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Disturbance and density-dependent processes (competition and facilitation) influence the fine-scale genetic structure of a tree species' population

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● **Background and Aims** Disturbances, dispersal and biotic interactions are three major drivers of the spatial distribution of genotypes within populations, the last of which has been less studied than the other two. This study aimed to determine the role of competition and facilitation in the degree of conspecific genetic relatedness of nearby individuals of tree populations. It was expected that competition among conspecifics will lead to low relatedness, while facilitation will lead to high relatedness (selection for high relatedness within clusters).
● **Methods** The stand structure and spatial genetic structure (SGS) of trees were examined within old-growth and second-growth forests (including multi-stemmed trees at the edge of forests) of *Nothofagus pumilio* following large-scale fires in Patagonia, Chile. Genetic spatial autocorrelations were computed on a spatially explicit sampling of the forests using five microsatellite loci. As biotic plant interactions occur among immediate neighbours, mean nearest neighbour distance (MNND) among trees was computed as a threshold for distinguishing the effects of disturbances and biotic interactions.
● **Key Results** All forests exhibited a significant SGS for distances greater than the MNND. The old-growth forest genetic and stand structure indicated gap recolonization from nearby trees (significantly related trees at distances between 4 and 10 m). At distances smaller than the MNND, trees of the second-growth interior forest showed significantly lower relatedness, suggesting a fading of the recolonization structure by competition, whereas the second-growth edge forest showed a positive and highly significant relatedness among trees (higher among stems of a cluster than among stems of different clusters), resulting from facilitation.
● **Conclusions** Biotic interactions are shown to influence the genetic composition of a tree population. However, facilitation can only persist if individuals are related. Thus, the genetic composition in turn influences what type of biotic interactions will take place among immediate neighbours in post-disturbance forests.

Key words: Biotic interactions, intraspecific aggregation, intraspecific facilitation, microsatellites, population genetics, multi-stemmed trees, *Nothofagus pumilio*, Patagonia.

INTRODUCTION

The structure and composition of most natural forests is the result of the occurrence of exogenous disturbances (e.g. fire, insect outbreaks), processes of pollen and seed dispersal and the action of endogenous processes (biotic interactions, e.g. competition). A disturbance, defined as natural or land-use-related events that remove biomass or individuals (Sousa, 1984; Veblen, 1992), makes resources available for seedling establishment, whereas competition, by definition, involves a struggle between individuals to pre-empt limiting resources that, together, determine rates of carbon acquisition. Disturbances and patterns of dispersal also have important consequences for the spatial distribution of genotypes within populations, i.e. the spatial genetic structure (SGS) (Epperson, 1993; Banks *et al.*, 2013). Large-scale, stand-replacing disturbances strongly modify the gene flow in a population (i.e. pollen and seed dispersal) through the reduction of population size, especially the number of seed sources, and the increase in distances between individuals (Bacles and Jump, 2011; Shohami and Nathan, 2014). After large-scale disturbances, the initial arrangement of genotypes of the new stand will depend on the spatial and genetic structure of the seed source and on seed dispersal (e.g. distance, direction), which will mostly create a random spatial distribution of genotypes (Hamrick and Godt, 1996). On the contrary, in undisturbed natural forests regeneration occurs through fine-scale disturbances, e.g. tree-fall gaps, that do not alter much the number of seed trees, and local seed dispersal is most probably responsible for colonization in gaps (Premoli and Kitzberger, 2005; Mathiasen and Premoli, 2013; Shohami and Nathan, 2014). This results in higher relatedness at short distances within populations, i.e. a fine-scale spatial genetic structure (Hamrick and Godt, 1996; Vekemans and Hardy, 2004), even in predominantly outcrossed species, as a consequence of shared parents or correlated mating (Hamrick and Nason, 1996). Thus, tree populations may have different genetic structures and diversity depending on the scale, frequency and intensity of disturbances.

However, density-dependent, biotic interaction processes may alter the genetic structure of plant populations generated by dispersal. This relationship has, however, been little explored (but see Hughes *et al.*, 2008; Vellend *et al.*, 2010). Plant interactions occur where plants overlap their 'zones of influence', e.g. among immediate neighbours at fine spatial scales (Stoll and Weiner, 2000). Negative interaction processes at the same trophic level, particularly competition, have long been considered the major drivers in the structuring and organization of natural communities (Hairston *et al.*, 1960; Tilman, 1982; Silvertown, 2004). For example, species coexistence mechanisms explaining community assembly are deeply rooted in the premise that intraspecific competition is stronger than interspecific competition (Chesson, 2000). This is because, according to the principle of competitive exclusion (Hardin, 1960; Hutchinson, 1961), competition will be stronger between species competing for similar resources than between species competing for different resources. Given that plants cannot move away from a bad neighbourhood, this necessarily implies lower survival or fitness for an individual if a near neighbour is a conspecific (i.e. has identical resource requirements). The spatial aggregation of conspecifics can thus promote species coexistence given that competitively dominant species limit their own abundance more than those of competitively inferior species, leading to stabilizing coexistence (Chesson, 2000). This theory of species coexistence, however, implicitly treats species as genetically (and functionally) invariant, which, according to Vellend *et al.* (2010), 'may produce faulty predictions if the outcome of species interactions depends on the genetic composition and diversity of the interacting populations'.

Species coexistence theory also does not consider positive plant interaction as having any significant role. Positive plant interaction, or facilitation, has been defined as 'an interaction in which the presence of one species alters the environment in a way that enhances growth, survival and reproduction of a second species' (Bronstein, 2009). Facilitation has historically been perceived as unimportant or ignored not only by ecologists but also by evolutionary biologists (Bronstein, 2009), and yet it has recently been recognized as a ubiquitous driver of biodiversity in natural communities (McIntire and Fajardo, 2014). Moreover, positive density-dependence (e.g. intraspecific facilitation) among same-cohort conspecifics (i.e. with strong niche overlap) has been reported to occur in varied types of plant populations (Cerfonteyn *et al.*, 2011; Fajardo and McIntire, 2011; Leicht-Young *et al.*, 2011; File *et al.*, 2012; Segovia *et al.*, 2015). Although there is an increasing number of studies documenting the existence of facilitation at the intra-specific level, very few have related this pattern with the spatial genetic structure of the plants involved (Till-Bottraud *et al.*, 2012; Segovia *et al.*, 2015).

How should facilitation between conspecifics alter the genetic structure of a population? Till-Bottraud *et al.* (2012), working in Patagonia (Chile), found that multi-stemmed trees (two to six stems per cluster) of the deciduous and mast-seeding species *Nothofagus pumilio* (Nothofagaceae) were mainly composed of merged individuals that were highly related, but not identical. Here multi-stemmed trees were persistently found at the edge of second-growth post-fire forests but never in other sections of the forest, e.g. in the interior (Fajardo and McIntire,

2010). It was suggested that stressful environmental conditions at early stages of stand development constrained seedlings of this species to survive and grow better when growing in clusters at the edge of the stand or in open areas (Fajardo and McIntire, 2011; McIntire and Fajardo, 2011). Merging of individuals occurs at a later stage, resulting in the observed multi-stemmed trees. Here merging is understood as the process by which two or more independent functional units (stems) become one entity (see Fig. 1 in McIntire and Fajardo, 2011); cambium of spatially close neighbours fuses and thus new vascular tissues connect, ultimately acting as a single tree (Fajardo and McIntire, 2010). It is not known when and how this histological fusion occurs, but we presume that it starts in the roots. Although this tree merging may apparently seem odd, many woody species can physiologically merge, a phenomenon that is regularly exploited in horticulture and orchard production (grafting) (Hartmann *et al.*, 1996). This fusion is accompanied by selection for kin individuals as merged trees are significantly more related than randomly expected [Till-Bottraud *et al.* (2012) found at least one pair of full sibs in all merged trees analysed], and as no mechanism of animal dispersal and storage in caches (dyszoochory) – which is often associated with high relatedness between close neighbours – was identified.

