

Rapid burial has differential effects on germination and emergence of small- and large-seeded herbaceous plant species

Sandra Burmeier*, Tobias W. Donath, Annette Otte and R. Lutz Eckstein

Institute of Landscape Ecology and Resource Management, Research Centre for Biosystems, Land Use and Nutrition (IFZ), Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

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Abstract

The dynamics of many plant populations essentially depend upon seed and seedling stages, and a persistent seed bank may give species an opportunity to disperse through time. Seed burial is a decisive prelude to persistence and may strongly influence seed-bank dynamics. The fate of buried seeds depends on species-specific traits, environmental conditions and possibly also burial mode. We tested seed germination, seedling emergence and growth of the co-occurring herbaceous flood-meadow species *Arabis nemorensis*, *Galium wirtgenii*, *Inula salicina*, *Sanguisorba officinalis* and *Selinum carvifolia* in response to the experimental manipulation of burial depth (0, 1, 2, 4, 8, 12 cm) and substrate type (sand, clay). Increasing burial depth led to decreased germination, emergence and growth in all species studied, and seedling growth differed significantly between substrate types. The responses of species differed on an individual basis, but also showed a higher-ranking pattern based on seed size. Larger-seeded species were able to emerge from greater depths and experienced less depth-mediated growth inhibition than smaller-seeded species, which, in turn, had higher survival rates during burial and were less likely to experience fatal germination. Based on these results, we suggest that herbaceous flood-meadow species have developed two different seed-size based strategies for coping with the extreme recruitment conditions prevailing in flood meadows, the balance of which seems to be maintained by disturbance events.

Keywords: burial experiment, desiccation cracks, dormancy, seed bank, seed mass, seed mortality, soil depth inhibition

*Correspondence
Fax: + 49-641-9937169
Email: sandra.burmeier@umwelt.uni-giessen.de

Introduction

The dynamics of many plant populations and communities essentially depend upon seed and seedling stages (Parker *et al.*, 1989), and a persistent seed bank enables species to disperse through time. This is particularly relevant in frequently disturbed habitats such as flood meadows, where a soil seed-bank may buffer populations against environmental variability and thus guarantee their long-term persistence (Thompson, 2000; Hölzel and Otte, 2004a).

As seeds generally cannot persist on the soil surface for long periods due to germination or predation, burial is an essential prelude to persistence (Thompson *et al.*, 1993; Grime, 2001). It may convey benefits such as reduction of air exposure, maintenance of high humidity levels and protection against extreme temperatures and foraging granivores and herbivores (Forcella *et al.*, 2000). However, it may also have inhibitory effects on germination and emergence (Zhang and Maun, 1994). Possible burial mechanisms include frost heave, earthworm activity and other naturally occurring soil disturbances (cf. Chambers and MacMahon, 1994), including entrapment by desiccation cracks (Elberling, 2000; Espinar *et al.*, 2005; Burmeier *et al.*, 2010) and coverage by flood-borne sediments.

Although seed burial and its impacts on seed germination and seedling emergence have been studied extensively, most investigations have either concentrated on weed seeds buried through land-use practices (e.g. Colosi *et al.*, 1988; Cussans *et al.*, 1996; Vleeshouwers, 1997; Benvenuti *et al.*, 2001; Grundy *et al.*, 2003; Oliveira and Norsworthy, 2006; Wilson *et al.*, 2006; Davis and Renner, 2007) or on plant species growing in dune environments where their seeds might become covered by moving sand (e.g. van der Valk, 1974; Maun and Lapiere, 1986; Zhang and Maun, 1990, 1994; Yanful and Maun, 1996; Chen and Maun, 1999; Ren *et al.*, 2002; Li *et al.*, 2006). The impacts of other burial mechanisms such as desiccation crack entrapment or sediment coverage are currently still unknown.

Generally, buried seeds may encounter any of five possible fates: (1) death prior to germination, e.g. due to pathogens (Davis and Renner, 2007), predation (Westerman *et al.*, 2003) or senescence (Telewski and Zeevaart, 2002); (2) death following germination, i.e. fatal germination where the seedling dies before reaching the soil surface; (3) successful germination where the seedling eventually emerges at the soil surface; (4) persistence in a non-dormant state (cf. Vleeshouwers *et al.*, 1995); or (5) dormancy. Only the latter two will contribute to a persistent seed bank (Thompson, 2000).

Which fate a particular seed experiences depends on a range of factors. Species-specific traits such as seed size and shape may influence the probability of burial (Chambers *et al.*, 1991; Thompson, 2000; Benvenuti, 2007; Schmiede *et al.*, 2009), the likelihood of a seed persisting in the soil (Thompson *et al.*, 1993) and the maximum depth of seedling emergence (van der Valk, 1974; Maun and Lapierre, 1986; Bond *et al.*, 1999; Leishman *et al.*, 2000; Grundy *et al.*, 2003; Li *et al.*, 2006). The prime environmental factors governing the germination of buried seeds are temperature (Thompson and Grime, 1983; Benech-Arnold *et al.*, 2000; Oliveira and Norsworthy, 2006), light quality (Baskin and Baskin, 2001), air quality and soil water potential (Forcella *et al.*, 2000). Soil particle composition substantially influences soil air quality and soil water potential (Benvenuti, 2003), which, in turn, may influence soil penetration resistance and thus also the rate of seedling emergence (Vleeshouwers, 1997).

