Seed ecology in dry sandy grasslands – an approach to patterns and mechanisms

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Abstract

Dry sandy grasslands occur throughout Central Europe and Southern Germany, and they host many rare and endangered plant species. To date, insufficient data on seed ecological aspect of this endangered vegetation type exist. Seed ecological studies attempt to develop ecological knowledge by identifying patterns and underlying mechanisms. Therefore, this thesis is an attempt to close knowledge gaps. Ch. 1 starts with an overview of seed ecology research, the linkage of current seed ecological studies to applied and theoretical ecology. In Ch. 2, occurrence of species along a soil pH gradient was related to aluminum toxicity, highlighting a correlation of species' regeneration niches and their sensitivity to according acidic soil conditions. In Ch. 3, influence of soil type and soil moisture and their interactive effects on seed survival was tested, indicating the important role of soil moisture. In Ch. 4, seed germination ecology of dry sandy grasslands revealed different seed ecological patterns in seed dormancy, germination traits and their mechanisms. The understanding of seed persistence mechanisms is advanced by the presented findings on how different seed traits and seed germination traits correlate with soil seed persistence. In Ch. 5, mechanisms of seed longevity in ex situ conditions with seed traits and germination traits was studied, showing the importance of seed traits in seed longevity similar to soil seed longevity mechanisms.

To bring all results together, it can be pointed out that environmental factors strongly shape seed ecological patterns. To find out the role of each environmental factor in seed ecological patterns, cross-interactions of different factors need to be considered which, in the case of seed persistence, showed that soil moisture is the strongest factor. Species with different mechanisms like those creating persistent seed banks for small sized seeds, faster germination speed and the development of physiological dormancy may tolerate sandy grasslands habitat conditions. Filtering effect of aluminum toxicity for germination of species from calcareous soil indicates the importance of regeneration niches in community assembly. Developing seed ecological traits as easy measurable traits would help to elucidate community assembly rules. Different factors like habitat conditions and seed responses to these environmental factors, seed reserves and germination limitation should be considered in restoration ecology and conservation issues. Seed ecological studies can help in habitat restoration planning and species reintroduction design making. Future investigations on conceptual frameworks for applications of seed ecological research in applied ecology are promising.

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Chapter 1

Introduction

Seed ecology (Patterns, processes, mechanisms)

Plants have developed different mechanisms to adapt to variable environmental conditions of habitats. Production of viable seeds, as well as dispersal and seed germination, are among the most vital processes during plant establishment. This, in turn, is one of the most crucial stages in a plant's life cycle (Grubb, 1977). Different seed ecological traits like seed germination, dormancy, dispersal, persistence have been broadly studied in order to understand the role of "regeneration niche" in different habitat conditions at global and local scales (Poschlod et al., 2013).

What is the seed ecologist's job? Seed ecological studies attempt first to figure out seed ecological patterns in response to the most important environmental factors influencing plant regeneration niches such as moisture, temperature, soil nutrients and soil reaction. Second, they let us understand the mechanisms, processes and function of these seed ecological patterns in relation to certain environmental conditions. Finally these patterns and functions could be used in theory and application linkages. Seed ecology could be used in community ecology assembly and also in applied ecology in restoration and conservation (Fig. 1).

Consideration of seed ecological patterns, different seed persistence types (Thompson et al., 1997), dormancy patterns (Baskin and Baskin, 1998), germination responses to light and temperatures (Thompson and Grime, 1983; Milberg et al., 2000), dispersal types (Poschlod et al., 2013) has been suggested to show the role of seeds in plant life cycles. Not only seed ecological traits, but also some seed anatomical and morphological traits can be an advantage at certain environmental conditions and also be used to find mechanisms and functions of seed ecological patterns. Seed size is the most important trait correlated to other seed ecological traits and environmental conditions. Seed size is negatively correlated to seed persistence (Bekker et al., 1998b) and light availability (Milberg et al., 2000). There is a tradeoff between seed production and seed size, species with large seeds having a lower seed production (Shipley and Dion, 1992). Seed size and dormancy may be also related to each other (Volis and Bohrer, 2012). Seed coat thickness

is also a good trait to explain soil seed persistence (Gardarin et al., 2010). Seed morphological characteristic may be correlated to the seed dispersal potential as well (Pakeman et al., 2002; Römermann et al., 2005).

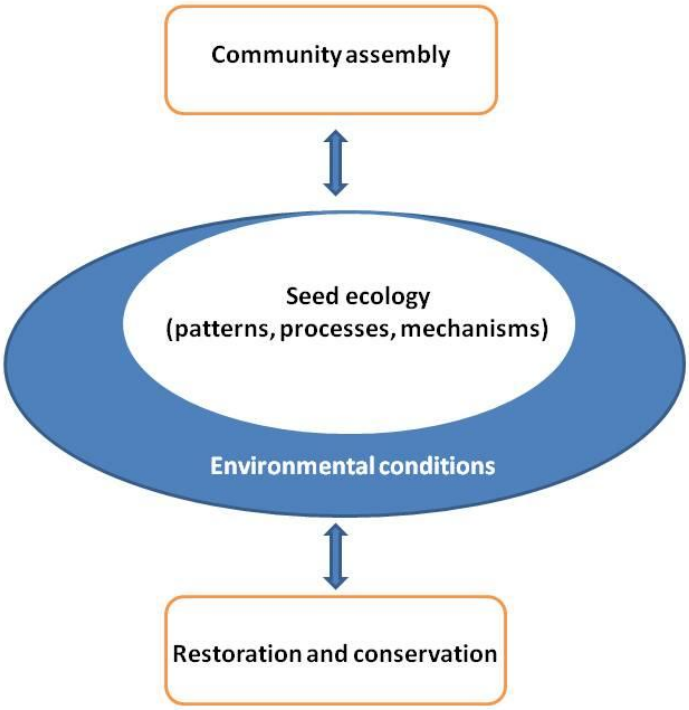


Figure1 Seed ecology and application in community ecology, conservation biology and restoration ecology

Linkages to community ecology and assembly rules

The answer to the question "Why does a species occur where it occurs" is a fundamental challenge in plant and vegetation ecology. It makes us try to understand not only the global and local distribution of a species, but also the species assembly in plant communities. Although many theories are discussed on different spatial scales such as the niche concept (Tilman, 1982, 1988; Chase and Leibold, 2003; Peterson et al., 2011), species pool theory (Zobel, 1997), the neutral theory (Hubbell, 2001), the metacommunity concept (Leibold et al., 2004) or other "mixed" models (Chase et al., 2005; Pavoine et al., 2011), most of these theories still remain to be validated. The same is true for the relevant environmental filters: they are essential for the formation of plant assemblages, because they sort the occurrence of species according to their physiological tolerances (Weiher and Keddy, 2001; Lortie et al., 2004). On a global scale, these are climatic parameters related to temperature (e.g. frost resistance) and precipitation (Woodward, 1987; Woodward and

Williams 1987), on a regional and local scale dispersal (Zobel, 1997; Poschlod et al., 2013; Ozinga et al., 2009), and on a local scale light, soil physical and chemical parameters (Weiher et al., 1998; Lortie et al., 2004) and management (Kahmen et al., 2002; Poschlod and WallisDeVries, 2002; Römermann et al., 2009) Previous research in this field has often failed to consider the roles of different aspect of seed ecology in species coexistence, community assembly and explanation of distribution patterns of plant species (Poschlod et al., 2013). On a global scale, seed dispersal (Morin et al., 2008) and seed germination and dormancy (Tweddle et al., 2003; Walck et al., 2011) can help in understanding species distribution. Seed dispersal plays an important role on a regional scale as displayed by the dispersal-assembly model (Myers and Harms, 2009; Reid and Holl, 2012). This model views local communities as "open- membership" assemblages in which species pools, dispersal potential and immigration history influence community assembly (Myers and Harms, 2011). In addition, niche assembly models which view local communities as deterministic can be also explained by seed ecological traits since germination and establishment strongly depend on local habitat conditions and disturbance regimes (Fig. 2).

