Patterns of grassland invasions by trees: insights from demographic and genetic spatial analyses

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Abstract

Aims

Woody invasions into grasslands have increased globally due to changing land use, climate and introduced woody species, but spatial processes generating and sustaining these invasions are not well understood. To gain insight into the patterns of spread of tree populations within grasslands, and to propose a full spatial analytical toolbox for studying native and non-native woody species spread when long-term data are not available, we tested if 50 years of grassland invasion in Western Carpathians by Norway spruce (*Picea abies* Karst.) proceeded by one of the two traditionally competing hypotheses of species spread: (i) by frontier expansion, or (ii) by advanced groups established ahead of the population frontier. We also tested whether the pattern of invasion changed over time.

Methods

We analyzed the spatial demographic and genetic patterns of a Norway spruce population invading a Western Carpathian grassland using Ripley's $L(t)$ and genetic kinship coefficients (F_{ij}). We mapped and genotyped spruce trees across the invasion front (from the invasion leading edge to fully colonized grassland near the source population) using three demographic classes (adults, juveniles and seedlings) to approximate the temporal aspects of the invasion. We studied how the spatial patterns of invasion by individual demographic classes and their genetic kinship varied among adjacent plots established at different distances from the source population (ranging from 0 to 160 m, in 40-m distance increments).

Important Findings

Juveniles were positively genetically related to adults on fine scales (<4 m), suggesting that adults within the grassland acted as a seed source and accelerated early invasion. However, adults did not act as nucleation centers for the formation of advanced juvenile groups. Instead, genetically unrelated juveniles formed groups independently of adults. These groups were small and separate at the leading edge but they increased in size and graded into a continuous zone near the source population. Thus, juvenile recruitment occurred as a frontier expansion near the source population and as advanced groups controlled by environmental variation at the leading edge. Unlike juveniles, seedlings were clustered on all scales across the invasion front and formed groups around adult crowns at the invasion leading edge. The bulk of seedling establishment occurred at intermediate distances from the source population, independently from the adults, suggesting that the invasion front continued to expand as a frontier, gradually coalescing with the advanced groups at the leading edge. Thus, the grassland invasion was driven by a gradual frontier expansion of the original population during the first 50 years, with advanced groups enhancing but not driving the invasion process. Frontier expansion appeared more important as a mechanism of woody species spread early in the invasion process in this study, while advanced groups may play a larger role over longer temporal scales.

Keywords: genetic spatial structure, mountain meadows, point pattern analysis, population structure, secondary succession

INTRODUCTION

Changing climate, land use and introductions of non-native woody plants can lead to woody species spread across landscapes (Fang 2005; Harsch *et al.* 2009; Moore and Huffman 2004; Richardson 1998). The spread of native or non-native woody species frequently occurs in fragmented forest landscapes where woody species colonize and spread

through adjacent herbaceous communities such as postagricultural old fields or abandoned grasslands (e.g. Doležal *et al.* 2004; Dovčiak *et al.* 2005). Despite the well-documented inhibition of woody species establishment in herbaceous communities or grasslands in temperate zones (e.g. Davis *et al.* 2005; Harmer *et al.* 2001; Lawson *et al.* 1999; Prach *et al.* 1996), some woody species can colonize such communities rapidly and cause considerable changes in their productivity, carbon storage or biodiversity (Jackson *et al.* 2002; Reich *et al.* 2001; Wiezik *et al.* 2013; Yurkonis and Meiners 2004). The rate of species spread has been linked to population spatial patterns, especially the presence of advanced individuals (or groups) established ahead of the population frontier that can considerably accelerate species spread compared to a slower spread by a gradual expansion of the continuous population frontier (Clark *et al.* 2001; Ramakrishnan *et al.* 2010; Shigesada *et al.* 1995). These two contrasting patterns of spread (i.e. by advanced groups or by expansion of population frontier) have been identified at broad spatiotemporal scales (Hastings *et al.* 2005), but our understanding of the fine-scale processes that generate shifts in species distributions (cf. Lenoir and Svenning 2015) is much less clear.

Advanced colonies established ahead of the invasion front can act as seed dispersal nuclei that accelerate species spread compared to the gradual expansion of the population frontier (Clark *et al.* 2001; Shigesada *et al.* 1995); positive density-dependent feedbacks *via* increased seed rain or environmental amelioration can further accelerate species spread once a critical population density is reached (cf. Loehle *et al.* 1996; Malanson 1997; Milne *et al.* 1996; Rietkerk *et al.* 2004). On the other hand, the decrease in individual fitness or population growth at low population densities (Allee effects) can inhibit species spread if a species cannot establish at a density critical for its persistence and spread at or ahead of the invasion front (Courchamp *et al.* 2008; Davis *et al.* 2004; Keitt *et al.* 2001; Taylor and Hastings 2005). Species spread inhibited by Allee effects is more likely to be 'pushed' by a slow gradual frontier expansion of the source population rather than 'pulled' forward by advanced individuals or groups established ahead of the invasion front (Kot *et al.* 1996). Density dependence can cause nonlinear population demographic (vital) rates and dynamics across the spatial abundance gradients between population (or patch) centers and range (or patch) margins of spreading species, as reviewed recently by Guo (2014).

Woody seedling establishment within grass- or forb-dominated habitats (and thus woody invasions into these habitats) appear to be particularly inhibited by dense herbaceous vegetation (Prach *et al.* 1996; Šerá *et al.* 2000), microclimate (De Steven 1991), herbivory (Cadenasso *et al.* 2002; Lawson *et al.* 1999) or lack of mycorrhizal symbionts (Dickie *et al.* 2005). Yet, herbaceous communities are heterogeneous mosaics composed of patches that vary in species composition and soil characteristics (Purves and Law 2002; Seabloom *et al.* 2005) and in their suitability for woody seedlings (Barot *et al.* 1999; Magee and Antos 1992). Furthermore, woody plants

previously established within the herbaceous community can facilitate subsequent woody species establishment by suppressing herbaceous competition and moderating solar radiation and microclimate (heat stress) (Dovčiak and Brown 2014; Dovčiak *et al.* 2008). Species that modify their environment to support their own population growth (ecosystem engineers) tend to exhibit faster rates of spread compared to other species (Cuddington and Hastings 2004).