Further evidence for facilitation was found later in stand development at the second-growth edge (SGE) forest. McIntire and Fajardo (2011) observed that stems belonging to multi-stemmed trees had higher survival and growth rates than single-stemmed trees. They also found evidence for competition within the second-growth interior (SGI) forest, e.g. even distribution of alive individuals (Fajardo and McIntire, 2010) and death of seedlings growing in clusters (McIntire and Fajardo, 2011). Consequently, in this forest system, in a matter of a few hundreds of metres one passes from old-growth (OG) forest of *N. pumilio* undergoing a gap-phase regeneration dynamic to a highly dense, even-aged, second-growth, post-fire forest of the same species where competition prevails (Fajardo and McIntire, 2010; McIntire and Fajardo, 2011), and finally to the second-growth edge forest, where facilitation has been demonstrated to occur both early between seedlings and later between trees (Fajardo and McIntire, 2011; McIntire and Fajardo, 2011) (Fig. 1), and where there is evidence of selection for related individuals in merged trees (Till-Bottraud *et al.*, 2012). We believe this unique natural experiment lends itself to expansion of the study of the impact of density-dependent processes on a tree population's genetic structure. In particular, no study so far has simultaneously assessed the relationship between biotic interactions (competition and facilitation) and the genetic structure of a particular population.

The objective of the present study was to measure the impact of biotic interactions on the fine-scale spatial genetic structure (f-SGS) of OG, SGI and SGE forests of *N. pumilio* in four areas in the Southern Andes of Chile (Patagonia). We thus first evaluated the SGS due to abiotic disturbances (tree falls and large-scale disturbances) and forest stand structure to account for differences in stand stage development among forest types (e.g. OG versus SGI). We then studied the genetic structure at very short spatial scales (f-SGS) within the zones of interaction of trees. Our hypothesis was that biotic interactions (i.e. competition and facilitation) will lead to significant changes in f-SGS (Fig. 1). In particular, we hypothesized that competition will
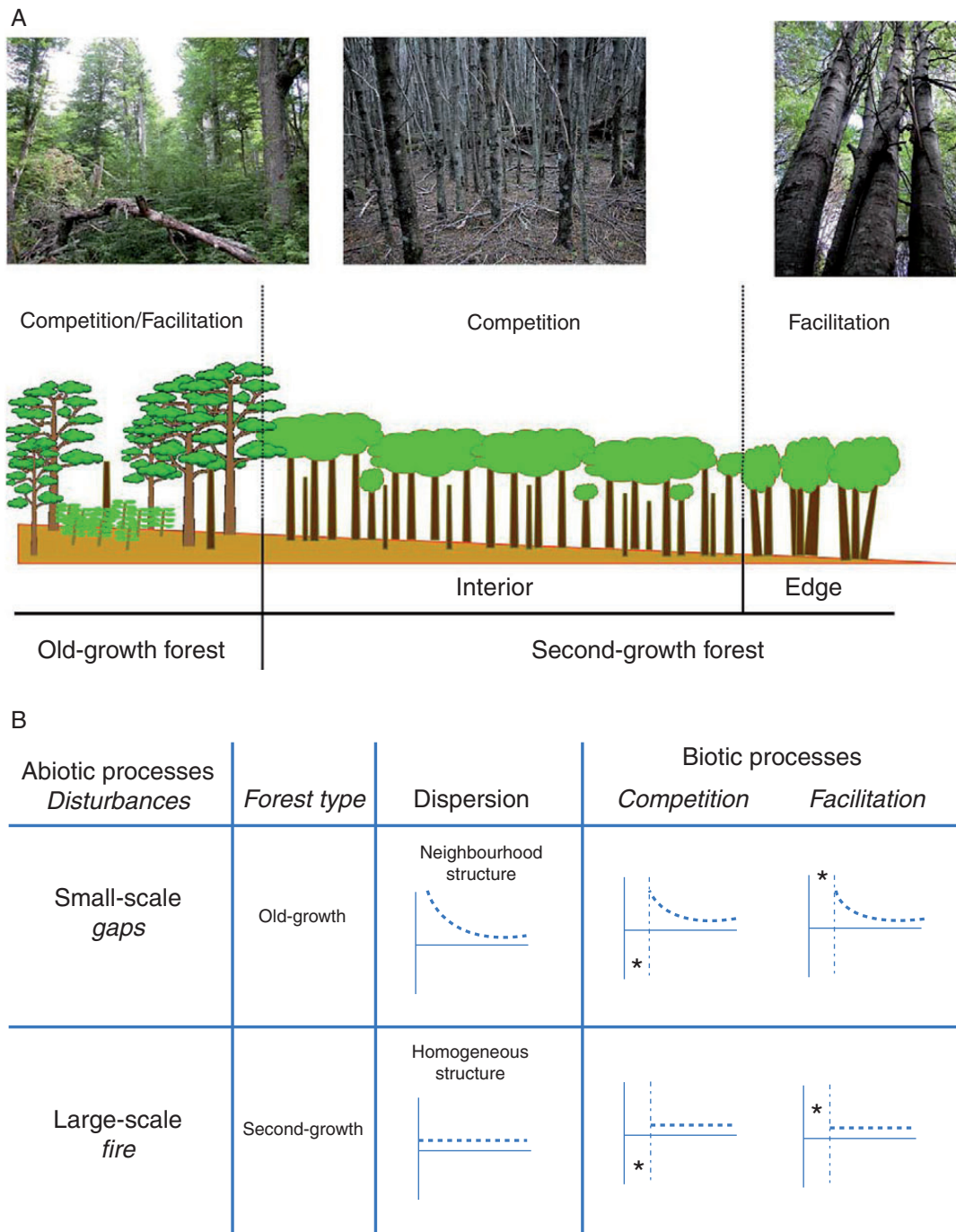
FIG. 1. (A) Old-growth forest (OG), second-growth forest interior (SGI) and second-growth forest edge (SGE) of *Nothofagus pumilio* and their schematic representation in a forest colonization gradient. The second-growth forest interior and edge are of fire origin. (B) In order to make an inference we used space as a surrogate for unmeasured processes (McIntire and Fajardo, 2009), where we tied specific ecological processes (e.g. biotic interactions) to the spatial genetic structure (SGS) patterns they most probably create in an *a priori* way, based on theory, empirical literature and observation. We first predicted the SGS due to abiotic factors (disturbance and dispersal). In OG we expect a significant SGS resulting from gap recolonization by seeds from nearby trees (Premoli and Kitzberger 2005; Shohami and Nathan 2014), i.e. a negative exponential relationship between genetic relatedness and distance (similar to a dispersal kernel); in SGI we expect no structure resulting from flash recolonization from a large seed source (Premoli and Kitzberger 2005; Mathiasen and Premoli 2013), i.e. no relationship between genetic relatedness and distance (a flat curve); alternatively, some remnant trees could create a fine-grain SGS of some magnitude in SGI (Shohami and Nathan 2014). We then tried to infer the genetic structure resulting from the combination of abiotic and biotic processes. In SGI we assume competition among individuals is occurring (Fajardo and McIntire 2010; McIntire and Fajardo 2011); thus, we expect lower genetic relatedness among trees located within the mean nearest neighbour distance (MNND) than immediately beyond; in SGE we expect a stronger genetic relatedness within the MNND than immediately beyond, due to facilitation between related individuals forming multi-stemmed trees in stressful conditions. Finally, we do not have strong predictions as to which way biotic interactions among nearest neighbours in OG should affect their fine-scale SGS as both competition between immediate neighbours and sheltering of smaller trees by larger related ones can be expected.

lead to a weakening of the f-SGS within the zone of interaction, whereas facilitation will result in strong f-SGS.

