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APPLICATION OF NESTED SAMPLES TO STUDY THE SOIL SEED BANK IN SEMIARID SANDY GRASSLAND

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The aim of the present work was to study the soil seed bank of the semiarid sandy grassland community using the nested sampling procedure. The samples consisted of six concentric cores with diameters ranging from 5 to 22 cm and surface area ratio of the outermost to the innermost ring of 19. Investigations were directed to establish the minimum core diameter to find the dominant and less frequent species in the seed bank and also to have an insight on distribution patterns of species. The smallest core (diameter: 5 cm) employed in 20 repetitions was adequate to find the dominant species in the seed bank, while increasing the sample area to 19 times resulted in doubling the number of species found. The seeds of the dominant species had clustered distribution even at the smallest applied sample scale, while patterns of seed clumps followed different (uniform, contagious, random) distributions.

Key words: distribution pattern of seeds, nested sampling, semiarid sandy grassland, soil seed bank

INTRODUCTION

Soil seed bank dynamics and degree of similarity between the species composition of the soil seed bank and coenological records are extensively studied in semiarid grassland and desert (Reichman 1984, Henderson *et al*. 1988, Coffin and Lauenroth 1989, Kemp 1989, Musil 1991, Pake and Venable 1996, Ghermandi 1997, Aguiar and Sala 1997). Seed banks of the open sandy grassland vegetation are characterised by strong aggregation tendencies, highly differing seed densities between open patches and closed stands and by transiency (Kellman 1978, Schenkeveld and Verkaar 1984). The detected seed distribution is affected by several factors, e.g. the soil micromorphology, distance from the parent, wind effects and dispersion by animals (Pulliam and Brand 1975, Bullock 1976, Nelson and Chew 1977, Reichman 1984, Baptista and Shumway 1998). The majority of the annual sandy grassland species do not have special dispersion organs, the dispersion pattern is determined largely by the seed weight (Symonides 1987) and 70% of the seeds is in the 5–10 cm vicinity of the parent plant (Harper 1977) resulting in aggregated distribu-

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tion (Major and Pyott 1966, Benoit *et al*. 1989). Several indices have been applied for studying the degree of dispersion like the variance to mean ratio (Thompson 1986, Benoit *et al*. 1989, Dessaint *et al*. 1991), Morisita's index (Johnson and Anderson 1986), Lloyd's mean crowding (Dessaint *et al*. 1991, Bigwood and Inouye 1988) and Lloyd's patchiness (Dessaint *et al*. 1991). The contiguous quadrat method was introduced by Greig-Smith (1952) to study the scale dependence of the patterns (Pielou 1977). However, there are only a few studies concerned with scale dependency of the spatial pattern by seeds (Forcella 1984, Bigwood and Inouye 1988).

The usual technique to determine the volume is to apply species-volume curves analog to species-area curves (Hayashi and Numata 1964, 1971, Chin 1973, Nakagoshi 1984). However, the general applicability of the method is questionable because of the non-random distribution of seeds and strong tendency of seeds to aggregate (Whipple 1978, Thompson 1986, Bigwood and Inouye 1988, Rusch 1992, Benoit *et al*. 1989).

The nested sampling procedure, contrary to the application of high number of samples of the same size, detects the scale dependence of the seed aggregation patterns at increasing scales (i.e. change of the distribution from random to aggregated when increasing the scale; Bigwood and Inouye 1988). The aim of the present study was to establish the species-area curve for the soil seed bank and to analysed the spatial distribution of the seed bank.

MATERIALS AND METHODS

The study area is situated 25 km west of the town of Kecskemét in the Hungarian Great Plain (Kiskunság National Park, near Fülöpháza). The frequency of water shortage periods is long, the soil is nutrient poor calcareous sand with low water holding capacity. Edaphic factors are also contributing to the temperate semidesert characteristics of the sandy grassland (Zólyomi 1958, Fekete *et al*. 1988). The sand hills are covered by grasslands, but some sand dunes are still moving. The mean annual precipitation is about 550 mm. The maximum vegetation cover is 50 to 70%, and the total species number is low (50 to 60). The constituent species are mainly hemicryptophytes and therophytes, but some chamaephytes and geophytes do also occur. Due to the small-scale variation in the ground exposition and the depth of water table the appearance of this vegetation is rather patchy. Our information on the seed bank of the grassland is rather sparse (Kincsek 1985, Halassy 2001).