Spatial population structure (demography) can provide insights into the dynamics of forests (Giencke *et al.* 2014; Xi *et al.* 2008; Zhang *et al.* 2013), tree–grass systems (Barot *et al.* 1999; Halpern *et al.* 2010), ecotones (Camarero *et al.* 2000; Eppinga *et al.* 2013) or invasion fronts of non-native or spreading native woody species (Fang 2005; McDonald *et al.* 2003). Spatial clustering of invading plants can suggest species spread by advanced groups, but it cannot differentiate retrospectively if the advanced groups formed due to seed rain from the advanced individuals or due to environmental patchiness. On the other hand, spatial genetic patterns can yield insights into past broad-scale tree migrations (Petit *et al.* 2004) and fine-scale site colonization histories (Chung *et al.* 2005; Gapare and Aitken 2005; Jones *et al.* 2006) because they can differentiate between long- and short-distance colonists (Hamrick and Trapnell 2011; Johansen and Latta 2003; Lesser and Jackson 2013). However, genetic similarity among individuals can arise due to gene flow (parent–offspring or sibling kinship) or environmental microselection (e.g. Slatkin and Arter 1991); thus, the study of coupled spatial genetic and demographic patterns can be a powerful tool for understanding plant invasions and spread.

In this study, we combined fine-scale spatial analyses of population demographic and genetic patterns across a Norway spruce (*Picea abies* Karst.) invasion front in a Western Carpathian grassland in order to (i) gain insight into the patterns of spread of tree populations within grasslands, and (ii) propose a full spatial analytical toolbox that can be used more generally to improve our understanding of native and non-native woody species spread when long-term data are not available. We are not aware of any other study that fully integrated fine-scale demography and genetics in a spatially explicit manner across an expanding invasion front. Using this approach, we tested (i) whether the tree invasion progressed by a gradual frontier expansion or by advanced groups; (ii) whether the early colonists facilitated subsequent invasion and (iii) whether invasion patterns varied with the distance to the source population (~invasion stage).

MATERIALS AND METHODS
Study area

The study site is a ~100 ha grassland situated within the forest matrix of the Poľana Biosphere Reserve in the Western Carpathian Mountains, Slovakia, Central Europe (Príslopy Pass, 48°38′10″N, 19°25′11″E, ~950 m above sea level). The moderately cool and moderately humid climate is

characterized by mean July temperatures of ca. 14°C, mean January temperatures of ca. −6°C, annual precipitation ~900–1200 mm and snow cover that lasts >100 days (Ministry of Environment of the Slovak Republic 2002). The soils are mostly deep and fertile cambisols derived from andesite lava flows (Hraško *et al.* 1980). Forests surrounding the grassland area are partly mixed or broadleaved of natural origin, and partly planted coniferous stands. Natural forests dominated by European beech (*Fagus sylvatica* L.) with an admixture of silver fir (*Abies alba* Mill.), sycamore maple (*Acer pseudoplatanus* L.), and less frequently (locally) *Fraxinus excelsior* L., *P. abies* Karst., *Ulmus glabra* Huds. and *Acer platanoides* L. are considered to be the native climax vegetation at this elevation in the region, while Norway spruce naturally dominates forest composition at elevations >1250 m (Ujházy 2003). This pattern is similar to the situation in the majority of Central European regions (cf. Ellenberg 1988; Korpeľ 1989; Oleksyn *et al.* 1998).

The grassland study site originated as a hay meadow in the 19th century after forest clearance. Reflecting a Europe-wide trend in the mechanization of agriculture and subsequent abandonment of montane grasslands, hay production (mowing) was replaced by cattle grazing in the 1950s which declined in the 1980s (Hrivnák and Ujházy 2005). Original species-rich grassland communities underwent gradual successional changes after abandonment. Competitive grasses *Avenula adsurgens*, *Brachypodium pinnatum*, *Calamagrostis arundinacea* and dwarf shrub *Vaccinium myrtilus* subsequently replaced smaller pasture species (such as *Agrostis capillaris*, *Nardus stricta*). The proportion of forb species was reduced and limited mostly to oligotrophic, shade tolerant or more competitive species (e.g. *Hypericum maculatum*, *Potentilla erecta*). Shrubs (*Juniperus communis*, *Rosa canina*) increased their cover and a dense moss layer developed in areas colonized by woody vegetation (Hrivnák and Ujházy 2005).

In the 1890s, a ~20-m wide strip of Norway spruce was planted along a portion of the north-facing forest edge. The spruce population planted along the forest edge started to invade the grassland in the 1950s. The invasion was well under way by 1975, and by 2005 a closed young spruce stand developed near the original planting and scattered spruce trees occurred as far as ~150 m into the grassland (Dovčiak *et al.* 2008).

Study design and field data collection

We established a 200×40 m permanent belt transect so that it was approximately perpendicular to forest edge and contour lines and crossed from the originally planted spruce population into the adjacent grassland. We divided the belt transect into five contiguous adjacent square plots (40×40 m large), one to characterize the spruce source population and four within the adjacent original grassland at progressively increasing distances from the source population (0–40, 40–80, 80–120 and 120–160 m; Fig. 1). We established a 10×10 m grid within the belt transect using an electronic tachymeter

(ELTA-4; Carl-Zeiss, Jena, Germany) and recorded the position (x, y, z coordinates), breast-height diameter (dbh, at 1.3-m height), height and the presence of cones for all trees >1.3-m tall within each plot. The position and height of all invading spruce seedlings (>1 year old and ≤1.3-m tall) were recorded within the original grassland portion of the belt transect in early spring (April) before the growth flush of the ground-layer vegetation. No large dead trees or logs were observed.

All mapped individuals were classified as adults (cone-producing, ≥19 cm dbh), juveniles (<19 cm dbh, >1.3-m height) or seedlings (>1 year old and ≤1.3-m height). Twigs with dormant winter buds were collected for genetic analyses from all juveniles and adults (i.e. from all trees ≥1.3-m tall) within the original grassland portion of the belt transect, yielding a total of 382 samples for genetic analyses. Samples were not collected from seedlings as the majority of them were too small to contain a sufficient quantity of well-developed dormant buds.