In the absence of dormancy, the position of seeds within the soil profile may exert a strong influence on whether germination will be successful or fatal, and emergence generally declines with increasing depth (Zhang and Maun, 1990; Redmann and Qi, 1992; Cussans *et al.*, 1996; Benvenuti *et al.*, 2001; Begum *et al.*, 2006; Oliveira and Norsworthy, 2006; Wilson *et al.*, 2006; Davis and Renner, 2007). As seedlings emerging from greater depths must penetrate a thicker layer of soil before eventually reaching the surface, they emerge later than siblings germinating in shallower depths (Cussans *et al.*, 1996; Benvenuti *et al.*, 2001; Ren *et al.*, 2002), are generally smaller (Li *et al.*, 2006) and thus in an inferior competitive position, which may lead to reduced biomass gains and can have large fitness consequences in later life stages (Cook, 1980).

The course or pathway of burial may also determine seed fate. Seeds that are entrapped by desiccation cracks (cf. Burmeier *et al.*, 2010) or covered with sediment are buried much more rapidly than those that are incorporated into the ground by slowly progressing burial mechanisms such as rain wash (van Tooren, 1988), frost heave (van Tooren, 1988) or earthworm activity (van der Reest and Rogaar, 1988;

Willems and Huijsmans, 1994). This could influence the effectiveness of depth-sensing mechanisms and thus have important consequences for the likelihood of fatal germination. However, data on the impacts of rapid burial mechanisms on seed germination and emergence are currently still lacking.

Here we present the results of an experimental investigation on the impacts of substrate type and burial depth on germination, emergence and growth of co-occurring herbaceous plant species. Our main objective was to shed light on the effects of rapid burial by desiccation crack entrapment and flood-borne sediment coverage – both of which are common to alluvial meadows (Hölzel and Otte, 2001; Burmeier *et al.*, 2010) – and assess its implications for community composition and dynamics. We specifically tested the following hypotheses:

- (1) The fate of buried seeds depends on burial depth, and the likelihood of survival during burial is expected to increase with increasing depth due to a lack of germination-triggering clues.
- (2) Post-burial seedling emergence depends on soil texture and is expected to be higher in coarse-textured sandy soil than in fine-textured clay soil, due to a lower penetration resistance.
- (3) Species differ in their response to the manipulated factors, depending on their seed mass:
 - (i) large-seeded species are expected to have higher emergence rates when buried in greater depths than small-seeded species, due to their greater energy resources;
 - (ii) large-seeded species are also expected to have higher rates of fatal germination and lower rates of survival during burial than small-seeded species, due to less effective depth-sensing mechanisms.

Methods

Study species

We selected five angiosperm plant species that differ in seed mass (Table 1). This allowed us to test the effects of burial depth and soil texture on germination, emergence and growth dependent on seed size and shape. The selected species are common to species-rich flood meadows along the northern Upper Rhine (cf. Donath *et al.*, 2003) and represent characteristic families of dicotyledons of these, as well as similar, grassland habitats. Their seed sizes cover about 70% of the total seed-size range of species growing in alluvial meadows (Hölzel and Otte, 2004b).

Table 1. Diaspore and germination characteristics of the species used in the burial experiments (mean \pm SE)

Species	Family	Seed mass (mg) ^a	Seed length (mm) ^b	Seed width (mm) ^b	Climate chamber experiment ^c		Germination in outdoor experiment (%) ^e
					Germination (%) ^d	Viability (%) ^d	
<i>Arabis nemorensis</i> (Hoffm.) Koch	Brassicaceae	0.05 \pm 0.001	0.90 \pm 0.02	0.59 \pm 0.01	72.6 \pm 2.7	91.2 \pm 3.1	82.2 \pm 2.8
<i>Inula salicina</i> L.	Asteraceae	0.12 \pm 0.003	1.53 \pm 0.02	0.38 \pm 0.01	5.0 \pm 0.2	79.6 \pm 2.7	60.8 \pm 6.3
<i>Galium wirtgenii</i> F.W. Schultz	Rubiaceae	0.5 \pm 0.01	1.16 \pm 0.02	0.86 \pm 0.02	89.3 \pm 1.9	94.0 \pm 2.3	63.6 \pm 5.2
<i>Selinum carvifolia</i> (L.) L.	Apiaceae	0.95 \pm 0.01	2.60 \pm 0.06	1.24 \pm 0.03	61.3 \pm 2.6	83.6 \pm 1.2	75.4 \pm 3.1
<i>Sanguisorba officinalis</i> L.	Rosaceae	2.08 \pm 0.07	3.11 \pm 0.04	1.66 \pm 0.04	17.7 \pm 3.8	89.6 \pm 2.1	75.6 \pm 3.0

^a Averages calculated from bulk data for 50 seeds ($n = 10$).

^b All appendices (e.g. pappus for *I. salicina*) removed prior to measuring ($n = 50$).

^c Six-week germination trial in a temperature-controlled incubator with 10/20°C (12/12 h) and 14 h photoperiod.

^d 50 seeds per replicate ($n = 5$).

^e 100 seeds per replicate ($n = 5$).

Seed collection, storage and germination tests

Seeds were collected between 15 July and 23 September 2008 (depending on the species and their degree of ripeness) in flood meadows along the northern Upper Rhine. For each species, seeds were collected from at least 20 individuals of at least five populations, with the exception of *Selinum carvifolia* of which only three populations of sufficient size were available. Seeds were air-dried, manually cleaned and stored in darkness at room temperature ($\sim 20^\circ\text{C}$) until sowing in November 2008. Before the beginning of the experiments seeds were tested for viability and germinability with climate-chamber and outdoor germination trials, and the results showed that most of the seeds ($> 79\%$) of all species were viable and that outdoor conditions were suitable for triggering germination (Table 1).