Seed dispersal not only has a role in dispersal assembly model, but also influences species coexistence in local community scale (Levine and Murrell, 2003). Concerning the role of biotic and abiotic factors on the local scale, seed ecological traits related to establishment can better explain species adaptation to certain habitat conditions. These traits are dormancy type, soil seed persistence, germination response to different soil properties such as soil moisture and also light and temperature variations as well as soil chemistry and physics (Poschlod et al., 2013). The germination response to certain environmental conditions acting as an ecological filter is often called the germination niche. Therefore, certain habitat conditions may prevent the occurrence of a species since they are not suitable at all for germination and establishment. Nevertheless, only few studies have described seed ecological traits for different habitats (Grime et al., 1981; Bakker et al., 1998a, Baskin and Baskin, 1998). These attempts to establish a link between seed ecological traits and species coexistence are at present not yet conclusive.

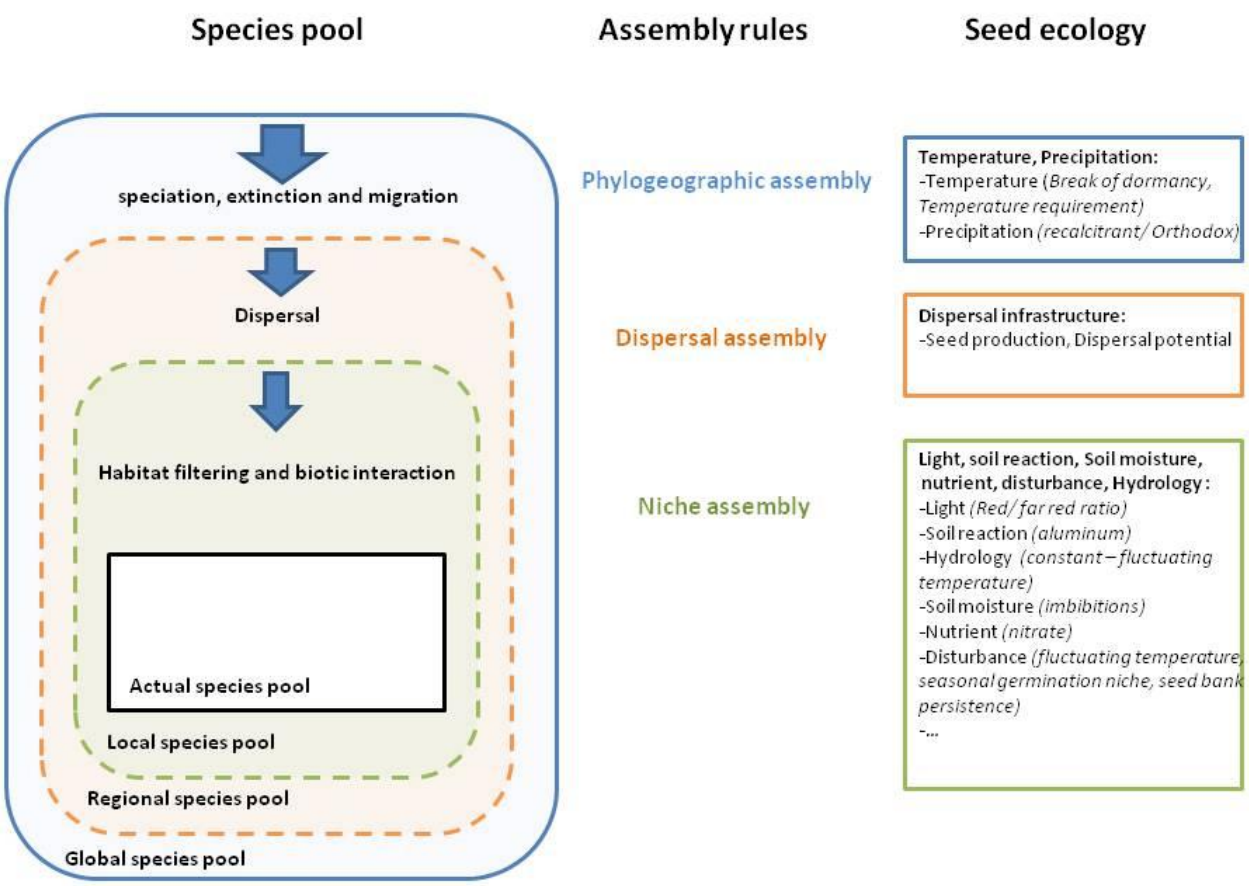


Figure 2 Seed ecological traits, species pool and community assembly rules (according to Poschlod et al., 2013). First, there is a global species pool that defines a regional species pool through the speciation, extinction and migration of species (phylogeographic assembly) with limiting seed temperatures and moisture requirement. At certain local site the plant community constitutes species from regional species pools that are able to successfully disperse there (Dispersal assembly). Finally, at the local scale, abiotic filtering and biotic interaction shape actual assemblage of plant species (niche assembly). Several abiotic factors such as moisture, light, soil reaction can limit species distribution in certain local habitat (Myers and Harms, 2011; Götzenberger et al., 2012; Poschlod et al., 2013).

Linkage to restoration and conservation

Seed ecology has an important role in conservation and restoration projects. Seed ecological data may help to plan restoration management (Bakker et al., 1996). Although several studies have attended to explained the relation between seed ecology and restoration projects (Clark et al., 2007; Bossuyt and Honnay, 2009), the relation between

different aspect of seed ecology and restoration ecology as system is not yet explained in a conceptual model.

Seed studies are not only important in ecological restoration, but ex situ seed conservation is also important for rare species conservation and "a tool" for preserving genetic diversity of plants. Successful re-establishment of extinct populations or establishing new populations of rare species may therefore depend on preserving seeds in gene banks (Khoury et al., 2010). Therefore, understanding seed persistence in dry storage can help to store seed for longer time in genebanks.

Thesis outline

To better understand mechanism of species coexistence and plant regeneration, it is necessary to consider seed (ecological) traits. Among different seed ecological traits, seed germination, dormancy and persistence have complicated mechanisms and strongly depend on local habitat conditions. Here we aimed at describing the seed ecological patterns and mechanisms and their linkage to community assembly and restoration, respectively in dry sandy grasslands.

Dry sandy grasslands occur throughout Central Europe including Southern Germany where sand was deposited, mostly during and after the last ice age. In Southern Germany dry sandy grasslands once covered large areas from the Rhine Valley in the west to Central Bavaria in the east. Sand was deposited either along rivers as terraces (Main, Naab, Regnitz, Rhine and others) or after wind-drift during the postglacial period or phases of intense land use (Upper Palatinate, Upper, Middle and Lower Franconia but also in the Rhine valley; Bork et al., 1998; Bateman and Godby, 2004). Depending on their geological origin and age, sand deposits range from being acidic (inland sand dunes) to slightly calcareous (river terraces). They host specific plant communities depending on soil pH (Korneck, 1978; Ellenberg, 1996; Mårtensson and Olsson, 2010) and disturbance regime such as grazing management (Ellenberg, 1996; Jentsch and Beyschlag, 2003; Poschlod et al., 2009). Grazing, mainly by sheep, was the dominant management on sandy deposits starting already in the Neolithic Age (Poschlod et al., 2009). It favoured either less palatable species or less competitive ones through disturbance by trampling (Jentsch, 2004, Poschlod et al., 2009).

The present study aims at elucidating various aspects in seed ecology of dry sandy grasslands. First, we wanted to show different seed ecological patterns in dormancy, germination and persistence related to habitat conditions. Second, we wanted to show if there are some seed ecological patterns, can any traits explain the mechanisms of these patterns. Third, we wanted to test if whether abiotic conditions limit species occurrence affecting seed germination and establishment. Finally, how we can apply seed ecology to applied restoration and conservation projects. Tab. 1 gives an overview about topics that presented in the individual chapters and how they are linked with basic seed ecological strategies.