Light conditions across the invasion front were characterized on a fine-scale 5×5 m grid within the belt transect using vertical hemispherical photographs taken at 20-cm height above the ground with a Nikon Coolpix 5400 digital camera equipped with a fisheye FC-E9 objective. Canopy openness (CO), and the amount of direct (R_{DIR}) and diffuse (R_{DIF}) solar radiation transmitted by the canopy were estimated from the photographs using Gap Light Analyser 2.0 (Frazer *et al.* 1999). Additional details of field data collection can be found in Dovčiak *et al.* (2008).

Analysis of demographic spatial structure

To generally characterize the overall trends in the abundance of spruce demographic stages (adults, juveniles and seedlings) with distance from the original population, we used ordinary least squares regression with a quadratic term to fit size-class abundance by distance class (e.g. Weisberg 2005). To describe the spatiotemporal patterns of the spruce invasion into the grassland, we tested the null hypothesis that spruce individuals are distributed in space equally at random regardless of their demographic class (seedlings, juveniles or adults) and invasion stage (approximated by distance to the source population). We tested the null hypothesis using Ripley's K function separately for each of the four study plots located at increasing distances (40-m distance bands) from the source population (Fig. 1). We characterized the spatial pattern of each demographic class within each distance band using the univariate version of the K function:

$$K_t = n^{-2}A\sum_i \sum_{j \neq i} w_{ij}^{-1} I_t(u_{ij}) \quad (1)$$

where *t* is the distance among spruce individuals for which K_t is calculated, *n* is the number of spruce individuals in the analyzed plot, *A* is the area of the plot, u_{ij} is the distance between the individuals *i* and *j*, $I_t(u_{ij})$ is a counter variable (I_t is 1 if $u_{ij} < t$ and 0 otherwise), and w_{ij} is a weighting factor for edge correction. We characterized the distribution of the three demographic classes relative to each other using a bivariate version of Equation 1 for each pair of demographic classes (Haase
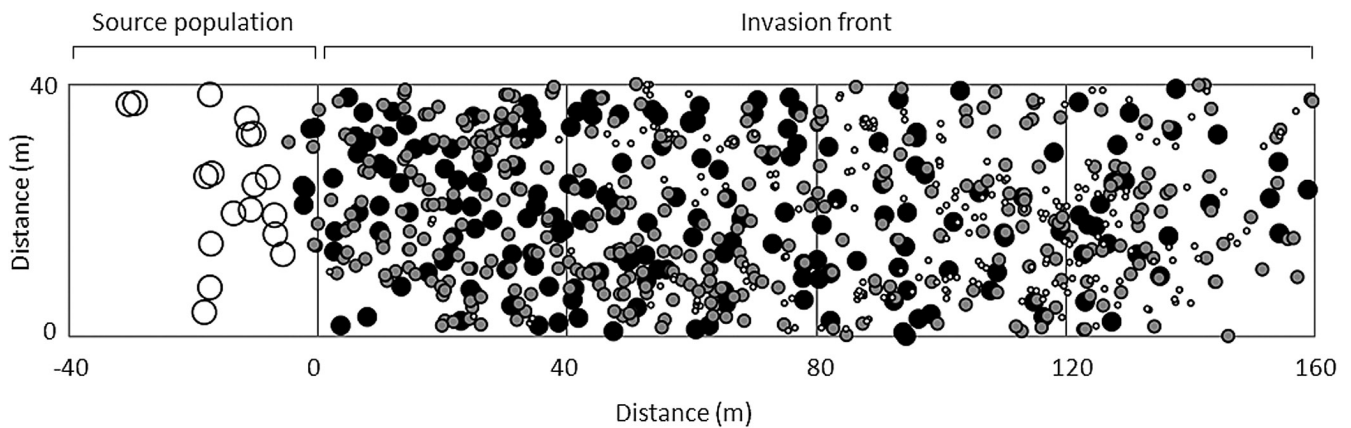
Figure 1: stem map of the spruce invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia. All planted spruce in the source population (large open circles) and all spruce >1-year-old invading the adjacent former hay meadow were mapped and the invading spruce were divided into three demographic classes based on their dbh and height: adults (dbh >19 cm; black closed circles), juveniles (height >1.3 m, dbh <19 cm; grey closed circles) and seedlings (height <1.3 m; small open circles).

1995; Peterson and Squiers 1995; Ripley 1977). The expected value of K_t under the null hypothesis of a random Poisson distribution of individuals is $K_t = \pi t^2$. We report results as the variance-stabilizing square root transformation of K_t:

$$L_t = t - \sqrt{K_t / \pi} \qquad (2)$$

where $L_t = 0$ indicates a random spatial pattern at distance t, $L_t > 0$ indicates an aggregated univariate spatial pattern for individual demographic classes or positive bivariate spatial association between two demographic classes and $L_t < 0$ indicates a uniform (overdispersed) univariate spatial pattern or negative bivariate spatial association (Haase 1995; Peterson and Squiers 1995). The statistical significance of the departure of Ripley's L_t from zero was tested using Monte Carlo simulated confidence intervals; a 95% confidence interval was constructed at each distance t from 1000 simulations of random spatial distribution. All calculations were performed using SPPA version 2.0.3 (Haase 2002) for 1-m distance classes with the maximum distance of 20 m (i.e. half the length of study plot side; Haase 1995) within each broader (40-m wide) distance band (study plot).