Experimental design

We used a three-factorial, completely randomized design to determine the effects of *species identity*, *substrate type* and *burial depth* on seed germination, seedling emergence and seedling biomass. Each combination of factors was replicated five times. Each replicate consisted of a 1-litre pot (diameter 11.7 cm, height 13.5 cm) filled with soil up to the rim, which was supplied with 50 seeds. The factor 'substrate type' had two levels, which were chosen to represent the two different burial modes: loamy sand, as found in the sediment layers that accumulate during flooding (called 'sand' in the following), and

silty clay typical of sites featuring desiccation cracks (called 'clay' in the following) (Table 2). The material was collected from the same sites along the northern Upper Rhine area where the seeds had been harvested and was steam sterilized prior to the experiments (6 h at 80°C , Sterilo 1 K, MAFAC/Schwarz, Alpirsbach, Germany). As for burial depth, seeds were either exposed at the surface or buried at 1, 2, 4, 8 or 12 cm depth, respectively.

The experiment was set up between 10 and 14 November 2008. This starting date allowed for ample time for cold stratification, which enhances germination of several of the study species (Baskin and Baskin, 2001; Hölzel and Otte, 2004b). As we assumed soil temperature fluctuation to be a main clue for depth-sensing in buried seeds, we refrained from setting the pots up in a greenhouse and chose to bury them in

Table 2. Characteristics of the two substrate types used in the experiment

Substrate type	Clay	Sand
Particle composition		
Sand (%)	9.9	81.8
Silt (%)	43.5	11.5
Clay (%)	46.5	6.7
Soil nutrients and pH		
C total (%)	6.735	2.214
N total (%)	0.492	0.087
S total (%)	0.1	0.039
P CAL (mg kg^{-1})	35.8	77.8
K CAL (mg kg^{-1})	44.1	66.4
pH	7.09	6.74

the ground in an experimental garden close to Giessen, Germany (50°32'12"N 8°41'35"E, 172 m above sea-level). The pots were positioned randomly beneath wooden frames (height: 50 cm), which were covered with wire mesh and gauze to prevent both access of larger herbivores and seed influx from outside. To prevent slug herbivory, slug pellets were distributed on the soil between the pots. From March 2009 onwards, all pots were watered regularly (one to two times per week, depending on weather conditions). Soil temperature was monitored with automatic data loggers (Tiny Tag Transit TG-0050, Gemini Data Loggers Ltd, Chichester, UK) at hourly intervals. As a safeguard against technical failure, we set up two loggers for each combination of soil type and burial depth. If both of them kept recording data throughout the experiment, their averages were used for the subsequent analyses.

The experiment was run in three parallel approaches focusing on germination (I), seedling emergence (II) and growth (III), respectively. In approach I, seeds were sown into bags made of nylon mesh fabric (mesh width 250 µm), which were then buried in the pots. This approach did not include a 0 cm treatment, i.e. we did not expose any bags at the soil surface. The bags were retrieved on 3 June 2009, and the seeds were transferred to Petri dishes equipped with moist filter paper, kept in a germination chamber for 4 weeks [10/20°C (12/12 h), 14 h photoperiod] and monitored weekly for germination. Seeds remaining at the end of this period were visually tested for viability, with viable seeds being defined as those containing a firm white embryo. They were assumed to be dormant. The sum of the number of seeds germinating in the climate chamber and those remaining in a dormant state were considered to have survived during burial. In approach II, seeds were sown directly at the respective burial depths, and seedlings penetrating the soil surface were counted and removed once a week from the onset of germination in late March 2009 until the experiment was terminated on 9 June 2009. In approach III, seeds were also sown directly, but seedlings were not removed. Instead, we measured average seedling length per pot at weekly intervals. On 9 June 2009 we determined the number of seedlings growing in the pots, and harvested, dried (48 h at 70°C) and weighed the entire above-ground biomass.

Data analyses

From the data from approach II, we calculated cumulative emergence over the entire duration of the experiment. Based on Houle *et al.* (2001), we also calculated an emergence velocity index (EVI)

according to the following formula:

$$EVI = \sum_{w=1}^W (G_w/w)/G_t \times 100$$

where G_w represents the number of seedlings emerging during week w , w is the number of weeks since the onset of emergence, W is the total number of weeks and G_t is the total number of seedlings emerging during the entire duration of the experiment. The higher the value of the index, the faster is emergence. Fatal germination (FG), i.e. germination in a depth from which the seedling could not reach the soil surface and died before emergence, was estimated for each combination of soil type and depth according to the following formula:

$$FG = n - \text{mean}_{\text{surviving seeds in approach I}} - \text{mean}_{\text{emerged seeds in approach II}}$$

where n is the number of seeds sown per pot (= 50), assuming that all of these were capable of germinating. This formula may slightly overestimate fatal germination as all seeds that had not survived during burial were considered to have germinated. However, since pre-trials had shown that seed viability was very high across species (see Table 1), we deemed this approach to be acceptable. As the calculations resulted in a single value for each combination of factors, we estimated confidence intervals with a bootstrap procedure, i.e. we drew five samples with replacement from the distributions of both surviving seeds and emerged seedlings for each combination of factors and used these values to calculate FG according to the formula above, repeated this procedure 3125 times (to cover all possible combinations of values) and used the resulting FG distribution to calculate the borders of the 95% confidence intervals. Univariate three-way analyses of variance (ANOVAs) were used to test for the effects of *species identity*, *burial depth* and *substrate type* on the dependent variables (final cumulative emergence, germination velocity, seedling length, survival during burial). To test for the effects on biomass, we used a three-way analysis of covariance (ANCOVA) with the *total number of seedlings per pot* as continuous predictor variable.

