

**Colonization processes and seed bank  
dynamics in flood meadows and their  
implications for ecological restoration**



# **Colonization processes and seed bank dynamics in flood meadows and their implications for ecological restoration**

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- 1) Burmeier, S., Eckstein, R.L., Donath, T.W. & Otte, A. (in press) Plant pattern development during early post-restoration succession in grasslands – a case study of *Arabis nemorensis*. Restoration Ecology, DOI: 10.1111/j.1526-100X.2010.00668.x.\*
- 2) Burmeier, S., Eckstein, R.L., Otte, A. & Donath, T.W. (in press) Spatially-restricted plant material application creates colonization initials for flood-meadow species. Biological Conservation, DOI: 10.1016/j.biocon.2010.08.018.\*
- 3) Burmeier, S., Eckstein, R.L., Otte, A. & Donath, T.W. (2010) Desiccation cracks act as natural seed traps in flood-meadow systems. Plant and Soil 333: 351-364.\*
- 4) Burmeier, S., Donath, T.W., Otte, A. & Eckstein, R.L. (2010) Rapid burial has differential effects on germination and emergence of small- and large-seeded herbaceous plant species. Seed Science Research 20: 189-200.\*

In paper 1, I had the main responsibility for field work, data analysis and writing while the co-authors were involved in planning the study and gave helpful comments. In paper 2, 3 and 4, I was responsible for the study design, field work, data analysis and writing. The co-authors contributed constructive suggestions and helpful comments.

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*„Very few people, however, come to appreciate the complexity hidden in the soil beneath a grassland community.”*

KEVIN J. RICE (1989)



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*Iris spuria* flowering in a restored flood meadow



# CHAPTER 1

## **Colonization processes and seed bank dynamics in flood meadows and their implications for ecological restoration: a synthesis**

*This chapter introduces the background and the key concepts underlying this thesis, states its aims and objectives and gives an outline of the four manuscripts the thesis is based on. It presents their main results and conclusions, puts them in a wider context and highlights the implications for flood-meadow restoration.*

## Introduction

### Background

#### *Flood meadows*

Flood meadows are typical plant communities of large lowland river valleys in subcontinental climatic conditions (Burkart 2001). These environments are highly dynamic and are characterized by the impacts of flooding events (Beltman et al. 2007), which may cause severe disturbances and can lead to significant nutrient intakes (Olde Venterink et al. 2006), but also by regular periods of severe summer drought which, in combination, account for highly variable soil water potentials (Leyer 2005; Toogood et al. 2008). This gives rise to distinct plant communities where species of mesic habitats are closely intermingled with species with a high flooding tolerance and species adapted to dry conditions (Hölzel et al. 2006). From a phytosociological point of view, the alliance *Cnidion* constitutes the vegetation of flood meadows *sensu strictu* (Balátová-Tuláčková 1969). However, ecologically related alliances such as *Molinion* and *Arrhenatherion* are often also associated with flood-meadow communities (Hölzel et al. 2006).

Flood meadows are strongly influenced by human land use, and regular mowing is crucial for protecting these rare communities and their typical plant and animal species (Ružičková et al. 2004). Intensified management, abandonment and conversion into arable fields have caused a drastic decline of flood meadows across Europe since the middle of the 20<sup>th</sup> century (Göbel 1995; McDonald 2001; Leyer 2002; Ružičková et al. 2004; Hölzel et al. 2006). As a result, they now belong to the most threatened plant communities in Europe (Korneck et al. 1996; Joyce & Wade 1998) and are considered to be of high conservation concern – in particular as they harbour many rare and endangered species (Schnittler & Günther 1999; Burkart 2001; Ružičková et al. 2004).

Larger Central European remnants still occur in the catchments of the rivers Elbe (Re-decker 2001; Leyer 2002; Härdtle et al. 2006), Oder (Korsch 1999) and Danube (Ružičková et al. 2004) and along the Northern Upper Rhine (Böger 1991; Göbel 1995). However, even in these areas fragments have become increasingly isolated (Donath et al. 2003; Van Looy & Meire 2009), which may have severe population genetic consequences for the concerned plant and animal species (Young et al. 1996 and references therein).

As *Cnidion* meadows are listed in Appendix 1 of the EU Habitats Directive (92/43/ECC), all member states are requested to take action to maintain them and, where appropriate, restore their 'favourable conservation status'. Restoration of degraded sites could help to re-connect isolated remnants, decrease the risk of local extinction for typical species and thus improve the overall conservation status of flood meadows.

#### *Ecological restoration*

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (Harris & van Diggelen 2006). It implies directing ecosystem development towards a target ecosystem by altering and/or accelerating processes such as dispersal and colonization or community assembly (Palmer et al. 1997; Bakker & Berendse 1999; Bakker et al. 2000). The response of communities and

the course of succession are determined by the habitat quality, and restoration success ultimately depends on creating a suitable environment for the target species and communities (Bakker et al. 1996; Strykstra et al. 1998).

Restoration projects are often impeded by the fact that biotic and abiotic site conditions of the restoration sites differ considerably from the former environment and thus from the demands of the target species (Bakker & Berendse 1999; Walker et al. 2004). This includes hydrological conditions and nutrient availability (Patzelt et al. 1998; Walker et al. 2004) as well as a shortage of suitable microsites for seed germination and seedling establishment (Isselstein et al. 2002). Even if abiotic site conditions are benign, it is often necessary to actively re-introduce target species to achieve restoration goals due to a shortage of dispersal units such as seeds, fruits or vegetative dispersal structures (Bischoff 2002; Pywell et al. 2002). This dispersal limitation is a particularly serious problem in isolated sites where colonization is hampered by distance (Palmer et al. 1997; van Dorp et al. 1997; Bossuyt & Hermy 2003). In flood meadows, for instance, seed dispersal by flood water is nowadays often insufficient for enabling restoration (Vécrin et al. 2007), and dispersal limitation has been identified as a chief obstacle for their restoration (Bischoff 2002; Donath et al. 2003; Bissels et al. 2004).

Several approaches have been suggested for re-introducing species in grassland ecosystems in order to overcome dispersal limitation. They include sowing of commercially available or locally gathered seed mixtures (e.g. Jones & Hayes 1999; Losvik & Austad 2002; Pywell et al. 2002; Vécrin et al. 2002), plug planting (Wallin et al. 2009), micro- (Pärtel et al. 1998) or macroturf transplantation (Bruehlheide & Flintrop 2000) and the transfer of diaspore-rich plant material from sites containing the desired target species or communities (e.g. Kiehl & Wagner 2006; Rasran et al. 2006; Klimkowska et al. 2007). The latter is one of the most popular techniques and has already been applied in the restoration of various types of grassland such as wet and fen meadows (Patzelt et al. 1998; Rasran et al. 2007), calcareous grassland (Kiehl et al. 2006), sand grassland (Kirmer & Mahn 2001; Stroh et al. 2002) and flood meadows (Hölzel & Otte 2003; Donath et al. 2007).

If at least some species-rich remnant sites still exist in the area where restoration shall take place, they can serve as donor sites for plant material transfer. In that case, the sites are mown (preferably at a time when many of the target species have ripe seeds), and the fresh plant material is subsequently transferred to the restoration sites. As these are often more abundant than donor sites, the plant material is usually not sufficient for covering the entire restoration sites and is thus applied in the form of square or rectangular patches (Kirmer & Mahn 2001; Stroh et al. 2002; Rasran et al. 2007) or strips (Donath et al. 2007) – with the implicit assumption that these will then act as colonization initials from which transferred target species may spread and eventually cover the entire restoration site (Patzelt et al. 1998; Kiehl et al. 2006).

Results of previous studies show that plant material transfer is generally very successful in transferring species and thus overcoming dispersal limitation if initial site conditions are suitable (Kiehl et al. 2010 and references therein). However, monitoring has so far been primarily restricted to comparing pre- and post-restoration states rather than comparing restored and remnant sites or testing whether sites treated with plant material really act as colonization initials for transferred target species. Further research is needed to test this implicit assumption and to evaluate whether plant material transfer really succeeds in

establishing communities that are similar to remnant reference sites (cf. Harris & van Diggelen 2006).

### *Soil seed banks*

Meaningful flood-meadow conservation and restoration requires a thorough understanding of the underlying ecological processes. This explicitly includes information about dispersal processes and soil seed bank dynamics (Bakker et al. 1996).

The term 'soil seed bank' refers to an underground reservoir of viable, yet ungerminated seeds. Its size is determined by input through the seed rain – as a result of Phase I and Phase II dispersal *sensu* Chambers & MacMahon (1994) – and loss resulting from a range of factors such as fungal disease, predation and, of course, germination (Simpson et al. 1989). These inputs and outputs control seed density and species composition, and shifts in the relative importance of these processes over time govern seed bank dynamics (Simpson et al. 1989). Buried seed densities vary widely and differ between ecosystems. They may range between virtually no viable seeds in the ground beneath Arctic tundra to up to well over 100 000 seeds/m<sup>2</sup> in some wetlands (Fenner & Thompson 2005 and references therein).

Several different systems for classifying soil seed banks have been published so far (Csontos & Tamas 2003). One of the most widely employed systems in temperate regions has been brought forward by Thompson et al. (1997), who distinguish three seed bank types based on seed longevity: Seeds of *transient* species persist in the soil for less than one year, seeds of *short-term persistent* species persist for at least one year but less than five years, and seeds of *long-term persistent* species persist for at least five years. As seeds generally cannot persist on the soil surface for long periods due to germination or predation, seed burial is an essential prelude to persistence and thus a major component influencing seed bank dynamics (Thompson et al. 1993; Grime 2001). Possible burial mechanisms include frost heave, bioturbation by animals (Willems & Huijsmans 1994; Wijnhoven et al. 2006) or entrapment by soil cracks (Elberling 2000; Espinar et al. 2005) as well as coverage by shifting substrate (van der Valk 1974; Yanful & Maun 1996).

Post-burial seed fate 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 & Lapierre 1986; Bond et al. 1999; Leishman et al. 2000; Grundy et al. 2003; Li et al. 2006). Environmental factors such as temperature (Thompson & Grime 1983; Benech-Arnold et al. 2000; Oliveira & Norsworthy 2006), light quality (Baskin & Baskin 2001), air quality and soil water potential (Forcella et al. 2000) may govern the germination of buried seeds. 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).

Generally, soil seed banks play an important role in determining the composition and dynamics of plant communities as they give species an opportunity to disperse not only in space, but also in time (Parker et al. 1989). A persistent soil seed bank may, for instance, guarantee the long-term survival of typical flood-meadow plant species by buffering their populations against environmental variability (Thompson 2000; Hölzel & Otte 2004) as it enables them to regenerate if the above-ground plants are destroyed, e.g. in the course

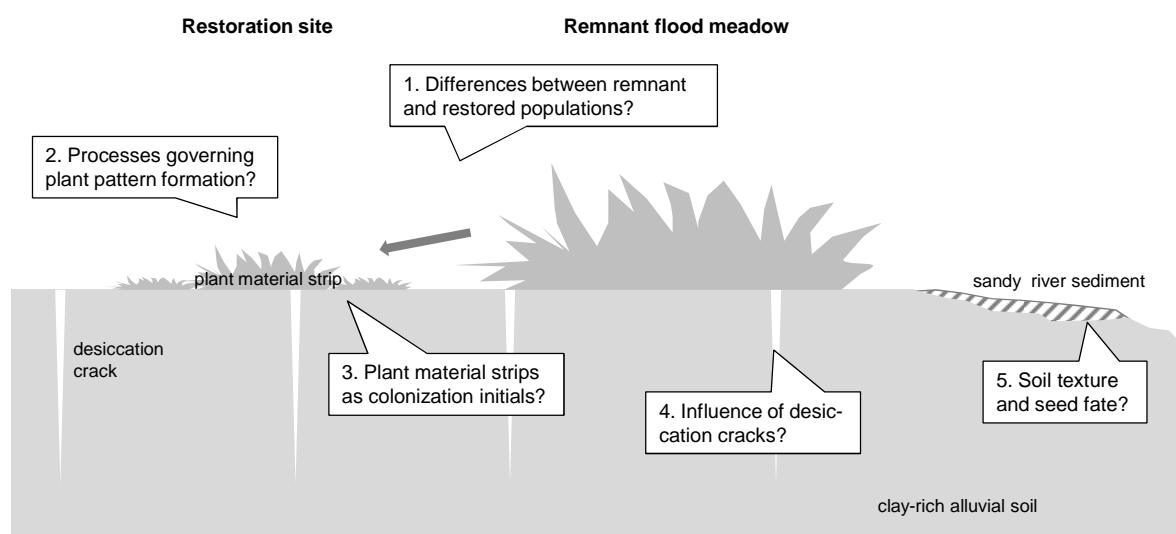
of a severe flooding event (cf. Richardson et al. 2007). Knowledge of seed bank dynamics may thus increase our understanding of important limiting factors and processes that occur within such communities (Leck et al. 1989).

## Objectives and study questions

The main objectives of this thesis were to evaluate the success of plant material transfer during flood-meadow restoration with a particular emphasis on seed bank formation and to explore the factors governing seed bank dynamics in a flood-meadow system (Figure 1.1).

The following questions are addressed:

1. Do remnant and restored plant populations in flood meadows differ with regard to their habitat structure, population dynamics and spatial patterns?
2. What processes govern plant pattern formation during early post-restoration succession?
3. Do plant material strips act as colonization initials for target species of flood-meadow restoration?
4. Do desiccation cracks influence seed bank dynamics?
5. Does the fate of buried seeds depend on soil texture and thus presumably also on the mode of burial (e.g. desiccation crack entrapment or sediment coverage)?



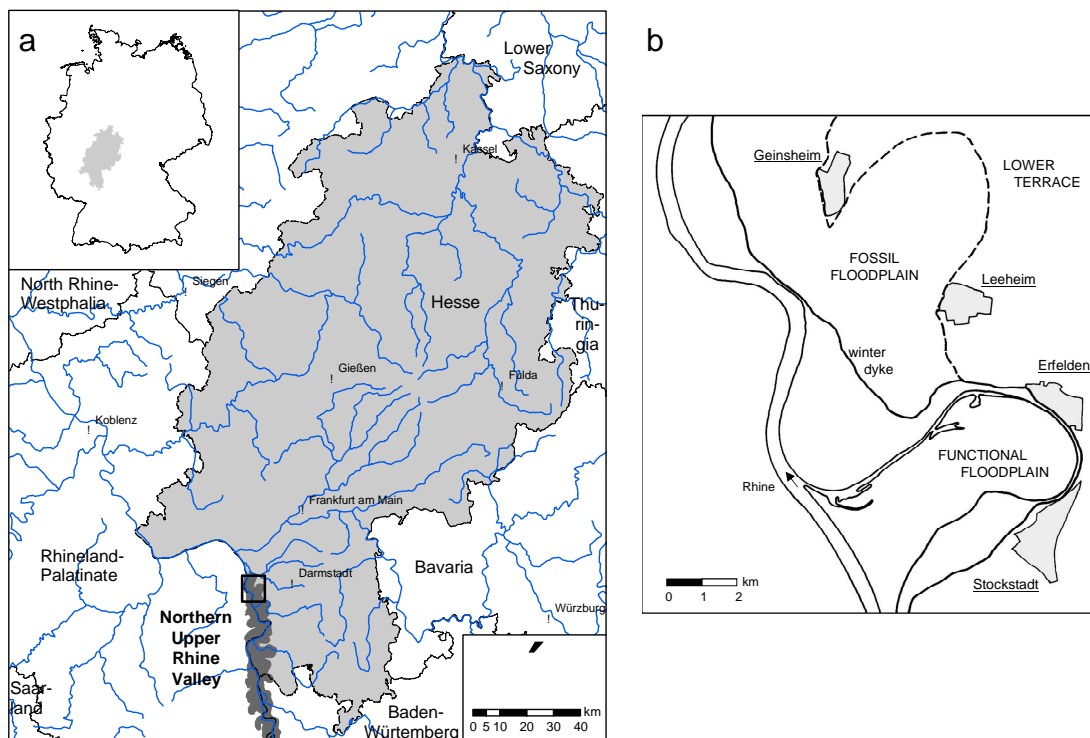
**Figure 1.1** Schematic representation of the main elements of the investigated flood-meadow system and the questions addressed in this thesis.

## Study area

This study was conducted in flood meadows of a Holocene floodplain within the biogeographical unit *Northern Upper Rhine Valley* (Figure 1.2a), approx. 35 km southwest of Frankfurt, Germany (49° 51' N, 8° 24' E). The region is characterized by strong seasonal and inter-annual fluctuations of the ground-water table (Bissels et al. 2005) and has relatively warm and dry climatic conditions with a mean temperature of 10.3 °C and a mean annual precipitation of 580 mm (Müller-Westermeier 1990). Fine-grained calcareous alluvial soils topping sandy sediments of the Rhine are predominant throughout the entire area (Böger 1991).

The area can be coarsely subdivided into two hydrological compartments (Hölzel & Otte 2001): (1) the functional floodplain riverwards the dykes, and (2) the fossil floodplain landwards the dykes (Figure 1.2b). While the functional floodplain is frequently exposed to direct flooding during high water of the Rhine, the fossil floodplain is merely indirectly affected when low depressions are submerged by ascending groundwater. Irregular flooding in conjunction with the climatic conditions results in highly variable soil water potentials in both compartments: While winter, spring and early summer may bring about long-lasting floods, summers are often very dry. This summer drought often leads to the formation of large desiccation cracks in the ground (as illustrated in Figure 1.1) because the fine-grained alluvial soils swell during wetting and shrink during drying.

Until the 1950s, the entire area was dominated by species-rich alluvial grasslands that were managed extensively as hay meadows (Knapp 1954). Due to intensified drainage



**Figure 1.2** Map of the geographical location of (a) the Northern Upper Rhine Valley and (b) the study area (based on Hölzel et al. 2006). The black rectangle in Figure (a) indicates the location of the study area within the Northern Upper Rhine Valley.

and structural changes in agriculture many sites were subsequently converted into arable fields (Böger 1991). In the 1980s, however, re-conversion into grassland began after severe flooding events had rendered agricultural use uneconomical and land was set aside for conservation purposes. As restoration by natural succession turned out to be strongly dispersal limited (Donath et al. 2003; Bissels et al. 2004), a large-scale restoration project was initiated in the late 1990s with the aim of re-establishing typical flood-meadow vegetation on former agricultural land by means of plant material transfer (Donath et al. 2007). This sets the scene for the investigations presented in this thesis.

## Chapter outline

This thesis is based on four manuscripts, which have all been submitted to peer-reviewed international scientific journals and are either already published or accepted for publication, respectively, or are currently under review. This section gives a brief outline of the contents and methods of the manuscripts, which form chapters 2, 3, 4 and 5 of this thesis. Their main results and conclusions are presented and discussed in the following section.

### **Chapter 2:** *Plant pattern development during early post-restoration succession in grasslands – a case study of *Arabis nemorensis**

This manuscript presents a case study of the flood-meadow plant species *Arabis nemorensis*. Its objectives were to evaluate the success of grassland restoration via plant material transfer by comparing restored and remnant sites with regard to population dynamics and spatial patterns and to analyse the processes governing plant pattern formation during early post-restoration succession, with a special emphasis on seed bank dynamics. To this end, I investigated spatial patterns of seedlings, juveniles and adults as well as the small-scale horizontal and vertical distribution of seeds in the soil, followed the fate of individual plants and recorded structural habitat parameters such as vegetation and litter cover.

### **Chapter 3:** *Spatially-restricted plant material application creates colonization initials for flood-meadow restoration*

This manuscript deals with an investigation of vegetation development on restoration sites 7-8 years after plant material application. The aim was to test the assumption that plant material strips act as colonization initials for transferred species and to evaluate whether it is feasible to restore entire sites by spatially-restricted plant material application in a flood-meadow ecosystem. I compared three different components of the emerging flood-meadow community – (i) the above-ground vegetation development, (ii) the seed rain and (iii) the composition of the soil seed bank – along five transverse transects that stretched from the centre of a plant material strip into its surroundings.

**Chapter 4: Desiccation cracks act as natural seed traps in flood-meadow systems**

This manuscript presents empirical and experimental investigations on desiccation cracks, which are a common feature of flood meadows. The aim was to test the seed trapping potential of such cracks and assess its impact on seed bank formation in a flood-meadow ecosystem. I documented crack patterns on permanent plots, analysed the soil seed content along and adjacent to cracks, tested seed translocation via cracks with a mark-recapture experiment and tested post-entrapment seed fate of five co-occurring herbaceous flood-meadow species with an *in situ* burial experiment.

**Chapter 5: Rapid burial has differential effects on germination and emergence of small- and large-seeded herbaceous plant species**

This manuscript presents an additional, *ex situ* burial experiment on post-entrapment seed fate. Its purpose was to test whether the results gained in the previous study also hold for a finer depth resolution and to investigate whether substrate type – as a proxy for burial mode – affects seed fate. I therefore tested seed germination, seedling emergence and growth of the same five species as in Chapter 4 in response to the experimental manipulation of burial depth (0, 1, 2, 4, 8, 12 cm) and substrate type (sand, clay). The substrate types were chosen to represent burial via sediment coverage (sand) and desiccation crack entrapment (clay).

**Main results and conclusions**

The different studies that were conducted within the framework of this thesis show that plant material transfer is a suitable method for flood-meadow restoration, which eventually also fosters seed bank formation (Chapters 2 & 3). Furthermore, seed bank dynamics in this disturbance-prone ecosystem are strongly influenced by abiotic conditions such as desiccation cracks (Chapters 4 & 5). These findings have important implications for flood-meadow restoration, as discussed in the final part of this section.

**Evaluation of plant material transfer**

The available propagule pool is one of the basic determinants of plant community composition (Pakeman et al. 1998; Zobel et al. 2000; Foster et al. 2004) and thus also of the course of succession (Lanta & Lepš 2009). Differential seed input may lead to different pathways of succession, even when all other environmental conditions are equal (Lanta & Lepš 2009). It is well-established that plant material transfer may successfully overcome dispersal limitation and thus boost the available propagule pool during grassland restoration (e.g. Kiehl & Wagner 2006; Donath et al. 2007; Klimkowska et al. 2009). Structural habitat conditions, spatial patterns and population dynamics, however, had so far received only little attention during the evaluation of such restoration measures.

The results of the case study with *Arabis nemorensis* show that plant material transfer rapidly triggered the formation of spatially-structured populations that closely resembled those of remnant sites (Chapter 2). Furthermore, population dynamics in restored and remnant populations of *A. nemorensis* could no longer be distinguished from each other

only three years after restoration measures were carried out. This study does not only contribute to the evaluation of plant material transfer as a restoration tool, but also provides valuable autecological information about a rare flood-meadow species, which in many cases still represent “terrae incognitae” with respect to their population biology (Burkart 2001).

Restoration often primarily focuses on the re-introduction of plant species, assuming that other taxa will follow. However, this is not necessarily the case (Kiehl & Wagner 2006; Woodcock et al. 2008), and the re-assembly of, for instance, invertebrate communities appears to be a much slower process than the re-creation of merely ‘botanically intact’ species-rich grasslands (Walker et al. 2004 and references therein). Structural parameters are important in this regard as they may determine whether restored sites provide suitable habitat conditions for other taxa (Dahms et al. 2008). The case study with *A. nemorensis* showed that the overall habitat structure of sites treated with plant material rapidly approximated that of remnant sites in the course of early post-restoration succession (Chapter 2). This indicates that plant material transfer may be a suitable method for restoring not only plant communities, but also structural conditions of flood meadows and presumably grasslands in general. It can thus be expected that it will eventually lead to the re-establishment of entire communities (cf. Hölzel et al. 2006) – given that restored and remnant sites are situated sufficiently close to each other to overcome dispersal limitation of other taxa (cf. Mortimer et al. 2002).

Transferred species, which were absent from the sites until restoration measures were carried out (cf. Donath et al. 2007), could be found in the above-ground vegetation, the seed rain and the soil seed bank on and adjacent to plant material strips 7-8 years after the strips had been established (Chapter 3). Furthermore, the above-ground vegetation along the transects had become more similar from 2007 to 2009, which could be the result of ongoing colonization by transferred species. All in all, almost 90 % of all species that had established on the plant material strips had already spread into their immediate surroundings (Chapter 3). The strips apparently acted as colonization initials from which species that had established due to the restoration measures had subsequently spread and colonized the surrounding area, i.e. the implicit assumption underlying the layout of plant material transfer indeed seems to hold. However, further research is necessary to determine whether this will eventually lead to the colonization of the entire restoration sites.

Management intervention turned out to be the most important factor determining the spread and distribution of transferred species on the scale considered in the colonization study (Chapter 3). The temporal pattern of the seed rain, which was investigated with seed traps, showed that mowing was responsible for a distinct peak in the seed input on the restoration sites. During haymaking, farmers usually leave the mown material on the site to dry for 2-3 days, during which it is turned several times. This implies that the drying plant material may be moved for several meters from the position where the plants were standing before cutting. As seeds may be disseminated from the plants during this process, it may result in considerable dispersal from the parent plant. Furthermore, seeds may also be transported by the mowing machinery itself (Strykstra et al. 1997; Coulson et al. 2001), both within and between sites. This mode of dispersal does not select for seed size (Strykstra et al. 1997), which may partly explain why no relationship between dispersal success and seed mass or shape of transferred target species was found in the colonization study. These results are supported by the findings of Schmiede et al. (2009),

who also did not find a significant relationship between seed size and post-restoration seed bank formation in a related study. Eriksson & Jakobsson (1998), in contrast, found seed size to be a key trait for colonizing ability in a field study that included 81 grassland plants in Sweden. As they conducted their study in pastures rather than meadows, this divergence presumably reflects the fact that different management regimes have different prevailing dispersal processes which, in turn, have caused a different selection of traits.

### **Soil seed bank dynamics in flood meadows**

The case study with *A. nemorensis* gave evidence for rapid seed bank formation during early post-restoration succession (Chapter 2), which is in line with Neff et al. (2009), who observed rapid seed bank development in their study of restored tidal freshwater wetlands. Willems & Bik (1998), however, who evaluated the restoration of species-rich, dry calcareous grasslands, suggest that the restoration of seed bank quality is a long-term process. The speed and extent of seed bank formation following restoration thus seems to be strongly community- and species-dependent.

For *A. nemorensis*, seed fate differed between restored and remnant populations: On remnant sites, seeds were more likely to be incorporated into the soil seed bank, whereas on restored sites they were more likely to germinate rapidly, followed by comparatively high seedling mortality (Chapter 2). These differences will presumably disappear as the habitat structure of restored populations further approximates that of remnant ones in the course of post-restoration succession (cf. Pywell et al. 2002). It seems likely that the restoration measures will eventually result in the formation of a typical flood-meadow soil seed bank and thus contribute to the establishment of self-sustaining populations of the target species – although seed bank formation turned out to be more time-consuming than the restoration of the above-ground vegetation cover (Chapter 3).

Vertical seed translocation, in contrast, occurred surprisingly rapidly, both for the model species *A. nemorensis* (Chapter 2) and for other species (Chapter 3). This could be due to bioturbation by animals (Willems & Huijsmans 1994; Welander 2000; Wijnhoven et al. 2006), but also to the impact of the desiccation cracks that regularly form on the sites during periods of extended summer drought. The results of the respective investigations have indeed shown that desiccation cracks act as natural seed traps and contribute to a rapid incorporation of shed seeds into the soil (Chapter 4). In the system studied here, seeds were translocated to an average depth of 10-20 cm, which is beyond the maximum depth of emergence of typical herbaceous flood-meadow species (Chapter 5). This depth presumably depends on the average local crack depth (cf. Espinar et al. 2005), which, in turn, is likely to depend on the soil particle composition and partly also on the land use history. It can thus be expected to be different in other systems featuring desiccation cracks.

Mapping of crack patterns in consecutive dry periods revealed that desiccation cracks were roughly spatially constant, at least over the short time period studied here (Chapter 4). This has important consequences for their trapping potential: If cracks frequently open in the same positions they can accumulate a large number of seeds, which may result in a clumped distribution of the soil seed bank, as found in the case study with *A. nemorensis* (Chapter 2). In the longer term, however, cracks may shift positions and thus eventually contribute to an increased seed density at their average depth throughout the entire site (Chapter 4, cf. also Espinar et al. 2005).

The fate of trapped seeds was investigated by means of two burial experiments with a set of five typical flood-meadow species. It turned out that seed fate during burial differed with burial depths, but also between species (Chapters 4 & 5). Survival during burial generally increased with increasing depth, which could indicate that the seeds of the species tested possess depth-sensing mechanisms that lead to germination inhibition when seeds are buried so deeply that successful seedling emergence would be unlikely. This is in line with Benvenuti et al. (2001), who also observed depth-mediated germination inhibition in seeds of 20 weed species. 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 & Maun 1994; Ren et al. 2002).

Seed fate differed pronouncedly between species and seemed to be connected with seed size. Smaller-seeded species had a higher likelihood of survival during burial than larger-seeded species, whereas the latter were more likely to emerge successfully after germination in greater depths (Chapters 4 & 5). The different survival likelihood of small- and large-seeded species could be the result of differential selection pressures: Small seeds have less resources and accordingly very shallow maximum emergence depths. Germination from even very shallow burial depths 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.

These results suggest that herbaceous flood-meadow species may have developed two seed-size based strategies for coping with the extreme recruitment conditions prevailing in their habitat (Chapter 5): Strategy 1 is characterised by producing comparatively larger and thus presumably fewer 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 characterised by producing many small seeds that can be incorporated into the soil easily and have effective depth-sensing mechanisms which trigger germination only under benign conditions and thus lower the risk of fatal germination. Species featuring this strategy may rapidly form long-term persistent seed banks and bet on persistence rather than competitive ability. However, their strategy only works in the presence of disturbances (e.g. due to rooting wild boars) that relocate buried seeds back on the soil surface where they may then germinate and establish.

The *ex situ* burial experiment also showed that substrate type did not have a significant effect on seedling emergence and seed survival during burial (Chapter 5). This is contrary to the findings of other studies, which 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). Benvenuti (2003) furthermore observed that depth-mediated germination inhibition of *Datura stramonium* seeds was higher in clay than in sandy soils. In the flood-meadow system investigated in this thesis, however, both substrate types seem to be equally likely to contribute to the formation of a persistent soil seed bank – which implies that the two modes of burial associated with the substrate types tested (i.e. desiccation crack entrapment and sediment coverage) may both contribute to the formation of a persistent soil seed bank.

All in all, it seems that desiccation cracks act as natural seed traps in flood meadows and thus contribute to the rapid accumulation of a patchy soil seed bank (Chapters 4 & 5). Moreover, cracks may cause a selection pressure towards developing long-term persistent seed banks (cf. Thompson et al. 1998; Grime 2001) – at least for small-seeded species with shallow maximum emergence depths. This assumption is supported by the findings of Hölzel & Otte (2004), who assessed soil seed bank persistence in flood meadows and concluded that a relatively large proportion of rare and endangered plant species in flood meadows could indeed be expected to form long-term persistent seed banks.

### **Implications for flood-meadow restoration**

Successful grassland restoration depends on the interaction between several conditions such as seed availability, favourable germination conditions and successful development of mature, seed producing individuals (Willems & Bik 1998), and its ultimate aim should be to reinstate communities and ecological functions (Walker et al. 2004). The results presented in this thesis give rise to the hope that plant material transfer is a suitable method for achieving this goal in the flood-meadow ecosystem investigated here: The case study of *Arabis nemorensis* has shown that it triggers the formation of populations with similar structural and spatial patterns to those on donor sites (Chapter 2), and the colonization study has revealed that species may spread and subsequently colonize wider parts of the restoration sites after they have established on plant material strips (Chapter 3). I therefore recommend the continued use of this technique for the restoration of flood meadows and other species-rich grasslands.

The results of the studies compiled within the framework of this thesis also give rise to some practical considerations for the spatial layout of such restoration schemes:

- As management turned out to be of overwhelming importance for small-scale dispersal processes on restoration sites (Chapter 3), it could be beneficial to establish plant material strips perpendicular to the prevailing management direction in order to maximise the extent of seed dispersal during mowing.
- Even though post-application colonization processes are rather time-consuming (Chapter 3), it seems nonetheless advisable to establish several plant material strips or patches on neighbouring sites rather than covering one contiguous site as a whole. Firstly, this would – in terms of a bet-hedging strategy – reduce the risk that the entire restoration project fails due to stochastic disturbance events. Secondly, the simultaneous creation of several subpopulations could benefit the re-establishment of metapopulation structures and processes, in particular with regard to animal species (Betzholtz et al. 2007; Pöyry et al. 2009).
- As the soil seed bank may contribute to the long-term persistence of the restored populations (cf. Thompson 2000; Hölzel & Otte 2004), management should also focus on enhancing seed production and seed bank formation (cf. Smith et al. 2002). To this end, restored sites should be mulched in the early phase of post-treatment vegetation development to minimize seed exports. Mulching may also be beneficial for the seedling establishment of some target species (Jögar & Moora 2008). Although plant material strips can be fully incorporated into existing extensive land-use systems after 2-3 years (Donath et al. 2004), it could nonetheless be beneficial to further omit small areas with successfully established transferred species from mowing.

These should then either be mulched in autumn or spared altogether and re-incorporated into the mowing scheme in the following year. If different areas were omitted each year, seed production and potential seed bank input of transferred species could be increased considerably with only marginal extra effort for farmers.

This thesis has also shown that desiccation cracks need to be accounted for as an important abiotic factor for conservation and restoration schemes in flood meadows and other systems featuring them, e.g. tidal marshes, mudflats or arctic ecosystems. Due to their seed-trapping potential (Chapter 4) they may accelerate the post-treatment development of self-supporting populations on restoration sites which are able to regenerate autonomously if a disturbance event destroys the above-ground vegetation – granted that the disturbance simultaneously causes a re-surfacing of the seeds trapped in the desiccation cracks. However, they may also hinder the development of a closed vegetation cover as nothing can grow in positions where cracks frequently open. Since community composition in flood meadows in the presence of rapid-burial mechanisms such as desiccation crack entrapment and sediment coverage seems to be at least partially upheld by disturbance events (Chapter 5), flood-meadow restoration should also seek to maintain and – if necessary – re-establish typical disturbance mechanisms.

## Perspectives

The overall aim of flood-meadow restoration should be to restore entire sites, reconnect isolated remnants and eventually re-establish a large-scale habitat network for the rare and endangered plant and animal species of this ecosystem (cf. Muller et al. 1998). This thesis has shown that plant material transfer can successfully lay the foundation for this endeavour. However, further investigations and in particular long-term monitoring are urgently required to assess whether it will eventually also succeed in reaching the overall aim.

Future studies should, for instance, focus on the ongoing post-treatment dispersal and colonization processes on the restoration sites. As succession proceeds, colonization may slow down as the vegetation matrix surrounding the plant material strips becomes increasingly denser and may thus offer increasing resistance to the ‘invasion’ of transferred species dispersing from the initial populations that have established on the strips (Tilman 1997). Furthermore, it would be worthwhile to investigate which modes of dispersal dominate during different stages of post-treatment colonization and succession, i.e. assess the relative importance of clonal spread versus dispersal via seeds and subsequent germination. This could generate important information with regard to the optimal set-up of the restoration measures and subsequent management of the restoration sites.

Moreover, the long-term seed bank development on the restoration sites should also be explored further. Both this study (Chapter 3) and other investigations (Willems & Bik 1998; Schmiede et al. 2009) have shown that seed bank development is a long-lasting process and may lack considerably behind the development of the above-ground vegetation. Yet it is a vital component of flood-meadow restoration and thus an essential element of evaluating the restoration success, which should be given due attention.

A possible future challenge for flood-meadow restoration may arise from the anticipated climatic changes and their implications. Regional climate modelling for the federal state of Hesse predicts rising summer temperatures and decreasing mean summer precipitation as well as increasing winter precipitation (Koschel et al. 2006). This implies that soil water potentials may become even more variable in the future, that the magnitude and duration of summer droughts may rise and that, consequently, the number, size and extent of desiccation cracks may drastically increase. This could have important consequences for the regeneration potential of flood-meadow species as an increasing number of seeds may become trapped and thus removed from the active propagule pool for a considerable length of time. Winter floods, in contrast, are expected to increase in the future, which could lead to a reduction in species richness in floodplain grasslands (Beltman et al. 2007). Further investigations are necessary to assess the impacts of these anticipated climatic changes on flood-meadow conservation and restoration.

