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Effect of three mound-building ant species on the formation of soil seed bank in mountain grassland

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Abstract

The effect of three ant species (*Lasius flavus*, *Formica* spp., *Tetramorium caespitum*) on soil seed bank formation was studied in temperate mountain grassland. Seed removal experiments, analysis of soil seed content and seed survival experiments were carried out to evaluate the influence of ground ants on the seed fate. In the seed removal experiment seeds of 16 species, including 5 species with elaiosome-bearing seeds (myrmecochores), were exposed and their removal followed for 39 h. On average, ants removed 63.8% of myrmecochorous seeds and 10.9% of seeds without adaptation to ant dispersal. Analysis of soil seed content revealed that myrmecochores, in spite of expectations that they would accumulate in nests of seed dispersing ants, were most abundant in the soil of control plots. Evidence on seed relocation to the ant nests was obtained from a comparison of mounds of seed dispersing and seed non-dispersing ant species, as more seeds were found in the mounds of *Formica* spp. and *Tetramorium caespitum* (seed dispersers) in comparison with the mounds of *Lasius favus* (non-disperser).

The soil seed bank of the compared microhabitats (control plots and mounds of 3 ant species) differed in their species composition, seed abundance and vertical distribution. The most distinct qualitative differences were between seed flora of control plots and mounds of *Tetramorium caespitum*. Control plots had approximately 30,000 propagules per m^2, which was double the number of seeds found in the ant mounds. In control plots, abundance and diversity of seeds steeply declined with depth; this trend was not observed in the mounds probably due to bioturbation. In the seed survival experiment, more seeds (2 out of 3 species) survived in control plots, which may also contribute to the higher seed abundance in this microhabitat.

This study showed that seed relocation by ants does not contribute significantly to seed bank build-up at this study site. Ants may, however, increase the regeneration success of myrmecochores, mainly by dispersal for distance and placement in a larger spectrum of microsites, in contrast to species not adapted for myrmecochory.

Keywords: Bioturbation; *Lasius flavus*; Myrmecochory; Pasture; Seed density

Introduction

Although regeneration by means of clonal expansion dominates in perennial grasslands (Arnthórsdóttir, 1994; Eriksson, 1989, 1993), regeneration by seeds is important for the maintenance of plant populations as

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well as for community structure (Bullock et al., 1995; Edwards and Crawley, 1999; Eriksson and Eriksson, 1997; Kalamees and Zobel, 2002). One of the most important limitations of successful seedling establishment is the availability of suitable microsites. Seedling recruitment is sensitive to the effect of competition and therefore depends on the presence of gaps in vegetation cover (Amiaud and Touzard, 2004; During and van Tooren, 1990; Hölzel and Otte, 2004; Kitajima and Tilman, 1996; Špačková et al., 1998; Zamfir, 2000; Zobel et al., 2000). Gaps emerge as a result of small-scale disturbances, for instance from plant deaths, substrate movement or animal activity such as trampling or burrowing (Edwards and Crawley, 1999; Jentsch and Beyschlag, 2003; Pickett and White, 1985; van Tooren, 1988). Diaspores that colonize gaps are derived from several sources, seeds present in the persistent seed bank, seeds produced in current year by adjacent vegetation, or seeds actively relocated by animals to the gaps over longer distances (Bullock et al., 1994; Edwards and Crawley, 1999; Kalamees and Zobel, 2002; Pakeman et al., 1998). Animal-dispersed seeds can form a large proportion of propagules deposited in the gaps and grazers are documented to deposit via droppings 1–45% of seeds found in the gaps (Malo et al., 1995; Pakeman et al., 1999; Russi et al., 1992; Zedler and Black, 1992). Some animals may have a dual role during seed regeneration as they are active in both seed dispersal and gap formation. Along with grazing animals, ants in temperate grasslands are another group of animals that may influence seed placement in microsites suitable for germination (Beattie, 1985; Peters et al., 2003).

Ants disturb vegetation cover by building soil nests, which have a pronounced above-ground part in some ant species (Hole, 1981; Paton et al., 1995). Besides disturbance, nest construction and maintenance also increases soil fertility (Folgarait, 1998). Ants' dispersal activity primarily involves species with seeds equipped with elaiosomes (myrmecochores). After seeds are taken to the nests, the elaiosomes are consumed whilst seed bodies usually remain intact. They may stay in the nests or are discarded by the workers in their vicinity (Beattie, 1985; Kjellson, 1985; Oostermeijer, 1989; Peters et al., 2003). Even species whose seeds do not bear elaiosomes may be occasionally relocated to ant nests and benefit from more suitable microsite conditions (Gorb et al., 1997). It is documented that seedlings in the nests perform better than seedlings growing outside of them through lower competition pressure and the qualitative superiority of nest soil (Culver and Beattie, 1980; Oostermeijer, 1989).

Although seed dispersal by ants is well-studied phenomenon (Beattie, 1985), there are only several studies documenting the effect of ants on soil seed bank formation in temperate grasslands (King, 1976; Gorb et al., 2000; van Tooren, 1988). Ants are expected to change seed abundance and species composition of the soil seed bank in their nests by seed relocation, but also by modification of soil environment. As stated above, ants change the soil chemistry of the mounds, and also prevent formation of soil horizons by permanent mounding and soil mixing (Paton et al., 1995).

This study aims to assess the effect of three mound-building ant species (*Lasius flavus*, *Formica* spp. and *Tetramorium caespitum*) on the quantitative and qualitative characteristics of soil seed bank in temperate mountain grassland. In addition to the seed bank survey, experiments on seed removal and seed survival were carried out (i) to compare microhabitats (nest mounds with their surroundings) in qualitative and quantitative parameters (species composition, seed density, vertical distribution) of soil seed bank, (ii) to evaluate the role of myrmecochory in seed bank build-up at study site, (iii) to investigate microhabitat differences on long-term seed survival and (iv) to discuss the role of ants in regeneration by seeds in the studied grassland.

Materials and methods

Study site

This study was carried out in the Slovenské Rudohorie Mts., Slovakia at an elevation of ca. 950 m a.s.l. near Obrubovanec point (1020 m a.s.l.; 48°41′N, 19′39′E). This area is characterized by a complex of mown and grazed grasslands. This study took place in the grazed grassland. The vegetation at the site is rather poor due to acidic bedrock (magmatites and amphibolites), with the dominance of the grass species *Festuca rubra*, *Festuca pratensis*, *Agrostis capillaris*, and *Dactylis glomerata*. It can be classified as the alliance Nardo-Agrostion tenuis Sillinger 1933. Nomenclature of vascular plants follows Flora Europaea (Tutin, 1964–1980).

