Small-scale seed-bank patterns in a forest soil

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Abstract

The forest seed bank has been demonstrated to vary spatially at scales from 2 to 10 m. To our knowledge, the fine-scale spatial structure, i.e. < 2 m, has not been studied before. This study aims to make a thorough investigation of fine-scale spatial structure. Soil samples (128) were collected from each of five 2.1 m × 2.1 m plots, using a combined systematic (64) and random design (64). This allowed investigation of the fine-scale spatial structure of individual species–plot combinations using indicator-variograms. Our results indicated that over half of all species recorded in a particular plot were spatially structured. Remarkably, the presence of spatial structure seemed independent of species frequency. Visualization of the spatial structure showed an irregular spatial pattern, i.e. seed clusters that were randomly distributed in space. Spatial dependence occurred over small distances, possibly suggesting that a significant proportion of seeds was deposited near the mother plant. We conclude by presenting the relevance and implications of small-scale spatial seed-bank patterning for seed-bank sampling.

Introduction

Living organisms are usually distributed neither uniformly nor randomly, but form all kinds of spatial structures (Legendre and Fortin, 1989). Understanding these spatial structures and the spatial heterogeneity – i.e. the tendency of things to be unevenly distributed in space (Dutilleul, 1993) – they create, is of vital importance to plant ecologists, particularly as the distribution of viable seeds in the soil affects the probability of a plant establishing in a given place (Thompson, 1986). Indeed, as seedlings may be recruited from the seed bank in small gaps in the vegetation of grasslands (Pakeman *et al.*, 1998; Kalamees and Zobel, 2002; Pakeman and Small, 2005), old fields (Lavorel *et al.*, 1994) and forests (Rydgren *et al.*, 1998; Jankowska-Blaszczuk and Grubb, 2006; Hautala *et al.*, 2008), persistent seed banks may not only have a considerable functional role to play in maintaining population dynamics (Kalamees and Zobel, 2002) and the re-vegetation of small-scale gaps in the herbaceous vegetation (e.g. Kalamees and Zobel, 2002; Pakeman and Small, 2005) but also in determining community composition at any given location (e.g. Lavorel *et al.*, 1994; Pakeman and Small, 2005), via the fine-scale spatial distribution of viable seeds.

Yet, the scarcity of information on spatial seed-bank patterns is remarkable. Most past research on spatial seed-bank pattern has focused on seed banks of open and/or disturbed habitats (e.g. Thompson, 1986; Bigwood and Inouye, 1988; Dessaint *et al.*, 1991; Ambrosio *et al.*, 2004; Reiné *et al.*, 2006; Makarian *et al.*, 2007; Caballero *et al.*, 2008). Spatial patterning in forest seed banks appears understudied, likely because of the intrinsic, highly heterogeneous character of both the vegetation and seed bank. Indeed, since spatially variable factors such as tree species (Van Oijen *et al.*, 2005), nutrient availability (Fraterrigo *et al.*, 2006) and micro-topography (Beatty, 1984) control the small-scale spatial distribution of forest understorey plants, their distribution is often highly spatially heterogeneous. Combined with the spatial dependence of pre-dispersal seed predation (Ehrlén, 1996), primary (Nathan and Muller-Landau, 2000) and secondary (Vander Wall *et al.*, 2005) seed dispersal and post-dispersal predation (Hulme, 1998), this will likely result in a spatially structured, yet even more heterogeneous seed-bank pattern. Unfortunately, the