MATERIALS AND METHODS

Species description and study sites

Nothofagus pumilio (Nothofagaceae) is a dominant tree species of the Southern Andes forests of Chile and Argentina. It is a widespread deciduous, mast-seeding, wind-pollinated, monoecious tree species that mostly forms monospecific forests. It extends through a wide latitudinal and altitudinal distribution from a Mediterranean-influenced climate in central Chile at 35°S to a humid climate in Tierra del Fuego at the southernmost point of the continent at 56°S, and from sea level to mid-elevation forests and to the treeline (Veblen *et al.*, 1996; Fajardo *et al.*, 2011). *Nothofagus pumilio* exhibits mast seeding at intervals of up to 7 years or more (10 years, A. Fajardo, pers. observations). Seeds of *N. pumilio* have small wing-like structures (similar to those of beech seeds) and are dispersed by either gravity or wind (Veblen *et al.*, 1996). We selected four areas where we found, very close to each other, OG, SGI and SGE forests of *N. pumilio*. All forests constituted pure forests of *N. pumilio* and were within the Southern lineage defined by Mathiasen and Premoli (2010). The selected areas were: Mano Negra (45°27′ S, 71°58′ W, ~930 m a.s.l.), Laguna Venus (45°31′ S, 72°03′ W, ~815 m a.s.l.), Laguna Verde (45°32′ S, 72°00′ W, ~896 m a.s.l.) and Cerro Castillo (46°06′ S, 72°03′ W, ~920 m a.s.l.). All these areas are located in the Coyhaique Province within the Aysén Region, Chilean Patagonia. Laguna Venus and Laguna Verde are within the Coyhaique Reserve and Cerro Castillo is within the Cerro Castillo Reserve, while Mano Negra is in a private property. All SGI and SGE forests are the consequence of large-scale human-provoked fires. Fire is not common in the dynamics of *N. pumilio* in central Patagonia. In ~1950 some 2 million hectares of *N. pumilio* OG forest were burned to develop cattle production. A fire boundary was then created, from where the unaffected OG forest of *N. pumilio* spread seeds down the slope. Thus, a fringe of regeneration was formed that led, within decades, to SGI and SGE forest composed of even aged individuals because of the strong mast-seeding habit of the species. This sequence of recolonization towards the formation of second-growth forest in *N. pumilio* is pervasive around this region (Fajardo and McIntire, 2010). It is important to note that the post-fire SGI and SGE forests are still reproductively immature (no seed production).

Sampling

In Mano Negra, Laguna Venus, Laguna Verde and Cerro Castillo areas, OG, SGI and SGE forests were identified and sampled. In order to ensure that all site conditions within each area were affected by the same fire event and were therefore comparable, we selected sites that were not more than ~1 km apart and no more than 20 m different in elevation. We also ensured that sites had not experienced any fire or management activities for the last 30 years. We defined SGE as the last 50 m of the second-growth forest before the treeless area (e.g.

prairie). This fringe has a high frequency of multi-stemmed trees, with, on average, 61 % of all stems in second-growth forest belonging to multi-stemmed trees (Fajardo and McIntire, 2010). The SGI corresponds to the forest stand located >100 m from the edge, where the presence of multi-stemmed trees is nil (Fajardo and McIntire, 2010). Finally, the OG that was not affected by fire is located some 300 m away from any forest border. In general, the structure of this forest differs significantly from the second-growth forest; it is uneven-aged, has noticeable canopy gaps with regeneration and has large woody debris from tree falls (Fajardo and de Graaf, 2004; Fajardo and McIntire, 2010). In each forest, samples were collected using a random walk design with a random start point. A unique list of sample coordinates for each forest was created by randomly generating bearing directions and distances. Bearing directions were constrained to 180° to ensure that the random walk did not immediately go back on itself; distances were constrained between 5 and 30 m. We sampled 25 individual trees in each forest; 13 of them were found by the bearing direction and distance method and the other 12 were located as the nearest neighbour of each of the first 12 individuals. For the SGE, we proceeded with the same random walk design but worked with only ten multi-stemmed trees (three stems each). Horizontal distances were estimated using a handheld laser rangefinder (Forest Pro, Laser Technology, CO, USA), whereas bearing directions were estimated using a compass (Suunto, Finland). For each individual, we determined the coordinates with a multiple averaged global positioning system (GPS, Garmin), and measured the diameter at breast height (DBH, 1·35 m). In the SGE, we also used a GPS for multi-stemmed location, and worked with a distance tape to record the distance among the three stems sampled in each multi-stemmed tree; thus, here we had 30 individuals per forest. We collected a small woody tissue sample (4 × 4 cm) from each individual using a chisel and a rubber mallet; the sample consisted of bark, cambium and wood. The tissue sample was immediately placed in a labelled Ziploc bag with silica gel in its interior to promote rapid tissue desiccation to avoid DNA degradation. Bags were then kept cold in a portable cooler and transported to the laboratory for genetic analysis.

We used population size structures (stem diameter distributions, stem density and basal area) to infer stand development and disturbance history in the different forests. For this, we established three 400-m² plots that were located randomly within each of the 12 forests. After plot delimitation (20 × 20 m), all trees >1 cm in DBH were tallied and the DBH was measured on each individual. We then compared DBH distributions among the different forest types (see the Statistical analyses section).

DNA extraction and microsatellite genotyping

A total of 320 tissue samples were collected (25 for OG, 25 for SGI and 30 for SGE for each of four areas). Approximately 0·5 g of dry tissue (cambium previously separated from bark) was used to extract DNA from each individual. Total DNA was extracted according to the protocol of Doyle and Doyle (1987) and treated with RNAse A (30 min at 37 °C). A total of six polymorphic microsatellite markers (Npum01, Npum09,

Npum11, Npum13, Npum17a and Npum18) previously developed by Soliani *et al*. (2010) and one (NnBio111) developed by Marchelli *et al*. (2008) were amplified for all individuals. PCR reactions were performed following the conditions described by Soliani *et al*. (2010) and Marchelli *et al*. (2008). For the first set of markers, PCR reactions were conducted in a total volume of 15 μL, containing 1× PCR buffer, 1·5–3·0 mM MgCl$_2$, 0·14 mM dNTP, 0·2 μM of each primer, 0·55 U of Taq polymerase and 25 ng of template DNA. The thermal profile was as follows: denaturation at 94 °C for 4 min followed by 35 cycles at 94 °C for 30 s, $T^{\circ}_{\text{annealing}}$ for 30 s, 72 °C 30 s and final extension at 72 °C for 10 min. $T^{\circ}_{\text{annealing}}$ was at 53 °C for Npum01, 60 °C for Npum09, Npum13 and Npum17a, and 62 °C for Npum11 and Npum18. NnBio111 was amplified in a total volume of 20 μL, containing 1× PCR buffer, 2·0 mM MgCl$_2$, 0·1 mM dNTPs, 0·2 μM of each primer, 0·6 % BSA, 1 U of Taq polymerase and 25 ng of template DNA. The thermal profile was as follows: denaturation at 95 °C for 5 min followed by 35 cycles at 94 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min and final extension at 72 °C for 7 min. Forward primers were labelled with Applied Biosystems fluorochromes (6-FAM, NED, PET and VIC). The PCR products were run in an automatic sequencer (Applied Biosystems 3120, 16 capillaries) at the Laboratorio de Genómica Forestal, Centro de Biotecnología, Universidad de Concepción, Chile. Finally, electropherograms were analysed using GeneMarker 6.0.