## References

- Bakker J.P. & Berendse F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution* 14: 63-68.
- Bakker J.P., Grootjans A.P., Hermy M. & Poschlod P. (2000) How to define targets for ecological restoration? *Applied Vegetation Science* 3: 3-6.
- Bakker J.P., Poschlod P., Strykstra R.J., Bekker R.M. & Thompson K. (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45: 461-490.
- Balátová-Tuláková E. (1969) Beitrag zur Kenntnis der tschechoslowakischen *Cnidion vernosi*-Wiesen. *Vegetatio* 17: 200-207.
- Baskin C.C. & Baskin J.M. (2001) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*, Academic Press, San Diego.
- Beltman B., Willems J.H. & Güsewell S. (2007) Flood events overrule fertiliser effects on biomass production and species richness in riverine grasslands. *Journal of Vegetation Science* 18: 625-634.
- Benech-Arnold R.L., Sanchez R.A., Forcella F., Kruk B.C. & Ghersa C.M. (2000) Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* 67: 105-122.
- Benvenuti S. (2003) Soil texture involvement in germination and emergence of buried weed seeds. *Agronomy Journal* 95: 191-198.
- Benvenuti S. (2007) Natural weed seed burial: effect of soil texture, rain and seed characteristics. *Seed Science Research* 17: 211-219.
- Benvenuti S., Macchia M. & Miele S. (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science* 49: 528-535.
- Betzholtz P.E., Ehrig A., Lindeborg M. & Dinnétz P. (2007) Food plant density, patch isolation and vegetation height determine occurrence in a Swedish metapopulation of the marsh fritillary *Euphydryas aurinia* (Rottemburg, 1775) (Lepidoptera, Nymphalidae). *Journal of Insect Conservation* 11: 343-350.
- Bischoff A. (2002) Dispersal and establishment of floodplain grassland species as limiting factors in restoration. *Biological Conservation* 104: 25-33.
- Bissels S., Donath T.W., Hölzel N. & Otte A. (2005) Ephemeral wetland vegetation in irregularly flooded arable fields along the northern Upper Rhine: the importance of persistent seed-banks. *Phytocoenologia* 35: 469-488.
- Bissels S., Hölzel N., Donath T.W. & Otte A. (2004) Evaluation of restoration success in alluvial grasslands under contrasting flooding regimes. *Biological Conservation* 118: 641-650.

- Böger K. (1991) *Grünlandvegetation im Hessischen Ried - pflanzensoziologische Verhältnisse und Naturschutzkonzeption*, Botanische Vereinigung für Naturschutz in Hessen e.V. (BVNH), Frankfurt a. M.
- Bond W.J., Honig M. & Maze K.E. (1999) Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia* 120: 132-136.
- Bossuyt B. & Hermy M. (2003) The potential of soil seed banks in the ecological restoration of grassland and heathland communities. *Belgian Journal of Botany* 136: 23-34.
- Bruehlheide H. & Flintrop T. (2000) Evaluating the transplantation of a meadow in the Harz Mountains, Germany. *Biological Conservation* 92: 109-120.
- Burkart M. (2001) River corridor plants (Stromtalpflanzen) in Central European lowland: a review of a poorly understood plant distribution pattern. *Global Ecology and Biogeography* 10: 449-468.
- Chambers J.C. & MacMahon J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics* 25: 263-292.
- Chambers J.C., MacMahon J.A. & Haefner J.H. (1991) Seed entrapment in alpine ecosystems: effects of soil particle size and diaspore morphology. *Ecology* 72: 1668-1677.
- Coulson S.J., Bullock J.M., Stevenson M.J. & Pywell R.F. (2001) Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. *Journal of Applied Ecology* 38: 204-216.
- Csontos P. & Tamas J. (2003) Comparisons of soil seed bank classification systems. *Seed Science Research* 13: 101-111.
- Cussans G.W., Raudonius S., Brain P. & Cumberworth S. (1996) Effects of depth of seed burial and soil aggregate size on seedling emergence of *Alopecurus myosuroides*, *Galium aparine*, *Stellaria media* and wheat. *Weed Research* 36: 133-141.
- Dahms H., Lenoir L., Lindborg R., Wolters V. & Dauber J. (2008) Restoration of seminatural grasslands: what is the impact on ants? *Restoration Ecology* 18: 330-337.
- Donath T.W., Bissels S., Hölzel N. & Otte A. (2007) Large scale application of diaspore transfer with plant material in restoration practice - Impact of seed and microsite limitation. *Biological Conservation* 138: 224-234.
- Donath T.W., Hölzel N., Bissels S. & Otte A. (2004) Perspectives for incorporating biomass from non-intensively managed temperate flood-meadows into farming systems. *Agriculture Ecosystems & Environment* 104: 439-451.
- Donath T.W., Hölzel N. & Otte A. (2003) The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Applied Vegetation Science* 6: 13-22.
- Elberling H. (2000) Spatial pattern of *Lesquerella arctica*: Effects of seed bank and desiccation cracks. *Ecoscience* 7: 86-91.
- Eriksson O. & Jakobsson A. (1998) Abundance, distribution and life histories of grassland plants: a comparative study of 81 species. *Journal of Ecology* 86: 922-933.
- Espinar J.L., Thompson K. & Garcia L.V. (2005) Timing of seed dispersal generates a bimodal seed bank depth distribution. *American Journal of Botany* 92: 1759-1763.
- Fenner M. & Thompson K. (2005) *The ecology of seeds*, Cambridge University Press, Cambridge, UK.
- Forcella F., Arnold R.L.B., Sanchez R. & Ghera C.M. (2000) Modeling seedling emergence. *Field Crops Research* 67: 123-139.
- Foster B.L., Dickson T.L., Murphy C.A., Karel I.S. & Smith V.H. (2004) Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *Journal of Ecology* 92: 435-449.
- Göbel W. (1995) Die Vegetation der Wiesen, Magerrasen und Rieder im Rhein-Main-Gebiet. *Dissertationes Botanicae* 237: 1-456.
- Grime J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons, Chichester.
- Grundy A.C., Mead A. & Burston S. (2003) Modelling the emergence response of weed seeds to burial depth: interactions with seed density, weight and shape. *Journal of Applied Ecology* 40: 757-770.

- Härdtle W., Redecker B., Assmann T. & Meyer H. (2006) Vegetation responses to environmental conditions in floodplain grasslands: Prerequisites for preserving plant species diversity. *Basic and Applied Ecology* 7: 280-288.
- Harris J.A. & van Diggelen R. (2006) Ecological restoration as a project for a global society. In: van Andel J. & Aronson J. (eds.) *Restoration Ecology*, pp. 3-15. Blackwell, Oxford.
- Hölzel N., Bissels S., Donath T.W., Handke K., Harnisch M. & Otte A. (2006) *Renaturierung von Stromtalwiesen am hessischen Oberrhein*, Bundesamt für Naturschutz, Bonn - Bad Godesberg.
- Hölzel N. & Otte A. (2001) The impact of flooding regime on the soil seed bank of flood-meadows. *Journal of Vegetation Science* 12: 209-218
- Hölzel N. & Otte A. (2003) Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. *Applied Vegetation Science* 6: 131-140.
- Hölzel N. & Otte A. (2004) Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. *Journal of Vegetation Science* 15: 93-100.
- Isselstein J., Tallowin J.R.B. & Smith R.E.N. (2002) Factors affecting seed germination and seedling establishment of fen-meadow species. *Restoration Ecology* 10: 173-184.
- Jögar Ü. & Moora M. (2008) Reintroduction of a rare plant (*Gladiolus imbricatus*) population to a river floodplain - How important is meadow management? *Restoration Ecology* 16: 382-385.
- Jones A.T. & Hayes M.J. (1999) Increasing floristic diversity in grassland: the effects of management regime and provenance on species introduction. *Biological Conservation* 87: 381-390.
- Joyce C.B. & Wade M.W. (1998) Wet grasslands: a European perspective. In: Joyce C.B. & Wade P.M. (eds.) *European Wet Grasslands: Biodiversity, Management and Restoration*, pp. 1-12. John Wiley, Chichester.
- Kiehl K., Kirmer A., Donath T.W., Rasran L. & Hölzel N. (2010) Species introduction in restoration projects - evaluation of different techniques for the establishment of semi-natural grasslands in Central and North-western Europe. *Basic and Applied Ecology* 11: 285-299.
- Kiehl K., Thormann A. & Pfadenhauer J. (2006) Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. *Restoration Ecology* 14: 148-156.
- Kiehl K. & Wagner C. (2006) Effect of hay transfer on long-term establishment of vegetation and grasshoppers on former arable fields. *Restoration Ecology* 14: 157-166.
- Kirmer A. & Mahn E.G. (2001) Spontaneous and initiated succession on unvegetated slopes in the abandoned lignite-mining area of Goitsche, Germany. *Applied Vegetation Science* 4: 19-27.
- Klimkowska A., Kotowski W., van Diggelen R., Grootjans A.P., Dzierża P. & Brzezińska K. (2009) Vegetation re-development after fen meadow restoration by topsoil removal and hay transfer. *Restoration Ecology*, DOI: 10.1111/j.1526-100X.2009.00554.x.
- Klimkowska A., Van Diggelen R., Bakker J.P. & Grootjans A.P. (2007) Wet meadow restoration in Western Europe: a quantitative assessment of the effectiveness of several techniques. *Biological Conservation* 140: 318-328.
- Knapp R. (1954) Über Pflanzengesellschaften der Wiesen in Trockengebieten Deutschlands. *Angewandte Pflanzensoziologie (Aichinger Festschriften)* 2: 1145-1186.
- Korneck D., Schnittler M. & Vollmer J. (1996) Rote Liste der Farn- und Blütenpflanzen (Pteridophyta et Spermatophyta) Deutschlands. *Schriftenreihe für Vegetationskunde* 28: 21-187.
- Korsch H. (1999) Chorologisch-ökologische Auswertung der Daten der Floristischen Kartierung Deutschlands. *Schriftenreihe für Vegetationskunde* 30: 3-200.
- Koschel H., Moslener U., Sturm B., Fahl U., Rühle B. & Wolf H. (2006) Integriertes Klimaschutzprogramm Hessen InKlim 2012. Endbericht. Wiesbaden.
- Lanta V. & Lepš J. (2009) How does surrounding vegetation affect the course of succession: A five-year container experiment. *Journal of Vegetation Science* 20: 686-694.
- Leishman M.R., Wright I.J., Moles A.T. & Westoby M. (2000) The evolutionary ecology of seed size. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 31-57. 2nd ed. CABI Publishing, Wallingford.

- Leyer I. (2002) Auengrünland der Mittel- und Niederelbe: Vegetationskundliche und -ökologische Untersuchungen in der rezenten Aue, der Altaue und am Auenrand der Elbe. *Dissertationes Botanicae* 363: 1-193.
- Leyer I. (2005) Predicting plant species' responses to river regulation: the role of water level fluctuations. *Journal of Applied Ecology* 42: 239-250.
- Li Q.Y., Zhao W.Z. & Fang H.Y. (2006) Effects of sand burial depth and seed mass on seedling emergence and growth of *Nitraria sphaerocarpa*. *Plant Ecology* 185: 191-198.
- Losvik M.H. & Austad I. (2002) Species introduction through seeds from an old, species-rich hay meadow: Effects of management. *Applied Vegetation Science* 5: 185-194.
- Maun M.A. & Lapierre J. (1986) Effects of burial by sand on seed germination and seedling emergence of four dune species. *American Journal of Botany* 73: 450-455.
- McDonald A.W. (2001) Succession during the re-creation of a flood-meadow 1985-1999. *Applied Vegetation Science* 4: 167-176.
- Milberg P., Andersson L. & Thompson K. (2000) Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* 10: 99-104.
- Mortimer S.R., Booth R.G., Harris S.J. & Brown V.K. (2002) Effects of initial site management on the Coleoptera assemblages colonising newly established chalk grassland on ex-arable land. *Biological Conservation* 104: 301-313.
- Müller-Westermeier G. (1990) *Klimadaten der Bundesrepublik Deutschland*, Deutscher Wetterdienst, Offenbach.
- Muller S., Dutoit T., Alard D. & Gréville F. (1998) Restoration and rehabilitation of species-rich grassland ecosystems in France: a review. *Restoration Ecology* 6: 94-101.
- Neff K.P., Rusello K. & Baldwin A.H. (2009) Rapid seed bank development in restored tidal freshwater wetlands. *Restoration Ecology* 17: 539-548.
- Olde Venterink H., Vermaat J.E., Pronk M., Wiegman F., van der Lee G.E.M., van den Hoorn M.W., Higler L. & Verhoeven J.T.A. (2006) Importance of sediment deposition and denitrification for nutrient retention in floodplain wetlands. *Applied Vegetation Science* 9: 163-174.
- Oliveira M.J. & Norsworthy J.K. (2006) Pitted morningglory (*Ipomoea lacunosa*) germination and emergence as affected by environmental factors and seeding depth. *Weed Science* 54: 910-916.
- Pakeman R.J., Attwood J.P. & Engelen J. (1998) Sources of plants colonizing experimentally disturbed patches in an acidic grassland, in eastern England. *Journal of Ecology* 86: 1032-1041.
- Palmer M.A., Ambrose R.F. & Poff N.L. (1997) Ecological theory and community restoration ecology. *Restoration Ecology* 5: 291-300.
- 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*, pp. 367-384. Academic Press, San Diego.
- Pärtel M., Kalamees R., Zobel M. & Rosén E. (1998) Restoration of species-rich limestone grassland communities from overgrown land: the importance of propagule availability. *Ecological Engineering* 10: 275-286.
- Patzelt A., Wild U. & Pfadenhauer J. (1998) Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. *Restoration Ecology* 9: 127-136.
- Pöyry J., Paukkunen J., Heliölä J. & Kuussaari M. (2009) Relative contributions of local and regional factors to species richness and total density of butterflies and moths in semi-natural grasslands. *Oecologia* 160: 577-587.
- Pywell R.F., Bullock J.M., Hopkins A., Walker K.J., Sparks T.H., Burke M.J.W. & Peel S. (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* 39: 294-309.
- Rasran L., Vogt K. & Jensen K. (2006) Seed content and conservation evaluation of hay material of fen grasslands. *Journal for Nature Conservation* 14: 34-45.
- Rasran L., Vogt K. & Jensen K. (2007) Effects of topsoil removal, seed transfer with plant material and moderate grazing on restoration of riparian fen grasslands. *Applied Vegetation Science* 10: 451-493.

- Redecker B. (2001) Schutzwürdigkeit und Schutzperspektive der Stromtal-Wiesen an der unteren Mittelbe. *Archiv naturwissenschaftliche Dissertationen* 13: 1-164.
- Ren J., Tao L. & Liu X.M. (2002) Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *Journal of Arid Environments* 51: 603-611.
- Richardson D.M., Holmes P.M., Esler K.J., Galatowitsch S.M., Stromberg J.C., Kirkman S.P., Pysek P. & Hobbs R.J. (2007) Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions* 13: 126-139.
- Ružičková H., Banášová V. & Kalivoda H. (2004) Morava River alluvial meadows on the Slovak-Austrian border (Slovak part): plant community dynamics, floristic and butterfly diversity - threats and management. *Journal for Nature Conservation* 12: 157-169.
- Schmiede R., Donath T.W. & Otte A. (2009) Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404-413.
- Schnittler M. & Günther K.-F. (1999) Central European vascular plants requiring priority conservation measures - an analysis from national Red Lists and distribution maps. *Biodiversity and Conservation* 8: 891-925.
- Simpson R.L., Leck M.A. & Parker V.T. (1989) Seed banks: general concepts and methodological issues. In: Leck M.A., Parker V.T. & Simpson R.L. (eds.) *Ecology of soil seed banks*, pp. 3-8. Academic Press, San Diego.
- Smith R.S., Shiel R.S., Millward D., Corkhill P. & Sanderson R.A. (2002) Soil seed banks and the effects of meadow management on vegetation change in a 10-year meadow field trial. *Journal of Applied Ecology* 39: 279-293.
- Stroh M., Storm C., Zehm A. & Schwabe A. (2002) Restorative grazing as a tool for directed succession with diaspore inoculation: the model of sand ecosystems. *Phytocoenologia* 32: 595-625.
- Strykstra R.J., Bekker R.M. & Bakker J.P. (1998) Assessment of dispersule availability: its practical use in restoration management. *Acta Botanica Neerlandica* 47: 57-70.
- Strykstra R.J., Verweij G.L. & Bakker J.P. (1997) Seed dispersal by mowing machinery in a Dutch brook valley system. *Acta Botanica Neerlandica* 46: 387-401.
- Thompson K. (2000) The functional ecology of soil seed banks. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 215-235. CABI Publishing, Wallingford.
- Thompson K., Bakker J.P. & Bekker R.M. (1997) *The soil seed banks of North West Europe: methodology, density and longevity*, University Press, Cambridge.
- Thompson K., Bakker J.P., Bekker R.M. & Hodgson J.G. (1998) Ecological correlates of seed persistence in soil in the north-west European flora. *Journal of Ecology* 86: 163-169.
- Thompson K., Band S.R. & Hodgson J.G. (1993) Seed size and shape predict persistence in soil. *Functional Ecology* 7: 236-241.
- Thompson K. & Grime J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology* 20: 141-156.
- Tilman D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78: 81-92.
- Toogood S.E., Joyce C.B. & Waite S. (2008) Response of floodplain grassland plant communities to altered water regimes. *Plant Ecology* 197: 285-298.
- van der Valk A.G. (1974) Environmental factors controlling the distribution of forbs on coastal foredunes in Cape Hatteras National Seashore. *Canadian Journal of Botany* 52: 1057-1073.
- van Dorp D., Schippers P. & van Groenendael J.M. (1997) Migration rates of grassland plants along corridors in fragmented landscapes assessed with a cellular automation model. *Landscape Ecology* 12: 39-50.
- Van Looy K. & Meire P. (2009) A conservation paradox for riparian habitats and river corridor species. *Journal for Nature Conservation* 17: 33-46.
- Vécrin M.P., Gréville F. & Muller S. (2007) The contribution of persistent soil seed banks and flooding to the restoration of alluvial meadows. *Journal for Nature Conservation* 15: 59-69.
- Vécrin M.P., van Diggelen R., Gréville F. & Muller S. (2002) Restoration of species-rich floodplain meadows from abandoned arable fields in NE France. *Applied Vegetation Science* 5: 263-270.

- Vleeshouwers L.M. (1997) Modelling the effect of temperature, soil penetration resistance, burial depth and seed weight on pre-emergence growth of weeds. *Annals of Botany* 79: 553-563.
- Walker K.J., Stevens P.A., Stevens D.P., Mountford J.O., Manchester S.J. & Pywell R.F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119: 1-18.
- Wallin L., Svensson B.M. & Lönn M. (2009) Artificial dispersal as a restoration tool in meadows: sowing or planting? *Restoration Ecology* 17: 270-279.
- Welander J. (2000) Spatial and temporal dynamics of wild boar (*Sus scrofa*) rooting in a mosaic landscape. *Journal of Zoology* 252: 263-271.
- Wijnhoven S., Thonon I., Velde G.D., Leuven R., Zorn M., Eijsackers H. & Smits T. (2006) The impact of bioturbation by small mammals on heavy metal redistribution in an embanked floodplain of the River Rhine. *Water Air and Soil Pollution* 177: 183-210.
- Willems J.H. & Bik L.P.M. (1998) Restoration of high species density in calcareous grassland: the role of seed rain and soil seed bank. *Applied Vegetation Science* 1: 91-100.
- Willems J.H. & Huijsmans K.G.A. (1994) Vertical seed dispersal by earthworms: a quantitative approach. *Ecography* 17: 124-130.
- Woodcock B.A., Edwards A.R., Lawson C.S., Westbury D.B., Brook A.J., Harris S.J., Brown V.K. & Mortimer S.R. (2008) Contrasting success in the restoration of plant and phytophagous beetle assemblages of species-rich mesotrophic grasslands. *Oecologia* 154: 773-783.
- Yanful M. & Maun M.A. (1996) Effects of burial of seeds and seedlings from different seed sizes on the emergence and growth of *Strophostyles helvola*. *Canadian Journal of Botany* 74: 1322-1330.
- Young A., Boyle T. & Brown T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* 11: 413-418.
- Zhang J. & Maun M.A. (1994) Potential for seed bank formation in seven Great Lakes sand dune species. *American Journal of Botany* 81: 387-394.
- Zobel M., Otsus M., Liira J., Moora M. & Möls T. (2000) Is small-scale species richness limited by seed availability or microsite availability? *Ecology* 81: 3274-3282.

Seed bank samples in the greenhouse



## CHAPTER 2

# **Plant pattern development during early post-restoration succession in grasslands – a case study of *Arabis nemorensis***

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*While it is well-established that plant material transfer is a suitable tool for overcoming dispersal limitation during grassland restoration, it is still unknown if structural and spatial patterns of newly-established populations on the restoration sites resemble those on remnant sites. Furthermore, the process of seed bank formation on restoration sites has not been sufficiently investigated. This chapter addresses these topics.*

## Abstract

The main objective of this study was to analyze whether plant material transfer is a suitable approach for establishing plant populations with spatial patterns and population structures resembling those of remnant populations. We studied pattern formation and population characteristics in three remnant and two restored populations of the biennial plant species *Arabis nemorensis* in the upper Rhine valley in southwestern Germany over a period of two years. We investigated spatial patterns of seedlings, juveniles and adults as well as the small-scale horizontal and vertical distribution of seeds in the soil, followed the fate of individual plants and recorded structural habitat parameters such as vegetation and litter cover. Population dynamics differed between the study sites, but there was no pronounced difference between restored and remnant sites. Seedlings, juveniles and adults as well as seeds in the seed bank showed aggregated spatial patterns on all study sites, with positive autocorrelation on a scale of 20-60 cm. Within sites, patterns remained approximately stable through time. Restored sites experienced rapid seed bank formation as a result of the restoration measures. Our results suggest that the restoration measures were not only successful in transferring the target species, but also triggered rapid formation of spatially-structured populations that, two years after restoration, closely resembled those of remnant sites.

## Keywords

flood meadows; grassland restoration; hay transfer; plant material transfer; population dynamics; soil seed bank; spatial autocorrelation

## Introduction

Species-rich grasslands have declined drastically across Europe since the middle of the 20<sup>th</sup> century due to intensified management, abandonment and conversion into arable fields (Joyce & Wade 1998; Blackstock et al. 1999). Flood meadows have been particularly affected since they were not common in the first place due to the limited spatial extent of the abiotic conditions necessary for their occurrence. These alluvial grasslands harbour many rare and endangered species (Burkart 2001) and are therefore considered to be of great conservation value. In many regions, however, only a few isolated remnants are left, which are particularly endangered (Blackstock et al. 1999). Restoration of degraded sites could help to overcome the isolation of these fragments and thus decrease the risk of local extinction for the typical species.

Restoration is generally considered to be a key approach to the conservation of European species-rich grasslands (Bakker & Berendse 1999; Blackstock et al. 1999, Donath et al. 2007), and restoration schemes are now implemented in many places (e.g. Vécrin et al. 2002; Klimkowska et al. 2009). The aim of such projects should be to establish viable populations of the species forming the target community. This requires a thorough understanding of ecological processes, including both structural and spatial components (Bruehlheide & Flintrop 2000; Woodcock et al. 2006) and information about population dynamics, including seedling survival and recruitment (e.g. Willems & Bik 1998). We propose that these criteria should also be considered during the evaluation of the restoration success. Currently, most projects are primarily evaluated by presence/absence and abundance of target species without explicitly considering structural and spatial components. Furthermore, evaluation generally focuses on comparing the 'pre-restoration' state of the target sites with their 'post-restoration' state, as opposed to comparing restored with remnant sites (Aronson & Vallejo 2006).

Restoration projects need to address site availability, differential species availability and differential species performances and could include measures such as designed disturbances or controlled colonization (Hobbs & Norton 1996). The latter may be achieved by plant material transfer, where restoration sites are stocked with diaspore-rich material from remnant sites (Kiehl et al. 2006; Donath et al. 2007). This successfully overcomes dispersal limitation, which has been identified as a key obstacle during grassland restoration (Bakker & Berendse 1999; Vécrin et al. 2002; Donath et al. 2003). Furthermore, it might also trigger the formation of spatial patterns because a large part of the diaspores in the plant material are still attached to the mother plants during the application process and the plant material is usually distributed in a slightly uneven fashion. This may cause the formation of a patchy litter layer as frequently found in natural systems (Facelli & Pickett 1991) and create patterns that reflect the clustered spatial structure of the donor populations (cf. Pottier et al. 2007). We assume that plant material transfer mimics the availability of seeds and regeneration niches (*sensu* Grubb 1977) of natural plant communities and fosters pattern formation. To test this assumption, we conducted a case study with the biennial plant *Arabis nemorensis* (Hoffm.) Koch, a typical species of flood meadows (Burkart 2001).

Our main objectives were to evaluate the success of grassland restoration via plant material transfer by comparing restored and remnant sites with regard to population dynamics and spatial patterns and to analyze the processes governing plant pattern formation during early post-restoration succession. We placed special emphasis on seed bank forma-

tion and dynamics as they are considered to be particularly relevant for understanding ecological processes in grasslands (Bakker et al. 1996). Furthermore, the flood meadows we used as our study system are prone to severe natural disturbances, and previous studies have shown that their long-term persistence is at least partially dependent on soil seed banks (Hölzel & Otte 2004). Successful restoration in this habitat should therefore also foster seed bank formation.

We addressed the following questions:

- 1) Do remnant and restored populations differ with regard to their a) habitat structure, b) population structure and c) spatial patterns?
- 2) If so, do these difference decrease in the course of early post-restoration succession?
- 3) How does seed bank formation of the model species *A. nemorensis* proceed after restoration via plant material transfer?

## Methods

### Study region

The study was conducted in flood meadows in the Holocene floodplain of the northern upper Rhine (49° 51' N, 8° 24' E). The region is one of the last and most important strongholds of many rare and endangered alluvial grassland species in Europe (Burkart 2001). It is characterized by strong seasonal and inter-annual fluctuations of the ground-water table (Bissels et al. 2005) and has relatively warm and dry climatic conditions with a mean temperature of 10.3 °C and a mean annual precipitation of 580 mm (Müller-Westermeier 1990). In 2000, a large-scale restoration project was initiated in the region with the aim of re-establishing typical flood-meadow vegetation on former agricultural land (Donath et al. 2007).

### Study species

We selected the biennial species *Arabis nemorensis* (Brassicaceae) since it is a typical representative of flood meadows (Burkart 2001) and considered to be of concern for Central European conservation (Schnittler & Günther 1999). We deliberately chose a forb instead of a grass species because grasses generally establish well and because the key difficulty in restoring grasslands is to enhance the performance of forb species (Pywell et al. 2003). *Arabis nemorensis* is able to build up a persistent seed bank (Hölzel & Otte 2004) and is microsite- rather than seed-limited (Hölzel 2005). It germinates in spring, and the seedlings grow into rosettes in the first year (called juveniles from here on), which usually turn into flowering individuals (called adults from here on) in the second year. These are on average  $58.4 \pm 3.1$  cm high, have  $2.8 \pm 0.7$  flowering stalks and produce  $22850.1 \pm 5936.4$  smooth, dark-brown seeds that weigh  $0.05 \pm 0.001$  mg and do not have any specific dispersal structures (S. Burmeier, unpublished data).

## Study design

We selected five sites within the study region to investigate spatial patterns and population dynamics of *A. nemorensis*. The largest distance between any of the sites was 12 km. Two of the sites (A-B, 'restored sites') were former agricultural fields that were left to natural colonization 25 years ago and had turned into species-poor grasslands. In 2005, they became part of a restoration scheme designed to enhance the sites and recreate species-rich flood meadows. They were ploughed (approx. 30-40 cm deep) and then treated with plant material from remnant flood meadows (for details see Donath et al. 2007), which resulted in the establishment of *A. nemorensis*. The three other sites (C-E, 'remnant sites') were *Cnidion* meadows existing for at least several decades. Data on the vegetation composition of these sites are given by Donath et al. (2007). All sites are usually mown once or twice each year, and during the study period they were mown annually in autumn with a scythe mower.

On each site we established one 1x4-m transect, which was subdivided into 100 cells using a 20x20-cm grid. In June 2007 and June 2008 we estimated the percentage of total plant cover, litter cover and bare soil for each grid cell. Estimation was done visually with an accuracy of 5 %. Furthermore, we mapped all juveniles and adults of *A. nemorensis* in the plots. We measured the rosette diameter of the juveniles and the number of flower stalks and the total height of the adults. In April 2008, we counted all *A. nemorensis* seedlings in the plots on a 10x10-cm grid scale.

To assess the reproductive output of *A. nemorensis* we randomly sampled 10 plants per site adjacent to the transects in early June 2007, measured their height and counted the flower stalks and pods. In July 2007 we randomly sampled 100 pods and counted the seeds per pod. We regressed the number of pods against the product of plant height and number of flower stalks and used this model to predict the number of pods produced by each plant within the study plots from its height and number of flower stalks. This figure was multiplied by the average number of seeds per pod to give an estimate of the number of seeds produced by each plant within the plots.

After spring germination had ceased in late May 2007 and 2008, we took soil samples for assessing the seed bank composition. We took one core (2.5 cm diameter, 10 cm depth) per grid cell and separated it into three depth layers (0-1 cm, 1-5 cm, and 5-10 cm). In 2007, samples were taken slightly above the centre point of the cell, and in 2008 they were taken slightly below the centre point to safeguard against sampling exactly the same location twice. We determined the seed content with the seedling emergence method (Roberts 1981). As preliminary tests had shown that cold-wet stratification significantly increased germination success of *A. nemorensis* (S. Burmeier 2009, unpublished data), samples were kept in a 4 °C-incubator for five weeks prior to germination testing. Afterwards, samples were spread thinly (~ 0.5 cm) in separate pots on moistened, sterilized potting soil. The pots were placed in a temperature-controlled glasshouse and watered regularly. As we were primarily interested in the distribution of the viable *A. nemorensis* seed bank, incubation conditions in the glasshouse were adjusted to provide optimum germination conditions for *A. nemorensis* (20 °C during daytime and 10 °C at night; S. Burmeier, unpublished data). Pots were monitored regularly, and seedlings were counted and removed as soon as they could be identified. We distinguished only between seedlings of *A. nemorensis*, other dicotyledonous species and monocotyledonous spe-

cies. After six weeks, pots were allowed to air dry for two weeks, whereupon the soil was carefully stirred and the pots were incubated for another 10 weeks.

## Data analysis

*Habitat structure.* We used individual two-factorial ANOVAs to compare vegetation cover, litter cover and soil cover between study sites and years. To limit pseudoreplication, we randomly selected 20 out of the 100 subplots per site for the analyses.

*Population structure and dynamics.* As we mapped all *A. nemorensis* individuals within the plots in 2007 and 2008, we could follow the fate of individual plants (survival in the same stage, regression into an earlier stage, growth, death) from one season to the next. We used this data to prepare a Lefkovitch matrix based on life-cycle stages (Caswell 2001) for each population and to construct life-cycle diagrams. We used log-linear analyses to test for the effects of population (A-E) or population type (restored/remnant), respectively, and year (2007/2008) on population composition by life stages (seeds/seedlings/juveniles/adults) (cf. Caswell 2001).

*Spatial patterns.* We chose a hierarchical approach to compare the distribution patterns of the different life stages of *A. nemorensis*. As a first step, we visualized the data. We then calculated the variance-to-mean ratio (VMR) to examine the overall dispersion patterns of the different life stages. The expected ratio for a random distribution is 1, and ratios greater than 1 indicate aggregated patterns. If VMR revealed an aggregated pattern, we subsequently calculated Moran's I (Moran 1950) to examine the extent of aggregation. This index takes values between -1 and +1, with positive values indicating positive autocorrelation in the data and negative values indicating negative autocorrelation. Assuming that the intrinsic assumption (cf. Legendre & Legendre 1998) was met, the spatial autocorrelation coefficients were tested for significance against the null hypothesis of no autocorrelation at the given distance class. Euclidean distances were computed among the centres of the grid cells, and all data were aggregated on a 20x20-cm-subplot scale. The distance data were divided into 20 classes of equal width, i.e. we used a lag distance of 20 cm. To account for multiple testing we applied a progressive Bonferroni correction (cf. Legendre & Legendre 1998). As Moran's I only allowed comparison of the spatial distributions of the different life stages with regard to their structure but could not reveal whether the detected clusters above- and below-ground actually occurred in the same places, we applied a spatially constrained correlation approach as a fourth step. For this we used density data, i.e. we aggregated all data on the 20x20-cm subplot scale and arranged them as 5x20-matrices according to the layout of the subplots within the plots. To test the relationship between any two patterns of interest, we correlated the respective matrices and calculated Pearson's correlation coefficient. Being fully aware that this ignores spatial autocorrelation, we then took a constrained permutation approach to generate a distribution of correlation coefficients: Both matrices were permuted row- and column-wise, i.e. we generated new matrices while conserving the spatial information present in the original matrices. Each of the resulting 100 versions of matrix 1 was correlated against each of the 100 resulting versions of matrix 2, yielding a total of 10,000 correlation coefficients. The original coefficient was then tested for significance against the distribution of the permuted coefficients.

Software ANOVA and spatially constrained correlation analysis were performed with R (version 2.8.1, R Development Core Team 2008), which was also used for visualizing the

spatial pattern with the package ggplot2 (Wickham 2009). Life-cycle analysis was done with PopTools (version 2.6.9, Hood 2005), and log-linear analyses were performed with Statistica (version 6.0, StatSoft 2001). Moran's I was calculated with the R Package for multidimensional and spatial analysis (Legendre & Vaudor 1991).

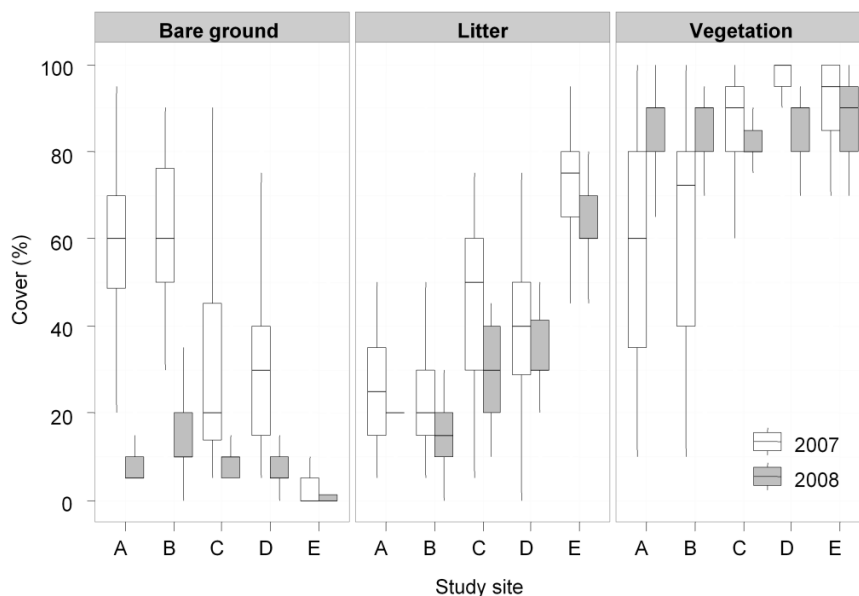
## Results

### Habitat structure

The study sites differed in terms of vegetation cover ( $F_{4,190} = 17.14$ ,  $p < 0.0001$ ), litter cover ( $F_{4,190} = 95.83$ ,  $p < 0.0001$ ) and bare soil cover ( $F_{4,190} = 75.96$ ,  $p < 0.0001$ ). The effect of time was significant for litter and soil cover ( $p < 0.0001$ ), but not for vegetation cover. While the restored sites (A-B) had a considerably lower vegetation cover and a considerably larger cover percentage of bare soil than the remnant sites (C-E) in 2007, these differences were much less pronounced in 2008 (Figure 2.1).

### Population structure and dynamics

In 2007, above-ground plant density across sites ranged between 1 and 25 individuals per m<sup>2</sup> for juveniles and between 11 and 67 individuals per m<sup>2</sup> for adults (Table 2.1). Restored populations had lower juvenile densities than remnant populations, but there was no consistent trend for adult densities. Seedling densities ranged between 45 and 961 individuals per m<sup>2</sup>. Seed production was higher on restored than on remnant sites in both years (Table 2.1). A total of 7904 seedlings emerged from the 1500 seed bank samples in 2007. Of these, 1777 (22.5 %) belonged to *A. nemorensis*, 5334 (67.5 %) to other dicotyledonous species, and 793 (10 %) to monocotyledonous species. In 2008, a total of 10 259 seedlings emerged, with 5052 (49.3 %) individuals of *A. nemorensis*, 4719 (46 %)



**Figure 2.1** Habitat structure of the study sites. Boxes show 25- and 75-percent quantiles and the median, whiskers show minimum and maximum values.

other dicotyledons, and 488 (4.8 %) monocotyledons. In both years, seed bank size and density differed immensely between the five study populations. Seed densities of *A. nemorensis* were higher at remnant sites in 2007, but higher at restored sites in 2008 (Table 2.1). Remnant sites had a much higher seed rain (i.e. seeds in 0-1 cm depth) than restored sites in 2007, whereas this relationship was reversed in 2008 (Figure 2.2). Furthermore, we observed a large increase in the number of seeds in 5-10 cm depth from 2007 to 2008 on both restored sites.