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spatial seed-bank pattern remains understudied at a fine scale, i.e. < 2 m (but see Thompson, 1986; Bigwood and Inouye, 1988), as most studies work with larger distance steps to study spatial seed-bank variation (e.g. Olano *et al.*, 2002; Makarian *et al.*, 2007; Caballero *et al.*, 2008). Yet, both Thompson (1986) and Bigwood and Inouye (1988) have found strong spatial structure at a fine scale (< 2 m). Although no similar studies address the fine-scale forest seed-bank structure, some sources on spatial seed-bank patterns in forests are available, with Matlack and Good (1990), Olano *et al.* (2002) and Schelling and McCarthy (2007) as notable examples. The latter two revealed significant spatial autocorrelation for the seed-bank composition, with significant differences in species composition at 6–8 m and 10–13.5 m, respectively, related to canopy changes. Olano *et al.* (2002) also investigated individual species spatial patterns, concluding that only two species (*Ericaceae* spp. and *Juncus effusus*) showed spatial autocorrelation (> 2 m). Matlack and Good (1990) equally found spatial clustering of individual species in nine out of ten species on a scale of several tens of metres. Consequently, despite a general consensus that seeds have irregularly clustered spatial distributions (Bigwood and Inouye, 1988), little is known about the actual fine-scale spatial structure of individual seed-bank species in temperate, deciduous forests.

Therefore, with this paper, we aimed at examining the spatial structure of individual seed-bank species at a fine scale, i.e. < 2 m. Semivariogram modelling – a technique already used to study spatial seed-bank patterns of agricultural lands (Ambrosio *et al.*, 2004; Makarian *et al.*, 2007) – was used to test whether individual forest seed-bank species are spatially structured at a fine scale. As this scale is relevant to seed-bank sampling, the implications of spatial autocorrelation of forest seed-bank species will be discussed briefly.

Material and methods

Study site

The study area was a 20 ha forest situated 25 km south-west from Nancy, north-eastern France. The oak–beech forest covers a limestone plateau and is managed as coppice-with-standards, with oak (*Quercus robur*) and beech (*Fagus sylvatica*) as standards (trees of large timber size) and hornbeam (*Carpinus betulus*) as coppice. Soils are rendzic leptosols, i.e. shallow (±17 cm), base-rich (mean pH H_2O = 6.9 ± 0.23), clay-textured soils which are biologically highly active. The dominant plant communities belong to the *Stellario-Carpinetum orchietosum* and *typicum* (Stortelder *et al.*, 1999). The former is differentiated by,

amongst others, *Viola hirta*, *Brachypodium sylvaticum*, *Fragaria vesca* and *Primula veris*, while the *Stellario-Carpinetum typicum* is differentiated by species such as *Daphne mezereum*, *Potentilla sterilis*, *Carex digitata*, *Orchis mascula*, *Campanula trachelium* and *Vinca minor*.

Data collection

Five 10 m × 10 m plots were selected from an earlier study (Plue *et al.*, 2009). Each plot, situated between 100 and 600 m from each other, had similar canopy conditions, i.e. both in tree species composition (oak and hornbeam) and canopy closure (> 80% cover). Although all were situated in the same vegetation type, limited inter-plot variation in understorey vegetation due to, for example, soil heterogeneity could not be excluded entirely (see Appendix 1 for a vegetation description per plot). In each of these plots a 2.1 m × 2.1 m plot was randomly placed within the boundaries of the original plot. Each subplot was subdivided into 49 0.3 m × 0.3 m plots. Strings were used to set up and visualize the grid in the field. In August 2007, seed-bank samples (5 cm deep, core diameter = 3.5 cm) were collected both systematically on the grid nodes (64 samples) and scattered randomly across the plot (64 samples), adding up to 128 samples per plot, 640 samples over all plots. The combined systematic–random sampling design per plot was conceived to maximize the number of distance couples at short lag-distances, which are crucial to allow optimal variogram modelling (Stein, 1988). Because of the partial random sampling design, sampling was different for each plot, but prior to application each design was checked to confirm that the design had sufficient distance couples per lag-distance to construct a meaningful variogram. Each seed-bank sample was stored individually in a small plastic container until further processing.