For each forest, we checked for deviations from Hardy–Weinberg (HW) equilibrium using GenAlEx v6.4 (Peakall and Smouse, 2006). Two loci (Npum01 and Npum11) showed consistent deviations across most forests and were therefore discarded in further analyses. Homozygote excess compared with HW expectations, together with the fact that some samples did not amplify for one locus, suggested potential null alleles for several loci in some stands (Supplementary Data Table S1). This is in accordance with Mathiasen and Premoli (2010), who studied populations of *N. pumilio* using isozymes and microsatellites and found deviation from HW expectations in 60 % of the tests (populations × loci). This was further confirmed using Micro-Checker 2.2 (van Oosterhout *et al*., 2004). However, as populations showed deviations from equilibrium at several loci, an alternative explanation is population substructure. In each forest, the number of alleles per locus ranged from 2 to 12 and unbiased expected heterozygosity per locus (UH$_e$) from 0·23 to 0·84 (Table 1) (average UH$_e$ 0·6–0·73), thus showing higher genetic variation than other studies on the same species; e.g. Mathiasen and Premoli (2010) obtained an H_e range of 0·41–0·59.

Nearest neighbour determination

Biotic plant–plant interactions are inherently local in nature. They mostly occur among immediate neighbours, in which case an individual's direct effect on another (negative or positive) will have a delimited influence in space, i.e. a 'zone of interaction'. For our purposes, we pragmatically considered that the current mean nearest neighbour distance (MNND) between stems in the forests under study was the result of biotic interactions (assuming that the original density and spatial pattern were no longer an influence). We computed the MNND for OG

TABLE 1. *Average number of trees sampled (*n*), number of alleles (*N$_a$*) and unbiased expected heterozygosity (*UH$_e$*) for each stand (site × forest type) and locus. Forest types were old-growth forest (OG), second-growth forest interior (SGI) and second-growth forest edge (SGE).*

Site	Forest	n	N_a	UH$_e$
1	OG	23·80	8·60	0·70
	SGI	23·40	7·20	0·72
	SGE	26·40	6·20	0·71
2	OG	23·60	8·80	0·73
	SGI	20·60	6·80	0·69
	SGE	26·60	8·20	0·72
3	OG	20·40	10·00	0·76
	SGI	26·60	8·80	0·71
	SGE	27·60	8·40	0·77
4	OG	22·00	9·20	0·79
	SGI	23·60	9·40	0·71
	SGE	27·20	8·80	0·71

based on the total number of pairs of distances in this forest type as a proxy for the zone of interaction. We expected similar zones of interaction in SGI and SGE because both arose during the same colonization event. We therefore computed the MNND for SGI and SGE based on the pooled pairs of distances from these two forest types. MNND can be considered a good surrogate for effective interaction distance, given that in a dense forest crowns and roots of trees are most likely to have been interacting for some time already. Thus, we confidently assumed that any genetic structure found within this MNND can be attributed to biotic interactions, although we are aware that MNND may underestimate the zone of interaction as biotic interactions may still be occurring in the vicinity of the MNND (see Fig. 1 for more details).

Statistical analyses

Forest structure assessment We compared DBH distributions for OG, SGI and SGE forests, fitting the empirical distributions to exponential and normal models (Ricci, 2005) and compared their relative fit (model selection) using Akaike's information criterion for small samples (AIC$_c$) (Burnham and Anderson, 2002). For these analyses we used the MASS package (Venables and Ripley, 2002) in R (R Development Core Team, 2013).

Spatial genetic structure: spatial autocorrelation analysis and Moran's I To determine the SGS among trees for the different forest types, we performed spatial autocorrelation analyses and computed Moran's *I* using the software SPAGeDi 1·4 c (Hardy and Vekemans, 2002). For each forest type (OG, SGI and SGE), we pooled the data from the four areas and restricted the pairwise comparison to all pairs of individuals within the same forest (area × forest type) using the within-stand allele diversity as reference allele frequency. In each forest type, the first distance class was defined as all distances within the zone of interaction (computed using the MNND). The subsequent distance classes were determined in order to have a sufficient number of pairs in each class (Vekemans and Hardy, 2004) and still be as similar as possible among forest types. The number of pairs in

Table 2. *Number of pairs of individuals in each distance class for each forest type (SPAGeDi analysis). Forest types correspond to old-growth (OG), second-growth interior (SGI) and second-growth edge (SGE) forests of* Nothofagus pumilio *in Patagonia, Chile.*

Number of pairs in	Maximum pair distance (m)								
	1·6	2·5	4	10	15	20	30	40	50
OG			28	27		77	111	139	99
SGI	9	15		46		83	114	114	109
SGE	106				118		217	186	134

each distance class is given in Table 2 and Supplementary Data Fig. S1. Moran's *I* index is equivalent to a relationship coefficient (Hardy and Vekemans, 1999) and ranges from −1 to +1, with 0 being the null hypothesis of no genetic relationship between pairs of individuals at a specific distance. Significance of autocorrelation was tested by Monte Carlo permutations (20 000) for the genetic data. For a specific distance class, values of Moran's *I* index greater than, equal to or lower than simulation envelopes indicate significant positive spatial autocorrelation, spatial independence and significant negative spatial autocorrelation, respectively (Legendre and Fortin, 1989). To estimate the SGS due to abiotic factors, we computed the slope of the regression with distance log-transformed as recommended in Vekemans and Hardy (2004), using the distance classes greater than the MNND.

Genetic relatedness pairwise relationship coefficient We computed the pairwise relationship coefficient r_{ij} (identity in state) within each forest following Hardy (2003) and using SPAGeDi 1·4 c (Hardy and Vekemans, 2002), similarly to Till-Bottraud *et al.* (2012). To test for the influence of biotic interactions in the OG and SGI forests, we compared r_{ij} between pairs of individuals within the MNND to those in the first distance class greater than MNND using a Kruskal–Wallis non-parametric test. However, pairwise relationship coefficients are not independent when individuals are related to each other. Therefore, to test whether the within- and among-cluster (or between the first two distance classes) relationship coefficients were different in the SGE forests we randomly sampled all the possible independent pairs within and among clusters and compared the average relationship coefficients using a Wilcoxon rank test (alternative hypothesis: r_{ij} among clusters is lower than r_{ij} within clusters). The procedure was repeated 9999 times. Results of the Wilcoxon rank tests were then combined using the Stoufer test (Whitlock, 2005). To have a finer picture of the effect of biotic interactions in the SGE forests, we compared the distribution of r_{ij} among and within clusters using the χ^2 test. In order to differentiate positive biotic interactions from seed dispersal, we compared r_{ij} within clusters in the SGE forests with r_{ij} in the first distance class greater than MNND of the OG forests using the same procedure (sampling all the pairs in the OG forests as they were all independent). We also compared the r_{ij} within clusters to the expected value for half-sibs (0·25). For this, we randomly sampled one pair per cluster and repeated the procedure 9999 times. The theoretical value (0·25) was then compared with this distribution and its probability was estimated.