Table 1 Seed ecological studies and their relation with our scientific research

Habitat conditions and seed ecological patterns	Ch.2 Germination and soil pH Ch.3 Seed longevity and soil moisture and properties. Ch.4 Seed persistence and light and temperature fluctuation
Seed patterns and their mechanisms	Ch.4 Seed persistence and seed ecological traits Ch.5 Seed ex situ longevity and seed ecological traits
Seed ecology and community assembly	Ch.2 Species coexistence and Aluminum toxicity Ch.1 community assembly Ch.6 Seed ecology and community assembly
Implication for restoration and conservation	Ch.6 Seed ecology and restoration planning

In Ch. 2 (Aluminium toxic effects on seedling root survival affect plant composition along soil reaction gradients) germination ecology and early root growth of dry sandy grasslands along a pH gradient simulated by different Aluminum concentrations were analysed. The aim of this study was to show the influence of abiotic filtering, in this case a pH gradient on germination and establishment and consequently on community assembly.

Different factors affect soil seed bank longevity (Fig. 3). In Ch. 3 (Soil moisture and soil types affect soil seed survival) the role of different habitat conditions (different soil types and soil moisture levels) on soil seed longevity was studied. The aim of this part was to show how soil seed bank longevity could be influenced by environmental factors.

In Ch. 4 (germination ecology and local assembly of dry sandy grasslands) we studied the germination ecology of dry sandy grassland species. Dormancy types, species reaction to light and darkness and constant and fluctuating temperatures were tested and their seed ecological patterns were described. Furthermore, to figure out the role of species specific factors in seed persistence (Fig. 3), the relation between seed persistence with germination traits and seed traits was analysed.

In Ch. 5 (Seed traits explain ex situ seed longevity) we tested if soil seed bank persistence is correlated to seed longevity under ex situ conditions. Different seed traits and seed germination traits were studied how they are correlated to ex-situ seed longevity.

Finally, the results of the previous chapters were reviewed with regard to their implications for community ecology and restoration practice (Ch. 6: Conclusion and perspectives).

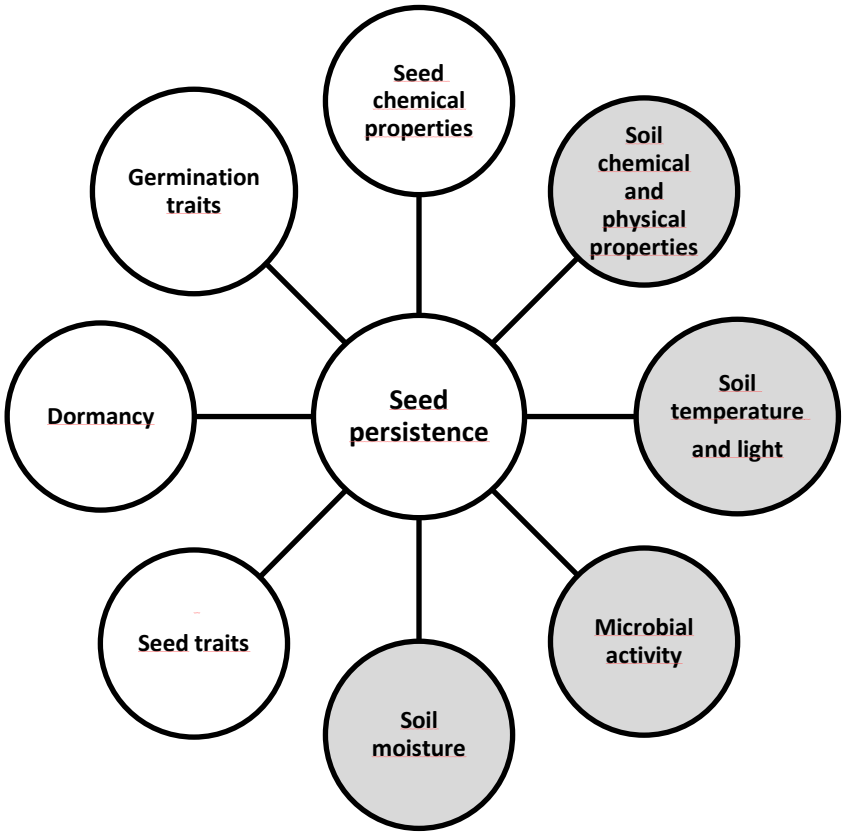


Figure 3 Seed persistence patterns influenced with different environmental factors (gray shading) explained in Ch. 3 and species specific factors (white shading) explained in Ch. 4.

Chapter 2

Aluminium toxic effects on seedling root survival affect plant composition along soil reaction gradients

Abstract

Aluminium (Al) toxicity is thought to be one of the most important factors restricting plant establishment on acidic soils, but its ecological significance for the occurrence of species along natural pH gradients is still under investigation. Are species occurring on acidic sandy soils less susceptible to Al toxic effects on germination and seedling root growth rate than species from calcareous sandy soils? How strong is the explanatory power of species' Al susceptibility for their occurrence along a pH-gradient, as represented by their Ellenberg indicator value (EIV) for soil reaction (R)? Can Al-tolerance of species be used as an independent trait to support Ellenberg's empirically derived reaction indicator values? Dry sandy grasslands in Southern Germany have soil reactions ranging from acidic to calcareous. We tested early seedling responses to different Al concentrations in 15 species from dry sandy grasslands. A filter paper-based system was used to germinate seeds under Al concentrations of up to 10 m*M*. Germination, absolute root growth and the length of the root hair zone were recorded seven and fourteen days after first germination. Al concentrations that reduced root growth by 50% or 95% (ED50 and ED95), respectively, were correlated with Ellenberg Indicator Values (EIV) for soil reaction. EIV was found to explain 66% of the variance in species' Al sensitivity. Tolerated Al concentrations resemble those concentrations the individual species are exposed to in their natural habitats. Among all soil factors varying with soil pH, Al is one of the strongest restrictions to species' occurrence in acidic soils. Al acts as an environmental filter by allowing only Al-tolerant seedlings to grow roots and establish. Al sensitivity is a measurable objective trait that could form a crucial physiological component in defining R-indicator values.

Introduction

Previous research in community ecology and assembly rules has often failed to consider the roles of different aspect of seed ecology in species coexistence, community assembly and explanation of distribution patterns of plant species (See Ch. 1).Aiming at a mechanism underlying species occurrence and distribution, we target Al-toxicity to evaluate in how far it can be named a principal environmental filter for species occurrence along a pH gradient in grassland ecosystems.

Species co-existence along ecological gradients

Understanding mechanisms of species occurrence and assembly enables ecologists to draw conclusions about environmental conditions prevailing at a site. Quantitative indicator systems, in particular ecological indicator values (Ellenberg et al., 1991), have been used extensively in different fields of applied vegetation ecology (Diekmann, 2003). These values assist the interpretation of species occurrence and performance along ecological gradients (Schaffers and Sýkora, 2000). Several validations of the EIV have shown their utility (Diekmann, 2003). Therefore, EIV are now widely accepted in European countries (Schaffers and Sýkora, 2000, Diekmann, 2003). However, only for Germany and Switzerland, EIV were specifically worked out and are available for all components of the vascular plant flora (Ellenberg et al., 1991; Landolt, 2010), for Germany even for bryophytes and lichens (Ellenberg et al., 1991). Despite this fact, EIV and habitat estimations based on them are rather often criticized for subjectivity and circularity (Diekmann, 2003).

EIVs have been validated only rarely. Even more rarely, the functional mechanisms behind a species' behavior were studied. However, both would strengthen the application of indicator values. The last approach would not only deepen our understanding of the mechanisms driving a species' occurrence, but could provide an independent trait for a species' ecological niche without any circular argumentation.