Plastic containers (9 cm × 9 cm × 10 cm) were filled with steam-sterilized potting soil, on top of which one seed-bank sample was spread out. All containers were placed in a greenhouse under a 16-h day/8-h night regime with daytime temperatures between 20 and 25°C. The containers were kept moist through capillary rise and were drained by gravity. Seeds were left to germinate for 42 weeks. The germination period was terminated after two consecutive weeks of no new seedling records. No additional chilling treatment was done, as results in terms of species and seed numbers from the current germination trial were clearly in line with the seed-bank data from Plue *et al.* (2009). All identified seedlings were counted and removed, while unidentified seedlings were transplanted and brought to flower for later identification. Species identification and plant nomenclature follows Lambinon *et al.* (1998).

Data analysis

Unravelling the small-scale spatial structure

Indicator-variograms were calculated to test whether individual seed-bank species exhibited fine-scale spatial autocorrelation, assuming that seed-bank patterns of individual species were indeed spatially dependent at a fine scale (cf. Bigwood and Inouye, 1988).

A variogram $\gamma(\mathbf{h})$ is a function describing the variance of the difference between two observations separated by a lag-distance, $\mathbf{h}$:

$$\gamma(\mathbf{h}) = \frac{1}{2n(\mathbf{h})} \sum_{\alpha=1}^{n(\mathbf{h})} (z_s(\mathbf{x}_\alpha) - z_s(\mathbf{x}_\alpha + \mathbf{h}))^2 \qquad (1)$$

with $z_s(\mathbf{x}_\alpha)$ being the count of seedlings of species s at location $\mathbf{x}_\alpha$, $\mathbf{x}$ being the position vector, and $n(\mathbf{h})$ the number of pairs separated by $\mathbf{h}$.

However, the narrow and low range of the counts of seedlings per sample, combined with the low frequencies (i.e. a non-normal and skewed distribution of variable measurements), did not allow the calculation of the Z-variogram. Therefore, we first applied an indicator coding, according to the binary presence–absence of each species. Seventeen species records (i.e. a species per plot) with a frequency larger than five were transformed from a count into a binary indicator $i_s(\mathbf{x}_\alpha)$ according to:

$$i_s(\mathbf{x}_\alpha) = \begin{cases} 1 & \text{if } z_s(\mathbf{x}_\alpha) > 0 \\ 0 & \text{if } z_s(\mathbf{x}_\alpha) = 0 \end{cases}. \qquad (2)$$

Next, the indicator-variogram $\gamma_{I_s}(\mathbf{h})$ is computed from these indicators:

$$\gamma_{I_s}(\mathbf{h}) = \frac{1}{2n(\mathbf{h})} \sum_{i=1}^{n(\mathbf{h})} (i_s(\mathbf{x}_\alpha) - i_s(\mathbf{x}_i + \mathbf{h}))^2. \qquad (3)$$

For each of the 17 species an omni-directional indicator-variogram was calculated. These were modelled using a spherical model (for all models $\gamma(0) = (0)$):

$$\gamma(\mathbf{h}) = \begin{cases} c_0 + c_1 \times \left[1.5\frac{\mathbf{h}}{a} - 0.5\left(\frac{\mathbf{h}}{a}\right)^3\right], & \text{if } 0 < \mathbf{h} \le a \\ c_0 + c_1, & \text{if } \mathbf{h} > a \end{cases} \qquad (4)$$

or an exponential function:

$$\gamma(\mathbf{h}) = c_0 + c_1(1 - \exp^{-\mathbf{h}/a}) \quad \text{if } \mathbf{h} > 0 \qquad (5)$$