The most common ant species at the study site is *Lasius flavus*; other ant species that build pronounced hills are *Tetramorium caespitum* and several species of the genus *Formica* (*F. fusca* L., *F. lemani* Bondroit, *F. pratensis* Retz., and *F. rufa* L.); due to problems in identification, *Formica* spp. refers to all named taxa. Two other ant species present, *Lasius niger* and *Myrmica ruginodis*, do not form soil mounds and were omitted from the study. Approximately one mound per 2 m^2 occurs in the study area (Raabová, unpublished data).

The ant species used in the study (*Lasius flavus*, *Formica* spp. and *Tetramorium caespitum*) differ in their ecology. *Formica* spp. and *Tetramorium caespitum* are documented to be seed dispersers, in contrast to *Lasius* *flavus* that lives mostly below ground and feeds on the honeydew of root aphids (Gorb et al., 2000; Oostermeijer, 1989; Woodell and King, 1991). Nests of *Lasius flavus* and *Formica* spp. are long-lasting structures (mounds of *Lasius flavus* were documented by Woodell and King (1991) to last for 22 years at minimum), whereas mounds of *Tetramorium caespitum* are rather ephemeral (Kovář and Kovářová, unpublished data).

Seed removal experiment

The seed removal experiment was carried out to demonstrate the presence of seed dispersal in the study system and to test the effect of adaptation for myrmecochory on seed removal rates. The experiment was initiated on July 30, 1999 at 6 p.m. and was terminated on August 1, 1999 at 9 a.m. (39 h in total) when no further significant removal of diaspores was observed.

The experiment was undertaken at the peak of the fruiting period. Seeds of 16 species, including myrmecochores ($n = 5$) and non-myrmecochores ($n = 11$) were collected at the site and used in the experiment (for a list of species see Fig. 1). Seeds were exposed on plastic trays (diameter = 10 cm) caged by wire mesh (mesh size = 1 cm). Such treatment allowed the ants to inspect displayed seeds but prevented larger insects or rodents from predate seeds. Twenty-five caged trays were placed outside the mounds in a regular grid with a distance of 3 m, covering an area of approximately 150 m^2. At the beginning of the experiment, 10 seeds per species were placed on each of 5 trays, giving 50 displayed propagules per species in total. To distribute 800 seeds (16 species × 50 seeds) over 25 trays, each tray contained a random combination of 3 or 4 species with 30 or 40 diaspores displayed. The trays were checked after 16, 18, 20, 24 and 39 h for the number of remaining seeds and ant activity. Eight trays were lost due to disturbance reducing the number of seeds used in further analysis (for their numbers see Fig. 1).

The results of this experiment were analyzed using Survival analysis (Cox proportional hazards) with Wald test. I tested the effect of (i) species and (ii) adaptation for myrmecochory on the time needed for seed removal.

Soil seed bank analysis

The soil seed bank was surveyed in 4 microhabitats [(i) control plots, (ii) mounds of *Formica* spp., (iii) mounds of *Lasius flavus* and (iv) mounds of *Tetramorium caespitum*] to test the effect of microhabitat on species composition and seed density (namely of myrmecochores). On September 30, 1999, 10 blocks (approximately 3 × 3 m large) with the presence of all 4 types of microhabitat were set up at the study site on an area approximately 100 × 100 m. In each block, a pair of samples (ca. 15 cm apart) were taken from each microhabitat. Soil taken from the mounds was sampled from the top of the nests. Samples from control plots were taken approximately 1 m from the edge of the nearest mound to avoid the refuse piles of the ant colonies. Prior to soil collection, a list of plants was recorded within a circle of radius = 15 cm around each sample. This list of plants is further referred as ‘VEGETATION’.

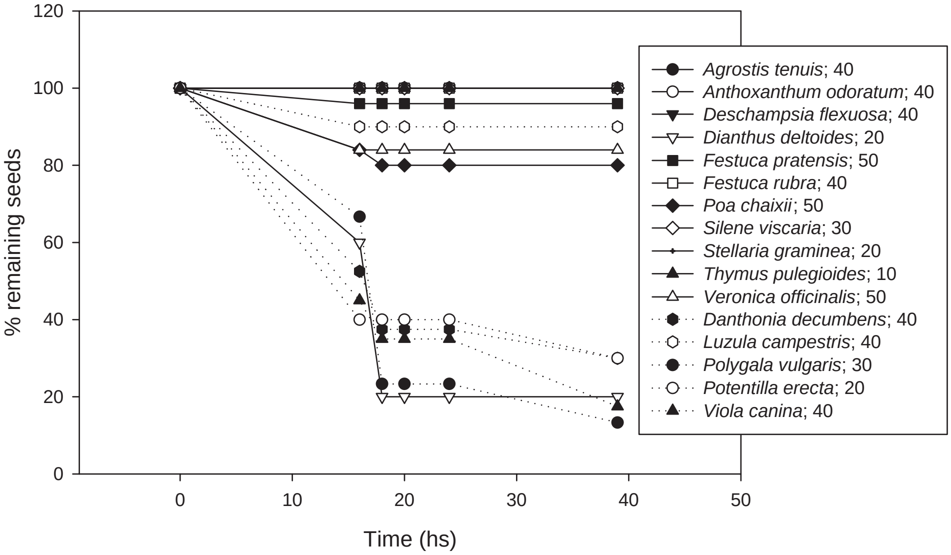

Fig. 1. Seed removal experiment – percentage of seeds not removed during 39 h of experiment. The numbers following species names indicate the amount of seeds used for the calculation of removal rates. Solid lines refer to non-myrmecochores, dotted lines to myrmecochores.

Soil samples, 10 cm deep, were obtained with a soil corer (diameter = 5 cm). After excavation, they were sliced to 0–5 and 5–10 cm layers and then stored in refrigerator for a month at 7 °C to break dormancy of present seeds. After stones and roots had been removed by sieving through 5 mm mesh, samples were laid out in a layer of 4 mm at maximum over sterilized sand in plastic trays (diameter 24 cm) and placed in the greenhouse. Seed content was studied using the seedling emergence method, which is able to detect most of the present seeds (Bekker et al., 2000). The trays were kept in the greenhouse for a period of 4 months (November 1999–February 2000) at 20 ± 5 °C and watered regularly and their position in the greenhouse was changed fortnightly. Emerging seedlings were counted and removed fortnightly. After 2 months the surface of each soil sample was stirred to stimulate further germination.