Analysis of genetic spatial structure

We tested if the spruce invasion progressed into the grassland by forming advanced groups of genetically related siblings associated with maternal (seed-bearing) trees established within the grassland (pollen dispersal does not affect genetic similarity strongly at the scale of an invasion front; cf. Burczyk *et al.* 2004). Collected dormant winter buds were analyzed using allozyme gene markers (cf. Jones *et al.* 2006; Pardini and Hamrick 2008). Twelve common enzyme systems controlled by 20 loci were assayed. Enzyme extraction, electrophoretic separation and staining procedures followed Longauer *et al.* (2004). Genetic interpretation of zymograms in terms of the translation of protein fractions into gene loci and alleles followed Muona *et al.* (1987) and Lagercrantz *et al.*

(1988). We calculated genetic relatedness among juveniles (sibling kinship) and between adults and juveniles (parent–offspring kinship) using kinship coefficient F_{ij} (Loiselle *et al.* 1995) which was shown to be robust when levels of polymorphism are relatively low (Vekemans and Hardy 2004). To avoid inflation of sampling variance of the kinship coefficient, monomorphic and nearly monomorphic loci were omitted from further analysis (cf. Hardy and Vekemans 2002; Loiselle *et al.* 1995) and only the five most polymorphic loci (frequency of the most common allele <0.9; *Lap-B, Got-C, G6pd-A, Pgi-B* and *Pepc-A*) were used to calculate genetic relatedness (kinship coefficient, F_{ij}) among individuals (sibling and parent–offspring kinships) following Loiselle *et al.* (1995):

$$F_{ij} = \frac{\sum_k (p_{ik} - \bar{p}_k) \cdot (p_{jk} - \bar{p}_k)}{\sum_k \bar{p}_k (1 - \bar{p}_k)} + \frac{1}{2(N-1)} \qquad (3)$$

where p_{ik} and p_{jk} are frequencies of the k-th allele in the i-th and j-th individual respectively, $\bar{p}_k$ is the frequency of the k-th allele in the sample, and N is the sample size. The first member of Equation 3 provides the actual estimator based on the correlation of allelic states and the second member provides a correction for sampling bias (Loiselle *et al.* 1995).

We calculated mean kinship coefficient F_{ij} for all pairs of individuals for 1-m distance classes separately for each study plot (see 'Analysis of demographic spatial structure'). We report F_{ij} values for each plot for distances between 0 and 10 m for clarity since all statistically significant relationships occurred only at fine spatial scales (<5 m). Individuals were considered genetically related (i.e. more similar than expected by chance) when $F_{ij} > 0$, genetically randomly related when $F_{ij} = 0$ and genetically unrelated (less similar than expected by chance) when $F_{ij} < 0$. The statistical significance of F_{ij} at each distance class was tested relative to a 95% confidence envelope derived from 1000 random permutations. All calculations were performed using the program SPAGeDi version 1.1 (Hardy and Vekemans 2002).

RESULTS

Progress of spruce invasion

The plots placed across the invasion front at different distances from the source population characterized the progress of spruce invasion into the grassland. The number of adults and juveniles was highest near the source population and it declined considerably with distance from the source population (Fig. 1, Fig. 2a and b). Correspondingly, canopy openness and solar radiation near the ground surface increased with distance from the source population (Table 1). Seedling abundance increased from near zero in areas already colonized by adults and juveniles near the source population to a distinct peak at intermediate distances (80–120 m), but it declined considerably (by ~50%) at the invasion leading edge (120–160 m) (Fig. 2c). While invading adults and juveniles were dominant near the source population (<80 m; accounting for ~75–95% of individuals), seedlings were the most abundant
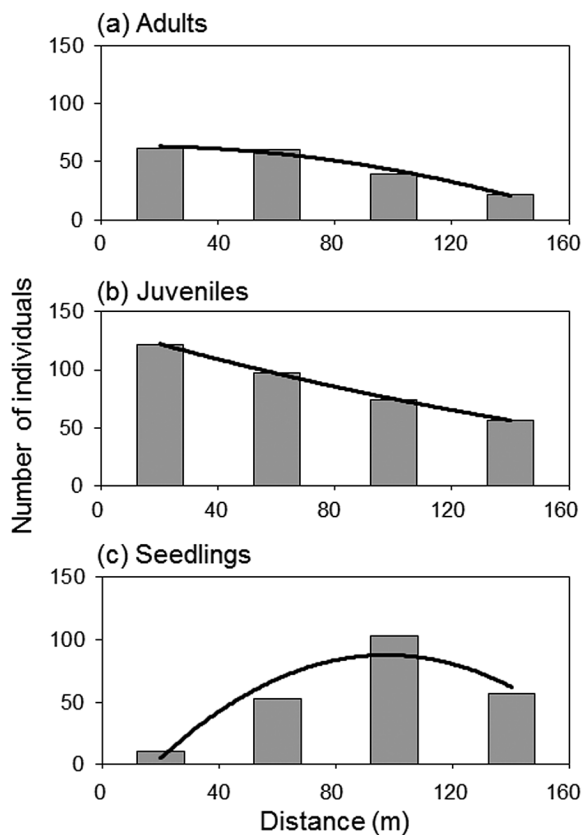
demographic class in the outer zones of the invasion front (>80 m, accounting for ~50% of individuals). Thus, spruce has fully colonized the area adjacent to the source population (Fig. 2a and b), and the invasion appears to be expanding into the meadow by a wave of seedling establishment at intermediate distances from the source population and near the invasion leading edge (Fig. 2c).

Spatial patterns of individual demographic classes

Spatial patterns of individual demographic classes differed from each other and varied with the distance from the source population. Adults tended to be distributed randomly within the plots across the invasion front; except within the fully colonized area adjacent to the source population (0–40 m distances), where their fine-scale overdispersion (~3 m) indicated potential negative effects of intracohort competition, and their larger scale clustering (>15 m) suggested that the initial colonization was spatially clustered near the source population (Fig. 3a). In contrast, juveniles tended to be clustered at all distances from the source population—on most scales in more heavily colonized areas near the source population (0–40 and 40–80 m distances), and in small groups (<3 m) in less advanced invasion stages in the outer portions of the invasion front (80–120 and 120–160 m) (Fig. 3b). Seedlings were too infrequent for spatial analyses near the source population (0–40 m was fully colonized by adults and juveniles; Fig. 2), but they tended to be more clustered than juveniles in the outward portion of the invasion front (80–120 and 120–160 m) (Fig. 3c) where their spatial patterns resembled those of juveniles in more advanced invasion stages closer to the source population (0–40 and 40–80 m) (Fig. 3b).