with c_0 the nugget or unstructured variance, c_1 the structured variance, $c_0 + c_1$ = the sill and a is the range coefficient. When the variogram reaches its sill at a finite lag-distance, the variogram has a range, which marks the average limit of spatial dependence between two observations. Finally, the nugget accounts for both the sampling error and the micro-scale variation which was not captured by the sampling design. Based on the sill and the nugget variance, the relative nugget effect [RNE = $c_0/(c_0 + c_1)$] can be calculated. The RNE is a relative measure of the strength of the spatial dependence as it determines to which extent the nugget variance contributes to the overall variance. According to Cambardella *et al.* (1994) this ratio can be interpret as: weak if RNE > 0.75, medium if $0.25 < \text{RNE} \le 0.75$ and strong if RNE ≤ 0.25. A RNE of 100% is called a 'pure nugget effect'. Variogram calculation and interactive trial-and-error modelling were performed with Variowin 2.21 (Pannatier, 1996), following the guidelines suggested by Olea (2006). Final selection of the best model was based on the Indicative Goodness of Fit (IGF) (see Pannatier, 1996, p. 56): the model with an IGF closest to zero provided the best fit.

For visualization purposes only, the indicators were interpolated using ordinary kriging with the modelled indicator-variograms using Surfer version 8.03 software (2003, Golden Software Inc., Golden, Colorado, USA).

The interpolated indicator values could be interpreted as the probability of finding a seed of species s at that location (Lark and Ferguson, 2004). For the theoretical background of ordinary kriging, we refer to standard books such as Goovaerts (1997) or Webster and Oliver (2007).

Results

General characteristics

The germination experiment yielded 27 species among the 413 germinated seeds. Germinating seeds were found in 309 of the 640 core samples (48.3%). Twenty-six seedlings (6.3%) died prior to identification. Two individuals remained unidentified. Seed densities ranged from 618 to 3254 seeds m^{-2} per plot, while species richness per plot ranged from 6 to 17 species. All important seed-bank characteristics are listed per plot in Table 1. A description of the seed-bank composition is given in full in Appendix 2.

Spatial structure of the seed banks of individual species

Modelled parameters of the experimental indicator-variograms can be found in Table 2. Eight species records were best fit by a pure nugget effect (not shown here), suggesting that their seeds are

Table 1. General seed-bank characteristics of the five investigated plots

	Plot				
	1	2	3	4	5
Species richness	17	6	6	13	8
Exclusive seed bank species	14	5	5	9	8
Seed number	112	64	42	221	74
Seed density (number m^{-2})	1649	942	618	3254	1089
Died	9	0	0	13	4
Frequency (number/128)	74	51	29	99	56

Frequency: the number of samples containing germinated seeds; exclusive seed-bank species: species richness of species not recorded in the vegetation of the respective plot.

spatially distributed at random at the fine scale. However, nine species records could be modelled with either a spherical (2) or exponential (7) model. The nine species records showed medium to strong spatial dependence (RNE between 0.06 and 0.38; Table 2) at short distances, ranging from 10 to 35 cm. The average range over which spatial autocorrelation was recorded was 19.2 (± 8.1) cm. Visual representations of the spatial seed-bank structure of five species are shown in Fig. 1.

At least one species record per plot displays spatial structure, notwithstanding the large variation in plot seed-bank characteristics (Table 1). No species showed consistent spatial structure over all plots. At the same time, spatial structure seemed independent of species frequency (Table 2).

Discussion

Spatial structure of individual seed-bank species

In general, the result of initial dispersal is a clustered distribution of seeds centred more or less around the mother plant (Bigwood and Inouye, 1988). These mother plants are, in turn, spatially distributed (see, for example, Webster and Jenkins, 2008), very often in a clustered pattern related to spatial variation in abiotic conditions and processes in the forest understorey (e.g. Lechowicz and Bell, 1991; Bengtson *et al.*, 2005). This will subsequently result in overlapping seed clusters of a plant species, both in space (via different individuals) and time (via the same individual). However, deposited seeds are far from immobile and can be displaced by ants, (burrowing) animals or wind (Vander Wall *et al.*, 2005; Milcu *et al.*, 2006). In addition, as few species of persistent forest seed banks occur in the vegetation (Bossuyt and Hermy, 2001), their seed clusters are subjected to disaggregative processes such as seed predation, failed germination and seed senescence, which slowly deplete the old seed clusters over time. Adding up the seed displacement by soil macrofauna, such as earthworms (Milcu *et al.*, 2006), the original fine-scale pattern is likely to be at least obscured or even destroyed. Nevertheless, even in this highly biologically active forest soil (mean pH $H_2O = 6.9$), at least some of the fine-scale spatial structure ($<2\,m$) remained intact, as nine species records could be modelled successfully using variograms (Table 2). Their spatial structure is both