RESULTS

Stand structure and dynamics

Consistent with casual observation, we found that the OG and second-growth forests (SGI and SGE) differed significantly in their structure (Fig. 2). The OG forests had on average a density of 1065 trees ha^{-1} (s.e. 349) and a basal area of 57·02 m^2 ha^{-1} (s.e. 7.32), while the SGI and SGE forests had densities of 3048 (s.e. 473) and 1932 trees ha^{-1} (s.e. 311) and basal areas of 41·68 (s.e. 4·31) and 43·38 m^2 ha^{-1} (s.e. 8·61), respectively. At the SGE, we found that nearly 100 % of all stems belonged to multi-stemmed trees. On the contrary, no multi-stemmed trees were found in the SGI or OG forests. Finally, we found, as expected, different MNNDs for each forest type: the OG forests had an MNND of 3·97 m (s.e. 0·25, $n = 47$), whereas the SGI and SGE forests had MNND of 1·61 m (s.e. 0·15, $n = 95$). In SGI, all distances between stems of the same cluster were <1·61 m. In SGE, this distance corresponded to a gap in the distribution of pairwise distances (no pairs within distances between 1·3 and 1·6 m; Supplementary Data Fig. S1).

Population-level genetic structure

We found that all three forest types showed a significant decrease in relatedness with increasing distance between trees, i.e. a significant SGS (slope of the regression of Moran's *I* with log-distance for distances greater than the MNND: $b = -0·029$, $p = 0·015$ for OG; $b = -0·027$, $p = 0·013$ for SGI; $b = -0·073$, $p < 0·001$ for SGE; Fig. 3). In particular, in the first distance class greater than MNND, pairs of trees were significantly more related than they were on average in all three forest types (Moran's $I = 0·156$, $p = 0·002$ for OG; $I = 0·311$, $p < 0·001$ for SGI; $I = 0·625$, $p < 0·001$ for SGE; Fig. 3). In general, the SGS signal was strong and significant despite a relatively small sample size.

Fine-scale genetic structure

In the OG forests, the relationship coefficient (r_{ij}) between pairs of individuals within the MNND was not significantly different from that in the first distance class greater than the MNND (Kruskal–Wallis $\chi^2 = 2$, d.f. = 1, $p = 0·157$; Fig. 3), while it was significantly lower within the MNND in the SGI forests than in the first distance class greater than the MNND in the OG forests (Kruskal–Wallis $\chi^2 = 7·69$, d.f. = 1, $p = 0·006$; Fig. 3). In the SGE forests, all trees within the MNND belonged to the same cluster and relatedness was very high and highly significant (Moran's $I = 0·625$, $p < 0·001$; Fig. 3).

The mean r_{ij} of pairs of trees within the MNND (i.e. within clusters) was significantly higher than immediately beyond or among clusters of the same forest (within clusters, $r_{ij} = 0·399$, s.e. 0·006; among stems within the first distance class beyond the MNND, $r_{ij} = 0·054$, s.e. 0·018; among clusters, $r_{ij} = -0·031$, s.e. 0·004; both comparisons $p < 0·001$; Fig. 4). Among clusters, the relatedness values (r_{ij}) were distributed between −0·5 and 0·7 with a peak at ∼0 (i.e. unrelated individuals). Within clusters the relatedness values were almost evenly distributed between 0 and 1 (Fig. 4), i.e. there was a significant deficit in the proportion of unrelated pairs and an excess in the proportion
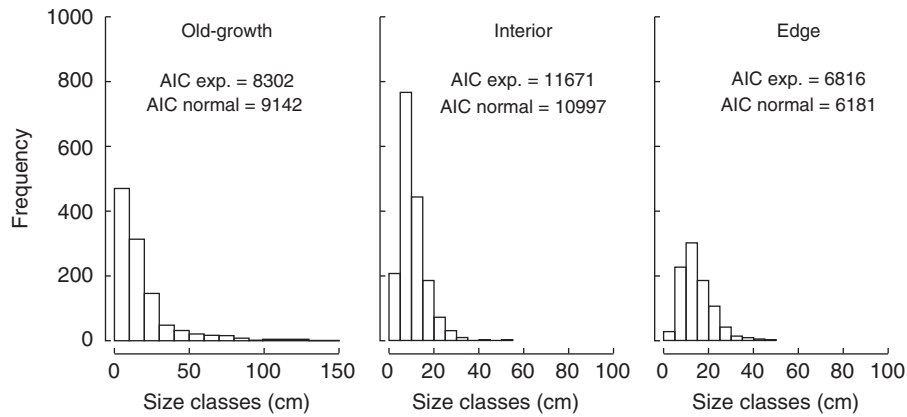
FIG. 2. Tree size distributions for the old-growth, second-growth interior and second-growth edge forests of *Nothofagus pumilio* in Patagonia, Chile. Frequency distributions of size-classes (DBH in cm) were compared by fitting theoretical models (normal and exponential). The frequency distributions differ significantly among forest types (see Results).
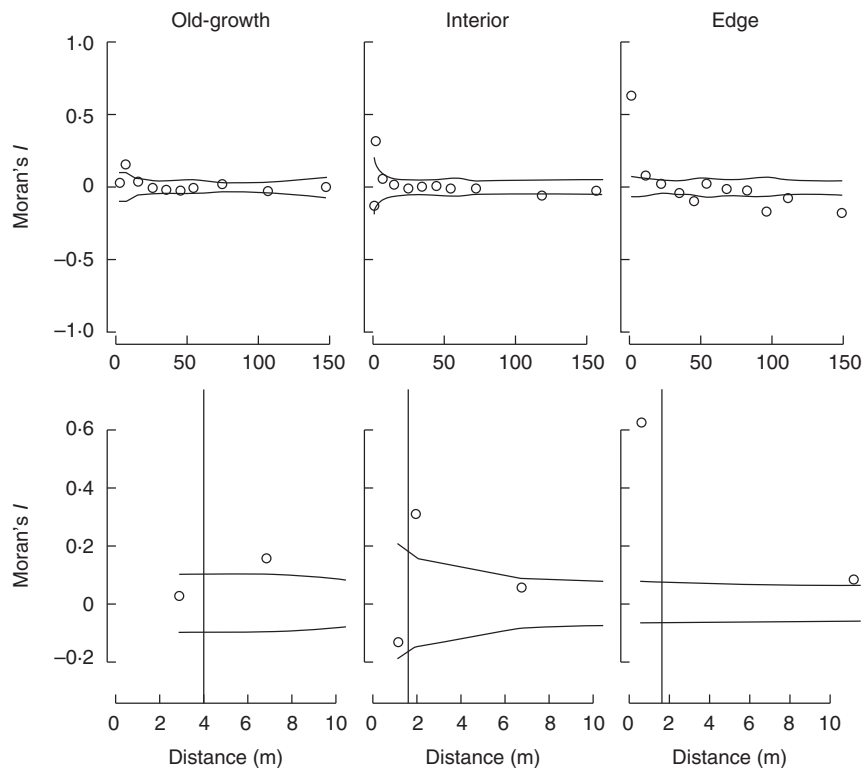
FIG. 3. Moran's *I* index (circles) for genetic relatedness as a function of lag distance between tree individuals in old-growth, second-growth interior and second-growth edge forests of *Nothofagus pumilio* in Patagonia, Chile. Simulation envelopes (solid lines) represent the fifth-lowest and fifth-highest values of the Monte Carlo simulations (20 000) of the null model of no spatial autocorrelation. Circles outside the simulation envelopes represent deviance from the null model at a specific lag distance, and are interpreted as positive (above the envelope) or negative (below the envelope) genetic relatedness. For clarity, we show the same figures on a fine scale representing the first 10 m of distance, where the zone of interaction's threshold (vertical line, mean nearest neighbour distance, MNND) is displayed.

of related pairs compared with among clusters ($\chi^2 = 303 \cdot 6$, d.f. $= 4$, $p < 0 \cdot 001$,). The mean r_{ij} within clusters was also significantly higher ($p < 0 \cdot 001$) than that of the significant distance class of the OG forest (4–10 m; $r_{ij} = 0 \cdot 088$, s.e. $0 \cdot 032$), indicating that this relatedness was not due (or not solely due) to local seed dispersal. In addition, the mean r_{ij} within clusters was significantly higher than the expected value for half-sibs ($0 \cdot 25$; $p < 0 \cdot 001$).