Population dynamics differed between study sites (Figure 2.3), but there was no pronounced difference between restored and remnant sites. It was, however, conspicuous that only remnant populations had juveniles remaining in that life stage for a second growing season. Furthermore, remnant sites had lower seedling mortality than restored sites. Log-linear analyses revealed that population or population type and year had a significant effect on population composition by life stages, even when the respective other effect was already accounted for in the model ( $p < 0.001$  in all cases, data not shown). The interactions between these effects were also highly significant ( $p < 0.001$ ).

## Spatial patterns

All life stages showed aggregated spatial patterns (Table 2.1), which were similar across sites and years (Figure 2.4). On sites with high seedling densities the aggregation levels indicated by the V/M-ratio decreased from seedling to juvenile to adult (Table 2.1). At all sites, we found positive spatial autocorrelation as indicated by significant values of Moran's  $I$  on a scale of 20-60 cm for almost all life stages (Figure 2.4). The distributional patterns of adults in 2007 were strongly positively correlated with those in 2008 at all sites (Table 2.2). In both new populations, the 2007 juvenile patterns were negatively correlated with the 2008 adult patterns, whereas they were positively correlated at site C. At the two other remnant sites this correlation was not significant. At site A, seedling patterns in 2008 were positively correlated with the 2007 seed rain patterns, whereas this correlation was not significant for all other sites. At site C we found a negative correlation

**Table 2.1** Density and aggregation patterns of different life stages and estimated seed production of *Arabis nemorensis*. A-B = restored sites, C-E = remnant sites, VMR = variance-to-mean ratio. Seedling data was only collected in 2008.

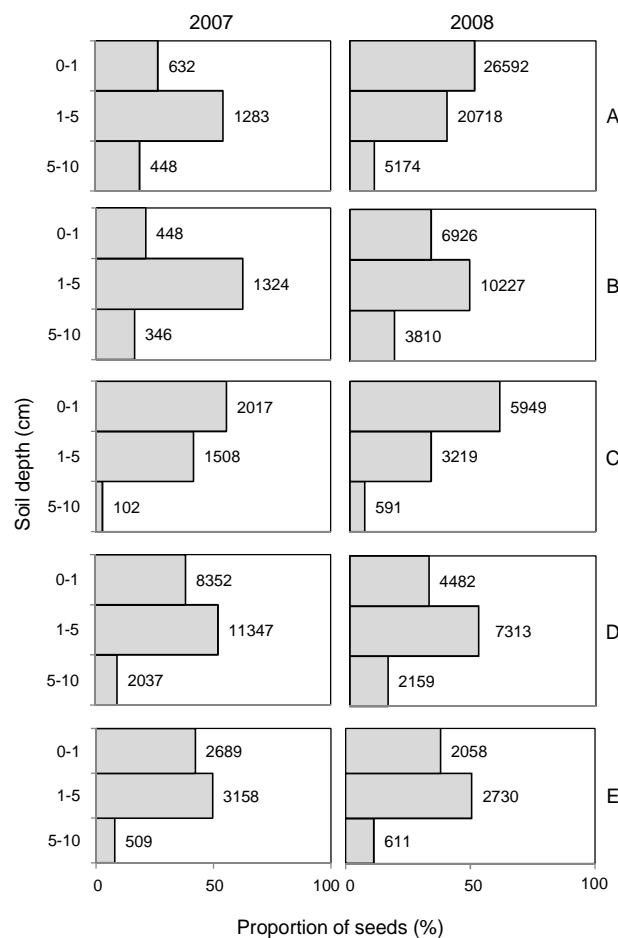
Year	Site	Seedlings		Juveniles		Adults		Seed bank (0-10 cm)		estimated seed production $\pm$ SE
		n/m <sup>2</sup>	VMR	n/m <sup>2</sup>	VMR	n/m <sup>2</sup>	VMR	n/m <sup>2</sup>	VMR	
2007	A			1	0.99	35	6.80	2363	3.23	12 590.5 $\pm$ 1812.7
	B			8	2.77	11	2.17	2119	3.67	24 006.2 $\pm$ 3858.3
	C			17	2.44	67	4.67	3626	2.32	5743.8 $\pm$ 125.7
	D			14	3.89	15	2.32	21737	5.94	5348.4 $\pm$ 1416.6
	E			25	4.53	14	2.90	6356	2.83	8817.6 $\pm$ 2262.9
2008	A	961	31.02	84	7.19	13	2.67	52845	37.71	11 479.7 $\pm$ 1621.5
	B	517	25.97	67	5.74	25	1.64	20963	10.38	15 826.9 $\pm$ 1647.3
	C	532	21.65	136	8.47	30	3.79	9758	12.86	5842.3 $\pm$ 251.1
	D	45	2.71	11	1.32	11	1.81	13955	3.92	7904.7 $\pm$ 904.7
	E	66	5.15	49	4.78	19	5.42	5399	2.56	9819.2 $\pm$ 1377.8

between the distributional patterns of adults in 2007 and seedlings in 2008, and at site E the distributional patterns of the 2007 seed rain were positively correlated with the 2008 seed bank.

The overall depth distribution patterns of seeds in the soil seed bank were consistent between years on all sites but site A (Figure 2.2). By contrast, horizontal seed distribution patterns differed between study years at both restored sites (Figure 2.5), and constrained correlation analysis revealed that the 2007 patterns were not correlated with the 2008 patterns. At the remnant sites, however, the 2007 horizontal distribution patterns of the seed bank of the lower soil layers of two of the sites were correlated with the 2008 patterns (Table 2.3). The spatial distribution of the seeds of *A. nemorensis* was positively correlated with those of other dicotyledonous species on two of the remnant sites in 2007 (site D:  $r = 0.22$ ,  $p < 0.001$ ; site E:  $r = 0.28$ ,  $p < 0.001$ ) and on one restored and two remnant sites in 2008 (site B:  $r = 0.33$ ,  $p < 0.05$ ; site C:  $r = 0.2$ ,  $p < 0.05$ ; site D:  $r = 0.38$ ,  $p < 0.001$ ).

## Discussion

Successful grassland restoration depends on the interaction between several conditions such as seed availability, favourable germination conditions and successful development of mature, seed producing individuals (Willems & Bik 1998). It is well-established that

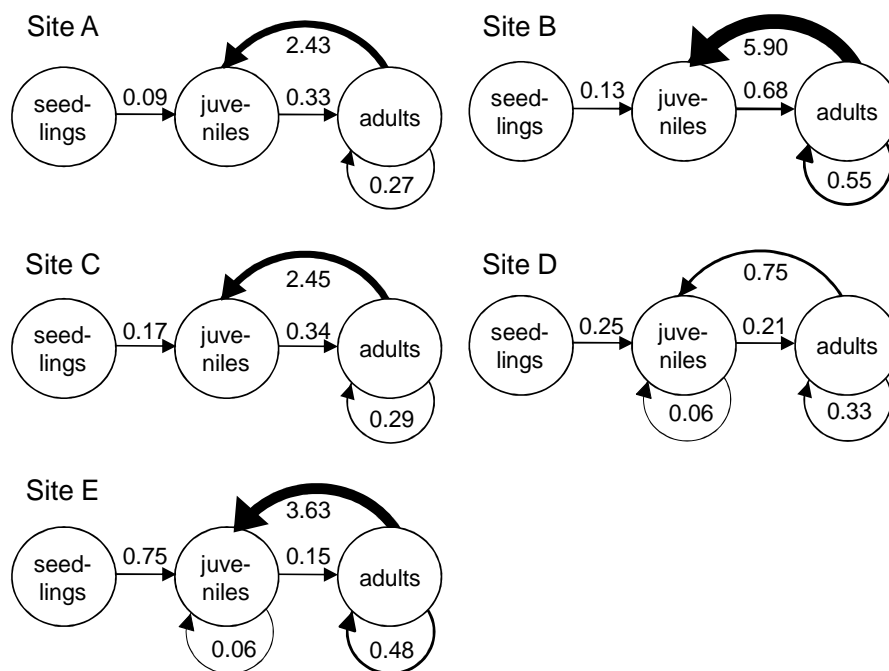


**Figure 2.2** Depth distributions of viable *Arabis nemorensis* seeds at the study sites in 2007 and 2008. Figures next to the bars give the absolute amount of seeds present (seeds per m<sup>2</sup>).

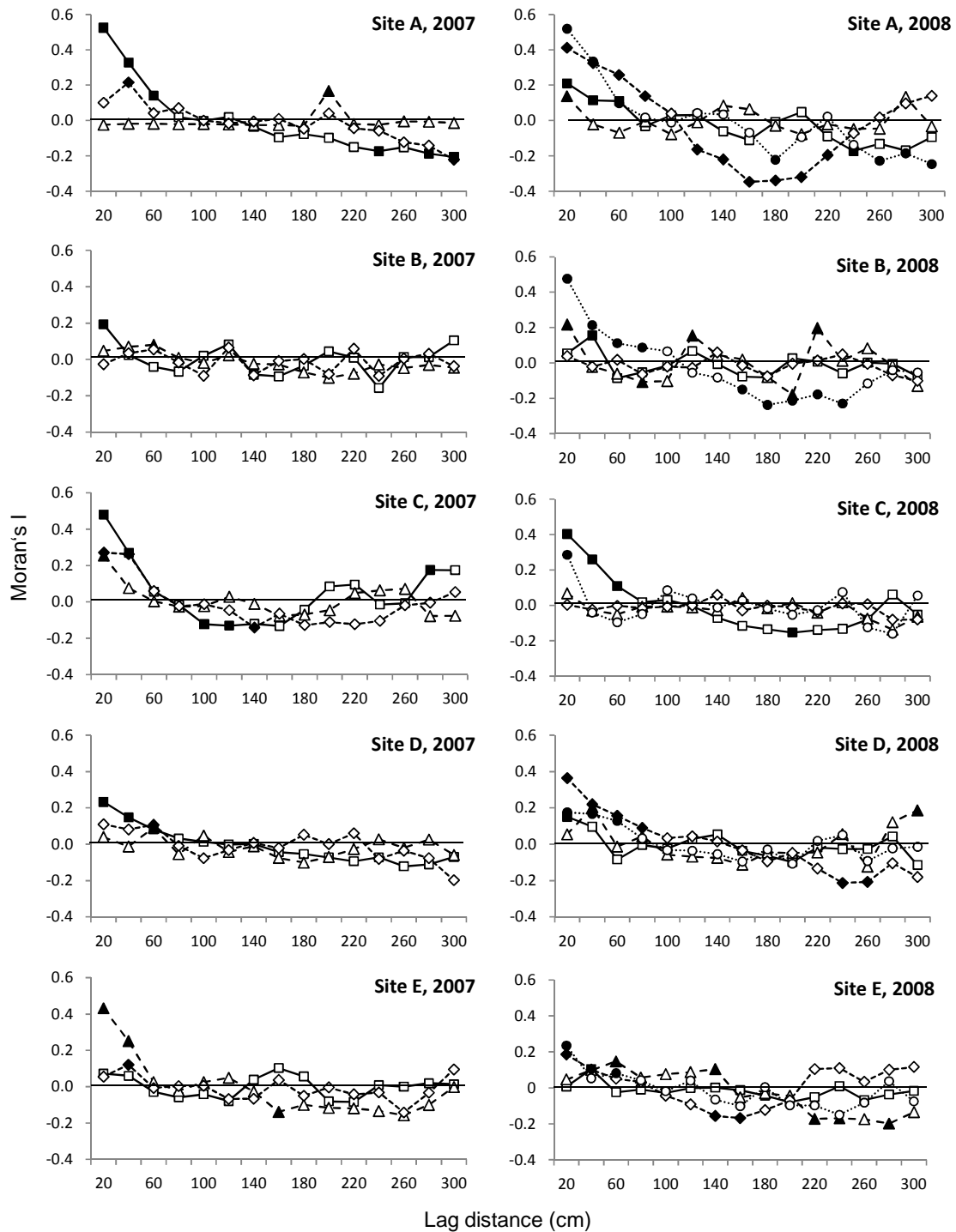
plant material transfer may provide these conditions and successfully re-introduce grassland species (e.g. Kiehl et al. 2006; Donath et al. 2007; Klimkowska et al. 2009). Spatial patterns and population dynamics, however, have received surprisingly little attention during the evaluation of such restoration measures. Our study has shown that plant material transfer triggered the formation of spatially-structured populations of the model species *A. nemorensis*.

Restoration often primarily focuses on the re-introduction of plant species, assuming that other taxa will follow. However, this is not necessarily the case (Kiehl & Wagner 2006; Woodcock et al. 2008), and structural parameters may be important for determining whether restored sites provide suitable habitat conditions for other taxa (Dahms et al. 2008). We found that the overall habitat structure of the restored sites rapidly approximated that of remnant sites in the course of early post-restoration succession. This indicates that plant material transfer may be a suitable method for restoring not only plant communities, but also structural conditions of flood meadows and presumably grasslands in general. We thus expect that it will eventually lead to the re-establishment of entire communities, given that restored and remnant sites are situated sufficiently close to each other to overcome dispersal limitations (cf. Mortimer et al. 2002).

Population dynamic processes in the study populations were overall very similar, and only three years after restoration had taken place the population dynamics of restored



**Figure 2.3** Results of a life-cycle analysis for the five *Arabis nemorensis* populations. The numbers adjacent to the arrows from seedlings to juveniles give the survival rate (expressed as number of juveniles in June 2008 per number of seedlings in April 2008), the numbers adjacent to the arrows from adults to juveniles give the fecundity (expressed as number of juveniles in 2008 per adults in 2007) and the other numbers give the transition probability between the respective life stages (cf. Caswell 2001). The diagrams are based on a simplified life cycle which does not include processes involving the seed bank.



**Figure 2.4** Spatial correlograms for the different life stages of *Arabis nemorensis*. Filled symbols indicate significant values (progressive Bonferroni correction).  $\square$  = adults,  $\triangle$  = juveniles,  $\diamond$  = seed bank (1-10 cm),  $\circ$  = seedlings. Spatial autocorrelation coefficients are not reported for the five distance classes beyond a lag distance of 300 cm since these included too few point pairs to yield reliable information.

populations could no longer be distinguished from remnant ones. This underscores the rapid success of the restoration measures. A year earlier, restored populations still had had lower juvenile densities than remnant populations, presumably due to the life cycle of *A. nemorensis*: As plants generally need two years until they flower, 2007 had been the first year of autonomous seed production on the restored sites. Any juveniles present on the sites at that time must have originated from seeds that had been brought to the sites during the restoration measures – and this ‘instant seed bank’ had already been depleted by the previous year’s germination. This underscores that short-term evaluation schemes for restoration projects should be adapted to the life cycle of the respective target species.

Restored and remnant populations differed with regard to seedling mortality, which was higher on restored sites. This could have been caused by density-dependent effects (cf. Moody-Weis & Alexander 2007), which may regulate the spatial structure within communities (Houle et al. 2001). High seedling densities had presumably been caused by higher light availability due to a more open vegetation cover, which triggered germination of a large number of seeds. Apparently, seed fate differed between restored and remnant populations: On remnant sites, seeds were more likely to be incorporated into the soil seed bank, whereas on restored sites they were more likely to germinate rapidly, followed by a comparatively high seedling mortality. These differences will presumably disappear as the habitat structure of restored populations further approximates that of remnant ones in the course of post-restoration succession (cf. Pywell et al. 2002).

Although *A. nemorensis* is a short-lived biennial whose population characteristics are likely to be different from those of the perennial species generally prevailing in grasslands (Eriksson & Jakobsson 1998), we believe that our findings on its population structure and dynamics are meaningful beyond a mere autecological perspective. As opposed to other grassland systems, the vegetation of the flood meadows we studied consists of approximately equal proportions of short-lived annuals and biennials on the one hand and long-lived perennials on the other hand (Schmiede et al. 2009). Our results may therefore be representative for many other species and thus contribute to an increased understanding of the patterns and processes of flood-meadow restoration.

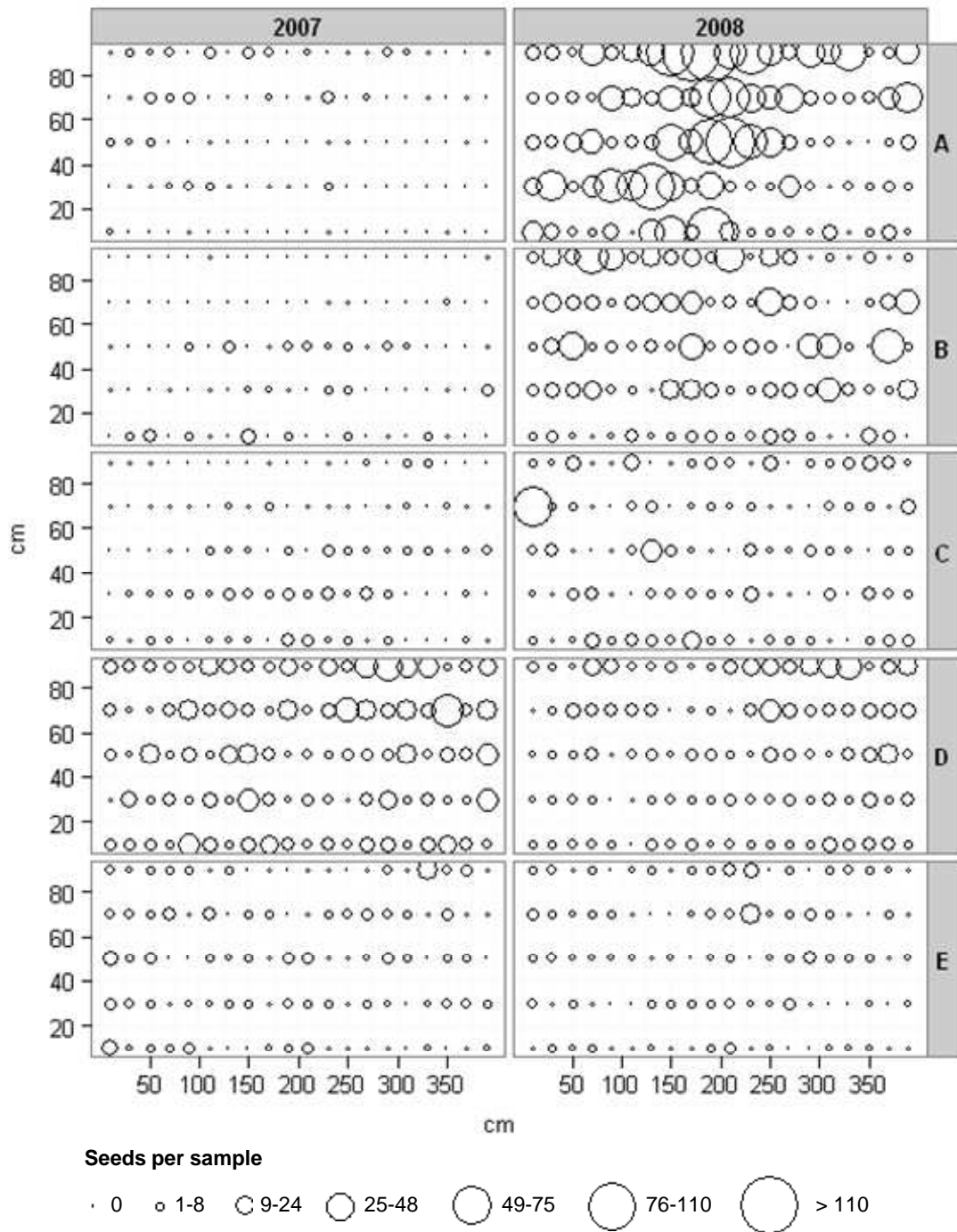
Grassland communities are often characterized by a strong spatial structure (Law et al. 2001), and successful restoration in these habitats should not only seek to re-introduce target species, but also aim at re-establishing their specific patterns and spatial arrangements. We found that the spatial patterns of our model species *A. nemorensis* showed a striking similarity between sites and that restored sites could no longer be distinguished from remnant ones in terms of their spatial patterns after only three years. Apparently, restoration had managed to trigger the formation of populations with a similar spatial structure to those on remnant sites.

Caballero et al. (2008) have proposed a spiral model to describe the relationship between the spatial patterns of above- and below-ground compartments of plant populations. The model predicts that this relationship will generally be maintained through time, despite small changes in the system and inter-year variations in composition and structure of the seed bank and the standing vegetation (Caballero et al. 2008). Transferred to a restoration context, this implies that the ultimate goal of restoration should be to re-establish populations for which the spiral model holds. We found that the patterns of different life

**Table 2.2** Results of a correlation analysis testing the relationships between the spatial distribution patterns of different life stages of *Arabis nemorensis*. Numbers in the top row and first column of the data entry give the number of individuals ( $n$ ) per m<sup>2</sup> observed in 2007 and 2008, respectively. The remaining matrix elements ( $a_{ij}$ ) represent the correlation coefficient (Pearson's R) for the relationship between stage  $j$  in 2007 and stage  $i$  in 2008. Significance was tested with a spatially-constrained correlation approach, and significant values are printed in bold and marked with asterisks (\*  $p > 0.05$ , \*\*  $p > 0.01$ , \*\*\*  $p > 0.001$ ).

Site A (new)		2007			
		Juveniles	Adults	Seed rain (0-1 cm)	Seed bank (1-10 cm)
2008	$n$	1	35	632	1732
	Juveniles	84	-0.03	0.07	0.11
	Adults	13	<b>-0.06***</b>	<b>0.64***</b>	0.22
	Seed rain	26952	<b>-0.1***</b>	0.16	0.13
	Seed bank	25893	-0.08	0.16	0.18
	Seedlings	961	-0.02	<b>0.18*</b>	0.21
Site B (new)		2007			
		Juveniles	Adults	Seed rain (0-1 cm)	Seed bank (1-10 cm)
2008	$n$	8	11	448	1670
	Juveniles	67	<b>-0.2***</b>	-0.05	-0.08
	Adults	25	<b>-0.42***</b>	<b>0.15*</b>	<b>0.22*</b>
	Seed rain	6926	-0.002	-0.04	-0.11
	Seed bank	14036	<b>-0.15*</b>	-0.05	-0.04
	Seedlings	517	-0.13	0.1	-0.05
Site C (old)		2007			
		Juveniles	Adults	Seed rain (0-1 cm)	Seed bank (1-10 cm)
2008	$n$	17	67	2017	1609
	Juveniles	136	0.07	-0.08	0.06
	Adults	30	<b>0.33*</b>	<b>0.61***</b>	0.01
	Seed rain	5949	-0.01	-0.06	0.01
	Seed bank	3810	0.15	0.01	-0.007
	Seedlings	532	-0.15	<b>-0.24***</b>	0.07
Site D (old)		2007			
		Juveniles	Adults	Seed rain (0-1 cm)	Seed bank (1-10 cm)
2008	$n$	14	15	8352	13384
	Juveniles	11	0.17	-0.001	-0.02
	Adults	11	0.18	<b>0.53***</b>	-0.08
	Seed rain	4482	0.12	0.26	<b>0.19*</b>
	Seed bank	9473	<b>0.33***</b>	0.002	<b>0.47***</b>
	Seedlings	45	<b>0.37***</b>	-0.13	-0.08
Site E (old)		2007			
		Juveniles	Adults	Seed rain (0-1 cm)	Seed bank (1-10 cm)
2008	$n$	25	14	2689	3667
	Juveniles	49	<b>0.3*</b>	<b>-0.15*</b>	0.02
	Adults	19	0.05	<b>0.73***</b>	-0.1
	Seed rain	2058	-0.07	0.07	-0.04
	Seed bank	3341	-0.06	0.12	<b>0.19*</b>
	Seedlings	66	0.12	-0.11	0.2

stages in restored and remnant populations of *A. nemorensis* showed similar structures within sites in both study years. This is generally in line with the spiral model and could imply that restoration has indeed achieved rapid success in terms of re-establishing spatially structured populations. However, to draw definite conclusions on this point, longer-term investigations covering a range of species would be necessary.



**Figure 2.5** Map of the soil seed bank densities of *Arabis nemorensis* at the study sites in 2007 and 2008. Symbol size is determined by the number of seeds per soil core (2.5 cm Ø, 10 cm depth). Respective samples from 2007 and 2008 were separated by a distance of approx. 3 cm distance (see text).

**Table 2.3** Results of a correlation analysis testing the relationships between the spatial distribution patterns of different seed bank layers of *Arabis nemorensis*. Numbers below and to the right of the stages give the number of individuals (*n*) observed in 2007 and 2008, respectively. Matrix elements ( $a_{ij}$ ) represent the correlation coefficient (Pearson's R) for the relationship between stage *j* in 2007 and stage *i* in 2008. Significance was tested with a spatially-constrained correlation approach, and significant values are printed in bold and marked with an asterisk (\*  $p > 0.05$ , \*\*  $p > 0.01$ , \*\*\*  $p > 0.001$ ).

Site A (new)			2007			
			0-1 cm	1-5 cm	5-10 cm	0-10 cm
2008		n	31	63	22	116
	0-1 cm	1323	0.16	0.1	0.12	0.16
	1-5 cm	1017	0.18	0.22	0.09	0.23
	5-10 cm	254	-0.03	-0.02	-0.03	-0.02
	0-10 cm	2594	0.16	0.15	0.1	0.19
Site B (new)			2007			
			0-1 cm	1-5 cm	5-10 cm	0-10 cm
2008		n	22	65	17	104
	0-1 cm	340	-0.04	-0.12	-0.05	-0.11
	1-5 cm	502	-0.12	-0.01	0.04	0.001
	5-10 cm	187	-0.09	-0.09	-0.07	-0.11
	0-10 cm	1029	-0.05	-0.09	-0.02	-0.08
Site C (old)			2007			
			0-1 cm	1-5 cm	5-10 cm	0-10 cm
2008		n	99	74	5	178
	0-1 cm	292	-0.02	-0.001	0.05	-0.01
	1-5 cm	158	0.05	0.05	-0.09	0.06
	5-10 cm	29	-0.13	-0.11	<b>-0.1***</b>	<b>-0.17*</b>
	0-10 cm	479	-0.02	0.003	0.01	-0.01
Site D (old)			2007			
			0-1 cm	1-5 cm	5-10 cm	0-10 cm
2008		n	410	557	100	1067
	0-1 cm	220	0.09	<b>0.2*</b>	0.08	0.19
	1-5 cm	359	0.02	<b>0.41***</b>	<b>0.27*</b>	<b>0.32***</b>
	5-10 cm	106	0.01	<b>0.32***</b>	<b>0.3**</b>	<b>0.26*</b>
	0-10 cm	685	0.06	<b>0.43***</b>	<b>0.26*</b>	<b>0.36**</b>
Site E (old)			2007			
			0-1 cm	1-5 cm	5-10 cm	0-10 cm
2008		n	132	155	25	312
	0-1 cm	101	-0.11	-0.03	-0.03	-0.09
	1-5 cm	134	<b>0.19*</b>	<b>0.23***</b>	<b>0.17*</b>	<b>0.29***</b>
	5-10 cm	30	0.02	0.14	-0.05	0.1
	0-10 cm	265	0.07	0.16	0.08	0.16

Since flood meadows are a disturbance-prone habitat where many species rely on persistent seed banks for their long-term survival (Hölzel & Otte 2004), successful restoration should also foster seed bank formation. We found evidence for a rapid seed bank forma-

tion of *A. nemorensis* during early post-restoration succession, which is in line with Neff et al. (2009), who also observed rapid seed bank development in their study of restored tidal freshwater wetlands. Willems & Bik (1998), however, who evaluated the restoration of species-rich, dry calcareous grasslands, suggest that the restoration of seed bank quality is a long-term process. The speed and extent of seed bank formation following restoration seems to be strongly community-dependent.

Seed bank formation generally depends on seed production, seed dispersal and subsequent seed fate. We found that the seed production of *A. nemorensis* in 2007 was not correlated with the seed rain in 2008, i.e. the number of seeds found in the uppermost layer of the ground. This may have been caused by seed predation (Cabin et al. 2000), but could also have been an artefact of the study design: We counted viable seeds only while determining the seed rain whereas our estimation of the reproductive output was based on all seeds present, including non-viable and aborted ones. It could also indicate that seeds undergo Phase II dispersal (i.e. horizontal or vertical movements) after an initial Phase I dispersal (i.e. movement from the parent plant to the surface, Chambers & MacMahon 1994). This assumption is supported by the fact that only one out of five study sites showed a positive correlation between the seed rain in 2007 and the seedling patterns in 2008. Furthermore, seed distribution patterns of *A. nemorensis* were correlated with those of other dicotyledonous species, which could indicate that these were caused by non-specific dispersal agents such as bioturbation by wild boars, moles or mice.

Incorporation of seeds into the soil and relocation back to the soil surface presumably occurs mainly through bioturbation by animals (Willems & Huijsmans 1994; Wijnhoven et al. 2006). However, the rapid incorporation of *A. nemorensis* seeds into deeper soil layers in the course of early post-restoration succession (cf. Schmiede et al. 2009) was probably also a side effect of the site history: The restoration sites were ploughed prior to restoration, and the plant material was applied on rough ground with clods and cracks. The small seeds of *A. nemorensis* could have easily been incorporated into these cracks and thus translocated to deeper soil layers, where they would enter dormancy and persist for extended periods (Burmeier et al. 2010). This presumably caused the short-term stable vertical patterns of seeds in the soil that we observed. We also found clustered horizontal seed bank patterns, which is in line with the findings of Sletvold & Rydgren (2007) for the facultative biennial *Digitalis purpurea*. Indeed, all of the studies that have so far dealt with small-scale spatial patterns of seed banks have reported clustered patterns (e.g. Thompson 1986; Shaukat & Siddiqui 2004), which could imply that patchiness might be a characteristic of soil seed banks in general. With regard to restoration, this implies that it is necessary to take a large number of seed bank samples in order to evaluate the restoration success on the seed bank level.

In summary, we found that restoration via plant material transfer rapidly triggered the formation of spatially-structured populations that closely resembled those of remnant sites. As we have only studied the initial phase of restoration we cannot be absolutely sure that our study species will persist as post-restoration succession continues. However, since it has already established a substantial seed bank and seed bank persistence is known to be an important determinant of restoration success (Pywell et al. 2003), we are confident that *A. nemorensis* will be able to maintain itself on the restored sites in the future. We conclude that restoration has been highly successful for this species and recommend the further use of plant material transfer in the restoration of grassland ecosystems.

## Implications for Practice

- Successful restoration of grassland systems should aim at re-establishing community-specific patterns and spatial arrangements.
- Criteria concerning spatial aspects should be included in target definitions and monitoring protocols for restoration projects.
- Plant material transfer – besides bypassing dispersal limitation – triggers the formation of populations with similar structural and spatial patterns to those on donor sites, and we recommend its further application and study during grassland restoration
- In the early phase of vegetation development after restoration, management should focus on enhancing seed production and seed bank formation, e.g. in flood meadows by mowing/mulching only late in the season.

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## References

- Aronson J. & Vallejo R. (2006) Challenges for the practice of ecological restoration. In: van Andel J. & Aronson J. (eds.) *Restoration Ecology*, pp. 234-247. Blackwell, Oxford.
- Bakker J.P. & Berendse F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution* 14: 63-68.
- Bakker J.P., Poschlod P., Strykstra R.J., Bekker R.M. & Thompson K. (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45: 461-490.
- Bissels S., Donath T.W., Holzel N. & Otte A. (2005) Ephemeral wetland vegetation in irregularly flooded arable fields along the northern Upper Rhine: the importance of persistent seed-banks. *Phytocoenologia* 35: 469-488.
- Blackstock T.H., Rimes C.A., Stevens D.P., Jefferson R.G., Robertson H.J., Mackintosh J. & Hopkins J.J. (1999) The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978-96. *Grass and Forage Science* 54: 1-18.
- Bruelheide H. & Flintrop T. (2000) Evaluating the transplantation of a meadow in the Harz Mountains, Germany. *Biological Conservation* 92: 109-120.
- Burkart M. (2001) River corridor plants (Stromtalpflanzen) in Central European lowland: a review of a poorly understood plant distribution pattern. *Global Ecology and Biogeography* 10: 449-468.
- Burmeier S., Eckstein R.L., Otte A. & Donath T.W. (2010) Desiccation cracks act as natural seed traps in flood-meadow systems. *Plant and Soil* 333: 351-364.
- Caballero I., Olano J.M., Loidi J. & Escudero A. (2008) A model for small-scale seed bank and standing vegetation connection along time. *Oikos* 117: 1788-1795.

- Cabin R.J., Marshall D.L. & Mitchell R.J. (2000) The demographic role of soil seed banks. II. Investigations of the fate of experimental seeds of the desert mustard *Lesquerella fendleri*. *Journal of Ecology* 88: 293-302.
- Caswell H. (2001) *Matrix population models*, Sinauer Associates, Sunderland.
- Chambers J.C. & MacMahon J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics* 25: 263-292.
- Dahms H., Lenoir L., Lindborg R., Wolters V. & Dauber J. (2008) Restoration of seminatural grasslands: what is the impact on ants? *Restoration Ecology* 18: 330-337.
- Donath T.W., Bissels S., Hölzel N. & Otte A. (2007) Large scale application of diaspore transfer with plant material in restoration practice - Impact of seed and microsite limitation. *Biological Conservation* 138: 224-234.
- Donath T.W., Hölzel N. & Otte A. (2003) The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Applied Vegetation Science* 6: 13-22.
- Eriksson O. & Jakobsson A. (1998) Abundance, distribution and life histories of grassland plants: a comparative study of 81 species. *Journal of Ecology* 86: 922-933.
- Facelli J.M. & Pickett S.T.A. (1991) Plant litter: its dynamics and effects on plant community structure. *The Botanical Review* 57: 1-31.
- Grubb P.J. (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* 52: 107-145.
- Hobbs R.J. & Norton D.A. (1996) Towards a conceptual framework for restoration ecology. *Restoration Ecology* 4: 93-110.
- Hölzel N. (2005) Seedling recruitment in flood-meadow species: The effects of gaps, litter and vegetation matrix. *Applied Vegetation Science* 8: 115-124.
- Hölzel N. & Otte A. (2004) Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. *Journal of Vegetation Science* 15: 93-100.
- Hood G.M. (2005) PopTools Version 2.6.9.
- Houle G., McKenna M.F. & Lapointe L. (2001) Spatiotemporal dynamics of *Floerkea proserpinacoides* (Limnanthaceae), an annual plant of the deciduous forest of eastern North America. *American Journal of Botany* 88: 594-607.
- Joyce C.B. & Wade M.W. (1998) Wet grasslands: a European perspective. In: Joyce C.B. & Wade P.M. (eds.) *European Wet Grasslands: Biodiversity, Management and Restoration*, pp. 1-12. John Wiley, Chichester.
- Kiehl K., Thormann A. & Pfadenhauer J. (2006) Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. *Restoration Ecology* 14: 148-156.
- Kiehl K. & Wagner C. (2006) Effect of hay transfer on long-term establishment of vegetation and grasshoppers on former arable fields. *Restoration Ecology* 14: 157-166.
- Klimkowska A., Kotowski W., van Diggelen R., Grootjans A.P., Dzierża P. & Brzezińska K. (2009) Vegetation re-development after fen meadow restoration by topsoil removal and hay transfer. *Restoration Ecology*, DOI: 10.1111/j.1526-100X.2009.00554.x.
- Law R., Purves D.W., Murrell D.J. & Dieckmann U. (2001) Causes and effects of small-scale spatial structure in plant populations. In: Silvertown J. & Antonovics J. (eds.) *Integrating Ecology and Evolution in a Spatial Context*, pp. 21-44. Blackwell Science, Oxford.
- Legendre P. & Legendre L. (1998) *Numerical ecology*, Elsevier, Amsterdam.
- Legendre P. & Vaudor A. (1991) *The R Package: Multidimensional analysis, spatial analysis*, Département de sciences biologique, Université de Montréal, Montréal.
- Moody-Weis J. & Alexander H.M. (2007) The mechanisms and consequences of seed bank formation in wild sunflower (*Helianthus annuus*). *Journal of Ecology* 95: 851-864.
- Moran P.A.P. (1950) Notes on continuous stochastic phenomena. *Biometrika* 37: 17-23.
- Mortimer S.R., Booth R.G., Harris S.J. & Brown V.K. (2002) Effects of initial site management on the Coleoptera assemblages colonising newly established chalk grassland on ex-arable land. *Biological Conservation* 104: 301-313.
- Müller-Westermeier G. (1990) *Klimadaten der Bundesrepublik Deutschland*, Deutscher Wetterdienst, Offenbach.