Table 2. Modelled parameters of the 17 estimated indicator-variograms

		Frequency (number/128)	Model	IGF	Nugget c_0	Sill c_1	Range (m) a	Relative nugget effect $c_0/c_0 + c_1$
Plot 1	*Digitalis lutea*	6	–	4.67×10^{-2}	0.04	–	–	1.00
	Euphorbia cyparissias	5	Spherical	6.85×10^{-2}	0.00	0.04	0.35	0.09
	Hypericum hirsutum	23	Spherical	4.97×10^{-3}	0.04	0.11	0.16	0.25
	Hypericum perforatum	13	Exponential	2.71×10^{-2}	0.01	0.08	0.10	0.15
	Milium effusum	19	Exponential	1.57×10^{-2}	0.01	0.12	0.24	0.09
	Moehringia trinervia	8	–	3.71×10^{-2}	0.06	–	–	1.00
	Verbascum thapsus	8	–	2.09×10^{-2}	0.06	–	–	1.00
Plot 2	*Verbascum thapsus*	47	Exponential	2.89×10^{-3}	0.06	0.18	0.13	0.30
Plot 3	*Hypericum hirsutum*	25	Exponential	6.97×10^{-3}	0.02	0.11	0.18	0.14
Plot 4	*Carex digitata*	8	–	4.77×10^{-2}	0.06	–	–	1.00
	Hypericum hirsutum	73	–	1.53×10^{-2}	0.23	–	–	1.00
	Hypericum perforatum	30	–	2.17×10^{-2}	0.18	–	–	1.00
	Verbascum thapsus	5	Exponential	5.75×10^{-2}	0.00	0.03	0.27	0.06
	Veronica officinalis	14	Exponential	1.04×10^{-2}	0.01	0.092	0.15	0.38
Plot 5	*Hypericum hirsutum*	9	–	1.51×10^{-1}	0.07	–	–	1.00
	Hypericum perforatum	26	Exponential	7.88×10^{-3}	0.05	0.12	0.15	0.30
	Verbascum thapsus	22	–	6.33×10^{-3}	0.14	–	–	1.00