DISCUSSION

SGS due to disturbance and dispersal

Although the stand structures of the OG and second-growth forests of *N. pumilio* were very different, both types of forest exhibited a significant SGS with higher relatedness at short distances, indicating non-homogeneous (restricted) gene flow. For the OG forests, this result suggests a neighbourhood
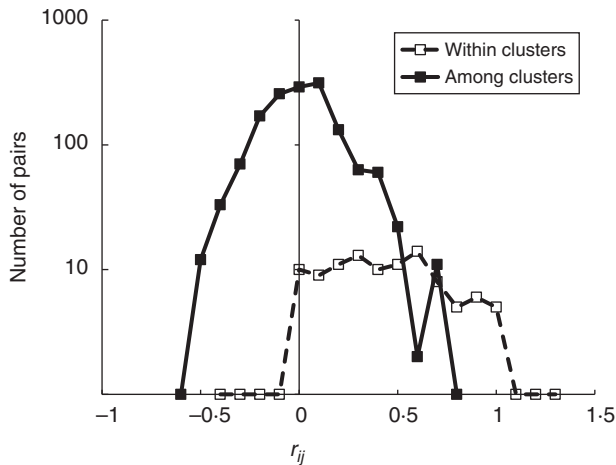
FIG. 4. Distribution of pairwise relationship coefficients (r_{ij}) in the second-growth edge forest of *Nothofagus pumilio* in Patagonia, Chile. Each pair was identified as 'within cluster' when both samples came from the same cluster, and 'among clusters' when the two samples came from different clusters. Note that a log-scaled *y*-axis is used for greater clarity.

structure due to gap recolonization by seeds from nearby trees with fine-scale dispersal associated with a gap-phase dynamic forest. When we compared the frequency distributions of size classes (DBH) with theoretical models, we indeed found that the OG forests were best fitted by a negative exponential distribution (Fig. 2), i.e. the highest size-class frequency for OG was trees of small diameter, which means that the forest is regenerating according to gap-phase dynamics (Oliver, 1981; Veblen, 1992; Fajardo and de Graaf, 2004). The SGI forests showed an even-aged structure (normal frequency distribution of size classes; Fig. 2) coincident with flash colonization from a large seed source, seeds likely having come from the OG forests that survived the fires. According to Oliver's classification of stand dynamics (Oliver, 1981), for these forests to have a normal frequency distribution of size classes (i.e. a low representation of extreme size classes) means that the forest is the result of a large-scale disturbance, being still quite homogeneous (e.g. even-aged), and in its optimal growth development phase. The higher relatedness at short distances (significant SGS) could be caused by the existence of few remnant trees located in sheltered areas producing a local seed rain, adding to the large seed source coming from the OG forests (see below).

These results are at least partly similar to other studies. In general, a significant SGS has been found in OG forests of *N. pumilio* and *Nothofagus dombeyi* in Argentina (Premoli and Kitzberger, 2005; Mathiasen and Premoli, 2013), *Larix decidua* in the Swiss Alps (Pluess, 2011), *Larix kaempferi* in Japan (Nishimura and Setoguchi, 2011) and *Pinus halepensis* in Israel (Shohami and Nathan, 2014). Interestingly, none of the previous studies found significant SGS for their second-growth forests. As stated earlier, genetic structure is strongly dependent on pollen and seed movement. *Nothofagus pumilio*, *N. dombeyi*, *L. decidua* and *L. kaempferi* are all wind-pollinated tree species; however, seed dispersal is limited in *Nothofagus* (masting species, wingless seeds), whereas seeds are wind-dispersed over large distances in *Larix*. As a final point, selfing is suggested to

be higher (although still limited) in *N. dombeyi* compared with *N. pumilio* (Mathiasen and Premoli, 2010).

Biotic interactions

Within the zone of interaction, i.e. where tree–tree interactions should most probably occur, the genetic relationship did not follow the global pattern. First, for the SGI forests we found relatedness values that were significantly lower at MNND than immediately beyond this zone. This result can be explained in two ways: (1) the signal of localized dispersal from remnant trees observed beyond the zone of interaction is cancelled out by competition within the zone of interaction; and (2) if our estimate of the zone of interaction is erroneous (and too small), there is a homogeneous genetic structure due to flash recolonization along with facilitation at the edge of the zone of interaction. It was clear from previous studies that competition is the process acting in these SGI forests (Fajardo and McIntire, 2010; McIntire and Fajardo, 2011). According to Oliver's (1981) model of forest stand development, this type of forest should be in the self-thinning stage, which is a period of time when competition, particularly for light, is higher and tree density is drastically reduced. With this in mind, we tend to discard the second explanation (i.e. facilitation) as a possible option. Thus, we think that there is dispersal both at large scale from the OG forest and at fine scale from remnant trees that survived the fires, and that this signal has been compensated by competition within the zone of interaction, i.e. that the significantly lower relatedness than that of the second distance class is a result of negative plant interactions in progress at the very fine scale. The same pattern (i.e. lower genetic relationship within MNND) was observed in the OG forests.

To find significant positive genetic relatedness within the zone of interaction in the SGE forests was not surprising given previous ecological and genetic studies (Fajardo and McIntire, 2010, 2011; McIntire and Fajardo, 2011; Till-Bottraud *et al.*, 2012). We have here confirmed that the pattern found by Till-Bottraud *et al.* (2012) is a general pattern as relatedness was tested with a codominant, highly variable set of markers (microsatellites versus amplified fragment length polymorphism (AFLP) in the previous work) and found to be significantly higher within than among clusters in all four areas studied (data not shown). Although in most cases facilitation has been claimed to be a transient phenomenon, McIntire and Fajardo (2011) proved that ∼45-year-old stems of *N. pumilio* within clusters continue facilitating one another even when abiotic stress is no longer a hazard. Till-Bottraud *et al.* (2012) furthermore suggested that selection for merging of highly related individuals was occurring as the merged stems were significantly more related than random pairs from the population. This hypothesis was confirmed here by the fact that relatedness within clusters was higher than that produced by local seed dispersal in the OG forests, and greater than that of half-sibs, indicating selection for highly related individuals during the facilitative interaction (Fig. 4). Possible mechanisms explaining this selection for high relatedness are kin cooperation and kin selection. Kin cooperation is a reciprocal beneficial interaction between related individuals. Kin selection is a mechanism in which altruism might be selected for as long as it is directed towards

kin. In both cases, relatedness lowers the cost or the probability of cheaters in the system and both mechanisms require kin recognition, i.e. the ability to assess the shared ancestry (and thus genetic relatedness) with another individual (Hamilton, 1964). Studies have recently shown the existence of kin recognition mechanisms in plants, through root exudates, root interactions or volatile compounds (Dudley and File, 2007; File *et al.*, 2012; Fang *et al.*, 2013; Semchenko *et al.*, 2014). Finally, the ecological mechanism involved may be facilitative (altruistic or cooperative) fusion, which would be selectively advantageous for resource acquisition or stability against wind.