- Neff K.P., Rusello K. & Baldwin A.H. (2009) Rapid seed bank development in restored tidal fresh-water wetlands. *Restoration Ecology* 17: 539-548.
- Pottier J., Marrs R.H. & Bedecarrats A. (2007) Integrating ecological features of species in spatial pattern analysis of a plant community. *Journal of Vegetation Science* 18: 223-230.
- Pywell R.F., Bullock J.M., Hopkins A., Walker K.J., Sparks T.H., Burke M.J.W. & Peel S. (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* 39: 294-309.
- Pywell R.F., Bullock J.M., Roy D.B., Warman L.I.Z., Walker K.J. & Rothery P. (2003) Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology* 40: 65-77.
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Roberts H.A. (1981) Seed banks in soils. In: Coaker T.H. (ed.) *Advancements in Applied Biology* 6, pp. 1-55. Academic Press, Cambridge.
- Schmiede R., Donath T.W. & Otte A. (2009) Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404-413.
- Schnittler M. & Günther K.-F. (1999) Central European vascular plants requiring priority conservation measures - an analysis from national Red Lists and distribution maps. *Biodiversity and Conservation* 8: 891-925.
- Shaukat S.S. & Siddiqui I.A. (2004) Spatial pattern analysis of seeds of an arable soil seed bank and its relationship with above-ground vegetation in an arid region. *Journal of Arid Environments* 57: 311-327.
- Sletvold N. & Rydgren K. (2007) Population dynamics in *Digitalis purpurea*: the interaction of disturbance and seed bank dynamics. *Journal of Ecology* 95: 1346-1359.
- StatSoft (2001) STATISTICA für Windows. Version 6.0.
- Thompson K. (1986) Small-scale heterogeneity in the seed bank of an acidic grassland. *Journal of Ecology* 74: 733-738.
- Vécrin M.P., van Diggelen R., Gréville F. & Muller S. (2002) Restoration of species-rich floodplain meadows from abandoned arable fields in NE France. *Applied Vegetation Science* 5: 263-270.
- Wickham H. (2009) ggplot2: An implementation of the Grammar of Graphics. R package version 0.8.2.
- Wijnhoven S., Thonon I., Velde G.D., Leuven R., Zorn M., Eijsackers H. & Smits T. (2006) The impact of bioturbation by small mammals on heavy metal redistribution in an embanked floodplain of the River Rhine. *Water Air and Soil Pollution* 177: 183-210.
- Willems J.H. & Bik L.P.M. (1998) Restoration of high species density in calcareous grassland: the role of seed rain and soil seed bank. *Applied Vegetation Science* 1: 91-100.
- Willems J.H. & Huijsmans K.G.A. (1994) Vertical seed dispersal by earthworms: a quantitative approach. *Ecography* 17: 124-130.
- Woodcock B.A., Edwards A.R., Lawson C.S., Westbury D.B., Brook A.J., Harris S.J., Brown V.K. & Mortimer S.R. (2008) Contrasting success in the restoration of plant and phytophagous beetle assemblages of species-rich mesotrophic grasslands. *Oecologia* 154: 773-783.
- Woodcock B.A., Lawson C.S., Mann D.J. & McDonald A.W. (2006) Effects of grazing management on beetle and plant assemblages during the re-creation of a flood-plain meadow. *Agriculture Ecosystems & Environment* 116: 225-234.

Flood meadow after mowing



## CHAPTER 3

# **Spatially-restricted plant material application creates colonization initials for flood-meadow restoration**

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*Chapter 2 has shown that plant material transfer may rapidly trigger the formation of spatially-structured populations of transferred species. However, do these species also spread from the plant material strips and eventually colonize the entire restoration sites? This is the subject of the present chapter.*

## **Abstract**

Plant material transfer is a well-established technique for overcoming dispersal limitation during grassland restoration. As restoration sites are frequently more abundant than donor sites, the plant material is often applied as patches or strips, with the assumption that these will act as colonization initials from which transferred species will spread and eventually cover the entire sites. Our aim was to test this assumption and to evaluate whether it is feasible to restore entire sites by spatially-restricted plant material application in a flood-meadow ecosystem. We established transverse transects consisting of eight 2x2 m plots on five plant material strips 7-8 years after plant material application. We monitored the above-ground vegetation development, analyzed the seed rain and determined the composition of the soil seed bank, i.e. we compared three different components of the emerging flood-meadow community. Transferred species were present in all three community components studied, and 88.6 % of the 79 species we found in total had already spread from the plant material strips and colonized their surroundings. Detected dispersal distances differed between community components, and the share of colonizers was highest for the above-ground vegetation and lowest for the soil seed bank. We conclude that plant material transfer is a suitable technique for restoring flood meadows as transferred species not only establish on the sites supplied with plant material, but also colonize their surroundings.

## **Keywords**

alluvial grassland; dispersal; floodplain; hay transfer; seed traps; soil seed bank

## Introduction

Alluvial grasslands harbour many rare and endangered species (Burkart 2001) and are considered to be of great conservation value. Like other species-rich grasslands, however, they have declined drastically across Europe since the middle of the 20th century due to intensified management, abandonment and conversion into arable fields (Joyce & Wade 1998; Blackstock et al. 1999). Restoration of degraded sites could help to reconnect isolated remnants and thus decrease the risk of local extinction for typical flood-meadow species.

Ecological restoration implies directing ecosystem development towards a target ecosystem by altering and/or accelerating successional processes (Palmer et al. 1997; Bakker & Berendse 1999; Bakker et al. 2000). The response of communities and the course of succession are determined by the habitat quality, and restoration success depends on creating a suitable environment for the target species and communities. This includes hydrological conditions and nutrient availability (Patzelt et al. 1998; Walker et al. 2004) as well as microsite limitation (Isselstein et al. 2002) and dispersal limitation, i.e. a shortage of dispersal units such as seeds, fruits or vegetative dispersal structures (Bischoff 2002; Pywell et al. 2002).

Even if abiotic site conditions are benign, it is therefore often necessary to actively reintroduce target species to achieve restoration goals, especially in isolated sites where colonisation is hampered by distance (Palmer et al. 1997; Bossuyt & Hermy 2003). Several approaches have been suggested for re-introducing species into grassland systems, including sowing of commercially available and locally gathered seed mixtures, turf transplantation and transfer of diaspore-rich plant material from sites containing the desired target species or communities (Kiehl et al. 2010 and references therein).

Plant material transfer has been applied in the restoration of different types of grassland such as calcareous grassland, mesic grassland, fens and flood meadows (Kiehl et al. 2010 and references therein). Generally, donor sites are mown in autumn to maximize seed content, and the fresh plant material is subsequently transferred to the restoration sites. As restoration sites are often more abundant than donor sites, the available plant material is generally not sufficient for covering the entire restoration sites and is applied in the form of square or rectangular patches (Kirmer & Mahn 2001; Stroh et al. 2002; Rasran et al. 2007) or strips (Donath et al. 2007) – with the implicit assumption that these patches or strips will then act as colonization initials from which the transferred target species can spread and eventually cover the entire restoration site (Patzelt et al. 1998; Kiehl et al. 2006).

Results of previous studies have shown that plant material transfer is generally very successful in transferring species and thus overcoming dispersal limitation if initial site conditions are suitable (Kiehl et al. 2010 and references therein). However, monitoring is generally restricted to evaluating species establishment in the above-ground vegetation on the sites that were treated with plant material (e.g. Patzelt et al. 1998; Stroh et al. 2002; Kiehl et al. 2006; Rasran et al. 2007). The assumed role of the plant material patches or strips as colonization initials and accompanying dispersal processes have received surprisingly little attention so far (but see Schmiede et al. 2009).

Our aim was to test the assumption that plant material strips act as colonization initials for transferred species and to evaluate whether the restoration of entire sites by spatially-restricted plant material application is actually feasible in a flood-meadow ecosystem. We (i) monitored above-ground vegetation development for three consecutive years, (ii) analysed the seed rain and (iii) determined the composition of the soil seed bank 7-8 years after plant material application, i.e. we compared three different components of the emerging flood-meadow community. The seed bank was of particular interest in this regard as it may guarantee the long-term persistence of plant species in disturbance-prone systems such as flood meadows by buffering their populations against environmental variability (Thompson 2000; Hölzel & Otte 2004). Accordingly, successful restoration should not only result in the re-establishing of the above-ground vegetation, but also of the soil seed bank.

We addressed the following questions:

- 1) Do plant material strips act as colonization initials for target species of flood-meadow restoration, i.e. are transferred species present in the above-ground vegetation, the seed rain and the soil seed bank of plant material strips and adjacent plots?
- 2) If transferred species can be detected adjacent to plant material strips, how far have they spread 7-8 years after restoration measures were carried out?
- 3) Is the colonization success of individual species linked to specific traits?
- 4) What are the implications for future restoration projects?

## **Methods**

### **Study area**

The study was conducted in flood meadows in the Holocene floodplain of the northern Upper Rhine, approx. 30 km southwest of Frankfurt, Germany (49° 51' N, 8° 24' E). The region is one of the last and most important strongholds of many rare and endangered alluvial grassland species in Europe (Burkart 2001). It is characterized by strong seasonal and inter-annual fluctuations of the ground-water table (Bissels et al. 2005) and has relatively warm and dry climatic conditions with a mean temperature of 10.3 °C and a mean annual precipitation of 580 mm (Müller-Westermeier 1990).

Until the 1950s, the area was dominated by species-rich alluvial grasslands that were managed extensively as hay meadows (Knapp 1954). Due to intensified drainage and structural changes in agriculture many sites were subsequently converted into arable fields (Böger 1991). In the 1980s, however, re-conversion into grassland began after severe flooding events had rendered agricultural use uneconomical and land was set aside for conservation purposes. As restoration by natural succession turned out to be strongly dispersal limited (Donath et al. 2003), a large-scale restoration project was initiated in the year 2000 with the aim of re-establishing typical flood-meadows on former agricultural land by means of plant material transfer (Donath et al. 2007).

## Study design

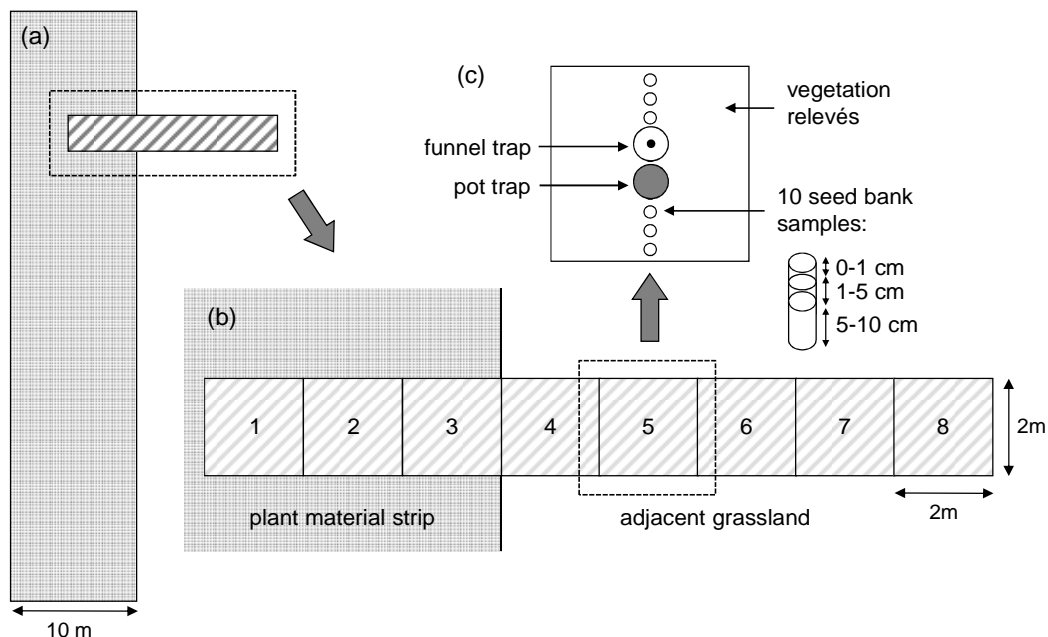
We selected five plant material strips that had been established in 2000 and 2001 (for details see Donath et al. 2007). Each strip was situated on a separate site, with the largest distance between any of the sites being 1.8 km, and all sites were mown once or twice a year (in mid-June and, occasionally, late autumn). Assuming dispersal from the plant material strips into the surrounding vegetation matrix, we established a transverse transect of eight contiguous 2 x 2 m plots on each of the strips (Figure 3.1), yielding a total of 40 study plots. In each plot, we conducted vegetation relevés, took seed bank samples and installed seed traps for monitoring the seed rain.

### *Vegetation relevés*

Vegetation data were collected in 2007, 2008 and 2009. In each subplot, the abundance of all species was estimated using a modified Braun-Blanquet-scale (van der Maarel 1979). Relevés were taken twice a year, in early June and mid-September. In 2007, however, logistical constraints prevented us from taking relevés in June so that only data from September are available for that year.

### *Seed bank sampling*

Seed bank samples were taken in February 2008. In each subplot, we extracted ten soil cores with an auger of 2.5 cm diameter down to a depth of 10 cm. The samples were divided into three depth layers (0-1 cm, 1-5 cm, 5-10 cm) which were then pooled per subplot, i.e. each layer in each subplot was represented by one mixed sample consisting of ten subsamples. The soil surface area sampled in each sub-plot was 49.1 cm<sup>2</sup>, and the volume sampled was 491 cm<sup>3</sup>. Within 48 h after collection, samples were spread thinly (0.5-1 cm) in Styrofoam trays over a base of moistened, sterilized standard potting soil. Trays were exposed to outside weather conditions in a common



**Figure 3.1** Layout of the sampling scheme, showing (a) one of the five plant material strips with (b) a transect of eight 2 x 2 m plots and (c) the locations of the different samples.

garden close to Giessen, Germany (50° 32' 12" N 8° 41' 35" E, 172 m above sea-level) and watered regularly. To prevent diaspore input they were covered with flat gauze lids. Control trays containing sterile soil were set up between the sample trays to allow quantification of any seed input from outside. Emerging seedlings were identified to species level as soon as possible after germination, counted and removed. Those specimens which could not be identified at the seedling stage were transferred to pots and grown until identification was possible. Species that were recorded in both the control trays and the experimental trays were excluded from the analysis. When germination ceased, the soil material was carefully stirred to stimulate germination of the remaining seeds. The germination trial continued for 18 months.

### *Seed rain monitoring*

We used two types of seed traps to monitor the seed rain: pitfall funnel traps, which are generally considered to be the most effective trap design (Kollmann & Goetze 1998; Page et al. 2002), and pot traps, which have the advantage of providing an integrated picture of the various processes involved in seed dispersal and seedling emergence (Kollmann & Goetze 1998).

Pitfall funnel traps were based on a design by Page et al. (2002), but modified slightly. To prevent larger animals, leaves etc. from falling into the traps and blocking them, funnels were covered with a wire grid (10 mm mesh width). Contrary to Page et al. (2002), we collected seeds in polyamide bags (ProSox® try-on socks, HFS, Buchholz, Germany) which were fixed at the mouth of the funnels with elastic bands. Bags were replaced every two weeks, and their contents were dried (14 h at 35 °C) and stored in paper bags until processing. Seeds were later investigated under a stereomicroscope and identified to species level with help of a reference collection and literature (Cappers et al. 2006). Unidentifiable specimens were sown on standard potting soil, and the resulting seedlings were cultivated until identification was possible.

Pot traps consisted of small pots (10 cm ø, 7 cm depth) filled with soil that had been taken from the sites and steam-sterilised in the laboratory (6 h at 80 °C, *Sterilo 1 K*, MAFAC/Schwarz, Alpirsbach, Germany) two weeks prior to trap installation. The pots were installed level to the soil surface and left exposed for 12 months. After retrieval, all plants growing in the pots were identified and removed, whereupon the pots were transferred to an unheated glasshouse and monitored for another 6 months. To prevent diaspore input they were covered with flat gauze lids, and control pots containing sterile soil were set up between them to allow quantification of any seed input from outside. All emerging seedlings were identified, counted and removed from the pots. Species that were recorded both in the control pots and the seed traps were excluded from the analysis.

### **Data analysis**

Based on Donath et al. (2007) and Schmiede et al. (2009), all plant species recorded in vegetation, seed bank and seed rain samples were classified as

- 1) *agrestal and ruderal species*: annuals or short-lived species typical for arable fields or disturbed areas (e.g. *Chenopodium polyspermum*, *Tripleurospermum perforatum*),

- 2) *resident grassland species*: herbs and grasses that were either present on the sites prior to restoration or that could reasonably be assumed to have colonised the restoration sites from the immediate surroundings rather than via plant material transfer (e.g. *Achillea millefolium*, *Elymus repens*, *Poa trivialis*),
- 3) *transferred species*: species that were present at the donor site, but not in the established vegetation or seed bank of the restoration sites and their immediate surroundings prior to restoration (e.g. *Centaurea jacea*, *Galium album*, *Inula salicina*).

Data from vegetation relevés were transformed to percentage cover values, and data from seed traps and seed bank samples were converted into seed densities (seeds/m<sup>2</sup>). Seed loss within the study plots was estimated as the difference between the number of seeds caught in the funnel traps and the number detected in the respective pot traps. Catches of funnel traps and pot traps were compared with a t-test for paired samples. The influence of plot position along the transects on number and abundance of transferred species in the above-ground vegetation, seed rain and seed bank of the plots was analysed through linear regression.

To trace vegetation development from 2007 to 2009, we calculated distance matrices based on the Sørensen dissimilarity measure and compared the Sørensen dissimilarity values between the centre plot of a transect and all others. For this we only considered species that were present in more than 5 % of the relevés. We used linear regression to analyse the influence of plot position on the dissimilarity of the vegetation composition between the centre plots of each transect and all others and tested whether this relationship was different between years by comparing the slopes of the regression lines (Zar 1999).

To test whether those transferred species that had spread from the plant material strips and colonised the surroundings differed from those that had remained on the strip with regard to their traits, we gathered data on seed bank type (transient, short-term persistent, long-term persistent), seed mass, seed shape (length/width-ratio), CSR-strategy type, species longevity, reproduction type, and releasing height for all observed species. The information was derived from the databases BIOPOP (Poschlod et al. 2003) and BIOLFLOR (Klotz et al. 2002) and supplemented by own measurements where necessary. We used Hotellings T<sup>2</sup>-test (Zar 1999) to test whether the vectors of means of all traits differed between colonizing and non-colonizing transferred species.

We used PopTools (version 2.6.9, Hood 2005) for Microsoft® Office Excel® 2007 (Microsoft Corporation 2008) to create Sørensen distance matrices. All other analyses were done with Statistica 6 (StatSoft 2001).

## Results

### Composition of above-ground vegetation, seed rain and soil seed bank

Above-ground vegetation, seed rain and seed bank of the study plots comprised a total of 169 vascular plant species. Of these, 130 species were recorded in the above-ground vegetation. In the seed rain, we detected 84 species of which 90.5 % also occurred in the

vegetation. The seed bank samples contained 111 species, of which 66.1 % also occurred in the vegetation and 54.5 % also occurred in the seed rain. The frequency of species in the above-ground vegetation was positively correlated with their frequency in the seed rain ( $R^2 = 0.67$ ,  $p < 0.001$ ) and in the soil seed bank ( $R^2 = 0.15$ ,  $p < 0.001$ ). Furthermore, we found a weak positive correlation between the latter and the frequency of species in the seed rain ( $R^2 = 0.19$ ,  $p < 0.001$ ).

### *Above-ground vegetation*

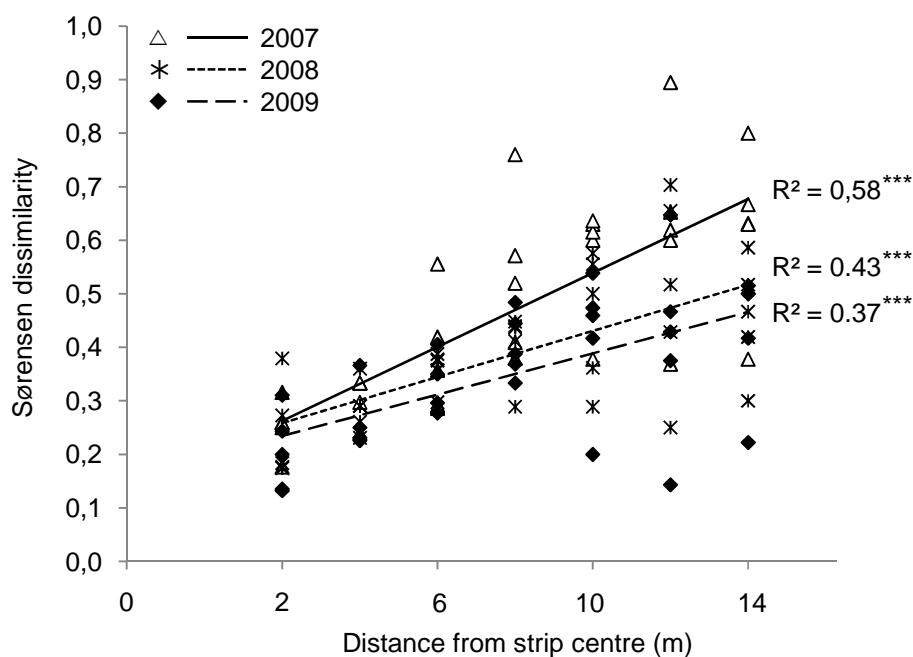
Species numbers per plot in the above-ground vegetation across sites and years ranged between 6 and 38 (mean:  $20.1 \pm 0.4$ ). Sørensen dissimilarity of the above-ground vegetation between the central plot of each transect and the others increased significantly with increasing distance in all three years (Figure 3.2). Regression slopes differed significantly between 2007 and 2009 ( $t = 2.27$ ,  $df = 66$ ,  $p < 0.05$ ), indicating an increase in similarity of the plots' vegetation composition.

### *Seed rain*

The number of species detected in the seed rain across sites ranged between 4 and 19 for funnel traps (mean:  $11 \pm 0.6$ ) and between 1 and 14 for pot traps (mean:  $5.3 \pm 0.4$ ). Funnel trap catches showed a clear peak in early July, when  $53.4 \pm 5$  % of the total number of seeds and  $36.9 \pm 3.1$  % of the total number of species were caught (Figure 3.3).

### *Soil seed bank*

The number of species present in the soil seed bank (0-10 cm depth) across sites was very similar to that of the above-ground vegetation and ranged between 6 and 33 per



**Figure 3.2** Relationship between Sørensen dissimilarity and plot position along the transect across study sites in 2007, 2008 and 2009. Sørensen dissimilarity was calculated between the central plot and all others.

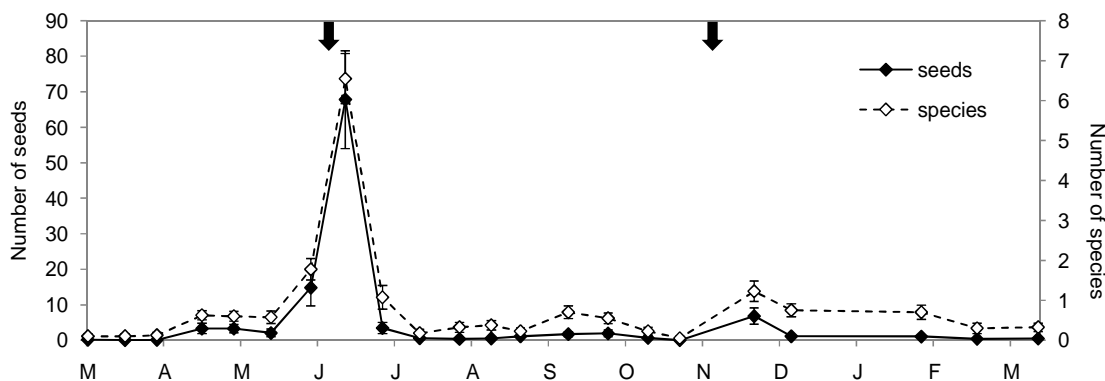
plot, with a mean of  $20.8 \pm 1.1$  ( $n = 40$ ). Overall, the soil seed bank comprised  $172\,300 \pm 48\,690$  seeds/m<sup>2</sup>, most of which were located in the upper 5 cm of the soil. The most abundant species was *Juncus bufonius*, which accounted for 66 % of all seeds found. Overall, the soil seed bank was clearly dominated by agrestal and ruderal species, which accounted for 79.5 % of the total abundance on the plant material strips and 89.2 % of the total abundance on the plots adjacent to the strips (Figure 3.4).

### Patterns of transferred species

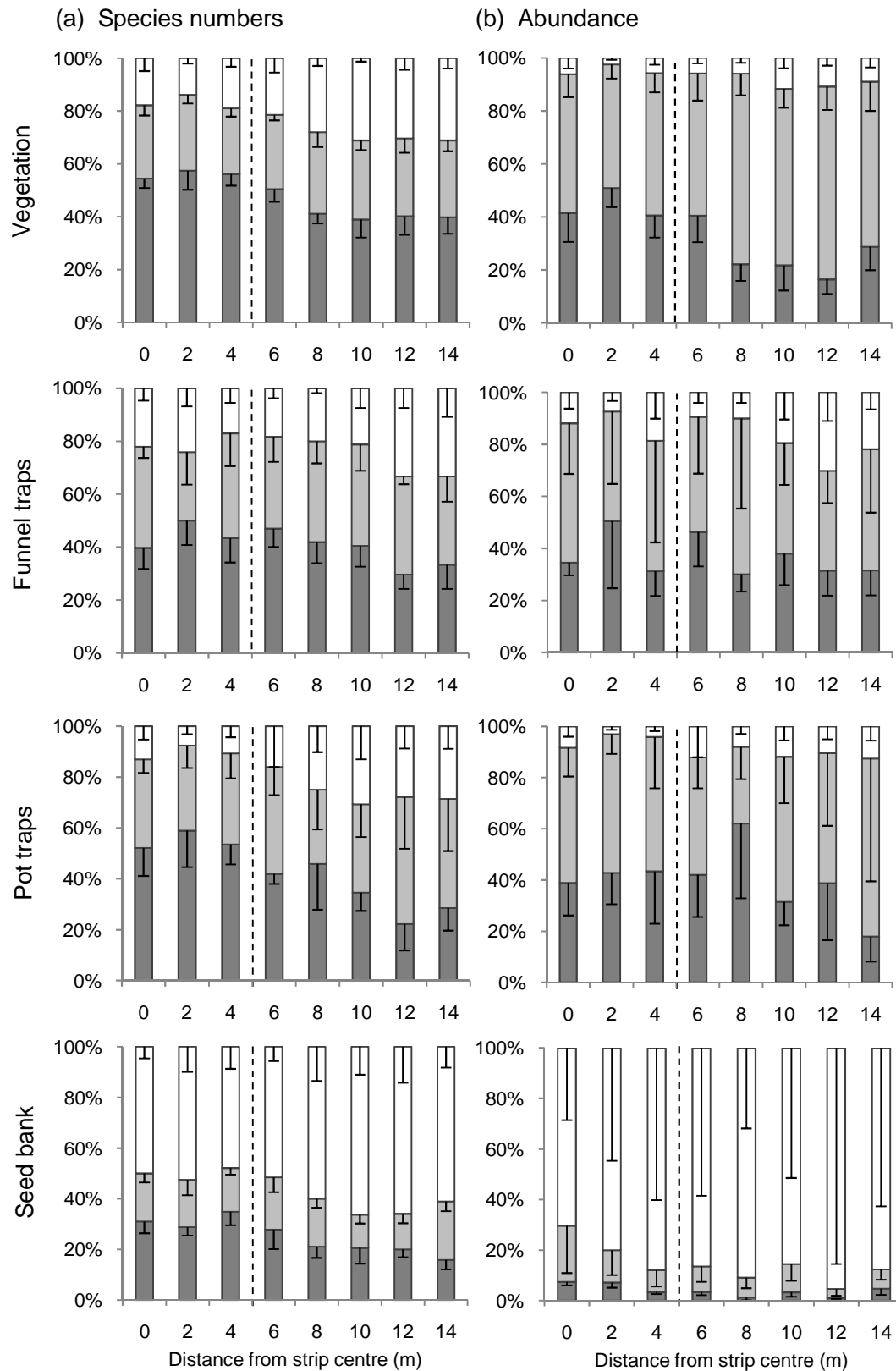
Transferred species were present in all community components, i.e. in the above-ground vegetation as well as in the seed rain and in the soil seed bank (Figure 3.4). We found a total of 79 transferred species, of which 88.6 % had already spread from the plant material strips and colonised their surroundings (Table 3.1). The others occurred only on the first three plots of the transects, i.e. within the area where plant material had been applied in 2000 or 2001, respectively. Strikingly, some of the rarest and most endangered flood-meadow species such as *Iris spuria* and *Viola pumila* belonged to this group. Detected dispersal distances differed between community components, and the share of transferred species that could be detected outside the plant material strip was highest for the above-ground vegetation and lowest for the soil seed bank (Table 3.1).

#### Above-ground vegetation

Across years, a total of 72 transferred species could be detected in the above-ground vegetation (see Appendix 3.1). Of these, 94.4 % had spread from the strips and colonized their surroundings. The number of transferred species per plot decreased significantly with increasing distance from the centre of the strip (June 2008:  $y = 13.4 - 0.5 \cdot \text{distance}$ ,  $F_{1,38} = 30.63$ ,  $p < 0.0001$ ). In terms of abundance, plots on the plant material strips were covered in approximately equal parts by transferred species and resident grassland species whereas plots adjacent to the strips were dominated by the latter (Figure 3.4). Accordingly, the abundance of transferred species in the above-ground vegetation also decreased significantly with increasing distance from the strip centre (June 2008:  $y = 66.42 - 3.4 \cdot \text{distance}$ ,  $F_{1,38} = 23.97$ ,  $p < 0.0001$ ).



**Figure 3.3** Number of seeds and species caught in the funnel traps between March 2008 and March 2009 (mean  $\pm$  SE,  $n = 5$ ). Markings on the horizontal axis denote the middle of the month, and arrows indicate mowing times.



**Figure 3.4** Share of agrestal and ruderal species (white bars), resident grassland species (light-grey bars) and transferred species (dark-grey bars) with respect to (a) species numbers and (b) abundance in vegetation (June 2008), seed rain caught in funnel and pot traps, and soil seed bank (0-10 cm depth) of the study plots in dependence of their position along the transects (mean - SE,  $n = 5$ ). The dashed lines indicate the border of the plant material strips.

**Table 3.1** Total number of transferred species that could be detected across sites in the different community components on the plant material strips (plots 1-3) and in the adjacent transect plots. Figures give the number of species that had spread to the respective plot, but not any further. Figures in brackets give the percentage of transferred species detected on (plots 1-3) and adjacent (plots 4-8) to the plant material strips.

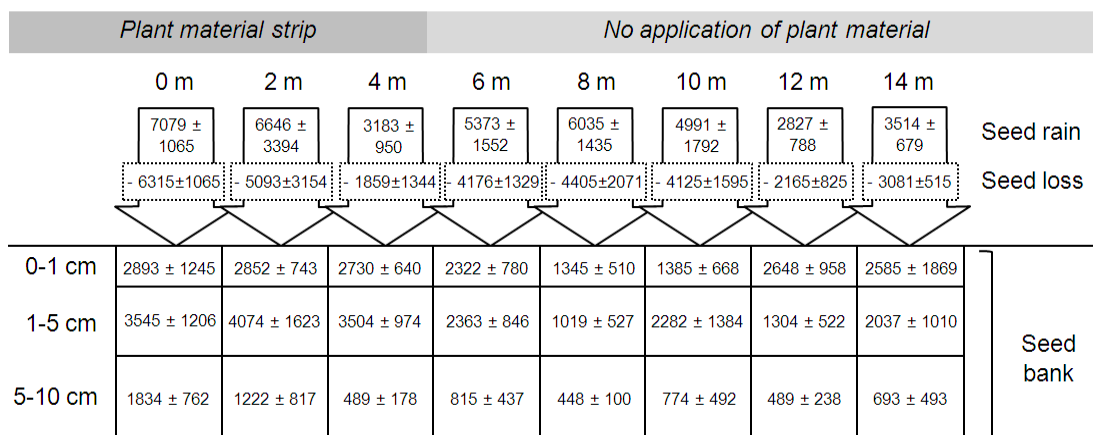
	Veg <sub>2007</sub>		Veg <sub>2008</sub>		Veg <sub>2009</sub>		Veg <sub>2007-2009</sub>		Seed rain		Seed bank		Total	
plots 1-3	15	(30.0)	2	(3.2)	5	(7.9)	4	(5.6)	12	(32.4)	20	(47.6)	9	(11.4)
plot 4	4		6		6		3		3		2		2	
plot 5	2		15		13		12		3		1		13	
plot 6	5	(70.0)	11	(96.8)	12	(92.1)	14	(94.4)	6	(67.6)	1	(52.4)	14	(88.6)
plot 7	9		5		7		7		3		6		7	
plot 8	15		24		20		32		10		12		34	

### Seed rain

The seed rain contained a total of 37 transferred species (see Appendix 3.1), which accounted for 40.7 % (funnel traps) and 42 % (pot traps), respectively, of all species detected in the seed rain. The most abundant species of this group were *Vicia tetrasperma*, *Plantago lanceolata*, *Galium verum*, *Vicia angustifolia* and *Galium album*. Of the 37 species, 67.6 % were also detected in plots adjacent to the plant material strips. Significantly more seeds of transferred species were caught in funnel traps than in pot traps (paired t-test;  $t = 6.72$ , d.f. = 39,  $p < 0.00001$ ), indicating large seed losses (Figure 3.5). The number of transferred species trapped decreased with increasing distance from the centre of the plant material strips (Figure 3.4; funnel traps:  $y = 11.1 - 0.9 \cdot \text{distance}$ ,  $F_{1,38} = 6.79$ ,  $p < 0.05$ ; pot traps:  $y = 10.4 - 1.5 \cdot \text{distance}$ ,  $F_{1,38} = 14.4$ ,  $p < 0.001$ ). However, seed rain abundance of transferred species did not decline significantly with increasing distance.

### Soil seed bank

The soil seed bank contained a total of 44 transferred species (see Appendix 3.1), of which 52.4 % were also detected on plots adjacent to the plant material strips (Table 3.1).



**Figure 3.5** Estimated pooled seed numbers of transferred species averaged across study sites 7-8 years after restoration (seeds/m<sup>2</sup>, mean ± SE,  $n = 5$ ). Arrows indicate average seed rain and seed losses (estimated as the difference between the amount of seeds trapped in funnel traps and the amount detected in pot traps) per plot in dependence of the distance to the strip centre.

The number of transferred species decreased significantly with increasing distance from the centre of the strip ( $y = 12.1 - 0.99 \cdot \text{distance}$ ,  $F_{1,38} = 15.96$ ,  $p < 0.001$ ), and they accounted for 31.5 % of all species found in the soil seed bank on the strip, but only for 24.4 % in plots 4-5 and 18.8 % in plots 6-8 (Figure 3.4). Nonetheless, transferred species had already established a considerable seed bank on the restoration sites, with some seeds even present in 5-10 cm depth (Figure 3.5). Overall seed density of transferred species, however, decreased significantly with increasing distance from the strip centre ( $y = 38.1 - 1.45 \cdot \text{distance}$ ,  $F_{1,38} = 4.28$ ,  $p < 0.05$ ).

### Species traits

Colonizing and non-colonizing transferred species, i.e. those that had and had not spread from the plant material strips into their surroundings, did not differ significantly in the vectors of their trait means, neither for all community components combined ( $T^2 = 13.9$ ;  $F_{11,67} = 1.10$ ,  $p < 0.37$ ) nor for them considered separately (Vegetation<sub>2007-2009</sub>:  $T^2 = 14.39$ ;  $F_{11,60} = 1.12$ ,  $p < 0.36$ ; seed rain:  $T^2 = 8.69$ ;  $F_{11,25} = 0.56$ ,  $p < 0.84$ ; seed bank:  $T^2 = 11.1$ ;  $F_{11,30} = 0.76$ ,  $p < 0.68$ ). Furthermore, none of the individual traits analysed showed a significant relationship with the species' colonization success (data not shown).