Some samples were lost during the transport, but each microhabitat was still well represented (minimum number of 11 samples in case of the mounds of *Tetramorium caespitum*).

The effect of microhabitat, soil layer (0–5 and 5–10 cm), and their interaction on the number of species and seed density was analyzed with a two-way ANOVA. Data were log-transformed prior to analysis. Differences among microhabitats in the seed density of the 10 most abundant species and of 5 myrmecochores found in the soil seed bank were analyzed by GLM with Poisson distribution. The effect of microhabitats on the presence of 5 myrmecochores in the seed bank and vegetation was analyzed by GLM with binomial distribution.

Species composition of the seed bank and the effect of microhabitats were investigated using redundancy analysis. To remove the effect of vegetation composition, above-ground species presence records were used as a covariate. Significance of the effect was tested by unrestricted Monte-Carlo permutation.

Seed survival experiment

The seed survival experiment was carried out using three myrmecochores to test the effect of edaphic conditions of four microhabitats on seed survival. Seeds from the myrmecochores *Potentilla erecta*, *Danthonia decumbens* and *Viola canina* were selected since they are very attractive to ants (see Results of seed removal experiment) and have higher probability to be dispersed to a wider range of microhabitats than non-myrmecochores. On September 25, 1998 each of 20 nylon mesh bags (mesh size = 0.2 mm) were filled with a mixture of all 3 species, 30 seeds per species. Seeds were collected directly at the study site on the same day as the experiment was initiated. One bag was placed in each of the 5 blocks (approximately 3 × 3 m) and 4 microhabitats (giving 20 bags in total). The microhabitats were identical to those in the soil seed bank analysis [(i) control plots, (ii) mounds of *Formica* spp., (iii) mounds of *Lasius flavus* and (iv) mounds of *Tetramorium caespitum*]. Bags were buried to a depth of 5 cm on September 25, 1998, and retrieved 2 years later (on October 2, 2000). After a period of 2 years, no change in microhabitat type was observed; i. e. mounds of specific ant species were occupied by the same ant 2 years later. Even mounds of *Tetramorium caespitum* did not collapse during the burial period and were occupied by this species on the retrieval day. The proportion of viable seeds was tested by the germination method. After storage in refrigerator for a month at 7 °C, exhumed seeds were placed in Petri dishes at room temperature and watered regularly for a period of 4 months. Seedlings that germinated were counted and removed weekly.

The effect of microhabitat on seed survival (expressed by viability of retrieved seeds) was tested by GLIM with Poisson distribution.

All univariate analyses were run in S-PLUS, Lucent Technologies; RDA analysis was implemented in CANOCO (Ter Braak and Šmilauer, 1998).

Results

Seed removal experiment

Seed removal rates differed significantly among species (Wald test = 90.3, d.f. = 15, $P<0.001$). There were species with seeds entirely ignored by ants during the experiment (e.g. *Agrostis capillaris*, *Stellaria graminea*) as well as species with 80% of seeds taken away (e.g. *Polygala vulgaris*, *Viola canina*).

Ants showed a strong preference for seeds of myrmecochores in comparison with non-myrmecochores (Fig. 1; Wald test = 47.7, d.f. = 1, $P<0.001$). On average, ants removed 63.8% of myrmecochorous seeds and 10.9% of seeds without adaptation to ant dispersal during the 39 h of experiment. There were exceptions in both groups. Ants removed only 10% of *Luzula campestris* (myrmecochore) but removed 80% of the seeds of *Dianthus deltoides* (Fig. 1).

Whilst checking the trays, *Formica* spp. and *Myrmica ruginodis* (ant species not involved in this study) were observed to examine and remove the seeds. Any activity or presence of the other two ant species whose mounds were surveyed for their seed bank (*Lasius flavus* and *Tetramorium caespitum*) was not recorded during the experiment.

Soil seed bank analysis

A total of 4391 seedlings of 35 species were found in the soil seed bank; two species *Agrostis capillaris* and

Table 1. Number of species in vegetation, number of species in seed bank, total seed density in seed bank and seed densities of the 10 most abundant species found in 0–10 cm expressed per m^2 (mean ± SE). Significances refer to the differences between microhabitats

	Control ($n = 17$)	*Formica* ($n = 20$)	*Lasius* ($n = 19$)	*Tetramorium* ($n = 11$)	P-value	P-value[a]
Agrostis capillaris	7643 ± 1234	4815 ± 1594	7321 ± 3484	6393 ± 4025	***	***
Deschampsia flexuosa	209 ± 88	586 ± 464	27 ± 27	0 ± 0	***	***
Campanula patula	839 ± 261	662 ± 384	80 ± 57	741 ± 538	***	***
Carex pillulifera	2787 ± 881	433 ± 166	215 ± 98	324 ± 124	***	***
Fragaria vesca	1259 ± 504	203 ± 154	268 ± 188	510 ± 322	***	***
Hypericum maculatum	779 ± 259	739 ± 292	456 ± 186	649 ± 395	***	***
Juncus buffonius	2218 ± 498	484 ± 175	402 ± 114	46 ± 46	***	***
Sagina procumbens	7164 ± 6205	1223 ± 487	2119 ± 1056	2733 ± 1239	***	***
Stellaria graminea	1409 ± 896	994 ± 710	1046 ± 535	324 ± 142	***	***
Pimpinella saxifraga	569 ± 238	76 ± 56	107 ± 107	185 ± 103	***	***

$^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$; n.s., not significant.

[a] Significances refer to the differences between mounds of different ant species only, without control plots.

Table 2. Results of two-way ANOVAs on the effect of microhabitat, soil layer and their interaction on density and species diversity of seeds in soil seed bank

	Factor	MS	DF	F	P-value
Seed density	Habitat	1.37	3	3.15	*
	Depth	2.47	1	5.66	*
	Habitat*Depth	1.19	3	2.74	*
	Error	0.44	126		
Species diversity	Habitat	0.300	3	6.51	***
	Depth	0.53	1	11.51	***
	Habitat*Depth	0.124	3	2.70	*
	Error	0.05	126		

$^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$.