To quantify the relative contribution of each factor and their interactions to total variability we calculated the ratio of the sum of squares of the respective factor or interaction to the total sum of squares. Two-way ANOVAs were used to test for the effects of *burial depth* and *substrate type* on cumulative seedling emergence and survival during burial at the species level. Percentage data were arcsin-square root transformed prior to analysis (Quinn and Keough, 2002). We used repeated-measures analysis (RM-ANOVA) to assess the overall effects of time, each of the factors and their interactions on the average seedling length per pot at each date. As the data did not meet the assumptions of

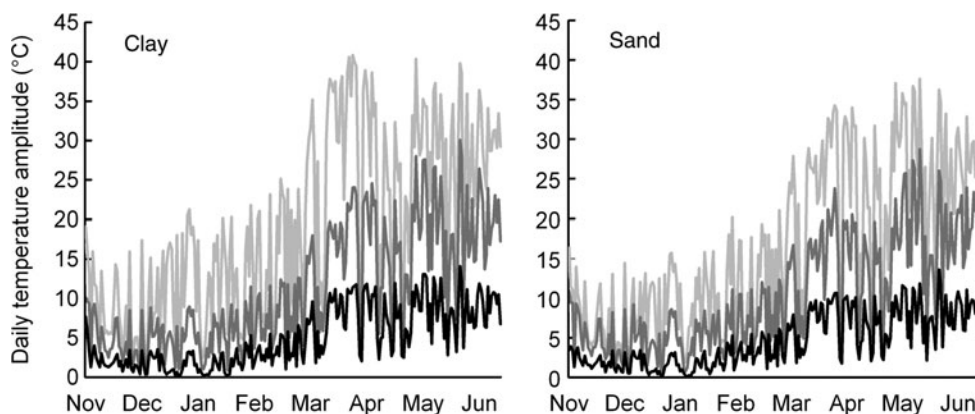


Figure 1. Daily soil temperature amplitudes in clay and sand throughout the course of the experiment (November 2008 to June 2009) at different depths (light grey line, 0 cm; dark grey line, 2 cm; black line, 8 cm). Temperatures were measured at hourly intervals. Data presented are averages from two loggers per combination of factors. Temperature amplitudes at 1, 4 and 12 cm were also measured, but are not shown.

sphericity and compound symmetry, P values were obtained using Pillai's trace, which has been found to be particularly robust (Scheiner, 2001). ANOVA analyses were performed using R, version 2.8.1 (R Development Core Team, 2008), and ANCOVA and RM-ANOVA were performed using Statistica 6 for Windows (2001; StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Soil temperatures

Daily temperature amplitudes across soils differed significantly between burial depths ($F_{5,2850} = 269.8$, $P < 0.0001$), whereas daily mean temperatures across soils did not ($F_{5,2850} = 0.5$, $P = 0.78$). Average daily temperature amplitudes drastically decreased with increasing depths, differed between substrate types ($F_{1,2854} = 17.34$, $P < 0.0001$) and were generally higher in clay than in sand (Fig. 1).

Survival of buried seeds

Survival during burial turned out to be strongly species-dependent, as species identity alone explained almost 70% of the total variation (Table 3). However, the main effect of burial depth and the interaction between species identity and burial depth were also significant. The small-seeded species *Arabidopsis nemorensis* and *Inula salicina* had the highest percentages of surviving seeds, whereas *Sanguisorba officinalis*, the species with the largest seed mass, had the lowest percentages (Fig. 2). Generally, the percentage of surviving seeds increased with increasing soil depths for all species except *Galium wirtgenii* (Fig. 2), resulting in a significant interaction between species identity and burial depth (Table 3).

Cumulative seedling emergence

Species identity and burial depth also had a significant effect on the total number of seedlings emerging (Table 4). Burial depth, together with its interactions with species identity and soil, accounted for more than 50% of the total variation (Table 4). Generally, emergence decreased with increasing depth, with 12 cm representing the limit from which seedlings could no longer emerge (Fig. 3). However, not all study species showed a monotonically decreasing relationship between emergence and burial depth, as indicated by a significant interaction between species identity and burial depth (Table 4). While the small-seeded species *A. nemorensis* and *I. salicina* showed maximum seedling emergence from seeds sown directly on the soil surface, emergence of *G. wirtgenii*, *S. carvifolia* and *S. officinalis* peaked at 1 cm in sand and 2 cm in clay (Fig. 3). The species also differed with regard to the maximum depth from which emergence was observed. *A. nemorensis* and *I. salicina* emerged from a maximum depth of 1 cm, *S. carvifolia* and *S. officinalis* emerged

Table 3. Results of a three-way ANOVA on the effects of species identity (I), substrate type (S) and burial depth (D) on the proportion of seeds surviving during burial. df = Degrees of freedom, MQ = mean sum of squares, vc (%) = relative contribution of individual factors and their interactions to total variation

Source of variation	df	MQ	P	vc (%)
I	4	22.4286	<0.0001	69.7
S	1	0.0414	0.1210	0.1
D	4	3.8545	<0.0001	12.0
I × S	4	0.1942	0.0251	0.6
I × D	16	1.7739	<0.0001	5.5
S × D	4	0.2026	0.0207	0.6
I × S × D	16	0.2875	0.4025	0.9
Residuals	200	3.4115		10.6

from up to 4 cm, and *G. wirtgenii* sporadically even emerged from a depth of 8 cm (Fig. 3). The interaction between substrate type and burial depth was also significant (Table 4). Emergence percentages across species were generally higher in sand than in clay for seeds lying on the soil surface, whereas they were higher in clay for seeds that had been sown at greater

depths (Fig. 3). At intermediate depths, the percentage of emerging seeds did not differ between soils.

Emergence velocity

Emergence velocity was significantly affected by species identity and burial depth, with burial depth alone accounting for more than 30% of the total variation (Table 4) and emergence velocity generally decreasing with increasing burial depth (Table 5). However, there was a significant interaction between burial depth and substrate type (Table 4). While emergence velocity was highest at the soil surface in most cases, it was highest in 1 cm depth for those seeds of *I. salicina*, *S. carvifolia* and *S. officinalis* that had been sown in clay (Table 5). Temporal emergence patterns differed between species, as indicated by a significant interaction between species identity and burial depth (Table 4). Whereas *A. nemorensis*, *S. carvifolia* and *S. officinalis* emerged comparatively rapidly with a pronounced peak at a fairly early stage, *I. salicina* kept emerging throughout the experiment, as indicated by much lower values for the emergence velocity index (Table 5). *G. wirtgenii* featured intermediate emergence velocity.