## Discussion

### Plant material strips as colonization initials

Transferred species, which were absent from the sites until restoration measures were carried out 7-8 years before our study took place (Donath et al. 2007), now occurred in all three community components on and adjacent to the strips (Figure 3.4). Furthermore, the above-ground vegetation along the transects had become more similar from 2007 to 2009 (Figure 3.2). We also found evidence for a significant seed input of target species (e.g. *Inula britannica*, *Inula salicina*, *Sanguisorba officinalis*) into plots adjacent to plant material strips as well as for beginning seed bank formation. All in all, almost 90 % of all species that had established on the plant material strips had already spread into their immediate surroundings 7-8 years after restoration (Table 3.1). As the methods we used are likely to underestimate the abundance of transferred species in the seed bank and the seed rain due to the small area sampled (cf. Strykstra et al. 1998), the real extent of post-treatment dispersal on the restoration sites might be even greater. We thus conclude that the strips do indeed act as colonization initials from which species that have established due to restoration measures can subsequently spread and colonize the surrounding area.

### Time frame for colonization

Despite the large proportion of 'colonizers' among the transferred species, colonization has so far proceeded rather slowly, and the above-ground vegetation of the plant material strips' surroundings is still dominated by resident grassland species (Figure 3.4). Furthermore, the most successful colonizers in terms of covered distance and plot frequency were common meadow species such as *Plantago lanceolata* or *Galium album*, whereas some of the rarest and most endangered species such as *Iris spuria* and *Viola pumila*

had not spread at all from the plant material strips. These findings are in line with Donath et al. (2003) and Bischoff et al. (2009) who have also observed low colonization velocities for typical flood-meadow species. It seems likely that the speed of the colonization process on our sites might slow down even further as succession proceeds. As the vegetation matrix surrounding the strips will become denser and more species rich during this process, it may offer increasing resistance to the 'invasion' of transferred species dispersing from the initial populations on the strips (Tilman 1997). The fact that common meadow species such as *Plantago lanceolata* or *Galium album* turned out to be fast and successful colonizers could be due to their capacity to regenerate both vegetatively and sexually (cf. Akinola et al. 1998; Rebele and Lehmann 2002).

Seed bank formation is apparently even more time-consuming than the restoration of the above-ground vegetation cover, as indicated by much lower species numbers and abundance values of transferred species in the seed bank compared with the above-ground vegetation (Figure 3.4, cf. also Schmiede et al. 2009). Currently, the soil seed bank of the restoration sites is still dominated by agrestal and ruderal species, which reflects their former use as agricultural fields. This is in line with findings of Jensen (1998) from abandoned wet meadows. Although seed densities have already declined significantly in the course of post-restoration succession (Schmiede et al. 2009), this will presumably remain so for a long time to come as agricultural weeds are notorious for their long-term persistent seed banks (Grime 2001). Moreover, the overwhelming majority of the seeds of transferred species that reach the area surrounding the plant material strips is subsequently lost (Figure 3.5), presumably due to causes such as seed predation (Hulme 1994; Crawley 2000) or Phase II dispersal (Chambers & MacMahon 1994). Nonetheless, typical flood-meadow species such as *Arabis nemorensis* or *Inula salicina* are already present in the seed bank of the restoration sites, even in deeper soil layers (Figure 3.5, cf. also Schmiede et al. 2009). This indicates that vertical seed translocation occurs comparatively rapidly, possibly due to desiccation cracks (Burmeier et al. 2010) or bioturbation by animals such as mice, moles or wild boars (Welander 2000; Wijnhoven et al. 2006). We thus expect that restoration measures will eventually result in the formation of a typical flood-meadow soil seed bank and thus contribute to the establishment of self-sustaining populations of the target species.

### Factors influencing dispersal success

Dispersal of transferred species from the plant material strips into their surroundings may occur either by seed or by clonal propagation. The fact that the percentage of colonizers (i.e. species that had spread from the plant material strips into their surroundings) among transferred species was higher in the vegetation than in the seed rain (Table 3.1) could indicate that small-scale post-treatment dispersal occurred mainly via clonal propagation. This is in line with Arnthórsdóttir (1994) who found that colonization of artificial gaps in a grassland occurred mostly by lateral vegetative growth rather than by seeds. However, our results could have also been an artefact of our sampling design as the area covered by the seed traps was much smaller than the area covered by the vegetation relevés. Further research is needed to clarify the interaction between dispersal by seed and by clonal propagation in the flood meadows studied here.

Irrespective of dispersal strategy, management intervention turned out to be the most important factor determining the spread and distribution of transferred species on the

scale considered in our study. The temporal pattern of the seed rain shows that mowing, which took place in mid-June, was responsible for a distinct peak in seed input (Figure 3.3). Undisturbed seed shedding, in contrast, would have occurred later in the year and extended over a longer time period (Göbel 2001). After mowing, farmers usually leave the cut material on the site to dry for 2-3 days, during which it is turned several times. In the course of this and the subsequent swathing the drying plant material may be moved for several meters from the position where the plants were standing before cutting (S. Burmeier, pers. obs.) and seeds may be disseminated from the cut plants, thus resulting in considerable dispersal from the parent plant.

Seeds may also be transported by mowing machinery (Strykstra et al. 1997; Coulson et al. 2001), both within and between sites. This mode of dispersal does not select for seed size (Strykstra et al. 1997), which may partly explain why we did not find a relationship between dispersal success and seed mass or shape. Our results are supported by the findings of Schmiede et al. (2009), who also did not find a significant relationship between seed size and post-restoration seed bank formation in a related study. Eriksson & Jakobsson (1998), in contrast, found seed size to be a key trait for colonizing ability in a field study that included 81 grassland plants in Sweden. However, as they conducted their study in pastures rather than meadows, this divergence presumably reflects the fact that different management regimes have different prevailing dispersal processes.

### **Implications for future restoration projects**

The overwhelming importance of management and land use for small-scale dispersal processes implies that natural factors such as the prevailing wind direction do not necessarily have to be considered for the spatial layout of restoration measures in mown grassland. It might, however, be beneficial to establish the strips perpendicular to the prevailing management direction in order to maximize the extent of seed dispersal during mowing, both with regard to dispersal via machinery and via subsequent turning and swathing of the cut material.

Since the soil seed bank presumably guarantees the long-term survival of the many of the newly established populations if the above-ground plants are destroyed in the course of a severe flooding event (cf. Richardson et al. 2007), management should also foster seed bank formation. This could, for instance, be achieved by selectively omitting small areas of the restoration sites with successfully established transferred species from mowing. These could then be mulched in autumn or spared altogether and re-incorporated into the mowing scheme in the following year. If different areas were omitted each year, seed production and thus also potential seed bank input of transferred species could be increased considerably while causing only marginal trouble for farmers.

Even though post-application colonization processes are rather time-consuming, we nonetheless recommend establishing several plant material strips or patches on neighbouring sites rather than covering one contiguous site as a whole. Firstly, this would – in terms of a bet-hedging strategy – reduce the risk that the entire restoration project fails due to stochastic events such as severe disturbance by wild boars or other agents. Secondly, the simultaneous creation of several subpopulations would benefit the re-establishment of metapopulation structures and processes, in particular with regard to animal species (Betzholtz et al. 2007; Pöyry et al. 2009).

## Conclusions

All things considered, our results show that plant material transfer is a suitable technique for restoring flood meadows as transferred species not only establish on the sites supplied with plant material, but eventually also colonize their surroundings. We thus conclude that it is feasible to restore entire sites by spatially-restricted plant material application and recommend the continued use of this technique for other restoration projects seeking to overcome dispersal limitation in grassland systems.

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## References

- Akinola, M.O., Thompson, K. & Hillier, S.H. (1998) Development of soil seed banks beneath synthesized meadow communities after seven years of climate manipulations. *Seed Science Research* 8: 493-500.
- Arnthórsdóttir S. (1994) Colonization of experimental patches in a mown grassland. *Oikos* 70: 73-79.
- Bakker J.P. & Berendse F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution* 14: 63-68.
- Bakker J.P., Grootjans A.P., Hermy M. & Poschlod P. (2000) How to define targets for ecological restoration? *Applied Vegetation Science* 3: 3-6.
- Betzholtz P.E., Ehrig A., Lindeborg M. & Dinnétz P. (2007) Food plant density, patch isolation and vegetation height determine occurrence in a Swedish metapopulation of the marsh fritillary *Euphydryas aurinia* (Rottemburg, 1775) (Lepidoptera, Nymphalidae). *Journal of Insect Conservation* 11: 343-350.
- Bischoff A. (2002) Dispersal and establishment of floodplain grassland species as limiting factors in restoration. *Biological Conservation* 104: 25-33.
- Bischoff A., Warthemann G. & Klotz S. (2009) Succession of floodplain grasslands following reduction in land use intensity: the importance of environmental conditions, management and dispersal. *Journal of Applied Ecology* 46: 241-249.
- Bissels S., Donath T.W., Hölzel N. & Otte A. (2005) Ephemeral wetland vegetation in irregularly flooded arable fields along the northern Upper Rhine: the importance of persistent seed-banks. *Phytocoenologia* 35: 469-488.
- Blackstock, T.H., Rimes, C.A., Stevens, D.P., Jefferson, R.G., Robertson, H.J., Mackintosh, J. & Hopkins, J.J. (1999) The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978-96. *Grass and Forage Science* 54: 1-18.
- Böger K. (1991) *Grünlandvegetation im Hessischen Ried - pflanzensoziologische Verhältnisse und Naturschutzkonzeption*, Botanische Vereinigung für Naturschutz in Hessen e.V. (BVNH), Frankfurt a. M.
- Bossuyt B. & Hermy M. (2003) The potential of soil seedbanks in the ecological restoration of grassland and heathland communities. *Belgian Journal of Botany* 136: 23-34.
- Burkart M. (2001) River corridor plants (Stromtalpflanzen) in Central European lowland: a review of a poorly understood plant distribution pattern. *Global Ecology and Biogeography* 10: 449-468.

- Burmeier S., Eckstein R.L., Otte A. & Donath T.W. (2010) Desiccation cracks act as natural seed traps in flood-meadow systems. *Plant and Soil* 333: 351-364.
- Cappers R.T.J., Bekker R.M. & Jans J.E.A. (2006) *Digital seed atlas of the Netherlands*, Barkhuis Publishing, Groningen.
- Chambers J.C. & MacMahon J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics* 25: 263-292.
- Coulson S.J., Bullock J.M., Stevenson M.J. & Pywell R.F. (2001) Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. *Journal of Applied Ecology* 38: 204-216.
- Crawley M.J. (2000) Seed predators and plant population dynamics. In: Fenner M. (ed.) *Seeds. The ecology of regeneration in plant communities*, pp. 167-182. 2 ed. CABI Publishing, Wallingford.
- Donath T.W., Bissels S., Hölzel N. & Otte A. (2007) Large scale application of diaspore transfer with plant material in restoration practice - Impact of seed and microsite limitation. *Biological Conservation* 138: 224-234.
- Donath T.W., Hölzel N. & Otte A. (2003) The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Applied Vegetation Science* 6: 13-22.
- Eriksson O. & Jakobsson A. (1998) Abundance, distribution and life histories of grassland plants: a comparative study of 81 species. *Journal of Ecology* 86: 922-933.
- Göbel P. (2001) Phänologische Untersuchungen in Stromtalwiesen am Hessischen Oberrhein. *Fachbereich Agrarwissenschaften, Ökotropologie und Umweltmanagement*. Diploma thesis, Justus Liebig University Giessen, Giessen.
- Grime J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons, Chichester.
- Hölzel N. & Otte A. (2004) Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. *Journal of Vegetation Science* 15: 93-100.
- Hood G.M. (2005) PopTools Version 2.6.9.
- Hulme P.E. (1994) Post-dispersal seed predation in grassland: its magnitude and source of variation. *Journal of Ecology* 82: 645-652.
- Isselstein J., Tallowin J.R.B. & Smith R.E.N. (2002) Factors affecting seed germination and seedling establishment of fen-meadow species. *Restoration Ecology* 10: 173-184.
- 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.
- Joyce, C.B. & Wade, M.W. (1998) Wet grasslands: a European perspective. In: Joyce, C.B. & Wade, P.M. (eds.) *European wet grasslands: Biodiversity, management and restoration*, pp. 1-12. John Wiley, Chichester.
- Kiehl K., Kirmer A., Donath T.W., Rasran L. & Hölzel N. (2010) Species introduction in restoration projects - evaluation of different techniques for the establishment of semi-natural grasslands in Central and North-western Europe. *Basic and Applied Ecology* 11: 285-299.
- Kiehl K., Thormann A. & Pfadenhauer J. (2006) Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. *Restoration Ecology* 14: 148-156.
- Kirmer A. & Mahn E.G. (2001) Spontaneous and initiated succession on unvegetated slopes in the abandoned lignite-mining area of Goitsche, Germany. *Applied Vegetation Science* 4: 19-27.
- Klotz S., Kühn I. & Durka W. (Eds.) (2002) BIOLFLOR - Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Bundesamt für Naturschutz, Bonn.
- Knapp R. (1954) Über Pflanzengesellschaften der Wiesen in Trockengebieten Deutschlands. *Angewandte Pflanzensoziologie (Aichinger Festschriften)* 2: 1145-1186.
- Kollmann J. & Goetze D. (1998) Notes on seed traps in terrestrial plant communities. *Flora* 193: 31-40.
- Microsoft Corporation (2008) Microsoft Excel.
- Müller-Westermeier G. (1990) *Klimadaten der Bundesrepublik Deutschland*, Deutscher Wetterdienst, Offenbach.

- Page M.J., Newlands L. & Eales J. (2002) Effectiveness of three seed-trap designs. *Australian Journal Of Botany* 50: 587-594.
- Palmer M.A., Ambrose R.F. & Poff N.L. (1997) Ecological theory and community restoration ecology. *Restoration Ecology* 5: 291-300.
- Patzelt A., Wild U. & Pfadenhauer J. (1998) Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. *Restoration Ecology* 9: 127-136.
- Poschold P., Kleyer M., Jackel A.K., Dannemann A. & Tackenberg O. (2003) BIOPOP - a database of plant traits and Internet application for nature conservation. *Folia Geobotanica* 38: 263-271.
- Pöyry J., Paukkunen J., Heliölä J. & Kuussaari M. (2009) Relative contributions of local and regional factors to species richness and total density of butterflies and moths in semi-natural grasslands. *Oecologia* 160: 577-587.
- Pywell R.F., Bullock J.M., Hopkins A., Walker K.J., Sparks T.H., Burke M.J.W. & Peel S. (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* 39: 294-309.
- Rasran L., Vogt K. & Jensen K. (2007) Effects of topsoil removal, seed transfer with plant material and moderate grazing on restoration of riparian fen grasslands. *Applied Vegetation Science* 10: 451-U93.
- Rebele, F. & Lehmann, C. (2002) Restoration of a landfill site in Berlin, Germany by spontaneous and directed succession. *Restoration Ecology* 10: 340-347.
- Richardson D.M., Holmes P.M., Esler K.J., Galatowitsch S.M., Stromberg J.C., Kirkman S.P., Pysek P. & Hobbs R.J. (2007) Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions* 13: 126-139.
- Schmiede R., Donath T.W. & Otte A. (2009) Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404-413.
- StatSoft (2001) STATISTICA für Windows. Version 6.0.
- Stroh M., Storm C., Zehm A. & Schwabe A. (2002) Restorative grazing as a tool for directed succession with diaspore inoculation: the model of sand ecosystems. *Phytocoenologia* 32: 595-625.
- Strykstra R.J., Bekker R.M. & Bakker J.P. (1998) Assessment of dispersule availability: its practical use in restoration management. *Acta Botanica Neerlandica* 47: 57-70.
- Strykstra R.J., Verweij G.L. & Bakker J.P. (1997) Seed dispersal by mowing machinery in a Dutch brook valley system. *Acta Botanica Neerlandica* 46: 387-401.
- Thompson K. (2000) The functional ecology of soil seed banks. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 215-235. CABI Publishing, Wallingford.
- Tilman D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78: 81-92.
- van der Maarel E. (1979) Transformation of cover-abundance values in phytosociology and its effects on community similarity. *Vegetatio* 39: 97-114.
- Walker K.J., Stevens P.A., Stevens D.P., Mountford J.O., Manchester S.J. & Pywell R.F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119: 1-18.
- Welander J. (2000) Spatial and temporal dynamics of wild boar (*Sus scrofa*) rooting in a mosaic landscape. *Journal of Zoology* 252: 263-271.
- Wijnhoven S., Thonon I., Velde G.D., Leuven R., Zorn M., Eijsackers H. & Smits T. (2006) The impact of bioturbation by small mammals on heavy metal redistribution in an embanked floodplain of the River Rhine. *Water Air and Soil Pollution* 177: 183-210.
- Zar J.H. (1999) *Biostatistical Analysis*, Prentice-Hall, Upper Saddle River.

## Appendix

**Appendix 3.1** Presence of transferred species in vegetation (v, relevés from June 2008), soil seed bank (sb, 0-10 cm depth) and seed rain (sr, funnel and pot traps combined) of the eight plots per transect and overall percentage plot frequencies for vegetation ( $f_v$ ), seed bank ( $f_{sb}$ ) and seed rain ( $f_{sr}$ ) along the entire transects.

<b>Table 3.1.1:</b> Transect 1	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	$f_v$	$f_{sb}$	$f_{sr}$
<i>Lathyrus pratensis</i>	v/sb/sr	v/sr	v/sr	v/sr	v	v	v	v	100	12.5	50
<i>Plantago lanceolata</i>	·	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	87.5	87.5	87.5
<i>Poa angustifolia</i>	v/sb/sr	v/sb	v/sb	·	v/sb	v/sb	v	v	87.5	62.5	12.5
<i>Carex spicata</i>	v/sb/sr	v/sb/sr	v	v	sb	v	·	v/sr	75	37.5	37.5
<i>Prunella vulgaris</i>	sb	v/sb/sr	v	v	v	·	v	v	75	25	12.5
<i>Bromus racemosus</i>	·	v	v/sr	v	v	·	v	v/sr	75	0	25
<i>Dactylis glomerata</i>	v/sr	sr	sr	v	sr	sr	v/sb	v/sb/sr	50	25	75
<i>Inula salicina</i>	v	v/sb/sr	v/sb	v	·	·	·	·	50	25	12.5
<i>Galium album</i>	sr	sr	·	sr	v	v/sr	v/sr	v	50	0	62.5
<i>Inula britannica</i>	v/sb	sb	v/sb	sb	v	·	sr	sr	37.5	50	25
<i>Mentha arvensis</i>	·	·	sr	v/sb	v/sb	·	v/sb	·	37.5	37.5	12.5
<i>Galium verum</i>	v/sb/sr	v/sb/sr	v/sr	sr	·	·	·	·	37.5	25	50
<i>Centaurea jacea</i>	v	v	sr	v	·	·	·	·	37.5	0	12.5
<i>Symphytum officinale</i>	v	·	·	sr	·	v	·	v	37.5	0	12.5
<i>Filipendula ulmaria</i>	v	v	·	·	·	·	·	v	37.5	0	0
<i>Iris spuria</i>	v	v	v	·	·	·	·	·	37.5	0	0
<i>Tragopogon pratensis</i>	v	v	·	v	·	·	·	·	37.5	0	0
<i>Lychnis flos-cuculi</i>	·	·	v/sb	v	·	·	sb	·	25	25	0
<i>Lysimachia vulgaris</i>	v/sb	·	v/sb	·	·	·	·	·	25	25	0
<i>Vicia tetrasperma</i>	·	·	sr	v/sr	v/sr	sr	sr	sr	25	0	75
<i>Vicia cracca</i>	·	·	sr	·	sr	v	·	v	25	0	25
<i>Pastinaca sativa</i>	·	sr	v	·	·	v	·	·	25	0	12.5
<i>Lythrum salicaria</i>	·	v	·	·	v	·	·	·	25	0	0
<i>Senecio aquaticus</i>	·	·	·	·	·	v	v	·	25	0	0
<i>Hypericum perforatum</i>	sr	·	·	v	·	sb	·	sb	12.5	25	12.5
<i>Potentilla reptans</i>	sb	v	·	·	·	·	·	·	12.5	12.5	0
<i>Geranium dissectum</i>	v/sr	·	·	·	·	·	·	·	12.5	0	12.5
<i>Cerastium holosteoides</i>	·	·	·	·	·	·	v	·	12.5	0	0
<i>Rubus caesius</i>	·	·	·	·	·	·	v	·	12.5	0	0
<i>Serratula tinctoria</i>	·	·	v	·	·	·	·	·	12.5	0	0
<i>Juncus inflexus</i>	sb	sb	sb	·	·	·	·	·	0	37.5	0
<i>Poa pratense</i>	sr	sb/sr	·	sb	·	·	·	·	0	25	25
<i>Carex distans</i>	sb	·	·	sb	·	·	·	·	0	25	0
<i>Medicago lupulina</i>	sb	·	·	·	·	·	·	sb	0	25	0
<i>Ranunculus acris</i>	·	·	·	·	·	·	sb	sb	0	25	0
<i>Trisetum flavescens</i>	sb	sr	·	·	·	·	·	·	0	12.5	12.5
<i>Carex disticha</i>	·	·	sb	·	·	·	·	·	0	12.5	0
<i>Mentha aquatica</i>	·	·	sb	·	·	·	·	·	0	12.5	0
<i>Bromus inermis</i>	·	sr	sr	sr	sr	·	·	sr	0	0	62.5
<i>Vicia angustifolia</i>	·	·	·	·	sr	·	·	·	0	0	12.5

<b>Table 3.1.2:</b> Transect 2	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	f <sub>v</sub>	f <sub>sb</sub>	f <sub>sr</sub>
<i>Vicia tetrasperma</i>	v/sb/sr	v/sr	v/sb/sr	v/sb/sr	v/sr	v/sb/sr	v/sb/sr	v/sr	100	62.5	100
<i>Galium album</i>	v/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sr	v/sb	.	v	87.5	50	62.5
<i>Festuca arundinacea</i>	v	v/sr	v/sr	v/sb/sr	v/sr	sr	v	v	87.5	12.5	62.5
<i>Trifolium campestre</i>	.	sr	v	v/sb	v/sr	v	v/sr	sr	62.5	12.5	50
<i>Dactylis glomerata</i>	sv/sb/sr	v	.	.	.	v	v	v	62.5	12.5	12.5
<i>Plantago lanceolata</i>	sr	.	.	v/sb	v/sb/sr	v	v/sb	.	50	37.5	25
<i>Trisetum flavescens</i>	sr	sr	sr	v/sr	v/sb/sr	sr	v/sr	v/sr	50	12.5	100
<i>Vicia cracca</i>	v/sr	v/sr	v/sr	v	sr	sr	.	sb/sr	50	12.5	75
<i>Galium verum</i>	sr	v/sr	v	v	v	.	.	sr	50	0	37.5
<i>Agrimonia eupatoria</i>	.	v	.	v	.	v	.	v	50	0	0
<i>Bromus inermis</i>	v	v	v	v	.	.	.	.	50	0	0
<i>Genista tinctoria</i>	v	v	v	v	.	.	.	.	50	0	0
<i>Poa pratensis</i> agg.	sb/sr	sb/sr	.	v/sb	v/sb/sr	v/sb/sr	sb	.	37.5	75	50
<i>Pseudolysimachion longifolium</i>	v/sb	v/sb	v/sb	.	.	.	.	.	37.5	37.5	0
<i>Securigera varia</i>	v	.	v	v	.	.	.	.	37.5	0	0
<i>Vincetoxicum hirundinaria</i>	v	v	v	.	.	.	.	.	37.5	0	0
<i>Cirsium tuberosum</i>	.	v	.	.	v	.	.	.	25	0	0
<i>Pastinaca sativa</i>	v	v	.	.	.	.	.	.	25	0	0
<i>Sanguisorba officinalis</i>	v	v	.	.	.	.	.	.	25	0	0
<i>Lotus corniculatus</i>	.	v/sb	.	sb	.	sr	.	.	12.5	25	12.5
<i>Lysimachia vulgaris</i>	v/sb	.	.	sb	.	.	.	.	12.5	25	0
<i>Viola pumila</i>	.	v/sb	.	.	.	.	.	.	12.5	12.5	0
<i>Ranunculus acris</i>	v/sr	.	.	.	.	.	.	sr	12.5	0	25
<i>Lathyrus pratensis</i>	.	sr	v	.	.	.	.	.	12.5	0	12.5
<i>Carex tomentosa</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Filipendula ulmaria</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Lythrum salicaria</i>	sb	sb	sb	sb	.	.	.	.	0	50	0
<i>Alopecurus pratensis</i>	sr	.	.	sb	.	.	.	.	0	12.5	12.5
<i>Allium angulosum</i>	sb	.	.	.	.	.	.	.	0	12.5	0
<i>Juncus inflexus</i>	.	.	sb	.	.	.	.	.	0	12.5	0
<i>Medicago lupulina</i>	.	.	sb	.	.	.	.	.	0	12.5	0
<i>Mentha arvensis</i>	.	.	.	sb	.	.	.	.	0	12.5	0
<i>Thalictrum flavum</i>	.	.	sb	.	.	.	.	.	0	12.5	0
<i>Trifolium repens</i>	.	.	.	.	sr	.	.	.	0	0	12.5

<b>Table A-3:</b> Transect 3	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	f <sub>v</sub>	f <sub>sb</sub>	f <sub>sr</sub>
<i>Plantago lanceolata</i>	v/sb/sr	v/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	v/sb/sr	v	100	75	87.5
<i>Galium album</i>	v	v/sb/sr	v/sr	v	v	v	v/sr	v	100	12.5	37.5
<i>Leucanthemum vulgare</i>	v/sb	v/sb	v/sb	v/sb/sr	v	v	.	v	87.5	50	12.5
<i>Galium verum</i>	v/sb	v	v/sr	v	v/sb	.	v/sr	.	75	25	25
<i>Centaurea jacea</i>	v/sb/sr	v/sb/sr	v/sb	v	v	.	.	.	62.5	37.5	25
<i>Vicia sepium</i>	v/sr	v	v/sr	v	v	.	.	.	62.5	0	25
<i>Lathyrus pratensis</i>	v	v	v	v	.	.	.	.	50	0	0
<i>Festuca ovina</i>	.	.	.	v	v	.	v	.	37.5	0	0

<b>Table A-3 continued</b>	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	f <sub>v</sub>	f <sub>sb</sub>	f <sub>sr</sub>
<i>Lotus corniculatus</i>	v/sb	v/sb	.	.	.	.	sb	sb	25	50	0
<i>Cerastium holosteoides</i>	.	.	.	.	.	.	v/sb/sr	v	25	12.5	12.5
<i>Vicia cracca</i>	v	.	.	v/sr	sr	.	.	.	25	0	25
<i>Trifolium pratense</i>	v	.	v	.	.	.	.	.	25	0	0
<i>Poa pratensis</i> agg.	v/sb/sr	.	.	.	.	.	.	sb	12.5	25	12.5
<i>Sanguisorba officinalis</i>	.	sr	v/sr	sr	.	sr	sr	.	12.5	0	62.5
<i>Ranunculus acris</i>	.	.	v/sr	.	.	.	.	.	12.5	0	12.5
<i>Agrimonia eupatoria</i>	.	.	v	.	.	.	.	.	12.5	0	0
<i>Bromus racemosus</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Galium boreale</i>	v	.	.	.	.	.	.	.	12.5	0	0
<i>Inula salicina</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Selinum carvifolia</i>	.	.	v	.	.	.	.	.	12.5	0	0
<i>Silaum silaus</i>	.	.	v	.	.	.	.	.	12.5	0	0
<i>Allium angulosum</i>	sb	.	.	.	.	.	.	sb	0	25	0
<i>Deschampsia cespitosa</i>	.	sr	.	.	.	.	.	.	0	0	12.5
<i>Prunella vulgaris</i>	.	sr	.	.	.	.	.	.	0	0	12.5

<b>Table 3.1.4: Transect 4</b>	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	f <sub>v</sub>	f <sub>sb</sub>	f <sub>sr</sub>
<i>Galium verum</i>	v/sb/sr	v/sb/sr	v/sb/sr	v/sr	v/sb/sr	v/sb/sr	v	v/sr	100	62.5	87.5
<i>Lythrum salicaria</i>	v/sb	v/sb	v/sb	v/sb	v/sb	v/sb	sb	v/sb	87.5	100	0
<i>Festuca ovina</i>	v	v	v	v	v	v/sr	v	sr	87.5	0	25
<i>Plantago lanceolata</i>	v/sb/sr	v/sb/sr	v/sb/sr	v/sb	sb	v/sb/sr	sb	v/sb/sr	75	100	62.5
<i>Lathyrus pratensis</i>	v/sr	v	v	sr	.	v	v	v	75	0	25
<i>Leucanthemum vulgare</i>	v/sb	v/sb	v/sb	v/sb/sr	v	sr	.	sb	62.5	62.5	25
<i>Galium album</i>	sr	.	.	v	v/sb/sr	v/sb	v	v/sb/sr	62.5	37.5	37.5
<i>Inula salicina</i>	v/sr	v/sr/sb	v/sr	v	sr	sr	.	.	50	12.5	62.5
<i>Centaurea jacea</i>	.	v	.	v	v	.	.	v	50	0	0
<i>Galium boreale</i>	v	v	v/sb/sr	.	.	sb	.	.	37.5	25	12.5
<i>Serratula tinctoria</i>	v/sr	.	v	v	.	.	.	.	37.5	0	12.5
<i>Bromus racemosus</i>	v	.	.	.	.	.	v	v	37.5	0	0
<i>Pastinaca sativa</i>	.	.	.	v/sr	v/sr	.	.	.	25	0	25
<i>Selinum carvifolia</i>	.	.	v	v	.	.	.	.	25	0	0
<i>Filipendula ulmaria</i>	.	.	.	.	.	.	.	v	12.5	0	0
<i>Lotus corniculatus</i>	.	.	sb	sb	.	.	sb	.	0	37.5	0
<i>Poa pratensis</i> agg.	sb	.	.	sb	.	sb	.	.	0	37.5	0
<i>Carex spicata</i>	.	.	.	.	.	sb	sr	.	0	12.5	12.5
<i>Rubus caesius</i>	.	.	.	.	.	sb	.	.	0	12.5	0
<i>Vicia cracca</i>	.	sr	.	.	sr	.	.	.	0	0	25
<i>Bromus inermis</i>	.	.	.	sr	.	.	.	.	0	0	12.5
<i>Tetragonolobus maritimus</i>	.	.	.	sr	.	.	.	.	0	0	12.5
<i>Trifolium campestre</i>	.	.	.	sr	.	.	.	.	0	0	12.5

<b>Table 3.1.5:</b> Transect 5	plot 1	plot 2	plot 3	plot 4	plot 5	plot 6	plot 7	plot 8	f <sub>v</sub>	f <sub>sb</sub>	f <sub>sr</sub>
<i>Daucus carota</i>	v/sb	v/sb	v/sb	v/sb	v/sb	v/sb/sr	v/sb	v/sb	100	100	12.5
<i>Galium verum</i>	v/sb	v	v/sb/sr	v/sr	v/sb/sr	v/sb	v/sb/sr	v	100	62.5	50
<i>Vicia angustifolia</i>	v/sb/sr	v/sr	v/sr	v/sb/sr	v/sb/sr	v/sr	v/sr	v/sr	100	37.5	100
<i>Carex spicata</i>	v/sb	v/sb/sr	v/sb	v	v/sb/sr	v/sb	sb	v	87.5	75	25
<i>Galium album</i>	.	v	v/sr	v	v/sb/sr	v/sb/sr	v/sb/r	v/r	87.5	37.5	62.5
<i>Plantago lanceolata</i>	sb	sb	v/sb	v/sb/sr	v/sb	v/sb/sr	v/sr	v/sb	75	87.5	37.5
<i>Leucanthemum vulgare</i>	v/sb	v/sr	sb	v	v/sb	v/sb	v	.	75	50	12.5
<i>Poa angustifolia</i>	v/sb	v/sb	v/sb	v/sb	v	.	sb	.	62.5	62.5	0
<i>Centaurea jacea</i>	v	v/sb	v	.	v	.	v	.	62.5	12.5	0
<i>Trifolium campestre</i>	v/sr	v/sr	v/sr	.	.	v	v	.	62.5	0	37.5
<i>Lotus corniculatus</i>	.	.	v	sr	v/sr	sr	v/sb	v/sb/sr	50	25	50
<i>Scutellaria hastifolia</i>	v/sb	v	v	.	.	.	.	.	37.5	12.5	0
<i>Agrimonia eupatoria</i>	v	v	v	.	.	.	.	.	37.5	0	0
<i>Iris spuria</i>	v	v	v	.	.	.	.	.	37.5	0	0
<i>Medicago lupulina</i>	.	sb/sr	.	.	sr	v/sb/sr	v/sr	.	25	25	50
<i>Lysimachia vulgaris</i>	.	v	v	.	.	.	.	.	25	0	0
<i>Prunella vulgaris</i>	.	.	.	.	v	v	.	.	25	0	0
<i>Arabis nemorensis</i>	.	.	sb	.	v	.	sb	.	12.5	25	0
<i>Vicia cracca</i>	v/sr	sr	.	sb/sr	.	sr	sr	sr	12.5	12.5	75
<i>Bromus inermis</i>	.	.	v/sr	.	.	.	.	sr	12.5	0	25
<i>Carex tomentosa</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Cerastium holosteoides</i>	.	.	v	.	.	.	.	.	12.5	0	0
<i>Inula salicina</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Mentha aquatica</i>	.	v	.	.	.	.	.	.	12.5	0	0
<i>Tragopogon pratensis</i>	.	.	.	v	.	.	.	.	12.5	0	0
<i>Trifolium repens</i>	.	.	.	.	.	.	.	v	12.5	0	0
<i>Vincetoxicum hirundinaria</i>	.	.	v	.	.	.	.	.	12.5	0	0
<i>Pseudolysimachion longifolium</i>	sb	sb	.	.	.	.	.	.	0	25	0
<i>Galium boreale</i>	.	.	sb	.	.	.	.	.	0	12.5	0
<i>Vicia sepium</i>	.	.	.	sr	sr	.	.	.	0	0	25
<i>Festuca ovina</i>	.	sr	.	.	.	.	.	.	0	0	12.5
<i>Ranunculus acris</i>	.	sr	.	.	.	.	.	.	0	0	12.5

Documenting desiccation crack patterns



# CHAPTER 4

## **Desiccation cracks act as natural seed traps in flood-meadow systems**

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*The previous chapters have shown that plant material transfer may trigger seed bank formation both on and adjacent to plant material strips and is thus likely to result in the establishment of self-sustaining populations of the transferred target species. However, what about the prevailing abiotic conditions in flood meadows? How do they influence seed bank formation and dynamics? These questions are addressed in this and the following chapter.*

## **Abstract**

Desiccation cracks are a natural phenomenon of clay-rich soils that form via soil shrinkage during dry conditions. Our aim was to test the seed trapping potential of such cracks and assess its impact on seed bank formation in a flood-meadow ecosystem. We documented crack patterns on permanent plots and analysed the soil seed content along and adjacent to cracks. Seed translocation via cracks was tested with a mark-recapture experiment, and post-entrapment seed fate was tested with a burial experiment. Most cracks re-opened in the same positions in consecutive dry periods. Along cracks, most seeds were found in 10-20 cm depth, whereas adjacent to cracks most seeds were found in 0-5 cm depth. The majority of seeds found in shallow depths adjacent to cracks belonged to species that were also present in the above-ground vegetation, whereas this rate was always under 50 % along desiccation cracks. The mark-recapture experiment gave evidence for vertical seed translocation through desiccation cracks. Post-entrapment seed fate differed between species and burial depth, with a trend towards increasing survival with increasing depth. We conclude that desiccation cracks act as natural seed traps, foster seed bank formation and thus influence plant community dynamics in flood-meadow systems.