Thus, facilitation at this step depends on pre-existing genetic relatedness between trees (merging of trees in a cluster requires at least one pair of related stems) and is enhanced by selecting for high relatedness within clusters. Interestingly, Mathiasen and Premoli (2013), studying the spatial autocorrelation pattern in one post-fire second-growth *N. pumilio* forest, found strikingly similar results (i.e. very high relatedness at close tree distances) to our SGE forests, although they do not mention sampling at the edge of the forest or the occurrence of multi-stemmed trees. High relatedness was also found in some clusters of merged trees of *Pinus flexilis* – average relatedness within clusters obtained using isozyme markers ranged between 0·19 (Schuster and Mitton, 1991) and 0·43 (Carsey and Tomback, 1994) – potentially suggesting a similar mechanism. The strong difference in relatedness values we found between immediate neighbours (<4 m) and further away, but still at short distances, underlines the importance of sampling a large range of distances to encompass all the ecological processes potentially acting on the SGS. This was already pointed at by Gaudeul and Till-Bottraud (2008) for pollen and seed dispersal in *Eryngium alpinum*.

How can we explain the observation that highly related seeds ended up next to one another at the edge of the second-growth forest? We tend to discard any animal dispersal and storage in caches (dyszoochory) – similar to what occurs in some pine species, like *Pinus albicaulis* or *P. flexilis* – because then we should have observed multi-stemmed trees not only in the SGE forests but also in the SGI forests, which was not the case. Given that *N. pumilio* is a gravity- or wind-dispersed species and given the masses of seeds produced during a masting event, we are more convinced that dispersal from remnant trees that survived the fire may explain the presence of a few related seeds in close proximity in a medium-range dispersal event. What is, then, the mechanism by which highly related individuals end up being merged in a multi-stemmed tree? McIntire and Fajardo (2011) showed that after a seedling regeneration period of facilitation, where growing in clusters led to higher survival under stressful conditions, individuals merged, potentially involving some grafting compatibility or kin recognition among highly related individuals. First, kin recognition via root exudates has been shown for several species (Dudley and File, 2007; Murphy and Dudley, 2009; Biedrzycki *et al.*, 2010; Fang *et al.*, 2013). Second, root grafting has been shown to improve access to, or the redistribution of, resources (Fraser *et al.*, 2007; Tarroux and DesRochers, 2010). This may occur at an early stage in *N. pumilio*, later followed by merging of stems. Then, even if survival is improved in clusters, mortality may still occur (McIntire and Fajardo, 2011), allowing a potential for selection through differential survival. Preliminary results on

seedlings planted at random with regard to their genotype by Fajardo and McIntire (2011) seem to indicate selection for related seedlings (Till-Bottraud and Fajardo, unpubl. res.). Given that no seeds have been produced yet in these forests, the size of trees is the only fitness estimate we can measure. Trees growing in clusters have higher basal areas than trees growing alone (McIntire and Fajardo, 2011), indicating that merging is selectively advantageous.

Conclusions

Till-Bottraud *et al.* (2012), studying merged trees at the edge of one single second-growth forest of *N. pumilio*, found that stems within multi-stemmed clusters were highly related to one another when compared with stems belonging to different clusters and to the population in general. They suggested that one of the causes of this peculiar genetic structure was selection for merging of highly related individuals and raised the question of how such highly related individuals could occur in close vicinity in a flash colonization from a large and distant seed source. Here, we corroborate their findings at four other sites in the same region (Patagonia, Chile), which gives generality to the peculiar genetic pattern previously found. We also provide a possible explanation for the high relatedness, as the global genetic structure strongly suggests the presence of remnant trees with a localized seed rain. This pre-existent genetic structure could be a prerequisite for positive interactions to take place, or at least to persist in time.

Simultaneously, this finding was crucial in generating the idea that, in addition to dispersal, genetic relatedness and biotic interactions are tightly linked in determining the fine scale SGS of populations. In particular, our results lead to the following suggestions. (1) Biotic interactions can significantly influence SGS at very short distances (within the zone of interaction of individuals). This fact is neglected in classical SGS studies and suggests in particular that greater care should be taken in studying SGS at very fine spatial scales. (2) The occurrence of positive interactions is determined by both stressful conditions (McIntire and Fajardo, 2011, 2014) and the pre-existing SGS of the population, i.e. merging mostly happens among highly related individuals (a form of kin cooperation or kin selection), and thus could only occur if highly related individuals were present in the original group of interacting individuals. Positive interactions would further increase relatedness in the merged groups of trees. We therefore suggest that the genetic composition of a population may have an influence on what type of biotic interactions will prevail among immediate neighbours in young populations that are still self-thinning. If merging is possible only when some stems in the cluster are highly related, then positive interactions can occur only with pre-existing SGS, but then the predicted negative density-dependent process of self-thinning will be altered. We may speculate that immediate neighbouring seedlings were also highly related in the SGI forests, which may lead to a competition delay. In any case, classical ecological theory based on the niche partitioning concept predicts that competition should be highest among individuals overlapping their niches, with kin individuals representing the extreme. We have shown that this is challenged by kin selection or kin cooperation theories. Finally, a question is posited for

community ecologists: what are the consequences for models predicting community structuring that ignore the genetic structure of interacting populations?

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: sample size (*n*), number of alleles (*N*$_a$), number of effective alleles (*N*$_e$), expected heterozygosity (*H*$_e$), fixation index (*F*), test of Hardy–Weinberg equilibrium (HWE) and potential presence of null alleles as suggested by Micro-Checker for each forest and locus. Fig. S1: distribution of pairwise geographic distances in each forest type.

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LITERATURE CITED

Bacles CFE, Jump AS. 2011. Taking a tree's perspective on forest fragmentation genetics. *Trends in Plant Science* **16**: 13–18.

Banks SC, Cary GJ, Smith AL, *et al.* **2013.** How does ecological disturbance influence genetic diversity? *Trends in Ecology and Evolution* **28**: 670–679.

Biedrzycki ML, Jilany TA, Dudley SA, Bais HP. 2010. Root exudates mediate kin recognition in plants. *Communicative and Integrative Biology* **3**: 28–35.

Bronstein JL. 2009. The evolution of facilitation and mutualism. *Journal of Ecology* **97**: 1160–1170.

Burnham KP, Anderson DR. 2002. *Model selection and multimodal inference: a practical information-theoretic approach.* New York: Springer.

Carsey KS, Tomback DF. 1994. Growth form distribution and genetic relationships in tree clusters of *Pinus flexilis*, a bird-dispersed pine. *Oecologia* **98**: 402–411.

Cerfonteyn ME, le Roux PC, Jansen van Vuuren B, Born C. 2011. Cryptic spatial aggregation of the cushion plant *Azorella selago* (Apiaceae) revealed by a multilocus molecular approach suggests frequent intraspecific facilitation under sub-Antarctic conditions. *American Journal of Botany* **98**: 909–914.

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* **31**: 343–366.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolating procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

Dudley SA, File AL. 2007. Kin recognition in an annual plant. *Biology Letters* **3**: 435–438.

Epperson BK. 1993. Spatial structure of genetic variation within populations of forest trees. *New Forests* **6**: 257–278.

Fajardo A, de Graaf R. 2004. Tree dynamics in canopy gaps in old-growth forests of *Nothofagus pumilio* in Southern Chile. *Plant Ecology* **173**: 95–106.

Fajardo A, McIntire EJB. 2010. Merged trees in second-growth, fire origin forests in Patagonia, Chile: positive spatial association patterns and their ecological implications. *American Journal of Botany* **97**: 1424–1430.