Frequency: the number of samples containing germinating seeds; IGF, indicative goodness of fit – the closer to zero the goodness of fit, the better the fit.
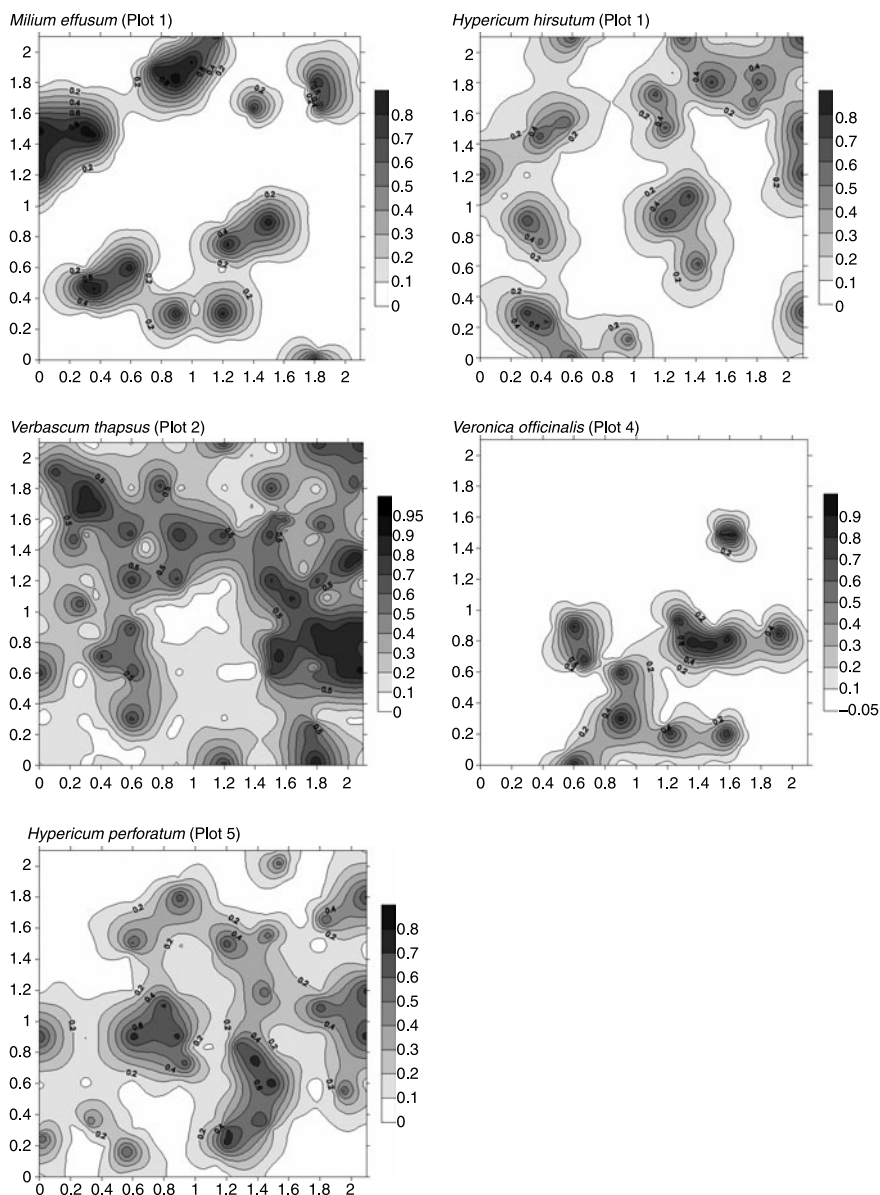
Figure 1. Contour maps visualizing the spatial distribution of different seed-bank species. Contour maps were constructed via indicator-kriging based on the parameters of the modelled indicator-variograms. Contours indicate the chance of finding a seed within the surface they delineate. Countour maps of the four remaining species that also showed spatial structure can be found in Appendix 3.

clustered, i.e. zones of high probability of seeds being present, and irregular, i.e. a random spatial distribution of the clusters (cf. Thompson, 1986; Bigwood and Inouye, 1988). However, our results also imply that the disaggregative effects of all previously mentioned processes on the spatial seed-bank structure may indeed erase the former, as in the remaining eight species records any initial spatial structure was likely obliterated.

Remarkably, no consistent trend in the presence or absence of spatial structure could be observed (cf. Thompson, 1986). Indeed, as most other species, even those species that were recorded many times over the different plots (i.e. *Hypericum hirsutum*, *H. perforatum* and *Verbascum thapsus*) were clustered at both low and high species frequencies. Hence, the presence or absence of spatial structure seemed to be independent of species frequency ($\approx$ seed density).

The distance over which spatial autocorrelation was recorded (i.e. the range), was generally small (0.10–0.35 m) (Table 2), possibly suggesting limited seed dispersal. Indeed, despite their adaptations to disperse away from the mother plant, the species still seem to have brought a notable portion of their seeds into the soil locally (cf. Clark *et al.*, 2005), likely near the former (*sensu* presently absent) mother plant.