EIVs for soil reaction (compare Tab. 2) were validated for pH itself (Seidling and Rohner , 1993; Ertsen et al., 1998; Wamelink et al., 2002; Ewald, 2003), available calcium (Schaffers and Sýkora, 2000) and base saturation (Ewald, 2009). Bogner (1968),

Gigon (1971) and Gigon (1987) experimentally compared the reaction of acidophytes and calciphytes to ammonium and nitrate. Al concentration in soil was often mentioned as a relevant trait to understand the composition of plant communities along pH gradients (e.g. for mat-grass grasslands, Peppler (1992). Ewald (2009) showed that average reaction values were more closely related to base saturation than to pH, which points to the importance of Al saturation. For species originating from a variety of plant communities, Rode (1988) found higher Al tolerance in five species from acidic soils as compared to five species from weakly acidic to alkaline soils. However, data on Al tolerance of different species from a specific plant community was, to date, published in just one single study (Grime and Hodgson, 1969). This is a gap of knowledge, because it is well conceivable that the Al sensitivity of a species could be a useful functional trait to understand mechanisms of species' occurrence along a pH gradient.

Effects of pH

Profound and complex effects of soil pH on vegetation cause variable distribution of species in acidic and calcareous soils. With the exception of extremely acidic soils, even high concentrations of protons *per se* are not considered to be harmful to plant growth, but indirect effects are much more important. Soil acidity is associated with deficiencies of magnesium, calcium, molybdenum and phosphorus (Tyler, 1992; Lee, 1998) and at the same time with increased solubility of iron (Lindsay, 1984), manganese (Mahmoud and Grime, 1977) and Al (Rorison, 1960a; Clarkson, 1969). It is also associated with a predominance of ammonium (Bogner, 1968; Gigon and Rorison, 1972; Britto and Kronzucker, 2002). By contrast, calcareous soils are typically characterized by high concentration of Ca^{2+}, Mg^{2+} and HCO_3^- (Woolhouse, 1966), low iron, manganese and Al availability (Wallihan, 1961) and a predominance of nitrate (Gigon and Rorison, 1972; de Graaf et al., 1998; Van Den et al., 2011). The impacts of many of these factors on plant growth have been examined extensively. However, studies directly relating the impacts of these factors to the occurrence of species along a pH-gradient are rare except for the effect of ammonium and nitrate (Bogner, 1968; Gigon and Rorison, 1972). Al is one of the strongest factors restricting cultivability of certain crop species on acidic soils (Kochian et al. 2004; Haling et al., 2010; Zheng, 2010). We therefore hypothesize that Al can be identified as a major factor restricting species occurrence along natural pH gradients.

Here, we examine the Al-susceptibility of 15 calcicole to calcifuge species from Central European dry sandy grasslands.

Aluminum toxicity

Among different soil chemical properties, aluminum toxicity is known to be a major limiting factor on plant growth in acidic soils (Rout et al., 2001; Poschenrieder et al., 2008, Stevens et al., 2011). Root growth inhibition and changes to the entire root architecture are the primary symptoms of Al toxicity (Delhaize and Ryan, 1995; Kochian et al., 2004). Plants establishing under high concentrations of soluble Al usually develop shallower root systems, often leading to reduced utilization of mineral nutrients (Ahonen-Jonnarth et al., 2000) and water (Marschner, 2002).

The topic of Al sensitivity was intensely studied in crop plants (Kochian et al., 2004; Haling et al., 2010; Zheng, 2010), but not in wild plant species. Until now, only a few selected wild terrestrial plant species were studied with respect to their Al sensitivity (Rorison, 1960a, b; Grime and Hodgson, 1969) and no larger set of species from one substrate or habitat type along a pH gradient was, to our knowledge, examined so far.

Dry sandy grassland species can occur along different soil pH, from very acidic to calcareous. High surface temperatures, low water storage, low nutrient contents, low organic matter content and litter cover on sandy substrate are the other main characteristics of this habitat (Jentsch and Beyschlag, 2003). Therefore, germination and root growth rate of dry sandy grassland species from a gradient of very acidic to calcareous sandy soils and with different EIV were exposed to different Al availabilities. We tested the hypothesis that species exclusively occurring in calcareous (high pH) sandy grasslands are more sensitive to high Al concentrations than species from acidic sandy grasslands. We also aimed at evaluating the usefulness of species Al sensitivity as a trait to mechanistically understand the pronounced effect of soil reaction on the presence and absence of plant species. Thus, we tried to answer the following questions:

How strongly are exchangeable aluminium stocks influenced by soil pH on sandy soils?

Are species occurring on acidic sandy soils less susceptible to aluminium toxic effects on germination and seedling root growth rate than species from calcareous sandy soils?

How strong is the explanatory power of species' aluminium susceptibility for their occurrence along a pH-gradient, as represented by their Ellenberg indicator value (EIV) for soil reaction?

Material and Methods

Study region

Dry sandy grasslands occur throughout Central Europe including Southern Germany where sand was deposited, mostly during and after the last ice age. In Southern Germany dry sandy grasslands once covered large areas from Central Bavaria in the east to the Rhine Valley in the west. Sand was deposited either along rivers as terraces (Main, Naab, Regnitz, Rhine and others) or after wind-drift during the postglacial period or phases of intense land use (Upper Palatinate, Upper, Middle and Lower Franconia but also in the Rhine valley; Bork et al., 1998; Bateman and Godby, 2004). Depending on their geological origin and age, sand deposits range from being acidic (inland sand dunes) to slightly calcareous (river terraces). They host specific plant communities depending on soil pH (Korneck, 1978; Ellenberg, 1996; Mårtensson and Olsson, 2010) and disturbance regime such as grazing management (Ellenberg, 1996; Jentsch and Beyschlag, 2003; Poschlod et al., 2009). Grazing, mainly by sheep, was the dominant management on sandy deposits starting already in the Neolithic Age (Poschlod et al., 2009). It favoured either less palatable species or less competitive ones through disturbance by trampling (Jentsch, 2004, Poschlod et al., 2009).

Soil sampling and analysis

To assess the correlation between soil pH and exchangeable aluminum in sandy soils we sampled eight different sites (Tab. 3) of dry sandy grasslands in Southern Germany ranging from acidic (min. pH 4.0) to neutral (max. pH 7.2). Each site is represented by eight individual samples. These were taken by pooling ca. 15 regularly distributed subsamples (Pürckhauer-cores, 2 cm diameter, 0 – 15 cm soil depth) from 2 x 2 m plots. This reflects the horizon where around 90% of the root biomass may be found and which

is consequently crucial for this study (Schenk and Jackson, 2002; Bartelheimer et al., 2006). For pH measurement 25 ml of $CaCl_2$ solution (0.01 *M*) was added to 10 g of dried soil, shaken repeatedly and measured by a pH-meter (multi 340i [WTW GMBH, Weilheim, Germany]).

Exchangeable Al from soil samples was extracted by adding 25 ml of KCl solution (1*M*) to 12 g of dried soil, shaking for 30 min and leaving to sediment for 30 min. After filtering the solution, 125 ml of KCl solution (1*M*) was added and Al concentration was measured by an Inductively Coupled Plasma Spectrometer (ICP-OES, JY-70plus [Jobin-Yvon, Longjumeau, France]).

Table 2 Description of Ellenberg Indicator Values for soil reaction (translated from Ellenberg et al. 2001).

EIV	Description
1	Indicator of strong acidity, never occurring on weak acidic to alkaline soils
2	between 1 and 3
3	Indicator of acidity, main distribution on acidic soils, exceptionally expanding into neutral conditions
4	between 3 and 5
5	Indicator of moderate acidity, rarely occurring on strongly acidic or on neutral to alkaline soils
6	between 5 and 7
7	Indicator of weak acidity to weakly alkaline conditions, never on strongly acidic soils
8	between 7 and 9
9	Indicator of alkaline and calcareous conditions, only on calcareous soils

Table 3 Overview of the study sites, where soil was sampled in sandy grasslands for the analysis of aluminium content. pH values given are means ± SE for n = 8.