## **Keywords**

alluvial meadows; burial experiment; grassland; seed fate; soil seed bank

## Introduction

Desiccation cracks are a natural phenomenon of clay-rich soils that form when the soils shrink during dry conditions. They have been described for a range of habitats such as river floodplains (Kazanci et al. 2001), temporary marshes (Bonis & Lepart 1994; Espinar et al. 2005; Espinar & Clemente 2007) and arctic ecosystems (Elberling 2000). So far, desiccation cracks have primarily received attention with regard to their impact on agriculture (e.g. Velde et al. 1996; Taki et al. 2006) and engineering (e.g. Yesiller et al. 2000; Rayhani et al. 2008). Their role as abiotic factor in natural ecosystems, however, has been largely neglected, and only a few studies have dealt with the impact of desiccation cracks on seed input (Elberling 2000; Espinar et al. 2005) and vertical seed movement in the soil (Espinar & Clemente 2007).

We assume that desiccation cracks may act as natural seed traps, i.e. that seeds may fall into the cracks and end up in depths too deep to emerge from, which would influence soil seed bank formation and thus also plant community dynamics in all systems featuring desiccation cracks. Previous studies have brought some first evidence that desiccation cracks could indeed influence seed bank formation. In their investigation on the diaspore banks of temporary marshes in south-western Spain, Espinar et al. (2005) found a bimodal, U-shaped distribution of seeds within the soil profile and suggested that this might have been caused by seed entrapment and vertical seed translocation via seasonal soil cracks. Schmiede et al. (2009), who analysed post-restoration soil seed bank development in floodplain grasslands, observed that seeds of some species were incorporated into deep soil layers within a surprisingly short time period and suggested that this could have been caused by seasonal soil cracks.

Generally, soil seed banks play an important role in determining the composition and dynamics of plant communities, particularly in frequently disturbed systems such as flood meadows where they guarantee the long-term persistence of plant species by buffering the populations against environmental variability (Thompson 2000; Hölzel & Otte 2004a). Knowledge of seed bank dynamics may thus increase our understanding of important limiting factors and processes that occur within communities (Leck et al. 1989). This is particularly relevant for habitats such as flood meadows, which belong to the most threatened plant communities in Europe (Joyce & Wade 1998), are listed in Appendix 1 of the EU Habitats Directive (92/43/ECC) and contain many rare and endangered plant species. Their conservation requires a thorough understanding of ecological processes including seed bank dynamics and recruitment (Bakker et al. 1996; Hölzel & Otte 2004a).

As seeds cannot persist on the soil surface for long periods due to germination or predation, burial is an essential prelude to persistence and thus a major component influencing seed bank dynamics (Thompson et al. 1993; Grime 2001). Burial may convey the benefit of reduced air exposure and thus maintain high humidity levels, protect the seeds against very high or very low temperatures as well as against granivores and herbivores dwelling on or near the soil surface (Forcella et al. 2000). However, it may also have an inhibitory effect on germination and emergence (Zhang & Maun 1994; Benvenuti et al. 2001). Possible burial mechanisms include frost heave, earthworm activity or other naturally occurring soil disturbances (cf. Chambers & MacMahon 1994), but also entrapment by desiccation cracks (Elberling 2000; Espinar et al. 2005). The probability of burial is generally higher for small and compact seeds than for large and wide seeds (Chambers et al. 1991; Thompson 2000; Benvenuti 2007).

Buried seeds may encounter any of five possible fates: (1) death prior to germination, e.g. due to pathogens (Davis & Renner 2007), predation (Westerman et al. 2003) or senescence (Telewski & 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 relating to both species-specific traits such as seed mass (Yanful & Maun 1996; Milberg et al. 2000) and environmental conditions such as temperature (Oliveira & Norsworthy 2006), light quality (Baskin & Baskin 2001), air quality and soil water potential (Forcella et al. 2000).

The consequences of seed entrapment by desiccation cracks on seed distribution patterns in the soil will be influenced by the spatial constancy of the cracks: if they frequently re-open in the same locations, they can accumulate a considerable number of seeds over time, resulting in clumped patterns of seeds. Although there are several papers focusing on the mathematical description of crack patterns (e.g. Chertkov 2002; 2005; Hallett & Newson 2005; Vogel et al. 2005a; Vogel et al. 2005b; Cornelis et al. 2006), their potential spatial constancy has received surprisingly little attention so far (but see Tang et al. 2008; Kishne et al. 2009). Apparently, more research is needed to elucidate the trapping potential of desiccation cracks and the influence on seed bank formation.

Here we present the results of empirical and experimental investigations on desiccation cracks and their impact on soil seed bank formation in a flood-meadow ecosystem. Our main objectives were to test the trapping potential of the desiccation cracks, to analyse the fate of trapped seeds and to assess their implications for community composition and dynamics. We specifically addressed the following questions:

- 1) Are desiccation cracks in flood-meadow soils spatially constant, i.e. do they re-open in the same locations in subsequent dry periods?
- 2) Do desiccation cracks act as natural seed traps, i.e. (a) do they contain more seeds in greater depths than directly adjacent locations without desiccation cracks, (b) are seeds translocated more rapidly to greater depths along desiccation cracks than adjacent to them, and (c) are seeds sorted according to seed size within the cracks?
- 3) Does seed fate of typical herbaceous flood-meadow species in desiccation cracks relate to seed mass and/or shape and crack depth?

## Methods

### Study sites

Five study sites were selected in flood meadows of the Holocene floodplain of the northern Upper Rhine (49° 50' N, 8° 24' E). The largest distance between any of the sites was 3.4 km. All sites had been used as agricultural fields until 2000 (site B), 2001 (site C), 2002 (sites A and D) or 2003 (site E), respectively, when they became part of a restora-

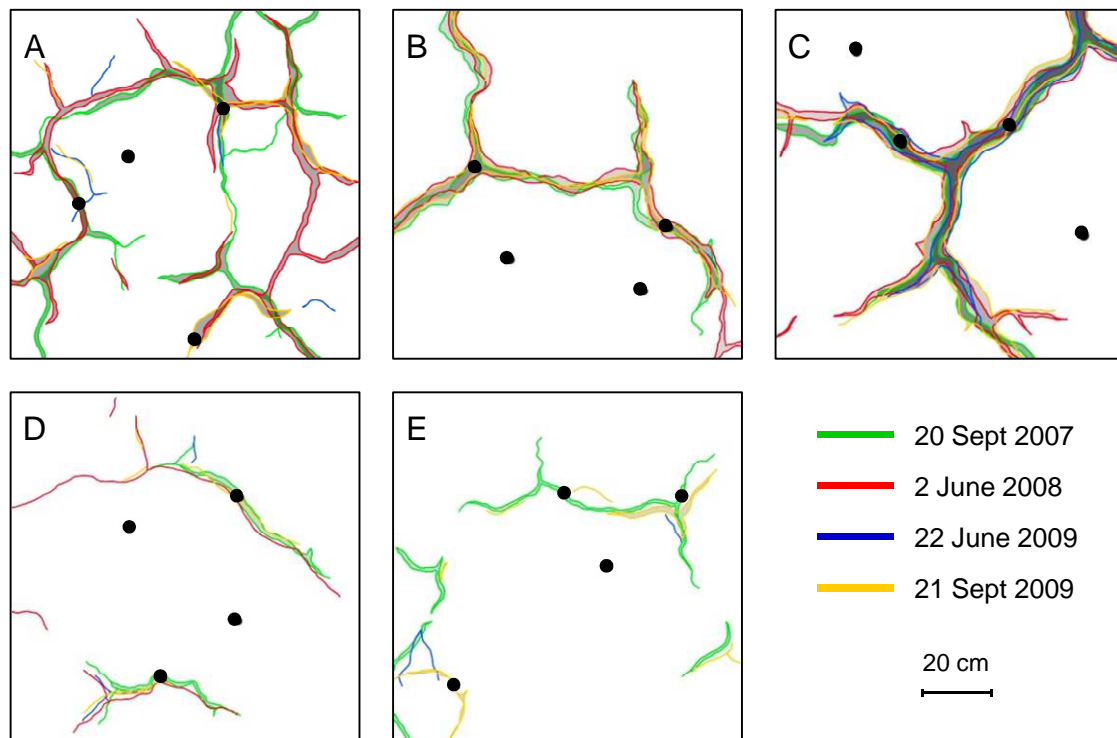
tion scheme seeking to re-establish typical flood-meadow vegetation via plant material transfer (cf. Donath et al. 2007). Accordingly, their present vegetation is a mixture of agrestal and ruderal species on the one hand and transferred flood-meadow species on the other hand. All sites are mown once or twice a year. The soils are calcic Vertisols characterized by high clay contents (see Appendix 4.1), which have developed from the latest Holocene aggradation of the Rhine and consist of calcic, clay-rich material containing many mollusk shells. While the upper parts of the investigated soils, which have all been ploughed regularly until restoration measures began, are free of lamination or signs of fluvial transport, such signs can be found in greater depths. Gleyic features such as oxidized stains in depth of 90-100 cm are apparently relic. Following WRB (IUSS Working Group WRB 2007), the horizon sequence of all study sites can be characterized as Ap/C.

### **Documentation of desiccation cracks**

In September 2007, we established one permanent 1 x 1 m plot on each site and documented the pattern of all desiccation cracks within this plot. This was done using a wooden frame (1 x 1 m, 5 cm high) that was covered with an acrylic pane. The frame was placed flat on the ground after the vegetation had been removed with shears, and the crack pattern was traced on the pane using a permanent marker. This procedure was repeated in June 2008, June 2009 and September 2009. The panes were subsequently photographed, and the patterns were digitised using the programme Photoshop CS3 (Adobe 2007).

### **Seed bank sampling**

In early March 2008, seed bank samples were taken on the study sites. On each permanent plot, two samples were taken along the previously documented desiccation cracks, and two samples were taken adjacent to the cracks (Figure 4.1). As the desiccation cracks were closed at that time of year, crack and non-crack sites were identified with help of the acrylic panes described above: With a small drill we made holes into the pane at the positions to be sampled, reinstalled the pane at the site and inserted small skewers into the drill holes. The pane was then removed, and samples were taken at the sites indicated by the skewers. Successive samples were taken with a soil corer (2.5 cm diameter) to a total depth of 50 cm, resulting in seven soil samples of 0-1, 1-5, 5-10, 10-20, 20-30, 30-40, and 40-50 cm soil depth. Great care was taken to avoid mixing of layers. Seed content was determined in the glasshouse with the seedling emergence method (Roberts 1981). Within 48 h after collection, samples were spread thinly (0.5-1 cm) in Styrofoam trays over a base of moistened, seed-free standard potting soil. The trays had holes at the bottom to prevent water logging, with a layer of horticultural fleece preventing loss of soil and seeds through the holes. The trays were placed in a common garden close to Giessen, Germany (50° 32' 12" N 8° 41' 35" E, 172 m above sea-level), exposed to outside weather conditions and watered regularly. To prevent diaspore input they were covered with flat gauze lids. Control trays containing seed-free soil only were set up between the sample trays to allow quantification of any seed influx from outside. The samples were monitored weekly, and emerged seedlings were identified to species level, counted and removed. Those seedlings which could not be identified were transferred to pots and grown until identification was possible. Species that were recorded both in the control trays and the experimental trays were excluded from the analysis (only *Salix* sp.).



**Figure 4.1** Patterns of desiccation cracks on the study sites (A-E) at four different dates. Black circles indicate the position of the soil samples taken in early March 2008, which were used for the soil seed bank analysis and experiment 1.

When germination ceased, the soil material was carefully stirred to stimulate germination of the remaining seeds. The germination trial was continued for 18 months.

### Vegetation sampling

Vegetation data were collected in early June 2008, late May 2009 and late September 2009. We identified all species and estimated their cover on 3 x 3 m plots with the 1 x 1 m plots, on which the cracks had been documented, in their centre to include all potential short-distance seed sources that may contribute to the soil seed bank. Species lists derived at the different dates were pooled for analysis, i.e. we compiled one overall species list for each site. Botanical nomenclature follows Wisskirchen & Haeupler (1998).

### Experiment 1: vertical seed translocation

We tested the extent and speed of vertical seed translocation via desiccation cracks with a mark-recapture experiment using seed mimics. We opted against using real seeds for this experiment as we would not have been able to distinguish between sown and naturally occurring seeds in the case of using seeds of local species and were reluctant to sow seeds of exotic species in such highly conservation-relevant sites. We thus used coloured glass beads of three different sizes as seed mimics (Table 4.1). In September 2007, we distributed 10 000 seed mimics per size class in each of the 1 x 1 m permanent plots, which resulted in an average density of one seed mimic per cm<sup>2</sup>. We chose this date since it lay well within the period of natural seed shedding in the study system (S. Burmeier, pers. obs.). For the recapture study we used the soil samples taken for the

**Table 4.1** Characteristics of the glass beads used as seed mimics in experiment 1 (mean  $\pm$  SE,  $n = 20$ ).

Size class	Colour	Mass (mg)	Length (mm)	Width (mm)	Height (mm)
small	red	2.94 $\pm$ 0.05	1.30 $\pm$ 0.02	1.28 $\pm$ 0.02	1.32 $\pm$ 0.02
medium	orange	13.08 $\pm$ 0.13	2.33 $\pm$ 0.03	2.34 $\pm$ 0.04	1.79 $\pm$ 0.04
large	blue	62.2 $\pm$ 0.46	3.76 $\pm$ 0.07	3.64 $\pm$ 0.08	3.14 $\pm$ 0.02

seed bank analysis described above and counted all seed mimics found during the processing of the samples. When seed bank monitoring was finished in October 2009, all samples were washed through sieves to extract any remaining beads. Pre-trials had shown that approx. 90 % of the seed mimics contained in a sample could be retrieved with this method (S. Burmeier, unpublished data).

## Experiment 2: fate of buried seeds

To determine the fate of seeds that have fallen into desiccation cracks we conducted a 2-year burial experiment. We selected five herbaceous plant species as model species, which differed in their seed masses (Table 4.2). This allowed us to test the effects of depth and duration of burial on seed fate subject to seed size and shape. All species are common to species-rich flood meadows along the northern Upper Rhine and represent characteristic families of dicotyledons of these as well as similar grassland habitats (cf. Donath et al. 2003). Their seed sizes cover about 70 % of the total seed size range of species growing in alluvial meadows (Hölzel & Otte 2004b). Seeds were collected between July and September 2007 along the Hessian 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 at darkness in the laboratory under room conditions until the beginning of the experiments in October 2007. Harvested seeds were tested for germinability by means of a 6-week-germination trial in a temperature-controlled incubator (10/20 °C (12/12 h), 14 h photoperiod) following a 5-week period of cold-wet stratification (5 °C) with five replicates of 50 seeds for each species. Viability was > 89 % for all species but *Sanguisorba officinalis* (Table 4.2). We used a three-factorial, completely randomized design to determine the effects of species identity, burial depth (1, 4, 12, 24 and 48 cm) and burial duration (6, 9, 12, 18, 21 and 24 months) on seed fate. Each combination of factors was replicated six times, and each replicate consisted of 50 seeds in a small nylon mesh bag (mesh width 250  $\mu$ m). Seeds were buried in October 2007 at one location in the Rhine floodplain (49° 50' 42.75" N, 8° 24' 58.41" E). After retrieval, they were examined, and all germinated seeds were counted. The remaining seeds were incubated for six weeks in a temperature-controlled incubator (10/20 °C (12/12 h), 14 h photoperiod), and germinated seeds (defined as those where the radicle was visible) were counted and discarded in weekly intervals. 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. To monitor soil temperatures throughout the course of the experiment, we installed an automatic data logger (Tiny Tag Transit TG-0050, Gemini Data Loggers Ltd, Chichester, UK) in each depth which measured and recorded soil temperatures in 4-hour intervals. To monitor the

**Table 4.2** Diaspore and germination characteristics of the species used in experiment 2 (mean  $\pm$  SE).

Species	Family	Seed mass (mg) <sup>a</sup>	Seed length (mm) <sup>b</sup>	Seed width (mm) <sup>b</sup>	Germination (%) <sup>c</sup>	Viability (%) <sup>c</sup>
<i>Arabis nemorensis</i> (Hoffm.) Koch	Brassicaceae	0.05 $\pm$ 0.001	0.90 $\pm$ 0.02	0.59 $\pm$ 0.01	85.6 $\pm$ 2.1	92.0 $\pm$ 1.7
<i>Inula salicina</i> L.	Asteraceae	0.12 $\pm$ 0.003	1.53 $\pm$ 0.02	0.38 $\pm$ 0.01	88.8 $\pm$ 1.4	89.6 $\pm$ 1.6
<i>Galium wirtgenii</i> F.W. Schultz	Rubiaceae	0.5 $\pm$ 0.01	1.16 $\pm$ 0.02	0.86 $\pm$ 0.02	86.4 $\pm$ 1.7	99.2 $\pm$ 0.8
<i>Selinum carvifolia</i> (L.) L.	Apiaceae	0.95 $\pm$ 0.01	2.60 $\pm$ 0.06	1.24 $\pm$ 0.03	89.2 $\pm$ 2.9	98.0 $\pm$ 1.1
<i>Sanguisorba officinalis</i> L.	Rosaceae	2.08 $\pm$ 0.07	3.11 $\pm$ 0.04	1.66 $\pm$ 0.04	64.8 $\pm$ 3.5	64.8 $\pm$ 3.5

<sup>a</sup> average data calculated from bulk data for 50 seeds ( $n = 10$ )<sup>b</sup>  $n = 50$ ; all appendices (e.g. pappus for *Inula salicina*) removed prior to measuring<sup>c</sup> averages per 50 seeds in climate chamber experiment ( $n = 5$ )

ground water level, we installed 9 gauges (perforated plastic tubes which were inserted into the ground to a depth of 1 m) on the burial site and measured water levels in fortnightly intervals.

## Data analysis

The soil seed bank data were analysed as a replicated block design with site as blocking factor, i.e. we conducted a three-factor mixed-effects ANOVA with site as random and crack and soil depths as fixed explanatory variables, and calculated F-ratios according to Underwood (1997). We transformed data on species numbers ( $\log(x+0.5)$ ) and seed numbers ( $1/\sqrt{x+0.5}$ ) prior to analysis to improve normality. To quantify the relative contribution of each factor and their interactions to the total variability in the response variables we calculated the ratio of the sum of squares of the respective factor or interaction to the total sum of squares. As only very few seeds were contained in samples from 30-40 and 40-50 cm depth, analyses only included data from 0-30 cm depth. To test whether the seed bank composition along and adjacent to desiccation cracks differed with regard to species traits, we gathered data on the following traits for all species found in the soil seed bank samples:

- (1) *Seed bank type*: Species were classified according to the longevity of their seeds as transient (< 1 year), short-term persistent (1-5 years) or long-term persistent (> 5 years). Data were derived from the database BIOPOP (Poschlod et al. 2003).
- (2) *Seed mass and seed shape*: Data were derived from the database BIOPOP (Poschlod et al. 2003) and supplemented by own measurements if necessary. Data on seed mass were square-root transformed prior to analysis to improve normality. Seed length/width ratio was calculated as a measure for seed shape.
- (3) *C-S-R strategy type*: The classification was derived from the database BIOLFLOR (Klotz et al. 2002), and values were calibrated by allocating a total of 3 points to each species divided among the three strategies C, S and R. A species recorded as CR-strategist was accordingly assigned 1.5 points for competitive ability, 0 points for stress tolerance and 1.5 points for ruderal adaptation. Percentage representation of strategy types in a sample was calculated by summing up the total number of points for the respective strategy types, dividing it by the total number of species contained in the sample and multiplying this figure with 100.

The data were analysed with a mixed-effects ANOVA as described above. For experiment 1, we conducted a four-factor mixed-effects ANOVA with site as random and crack, soil depths and seed mimic size class as fixed explanatory variables and calculated F-ratios according to Underwood (1997). As only very few seed mimics were contained in samples from 30-40 and 40-50 cm depth, we only included data from 0-30 cm depth. For experiment 2, we used a three-factor ANOVA with species identity, soil depth and burial duration as explanatory and the percentages of germinable and surviving seeds as response variables. All percentage data were arcsin-square root transformed prior to analysis (Quinn & Keough 2002). Statistical analyses were done with the programme Statistica 6 (StatSoft 2001), and graphics were compiled with Microsoft® Office Excel® 2007 (Microsoft Corporation 2008).

## Results

### Desiccation crack patterns

Documentation of crack patterns over three years showed that patterns had a high degree of spatial constancy, i.e. the majority of cracks re-opened in the same positions in consecutive dry periods (Figure 4.1). On two of the sites (A and E), however, cracks partially shifted locations between 2007 and 2009 so that the location of one of the 'non-crack' seed bank samples on each site later coincided with the position of a crack. Considering crack width, it was conspicuous that site E, which had the lowest clay content of all study sites (see Appendix 4.1), also had the narrowest cracks.

### Soil seed bank

We found a total of 60 species in the soil seed bank (see Appendix 4.2), which was clearly dominated by *Juncus bufonius* (91.2 % of all seeds found). This species was therefore excluded from all further analyses concerning seed numbers as these would have otherwise only provided information about the patterns of this species and not about the overall seed bank composition that we were interested in. The number of seeds and species differed significantly between study sites (Table 4.3), and the factor crack affected vertical distribution patterns (significant crack x soil depth interaction for both response variables). Samples that were taken along desiccation cracks contained most seeds and species in 10-20 cm depth, whereas samples taken adjacent to desiccation cracks contained most seeds and species in 0-5 cm depth (Figure 4.2). However, the latter also showed a pronounced subsidiary peak at 10-20 cm depth.

None of the species traits we analysed showed a significant relationship with either the factors depth and crack or their interaction. In some cases, however, we observed a trend, i.e. a pattern which was conspicuous, but not statistically significant. Both the average seed length/width ratio and the percentage of C-strategists decreased with increasing soil depth ( $F_{4,50} = 2.06$ ,  $p = 0.099$  and  $F_{4,50} = 2.42$ ,  $p = 0.061$ , respectively). The interaction between depth and crack showed a marginally non-significant effect on the percentage of species known to form a long-term persistent seed bank ( $F_{4,50} = 2.11$ ,  $p = 0.093$ ): samples taken along desiccation cracks contained a higher percentage of species with a long-term persistent seed bank in depths deeper than 10 cm than samples

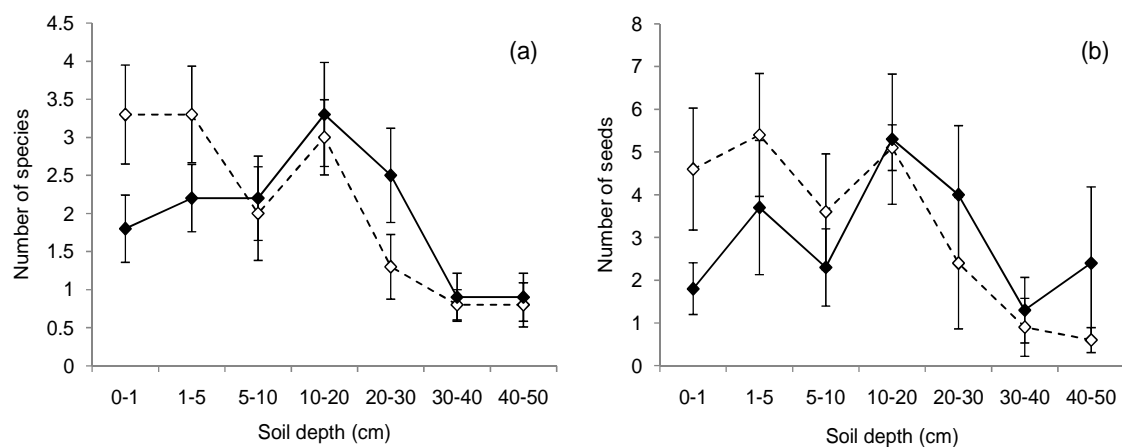
**Table 4.3** Results of a three-way mixed-effects ANOVA on the effects of site (S), crack (C) and soil depth (D) on the number of species and the number of seeds found in seed bank samples (0–30 cm) of the study sites. For the number of seeds, *Juncus bufonius* was excluded from the analysis (see text). df = degrees of freedom, MS = mean sum of squares, vc (%) = relative contribution of the individual factors and their interactions to the total variation.

Source of variation	df	Number of species			df	Number of seeds		
		MS	<i>p</i>	vc (%)		MS	<i>p</i>	vc (%)
Site (S)	4	0.77264	0.000006	29.6	4	0.78149	0.000032	26.6
Crack (C)	1	0.00194	0.744654	0.0	1	0.05023	0.194095	0.4
Depth (D)	4	0.18541	0.13802	7.1	4	0.23850	0.062751	8.1
S x C	4	0.01810	0.92027	0.7	4	0.02899	0.870812	1.0
S x D	16	0.10140	0.241438	15.5	16	0.09959	0.415289	13.6
C x D	4	0.18813	< 0.0000001	7.2	4	0.18727	0.000224	6.4
S x C x D	16	0.01402	0.999766	2.1	16	0.02813	0.994694	3.8
Error	50	0.07870			50	0.09392		

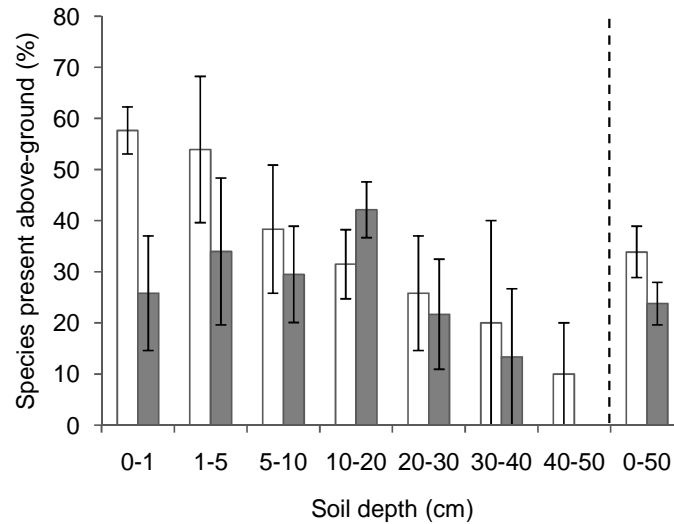
taken adjacent to desiccation cracks (data not shown). Furthermore, samples taken along desiccation cracks generally contained a higher percentage of R-strategists than samples taken adjacent to cracks ( $F_{1,50} = 3.44$ ,  $p = 0.07$ ), particularly in depths deeper than 20 cm (data not shown).

## Vegetation

Vegetation plots contained on average  $38.8 \pm 2.5$  species. The above-ground species pool accounted for  $23.8 \pm 4.2$  % of the seeds found in soil samples taken along desiccation cracks and for  $33.9 \pm 5.0$  % of the seeds found in samples taken adjacent to desiccation cracks (Figure 4.3). The majority of seeds present in shallow soil depths adjacent to desiccation cracks was also present in the above-ground vegetation, whereas this rate was always under 50 % for samples taken along desiccation cracks (Figure 4.3), i.e. the majority of seeds found there belonged to species that were absent from the above-ground vegetation on and immediately adjacent to the plots.



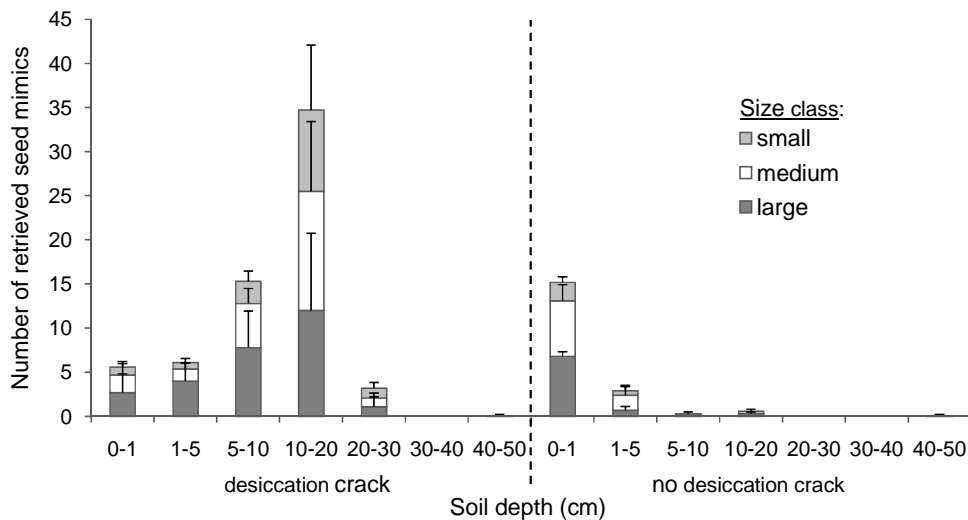
**Figure 4.2** Number of (a) species and (b) seeds (excluding *Juncus bufonius*, see text) present in soil seed bank samples of different depths taken along (◆) and adjacent to (◇) desiccation cracks (mean ± SE,  $n = 5$ ).



**Figure 4.3** Proportion of the above-ground species pool (on 3 x 3 m plots surrounding the 1 x 1 m permanent plots) present in soil seed bank samples of different depths taken along (grey bars) and adjacent to (white bars) desiccation cracks (mean  $\pm$  SE,  $n = 5$ ).

### Experiment 1: vertical seed translocation

Similar to the seed bank results, study sites differed significantly with regard to the number of retrieved seed mimics ( $F_{4,210} = 3.23$ ,  $p < 0.05$ ). The number of retrieved seed mimics differed between size classes ( $F_{2,210} = 9.76$ ,  $p < 0.0001$ ; Figure 4.4), and we found a significant interaction between size class, crack and depth ( $F_{12,210} = 1.96$ ,  $p < 0.05$ ). Along desiccation cracks, most seed mimics were found in 10-20 cm depth, whereas adjacent to cracks most were found immediately at the soil surface in 0-1 cm depth (Figure 4.4). Considering that we had distributed a total of 30 000 seed mimics on each of the 1 x 1 m plots and sampled the sites with a soil corer with a diameter of 2.5 cm, samples should have contained an average of 14.7 seed mimics. Those samples taken adjacent to desic-



**Figure 4.4** Number of seed mimics retrieved from soil seed bank samples of different depths taken along and adjacent to desiccation cracks (mean  $\pm$  SE,  $n = 5$ ).

cation cracks contained an average of  $19.1 \pm 2.6$  seed mimics across depths, which is close to the expected number. Those samples taken along cracks, however, contained the threefold amount ( $65 \pm 21.9$ ), and the factor crack had a significant effect on the number of seed mimics retrieved within a particular sample ( $F_{1,210} = 4.83$ ,  $p < 0.05$ ).

## Experiment 2: fate of buried seeds

Daily soil temperature amplitudes and their seasonal fluctuations decreased strongly with increasing burial depths (see Appendix 4.3). The highest ground water level measured throughout the experiment was 57.5 cm below the soil surface, i.e. none of the buried seeds ever experienced water-logged conditions. Seed fate differed between species and burial depth (Figure 4.5), and the ANOVA results showed that all factors and their interactions had a significant effect on the number of germinable and surviving seeds (Table 4.4). Species identity and burial duration explained a large part of the total variation in the number of germinable and surviving seeds. Soil depth only explained a comparatively low fraction of the total variation for both response variables. However, we observed a trend towards increasing survival with increasing burial depth, particularly for the larger-seeded species with low overall survival rates (Figure 4.5). Generally, seed-size was negatively connected with survival: whereas *Arabis nemorensis*, the species with the smallest seeds, had the highest percentages of surviving seeds, *Sanguisorba officinalis*, the species with the largest seeds, had the lowest percentages (Figure 4.5).

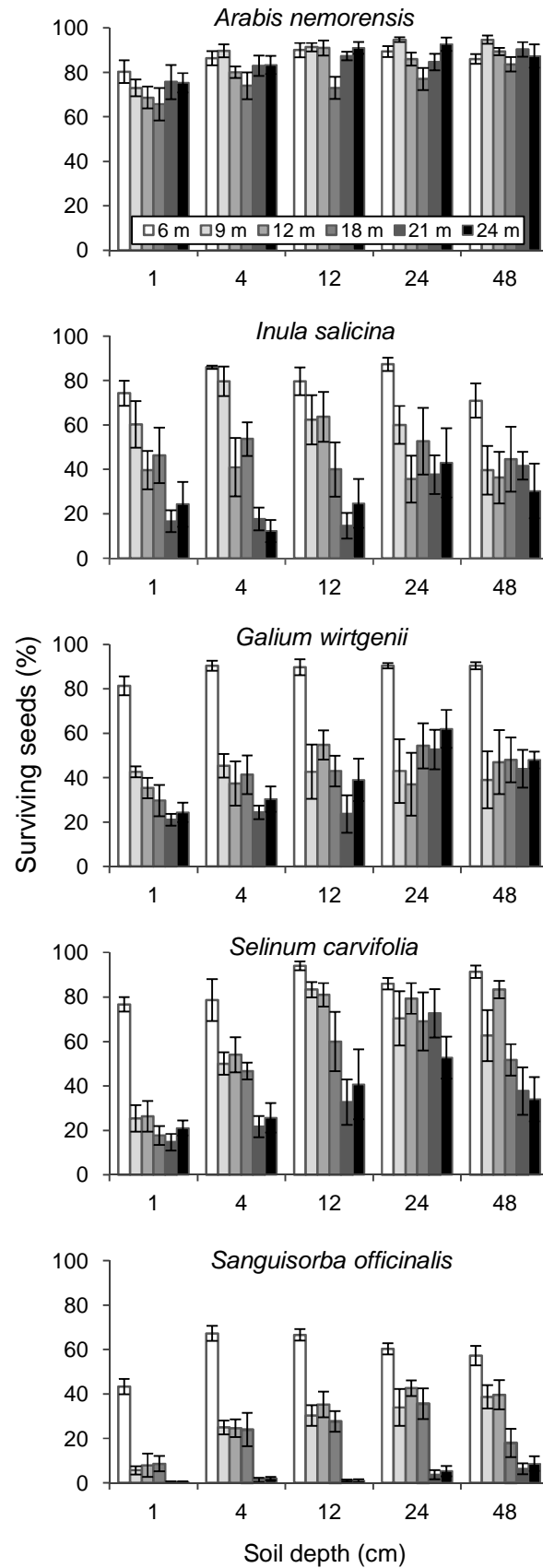
## Discussion

### Spatial constancy of crack patterns

We found that desiccation cracks were overall spatially constant over the time period studied. This result is in line with Tang et al. (2008), who observed that cracks appeared in the same positions in consecutive drying cycles in a laboratory experiment. Kishne et

**Table 4.4** Effects of species identity (S), soil depth (D) and burial duration (B) on the number of germinable and surviving seeds in experiment 2. df = degrees of freedom, MS = mean sum of squares, vc (%) = relative contribution of the individual factors and their interactions to the total variation.

Source of variation	Number of germinable seeds				Number of surviving seeds			
	df	MS	<i>p</i>	vc (%)	df	MS	<i>p</i>	vc (%)
Species identity (S)	4	12.7890	0.000000	36.49	4	4.3501	0.000000	14.63
Soil depth (D)	4	1.6148	0.000000	4.61	4	1.0539	0.000000	3.54
Burial duration (B)	5	5.4690	0.000000	19.51	5	6.0857	0.000000	25.58
S x D	16	0.1745	0.000002	1.99	16	0.1947	0.000001	2.62
S x B	20	0.5233	0.000000	7.47	20	0.7463	0.000000	12.55
D x B	20	0.1178	0.000353	1.68	20	0.1426	0.000082	2.40
S x D x B	80	0.0494	0.390610	2.82	80	0.0820	0.002035	5.51
Error	750	0.0475			750	0.0526		



**Figure 4.5** Percentages of surviving seeds of the study species (arranged according to increasing seed mass) in experiment 2 during burial at different depths for different periods of time (bar colour indicates burial duration in months). Data are means  $\pm$  SE ( $n = 6$ ).

al. (2009), however, report that soil cracks in a prairie Vertisol along the Central Texas Gulf Coast sometimes closed and re-opened at exactly the same places, but shifted locations other times. This might also be the case in our study system (as indicated by sites A and E, cf. Figure 4.1), but longer-term investigations of crack patterns would be necessary to draw definite conclusions on this point.

Spatial constancy has important implications for the trapping potential of desiccation cracks. If cracks frequently open in the same positions, they can accumulate a large number of seeds, which may result in a clumped distribution of the soil seed bank. Such a distribution has been found in an investigation of the small-scale vertical and horizontal seed bank patterns of *Arabis nemorensis* in the same flood-meadow system as studied here (Burmeier et al., in press). A clumped distribution may have both positive and negative consequences for the seeds. On the one hand, being positioned amidst many other seeds may reduce the predation likelihood for each individual seed. On the other hand, large accumulations of seeds may attract granivores and thus even increase the predation likelihood (Edwards & Crawley 1999; Cabin et al. 2000). Furthermore, they may increase the risk of pathogen infection. A dense neighbourhood may also induce secondary dormancy in some of the seeds and thus reduce the fraction of potentially germinable seeds (Tielbörger & Prasse 2009).