Sagina procumbens formed 50% of seeds present in the soil. Thirty species appeared in both the soil seed bank and vegetation whereas five species including two highly abundant species, *Sagina procumbens* and *Juncus bufonius*, were found in the seed bank only. Twenty species which were present in vegetation above the sampling points were absent from the soil seed flora. The 10 most abundant species found in the soil seed bank and their seed densities in respective microhabitats are shown in Table 1.

Seed density differed significantly among microhabitats and among upper (0–5 cm) and lower (5–10 cm) layers; the interaction between microhabitat and soil layer on seed density was also significant (Table 2). There were twice as many seeds in control plots as in the mounds; on average, there were 31,472 ± 6691 SE seeds per m^2 in control plots in comparison with mounds of *Formica* spp., *Lasius flavus* and *Tetramorium caespitum* with densities of 14,446 ± 2279 SE, 15,796 ± 3396 SE, 16,213 ± 5028 SE seeds per m^2 (to a depth of 10 cm). Considerably more seeds were concentrated in the upper layer (0–5 cm) in control plots whereas they were much more equally distributed among both layers in the mounds (Fig. 2). A similar pattern was found for the species diversity of seed flora: on average, seeds of 8.4 ± 0.8 SE species per soil sample (soil volume = 0.2 l) were found in control plots whilst in mounds of *Formica* spp., *Lasius flavus* and *Tetramorium caespitum* there were 5.0 ± 0.5 SE, 4.4 ± 0.6 SE and 5.6 ± 0.9 SE species per soil sample, respectively. Unlike in the mounds, species diversity was considerably higher in upper layer in control plots (Fig. 2, Table 2).

Distribution of myrmecochores in seed bank and vegetation

Seeds of five myrmecochorous species (*Carex pillulifera*, *Danthonia decumbens*, *Luzula campestris*, *Potentilla erecta* and *Viola canina*) were found in the soil seed bank. *Carex pillulifera* was the most frequent (found in 30 samples out of 67 samples) and abundant myrmecochorous species (with density 951 ± 262 seeds per m^2; mean ± SE); other myrmecochores were only marginally represented. Seeds of ant-dispersed species were most abundant in the control plots, with mean density 3447 ± 979 seeds per m^2 (mean ± SE) in contrast to the mounds where density of myrmecochores was much lower (322–787 seeds per m^2). Difference in seed densities of myrmecochores could be partly related to the differences in their frequency in the vegetation. However, in case of the most abundant myrmecochores, *Carex pillulifera* and *Danthonia decumbens*, frequency in vegetation did not differ significantly between microhabitats.

If control plots were omitted from the comparison of microhabitats, the density of ant-dispersed seeds was twice as high in the mounds of *Formica* spp. and *Tetramorium caespitum* than in the mounds of *Lasius flavus*, a species not involved in myrmecochory. The

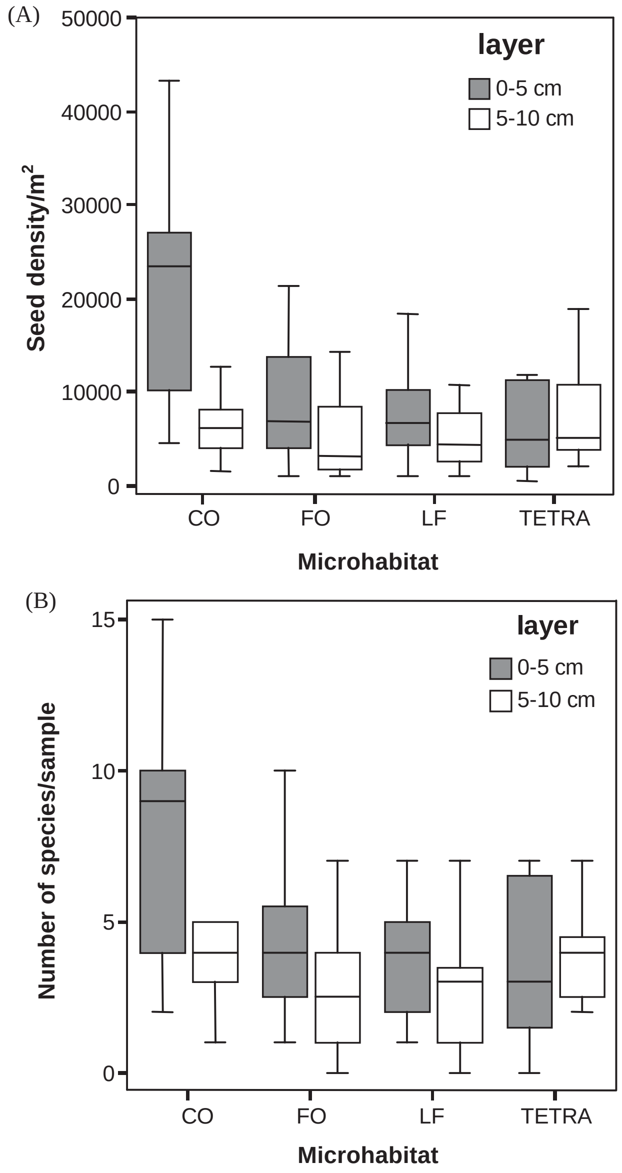

Fig. 2. Seed densities per m^2 (A) and number of species per soil sample (B) in the seed bank of the four different microhabitats and 2 layers (0–5 and 5–10 cm). CO – contol plots, FO – mounds of *Formica* sp., LF – mounds of *Lasius flavus*; TETRA – mounds of *Tetramorium caespitum*. Median, 25 and 75 percentiles, and minimum and maximum values are shown. The analysis was done using transformed data, but the figure presents data without transformation.

effect of ant species was significant for all five myrmecochores when analyzed separately; these differences were independent of species frequency in the vegetation (Table 3).

Effect of microhabitat on species composition of soil seed bank

The species composition of seed banks significantly differed between surveyed microhabitats. Differences between microhabitats explained 10.4% of the total variation in composition; proportion of explained variation increased to 13.1% if composition of aboveground vegetation was taken as covariate in the model. The main direction of variation (the first canonical axis) separated the seed bank of the control plot and seed bank of the mounds of *Tetramorium caespitum* (see Fig. 3a). Species associated with the control plots were *Juncus bufonius*, *Carex pillulifera* and *Hieracium pilosella*. Species whose seeds were more abundant in seed bank of the mounds of *Tetramorium caespitum* were *Sagina procumbens*, *Veronica chamaedrys* and *Hypericum maculatum*. The second canonical axis separated the seed bank of mounds of *Lasius flavus* from the seed bank of the remaining microhabitats. *Formica* spp. mounds contributed little to the total variation in seed bank composition (Fig. 3a).