Spatial associations between demographic classes

Spatial associations between demographic classes varied with the distance from the source population and with demographic classes considered. While juveniles tended to be associated with adults randomly across the invasion front (Fig. 4a), seedlings had random but also distinct negative and positive associations with adults (Fig. 4b). There were too few seedlings for analysis near the source population (0–40 m distances), but they were clearly negatively associated with adults on fine scales (<5 m) in the adjacent distance class (40–80 m) (Fig. 4b), suggesting that adults tended to exclude seedlings from underneath their crowns in this well-colonized invasion stage. Adults affected seedlings less negatively in the more open invasion stages (at >80 m distances) and seedlings were positively associated with adults at intermediate scales (~5–7 m) in the least advanced invasion stage (120–160 m), suggesting that adults may promote seedling establishment immediately adjacent to their crowns (but not directly underneath adults) (Fig. 4b). Spatial associations between seedlings and juveniles tended to be random, except at the least advanced invasion stage (120–160 m) where seedlings were positively associated with juveniles on intermediate scales

Figure 2: spruce demographic structure across the invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia, characterized by the number of adults (**a**), juveniles (**b**) and seedlings (**c**) at increasing distance bands (between 0 and 160 m, in 40-m increments) from the source population. The trend lines were fitted using ordinary least squares regression with a quadratic term to illustrate the overall changes in the abundance of spruce demographic stages (adults, juveniles and seedlings) with distance; R^2 range from 0.87 (c) to 0.99 (b). Definitions of the demographic classes (adults, juveniles and seedlings) are given in Fig. 1.

Table 1: canopy openness, and direct (R_{DIR}) and diffuse (R_{DIF}) solar radiation transmitted by tree canopy[a] across the spruce invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia

	Distance from source population (m)			
Variable	0–40	40–80	80–120	120–160
Canopy openness (%)	8.71 ± 1.24	9.93 ± 1.81	12.61 ± 4.84	21.38 ± 10.66
R_{DIR} (mol m^{-2} day^{-1})	1.71 ± 0.78	2.09 ± 1.25	2.29 ± 1.52	4.91 ± 3.89
R_{DIF} (mol m^{-2} day^{-1})	2.88 ± 0.45	3.25 ± 0.66	4.02 ± 1.67	6.48 ± 3.31

Values are means ± standard error.
[a]Derived from hemispherical photos taken at 20 cm above ground. See 'Study design and field data collection' for further details.
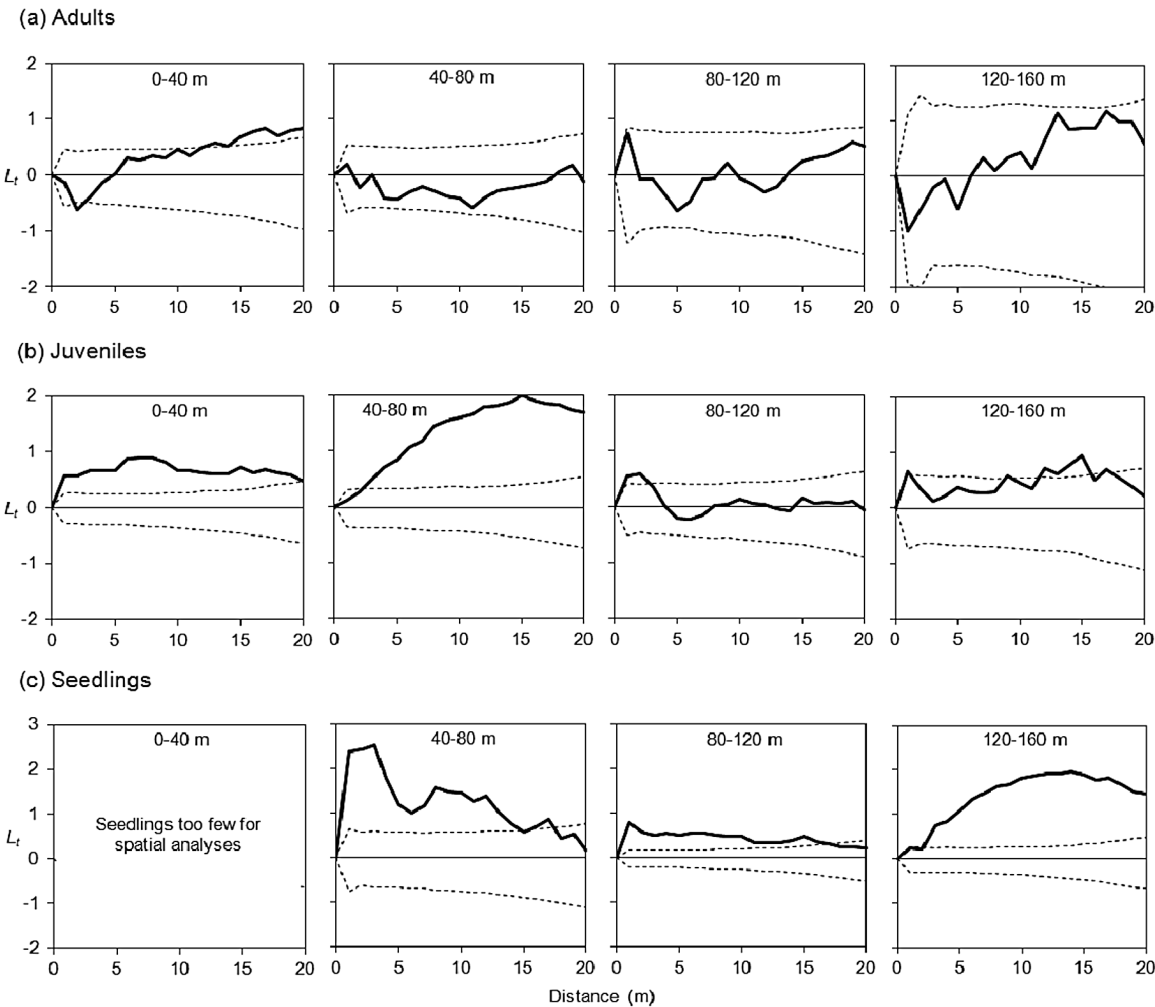

Figure 3: spatial demographic structure across the spruce invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia, characterized by univariate Ripley's L_t (thick lines) describing spatial patterns of adults (**a**), juveniles (**b**) and seedlings (**c**) at increasing distance bands from the source population (between 0 and 160 m, in 40-m increments). Ripley's L_t was calculated for distances among individuals of each size class at 1-m intervals within each broader (40-m wide) distance band. Maximum distance reported for each 40-m distance band is 20 m (see 'Analysis of demographic spatial structure'). Ripley's L_t suggests random spatial pattern at a distance t when it is within the confidence envelope (thin dashed lines), spatially aggregated patterns above the upper confidence limit and overdispersed patterns below the lower confidence limit. Definitions of the demographic classes (adults, juveniles and seedlings) are given in Fig. 1.

