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From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species

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Abstract

Seed germination and emergence are influenced by the position of seeds in the soil bank profile. Mediterranean grasslands are heavily dependent on seed banks, as these systems are mainly composed of annual species. Seed bank germination experiments in a greenhouse were conducted to analyse the role played by burial depth on seed bank dynamics in annual Mediterranean grasslands. Specifically, they addressed two objectives: (1) to assess the ability of seeds in the shallow layer of the soil bank to emerge when they are buried at different depths, and (2) to ascertain the ability of seeds from deep layers to germinate and emerge to the surface. The study also produced a depth profile of species and seeds. The results show that: (1) all species (100%) and the majority of viable seeds (98.9%) are situated in the first centimetre, with a significant fall in the number of species and seeds in the soil bank as depth increases; (2) for the majority of species (92%) and seeds (85.4%) in the shallow bank, the emergence percentage declines significantly with burial depth; and (3) seeds that are present in deep layers need to rise to the surface in order to produce seedlings. In conclusion, the function of the seed bank in Mediterranean grasslands depends on the number of species and seeds in it, but also on the seed position in the profile and vertical movements that enable them to reach the surface.

Keywords: burial depth, emergence, germination, Mediterranean grassland, seed bank

Introduction

One of the major features of soil seed banks in Mediterranean grasslands is the large numbers of seeds and species, primarily annuals (Levassor *et al.*, 1990; Russi *et al.*, 1992; Ortega *et al.*, 1997; Marañón, 1998). The soil bank is only functional if the seeds are in a viable state and ready for germination at the right time (Murdoch and Ellis, 2000).

Vertical distribution of seeds in the soil profile is very heterogeneous. A depth-related decrease in soil seed bank density has been documented (Roberts, 1981; Russi *et al.*, 1992; Hutchings and Booth, 1996), which, in some cases, has been linked to the ability of seeds from certain species to germinate and emerge (Freas, 1989; Grundy *et al.*, 1999; Smith *et al.*, 1999; Pons, 2000; Benvenuti *et al.*, 2001), although the emergence of seeds from the soil bank in Mediterranean grasslands related to depth has not been studied to date. In general, if seeds in the soil bank are permanently buried too deeply, they fail to act as an effective seed bank (Baker, 1989), forming what, in some cases, has been called the inactive component of the soil bank (McGraw and Vavrek, 1989). But how deep is too deep?

Research into the relationship between burial depth and seed emergence in Mediterranean grasslands is an important step in the definition of the real function of the soil bank. Therefore, the objectives of this study were: (1) to assess the ability of seeds from the shallow layer of the soil bank to emerge when they are buried at different depths; and (2) for the emergence of a seed, to ascertain the importance of it remaining buried or rising to the surface (vertical movement). The same analysis also produced a depth profile of species' and seeds' presence.

The depth of seed burial can affect seedling emergence: (1) by influencing germination via environmental factors (light, oxygen, temperature, moisture, etc.), which act on both dormancy and germination (see Bewley and Black, 1994; Baskin and Baskin, 2001), or (2) by hindering access of the hypocotyl to the surface. Both effects are species dependent. In the present paper, these two effects are not distinguished, and we evaluate the overall effect on seedling emergence.

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Materials and methods

Study site

The study area was a *Quercus ilex* subsp. *ballota* dehesa situated 20 km north of Madrid (40°38′N; 3°70′S) on soils with a gneiss substrate, and a herbaceous layer dominated by *Poa bulbosa* and a large number of annuals. The study area has an altitude range of 700–900 m; mean annual temperature 13°C, and average annual rainfall around 450–500 mm, with a heavy drought period in the summer and high interannual fluctuations. This dehesa has been under traditional management for centuries with extensive livestock grazing, a total lack of agricultural crops and some forestry activities. More details on the study area have been published in Peco *et al.* (2003).

Experiment 1. How deep can seeds from the shallow bank germinate and become established?