Fatal germination

The share of seeds dying through fatal germination differed between species and burial depths. In the two small-seeded species *A. nemorensis* and *I. salicina*, fatal germination decreased with increasing depths, whereas the opposite was the case for the larger-seeded species (Fig. 4). Across depths, estimated fatal germination was much lower for the small-seeded species than for the others.

Seedling biomass

Seedling density had a significant effect on the final seedling biomass per pot and explained more than 8% of the total variation. However, beyond density,

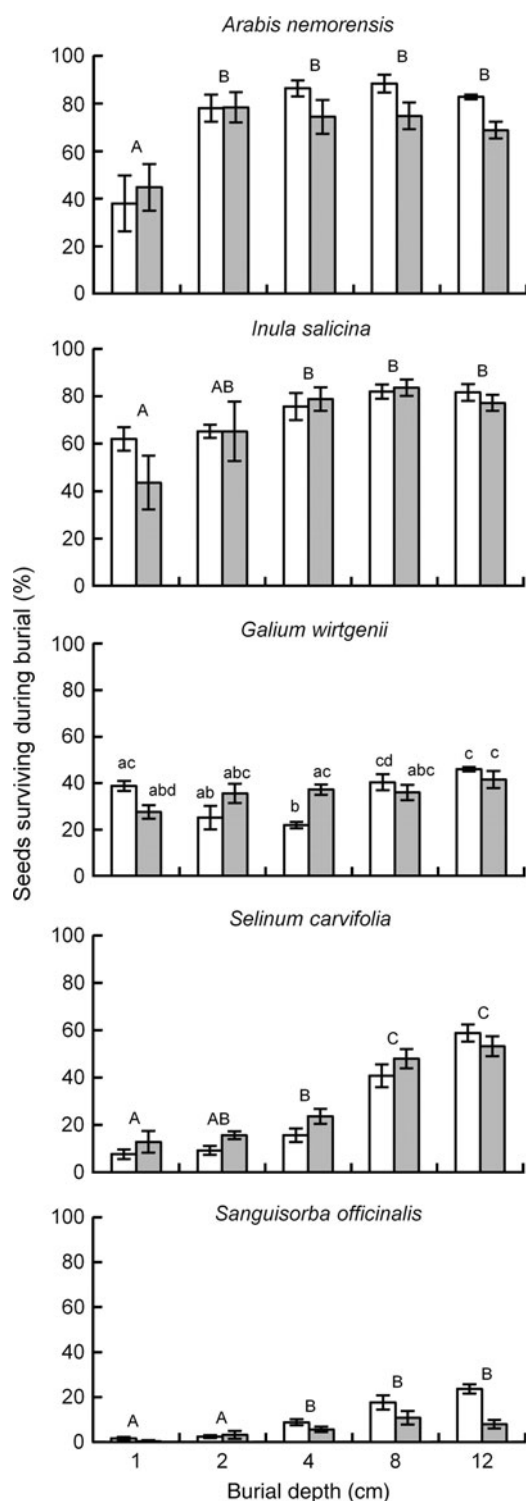


Figure 2. Final percentage of seeds remaining viable during burial at different depths in clay (open bars) and sand (shaded bars). Data are presented as means \pm SE ($n = 5$). Differences between means were tested *a posteriori* using Tukey's HSD test. For *G. wirtgenii* differences between all means were tested as the interaction between substrate type and burial depth was significant ($F_{4,40} = 6.63$, $P < 0.001$). For all other species only differences among burial depths across substrate types were tested because the main effect of burial type was significant ($F_{4,40} = 6.96$, $P < 0.001$). For *S. officinalis*, the main effect of substrate type was also significant ($F_{1,40} = 14.4$, $P < 0.001$). Bars and burial depth with different lower- or upper-case letters, respectively, are significantly different ($P < 0.05$).

Table 4. Results of a three-way ANOVA on the effects of *species identity* (I), *substrate type* (S) and *burial depth* (D) on cumulative emergence, emergence velocity and average final seedling length. df = Degrees of freedom, MQ = mean sum of squares, vc (%) = relative contribution of individual factors and their interactions to total variation. Burial depths from which no emergence occurred across species were excluded from the analysis (8 and 12 cm)

Source of variation	df	Cumulative emergence			Emergence velocity			Seedling length		
		MQ	P	vc (%)	MQ	P	vc (%)	MQ	P	vc (%)
I	4	6.3656	<0.00001	19.9	603.96	<0.0001	22.5	342.6	<0.0001	49.8
S	1	0.0071	0.5682	0.0	11.49	0.0707	0.4	5.3	0.0069	0.8
D	3	5.4651	<0.00001	17.0	865.45	<0.0001	32.2	56.2	<0.0001	8.2
I × S	4	0.9525	<0.00001	3.0	52.15	0.0060	1.9	21.7	<0.0001	3.2
I × D	12	8.0138	<0.00001	25.0	280.17	<0.0001	10.4	60.9	<0.0001	8.9
S × D	3	3.6298	<0.00001	11.3	170.15	<0.0001	6.3	43	<0.0001	6.3
I × S × D	12	1.1132	<0.00001	3.5	147.86	0.0001	5.5	45	<0.0001	6.5
Residuals	160	3.464		10.8	555.28		20.7	112.9		16.4

species identity and burial depth both had a significant effect on total biomass per pot, and together with their interaction explained almost 35% of the total variation (Table 6). The small-seeded species *A. nemorensis* and *I. salicina* reached maximum biomass production after emergence from the soil surface, whereas the larger-seeded *S. carvifolia* and *S. officinalis* reached maximum biomass production after emergence from intermediate depths (data not shown).