RDA analysis of the seed bank composition of ant mounds yielded significant differences only after the inclusion of vegetation as a covariate in the model. As expected from the first RDA analysis, the main differences were found between the mounds of *Tetramorium caespitum* and *Lasius flavus*. Myrmecochores *Potentilla erecta*, *Danthonia decumbens* and *Luzula campestris* were among the most typical species for the mounds of *Tetramorium caespitum* (Fig. 3b; Table 4).

Seed survival experiment

Seeds of myrmecochores retrieved after 2 years from four microhabitats differed in their viability. A significantly higher survival rate was recorded for seeds of *Danthonia decumbens* and *Potentilla erecta* buried in the control plots. A lower germination percentage of *Danthonia decumbens* was found in seeds retrieved from mounds of *Lasius flavus* in comparison with mounds of other the two species (Table 5).

Discussion

A survey of the seed bank of mountain grassland revealed on average 20,000 seeds per m^2 which is well within the range of densities found for this ecosystem in previous studies (10^3–10^6 of seeds per m^2; Fenner, 1985). Twenty species from the established vegetation were missing in the seed bank whereas two other species absent from the vegetation (*Sagina procumbens* and *Juncus bufonius*) were among the most abundant in seed bank. Seeds of these two species in seed bank were probably brought to the grassland by grazing cattle.

Most of the species in the seed bank were poorly represented whereas two species (*Agrostis capillaris*, *Sagina procumbens*) formed 50% of seeds present in the soil; dominance of several species in seed bank is a

Table 3. Myrmecochores found in the seed bank. Their frequency in the vegetation and in soil seed bank (SSB) and seed densities in 0–10 cm (per m^2; mean ± SE). Frequency indicates the percentage of samples with myrmecochore presence. Significances refer to the differences between microhabitats

Species	Frequency (density)	Control ($n = 17$)	*Formica* ($n = 20$)	*Lasius* ($n = 19$)	*Tetramorium* ($n = 11$)	*P*-value	*P*-value[a]
Carex pillulifera	Vegetation (%)	12	10	0	18	ns	ns
	SSB (%)	82	35	21	45	**	ns
	Density	2787 ± 882	433 ± 166	215 ± 98	324 ± 124	***	***
Luzula campetsris	Vegetation (%)	65	20	11	36	**	ns
	SSB (%)	18	10	0	27	ns	ns
	Density	90 ± 49	51 ± 35	0 ± 0	139 ± 72	***	***
Potentilla erecta	Vegetation (%)	65	10	11	36	**	ns
	SSB (%)	18	0	0	27	**	ns
	Density	120 ± 70	0 ± 0	0 ± 0	185 ± 104	***	***
Danthonia decumbens	Vegetation (%)	12	10	0	0	ns	ns
	SSB (%)	18	5	5	9	ns	ns
	Density	300 ± 240	25 ± 25	27 ± 27	46 ± 46	***	***
Viola canina	Vegetation (%)	82	40	53	36	*	ns
	SSB (%)	24	20	16	9	ns	ns
	Density	150 ± 73	127 ± 63	80 ± 44	93 ± 93	***	***
Total seed density of	myrmecochores	3447 ± 979	637 ± 184	322 ± 97	787 ± 242	***	***

***$P<0.001$; **$P<0.01$; *$P<0.05$; n.s., not significant.

[a] Significances refer to the differences between mounds of different ant species only, without control plots.

general feature of seed banks under meadow and pasture plant communities (Amiaud and Touzard, 2004; Bakker et al., 1996; Bekker et al., 1998; Jensen, 1998).

Ant presence significantly changed characteristics of the seed bank in studied grassland. Myrmecochory, soil heaping and modification of the soil environment contributed to differences in composition and seed abundance between studied microhabitats. Effect of these activities is discussed in detail.

The removal experiment proved the presence of myrmecochory at study site. Seeds of myrmecochores were more highly preferred to non-myrmecochores and therefore were expected to be more abundant in nests of seed-dispersing species. However, in opposite to expectations, seeds of myrmecochores were more abundant in the control plots than in the mounds of seed dispersing ant species, i.e. relocation did not significantly contribute to seed bank build up in ant nests. The myrmecochores *Carex pillulifera* and *Danthonia decumbens* were also among species characteristic of the vegetation of control plots. Accumulation of ant-dispersed seeds outside ant nests can be accounted for seed losses during the transport. Gorb and Gorb (1999) recorded that dropping rates of relocated propagules can be 8–10% per meter of trail for large-seeded myrmecochores and 20–100% for small-seeded myrmecochores. In other systems, however, no significant seed drops were observed and most seeds were transported to the nests (Higashi et al., 1989). The higher abundance of myrmecochores in control plots can be explained either by an only small effect of myrmecocohory on seed relocation or by seed predation in the nests. In spite of the proved presence of myrmecochory in the studied grassland, this experiment could not provide information on the seed removal dynamics during the whole fruiting season. As it was shown in previous studies, removal activity strongly depends on site characteristics, weather conditions and supply of other food resources (reviewed by Beattie, 1985). Seed predation in ant-nests was proposed by Culver and Beattie (1983) to explain the lower frequency of myrmecochores in vegetation of the mounds than in control plots. This might be the case of *Tetramorium caespitum*, with previous records on