While these seeds could be considered as unsuccessful dispersers (Cousens *et al.*, 2008), the seeds of persistent forest seed bank species do occupy a suitable habitat patch and consequently may have the highest probability of colonizing the site when suitable germination conditions arise. In the case of *Verbascum thapsus*, this has already been reported (Gross, 1980). Hence, for persistent forest seed-bank species, this can be regarded a successful strategy to assure their survival, additional to the benefits of successful dispersal, particularly in the spatiotemporally highly heterogeneous forest environment (Runkle, 1985). Combining both strategies, allows spatially distributed dormant species to enjoy a greater chance of encountering local disturbance.

Consequences of the small-scale spatial seed-bank patterns for sampling

The scarcity of information on small-scale spatial seed-bank patterns has resulted in a myriad of sampling designs (cf. Simpson *et al.*, 1989). The fine-scale seed-bank patterning this study visualized (Fig. 1) has significant relevance to future seed-bank sampling at similar scales, as the majority of seed-bank studies indeed sample the seed bank in 2 m × 2 m, 1 m × 1 m or even smaller (sub)plots. Next to a sufficiently large sampled volume or surface area – which is widely acknowledged to be of the utmost importance to obtain reasonably accurate seed-bank estimates (Thompson, 1986; Bigwood and Inouye, 1988) – we therefore argue that the sampling mode to gather these volumes should overcome the small-scale spatial seed-bank structure. One way to collect sufficiently large volumes within a plot, is to take few large samples rather than many small ones (Bigwood and Inouye, 1988). Many forest seed-bank studies (e.g. Sakai *et al.*, 2005; Dassonville *et al.*, 2006; Schelling and McCarthy, 2007) still sample forest seed banks in this way, likely due to practical and time limitations. Yet, the fine-scale patterns or clusters observed in our work (Fig. 1), may easily lead to biased estimates of all seed bank characteristics through 'unfortunate' plot placement (Bigwood and Inouye, 1988). Therefore, this study of small-scale seed-bank patterns corroborates the view that many small samples should be taken to achieve the pre-set volume or surface area (Bigwood and Inouye, 1988). However, the best way to collect these samples is by ensuring that their collection is independent (cf. Legendre, 1993), as they are likely to yield unbiased, precise plot seed-bank estimates. Linked to variograms, this means that core sampling needs to take place at a distance beyond the range, which defines the limit over which spatial dependence occurs (Webster and Oliver, 2007). However, it is difficult to formulate unequivocal advice on within-plot sampling distance, based on only nine ranges (Table 2). Nonetheless, most spatially structured species do have a small range, despite their large variation in plant traits. Therefore, we believe that within-plot sampling distances of 30–50 cm will likely suffice in most cases to return independent core samples, yielding unbiased and reasonably precise estimates of seed-bank composition and seed density.

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Appendix 1

Vegetation description per seed-bank plot (2.1 m × 2.1 m). Vegetation composition per plot expressed as frequency of occurrence of individual species on 49 subplots (0.3 m × 0.3 m). Plant nomenclature follows Lambinon *et al.* (1998)

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Acer campestre L.	13	5	7	2	3
Acer platanoides L.				1	
Acer pseudoplatanus L.	3	40	37	5	2
Anemone nemorosa L.			48	44	49
Agrimonia eupatoria	1				
Arum maculatum L.		10	1		1
Brachiopodum sylvaticum (Huds.) Beauv.	9			37	1
Bromus benekinii (Lange) Trimen		1		1	
Carex digitata L.			1	18	
Carex spp.			1		
Carex montana L.				2	
Carex sylvatica Huds.	2	1	3		
Cardamine pratensis L.					3
Carpinus betulus L.	32	32	34	44	47
Convallaria majalis L.		4	28	3	
Corylus avellana L.	3				
Crataegus monogyna Jacq.	2				
Crataegus laevigata (Poiret) DC.		1	6		7
Dactylis glomerata L.	1				
Euphorbia cyparissias L.	15				
Fagus sylvatica L.	2	2	1	1	
Fraxinus excelsior L.		2			1
Fragaria vesca L.				5	
Galium aparine L.					1
Galium odoratum (L.) Scop.		28			
Geranium robertianum L.	1	5			
Geum urbanum L.	10	1		17	