(≥7 m) (Fig. 4c), likely reflecting the position on the invasion leading edge.

Spatial genetic structure

The study of spatial genetic structure was limited to juveniles and adults (see Materials and Methods). Unlike the spatial distribution of individuals, genetic spatial structure varied little with the distance from the source population (Fig. 5). Juvenile kinship coefficients were random at all spatial scales and at all distance classes from the source population (Fig. 5a). Thus, the juvenile clusters within the invasion front (Fig. 3b) were formed from the individuals of variable
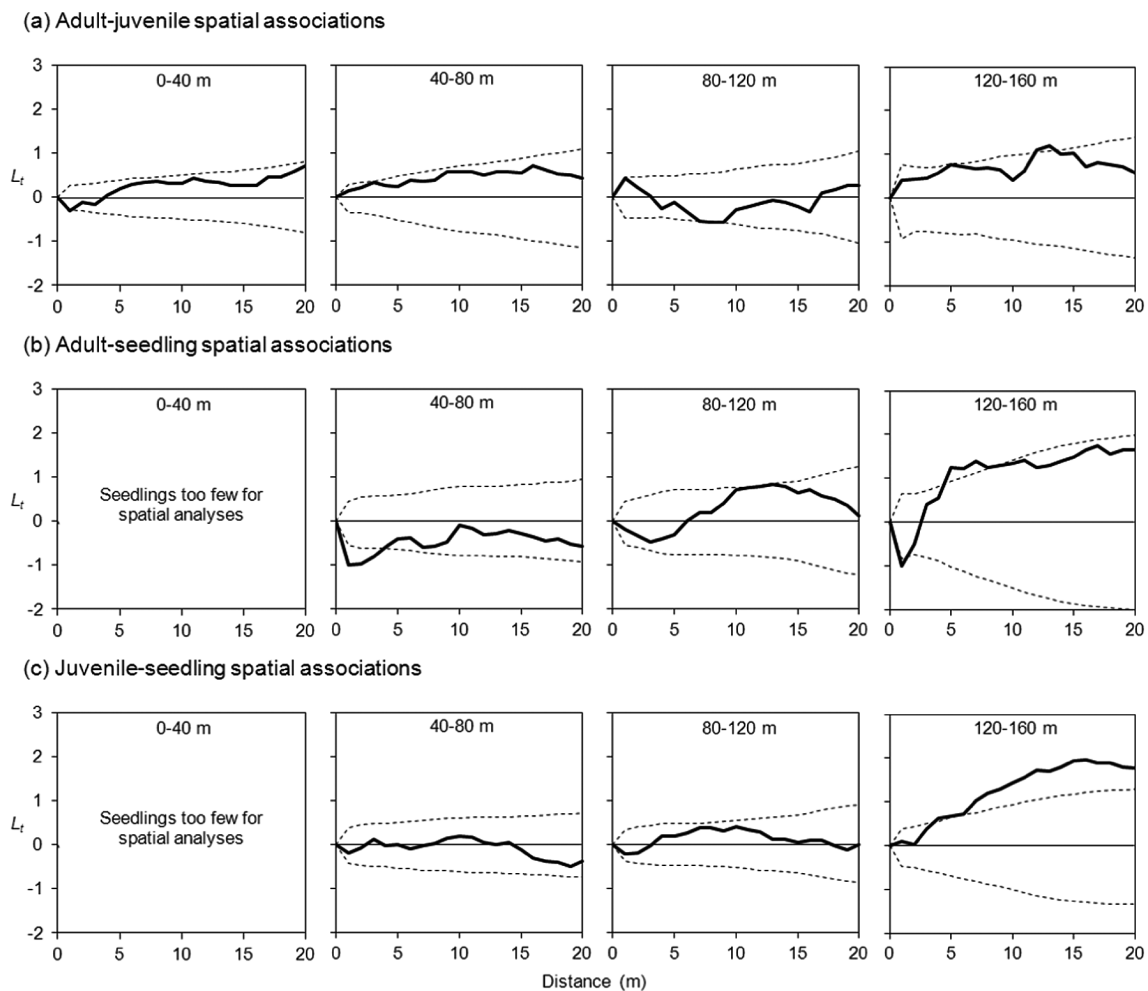
(a) Adult–juvenile spatial associations

(b) Adult–seedling spatial associations

(c) Juvenile–seedling spatial associations

Figure 4: spatial associations between the demographic classes across the spruce invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia, characterized by bivariate Ripley's L_t (thick lines) describing the associations between adults and juveniles (**a**), adults and seedlings (**b**) and juveniles and seedlings (**c**) at increasing distance bands from the source population (between 0 and 160 m, in 40-m increments). Ripley's L_t was calculated for distances among individuals from different size classes at 1-m intervals within each broader (40-m wide) distance band. Maximum distance reported for each 40-m distance band is 20 m (see 'Analysis of demographic spatial structure'). Ripley's L_t suggests random associations between the demographic classes at a distance t when it is within the confidence envelope (thin dashed lines), positive spatial association above the upper confidence limit and negative spatial association below the lower confidence limit. Definitions of the demographic classes (adults, juveniles and seedlings) are given in Fig. 1.

ancestry, indicating that seed dispersal at the time of establishment of current juveniles occurred over distances large enough to prevent the formation of their sibling (half-sib) groups.