Sites	pH ($CaCl_2$)		
	Mean ± SE	Min	Max
Siegenburg (Upper Palatinate, Bavaria)	4.4 ± 0.10	4.0	4.8
Astheim at Volkach (Lower Franconia, Bavaria)	4.6 ± 0.15	4.2	5.6
Bodenwöhr (Upper Palatinate, Bavaria)	4.4 ± 0.05	4.1	4.5
Kornburg, Nuremberg (Central Franconia, Bavaria)	5.5 ± 0.13	4.8	5.8
Elgersheimer Hof at Volkach (Lower Franconia, Bavaria)	5.0 ± 0,07	4.8	5.4
Hallstadt at Bamberg (Lower Franconia, Bavaria)	4.9 ± 0.12	4.4	5.3
Sandhausen South (Karlsruhe, Baden-Wuerttemberg)	6.8 ± 0.07	6.5	7.0
Sandhausen North (Karlsruhe, Baden-Wuerttemberg)	7.1 ± 0.01	7.0	7.2

Study design

We tested susceptibility of different species from sandy grassland towards Al by germinating them on different concentrations of $AlCl_3$. Species were selected based on their EIV for soil reaction (R, Tab. 2; Ellenberg et al., 1991). 15 species were selected that represent typical and very common species of dry sandy grasslands according to the phytosociological classification of South German vegetation (Korneck, 1978). Where possible, Ellenberg indicator values (EIV) for soil reaction from 1 (growing on very acidic soils) to 8 (growing on calcareous soils) were represented by two species each (Tab. 4). No species with the indicator value 9 for soil reaction is occurring in the dry sandy grasslands of the study region. Seeds were mostly collected in the same grasslands, where soil chemistry was studied (Tab. 4).

Only species with seed weight below 1mg were selected for this experiment (compare Tab. 4), because 1 mg is often viewed as a critical seed weight above which seedling establishment is largely supported by seed reserves (Schütz, 2000). Larger seeded species were not included in order to keep the species choice more homogeneous in that respect. Seeds were not pretreated except for *T. arvense*, which was scarified by use of sand paper.

A filter-paper-based system was used to germinate seeds and to cultivate seedlings in order to identify Al concentrations affecting different species (compare Tamas et al., 2006). In petri dishes 20 seeds were germinated on two 90-mm-diameter filter paper discs (Sartorius 3 hw). Filter papers were saturated with 4 ml solution. Al concentration of these solutions was varied in 10 steps from 0mM (control) to 10m*M* (0µ*M*, 10µ*M*, 100µ*M*, 500µ*M*, 1m*M*, 2m*M*, 3m*M*, 4m*M*, 5m*M*, 10m*M*). Due to the acidifying effect of Al cations, all solutions containing Al had acidic pH values with stepwise reduction from pH 5.2 (in 10µM) over 4.3 (in 1mM) to pH 4.06 (in 10mM). An additional test on the species *Verbascum lychnitis* (R=7), *Arenaria serpyllifolia* (R=7) and *Helichrysum arenarium* (R=5) comparing control treatments (pure water) to an acidic treatments (pure water with pH adjusted to 4.06) revealed no differences in root growth parameters ($p>0.05$ in T-test for n=5, unpublished data).

In order to prevent evaporation, petri dishes were tightly sealed with an impermeable parafilm. Number of replicates was five per species and Al concentration. We used $AlCl_3$

to achieve different Al concentrations. Unintended effects of chloride can be excluded, as according to Greenway and Munns (1980) concentrations like those occurring in the above experimental setup are non-toxic to all but 'the very sensitive non-halophytes'. Moreover, additional experiments with six species from Tab.4, varying $CaCl_2$ in a filter paper based system as described above showed that root parameters remained unaffected at Cl^--concentrations exceeding 30 m*M* (unpublished data).

Table 4 Overview of study species with EIV and respective locations of seed collections.

Species	EIV	Origin of seeds
Teesdalia nudicaulis (L.)R. BR	1	Neusath (Upper Palatinate, Bavaria)
Trifolium arvense L.	2	Ramsberg (Middle Franconia, Bavaria)
Deschampsia flexuosa (L.) TRIN	2	Siegenburg (Upper Palatinate, Bavaria)
Corynephorus canescens (L.) P. B.	3	Siegenburg (Upper Palatinate, Bavaria)
Jasione montana L.	3	Kirchheim/Ries (Swabia, Bavaria)
Vulpia bromoides (L.) S. F. GRAY	4	Bad Kissingen (Lower Franconia, Bavaria)
Arabidopsis thaliana (L.) HEYNH.	4	Hallstadt at Bamberg (Lower Franconia, Bavaria)
Petrorhagia prolifera (L.) P. W. BALL & HEYW.	5	Kornburg, Nuremberg (Central Franconia, Bavaria)
Helichrysum arenarium (L.) MOENCH	5	Schwetzingen (Karlsruhe, Baden-Wuerttemberg)
Armeria maritima ssp. *elongata (HOFFM.) BONNIER*	6	Kornburg, Nuremberg (Central Franconia, Bavaria)
Cerastium semidecandrum L.	6	Hallstadt at Bamberg (Lower Franconia, Bavaria)
Arenaria serpyllifolia L.	7	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Verbascum lychnitis L.	7	Kornburg, Nuremberg (Central Franconia, Bavaria)
Koeleria glauca (SPR.) DC.	8	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Erigeron acris L.	8	Sandhausen (Karlsruhe, Baden-Wuerttemberg)

Germination and root growth

Dishes were placed in a climate chamber (day/night cycle 12 h/12 h; temperature 22°C/14°C). Germination and absolute root growth (ARG) as well as length of the root hair zone (LRHZ) were studied during two weeks (day 3, 7 and 14, where day 0 was defined as the day when >5% of the seeds in the control treatment had started to germinate). To determine seed germination, a seed was considered to have germinated if the radicle had protruded at least 1mm. Final germination percentage was calculated per petri dish (n = 5). Root lengths were measured by use of a binocular with ocular scale in day 3 and 7 and by ruler on day 14 (also see Appendix S1 for an illustration of root reactions to Al). The length of the root hair zone (LRHZ) was measured in day 14, only. ARG was calculated as the difference between root length values obtained in day 14 and day 3.

Statistical analysis

Germination percentage data per petri dish was analyzed using SAS 9.2 statistical software (SAS, Cary North Carolina, USA). Data was checked for ANOVA assumptions (normality checked by Kolmogorov–Smirnov-test, homogeneity of variance checked by Bartlett's test) and no deviations from ANOVA assumptions were detected. Significant ANOVA results were followed by Duncan's Multiple Range Test for multiple comparisons. Effective doses (ED) of Al were calculated for ARG or LRHZ, respectively, where ED50 values mark 50% reduction and ED95 values mark 95% reduction. ED values were calculated using regression analysis (four-parameter loglogistic dose-response model) in R statistical software (drc add-on package) (Ritz and Streibig, 2005; R Foundation for Statistical Computing 2006). Furthermore, non-linear regression was carried out (using SigmaPlot 2000 for Windows (SPSS Inc., Chicago, IL)) to estimate the relation between soil pH and soluble aluminum in soil as well as the relation between EIV and the ED50 and ED95 values for each, ARG and LHRZ.

The results of non-linear regressions using EIV are regarded as reliable, considering that these indicator values were conceived by Ellenberg as quasi-metric data (Ellenberg, 1991) and have been extensively used as such (Diekmann, 2003; Käfer and Witte, 2004).

Validation of the regression results

To validate the regression results of EIV and the critical concentration for each, ARG and LHRZ we used an independent data set from the literature (Grime and Hodgson, 1969) and re-analysed the data by correlating the results to EIV.