In order to analyse the depth ranges at which effective germination from the seed bank occur, we selected ten flat 2 × 2 m plots outside the influence of trees. In October 1997 we took four randomly selected soil samples at 1 cm depth from each plot, using a 4 cm diameter cylindrical corer after removing the litter. Samples from the same plot were stored in the same paper bag, and were then crumbled and mechanically homogenized in plastic mixing trays. Homogenized samples were divided into four subsamples, which were assumed to have equal seed contents. Each subsample was put into germination pots, in thin 1 cm layers separated from a vermiculite base by sterile gauze. The treatment to simulate the effect of burial depth consisted of covering the soil subsamples with different layers (1, 2 and 3 cm deep) of sterile sand with similar particle size and texture to the original soil. We also set up a control treatment with no burial. There were ten replicates for each treatment and control.

Experiment 2. Can seeds buried at a certain depth germinate and establish from that depth?

This experiment tried to ascertain how important remaining buried at a given depth or rising to the surface (vertical movement) is for the effective germination of a buried seed. We selected another ten 2 × 2 m plots, where, in October 1997, we took two soil core samples (4 cm diameter; 4 cm deep) from each plot, carefully separating the soil cylinder contents into four equal layers: 0–1 cm (after removing the litter); 1–2 cm; 2–3 cm and 3–4 cm. The two fractions from the same plot and depth were stored in the same paper bag, laboratory homogenized and divided into two subsamples, one of which was sown at the surface level (equivalent to a control treatment) and the other at the same depth as the point from which it was extracted. The sowing method was identical to that described in Experiment 1.

Samples from both experiments were kept in the greenhouse for 18 months, watered regularly, and checked every day. There was no heating system in the greenhouse and temperatures ranged from 3 to 39°C during the germination period. As soon as they were identified, seedlings were removed or transplanted to individual pots, in order to avoid deleterious effects between seedlings. We regularly disturbed the surface layer to maximize emergence and ascertain the total viable seed content in the control samples. After concluding the greenhouse test, we checked 10% of the control samples under a binocular microscope for remaining seeds, but no sound seeds were found in either of the two experiments. This method has proven to be particularly effective for the analysis of the viable seed contents of the soil at the community scale (for details, see Traba *et al.*, 1998). In the present study, we did not differentiate between seedlings from seeds (*sensu stricto*) and other types of shoots (bulbs, rhizomes, etc.), although only three species could potentially have the latter (*Agrostis castellana*, *Poa bulbosa* and *Ranunculus paludosus*).

Statistics

For Experiment 1, differences in seedling density and number of species between control and treatment samples were tested using the Friedman ANOVA test, due to the lack of normality and homoscedasticity of the data even after log-transformation.

For Experiment 2, differences in seedling density and number of species between control and treatment for all the samples were tested using the Friedman ANOVA test. The Wilcoxon matched-pairs test was used to analyse differences in both parameters between buried and control samples, for each depth level. All analyses were undertaken using the Statistica statistical package (Statsoft, 1985).

Results

Experiment 1. How deep can seeds from the shallow bank germinate and become established?

A total of 632 seedlings from 25 species emerged from the samples processed in Experiment 1 (Appendix 1). The majority of species (92%) and individual seedlings (85.4%) emerged from the control sample (surface sown), while both the number of species (Friedman test, $F = 23.885$; $P < 0.0001$) and the number of individual seedlings (Friedman test, $F = 23.876$; $P < 0.0001$) declined drastically when the shallow soil seed banks were buried below 1 cm of sterile sand (Figs 1a, b).

Only five species were able to establish seedlings when buried experimentally at more than 1 cm depth: *Agrostis castellana*, *Aphanes microcarpa*, *Crassula tillaea*, *Poa bulbosa* and *Ranunculus paludosus* (Appendix 1). Of these species, only *A. castellana* and *P. bulbosa* emerged from the deepest layer (3–4 cm), while the other species managed to germinate from layers situated at 2–3 cm depth.

Experiment 2. Can seeds buried at a certain depth germinate and establish from that depth?