Average seedling length development was significantly affected by species identity, substrate type and burial depth (RM-ANOVA, data not shown). However, we also observed a clear time effect, and none of the patterns were consistent throughout the entire duration of the experiment (RM-ANOVA, significant factor × time interactions, data not shown). For *G. wirtgenii*, average length of seedlings emerging from different depths developed roughly parallel with time, whereas for all other species seedlings that had emerged from shallower depths generally did not only remain larger than their siblings from greater depths throughout the course of the experiment, but also extended their lead with time (data not shown). As for final seedling length, species identity explained by far the largest fraction of the total variation (Table 4). However, the main effects of substrate type and burial depth, as well as all interactions between the factors, were also significant. Across species, seedlings emerging from shallower depths were generally larger than those emerging from greater depths (data not shown).

Discussion

Effects of burial depth

We found that the likelihood of survival during burial generally increased with increasing depth (Fig. 2),

which supports our first hypothesis. This could indicate that the seeds of the species tested possess depth-sensing mechanisms that lead to germination inhibition when seeds are buried so deep that successful seedling emergence would be unlikely. Our findings are in line with those of Benvenuti *et al.* (2001), who observed that seeds of 20 weed species perceived unfavourable germination conditions and responded by depth-mediated germination inhibition. Such an inability to germinate at greater depths may have important ecological implications as a seed bank that is inactive because of its depth can be re-activated as soon as disturbances remove the cover layer and bring the seeds close to the surface again (Zhang and Maun, 1994; Ren *et al.*, 2002). In our study, this pattern was least pronounced for *G. wirtgenii* (Fig. 2), which, however, also had the highest maximum depth of emergence and thus presumably experienced the least selection pressure towards effective depth-sensing mechanisms. Most probably, soil temperature fluctuation acted as the main depth-sensing clue (Fig. 1) since light only penetrates the very top layer of the soil surface (Woolley and Stoller, 1978; Benvenuti, 1995) and may thus only be used to distinguish between 'surface' or 'buried', but does not provide any further information about the depth of burial.

Many studies have found a direct relationship between burial depth and emergence: the deeper the burial, the lower the germination (Zhang and Maun, 1990; Redmann and Qi, 1992; Cussans *et al.*, 1996; Benvenuti *et al.*, 2001; Begum *et al.*, 2006; Oliveira and Norsworthy, 2006; Wilson *et al.*, 2006; Davis and Renner, 2007). We, however, observed such a linear relationship only for the two small-seeded species, whereas the three species with larger seeds showed a parabolic response curve, i.e. they had highest emergence percentages from intermediate burial depths (Fig. 3). This is in line with several other studies (e.g. Maun and Lapierre, 1986; Colosi *et al.*,

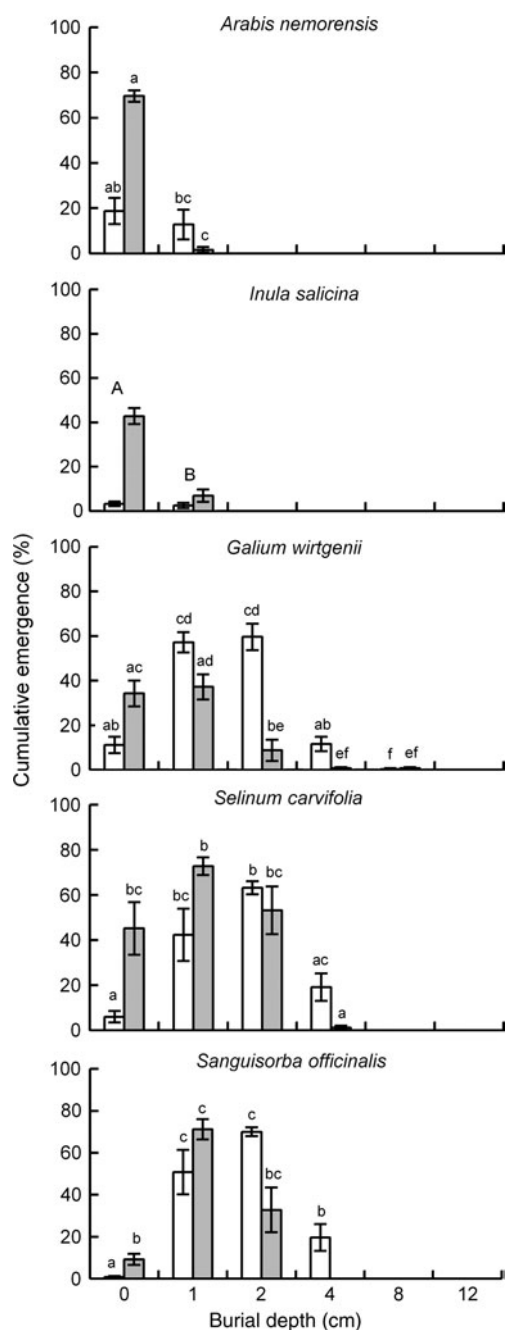


Figure 3. Cumulative seedling emergence of the study species from clay (open bars) and sand (shaded bars). Data are presented as means ± SE (*n* = 5). Differences between means were tested *a posteriori* using Tukey's HSD test. Interactions between substrate type and burial depth were significant for all species (*A. nemorensis*: $F_{1,16} = 12.9$, $P < 0.05$; *S. carvifolia* and *S. officinalis*: $F_{3,32} > 8.4$, $P < 0.001$; *G. wirtgenii*: $F_{4,40} = 10.5$, $P < 0.0001$) with the exception of *I. salicina*. Here, only differences among burial depths across substrate types were tested as the main effect of burial depth was significant ($F_{1,16} = 7.9$, $P < 0.05$). Bars and burial depths with different lower- or upper-case letters, respectively, are significantly different ($P < 0.05$).