### **Seed entrapment by desiccation cracks**

Both the empirical and the experimental investigation on vertical seed distribution and translocation in the soil gave evidence that desiccation cracks act as natural seed traps. Samples taken along desiccation cracks contained more seeds in greater depths than those taken adjacent to cracks, and the seed-mimic experiment showed that desiccation cracks caused rapid vertical seed translocation. Apparently, desiccation cracks contribute to a rapid incorporation of shed seeds into the soil and translocate them to an average depth of 10-20 cm in the flood-meadow system we studied. This depth presumably depends on the average crack depth (cf. Espinar et al. 2005), which, in turn, is likely to depend at least partly on the land use history and can thus

be expected to be different in other systems featuring desiccation cracks. The fact that samples taken adjacent to cracks also showed a subsidiary seed density peak in 10-20 cm depth supports the above-mentioned assumption that, in the longer term, cracks may shift positions and thus eventually contribute to an increased seed density at their average depth throughout the entire site.

Seed bank samples taken adjacent to desiccation cracks had a larger overall representation of the above-ground species pool than those taken along desiccation cracks. This could indicate that desiccation cracks act as a memory of the above-ground vegetation by storing seeds that were produced by previous plant generations (granted that the seeds are long-term persistent). In our case, samples taken along cracks still contained seeds of typical agricultural weeds such as *Euphorbia helioscopia*, *Kickxia elatine* or *Lepidium campestre* (see Appendix 4.2) which reflect the sites' former use as agricultural fields, whereas samples taken adjacent to cracks did not. Overall, the soil seed bank of the sites was still dominated by an arable weed flora with only few seeds of typical flood-meadow species, despite 5-8 years of post-restoration succession. However, land-use history and ploughing by themselves are not sufficient for explaining the current seed distribution: although highest densities of arable weed seeds were found in the former ploughing hori-

zon, we observed a pronounced distinction between samples taken along and adjacent to desiccation cracks. Apparently, land use history and crack dynamics have jointly influenced the seed bank composition of the study sites.

Average seed mass of the species present in the seed bank samples did not differ significantly between different depths and between samples taken along and adjacent to desiccation cracks. However, we observed a trend towards a vertical sorting of seeds based on their shape, with bulky seeds remaining at the soil surface and compact seeds being incorporated into the soil. This is in line with Benvenuti (2007), who observed that small and spherical seed shape favours incorporation into the ground, and with Chambers et al. (1991), who found that small diaspores had a higher probability of being entrapped by soil structures than larger diaspores. In experiment 1, however, we did not observe a clear trend towards a seed-size based depth sorting of the seed mimics, although the factor seed size significantly influenced the overall number of seed mimics retrieved. This deviation might have been caused by the fact that seed mimics had a larger seed mass/size ratio than real seeds, which led even large seeds to be rapidly translocated downwards due to their mass and the associated gravitational pull.

Samples taken along desiccation cracks contained higher percentages of species with a long-term persistent seed bank than samples taken adjacent to desiccation cracks. This could have been an artefact of our study design because the seedling emergence method we used only allowed us to detect seeds that were still viable and capable of germinating under the conditions we exposed them to. Seeds that were either already dead or in deep dormancy went undetected. Accordingly, our results may have been biased towards species with a long-term persistent seed bank. However, considering that we have also observed a comparatively high percentage of R-strategists in samples from greater depths along desiccation cracks, these findings could also point towards a set of traits pre-disposing seeds to accumulate in desiccation cracks. Ruderal species are, by definition, those that produce large numbers of small, persistent seeds (Grime 2001). These seeds have a higher likelihood of being incorporated into the soil due to their shape (Chambers et al. 1991; Benvenuti 2007). They also have a higher stochastic likelihood of being incorporated due to their mere abundance since vertical seed translocation can be conceptually regarded just like horizontal seed dispersal. That is, it can be described as a dispersal curve with a long, narrow tail (cf. Cain et al. 2000) stretching towards increasingly deeper soil layers. The likelihood of being incorporated deep into the ground should be approximately the same for any individual seed of a given size and shape, whereas the likelihood of having any of their seeds being incorporated into the ground should be higher for species producing a large number of seeds. This may explain why we found high percentages of R-strategist, but only low percentages of C-strategists (which, by definition, devote only a small proportion of their annual production to seeds, cf. Grime 2001) in greater depths.

### **Post-entrapment seed fate**

Desiccation cracks may rapidly translocate seeds to depths from which they are not able to emerge (cf. Redmann & Qi 1992; Cussans et al. 1996; Benvenuti et al. 2001; Davis & Renner 2007). Our analysis of post-entrapment seed fate of species frequently occurring on sites prone to desiccation cracks showed that seed survival during burial generally increased with increasing depth. 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. The results of Benvenuti et al. (2001), who observed depth-mediated germination inhibition in seeds of 20 weed species, support our findings. Most probably, soil temperature fluctuation acted as the main depth-sensing clue. Light only penetrates the very top layer of the soil surface (Woolley & Stoller 1978; Benvenuti 1995) and may thus only be used to distinguish between 'surface' or 'buried', whereas soil temperature amplitudes differ markedly between depths.

Generally, post-entrapment seed fate differed between species and seemed to be connected with seed size, with smaller-seeded species having higher survival rates than larger-seeded species. This could be the result of differential selection pressures as small seeds have only few resources and thus very shallow maximum emergence depths (Bond et al. 1999; Grundy et al. 2003). Hence, germination from even very shallow burial depth could already be fatal – which implies that small-seeded species should have experienced a high selection pressure towards developing effective depth-sensing mechanisms that cause depth-mediated germination inhibition (cf. Thompson & Grime 1983; Milberg et al. 2000). Large-seeded species, in contrast, can emerge from greater depths (Donath & Eckstein 2009), are thus not so threatened by fatal germination and should have experienced less selection pressure.

Zhang & Maun (1994) pointed out that the inability to germinate at greater depths has important ecological implications and stress that a seed bank that is inactive because of its depth would be re-activated as soon as disturbances remove the cover layer and bring the seeds close to the surface again. In the case of desiccation cracks, disturbances such as bioturbation by animals, sudden flooding events (cf. Espinar et al. 2005) or sediment shifting might cause a re-surfacing of the trapped seeds.

### **Implications for plant community composition and dynamics**

Our overall results indicate that desiccation cracks act as natural seed traps in flood meadows and may lead to the rapid accumulation of a patchy soil seed bank for those species that have developed long-term persistent seeds possessing depth-sensing mechanisms. For other species, desiccation cracks apparently represent seed sinks. This implies that such cracks may - in combination with other prevailing abiotic conditions in flood meadows (in particular disturbance by flooding, wild boar activity etc.) - generally cause a selection pressure towards developing long-term persistent seed banks (cf. Thompson et al. 1998; Grime 2001). This assumption is supported by the findings of Hölzel & Otte (2004a), who assessed the soil seed bank persistence in flood meadows and observed that a relatively large proportion of rare and endangered plant species in flood meadows could be expected to form long-term persistent seed banks.

With regard to flood-meadow restoration, our results imply that desiccation cracks may accelerate the development of self-supporting populations which are able to regenerate autonomously after disturbance events have destroyed the above-ground vegetation – granted that these disturbances simultaneously cause a re-surfacing of the seeds trapped in the desiccation cracks. However, desiccation cracks may also hinder the development of a closed vegetation cover as nothing can grow in positions where cracks frequently open. This phenomenon is likely to be most distinctive on sites with very high clay contents as these are particularly prone to the formation of large desiccation cracks (Rayhani

et al. 2008; Tang et al. 2008). Accordingly, desiccation cracks should be accounted for as an important abiotic factor in flood meadows and other systems featuring them and are worth being investigated further in the future.

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## References

- Adobe (2007) Photoshop CS3.
- Bakker J.P., Poschod P., Strykstra R.J., Bekker R.M. & Thompson K. (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45: 461-490.
- Baskin C.C. & Baskin J.M. (2001) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*, Academic Press, San Diego.
- Benvenuti S. (1995) Soil light penetration and dormancy of Jimsonweed (*Datura stramonium*) seeds. *Weed Science* 43: 389-393.
- Benvenuti S. (2007) Natural weed seed burial: effect of soil texture, rain and seed characteristics. *Seed Science Research* 17: 211-219.
- Benvenuti S., Macchia M. & Miele S. (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science* 49: 528-535.
- Bond W.J., Honig M. & Maze K.E. (1999) Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia* 120: 132-136.
- Bonis A. & Lepart J. (1994) Vertical structure of seed banks and the impact of depth of burial on recruitment in two temporary marshes. *Vegetatio* 112: 127-139.
- Burmeier S., Eckstein R.L., Donath T.W. & Otte A. (in press) Plant pattern development during early post-restoration succession in grasslands – a case study of *Arabis nemorensis*. *Restoration Ecology*, DOI: 10.1111/j.1526-100X.2010.00668.x.
- Cabin R.J., Marshall D.L. & Mitchell R.J. (2000) The demographic role of soil seed banks. II. Investigations of the fate of experimental seeds of the desert mustard *Lesquerella fendleri*. *Journal of Ecology* 88: 293-302.
- Cain M.L., Milligan B.G. & Strand A.E. (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217-1227.
- Chambers J.C. & MacMahon J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics* 25: 263-292.
- Chambers J.C., MacMahon J.A. & Haefner J.H. (1991) Seed entrapment in alpine ecosystems: effects of soil particle size and diaspore morphology. *Ecology* 72: 1668-1677.
- Chertkov V.Y. (2002) Modelling cracking stages of saturated soils as they dry and shrink. *European Journal of Soil Science* 53: 105-118.
- Chertkov V.Y. (2005) The shrinkage geometry factor of a soil layer. *Soil Science Society of America Journal* 69: 1671-1683.

- Cornelis W.M., Corluy J., Medina H., Hartmann R., Van Meirvenne M. & Ruiz M.E. (2006) A simplified parametric model to describe the magnitude and geometry of soil shrinkage. *European Journal of Soil Science* 57: 258-268.
- Cussans G.W., Raudonius S., Brain P. & Cumberworth S. (1996) Effects of depth of seed burial and soil aggregate size on seedling emergence of *Alopecurus myosuroides*, *Galium aparine*, *Stellaria media* and wheat. *Weed Research* 36: 133-141.
- Davis A.S. & Renner K.A. (2007) Influence of seed depth and pathogens on fatal germination of velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*). *Weed Science* 55: 30-35.
- Donath T.W., Bissels S., Hölzel N. & Otte A. (2007) Large scale application of diaspore transfer with plant material in restoration practice - Impact of seed and microsite limitation. *Biological Conservation* 138: 224-234.
- Donath T.W. & Eckstein R.L. (2009) Effects of bryophyte and grass litter on seedling emergence vary by vertical seed position and seed size. *Plant Ecology*
- Donath T.W., Hölzel N. & Otte A. (2003) The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Applied Vegetation Science* 6: 13-22.
- Edwards G.R. & Crawley M.J. (1999) Rodent seed predation and seedling recruitment in mesic grassland. *Oecologia* 118: 288-296.
- Elberling H. (2000) Spatial pattern of *Lesquerella arctica*: Effects of seed bank and desiccation cracks. *Ecoscience* 7: 86-91.
- Espinar J.L. & Clemente L. (2007) The impact of vertic soil cracks on submerged macrophyte diaspore bank depth distribution in Mediterranean temporary wetlands. *Aquatic Botany* 87: 325-328.
- Espinar J.L., Thompson K. & Garcia L.V. (2005) Timing of seed dispersal generates a bimodal seed bank depth distribution. *American Journal of Botany* 92: 1759-1763.
- Forcella F., Arnold R.L.B., Sanchez R. & Ghera C.M. (2000) Modeling seedling emergence. *Field Crops Research* 67: 123-139.
- Grime J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons, Chichester.
- Grundy A.C., Mead A. & Burston S. (2003) Modelling the emergence response of weed seeds to burial depth: interactions with seed density, weight and shape. *Journal of Applied Ecology* 40: 757-770.
- Hallett P.D. & Newson T.A. (2005) Describing soil crack formation using elastic-plastic fracture mechanics. *European Journal of Soil Science* 56: 31-38.
- Hölzel N. & Otte A. (2004a) Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. *Journal of Vegetation Science* 15: 93-100.
- Hölzel N. & Otte A. (2004b) Ecological significance of seed germination characteristics in flood-meadow species. *Flora* 199: 12-24.
- IUSS Working Group WRB (2007) World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports No. 103. FAO, Rome.
- Joyce C.B. & Wade M.W. (1998) Wet grasslands: a European perspective. In: Joyce C.B. & Wade P.M. (eds.) *European Wet Grasslands: Biodiversity, Management and Restoration*, pp. 1-12. John Wiley, Chichester.
- Kazanci N., Emre O. & Alcicek M.G. (2001) Animal burrowing and associated formation of large desiccation cracks as factors of a rapid restoration of soil cover in flooded farmlands. *Environmental Geology* 40: 964-967.
- Kishne A.S., Morgan C.L.S. & Miller W.L. (2009) Vertisol Crack Extent Associated with Gilgai and Soil Moisture in the Texas Gulf Coast Prairie. *Soil Science Society of America Journal* 73: 1221-1230.
- Klotz S., Kühn I. & Durka W. (eds.) (2002) BIOLFLOR - Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Bundesamt für Naturschutz, Bonn.
- Leck M.A., Parker V.T. & Simpson R.L. (eds.) (1989) *Ecology of soil seed banks*. Academic Press, San Diego.
- Microsoft Corporation (2008) Microsoft Excel.
- Milberg P., Andersson L. & Thompson K. (2000) Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* 10: 99-104.

- Oliveira M.J. & Norsworthy J.K. (2006) Pitted morningglory (*Ipomoea lacunosa*) germination and emergence as affected by environmental factors and seeding depth. *Weed Science* 54: 910-916.
- Poschold P., Kleyer M., Jackel A.K., Dannemann A. & Tackenberg O. (2003) BIOPOP - a database of plant traits and Internet application for nature conservation. *Folia Geobotanica* 38: 263-271.
- Quinn G.P. & Keough M.J. (2002) *Experimental design and data analysis for biologists*, Cambridge University Press, Cambridge.
- Rayhani M.H.T., Yanful E.K. & Fakher A. (2008) Physical modeling of desiccation cracking in plastic soils. *Engineering Geology* 97: 25-31.
- Redmann R.E. & Qi M.Q. (1992) Impacts of seeding depth on emergence and seedling structure in eight perennial grasses. *Canadian Journal of Botany* 70: 133-139.
- Roberts H.A. (1981) Seed banks in soils. In: Coaker T.H. (ed.) *Advancements in Applied Biology* 6, pp. 1-55. Academic Press, Cambridge.
- Schmiede R., Donath T.W. & Otte A. (2009) Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404-413.
- StatSoft (2001) STATISTICA für Windows. Version 6.0.
- Taki O., Godwin R.J. & Leeds-Harrison P.B. (2006) The creation of longitudinal cracks in shrinking soils to enhance seedling emergence. Part I. The effect of soil structure. *Soil Use and Management* 22: 1-10.
- Tang C.S., Shi B., Liu C., Zhao L.Z. & Wang B.J. (2008) Influencing factors of geometrical structure of surface shrinkage cracks in clayey soils. *Engineering Geology* 101: 204-217.
- Telewski F.W. & Zeevaart J.A.D. (2002) The 120-yr period for Dr. Beal's seed viability experiment. *American Journal of Botany* 89: 1285-1288.
- Thompson K. (2000) The functional ecology of soil seed banks. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 215-235. CABI Publishing, Wallingford.
- Thompson K., Bakker J.P., Bekker R.M. & Hodgson J.G. (1998) Ecological correlates of seed persistence in soil in the north-west European flora. *Journal of Ecology* 86: 163-169.
- Thompson K., Band S.R. & Hodgson J.G. (1993) Seed size and shape predict persistence in soil. *Functional Ecology* 7: 236-241.
- Thompson K. & Grime J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology* 20: 141-156.
- Tielbörger K. & Prasse R. (2009) Do seeds sense each other? Testing for density-dependent germination in desert perennial plants. *Oikos* 118: 792-800.
- Underwood A.J. (1997) *Experiments in ecology*, Cambridge University Press, Cambridge.
- Velde B., Moreau E. & Terribile F. (1996) Pore networks in an Italian Vertisol: Quantitative characterisation by two dimensional image analysis. *Geoderma* 72: 271-285.
- Vleeshouwers L.M., Bouwmeester H.J. & Karssen C.M. (1995) Redefining seed dormancy: An attempt to integrate physiology and ecology. *Journal of Ecology* 83: 1031-1037.
- Vogel H.J., Hoffmann H., Leopold A. & Roth K. (2005a) Studies of crack dynamics in clay soil II. A physically based model for crack formation. *Geoderma* 125: 213-223.
- Vogel H.J., Hoffmann H. & Roth K. (2005b) Studies of crack dynamics in clay soil I. Experimental methods, results, and morphological quantification. *Geoderma* 125: 203-211.
- Westerman P.R., Wes J.S., Kropff M.J. & Van der Werf W. (2003) Annual losses of weed seeds due to predation in organic cereal fields. *Journal of Applied Ecology* 40: 824-836.
- Wisskirchen R. & Haeupler H. (1998) *Standardliste der Farn- und Blütenpflanzen Deutschlands*, Ulmer, Stuttgart.
- Woolley J.T. & Stoller E.W. (1978) Light penetration and light-induces seed germination in soil. *Plant Physiology* 61: 597-600.
- Yanful M. & Maun M.A. (1996) Effects of burial of seeds and seedlings from different seed sizes on the emergence and growth of *Strophostyles helvola*. *Canadian Journal of Botany* 74: 1322-1330.
- Yesiller N., Miller C.J., Inci G. & Yaldo K. (2000) Desiccation and cracking behavior of three compacted landfill liner soils. *Engineering Geology* 57: 105-121.

Zhang J. & Maun M.A. (1994) Potential for seed bank formation in seven Great Lakes sand dune species. *American Journal of Botany* 81: 387-394.

## Appendix

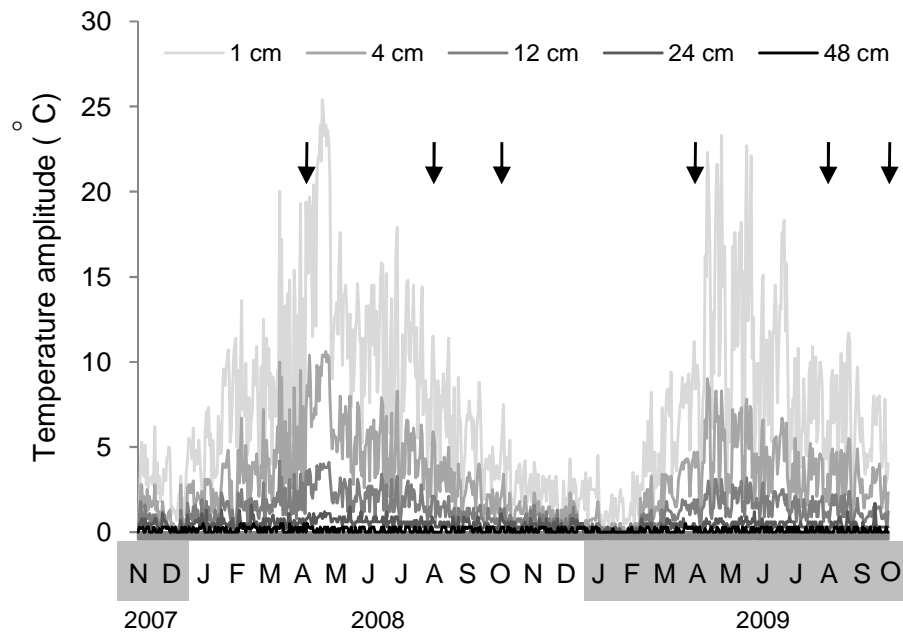
### Appendix 4.1: Soil characterization of the study sites.

Study site	Horizon	Depth (cm)	Soil fraction (%)		
			Clay	Silt	Sand
A	Ap	0-35	30.6	28.7	40.7
	C	35-100	52.5	40.4	7.2
B	Ap	0-35	57.4	37.2	5.4
	C	35-100	66.4	33.3	0.4
C	Ap	0-35	59.5	38.3	2.2
	C	35-100	60.9	38.0	1.1
D	Ap	0-35	55.4	41.2	3.5
	C	35-100	69.6	30.2	0.2
E	Ap	0-32	34.4	32.8	32.8
	C	32-100	29.0	31.3	39.7

**Appendix 4.2:** Seed abundance (no. of seeds/m<sup>2</sup>, mean  $\pm$  SE) and frequency (f; % occurrence in 140 samples) of the species present in the soil seed bank of the study sites.

Species	Seed bank								f
	0-1 cm	1-5 cm	5-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm	Sum	
<i>Juncus bufonius</i>	9167 $\pm$ 4580	37077 $\pm$ 18958	132621 $\pm$ 69223	207080 $\pm$ 111482	62542 $\pm$ 31368	5806 $\pm$ 2897	2648 $\pm$ 1762	9138803	31.4
<i>Juncus articulatus</i>	917 $\pm$ 376	306 $\pm$ 223	1019 $\pm$ 563	1426 $\pm$ 535	2241 $\pm$ 1054	102 $\pm$ 102	407 $\pm$ 187	128343	20.0
<i>Plantago major</i> ssp. <i>intermedia</i>	815 $\pm$ 374	2852 $\pm$ 1342	1324 $\pm$ 758	1019 $\pm$ 563	306 $\pm$ 223	0	102 $\pm$ 102	128343	15.0
<i>Poa trivialis</i>	1019 $\pm$ 479	509 $\pm$ 202	509 $\pm$ 291	306 $\pm$ 223	306 $\pm$ 223	0	102 $\pm$ 102	55004	12.9
<i>Epilobium tetragonum</i>	204 $\pm$ 140	509 $\pm$ 251	407 $\pm$ 187	509 $\pm$ 415	306 $\pm$ 167	102 $\pm$ 102	0	40744	11.4
<i>Arabidopsis thaliana</i>	204 $\pm$ 140	0	0	917 $\pm$ 522	1324 $\pm$ 578	102 $\pm$ 102	102 $\pm$ 102	52967	10.7
<i>Conyza canadensis</i>	509 $\pm$ 326	1222 $\pm$ 684	204 $\pm$ 140	0	0	0	0	38706	5.7
<i>Sonchus asper</i>	102 $\pm$ 102	306 $\pm$ 167	102 $\pm$ 102	204 $\pm$ 140	0	102 $\pm$ 102	0	16297	5.7
<i>Veronica catenata</i>	102 $\pm$ 102	204 $\pm$ 204	204 $\pm$ 204	917 $\pm$ 581	204 $\pm$ 140	0	0	32595	5.7
<i>Plantago lanceolata</i>	102 $\pm$ 102	102 $\pm$ 102	0	713 $\pm$ 370	204 $\pm$ 204	0	0	22409	5.0
<i>Polygonum aviculare</i>	0	102 $\pm$ 102	102 $\pm$ 102	204 $\pm$ 204	204 $\pm$ 140	102 $\pm$ 102	102 $\pm$ 102	16297	5.0
<i>Carex spicata</i>	102 $\pm$ 102	102 $\pm$ 102	102 $\pm$ 102	611 $\pm$ 514	0	0	102 $\pm$ 102	20372	4.3
<i>Inula britannica</i>	102 $\pm$ 102	815 $\pm$ 715	306 $\pm$ 306	407 $\pm$ 407	204 $\pm$ 204	0	0	36669	4.3
<i>Sonchus arvensis</i>	204 $\pm$ 140	102 $\pm$ 102	102 $\pm$ 102	306 $\pm$ 306	102 $\pm$ 102	0	0	16297	4.3
<i>Taraxacum</i> sect. <i>Ruderalia</i>	306 $\pm$ 306	204 $\pm$ 140	0	102 $\pm$ 102	509 $\pm$ 415	0	0	22409	4.3
<i>Chenopodium album</i>	0	102 $\pm$ 102	509 $\pm$ 509	0	0	713 $\pm$ 713	1834 $\pm$ 1834	63153	2.9
<i>Galium mollugo</i> agg.	306 $\pm$ 167	0	0	204 $\pm$ 204	0	0	0	10186	2.9
<i>Achillea millefolium</i>	102 $\pm$ 102	0	0	0	0	204 $\pm$ 140	0	6112	2.1
<i>Agrostis stolonifera</i>	102 $\pm$ 102	102 $\pm$ 102	102 $\pm$ 102	0	0	0	0	6112	2.1
<i>Alopecurus aequalis</i>	102 $\pm$ 102	102 $\pm$ 102	0	0	0	0	102 $\pm$ 102	6112	2.1
<i>Alopecurus myosuroides</i>	0	204 $\pm$ 204	102 $\pm$ 102	407 $\pm$ 407	0	0	0	14260	2.1
<i>Cirsium arvense</i>	102 $\pm$ 102	0	0	204 $\pm$ 140	0	0	0	6112	2.1
<i>Daucus carota</i>	0	204 $\pm$ 204	0	306 $\pm$ 223	0	0	0	10186	2.1
<i>Mentha aquatica</i>	0	102 $\pm$ 102	0	204 $\pm$ 140	0	0	0	6112	2.1
<i>Papaver rhoeas</i>	102 $\pm$ 102	204 $\pm$ 140	0	0	0	0	0	6112	2.1
<i>Anagallis arvensis</i>	102 $\pm$ 102	0	0	0	0	102 $\pm$ 102	0	4074	1.4
<i>Arabis nemorensis</i>	0	0	0	509 $\pm$ 509	0	0	102 $\pm$ 102	12223	1.4
<i>Cirsium vulgare</i>	0	0	102 $\pm$ 102	102 $\pm$ 102	0	0	0	4074	1.4
<i>Crepis setosa</i>	102 $\pm$ 102	102 $\pm$ 102	0	0	0	0	0	4074	1.4
<i>Holcus lanatus</i>	306 $\pm$ 223	0	0	0	0	0	0	6112	1.4
<i>Juncus inflexus</i>	0	102 $\pm$ 102	0	0	102 $\pm$ 102	0	0	4074	1.4
<i>Myosotis arvensis</i>	0	0	102 $\pm$ 102	102 $\pm$ 102	0	0	0	4074	1.4
<i>Pastinaca sativa</i>	0	0	102 $\pm$ 102	102 $\pm$ 102	0	0	0	4074	1.4
<i>Persicaria lapathifolia</i>	102 $\pm$ 102	0	102 $\pm$ 102	0	0	0	0	4074	1.4
<i>Picris hieracoides</i>	0	102 $\pm$ 102	102 $\pm$ 102	0	0	0	0	4074	1.4
<i>Senecio erucifolius</i>	0	0	0	102 $\pm$ 102	102 $\pm$ 102	0	0	4074	1.4
<i>Typha latifolia</i>	0	0	102 $\pm$ 102	102 $\pm$ 102	0	0	0	4074	1.4
<i>Agrostis capillaris</i>	306 $\pm$ 306	0	0	0	0	0	0	6112	0.7
<i>Bromus sterilis</i>	0	102 $\pm$ 102	0	0	0	0	0	2037	0.7
<i>Calamagrostis epigejos</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Cerastium holosteoides</i>	0	0	102 $\pm$ 102	0	0	0	0	2037	0.7
<i>Chaenorhinum minus</i>	0	0	0	0	102 $\pm$ 102	0	0	2037	0.7
<i>Chenopodium polyspermum</i>	0	204 $\pm$ 204	0	0	0	0	0	4074	0.7
<i>Eleocharis palustris</i>	0	102 $\pm$ 102	0	0	0	0	0	2037	0.7
<i>Euphorbia helioscopia</i>	0	0	0	0	0	0	102 $\pm$ 102	2037	0.7
<i>Fallopia convolvulus</i>	0	0	0	0	0	611 $\pm$ 611	0	12223	0.7
<i>Galium wirtgenii</i>	102 $\pm$ 102	0	0	0	0	0	0	2037	0.7
<i>Juncus compressus</i>	0	0	102 $\pm$ 102	0	0	0	0	2037	0.7
<i>Kickxia elatine</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Lepidium campestre</i>	0	102 $\pm$ 102	0	0	0	0	0	2037	0.7
<i>Lythrum salicaria</i>	0	0	0	0	102 $\pm$ 102	0	0	2037	0.7
<i>Persicaria maculosa</i>	0	102 $\pm$ 102	0	0	0	0	0	2037	0.7
<i>Plantago media</i>	0	0	102 $\pm$ 102	0	0	0	0	2037	0.7
<i>Poa palustris</i>	0	0	0	0	102 $\pm$ 102	0	0	2037	0.7
<i>Rubus caesius</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Sinapis arvensis</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Stellaria media</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Trifolium repens</i>	0	0	0	0	0	102 $\pm$ 102	0	2037	0.7
<i>Urtica dioica</i>	0	0	0	102 $\pm$ 102	0	0	0	2037	0.7
<i>Veronica chamaedrys</i>	0	0	0	0	102 $\pm$ 102	0	0	2037	0.7
Total	33512 $\pm$ 17695	207080 $\pm$ 102941	45022 $\pm$ 26322	11001 $\pm$ 3480	74561 $\pm$ 37512	123861 $\pm$ 80135	6112 $\pm$ 2551	10022940	

**Appendix 4.3:** Daily soil temperature amplitudes in different soil depths at the burial site throughout the course of experiment 2 (Oct 2007-Oct 2009). Temperatures were recorded in 4-hour intervals. Arrows indicate excavation dates.



Burial experiment in the common garden



## CHAPTER 5

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

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*The previous chapter has shown that desiccation cracks act as natural seed traps and that the fate of the trapped seeds differs with seed size. However, do these conclusions also hold for a finer depth resolution? And how much influence does the substrate type have on the fate of trapped seeds? These questions are addressed in the present chapter.*

## 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. Species' responses 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 upheld by disturbance events.

## Keywords

burial experiment; desiccation cracks; dormancy; seed bank; seed mass; seed mortality; soil depth inhibition

## 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 & 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 reduced 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 & Maun 1994). Possible burial mechanisms include frost heave, earthworm activity and other naturally occurring soil disturbances (cf. Chambers & 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 & Norsworthy 2006; Wilson et al. 2006; Davis & 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 & Lapierre 1986; Zhang & Maun 1990; Zhang & Maun 1994; Yanful & Maun 1996; Chen & 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 & Renner 2007), predation (Westerman et al. 2003) or senescence (Telewski & 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 & 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 & Grime 1983; Benech-Arnold et al. 2000; Oliveira & Norsworthy 2006), light quality (Baskin & 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 & Maun 1990; Redmann & Qi 1992; Cussans et al. 1996; Benvenuti et al. 2001; Begum et al. 2006; Oliveira & Norsworthy 2006; Wilson et al. 2006; Davis & 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 lower 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 & Rogaar 1988; Willems & 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 – which are both common to alluvial meadows (Hölzel & 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:
  - a) 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.
  - b) 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 5.1). 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 & Otte 2004b).

### 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 5.1).

### 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,

**Table 5.1** Diaspore and germination characteristics of the species used in the burial experiments (mean  $\pm$  SE).

Species	Family	Seed mass (mg) <sup>a</sup>	Seed length (mm) <sup>b</sup>	Seed width (mm) <sup>b</sup>	Climate chamber experiment <sup>c</sup>		Germination in outdoor experiment (%) <sup>e</sup>
					Germination (%) <sup>d</sup>	Viability (%) <sup>d</sup>	
<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

<sup>a</sup> averages calculated from bulk data for 50 seeds ( $n = 10$ )

<sup>b</sup> all appendices (e.g. pappus for *I. salicina*) removed prior to measuring ( $n = 50$ )

<sup>c</sup> 6-week germination trial in a temperature-controlled incubator with 10/20  $^\circ\text{C}$  (12/12 h) and 14 h photoperiod

<sup>d</sup> 50 seeds per replicate ( $n = 5$ )

<sup>e</sup> 100 seeds per replicate ( $n = 5$ )

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 that 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 for sites featuring desiccation cracks (called 'clay' in the following) (Table 5.2). The material was collected from the same sites along the northern Upper Rhine area where the seeds had been harvested and was steam-sterilised 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 cm, 2 cm, 4 cm, 8 cm 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 & Baskin 2001; Hölzel & 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 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 randomly positioned beneath wooden frames (height: 50 cm) that 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 (1–2 x 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) in 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 four

**Table 5.2** 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 <sup>-1</sup> )	35.8	77.8
K CAL (mg kg <sup>-1</sup> )	44.1	66.4
pH	7.09	6.74

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 in weekly intervals. On 9 June 2009 we determined the number of seedlings growing in the pots, and harvested, dried (48 hours 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 emergence is. 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 5.1), 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 5 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 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 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 & 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 sphericity and compound symmetry, *P*-values were obtained using Pillai's trace, which has been found to be particularly robust (Scheiner 2001). ANOVA analysis were performed using R, version 2.8.1 (R Development Core Team 2008), and ANCOVA and RM-ANOVA were performed using Statistica 6 (StatSoft 2001).

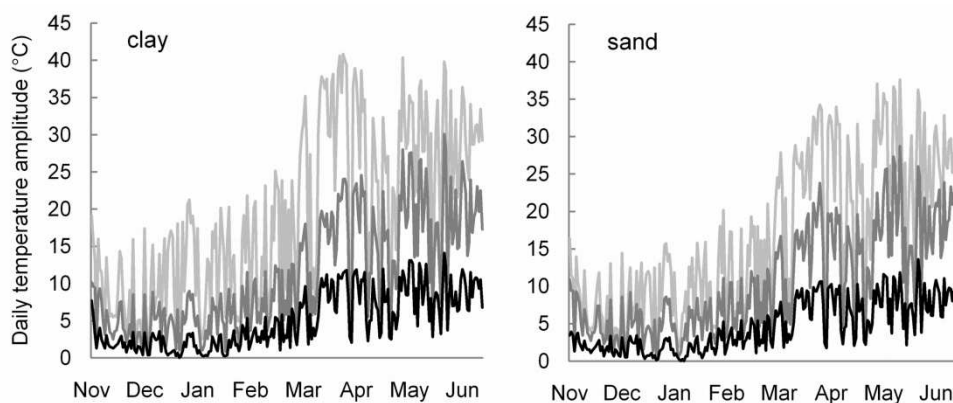
## Results

### Soil temperatures

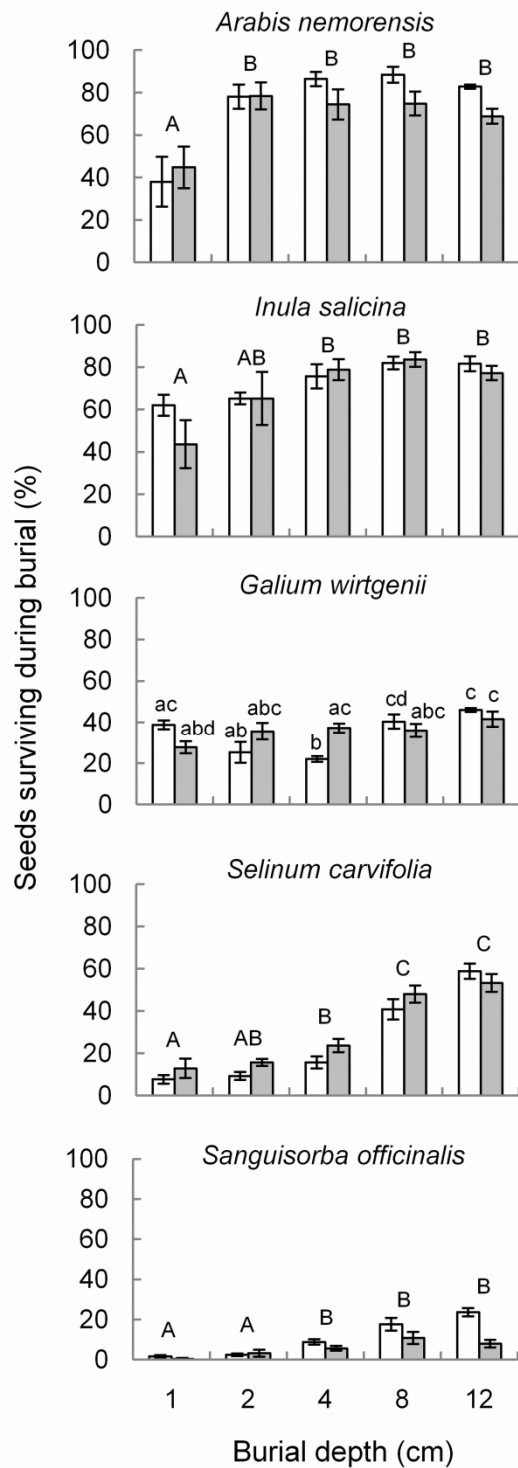
Daily temperature amplitudes across soils differed significantly between burial depths ( $F_{5,2850} = 269.8$ ,  $p < 0.0001$ ), whereas daily mean temperatures across soils did not ( $F_{5,2850} = 0.5$ ,  $p = 0.78$ ). Average daily temperature amplitudes drastically decreased with increasing depths, differed between substrate types ( $F_{1,2854} = 17.34$ ,  $p < 0.0001$ ) and were generally higher in clay than in sand (Figure 5.1).