A total of 844 seedlings from 24 species appeared in the set of all control samples and treatments in Experiment 2 (Appendix 2). There was a significant fall in the number of species and viable seeds as the depth of the soil bank increased (Friedman test for control samples; for density of emerged seeds: $F = 26.161$; $P < 0.0001$; for number of species: $F = 22.677$; $P < 0.0001$), although the main differences can be attributed to the large number of seeds in the first centimetre (100% of species and 98.9% of seeds). The control samples revealed that 14 species had viable seeds below the surface, four of which (*Crassula tillaea*, *Juncus bufonius*, *Poa bulbosa* and *Spergularia purpurea*) had seeds at the deepest layer (Appendix 2).

In spite of the presence of viable seeds below the first centimetre, emergence was almost completely prevented when the samples were buried at the same depth as they had been extracted from the soil, in comparison with the control samples (Figs 2a, b).

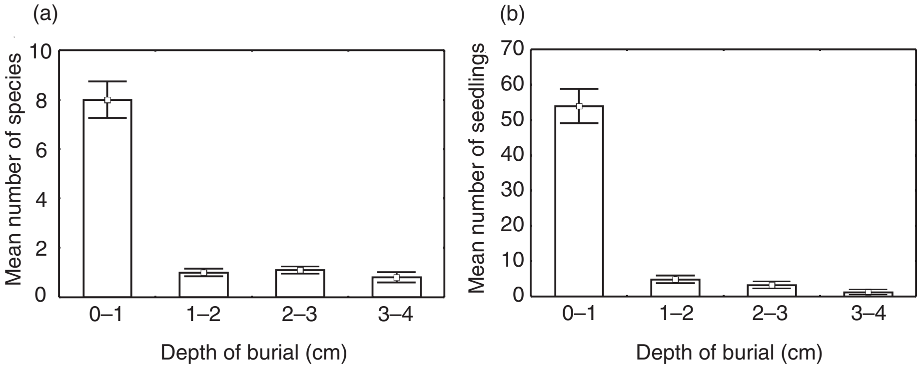

Figure 1. Experiment 1. (a) Mean number of species (± 1SE) and (b) mean number of seedlings (± 1SE) per sample (12.57 cm^2) emerged from surface soil bank samples buried at each depth.

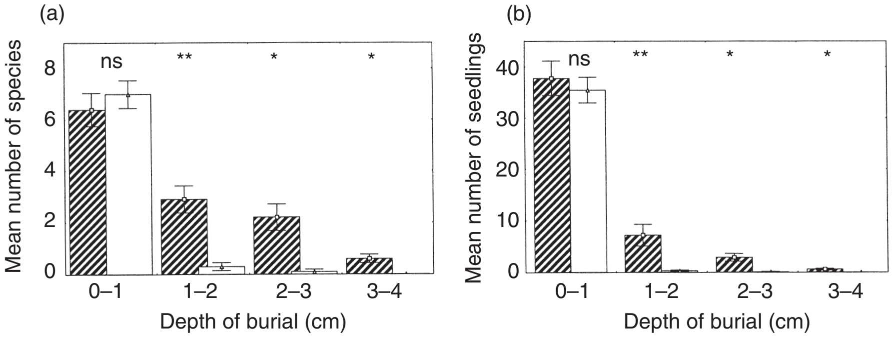

Figure 2. Experiment 2. (a) Mean number of species (± 1SE) and (b) mean number of seedlings (± 1SE) per sample (12.57 cm^2) emerged from control (hatched) and burial treatment (white) for the experimental depths. Symbols show results of the Wilcoxon matched-pairs test between control and treatment; ns, non significant; *, $P < 0.05$; **, $P < 0.01$.

Discussion

Several authors have mentioned that the soil seed bank is divided into two fractions according to its function – an active part that is ready for germination and another, inactive or storage, part (McGraw and Vavrek, 1989). Our results show that the majority of the seeds in Mediterranean grasslands are unable to emerge from depths below 1 cm, and the functional or active part of the Mediterranean grasslands bank seems to be restricted to the first centimetre, while the rest of the profile may be regarded as an inactive reservoir, from which any viable seeds have to rise to the surface in order to produce seedlings.