Results

Relation between soil pH and soluble Al contents

Sandy soils, exemplified by samples from eight different sites in Southern Germany, showed a close correlation between pH and extractable aluminum concentration in the soil solute (non-linear regression, R^2=0.81; $p<0.001$). While no differences in aluminum

content were found between pH 4.9 and 7 (typical values of around 0.3 m*M*), there was an exponential increase in extractable aluminum with decreasing soil pH, with typical Al-concentrations of around 1.5 m*M* between pH 4.5 and 4.9, and around 4.5 m*M* below pH 4.5 (Fig. 4).

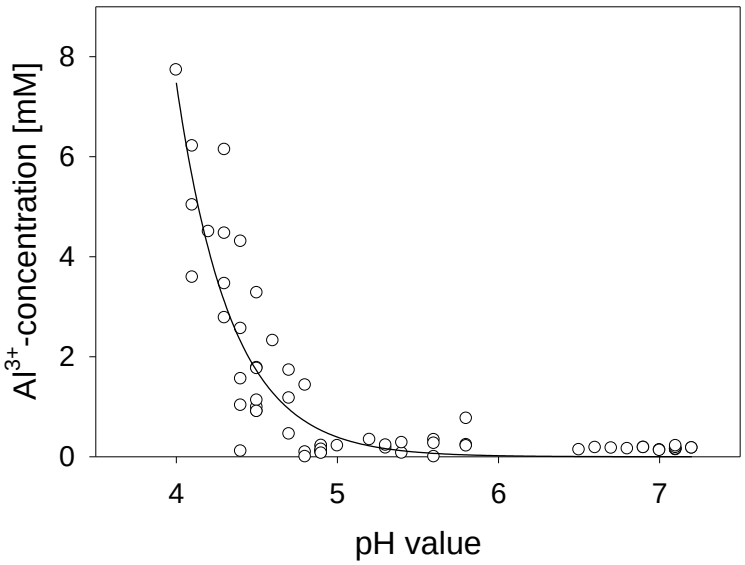


Figure 4 Non-linear regression of pH-value and content in exchangeable aluminium in sandy soils from eight different sites of dry sandy grasslands in Southern Germany ranging from acidic (min. pH 4.0) to neutral (max. pH 7.2); the fitted curve follows an exponential decay fit (two parameters) with R2=0.81; p<0.001, N=64. Individual dots represent soils from 2 x 2 m relevees (eight from each site).

Germination

In seven out of fifteen species (*Teesdalia nudicaulis, Corynephorus canescens, Petrorhagia prolifera, Helichrysum arenarium, Arenaria serpyllifolia, Verbascum lychnitis, Erigeron acris*) germination was not significantly affected by Al-concentration and was high in all treatments (Tab. 5). In the remaining eight species germination at high concentrations (10 m*M*) was reduced in comparison to the control or to the concentration where maximum germination occurred, respectively. Maximum values were in many species reached at Al-concentrations higher than zero, presumably due to positive ion effects at non-toxic Al concentrations. Species comparisons in ED50 or ED95 values could not be carried out, here, because with the exception of three species, germination was not reduced by 50% along the Al concentration gradient. Across species, no relation between EIV and germination response to Al was detectable.

Table 5 Effect of different aluminum concentrations on germination of 15 species from dry sandy grassland (mean percentage values ± SE for n = 5). Asterisks indicate significant ANOVA results with ***: p < 0.001; **: p < 0.01; *: p < 0.05 (ns: not significant). Dissimilar letters indicate significant differences (p < 0.05) within species among treatments in post-hoc Duncan's Multiple Range Test.

Species	Sig	control	0.01mM	0.1mM	0.5mM	1mM	2mM	3mM	4mM	5mM	10mM
T. nudicaulis	ns	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
T. arvense	***	99 ± 1.0 ^{ab}	97 ± 2.0 ^{ab}	100 ^{a}	98 ± 1.2 ^{ab}	98 ± 2.0 ^{ab}	86.2 ± 1.1 ^{b}	70 ± 6.1 ^{c}	53 ± 4.3 ^{d}	24 ± 5.1 ^{e}	7 ± 2.0 ^{f}
D. flexuosa	***	34 ± 3.3 ^{cd}	55 ± 5.7 ^{a}	44 ± 2.9 ^{abc}	44 ± 5.3 ^{abc}	49 ± 5.1 ^{ab}	52.5 ± 2.8 ^{ab}	40 ± 3.1 ^{bc}	28 ± 3.7 ^{ed}	10 ± 2.2 ^{f}	19 ± 1.8 ^{ef}
C. canescens	ns	53 ± 6.0 ^{ab}	60 ± 2.7 ^{ab}	63 ± 7.1 ^{ab}	64 ± 5.7 ^{ab}	63.7 ± 7.6 ^{a}	68 ± 3.7 ^{a}	60 ± 4.7 ^{ab}	52 ± 4.1 ^{ab}	59 ± 2.1 ^{ab}	47 ± 6.0 ^{b}
J. montana	***	98 ± 1.2 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	90 ± 1.8 ^{b}	94 ± 2.9 ^{a}	99 ± 1.0 ^{a}	98 ± 2.0 ^{a}	100 ^{a}
V. bromoides	***	81 ± 3.6 ^{a}	77 ± 4.0 ^{a}	73 ± 6.0 ^{a}	80 ± 4.1 ^{a}	71 ± 6.2 ^{a}	45 ± 5.1 ^{b}	35 ± 1.5 ^{b}	45 ± 5.7 ^{b}	33 ± 4.6 ^{b}	0 ^{c}
A. thaliana	**	75 ± 4.1 ^{abcd}	96 ± 2.4 ^{a}	84 ± 3.6 ^{abc}	89 ± 7.1 ^{ab}	88 ± 4.6 ^{ab}	77.5 ± 6.9 ^{bcd}	66 ± 7.9 ^{cd}	59 ± 1.0 ^{d}	77 ± 12.1 ^{abcd}	60 ± 6.2 ^{d}
P. prolifera	ns	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	99 ± 0.2 ^{a}	99 ± 0.2 ^{a}
H. arenarium	ns	82 ± 2.3 ^{ab}	85 ± 1.0 ^{ab}	85 ± 1.4 ^{ab}	72 ± 2.7 ^{b}	80 ± 2.1 ^{ab}	81.2± 0.5 ^{ab}	87 ± 1.5 ^{a}	81 ± 1.1 ^{ab}	78 ± 1.0 ^{ab}	74 ± 2.6 ^{b}
A.a elongata	*	87 ± 3.3 ^{a}	74 ± 4.8 ^{ab}	71 ± 6.4 ^{abc}	73 ± 5.8 ^{ab}	73 ± 4.2 ^{ab}	56 ± 8.1 ^{bc}	60 ± 8.8 ^{bc}	5 ± 6.5 ^{c}	63 ± 7.1 ^{bc}	66 ± 6.4 ^{abc}
C. semidecandrum	**	98 ± 2.0 ^{a}	95 ± 2.7 ^{a}	88 ± 2.0 ^{b}	93 ± 3.0 ^{a}	89 ± 3.6 ^{ab}	96 ± 2.9 ^{a}	95 ± 2.2 ^{a}	96 ± 1.0 ^{a}	100 ^{a}	98 ± 2.0 ^{a}
A. serpyllifolia	ns	99 ± 1.0 ^{a}	95 ± 1.5 ^{ab}	98 ± 1.2 ^{ab}	96 ± 1.8 ^{ab}	98.7 ± 1.1 ^{a}	97 ± 1.2 ^{ab}	91 ± 4.0 ^{b}	94 ± 2.9 ^{ab}	97 ± 2.0 ^{ab}	92 ± 3.0 ^{ab}
V. lychnitis	ns	97 ± 2.0 ^{a}	97 ± 1.2 ^{a}	89 ± 2.4 ^{a}	96 ± 1.8 ^{a}	90 ± 2.7 ^{a}	97.5 ± 1.2 ^{a}	95 ± 2.7 ^{a}	95 ± 1.5 ^{a}	98 ± 1.2 ^{a}	99 ± 1.0 ^{a}
K. glauca	***	92 ± 0.2 ^{a}	88 ± 0.9 ^{a}	93 ± 0.6 ^{a}	95 ± 0.5 ^{a}	87 ± 0.6 ^{a}	87.5 ± 0.5 ^{ab}	75 ± 1.0 ^{c}	76 ± 0.7 ^{bc}	69 ± 0.7 ^{c}	57 ± 0.8 ^{d}
E. acris	ns	88 ± 5.1 ^{a}	89 ± 1.1 ^{a}	89 ± 2.7 ^{a}	89 ± 2.7 ^{a}	84 ± 9.0 ^{a}	88 ± 1.9 ^{a}	87 ± 1.5 ^{a}	85 ± 2.5 ^{a}	84 ± 1.1 ^{a}	76 ± 2.7 ^{a}