### 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 5.3). However, the main effect of burial depth and the interaction between species identity and burial depth were also significant. The small-seeded species *A. nemorensis* and *I. salicina* had the highest percentages of surviving seeds, whereas *Sanguisorba officinalis*, the species with the largest seed mass, had the lowest percentages (Figure 5.2). Generally, the percentage of survi-



**Figure 5.1** Daily soil temperature amplitudes in clay and sand throughout the course of the experiment (Nov 08 - Jun 09) at different depths (light gray line: 0 cm, dark gray 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.



**Figure 5.2** 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 ( $p < 0.05$ ).

ving seeds increased with increasing soil depths for all species but *G. wirtgenii* (Figure 5.2), resulting in a significant interaction between species identity and burial depth (Table 5.3).

### Cumulative seedling emergence

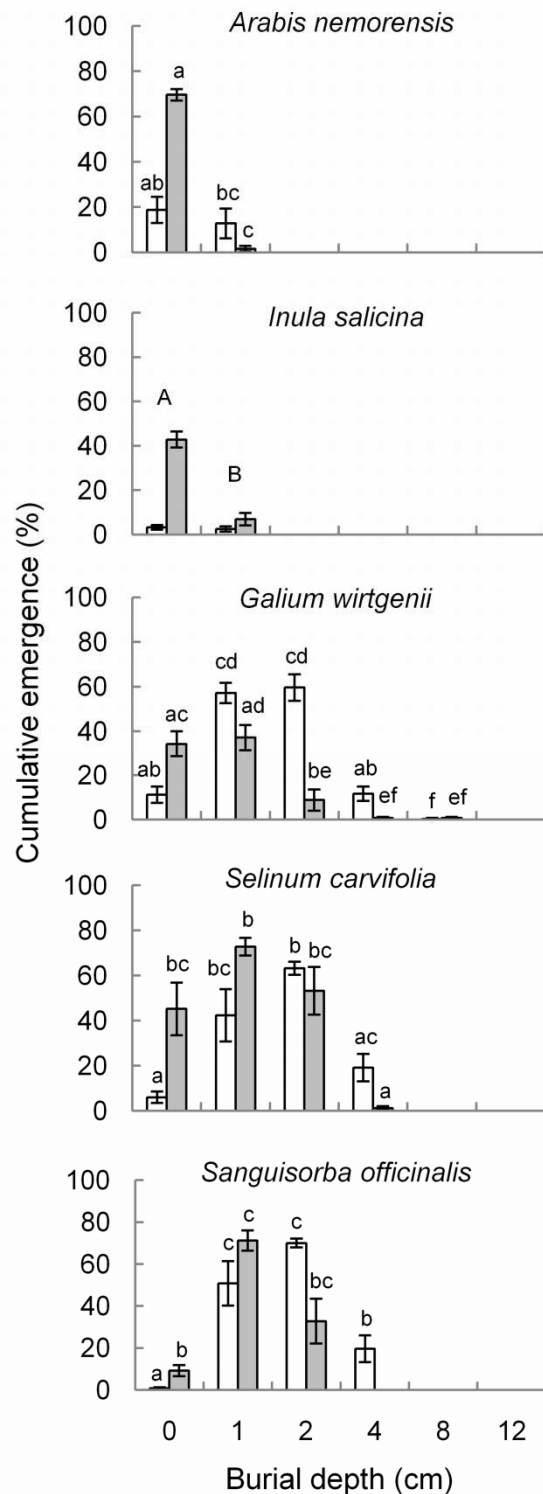
Species identity and burial depth also had a significant effect on the total number of seedlings emerging (Table 5.4). Burial depth, together with its interactions with species identity and soil, accounted for more than 50 % of the total variation (Table 5.4). Generally, emergence decreased with increasing depth, with 12 cm representing the limit from which no seedlings could emerge any more (Figure 5.3). 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 5.4): 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 (Figure 5.3). 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 from up to 4 cm, and *G. wirtgenii* sporadically even emerged from a depth of 8 cm (Figure 5.3). The interaction between substrate type and burial depth was also significant (Table 5.4): 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 (Figure 5.3). 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 5.4) and emergence velocity generally decreasing with increasing burial depth (Table 5.5). However, there was a significant interaction between burial depth and substrate type (Table 5.4). 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

**Table 5.3** 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 x S	4	0.1942	0.0251	0.6
I x D	16	1.7739	<0.0001	5.5
S x D	4	0.2026	0.0207	0.6
I x S x D	16	0.2875	0.4025	0.9
Residuals	200	3.4115		10.6



**Figure 5.3** Cumulative seedling emergence of the study species from 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. 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 5.4** 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 x S	4	0.9525	<0.00001	3.0	52.15	0.0060	1.9	21.7	<0.0001	3.2
I x D	12	8.0138	<0.00001	25.0	280.17	<0.0001	10.4	60.9	<0.0001	8.9
S x D	3	3.6298	<0.00001	11.3	170.15	<0.0001	6.3	43	<0.0001	6.3
I x S x 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

been sown in clay (Table 5.5). Temporal emergence patterns differed between species, as indicated by a significant interaction between species identity and burial depth (Table 5.4). 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 5.5). *G. wirtgenii* featured intermediate emergence velocity.

### Fatal germination

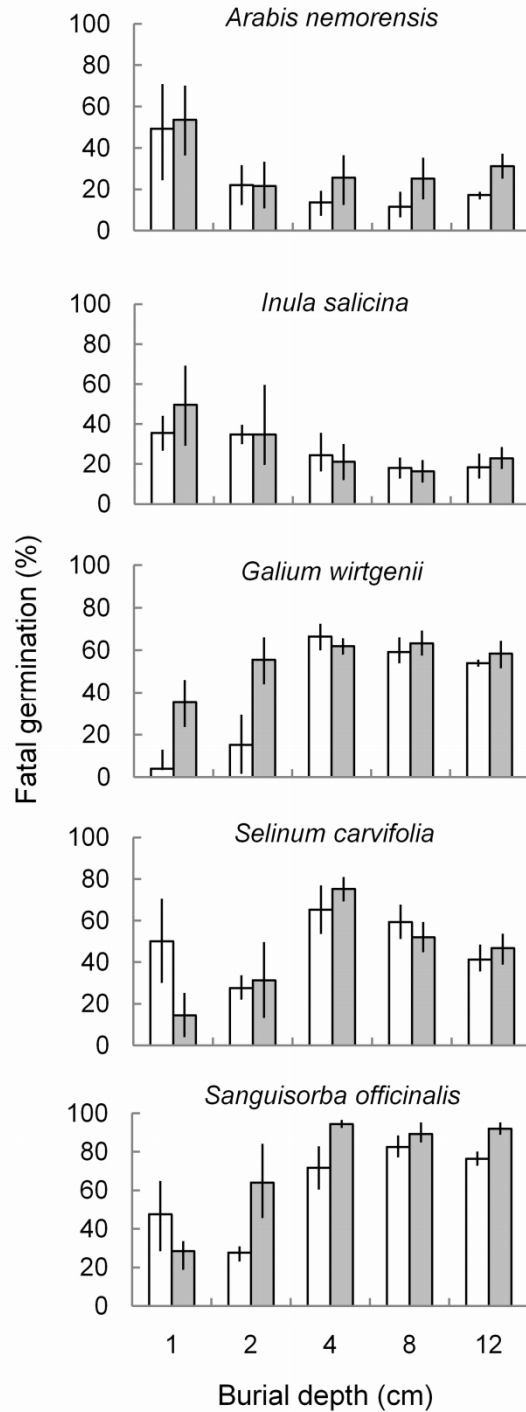
The share of seeds dying through fatal germination differed between species and burial depths. In the two small-seeded species *A. nemorensis* and *I. salicina*, fatal germination decreased with increasing depths, whereas the opposite was the case for the larger-seeded species (Figure 5.4). Across depths, 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 species identity and burial depth both had a significant effect on total biomass per pot, and together with

**Table 5.5** Emergence velocity in clay and sand (mean  $\pm$  SE). Numbers give values of the emergence velocity index (EVI). The higher EVI is, the faster emergence occurred.

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



**Figure 5.4** 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.

their interaction explained almost 35 % of the total variation (Table 5.6). 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 x 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 the by far largest fraction of the total variation (Table 5.4). 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 (Figure 5.2), 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 Benvenuti et al. (2001), who observed that seeds of 20 weed species perceived unfavourable germination conditions and responded by

**Table 5.6** 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	12605	0.8878	0.005
D	3	2002132	0.0260	2.48
I x S	4	4156670	<0.0001	6.87
I x D	12	2755835	<0.00001	13.66
S x D	3	2214239	0.0168	2.75
I x S x D	12	1113161	0.0587	5.52
Residuals	159	631529		41.49

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 & Maun 1994; Ren et al. 2002). In our study, this pattern was least pronounced for *G. wirtgenii* (Figure 5.2), 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 (Figure 5.1) since light only penetrates the very top layer of the soil surface (Woolley & 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 & Maun 1990; Redmann & Qi 1992; Cussans et al. 1996; Benvenuti et al. 2001; Begum et al. 2006; Oliveira & Norsworthy 2006; Wilson et al. 2006; Davis & 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 (Figure 5.3). This is in line with several other studies (e.g. Maun & Lapierre 1986; Colosi et al. 1988; Yanful & Maun 1996; Chen & 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 more attractive prey 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 5.5), 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: Firstly, germination occurred later in greater depths as seasonal soil temperature increases (as triggers of germination) set in later at increasing depths due to the insulating effect of the soil (Figure 5.1; cf. Begum et al. 2006). Secondly, 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 5.4 and 5.6). 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 asymmetric (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 & 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 5.3). 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 5.4), 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 in greater depth (Figure 5.3). The higher surface germination in sand may have been due to the fact that soil temperatures were generally higher in clay than in sand (Figure 5.1) – 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. In 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 5.4), we observed a trend towards lower emergence velocity in sand than in clay (Table 5.5). 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 (Figure 5.1). This could imply that critical temperature thresholds for germination were reached later in sand than in clay so that germination set in 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 (Figure 5.2), which is in line with our third hypothesis and supports the finding of Yanful & Maun (1996) and Davis & Renner (2007). This might be the result

of differential selection pressures as small seeds have less 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 (Figure 5.4).

Maximum depth of emergence was much lower for the two small-seeded species *A. nemorensis* and *I. salicina* than for the other species (Figure 5.3). 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 & 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 *Nitraria 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 seed-size based strategies for coping with the extreme recruitment conditions prevailing in their habitat. Strategy 1 is characterised 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 characterised by producing many small seeds that can be incorporated into the soil easily (cf. Thompson et al. 1993) and have effective depth-sensing mechanisms which 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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## References

- Baskin C.C. & Baskin J.M. (2001) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*, Academic Press, San Diego.
- Begum M., Juraimi A.S., Amartalingam R., Bin Man A. & Rastans S.O.B. (2006) The effects of sowing depth and flooding on the emergence, survival, and growth of *Fimbristylis miliacea* (L.) Vahl. *Weed Biology and Management* 6: 157-164.
- Benech-Arnold R.L., Sanchez R.A., Forcella F., Kruk B.C. & Ghera C.M. (2000) Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* 67: 105-122.
- Benvenuti S. (1995) Soil light penetration and dormancy of Jimsonweed (*Datura stramonium*) seeds. *Weed Science* 43: 389-393.
- Benvenuti S. (2003) Soil texture involvement in germination and emergence of buried weed seeds. *Agronomy Journal* 95: 191-198.
- Benvenuti S. (2007) Natural weed seed burial: effect of soil texture, rain and seed characteristics. *Seed Science Research* 17: 211-219.
- Benvenuti S., Macchia M. & Miele S. (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science* 49: 528-535.
- Bond W.J., Honig M. & Maze K.E. (1999) Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia* 120: 132-136.
- Burmeier S., Eckstein R.L., Otte A. & Donath T.W. (2010) Desiccation cracks act as natural seed traps in flood-meadow systems. *Plant and Soil* 333: 351-364.
- Chambers J.C. & MacMahon J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics* 25: 263-292.
- Chambers J.C., MacMahon J.A. & Haefner J.H. (1991) Seed entrapment in alpine ecosystems: effects of soil particle size and diaspore morphology. *Ecology* 72: 1668-1677.
- Chen H. & Maun M.A. (1999) Effects of sand burial depth on seed germination and seedling emergence of *Cirsium pitcheri*. *Plant Ecology* 140: 53-60.
- Colosi J.C., Cavers P.B. & Bough M.A. (1988) Dormancy and survival in buried seeds of proso millet (*Panicum miliaceum*). *Canadian Journal of Botany* 66: 161-168.
- Cook R.E. (1980) Germination and size-dependent mortality in *Viola blanda*. *Oecologia* 47: 115-117.
- Cussans G.W., Raudonius S., Brain P. & Cumberworth S. (1996) Effects of depth of seed burial and soil aggregate size on seedling emergence of *Alopecurus myosuroides*, *Galium aparine*, *Stellaria media* and wheat. *Weed Research* 36: 133-141.
- Davis A.S. & Renner K.A. (2007) Influence of seed depth and pathogens on fatal germination of velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*). *Weed Science* 55: 30-35.
- Donath T.W., Hölzel N. & Otte A. (2003) The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Applied Vegetation Science* 6: 13-22.
- Elberling H. (2000) Spatial pattern of *Lesquerella arctica*: Effects of seed bank and desiccation cracks. *Ecoscience* 7: 86-91.
- Espinar J.L., Thompson K. & Garcia L.V. (2005) Timing of seed dispersal generates a bimodal seed bank depth distribution. *American Journal of Botany* 92: 1759-1763.
- Fenner M. & Thompson K. (2005) *The ecology of seeds*, Cambridge University Press, Cambridge, UK.
- Forcella F., Arnold R.L.B., Sanchez R. & Ghera C.M. (2000) Modeling seedling emergence. *Field Crops Research* 67: 123-139.
- Grime J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons, Chichester.
- Grundy A.C., Mead A. & Burston S. (2003) Modelling the emergence response of weed seeds to burial depth: interactions with seed density, weight and shape. *Journal of Applied Ecology* 40: 757-770.
- Hölzel N. & Otte A. (2001) The impact of flooding regime on the soil seed bank of flood-meadows. *Journal of Vegetation Science* 12: 209-218.
- Hölzel N. & Otte A. (2004a) Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. *Journal of Vegetation Science* 15: 93-100.

- Hölzel N. & Otte A. (2004b) Ecological significance of seed germination characteristics in flood-meadow species. *Flora* 199: 12-24.
- Houle G., McKenna M.F. & Lapointe L. (2001) Spatiotemporal dynamics of *Floerkea proserpinacoides* (Limnanthaceae), an annual plant of the deciduous forest of eastern North America. *American Journal of Botany* 88: 594-607.
- Leishman M.R., Wright I.J., Moles A.T. & Westoby M. (2000) The evolutionary ecology of seed size. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 31-57. 2nd ed. CABI Publishing, Wallingford.
- Li Q.Y., Zhao W.Z. & Fang H.Y. (2006) Effects of sand burial depth and seed mass on seedling emergence and growth of *Nitraria sphaerocarpa*. *Plant Ecology* 185: 191-198.
- Maun M.A. & Lapierre J. (1986) Effects of burial by sand on seed germination and seedling emergence of four dune species. *American Journal of Botany* 73: 450-455.
- Milberg P., Andersson L. & Thompson K. (2000) Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* 10: 99-104.
- Oliveira M.J. & Norsworthy J.K. (2006) Pitted morningglory (*Ipomoea lacunosa*) germination and emergence as affected by environmental factors and seeding depth. *Weed Science* 54: 910-916.
- 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*, pp. 367-384. Academic Press, San Diego.
- Quinn G.P. & Keough M.J. (2002) *Experimental design and data analysis for biologists*, Cambridge University Press, Cambridge.
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Redmann R.E. & Qi M.Q. (1992) Impacts of seeding depth on emergence and seedling structure in eight perennial grasses. *Canadian Journal of Botany* 70: 133-139.
- Ren J., Tao L. & Liu X.M. (2002) Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *Journal of Arid Environments* 51: 603-611.
- Scheiner S.M. (2001) MANOVA: Multiple response variables and multispecies interactions. In: Scheiner S.M. & Gurevitch J. (eds.) *Design and analysis of ecological experiments*, pp. 99-115. Oxford University Press, New York.
- Schmiede R., Donath T.W. & Otte A. (2009) Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404-413.
- StatSoft (2001) STATISTICA für Windows. Version 6.0.
- Telewski F.W. & Zeevaart J.A.D. (2002) The 120-yr period for Dr. Beal's seed viability experiment. *American Journal of Botany* 89: 1285-1288.
- Thompson K. (2000) The functional ecology of soil seed banks. In: Fenner M. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 215-235. CABI Publishing, Wallingford.
- Thompson K., Band S.R. & Hodgson J.G. (1993) Seed size and shape predict persistence in soil. *Functional Ecology* 7: 236-241.
- Thompson K. & Grime J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology* 20: 141-156.
- van der Reest P.J. & Rogaar H. (1988) The effect of earthworm activity on the vertical distribution of plant seeds in newly reclaimed polder soils in the Netherlands. *Pedobiologia* 31: 211-218.
- van der Valk A.G. (1974) Environmental factors controlling the distribution of forbs on coastal foredunes in Cape Hatteras National Seashore. *Canadian Journal of Botany* 52: 1057-1073.
- van Tooren B.F. (1988) The fate of seeds after dispersal in chalk grassland: the role of the bryophyte layer. *Oikos* 53: 41-48.
- Vleeshouwers L.M. (1997) Modelling the effect of temperature, soil penetration resistance, burial depth and seed weight on pre-emergence growth of weeds. *Annals of Botany* 79: 553-563.
- Vleeshouwers L.M., Bouwmeester H.J. & Karssen C.M. (1995) Redefining seed dormancy: An attempt to integrate physiology and ecology. *Journal of Ecology* 83: 1031-1037.
- Weiner J. (1990) Asymmetric competition in plant populations. *Trends in Ecology and Evolution* 5: 360-364.

- Westerman P.R., Wes J.S., Kropff M.J. & Van der Werf W. (2003) Annual losses of weed seeds due to predation in organic cereal fields. *Journal of Applied Ecology* 40: 824-836.
- White R.E. (1997) *Principles and practice of soil science*, Blackwell, Oxford.
- Willems J.H. & Huijsmans K.G.A. (1994) Vertical seed dispersal by earthworms: a quantitative approach. *Ecography* 17: 124-130.
- Wilson D.G., Burton M.G., Spears J.E. & York A.C. (2006) Doveweed (*Murdannia nudiflora*) germination and emergence as affected by temperature and seed burial depth. *Weed Science* 54: 1000-1003.
- Woolley J.T. & Stoller E.W. (1978) Light penetration and light-induces seed germination in soil. *Plant Physiology* 61: 597-600.
- Yanful M. & Maun M.A. (1996) Effects of burial of seeds and seedlings from different seed sizes on the emergence and growth of *Strophostyles helvola*. *Canadian Journal of Botany* 74: 1322-1330.
- Zhang J. & Maun M.A. (1990) Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. *Canadian Journal of Botany* 68: 304-310.
- Zhang J. & Maun M.A. (1994) Potential for seed bank formation in seven Great Lakes sand dune species. *American Journal of Botany* 81: 387-394.



Pollarded willow surrounded by flood meadows



# Summary

Flood meadows – typical plant communities of large lowland river valleys – have declined drastically across Europe during the last decades and now belong to the most threatened plant communities. As they also harbour many rare and endangered species they are considered to be of high conservation concern, which entails the need to protect remnant and restore degraded sites. Both require detailed information about ecological relationships within the system.

This thesis deals with the ecology and restoration of flood meadows in the Northern Upper Rhine Valley in southwestern Germany. Until the 1950s, the entire region was dominated by species-rich alluvial grasslands, which were subsequently converted into arable fields. In the 1980s, some of the sites were left to natural succession to reconvert them into species-rich grasslands. However, typical flood-meadow species turned out to be strongly dispersal limited and could not establish on the sites. A large-scale restoration project was thus initiated in the late 1990s with the aim of re-establishing typical flood-meadow vegetation by means of plant material transfer. This implies mowing species-rich flood-meadow remnants in autumn and transferring the plant material to the restoration sites. As it contains seeds of many typical flood-meadow species, these may then establish on the restoration sites. Since the latter are more abundant than remnant sites, the plant material is generally not sufficient for covering the entire sites and is thus applied in the form of narrow strips.

The main objectives of this thesis were (1) to evaluate the success of plant material transfer as a restoration tool and (2) to explore the factors governing soil seed bank dynamics in flood meadows. The term ‘soil seed bank’ refers to a reservoir of viable, yet ungerminated seeds in the ground. It is of particular interest in flood meadows as it enables the rapid regeneration of plant populations after disturbance events and may thus contribute to their long-term persistence in this disturbance-prone system. This thesis comprises a total of four studies that deal with different aspects of the topic.

The first was a case study with the flood-meadow plant species *Arabis nemorensis*. Its objective was to evaluate the success of grassland restoration via plant material transfer by comparing restored and remnant sites with regard to population dynamics and spatial patterns. The results show that plant material transfer rapidly triggered the formation of spatially-structured populations that closely resembled those of remnant sites. Furthermore, population dynamics in restored and remnant populations of *A. nemorensis* could no longer be distinguished from each other only three years after restoration measures were carried out. The overall habitat structure of the restoration sites – which may deter-

mine whether they will also provide suitable habitat conditions for other taxa – rapidly approximated that of remnant sites in the course of early post-restoration succession.

The second study dealt with vegetation development on restoration sites 7-8 years after plant material application. Its aim was to test the assumption that plant material strips act as colonization initials for transferred species and to evaluate whether it is feasible to restore entire sites by spatially-restricted plant material application. It turned out that transferred species, which were absent from the sites until restoration began, now occurred in the above-ground vegetation, the seed rain and the soil seed bank on and adjacent to the plant material strips. Almost 90 % of all species that had established on the strips had also spread into their surroundings. Apparently, the strips really acted as colonization initials for flood-meadow restoration, i.e. the implicit assumption underlying the layout of plant material transfer seems to hold.

The first two studies also gave evidence for rapid seed bank formation during early post-restoration succession, and it seems likely that the restoration measures will eventually result in the establishment of self-sustaining populations of the target species. Vertical seed translocation occurred particularly rapidly, which could at least partially be explained by seed entrapment due to desiccation cracks. Such cracks regularly form on the study sites during periods of extended summer drought, when desiccation causes the fine-grained alluvial soils to shrink and thus leads to the formation of distinctive cracks.

The third study presented in this thesis comprises empirical and experimental investigations on the impact of desiccation cracks on soil seed bank formation and dynamics. Its results show that the cracks act as natural seed traps and contribute to a rapid incorporation of shed seeds into the soil. Mapping of crack patterns in consecutive dry periods revealed that the cracks were at least short-term spatially constant, which has important consequences for their trapping potential: If cracks frequently open in the same positions they can accumulate a large number of seeds, which may result in a clumped distribution of the soil seed bank, as indeed found in the case study with *A. nemorensis*.

The implications of seed entrapment by desiccation cracks were investigated by means of two burial experiments, which are presented in the third and fourth study compiled here. Seeds of five herbaceous flood-meadow species were buried in different depths and partly also different substrates and tested at regular intervals. It turned out that the likelihood of survival during burial generally increased with increasing depth. This indicates that the seeds possess depth-sensing mechanisms that lead to germination inhibition when they are buried so deep that successful seedling emergence would be unlikely. Furthermore, seed fate differed pronouncedly between species and seemed to be connected with seed size: Smaller-seeded species had a higher likelihood of survival during burial than larger-seeded species, whereas the latter were more likely to emerge successfully after germination in greater depths. Substrate type did not have a pronounced influence on the fate of seeds and seedlings. These results indicate that desiccation cracks may favour the development of long-term persistent soil seed banks and could thus indirectly influence the composition of the plant community.

All in all, the results presented in this thesis show (1) that plant material transfer is a suitable method for flood-meadow restoration and (2) that seed bank dynamics in this disturbance-prone ecosystem are strongly influenced by abiotic conditions such as desiccation cracks. These findings have important implications for flood-meadow restoration as they indicate that plant material transfer is not only suitable for transferring individual species,

but may also contribute to reinstating entire flood-meadow communities. I therefore recommend its continued use for the restoration of flood meadows and other species-rich grasslands and discuss some practical considerations for the spatial layout of such restoration schemes. Furthermore, I suggest that desiccation cracks should be accounted for as an important abiotic factor in flood-meadow systems. Finally, I outline some areas in need of further research and discuss upcoming challenges for flood-meadow conservation and restoration.



# Zusammenfassung

Stromtalwiesen – typische Pflanzengemeinschaften großer Flusstäler – sind in den letzten Jahrzehnten in Mitteleuropa stark zurückgegangen und gelten heute als gefährdeter Lebensraum. Da sie viele seltene Pflanzen- und Tierarten beherbergen, ist die Erhaltung verbliebener Restvorkommen sowie die Renaturierung ehemaliger, inzwischen ackerbaulich genutzter oder verbrachter Bestände von großer naturschutzfachlicher Relevanz. Dazu sind umfassende Informationen über die ökologischen Zusammenhänge innerhalb des System Stromtalwiese erforderlich.

Die vorliegende Arbeit präsentiert Untersuchungen zur Ökologie und Renaturierung von Stromtalwiesen im nördlichen Oberrheintal in Südwestdeutschland. Bis in die 50er Jahre hinein war das gesamte Gebiet durch artenreiches Auengrünland gekennzeichnet, das dann jedoch großflächig in Ackerland umgewandelt wurde. In den 80er Jahren begannen Versuche, einige der Ackerflächen wieder in artenreiches Grünland umzuwandeln, wozu die Flächen der natürlichen Sukzession überlassen wurden. Es stellte sich allerdings heraus, dass viele der typischen Stromtalarten stark ausbreitungslimitiert waren und sich nicht auf den Flächen etablieren konnten. Daher wurde Ende der 90er Jahre ein Renaturierungsprojekt ins Leben gerufen, um mithilfe der sogenannten Mahdgutübertragung großflächig artenreiche Stromtalwiesen wiederherzustellen. Dabei werden noch vorhandene Restbestände von Stromtalwiesen im Herbst gemäht, woraufhin das Mahdgut auf Renaturierungsflächen übertragen wird. Da darin zahlreiche Samen typischer Stromtalarten vorhanden sind, können sich diese in der Folge auf den Renaturierungsflächen etablieren. Da deren Zahl die der Restbestände deutlich übersteigt, wird das Mahdgut meist nicht flächendeckend, sondern in Form langgestreckter Streifen ausgebracht.

Hauptziele der Arbeit waren, (1) den Erfolg von Mahdgutübertragung als Renaturierungsmethode zu überprüfen und (2) die Faktoren zu erforschen, die die Samenbankdynamik in Stromtalwiesen steuern. Der Begriff ‚Samenbank‘ meint hier die Gesamtheit der keimfähigen Samen im Boden. Sie ist in Stromtalwiesen von besonderem Interesse, weil sie zur raschen Regeneration der Populationen nach Störereignissen beiträgt und so etlichen Pflanzenarten überhaupt erst ermöglicht, sich dauerhaft im störungsintensiven Auengrünland zu halten. Insgesamt wurden im Rahmen der vorliegenden Arbeit vier Untersuchungen bzw. Teilstudien durchgeführt, die sich mit unterschiedlichen Aspekten des Themas befassen.

Bei der ersten Untersuchung handelte es sich um eine Fallstudie mit der Pflanzenart *Arabis nemorensis*. Ziel war es, Populationsdynamik und räumliche Muster in ursprünglichen und renaturierten Populationen zu vergleichen und so den Erfolg der Mahdgutübertragung zu überprüfen. Es zeigte sich, dass Mahdgutübertragung rasch zur Bil-

derung räumlich strukturierter Populationen führte, die den ursprünglichen Restpopulationen stark ähnelten. Bereits drei Jahre nach der Mahdgutübertragung ließen sich renaturierte und ursprüngliche Populationen in Hinblick auf ihre Populationsdynamik nicht mehr voneinander unterscheiden. Auch die Habitatstruktur der Renaturierungsflächen – die entscheidend dafür sein kann, ob diese auch für andere Organismengruppen geeignete Lebensräume darstellen können – näherte sich rasch der der Restflächen an.

In der zweiten Untersuchung ging es um die Vegetationsentwicklung auf Flächen, auf denen vor sieben bis acht Jahren Mahdgutstreifen angelegt worden waren. Ziel war es zu überprüfen, ob die Streifen – wie üblicherweise postuliert – als Quellpopulationen für die übertragenen Arten dienen und ob es möglich ist, komplette Flächen durch eine räumlich begrenzte Ausbringung von Mahdgut zu renaturieren. Es zeigte sich, dass übertragene Arten in der oberirdischen Vegetation, dem Sameneintrag und der Samenbank nachweisbar waren, und zwar sowohl auf den Mahdgutstreifen selbst als auch in den angrenzenden Flächen. Fast 90 % aller Arten, die sich durch die Mahdgutübertragung auf den Streifen etablieren konnten, hatten mittlerweile auch deren Umgebung besiedelt. Die Streifen dienten also offenbar tatsächlich als Quellpopulationen, von denen aus die übertragenen Arten weitere Bereiche der Renaturierungsflächen besiedeln konnten.

Die ersten beiden Untersuchungen gaben auch Hinweise darauf, dass es schon während der Frühphase der Grünlandsukzession zu einer raschen Samenbankentwicklung kommt. Es ist daher wahrscheinlich, dass die Renaturierungsmaßnahmen letztlich dazu führen werden, dass sich die neu angelegten Populationen dauerhaft auf den Flächen etablieren können. Insbesondere die vertikale Samenverlagerung erfolgte sehr rasch, was zumindest teilweise durch die Auswirkungen von Trockenrissen erklärt werden kann. Solche Risse entstehen regelmäßig in sommerlichen Trockenphasen, da starke Austrocknung in den feinkörnigen Auenböden zu Schrumpfungsprozessen führt, durch die sich dann ausgeprägte Risse bilden.

Im Rahmen der dritten Teilstudie wurden empirische und experimentelle Untersuchungen zu den Auswirkungen solcher Trockenrissen auf die Samenbankentwicklung und -dynamik von Stromtalwiesen durchgeführt. Es stellte sich heraus, dass die Risse als Samenfallen wirken und zur raschen Tiefenverlagerung von Samen beitragen. Eine wiederholte Kartierung von Rissmustern zeigte zudem, dass die Risse wiederholt an den gleichen Stellen auftreten, was Auswirkungen auf ihr Fangpotential hat: Wenn sich die Risse in aufeinanderfolgenden Trockenphasen immer wieder an den gleichen Stellen öffnen, kann sich in ihnen eine große Zahl von Samen ansammeln, was zu einer geklumpten Verteilung der Samen im Boden führen kann. Eine derartige geklumpete Verteilung wurde auch in der Fallstudie mit *A. nemorensis* beobachtet.

Die Auswirkung des ‚Samenfangs‘ durch Trockenrisse wurden durch zwei Vergrabungsexperimente überprüft, die im Rahmen der dritten und vierten Teilstudie durchgeführt wurden. Dazu wurden Samen von fünf typischen Stromtalarten in unterschiedlichen Tiefen und teilweise auch Böden vergraben und in regelmäßigen Abständen kontrolliert. Es zeigte sich, dass die Überlebenswahrscheinlichkeit vergrabener Samen generell mit zunehmender Tiefe anstieg. Das deutet darauf hin, dass die Samen der getesteten Arten über Mechanismen zur Tiefenermittlung verfügen, die keimungshemmend wirken, wenn sich die Samen in so großer Tiefe befinden, dass der Keimling die Oberfläche voraussichtlich nicht erreichen würde. Daneben gab es auch deutliche artspezifische Unterschiede im Samenschicksal, die mit der Samengröße zusammenhingen: Kleinsamige

Arten überlebten mit höherer Wahrscheinlichkeit im Boden als großsamige Arten, wohingegen diese noch aus deutlich größeren Tiefen erfolgreich keimen konnten. Die Bodenart hatte dagegen keinen Einfluss auf die Überlebens- und Keimungswahrscheinlichkeit der Samen. Diese Befunde deuten darauf hin, dass Trockenrisse die Entwicklung einer langfristig persistenten Samenbank begünstigen und so indirekt die Zusammensetzung der Pflanzengemeinschaft beeinflussen könnten.

Insgesamt zeigen die Ergebnisse der vorliegenden Arbeit, (1) dass Mahdgutübertragung ein geeignetes Verfahren zur Renaturierung von Stromtalwiesen ist und (2) dass die Samenbankdynamik in diesem Lebensraum stark durch abiotische Umweltbedingungen wie Trockenrisse beeinflusst wird. Diese Befunde sind relevant für die Renaturierung von Stromtalwiesen, denn sie zeigen, dass Mahdgutübertragung nicht nur geeignet ist, um einzelne Arten wieder anzusiedeln, sondern auch dazu beitragen kann, ganze Lebensgemeinschaften und damit auch die ökologischen Funktionen von Stromtalwiesen wiederherzustellen. Ich empfehle daher, diese Methode auch weiterhin bei der Renaturierung von Stromtalwiesen und anderen artenreichen Grünlandbeständen einzusetzen und diskutiere einige praktische Aspekte zum optimalen Design der Mahdgutstreifen. Außerdem rate ich, Trockenrisse als wichtige abiotische Faktoren beim Schutz und der Renaturierung von Stromtalwiesen zu berücksichtigen. Abschließend skizziere ich einige Themengebiete, auf denen noch Forschungsbedarf besteht, und diskutiere künftige Herausforderungen für den Schutz und die Renaturierung von Stromtalwiesen.

Running for it on a freshly-mown flood meadow



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In the field



# List of publications

## International journals

- Burmeier S., Eckstein R.L., Donath T.W. & Otte A. (in press): Plant pattern development during early post-restoration succession in grasslands – a case study of *Arabis nemorensis*. Restoration Ecology, DOI: 10.1111/j.1526-100X.2010. 00668.x.
- Burmeier, S., Eckstein, R.L., Otte, A. & Donath, T.W. (in press): Spatially-restricted plant material application creates colonization initials for flood-meadow restoration. Biological Conservation, DOI: DOI: 10.1016/j.biocon.2010.08.018.
- Burmeier, S., Donath, T.W., Otte, A. & Eckstein, R.L. (2010): Rapid burial has differential effects on germination and emergence of small- and large-seeded herbaceous plant species. Seed Science Research 20: 189-200.
- Burmeier, S., Eckstein, R.L., Otte, A. & Donath, T.W. (2010): Desiccation cracks act as natural seed traps in flood-meadow systems. Plant and Soil 333: 351-364.
- Burmeier, S. & Jensen, K. (2009): Experimental ecology and habitat specificity of the endangered plant *Apium repens* (Jacq.) Lag. at the northern edge of its range. Plant Ecology & Diversity 2: 65-75.
- Burmeier, S. & Jensen, K. (2008): Is the endangered *Apium repens* (Jacq.) Lag. rare because of a narrow regeneration niche? Plant Species Biology 23: 111-118.

## National journals and reports

- Donath T.W., Schmiede R., Harnisch M., Burmeier S., Eckstein R.L. & Otte A. (2009): Renaturierung von Auenwiesen am hessischen Oberrhein - Perspektiven für die langfristige Entwicklung. Laufener Spezialbeiträge 2/09, 122-132.
- Burmeier, S. (2009): Merkblatt Artenschutz 17: Kriechender Sellerie *Apium repens* (Jacq.) Lag.. Bayerisches Landesamt für Umwelt, Augsburg.
- Burmeier, S., Eckstein, R.L., Donath, T.W. & Otte, A. (2008): Samenbankentwicklung und -dynamik in Stromtalwiesen am hessischen Oberrhein. Treffpunkt Biologische Vielfalt 8: 133-138.

# Versicherung

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.



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(Sandra Burmeier)

Gießen, 6. Mai 2010

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Ort, Datum