Only five species were able to emerge from a depth of more than 1 cm, although three showed the ability to resprout from bulbs (*R. paludosus*) or from thickened roots or similar organs (*P. bulbosa*, *A. castellana*). Thus, only two species originating unequivocally from seeds (*A. microcarpa* and *C. tillaea*) were able to emerge from below the 2 cm level (Appendix 1).

Other studies have yielded similar results, although none were performed in Mediterranean grasslands. Freas (1989), for example, mentions that the seeds of many annual species in desert areas cannot germinate and emerge from below a depth of 1 cm. In an analysis of the response of a grass in Great Britain, Smith *et al*. (1999) found percentages of emergence close to 100% down to a depth of 5 cm, with a drastic fall afterwards; Benvenuti *et al*. (2001) found uneven results, although beyond 3.6 cm seedling emergence was half that of the surface layer. Other studies with weeds in Britain have yielded similar results (Grundy and Mead, 1998; Grundy *et al*., 1999, 2003).

The profile of seed and species density indicates that there is a clear decline linked to an increase in depth. This coincides with the general idea that the maximum seed density lies close to the surface: in the first 5 cm (Akinola *et al*., 1998), in the first 4 cm (Hutchings and Booth, 1996), in the first 3 cm (Russi *et al*., 1992), in the first 2 cm (Moore and Wein, 1977; Bakker *et al*., 1991; O'Connor and Pickett, 1992), or in the first centimetre (Young *et al*., 1981). However, our results show that, in spite of the clear decline after the first centimetre, there are still many viable seeds in lower layers (more than 5700 seeds m^{-2} in the second centimetre and over 2300 seeds m^{-2} in the third centimetre) that are capable of germination when brought to the surface.

The poor emergence of seedlings from seeds found below the first centimetre may be due to a lack of germination. Nevertheless, the possibility that the seeds germinate in lower layers of the soil and are unable to emerge cannot be discarded, although burial generally induces dormancy rather than suicide germination (Benvenuti *et al*., 2001). The inability of seeds to germinate and emerge from deeper layers could also have been caused by dormancy, even when seeds were brought to the surface, although no viable seeds were found in the manual examination of the control subsamples using a binocular microscope. Physical extraction of seeds from buried subsamples would have been feasible, but this methodology is not effective in Mediterranean grasslands, where very small seeds are highly abundant (Traba *et al*., 1998).

The ability to emerge from deep layers has been related to seed size (Benvenuti *et al*., 2001; Grundy *et al*., 2003). Small seeds, such as the typical sizes found in Mediterranean grasslands (Azcárate *et al*., 2002; Peco *et al*., 2003), have less likelihood of emergence if they germinate from deep layers than large seeds (Grundy *et al*., 2003). In our case, this does not seem to be the reason for the lower emergence rate from deep layers, given that the analysed depth range (from 0–1 to 3–4 cm) does not seem to limit the emergence of the hypocotyl, and the two species that emerge from levels below 1 cm (*A. microcarpa* and *C. tillaea*) have seeds in the smallest size range found amongst Mediterranean grassland species (Azcárate *et al*., 2002; Peco *et al*., 2003). Nevertheless, more detailed studies of the incidence of suicide germination in these grasslands would be useful.

The inactivity found in the bank below the 1 cm level is probably due to the lack of germination in the species. Many factors that vary with depth affect germination capacity (for a review, see Baskin and Baskin, 2001), and our methodology does not permit the discernment of which ones, or which of their interactions, is the cause of the lack of germination in this case. Nevertheless, it is clear that the germination of these seeds is promoted by vertical movements that bring them to the surface. These movements may be caused by earthworms (Thompson *et al*., 1994; Willems and Huijsmans, 1994), ants (MacMahon *et al*., 2000), trampling by large herbivores (Harper, 1977; Chambers and MacMahon, 1994) or induced artificially by ploughing (Bakker et al., 1991). While the risk of surface germination in semi-arid environments such as this study area might put seedling survival at risk (Peart, 1984), our results seem to indicate that Mediterranean grassland species behave like typical gap detectors or bare-ground colonizers, requiring large amounts of sunlight to germinate (Pons, 2000). This effect may work alone or in conjunction with variations in soil temperature (Thompson *et al*., 1977; Benech-Arnold *et al*., 1988; Ghersa *et al*., 1992), because the deeper a seed is buried, the less it will be affected by temperature fluctuations.