Root Reactions and effective Al doses

Al had clear effects on root growth and root morphology in all species, though the respective concentrations of Al causing these effects differed between species. Typical symptoms like swollen and brown-colored root tips were observed along with stunted or curled lateral roots. ARG and LRHZ were reduced by Al and the derived ED50 and ED95 values varied strongly between species (Tab. 6, Fig. 5). Regression analyses of EIV and ED values revealed that EIV explained a considerable percentage of the variation in ED50 on LRHZ (R^2=0.46, p=0.0056, Fig. 5a), while the regression for EIV and ED50 on ARG was not significant (p=0.0835) (Fig. 5b). 42% of the variation in ED95 on LRHZ was explained by EIV (R^2=0.42, p=0.0092, Fig. 5c). EIV had the strongest explanatory power for ED95 on ARG (R^2=0.66, p=0.0003, Fig. 5d).

The corresponding regression curve (Fig. 5d) follows the form of a simple power function (two parameters) and has remarkable similarity to the curve in Fig. 4. It is deducible that species with EIVs greater than 3 (i.e. species that usually occur on max. moderately acidic soils, never on strongly acidic soils) are unable maintain more than 5% of their potential root elongation rate in Al concentrations higher than 3m*M*. Species with

EIVs smaller than 4 (indicators of acidity, compare Tab. 2) can tolerate considerably higher concentrations.

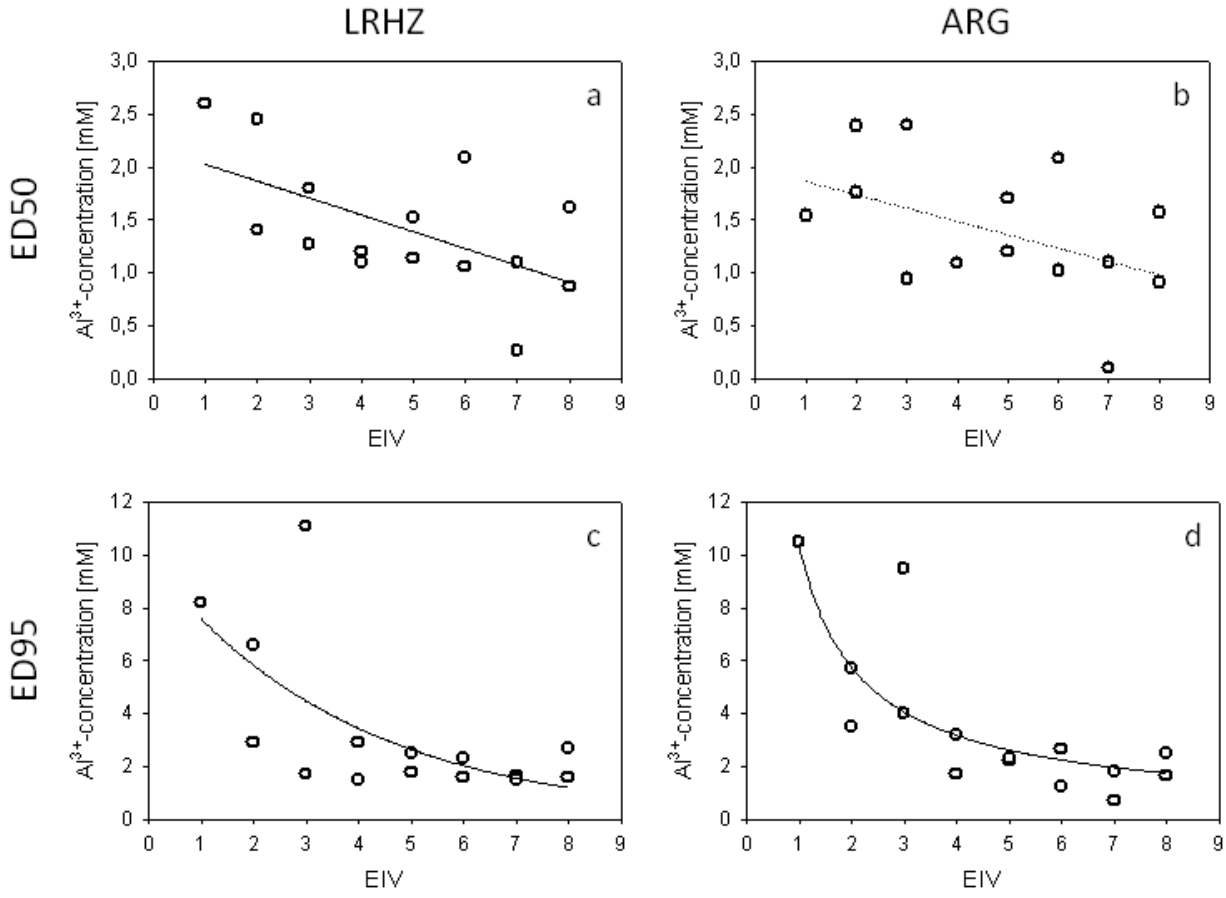


Figure 5 Regressions for species' EIV and effective doses (ED) of aluminium impacting on the length of the root hair zone (LRHZ) or absolute root growth (ARG), respectively. ED50 and ED95 are Al-concentrations that reduce root growth parameters by 50% or 95%, respectively. Regression lines derive from linear and non-linear curve-fitting including N=15 species and represent the best fit in each respective case. a: ED50 for LRHZ; linear regression (2 parameter); R2=0.458; P=0.0056. b: ED50 for ARG; linear regression (2 parameter); R2=0.213; P = 0.0835; note that at EIV=4 two dots are stacked. c: ED95 for LRHZ; the regression follows the form of an exponential decay function (2 parameter); R2=0.418; P=0.0092. d: ED95 for ARG; the regression follows the form of a power function (2 parameter); R2=0.655; P=0.0003; note that at EIV=5 two dots are stacked.

To validate the correlation of pH with EIV in our dataset we reanalyzed data available from literature (compare Grime and Hodgson, 1969). These data showed the same fit between EIV and species' Al-tolerance (Fig. 6). Variance in Al-tolerance (represented by the Al-concentration required for 50% inhibition of root growth in hydroponic culture)

was even better explained by EIV ($R^2 = 0.82$; $p < 0.001$). Once more, the hyperbolic shape of the curve points to a steep rise in Al-tolerance in species with EIV<4.