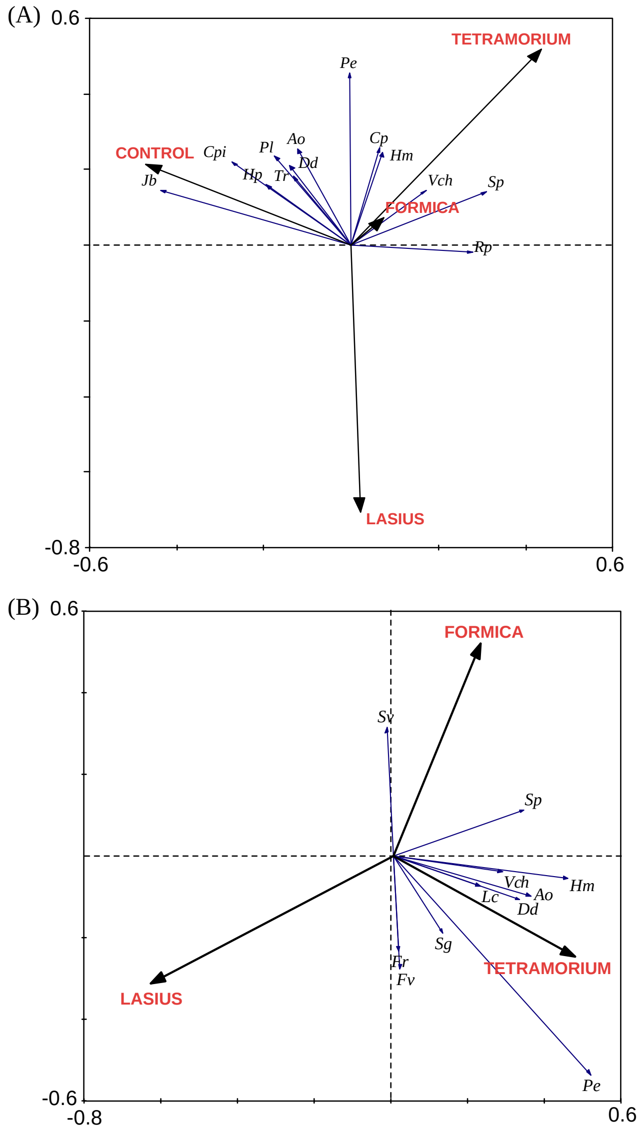

Fig. 3. (A) RDA of the soil seed bank species composition in relation to the 4 microhabitats: (i) control plots (CONTROL), (ii) mounds of *Formica* spp. (FORMICA), (iii) mounds of *Lasius flavus* (LASIUS), and (iv) mounds of *Tetramorium caespitum* (TETRAMORIUM). (B) Composition of seed bank in relation to mounds of the different ants only (without control plots). Only species with >5% fit are shown. Species abbreviations: Ao, *Anthoxanthum odoratum*; Cp, *Campanula patula*; Cpi, *Carex pillulifera*; Dd, *Danthonia decumbens*; Fr, *Festuca rubra*; Fv, *Fragaria vesca*; Hm, *Hypericum maculatum*; Hp, *Hieracium pilosella*; Jb, *Juncus bufonius*; Lc, *Luzula campestris*; Pe, *Potentilla erecta*; Pl, *Plantago lanceolata*; Rp, *Ranunculus polyanthemos*; Sp, *Sagina procumbens*; Sg, *Stellaria graminea*; Sv, *Silene viscaria*; Tr, *Trifolium repens*; Vch, *Veronica chamaedrys*.

granivory (Brian et al., 1967). Nevertheless, despite most myrmecochorous seeds being found in the soil seed bank of control plots, some evidence of seed transport to the nests was obtained from a comparison of the seed content of mounds of seed dispersing ant species with mounds of species not participating in myrmecochory. A significantly higher abundance of myrmecochorous seeds was found in nests of *Tetramorium caespitum* and *Formica* spp. than in nests of *Lasius flavus*. Unfortunately, seed removal activity during the removal experiment was observed in *Formica* spp. only.

There were also significant differences in seed densities between compared microhabitats. Seed density in the mounds was considerably lower than in control plots. This can be explained by smaller seed production resulting from the reduced vegetation cover on the mounds (Dean et al., 1997; King, 1977), but it may also be a result of soil transport within the mounds. One colony of *Lasius flavus* transports yearly on average more than 1 l of soil during construction of the mound (King, 1981). Soil particles are usually brought from the deeper layers where diaspores are absent or have low abundance, and soil mixing among layers may thin the density of present seeds in top layers. This was also the explanation given for the low density of seeds found in previous surveys of seed banks of ant mounds (King, 1976; van Tooren, 1988), gopher mounds (Hobbs and Mooney, 1985), or vole hills (Ryser and Gigon, 1985).

Soil transport within the mounds may also be responsible for the different proportion of seeds present in the upper (0–5 cm) and the lower layer (5–10 cm). Seed density and diversity usually declines significantly below 5 cm (Bakker et al., 1996; Kitajima and Tilman, 1996; Leck, 1989; Nicolson and Keddy, 1983; Roberts, 1981). This pattern was also found in the soil of control plots; seed density and species diversity declined by 70% and 50% respectively in the lower layer 5–10 cm. In ant mounds, namely in the mounds of *Tetramorium caespitum*, there was no significant decrease along the vertical gradient. Ants are not the only soil fauna recorded to change the vertical distribution of the seeds. Ground beetles or earthworms were found to have a similar effect on seed vertical distribution and it is supposed that vertical transport of the diaspores occurs in both directions, i.e. these animals not only bury seeds to deeper layers but also return them to the surface (Beattie and Culver, 1982; Bernhardt, 1994; Thompson et al., 1994; Willems and Huijsmans, 1994).

Different soil seed densities between microhabitats can also be explained by the differences in the soil environment and their effect on seed bank depletion. It is documented that soil environment parameters such as moisture, aeration, fertility or activity of soil microorganisms can influence seed survival and germination in the soil (Cavers and Benoit, 1989; Parker et al., 1989; Bekker et al., 1998; Qaderi et al., 2002). Therefore lower seed densities in the mounds can also be related to lower seed survival rates as it was found for two out the three species.

Table 4. Summary of species-environment relation determined by redundancy analyses. Species composition of the vegetation was related to four types of microhabitat: (i) control plots, (ii) mounds of *Formica* spp., (iii) mounds of *Lasius flavus* and (iv) mounds of *Tetramorium caespitum*

Environmental variable (s)	Covariate(s)	Variation explained by first canonical axis (%)	Variation explained by all canonical axes (%)
All microhabitats			
Microhabitat		8.5**	10.4**
Microhabitat	Sp. absence/presence in vegetation	7.4**	13.1**
Ant mounds only			
Microhabitat (ant species)		2.2^{ns}	3.1^{ns}
Microhabitat (ant species)	Sp. absence/presence in vegetation	10.4**	13.8**

$^{**}P<0.01$; $^{*}P<0.05$; n.s.–not significant.