These results may have several consequences for soil seed bank management in relation to the inability of the seed bank to germinate and/or emerge when buried under a thin layer of soil, mulch or the like. In addition, they suggest that soil seed bank numbers estimated from greenhouse germination treatments may be underestimated when samples have been placed in layers more than 1–2 cm deep.

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Appendix 1

Experiment 1. Samples collected from the surface layer (0–1 cm). Species and number of seedlings emerged from the different experimental burial depths

	Burial depths			
Species	0–1 cm	1–2 cm	2–3 cm	3–4 cm
Agrostis castellana	–	–	–	2
Aphanes microcarpa	32	1	1	–
Bromus tectorum	1	–	–	–
Cerastium ramossisimum	1	–	–	–
Cerastium semidecandrum	10	–	–	–
Crassula tillaea	68	–	2	–
Erophila verna	20	–	–	–
Evax carpetana	1	–	–	–
Herniaria hirsuta	9	–	–	–
Juncus bufonius	1	–	–	–
Leontodon taraxacoides	1	–	–	–
Linaria spartea	1	–	–	–
Mibora minima	1	–	–	–
Moenchia erecta	12	–	–	–
Parentucelia latifolia	3	–	–	–
Plantago bellardii	2	–	–	–
Poa bulbosa	309	47	26	10
Ranunculus paludosus	–	–	3	–
Sedum caespitosum	4	–	–	–
Spergula pentandra	1	–	–	–
Spergularia purpurea	45	–	–	–
Tolpis barbata	2	–	–	–
Tuberaria guttata	12	–	–	–
Veronica verna	3	–	–	–
Vulpia ciliata	1	–	–	–

Appendix 2

Experiment 2. Samples collected from different depths. Species and number of seedlings emerged from control (surface) and burial treatments. For 0–1 cm samples, control and burial are the same treatment

	Collection depths							
	0–1 cm		1–2 cm		2–3 cm		3–4 cm	
Species	Control	Burial	Control	Burial	Control	Burial	Control	Burial
Aphanes microcarpa	29	20	17	1	5	–	–	–
Cerastium ramossisimum	4	1	–	–	–	–	–	–
Cerastium semidecandrum	4	8	1	–	1	–	–	–
Crassula tillaea	54	26	18	–	7	–	1	–
Erophila verna	11	7	1	–	1	–	–	–
Heliotropium europaeum	1	–	–	–	–	–	–	–
Herniaria hirsuta	11	10	4	–	1	–	–	–
Juncus bufonius	–	2	–	–	3	–	1	–
Logfia gallica	–	1	–	–	–	–	–	–
Moenchia erecta	1	5	–	–	–	–	–	–
Parentucelia latifolia	1	1	–	–	–	–	–	–
Plantago bellardii	3	4	–	–	–	–	–	–
Poa bulbosa	186	209	5	2	2	1	2	–
Sagina apetala	2	–	–	–	1	–	–	–
Sedum caespitosum	11	16	7	–	2	–	–	–
Spergularia purpurea	12	7	13	–	3	–	2	–
Stellaria media	4	1	1	–	1	–	–	–
Tolpis barbata	–	4	–	–	–	–	–	–
Trifolium glomeratum	–	1	1	–	–	–	–	–
Trifolium suffocatum	1	4	–	–	–	–	–	–
Tuberaria guttata	36	24	3	–	2	–	–	–
Veronica arvensis	2	1	–	–	–	–	–	–
Veronica verna	1	3	1	–	–	–	–	–
Vulpia muralis	4	–	–	–	–	–	–	–

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