible that geographic isolation of the fragmented *Q. ellipsoidalis* populations has likely contributed to the development of more pronounced family structures and potential inbreeding than in the more widely distributed *Q. rubra* at the species' range edge. These effects of frequent disturbances on SGS might be limited in the main distribution range of *Q. ellipsoidalis*, warranting a comparison of core and peripheral populations to test this hypothesis.

Overall our study suggests that management regimes, natural disturbance, life history traits, and geographic isolation can affect SGS and genetic variation of peripheral populations. Isolated *Q. ellipsoidalis* populations showed low seed production, significant SGS, and potential evidence for inbreeding. However, it was not possible to disentangle the effects of management and natural disturbances on genetic variation and SGS. Furthermore, life history characteristics of the species in combination with natural disturbance regimes and human-mediated management likely leads to variable impacts on a population's SGS. In wind-pollinated forest tree species with generally large effective population sizes, the effects of management and disturbance on SGS and inbreeding are likely to be most pronounced in fragmented populations at the species' distribution edge. SGS is also likely to affect the mating patterns in tree populations increasing for example the likelihood of mating between related individuals even in wind-pollinated trees (Berg and Hamrick 1994; Chybicki et al. 2011). However, to study the long-term impact of SGS on evolutionary and ecological processes requires long-term monitoring in trees that need 40–50 years to mature in the environments studied.

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