On the other hand, juveniles tended to be genetically related to immediately adjacent adults as indicated by positive adult–juvenile kinship coefficients on fine scales at all distances from the source population (although a little less so at 120–160 m from the source population; Fig. 5b). Since juveniles themselves were not genetically related (Fig. 5a) or clustered around adult trees (Fig. 4a), it appears that current adults within the invasion front acted as an additional seed source, augmenting but not driving the early invasion in their vicinity. The juvenile groups within the invasion front (Fig. 3b) must have reflected environmental heterogeneity

rather than locally increased seed rain by previously established trees within the grassland.

DISCUSSION

Changing climate and invasions by non-native species highlight the importance of better understanding species migration rates and patterns of spread across the fragmented landscapes of the present day (Poschlod *et al.* 1996; Treyger and Nowak 2011). We tested three hypotheses addressing fine-scale spatial patterns and mechanisms of Norway spruce invasion into a postagricultural grassland over ~50 years, which allowed us to infer that: (i) the invasion was driven by a gradual frontier expansion of the original population; (ii) early colonists that established ahead of the continuous population
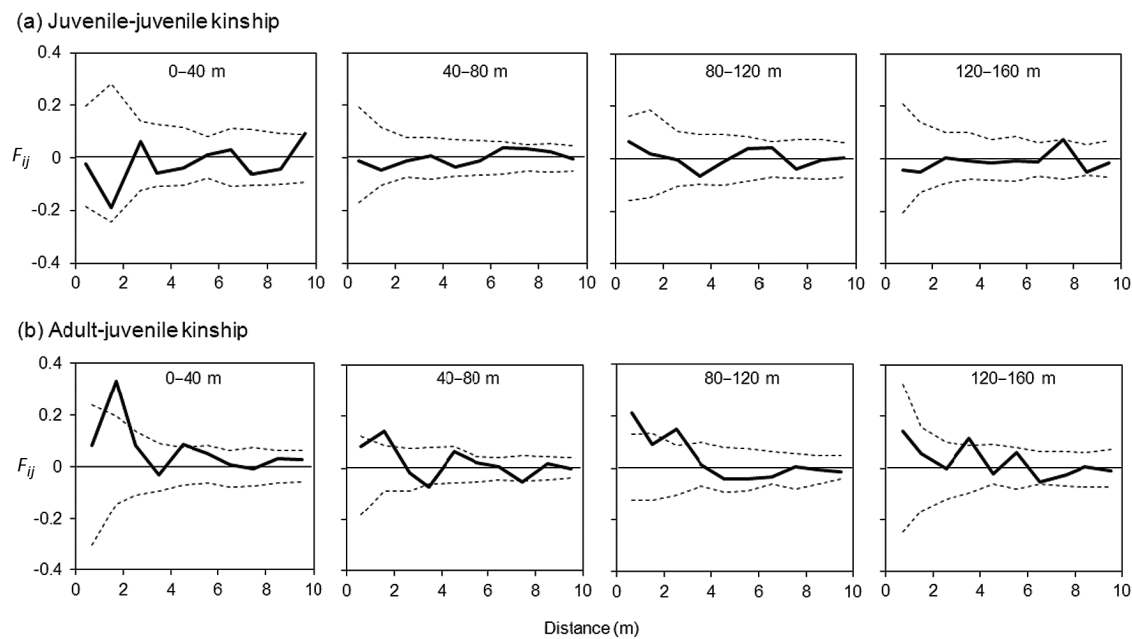
Figure 5: spatial genetic structure across the spruce invasion front in a Western Carpathian montane meadow in the Poľana Biosphere Reserve, Slovakia, characterized by kinship coefficient F_{ij} (thick lines) expressing genetic relatedness within juveniles (**a**) and between juveniles and adults (**b**) at increasing distance bands from the source population (between 0 and 160 m, in 40-m increments). Kinship coefficients F_{ij} were calculated following Loiselle *et al.* (1995) for distances among individuals at 1-m intervals within each broader (40-m wide) distance band. Maximum distance reported for each 40-m distance band is 10 m (see 'Analysis of genetic spatial structure'). Kinship coefficients F_{ij} within the confidence envelope (thin dashed lines) indicate random genetic relationships, while positive values above the upper confidence limit indicate kinship due to sibling (half-sib) genetic relatedness for juveniles, or due to parent–offspring genetic relatedness for the kinship between adults and juveniles. Definitions of the demographic classes are given in Fig. 1.

frontier as advanced individuals or groups enhanced the subsequent invasion, but they did not drive it and (iii) the size of advanced groups declined toward the outer portions of the invasion front. Thus, the first 50 years of the invasion could be characterized as an invasion by frontier expansion (typical when strong Allee effects are present; Kot *et al.* 1996; Taylor and Hastings 2005) with complementary (but small) effects of advanced groups, rather than as an invasion driven by advanced groups (Clark *et al.* 2001). However, the clustering of seedlings around adults at the invasion edge in our study suggests that the role of advanced individuals may increase over time with their size and ability to produce and disperse seeds and suppress competition from grasses (Dovčiak *et al.* 2008).

In our study, the Norway spruce invasion front traveled <200 m into the grassland over half a century, a rate much slower than the historical rates of Norway spruce migrations across forested landscapes (~200 m per year; Bradshaw and Lindbladh 2005), but faster than the inhibited woody colonization rates in some Central European montane grasslands (Prach *et al.* 1996). Woody invasions into grasslands occur in spatiotemporal windows of opportunity when seed rain, environmental amelioration (e.g. moderate canopy shade, grass suppression) and climatic conditions support woody seedling establishment (Dovčiak *et al.* 2005, 2008). We propose that a small source population of an ecosystem engineer

species can invade environments with inhibitive effects on its establishment (e.g. woody species invading grasslands) in the form of a traveling wave: the initial and relatively sparse invasion (Fig. 6, a) enhances the following more abundant invasion by additional seed rain and environmental amelioration (Fig. 6, b), over time generating a traveling invasion wave (Fig. 6, c and d) when most individuals establish progressively further from the source population in areas where seed rain and environmental amelioration are high, while only a few individuals establish at the outer invasion edge (where interspecific competition is high) and near the source population (where intraspecific competition is high). Species spread is thus mainly 'pushed' by frontier expansion and only partly 'pulled' forward by advanced individuals with facilitative effects at critical population densities (Cuddington and Hastings 2004; Dovčiak *et al.* 2005; Kot *et al.* 1996; Milne *et al.* 1996). The need to rely mainly on a slower frontier expansion *via* the traveling wave to invade grassland vegetation with strong inhibitory effects may provide an additional explanation for the slow rates of the woody colonization of grasslands documented in various ecosystems (e.g. Lawson *et al.* 1999; Prach *et al.* 1996; Smith and Olff 1998).