Table 5. Viability of seeds retrieved after 2 years of burial in four different microhabitats (mean ± SE). Significances refer to differences between microhabitats

	Control ($n = 5$)	*Formica* ($n = 5$)	*Lasius* ($n = 5$)	*Tetramorium* ($n = 5$)	*P*-value	*P*-value[a]
Danthonia decumbens	11.4 ± 3.3	5.2 ± 5.0	1.8 ± 1	6.0 ± 3.7	***	***
Potentilla erecta	7 ± 1.8	1.4 ± 0.9	2.6 ± 1.3	2.3 ± 1.5	***	ns
Viola canina	0.8 ± 0.4	0.2 ± 0.2	1 ± 0.4	0.75 ± 0.5	ns	ns

$^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$; n.s., not significant.

[a] Significances refer to the differences between mounds of different ant species only, without control plots.

Although this study did not explicitly deal with the effect of ants on seedling recruitment and establishment, ants' role in seed regeneration at the study site can be inferred from the seed removal experiment and survey of soil seed bank. In spite of higher seed densities and species diversity of the seed bank in control plots for both myrmecochores and non-myrmecochores, it could be expected that species adapted for myrmecochory may benefit from the presence of seed dispersing ants in the studied grassland. Non-myrmecochores in the seed removal experiment were left without ant attention and therefore had only a very small probability of secondary dispersal. Myrmecochores, though a large proportion is probably lost during relocation, may still be spread across a larger area and some may be even taken to the ant nests where more favorable conditions for germination are expected (Beattie, 1985). Dispersal for distance and occupation of new sites are the principal benefits of this relocation system (Gorb et al., 2000). This effect of ants on seed spreading is comparable with the effect of grazing animals on the distribution of endozoochores (e.g. Razanamandranto et al., 2004); they were found to occupy much larger area of grassland than species lacking this adaptation (Malo et al., 1995). To conclude this study, a highly possible influence of the ants on seed ecology in studied grassland is indicated, including both direct effects like dispersal and indirect influences by changing habitat properties.

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References

Amiaud, B., Touzard, B., 2004. The relationship between soil seed bank, above ground vegetation and disturbances in old embanked marshlands of Western France. Flora 199, 25–35.

Arnthórsdóttir, S., 1994. Colonization of experimental patches in a mown grassland. Oikos 70, 73–79.

Bakker, J.P., Bakker, E.S., Rosén, E., Verwij, G.L., 1996. Soil seed bank composition along a gradient from dry alvar grassland to *Juniperus* shrubland. J. Veg. Sci. 7, 165–176.

Beattie, A.J., 1985. The Evolutionary Ecology of Ant-plant Mutualisms (Cambridge Studies in Ecology). Cambridge University Press, Cambridge.

Beattie, A.J., Culver, D.C., 1982. Inhumation: how ants and other invertebrates help seeds. Nature 297, 627.

Bekker, R.M., Knevel, I.C., Tallowin, J.B.R., Troost, E.M.L., Bakker, J.P., 1998. Soil nutrient input effects on seed longevity: a burial experiment with fen-meadow species. Funct. Ecol. 12, 673–682.

Bekker, R.M., Verweij, G.L., Bakker, J.P., Fresco, L.F.M., 2000. Soil seed bank dynamics in hayfield succession. J. Ecol. 88, 594–607.

Bernhardt, K.G., 1994. Seed burial by soil burrowing animals. Nord. J. Bot. 15, 257–260.

Brian, M.V., Elmes, G., Kelly, A.F., 1967. Populations of the ant *Tetramorium caespitum* Latreille. J. Anim. Ecol. 36, 337–342.

Bullock, J.M., Hill, B.C., Dale, M.P., Silvertown, J., 1994. An experimental study of the effect of sheep grazing on vegetation change in a species-poor grassland and the role of seedling recruitment into gaps. J. Appl. Ecol. 31, 493–507.

Bullock, J.M., Hill, B.C., Silvertown, J., Sutton, M., 1995. Gap colonization as a source of grassland community change: effects of gap size and grazing on the rate and node of colonization by different species. Oikos 72, 273–282.

Cavers, P.B., Benoit, D.L., 1989. Seed banks in arable land. In: Leck, M.A., Parker, V.T., Simpson, R.L. (Eds.), Ecology of Soil Seed Banks. Academic Press, New York, pp. 309–328.

Culver, D.C., Beattie, A.J., 1980. The fate of *Viola* seeds dispersed by ants. Am. J. Bot. 67, 710–714.

Culver, D.C., Beattie, A.J., 1983. Effects of ant mounds on soil chemistry and vegetation patterns in a Colorado montane meadow. Ecology 64, 485–492.

Dean, W.R.J., Milton, S.J., Klotz, S., 1997. The role of ant nest-mounds in maintaining small scale patchiness in dry grasslands. Biodivers. Conserv. 6, 1293–1307.

During, H.J., van Tooren, B.F., 1990. Bryophyte interactions with other plants. Bot. J. Linn. Soc. 104, 79–98.

Edwards, G.R., Crawley, M.J., 1999. Herbivores, seed banks and seedling recruitment in mesic grassland. J. Ecol. 87, 423–435.

Eriksson, O., 1989. Seedling dynamics and life histories in clonal plants. Oikos 55, 231–238.

Eriksson, O., 1993. The species-pool hypothesis and plant community diversity. Oikos 68, 371–374.

Eriksson, A., Eriksson, O., 1997. Seedling recruitment in semi-natural pastures: the effects of disturbance, seed size, phenology and seed bank. Nord. J. Bot. 17, 469–482.

Fenner, M., 1985. Seed Ecology. Chapman & Hall, London, New York.

Folgarait, P.J., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7, 1221–1244.

Gorb, S.N., Gorb, E.V., 1999. Dropping rates of elaiosome-bearing seeds during transport by ants (*Formica polyctena* Foerst): Implications for distance dispersal. Acta Oecol. 20, 509–518.

Gorb, S.N., Gorb, E.V., Sindarovskaya, Y., 1997. Interaction between the non-myrmecochorous herb *Galium aparine* and the ant *Formica polyctena*. Plant Ecol. 131, 215–221.

Gorb, S.N., Gorb, E.V., Puntilla, P., 2000. Effects of redispersal of seeds by ants on vegetation pattern in a deciduous forest: a case study. Acta Oecol. 21, 293–301.