Soil samples were gathered from the microhabitat of the *Festucetum vaginatae danubialae* Soó (1929) 1933 community. The microhabitat is situated on the NE slope of a sand dune. The detailed coenological description of the area

reported several works (Magyar 1933, Hargitai 1940, Fekete and Tuba 1982, Bagi 1997, Kemény *et al*. 2001). The total cover of the microhabitat was 60–70% and the share of mosses and lichens was 10–15%. Abundant perennials were *Stipa borysthenica*, *Fumana procumbens* and *Poa bulbosa*. Among the therophyte species *Arenaria serpyllifolia* had the highest cover value.

A total of twenty samples were collected at 5 cm depths in a 12 m × 12 m area at 18 September, 1993. The surface area was 7600 cm^2. Each sample consisted of six concentric cores and were analysed seperately. The successively larger cores and their areas are referred to alphabetically from A (smallest) to F (largest) (Table 1).

Vegetative plant parts were removed from the samples by sieving. The cores were transferred to a plant growth chamber (14 h day length, 300 µmol/m^2/s light intensity, 23/15 °C day/night temperatures) and spread in 3 cm layers on trashes. Seedlings were identified, counted and removed after four successive germination periods in five months by mixing and spreading the soil again after each counting procedure (Thompson *et al*. 1997). No new individuals were detected in the 40 days after the fourth counting procedure. Species were identified after Csapody (1968) and using germinated seeds of species collected in the area. Species life forms and community nomenclature followed Horváth *et al*. (1995) and Soó (1964).

Statistical analysis

The "new species-area curves" (Forcella 1984) give the new number of species present in the successive cores of the nested sample *vs* the total area sampled.

Degree of dispersion was estimated by the variance to mean ratio (Greig-Smith 1952) and by Lloyd's patchiness (Lloyd 1967) considering the distribution as aggregated, random and uniform if the value of both the indices were larger, equal to or smaller than 1, respectively.

Analysis of spatial patterns is based on Iwao's method (Iwao 1968), based on the m^*–m relation, where m^* (Lloyd's mean crowding (Lloyd 1967)) is plotted against the mean density (m) as found with increasing sample size.

Table 1
Parameters of the nested sample

Diameter of the successive cores (cm)	5	7	10	14	18	22
Core's surface area (cm^2)	20	39	79	154	254	380
Surface area of the successive core (n = 20) (cm^2)	400	780	1580	3080	5080	7600
	A	B	C	D	E	F
Area differences between successive cores (n = 20) (cm^2)		380	800	1500	2000	2520

$$m^* = \frac{\sum_{n=1}^{Q} x_i (x_i - 1)}{\sum_{n=1}^{Q} x_i}$$

where Q is the number of samples, x_i is the number of individuals in the ith sample. In the present study the m^* and m values were calculated at every increasing sample size. The m^*–m relation is generally linear for the majority of the investigated insect and plant populations (Iwao 1968). The slope and the intercept can be used to characterise the distribution of individuals and clumps as follows:

for individual seeds

slope(β)	intercept(α)	distribution
1	0	Poisson (Iwao's line, Iwao 1968)
>1	>0	aggregated
1>	0>	underdispersion
>1	0>	change of pattern: detection of seed clumps, overdispersion

for seed clumps

1	>0	random distribution of seed clumps
>0	>0	contagious distribution of seed clumps

Linear regressions (m^* *vs* m) were carried out from contiguous samples' data for species exceeding the mean density of 0.1/20 cm^2 (Dessaint *et al*. 1991).

RESULTS

Nine annual and 9 perennial species were found in the seed bank (Table 2) with larger seed number (87% of the total) by the annual species, 39% belonging to *Arenaria serpyllifolia*. Further dominant species were *Erophila verna* (21%) and *Saxifraga tridactylites* (19%). Six species shared 19% of the total number of seeds found (*Minuartia verna* (7%), *Poa bulbosa* (4%), *Cerastium semidecandrum* (3%), *Holosteum umbellatum* (3%), *Conyza canadensis* (2%)), while the remaining 1% was belonging to ten species. Dominant species in the seed bank occurred in the smallest sample scale (Fig. 1). Further 9 species appeared with increasing the sample scale from A to F. Rare species caused the apparent noise in the species area curve.