Table 5. Emergence velocity in clay and sand (mean ± SE). Numbers give values of the emergence velocity index (EVI). The higher the EVI, the faster emergence occurred

Depth (cm)	<i>Arabis nemorensis</i>		<i>Inula salicina</i>		<i>Galium wirtgenii</i>		<i>Selinum carvifolia</i>		<i>Sanguisorba officinalis</i>	
	Clay	Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay	Sand
0	80.2 ± 6.0	84.8 ± 4.1	11.5 ± 3.9	30.6 ± 2.2	76.3 ± 9.3	55.5 ± 6.2	34.0 ± 15.8	98.0 ± 1.2	26.7 ± 19.4	89.7 ± 4.8
1	59.1 ± 21.1	12.2 ± 9.7	24.1 ± 19.1	22.2 ± 8.6	73.5 ± 5.2	54.1 ± 8.0	82.4 ± 2.9	58.4 ± 2.1	67.5 ± 8.9	47.9 ± 1.8
2	0	0	0	0	53.9 ± 5.5	30.2 ± 12.0	52.5 ± 4.9	30.2 ± 3.4	39.3 ± 2.4	30.2 ± 2.1
4	0	0	0	0	24.0 ± 2.8	5.7 ± 3.5	23.9 ± 6.1	17.0 ± 11.8	23.9 ± 1.1	0
8	0	0	0	0	20.0 ± 20.0	5.7 ± 3.5	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0

1988; Yanful and Maun, 1996; Chen and Maun, 1999; Ren *et al.*, 2002) and may have been caused by different water availability and increased predation pressure on the soil surface. Large seeds require more time to imbibe water prior to germination and are thus exposed to greater risks of dehydration at the soil surface than smaller seeds (Forcella *et al.*, 2000). Furthermore, they represent a more attractive food

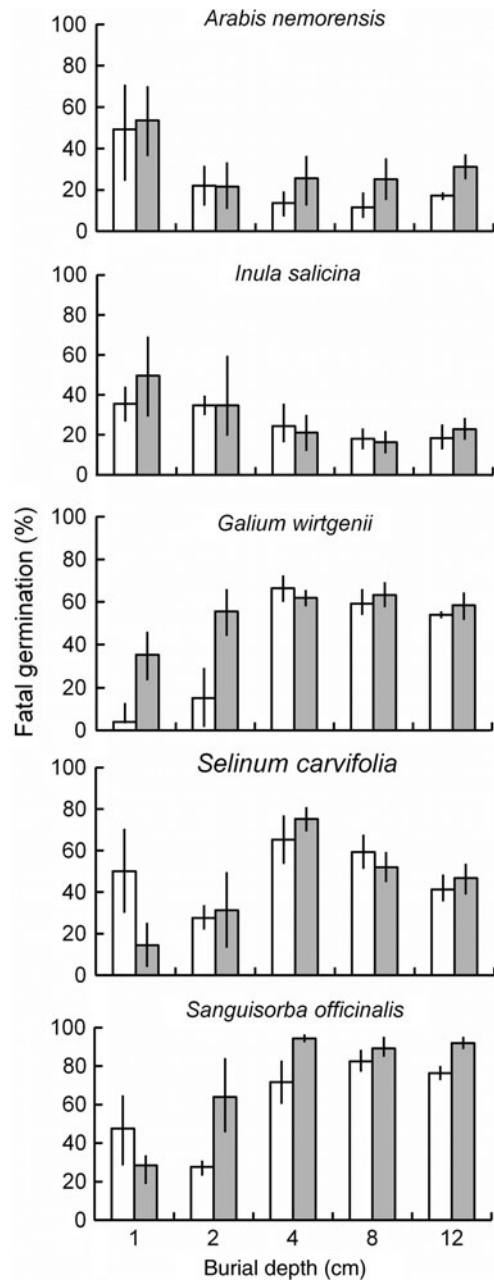


Figure 4. Average percentage of seeds dying through fatal germination (as calculated from the difference between seeds surviving during burial and seeds emerging) while germinating from different depths in clay (open bars) and sand (shaded bars). Error bars give the limits of bootstrapped 95% confidence intervals.

Table 6. Results of a three-way ANCOVA with seedling number (N) as continuous predictor variable on the effects of species identity (I), substrate type (S) and burial depth (D) on final seedling biomass. df = Degrees of freedom, MQ = mean sum of squares, vc (%) = relative contribution of individual factors and their interactions to total variation. Burial depths from which no emergence occurred across species were excluded from the analysis (8 and 12 cm)

Source of variation	df	MQ	P	vc (%)
N	1	20861033	<0.00001	8.62
I	4	11261204	<0.00001	18.61
S	1	12 605	0.8878	0.005
D	3	2002132	0.0260	2.48
I × S	4	4156670	<0.0001	6.87
I × D	12	2755835	<0.00001	13.66
S × D	3	2214239	0.0168	2.75
I × S × D	12	1113161	0.0587	5.52
Residuals	159	631529		41.49

source for granivores foraging on or close to the soil surface and may thus experience higher predation pressure (Westerman *et al.*, 2003).

Emergence velocity decreased with increasing depth (Table 5), which is also in line with findings from previous studies (e.g. Benvenuti *et al.*, 2001; Ren *et al.*, 2002) and may have been caused by two different mechanisms. First, germination occurred later at greater depths as seasonal soil temperature increases (as triggers of germination) commence later at increasing depths due to the insulating effect of the soil (Fig. 1; cf. Begum *et al.*, 2006). Second, seedlings also had to penetrate thicker soil layers after germination from greater depths and thus required more time before finally emerging on the soil surface.