Higashi, S., Tsuyuzaki, S., Ohara, M., Fuminori, I., 1989. Adaptive advantage of ant-dispersed seeds in the myrmecochorous plant *Trillium tschonoskii* (Liliaceae). Oikos 54, 389–394.

Hobbs, R.J., Mooney, H.A., 1985. Community and population dynamics of serpentine grassland annuals in relation to gopher disturbance. Oecologia 67, 342–351.

Hole, F.D., 1981. Effects of animals on soil. Geoderma 25, 75–112.

Hölzel, N., Otte, A., 2004. Ecological significance of seed germination characteristics in flood-meadow species. Flora 199, 12–24.

Jensen, K., 1998. Species composition of soil seed bank and seed rain of abandoned wet meadows and their relation to aboveground vegetation. Flora 193, 345–359.

Jentsch, A., Beyschlag, W., 2003. Vegetation ecology of dry acidic grasslands in the lowland area of Central Europe. Flora 198, 3–25.

Kalamees, R., Zobel, M., 2002. The role of the seed bank in gap regeneration in a calcareous grassland community. Ecology 83, 1017–1025.

King, T.J., 1976. The viable seed contents of ant-hill and pasture soil. New Phytol. 77, 143–148.

King, T.J., 1977. The plant ecology of ant-hills in calcareous grasslands. I. Patterns of species in relation to ant-hills in southern England. J. Ecol. 65, 235–256.

King, T.J., 1981. Ant-hills and grassland history. J. Biogeogr. 8, 329–334.

Kitajima, K., Tilman, D., 1996. Seed banks and seedling establishment on an experimental productivity gradient. Oikos 76, 381–391.

Kjellson, G., 1985. Seed fate in a population of *Carex pillulifera* L. I. Seed dispersal and ant-seed mutualism. Oecologia 67, 416–423.

Leck, M.A., 1989. Wetland seed banks. In: Leck, M.A., Parker, V.T., Simpson, R.L. (Eds.), Ecology of Soil Seed Banks. Academic Press, San Diego, pp. 283–308.

Malo, J.E., Bestabé, J., Suaréz, F., 1995. Seed bank build-up in small disturbances in a Mediterranean pasture: the contribution of endozoochorous dispersal by rabbits. Ecography 18, 73–82.

Nicolson, A., Keddy, P.A., 1983. The depth profile of a shoreline seed bank in Matchedash Lake, Ontario, Canada. Can. J. Bot. 65, 2028–2035.

Oostermeijer, J.G.B., 1989. Myrmecochory in *Polygala vulgaris* L, *Luzula campestris* (L.) DC and *Viola curtisii* Forster in a Dutch dune area. Oecologia 78, 302–311.

Pakeman, R.J., Attwood, J.P., Engelen, J., 1998. Sources of plants colonizing experimentally disturbed patches in an acidic grassland, in eastern England. J. Ecol. 86, 1032–1041.

Pakeman, R.J., Engelen, J., Attwood, J.P., 1999. Rabbit endozoochory and seedbank build-up in an acidic grassland. Plant Ecol. 145, 83–90.

Parker, V.T., Simpson, R.L., Leck, M.A., 1989. Pattern and process in the dynamics of seed banks. In: Leck, M.A., Parker, V.T., Simpson, R.L. (Eds.), Ecology of Soil Seed Banks. Academic Press, New York, pp. 367–384.

Paton, T.R., Humphreys, G.S., Mitchell, P.B., 1995. Soils. A New Global View. Yale University Press, New Haven.

Peters, M., Oberrath, R., Böhning-Gaese, K., 2003. Seed dispersal by ants: are seed preferences influenced by foraging strategies or historical constraints? Flora 198, 413–420.

Pickett, S.T.A., White, P.S., 1985. The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, Orlando.

Qaderi, M.M., Cavers, P.B., Bernards, M.A., 2002. Seed bank dynamics of *Onopordum acanthium*: emergence patterns and chemical attributes. J. Ecol. 90, 672–683.

Razanamandranto, S., Tigabu, M., Neya, S., Odén, P.C., 2004. Effects of gut treatment on recovery and germinability of bovine and ovine ingested seeds of four woody species from the Sudanian savanna in West Africa. Flora 199, 389–397.

Roberts, H.A., 1981. Seed banks in soils. Adv. Appl. Biol. 6, 1–55.

Russi, L., Cocks, P.S., Roberts, E.H., 1992. The fate of legume seeds eaten by sheep from a Mediterranean grassland. J. Appl. Ecol. 29, 772–778.

Ryser, P., Gigon, A., 1985. Influence of seed bank and small mammals on the floristic composition of limestone grassland (Mesobrometum) in northern Switzerland. Ber. Geobot. Inst. ETH. Zürich 52, 41–52.

Špačková, I., Kotorová, I., Lepš, J., 1998. Sensitivity of seedling recruitment to moss, litter and dominant removal in a oligotrophic wet meadow. Folia Geobot. 33, 17–30.

Ter Braak, C.J.F., Šmilauer, P., 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer Power, Ithaca.

Thompson, K., Green, A., Jewels, A.M., 1994. Seeds in soil and worm casts from a neutral grassland. Funct. Ecol. 8, 29–35.

Tutin, T.G., 1964–1980. Flora Europaea. Cambridge University Press, Cambridge.

Van Tooren, B.F., 1988. The fate of seeds after dispersal in chalk grassland: the role of bryophyte layer. Oikos 53, 41–48.

Willems, J.H., Huijsmans, K.G.A., 1994. Vertical seed dispersal by earthworms: a quantitative approach. Ecography 17, 124–130.

Woodell, S.R.J., King, T.J., 1991. The influence of mound-building ants on British lowland vegetation. In: Huxley, C.R., Cutler, D.F. (Eds.), Ant–Plant Interactions. Oxford University Press, Oxford, pp. 521–535.

Zamfir, M., 2000. Effects of bryophytes and lichens on seedling emergence of alvar plants: evidence from greenhous experiments. Oikos 88, 603–611.

Zedler, P.H., Black, C., 1992. Seed dispersal by a generalized herbivore: rabbit as dispersal vector in a semiarid California vernal pool landscape. Am. Midl. Nat. 128, 1–10.

Zobel, M., Otsus, M., Liira, J., Moora, M., Möls, T., 2000. Is small-scale richness in a calcareous grassland limited by seed availability? Ecology 82, 3274–3282.