The variance to mean ratio and Lloyd's patchiness were larger than one in the case of *Arenaria serpyllifolia, Saxifraga tridactylites, Minuartia verna, Erophila*

Table 2
Summary of data for seed bank of the sandy grassland

Life forms	Species	Total seed number ((7,600 cm^2, 2,407 pieces)	Frequency (%)	Average seed number (20 cm^2)
Th	*Arenaria serpyllifolia*	950	39	2.5
Th	*Erophila verna*	494	21	1.3
Th	*Saxifraga tridactylites*	453	19	1.2
H–Ch	*Minuartia verna*	159	7	0.4
H	*Poa bulbosa*	107	4	0.3
Th	*Cerastium semidecandrum*	79	3	0.2
Th	*Holosteum umbellatum*	69	3	0.2
Th–TH	*Conyza canadensis*	50	2	0.1
H	*Stipa borysthenica*	18	0.7	0.05
N	*Fumana procumbens*	9	0.3	0.02
Th	*Lithospermum arvense*	5	0.2	0.01
H	*Syrenia cana*	4	0.2	0.01
G	*Cleistogenes serotina*	3	<0.1	<0.01
H	*Festuca vaginata*	2	<0.1	<0.01
H	*Linaria genistifolia*	2	<0.1	<0.01
Th	*Chenopodium album*	1	<0.1	<0.01
H	*Bothriochloa ischaemum*	1	<0.1	<0.01
Th	*Veronica arvensis*	1	<0.1	<0.01

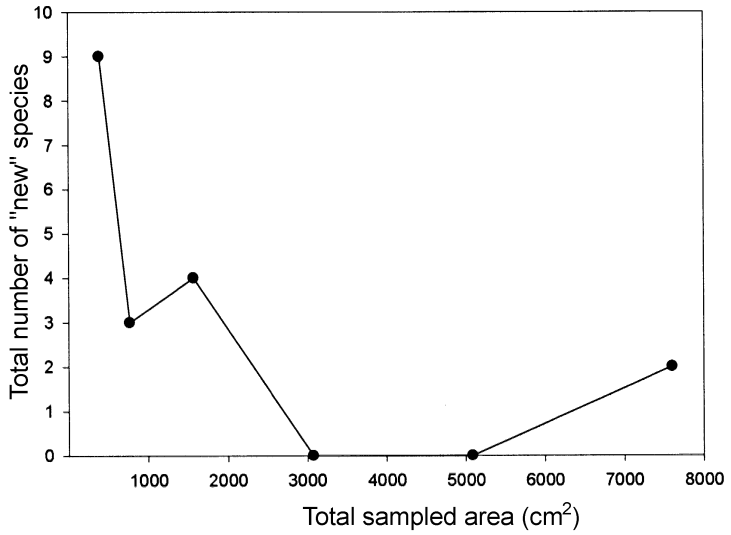

Fig. 1. The new species-area curve. The number of new species are appeared in the successive cores *vs* the total area sampled. (*x axis* = total area sampled; *y axis* = number of "new" species)

verna and *Conyza canadensis* showing the aggregated distribution for these species at the smallest sample scale (Table 3). Seed clumps of *Saxifraga tridactylites* and *Conyza canadensis* were distributed contagiously ($\alpha > 0$, $\beta > 0$) (Fig. 2). For *Arenaria serpyllifolia*, *Minuartia verna* and *Erophila verna* β values were close to unity, indicating the distribution was close to random (Fig. 2). Patterns of seed