Our results reinforce the theory of phase transitions (critical population density; Milne *et al.* 1996) and empirical observations that forest–grassland transitions can be relatively abrupt (Danz *et al.* 2013; Wiegand *et al.* 2006) and occur over time
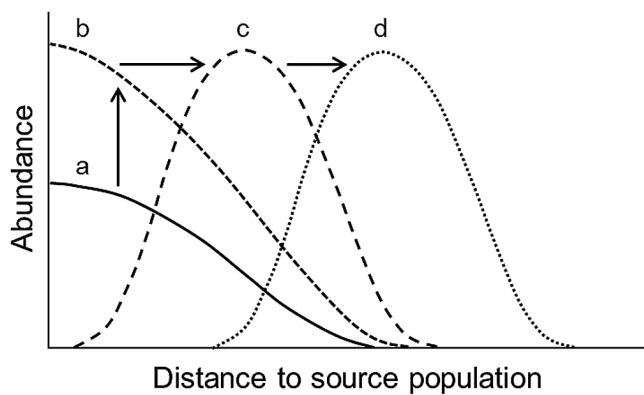
Figure 6: conceptual model illustrating the development of an invasion wave from a small population of an ecosystem engineer invading when strong Allee effects are present: (a) initial relatively sparse invasion near the source population, (b) enhanced subsequent invasion, (c) traveling invasion wave with the establishment of new individuals enhanced at intermediate distances from the source population but limited near the source population by intraspecific competition and near the outer invasion edge by interspecific competition within the invaded community, and (d) future location of the traveling invasion wave. Arrows indicate temporal progression of the invasion. Note that climatic fluctuations (positive or negative) can affect both peak abundance and width of any particular invasion wave.

via shifts in fine-scale spatial patterns by positive feedback mechanisms (cf. Rietkerk *et al.* 2004). We observed positive seedling–adult associations in the outer portions of the invasion front, although negative fine-scale associations developed among adults (and between adults and seedlings) within the densely colonized areas near the seed source population where competitive intraspecific interactions became important with the increase in individual size, population density and resource requirements of the initial colonists (cf. Álvarez-Yépiz *et al.* 2014; Valiente-Banuet and Verdú 2008). Tree seedling establishment in grasslands can be enhanced near trees by seed rain, environmental amelioration, suppression of grass or presence of mycorrhizae, but seedling growth and survival can be negatively affected in areas deeply shaded by canopy trees (Dickie *et al.* 2005; Dovčiak *et al.* 2005; Halpern *et al.* 2010). In our study, grass cover decreased with decreasing canopy openness (with proximity to the source population), while the cover of mosses and conifer litter (associated with only a sparse cover of shade-tolerant forbs) increased, facilitating high spruce seedling establishment in the central portions of the invasion front (Dovčiak *et al.* 2008; Janišová *et al.* 2007; Hrivnák and Ujházy 2005). We attribute the observed increase in population spatial clustering with proximity to the source population, and over time, to the gradual environmental amelioration and corresponding changes in understory vegetation. Patterns of tree invasion into grasslands also varied with vegetation composition in other studies; e.g. conifer invasion fronts were broader in grasslands with forbs and narrower in grasslands dominated by grasses and sedges (Magee and Antos 1992).

Fine-scale genetic structure (~10 m) has been found in woody plant populations due to parent–offspring genetic relatedness when parents were present, while no spatial genetic structure was found in populations established *via* long-distance dispersal even when trees were spatially aggregated in suitable environments (Chung *et al.* 2005). We did not find spatial genetic structure among juveniles even though they tended to be genetically related to adults at fine scales. Since juveniles formed groups that were independent of adults, background seed rain must have overwhelmed seed rain from the advanced individuals in our study. Spatial genetic structure can be near-random in continuous populations with overlapping seed shadows, while it can be aggregated in peripheral populations with low density of reproductive adults where offspring establish near their parents (Gapare and Aitken 2005). The fine scale of the genetic similarity between adults and juveniles in our study corresponds well to the distance of the maximum seed deposition from smaller seed-bearing individuals (<5 m; cf. dispersal kernels in Dovčiak *et al.* 2008), suggesting that local seed rain from advanced individuals can amend the background seed rain more significantly only at peak local seed deposition density. We saw no effects of potential microenvironmental selection given that juveniles formed groups of randomly related individuals (cf. Slatkin and Arter 1991).

Combined spatial genetic and demographic analyses can enhance our understanding of vegetation boundary dynamics compared to other static ('snap-shot') approaches (e.g. Eppinga *et al.* 2013). The wider applicability of our approach is corroborated by studies of site colonization histories that quantify spatial genetic and population patterns for individual demographic classes (e.g. Jones and Hubbell 2006). We argue that a full analytical toolbox for the study of invasion fronts should include two additional components: (i) spatial analysis of bivariate demographic associations (e.g. between adults and juveniles or seedlings), and (ii) explicit consideration of the distance from the source population. For example, despite the fine-scale genetic similarity between adults and juveniles in our study, the establishment of juveniles was driven by broader background seed rain and environmental heterogeneity as evidenced by the groups of genetically randomly related juveniles that were not associated with adults (with group size decreasing with the distance from the source population). Overall, our combined spatial demographic and genetic analyses support the idea that gradual changes in the population distribution in space and time can lead to shifts in ecosystems spatially structured by the positive feedback mechanisms of an ecosystem engineering species (Cuddington and Hastings 2004; Milne *et al.* 1996; Rietkerk *et al.* 2004); and that these changes occur *via* a traveling invasion wave generated by a gradual frontier expansion partly enhanced by advanced groups or individuals.

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