Fajardo A, McIntire EJB. 2011. Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. *Journal of Ecology* **99**: 642–650.

Fajardo A, Piper FI, Cavieres LA. 2011. Distinguishing local from global climate influences in the variation of carbon status with altitude in a tree line species. *Global Ecology and Biogeography* **20**: 307–318.

Fang S, Clark RT, Zheng Y, *et al.* **2013.** Genotypic recognition and spatial responses by rice roots. *Proceedings of the National Academy of Sciences of the USA* **110**: 2670–2675.

File AL, Murphy GP, Dudley SA. 2012. Fitness consequences of plants growing with siblings: reconciling kin selection, niche partitioning and competitive ability. *Proceedings of the Royal Society of London Series B: Biological Sciences* **279**: 209–218.

Fraser EC, Lieffers VJ, Landhäusser SM. 2007. The persistence and function of living roots on lodgepole pine snags and stumps grafted to living trees. *Annals of Forest Science* **64**: 31–36.

Gaudeul M, Till-Bottraud I. 2008. Genetic structure of the endangered perennial plant *Eryngium alpinum* (Apiaceae) in an alpine valley. *Biological Journal of the Linnean Society* **93**: 667–677.

Hairston NG, Smith FE, Slobodkin LB. 1960. Community structure, population control, and competition. *American Naturalist* **94**: 421–425.

Hamilton WD. 1964. Genetic evolution of social behaviour. *Journal of Theoretical Biology* **7**: 1–16.

Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **351**: 1291–1298.

Hamrick JL, Nason JD. 1996. Consequences of dispersal in plants. In: OE Rhodes, RK Chesser, MH Smith, eds. *Population dynamics in ecological space and time*. Chicago: University of Chicago Press.

Hardin G. 1960. The competitive exclusion principle. *Science* **131**: 1292–1298.

Hardy OJ. 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology* **12**: 1577–1588.

Hardy OJ, Vekemans X. 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetic models. *Heredity* **83**: 145–154.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Hartmann HT, Kester DE, Davies FT, Geneve RL. 1996. *Plant propagation: principles and practices*. Englewood Cliffs: Prentice Hall.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M. 2008. Ecological consequences of genetic diversity. *Ecology Letters* **11**: 609–623.

Hutchinson GE. 1961. The paradox of the plankton. *American Naturalist* **95**: 137–141.

Legendre P, Fortin M-J. 1989. Spatial pattern and ecological analysis. *Vegetatio* **80**: 107–138.

Leicht-Young S, Latimer AM, Silander JA. 2011. Lianas escape self-thinning: experimental evidence of positive density dependence in temperate lianas *Celastrus orbiculatus* and *C*. *scandens*. Perspectives in Plant Ecology Evolution and Systematics **13**: 163–172.

Marchelli P, Caron H, Azpilicueta MM, Gallo LA. 2008. A new set of highly polymorphic nuclear microsatellite markers for *Nothofagus nervosa* and related South American species. *Silvae Genetica* **2**: 45–92.

Mathiasen P, Premoli AC. 2010. Out in the cold: genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. *Molecular Ecology* **19**: 371–385.

Mathiasen P, Premoli AC. 2013. Fine-scale genetic structure of *Nothofagus pumilio* (lenga) at contrasting elevations of the altitudinal gradient. *Genetica* **141**: 95–105.

McIntire EJB, Fajardo A. 2009. Beyond description: the active and effective way to infer processes from spatial patterns. *Ecology* **90**: 46–56.

McIntire EJB, Fajardo A. 2011. Facilitation within species: a possible origin of group selected superorganisms. *American Naturalist* **178**: 88–97.

McIntire EJB, Fajardo A. 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* **201**: 403–416.

Murphy GP, Dudley SA. 2009. Kin recognition: competition and cooperation in *Impatiens* (Balsaminaceae). *American Journal of Botany* **96**: 1990–1996.

Nishimura N, Setoguchi H. 2011. Homogeneous genetic structure and variation in tree architecture of *Larix kaempferi* along altitudinal gradients on Mt. Fuji. *Journal of Plant Research* **124**: 253–263.

Oliver CD. 1981. Forest development in North America following major disturbances. *Forest Ecology and Management* **3**: 153–168.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535–538.

Peakall R, Smouse PE. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–295.

Pluess AR. 2011. Pursuing glacier retreat: genetic structure of a rapidly expanding *Larix decidua* population. *Molecular Ecology* **20**: 473–485.

Premoli AC, Kitzberger T. 2005. Regeneration mode affects spatial genetic structure of *Nothofagus dombeyi* forests. *Molecular Ecology* **14**: 2319–2329.

R Development Core Team. 2013. *R: a language and environment for statistical computing. Version 2.15.3.* R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Ricci V. 2005. *Fitting distributions with R.* http://cran.r-project.org/doc/contrib/Ricci-distributions-en.pdf.

Schuster WSF, Mitton JB. 1991. Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. *Heredity* **67**: 41–48.

Segovia NI, Vásquez JA, Faugeron S, Haye PA. 2015. On the advantage of sharing a holdfast: effects of density and occurrence of kin aggregation in the kelp *Lessonia berteroana*. *Marine Ecology*. In press. doi:10.1111/maec.12206.

Semchenko M, Saar S, Lepik A. 2014. Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytologist* **204**: 631–637.

Shohami D, Nathan R. 2014. Fire-induced population reduction and landscape opening increases gene flow via pollen dispersal in *Pinus halepensis*. *Molecular Ecology* **23**: 70–81.

Silvertown J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* **19**: 605–611.

Soliani C, Sebastiani F, Marchelli P, Gallo L, Vendramin GG. 2010. Development of novel genomic microsatellite markers in the southern beech *Nothofagus pumilio* (Poepp. et Endl.) Krasser. *Molecular Ecology Resources* **10**: 404–408.

Sousa WP. 1984. The role of disturbance in natural communities. *Annual Review of Ecology and Systematics* **15**: 353–391.

Stoll P, Weiner J. 2000. A neighborhood view of interactions among individual plants. In: U Dieckmann, R Law, JAJ Metz, eds. *The geometry of ecological interactions*. Cambridge: Cambridge University Press.

Tarroux E, DesRochers A. 2010. Frequency of root grafting in naturally and artificially regenerated stands of *Pinus banksiana*: influence of site characteristics. *Canadian Journal of Forest Research* **40**: 861–871.

Till-Bottraud I, Fajardo A, Rioux D. 2012. Multi-stemmed trees of *Nothofagus pumilio* second-growth forest in Patagonia are formed by highly related individuals. *Annals of Botany* **110**: 905–913.

Tilman D. 1982. *Resource competition and community structure*. Princeton: Princeton University Press.

Veblen TT. 1992. Regeneration dynamics. In: DC Glen-Lewin, RK Peet, TT Veblen, eds. *Plant succession: theory and prediction*. London: Chapman & Hall.

Veblen TT, Donoso C, Kitzberger T, Rebertus A. 1996. Ecology of Southern Chilean and Argentinean *Nothofagus* forests. In: TT Veblen, RS Hill, J Read, eds. *The ecology and biogeography of Nothofagus forests*. New Haven: Yale University Press.

Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**: 921–935.

Vellend M, Drummond EBM, Tomimatsu H. 2010. Effects of genotype identity and diversity on the invasiveness and invasibility of plant populations. *Oecologia* **162**: 371–381.

Venables WN, Ripley BD. 2002. Modern applied statistics with S. New York: Springer.

Whitlock MC. 2005. Combining probability from independent tests : the weighted z-method is superior to Fisher's approach. *Journal of Evolutionary Biology* **18**: 1368–1373.