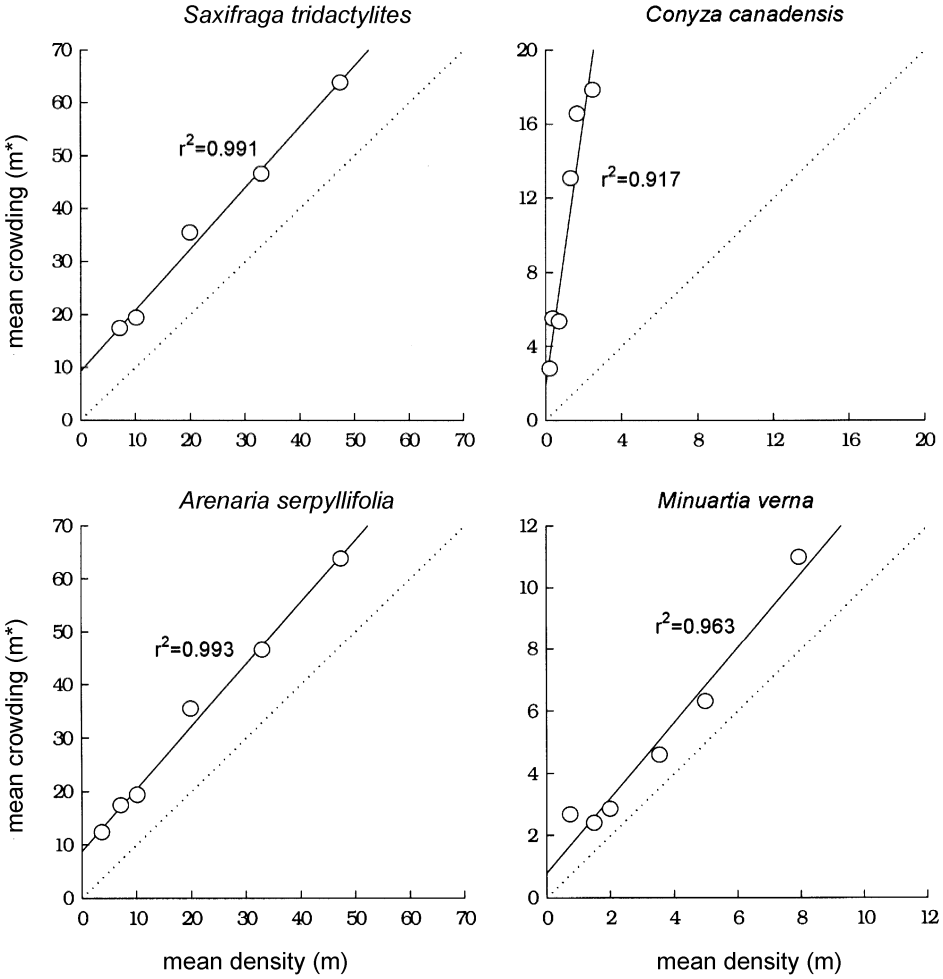

Fig. 2. The regression lines of mean crowding (m*) vs. mean density (m) with the intercept (α) and the slope (β) values for *Saxifraga tridactylites* (α = 0.94; β = 2.5), *Conyza canadensis* (α = 1.87; β = 7.17), *Arenaria serpyllifolia* (α = 8.71; β = 1.17), *Minuartia verna* (α = 0.78; β = 1.21), *Erophila verna* (α= –0.06; β = 1.29), *Poa bulbosa* (α = –1.22; β = 2.78), *Cerastium semidecandrum* (α = –0.2; β = 3.17) and *Holosteum umbellatum* (α = –0.74; β = 2.73)

clumps (overdispersion) were detected ($0 > \alpha$, $\beta > 1$) for *Poa bulbosa*, *Cerastium semidecandrum* and *Holosteum umbellatum* when increasing the sample scale (Fig. 2).

DISCUSSION

Dominance of perennial species characterised the area investigated. While the number of perennial and annual species were nearly the same, the