Final seedling length and total seedling biomass were both significantly influenced by burial depth (Tables 4 and 6). Species differed significantly from each other, as was to be expected when comparing species of different growth forms. However, we observed a general trend towards decreasing biomass development and growth with increasing burial depth, which is in line with the results of Li *et al.* (2006) who found that initial seedling sizes of the dune species *Nitraria sphaerocarpa* decreased with increasing depth of emergence. This 'shrinkage effect' of increased burial depth is presumably caused by the fact that seedlings originating from deeper-buried seeds require more energy resources and more time to reach the soil surface than those originating from seeds in shallower positions. As competition for light is known to be particularly asymmetrical (Weiner, 1990), this implies that 'shallow emergers' will have a disproportionate advantage over seedlings originating from greater depths because they can start assimilating before the others have even reached the

soil surface – which may drastically increase their establishment chances (Fenner and Thompson, 2005). We thus conclude that the position of a seed within the soil profile does not only influence the likelihood of its survival and germination, but may also have important consequences for seedling establishment and performance in later life stages.

Effects of substrate type

Previous studies have shown that soil particle size influences soil physical characteristics, which may in turn affect seed germination and emergence (Cussans *et al.*, 1996; Benvenuti *et al.*, 2001). In our experiment, substrate type did not affect the likelihood of survival during burial (Table 3). This is contradictory to the findings of Benvenuti (2003), who observed that depth-mediated germination inhibition of *Datura stramonium* seeds was higher in clay than in sandy soils. In the flood meadows we studied, however, both substrate types, and thus both modes of rapid burial (i.e. desiccation crack entrapment and sediment coverage), are apparently equally likely to contribute to the formation of a persistent soil seed-bank.

In contrast to our second hypothesis, substrate type also did not affect seedling emergence (Table 4), although the two substrates differed considerably in their response to irrigation and drying periods. Whereas desiccation led to the rapid formation of small cracks in the clay substrate, the sandy substrate merely shrank from the rim to the middle of the pot without any crack formation. However, we found a significant interaction between substrate type and burial depth and observed a trend for higher germination percentages in sand on the soil surface and higher emergence from clay for seeds sown at greater depth (Fig. 3). The higher surface germination in sand may have been due to the fact that soil temperatures were generally higher in clay than in sand (Fig. 1) – possibly due to differences in soil colour as the darker clay presumably absorbed more radiation energy and thus heated up faster than the brighter sand. This may have led to increased evaporation and thus reduced water availability of the top layer in clay compared to sand. At greater depths, however, water availability may have been higher in clay than in sand as soil water storage capacity is inversely linked with soil particle size (White, 1997), resulting in comparatively higher germination percentages in clay.

Although the main effect of substrate type on emergence velocity was not significant (Table 4), we observed a trend towards lower emergence velocity in sand than in clay (Table 5). This is contrary to the findings of Cussans *et al.* (1996), who reported that germination of seeds buried at different depths

became slower as the average aggregate size of the soil cover decreased, and that these differences increased with increasing depth of sowing. Our results may again have been caused by differences in soil temperature amplitudes, which were not only generally lower in sand than in clay, but also showed a certain time lag (Fig. 1). This could imply that critical temperature thresholds for germination were reached later in sand than in clay so that germination began at a later point in time. This delay could apparently not be compensated for by faster seedling growth and substrate penetration in the more coarsely aggregated sandy soil (cf. Cussans *et al.*, 1996), resulting in reduced emergence velocity for germination in sand.

Differences between species

Small-seeded species had higher percentages of seeds surviving during burial than larger-seeded species (Fig. 2), which is in line with our third hypothesis and supports the finding of Yanful and Maun (1996) and Davis and Renner (2007). This might be the result of differential selection pressures as small seeds have fewer resources and accordingly very shallow maximum emergence depths. Germination from even very shallow burial depth could therefore already be fatal – which implies that small-seeded species should experience a high selection pressure towards developing depth-sensing mechanisms that cause depth-mediated germination inhibition (cf. Milberg *et al.*, 2000). Large-seeded species, in contrast, can emerge from greater depths, are thus not so threatened by fatal germination and should have experienced less selection pressure. We indeed found that fatal germination decreased with increasing depth for small-seeded, but not for larger-seeded species (Fig. 4).

Maximum depth of emergence was much lower for the two small-seeded species *A. nemorensis* and *I. salicina* than for the other species (Fig. 3). This is in line with previous studies reporting that species with larger seeds emerged from greater depths than those with small seeds (van der Valk, 1974; Maun and Lapierre, 1986; Bond *et al.*, 1999; Grundy *et al.*, 2003) and that species with very small seeds show a sharp decline in emergence when burial exceeds 1 cm (Grundy *et al.*, 2003). In a study of the dune species *N. sphaerocarpa*, Li *et al.* (2006) found that seedling emergence differed with seed mass even within a single species.

Conclusions

Our overall results suggest that herbaceous flood-meadow species may have developed two different strategies based on seed size for coping with the extreme recruitment conditions prevailing in their

habitat. Strategy 1 is characterized by producing comparatively fewer, but larger seeds which contain a lot of energy reserves and have less effective depth-sensing mechanisms. Species featuring this strategy bet on direct germination and the competitive edge this implies. Strategy 2 is characterized by producing many small seeds that can be incorporated into the soil easily (cf. Thompson *et al.*, 1993) and have effective depth-sensing mechanisms that trigger germination only under benign conditions and thus lower the risk of fatal germination. As a result, species featuring this strategy may rapidly form long-term persistent seed-banks and bet on persistence rather than competitive ability. This strategy, however, only works in the presence of disturbances, such as bioturbation or flooding, that relocate buried seeds back on the soil surface where they may then germinate and establish. We thus conclude that community composition in flood meadows in the presence of prevailing rapid-burial mechanisms may at least partially be upheld by disturbance events which cause the re-surfacing of small seeds.

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