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Hedera helix L.	48	45	48	48	38
Helleborus foetidus L.					2
Hepatica nobilis Schreb.				10	
Hordelymus europeus (L.) Herz.				5	
Hypericum hirsutum L.				1	
Lamium galeobdolon (L.) L.	42	28		48	39
Luzula pilosa L.	9				
Melica uniflora L.	42	6	8	24	6
Mercurialis perennis L.					11
Milium effusum L.	42	39			1
Ornithogalum pyrenaicum L.					1
Polygonatum multiflorum (L.) All.			26		5
Prunus avium (L.) L.	1				
Prunus spinosa L.			1	1	1
Ranunculus auricomus L.	46	44			15
Ranunculus parviflorus L.	1				
Ribes alpinum L.		8	3	9	4
Rosa arvensis Huds.	3				
Scilla bifolia L.				1	
Sorbus aria (L.) Crantz.			1		
Sorbus torminalis (L.) Crantz.	2	2			
Taraxacum officinale agg.	4				
Vicia sepium L.	33	17	3		6
Vinca minor L.	49	48			49
Viola mirabilis L.			1		
Viola reichenbachiana Jord. Ex Boreau	19	9	40	17	1

Appendix 2

Seed-bank composition per seed-bank plot (2.1 m × 2.1 m) in terms of abundance (number of seeds/128 samples)/frequency (number of samples/128 samples) per species. Plant nomenclature follows Lambinon *et al.* (1998)

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Betula pendula	–	–	–	1/1	–
Carex digitata	–	–	5/4	14/8	–
Carex muricata	1/1	–	–	–	–
Centaurium pulchellum	–	–	–	3/3	–
Cirsium arvense	–	–	1/1	–	–
Daucus carota	1/1	–	–	–	–
Digitalis lutea	6/6	1/1	–	–	–
Epilobium parviflorum	–	–	1/1	1/1	1/1
Epilobium tetragonum	1/1	–	–	–	2/2
Euphorbia cyparissias	5/5	–	–	–	–
Fragaria vesca	5/4	1/1	4/4	2/2	2/2
Geum urbanum	–	–	–	1/1	–
Hypericum hirsutum	26/23	1/1	25/19	112/73	9/9
Hypericum perforatum	13/13	1/1	–	42/30	28/26
Luzula pilosa	1/1	–	–	–	–
Millium effusum	22/19	3/3	–	–	–
Moehringia trinervia	8/8	–	–	1/1	2/2
Poa spp.	–	–	–	1/1	–
Rumex crispus	1/1	–	–	–	–
Sonchus arvensis	–	–	1/1	–	–
Sonchus asper	–	–	–	–	1/1
Sonchus spp.	–	–	–	1/1	–
Typha latifolia	1/1	–	–	–	–
Verbascum thapsus	9/8	57/47	–	6/5	25/22
Veronica officinalis				19/14	–
Vicia cracca	1/1	–	–	–	–
Unknown sp. 1	1/1	–	–	–	–
Unknown sp. 2	1/1	–	–	–	–
Died	9/8	–	5/5	17/16	4/4

Appendix 3

Contour maps visualizing the spatial distribution of the four remaining spatially structured seed-bank species. Contour maps were constructed via indicator-kriging based on the parameters of the modelled indicator-variograms. Contours indicate the chance to find a seed within the surface they delineate.

Euphorbia cyparissias (Plot 1)

Hypericum perforatum (Plot 1)

Hypericum hirsutum (Plot 3)

Verbascum thapsus (Plot 4)