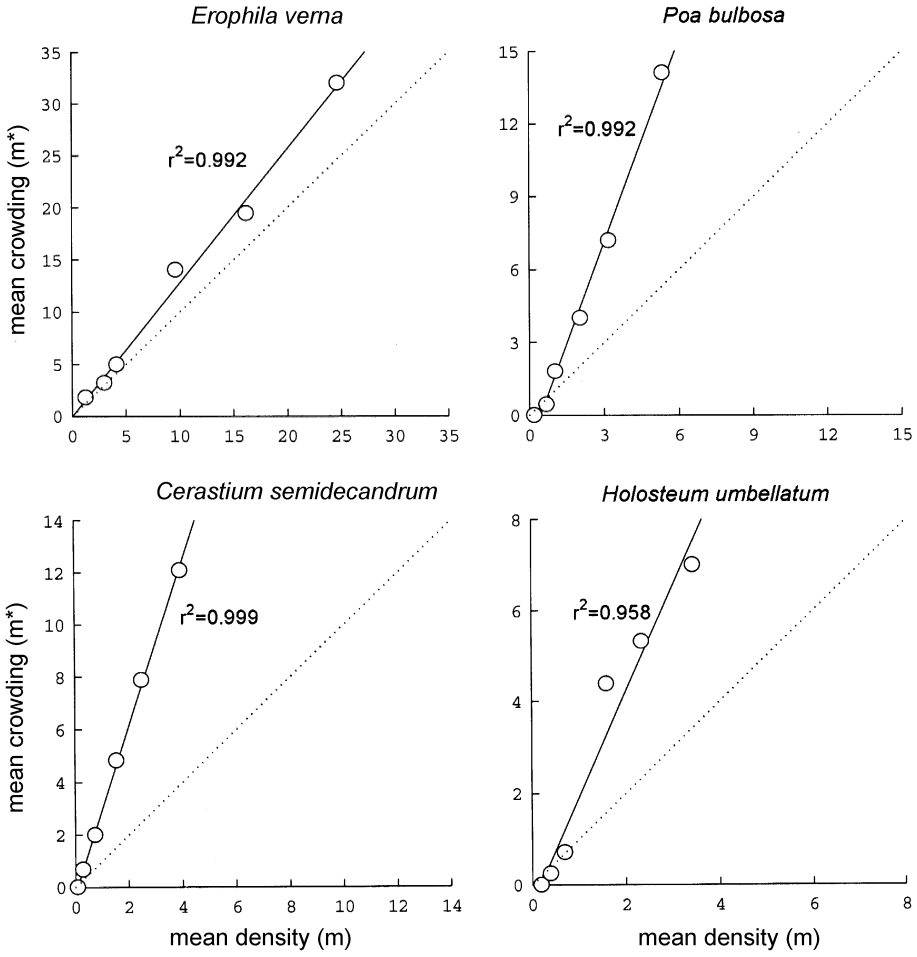

Fig. 2 (continued)

Table 3
Estimates of variance to mean ratio (V/m), patchiness (Ip) (mean crowding (m*)/mean density (m)) with standard errors. This values were calculated at the smallest sample size

Species	V/m	Ip (m*/m)	S. E. (Ip)
Arenaria serpyllifolia	9.63*	3.25	0.18
Saxifraga tridactylites	5.59	3.21	0.17
Minuartia verna	3.07	3.56	0.20
Erophila verna	1.55	1.36	0.07
Conyza canadensis	2.4**	7.5	0.47
Poa bulbosa	0.84	0.00	0.00
Cerastium semidecandrum	0.95	0.00	0.00
Holosteum umbellatum	0.84	0.00	0.00

* $P < 0.05$, ** $P < 0.01$

mean seed density was much higher for the annual species *Erophila verna*, *Arenaria serpyllifolia* and *Saxifraga tridactylites* than for the perennials which scarcely occurred in the seed bank except for *Poa bulbosa* and *Minuartia verna*.

The smallest core (A, diameter: 5 cm) applied in 20 repetitions was adequate to find the dominant annual species in the seed bank, while increasing the nested sample area to 19 times resulted in only doubling of the total number of species found. This seems to support the findings by Bigwood and Inouye (1988), namely that considering the rare species it is difficult if not impossible to reach number of samples adequate for analysis in a statistical sense. The general view when investigating seed patterns as to it is better to use more smaller than few larger samplers (Kropač 1966, Roberts 1981, Thompson 1986) may turn out to be of limited validity because of the unknown seed clumps even in the case of the frequent species. The main problem with most seed bank studies is the imprecise estimates of seed numbers as the seeds have clustered patterns (Dessaint *et al*. 1991). In the case of the present study species with high density in the seed bank (*Arenaria serpyllifolia, Erophila verna, Saxifraga tridactylites*) showed aggregated distribution even at the smallest sample scale similarly to the high-density species in other studies (Johnson and Anderson 1986, Henderson *et al*. 1988, Benoit *et al*. 1989, Dessaint *et al*. 1991). Increasing the sample scale provides information on the distribution pattern of seed clumps. In this respect seed clumps of *Arenaria serpyllifolia, Minuartia verna* and *Erophila verna* showed random, those of *Saxifraga tridactylites* and *Conyza canadensis* contagious distribution. However, there was no trend in the spatial distributions of seed clumps among species. The seed clumps of the low density species (*Poa bulbosa, Cerastium semidecandrum* and *Holosteum umbellatum*) were found when increasing the sample size.

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