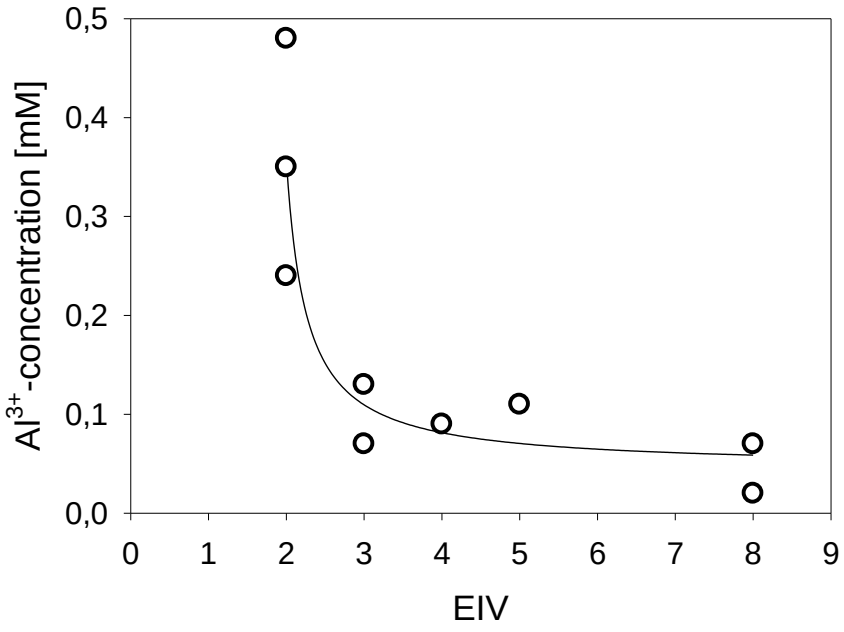


Figure 6 Regression for EIV and Al-tolerance, reanalyzed after Grime and Hodgson 1969. Displayed Al concentrations inhibited seedling root growth by 50%. Note that from the original data species with EIV = x (indifferent species) were omitted from the analysis. The non-linear regression follows the form of a hyperbolic curve (two parameters) with R2=0.82; p=0.0008.

Table 6 Effects of different aluminium concentrations on absolute root growth (ARG) and length of the root hair zone (LRHZ) of 15 species from dry sandy grassland. ED50 and ED95 are effective doses of Al that reduce root growth parameters by 50% or 95%, respectively (mean ± SE).

Species	ED50		ED95	
	ARG	***LRHZ***	***ARG***	***LRHZ***
Teesdalia nudicaulis	1.5 ± 0.4	2.6 ± 0.1	10.5± 6.5	8.2 ± 2.5
Trifolium arvense	1.8 ± 0.1	1.4 ± 0.1	3.5 ± 0.4	2.9 ± 0.4
Deschampsia flexuosa	2.4 ± 0.3	2.5 ± 0.4	5.7 ± 2.6	6.6 ± 4.0
Corynephorus canescens	2.4 ± 0.6	1.8 ± 0.4	9.5 ± 7.0	11. ± 7.5
Jasione montana	1.0 ± 0.1	1.3 ± 0.4	4.0 ± 2.4	1.7 ± 1.2
Vulpia bromoides	1.1 ± 0.1	1.2 ± 0.1	3.2 ± 0.6	2.9 ± 0.8
Arabidopsis thaliana	1.1 ± 0.1	1.1 ± 0.1	1.7 ± 0.6	1.5 ± 0.8
Petrorhagia prolifera	1.7 ± 0.4	1.5 ± 0.1	2.3 ± 0.8	2.5 ± 0.2
Helichrysum arenarium	1.2 ± 0.1	1.1 ± 0.1	2.2 ± 0.6	1.8 ± 0.9
Armeria elongata	2.1 ± 0.1	2.1 ± 0.2	2.7 ± 1.2	2.3 ± 1.3
Cerastium semidecandrum	1.0 ± 0.2	1.1 ± 0.1	1.3 ± 0.8	1.6 ± 1.1
Arenaria serpyllifolia	0.1 ± 0.1	0.3 ± 0.0	0.7 ± 0.6	1.7 ± 0.6
Verbascum lychnitis	1.1 ± 0.1	1.1 ± 0.2	1.8 ± 0.9	1.5 ± 1.1
Koeleria glauca	1.6 ± 0.3	1.6 ± 0.1	2.5 ± 1.2	2.7 ± 0.6
Erigeron acris	0.1 ± 0.0	0.9 ± 0.0	1.7 ± 0.3	1.6 ± 0.2

Discussion

Species Al-tolerance correlates with EIV

We found that Al had strong impacts on radicle development. Critical Al concentrations for the formation of a root hair zone and root growth are highly species-specific (Tab. 6). The development of the fine root zone as well as root growth in seedlings is among the most vital processes during seedling establishment. This in turn is one of the most crucial stages in a plant's life cycle (Grubb, 1977, Harper, 1977).

Tolerance of Al (i.e. their ED95 for ARG, Fig. 5d) differs considerably between species with different requirements regarding soil pH: species from calcareous to slightly acidic soils can only tolerate Al concentrations below 3m*M*, mostly only below 2m*M* (Fig. 5d). In species with EIVs of ≤4, i.e. in species from acidic soils, tolerance increases in an exponential fashion and thereby reaches critical concentrations of up to 10.5m*M* (Fig. 5d). 66% of the variation in species Al-tolerance is explained by the estimated species' optimum along the pH-gradient, represented by EIV (Fig. 5d). Species occurrence is not primarily constrained by the Al concentration at which root growth is significantly reduced, but by the level where it is entirely suppressed. Hence, Al seems to act as a sieve permitting or preventing survival rather than as a gradual inhibiting factor.

In fact, concentrations of exchangeable Al found under natural conditions correspond well to ED95 values measured here. We found a close correlation between soil pH and exchangeable Al in sandy soils (R^2=0.82). Below pH 4.9 the Al concentration rises in an exponential fashion, from max. 0.77 m*M* to up to 7.7 m*M*. It is striking that the shape of this curve and, with caution, also the range of Al concentrations much resemble the regression between EIV and ED95 on ARG (Fig. 5d). Judging from this pattern, there appears to be a close correspondence between the amount of exchangeable Al a species encounters in its natural habitat and the amount the species can tolerate when the young seedling's root system develops. This interesting point needs to be interpreted with caution, because there is still debate on the degree to which exchangeable Al (as measured in this study) is available to plant (Blume et al., 2011, Stahr pers. comm.). On the one

hand, Fischer and Lorenz (2011) measured Al concentrations in soil solutions in a long-term forest monitoring program in Bavaria and found concentrations to be about one order of magnitude lower than the critical levels in our germination experiments (Raspe pers. comm.). On the other hand, exchange mechanisms of plant roots (Hinsinger et al., 2003) may lead to locally and temporally higher concentrations in the rhizosphere, especially close to the root, necessitating physiological adaptation. Extractable and soluble Al have been found to increase in a similar fashion with decreasing pH (Tyler, 1996). It could therefore be argued that if different species can indeed tolerate Al concentrations of an order of magnitude higher than what they are usually exposed to in the soil solute, there is still strong correspondence to exchangeable Al. This might indicate that a plant needs to be tolerant to concentrations exceeding the usual (i.e. they need a tolerance buffer). Soluble Al in sandy soils was measured in at least one further study (Mulder et al., 1987, Scheffer et al., 2002), where the same exponential increase was found, while concentrations did not typically exceed 3mM in acidic soil pH. Even this amount of Al would have been lethal for any species with an R indicator value > 4.

The close correspondence of EIV and ED95 suggests that Al toxicity exerts a strong selection pressure. Increased Al-tolerance is only found in species typical of acidic soils, where Al concentrations are high. Plants invest considerable amounts of carbon to physiological mechanisms conferring Al-tolerance (especially exudation of organic acids, Ma et al., 2001; Conyers et al., 2005; Trejo-Téllez, 2010). Thus, an evolutionary tradeoff may be postulated for species along the pH gradient. Either, cost intensive tolerance mechanisms are evolved, allowing survival on acidic soils, or resources are invested otherwise, thus precluding the species from acidic soils. Interestingly, in contrast to many other environmental factors impacting on plants, mycorrhiza appears to have only limited influence on Al-sensitivity. Our filter paper-based array used seedlings without inoculum of arbuscular mycorrhiza (AM) and yielded a pretty clear result. What is more, Göransson et al., (2008) found that species from acidic soils had lower AM-colonization rates than species from less acidic soils, so that a prominent role of AM in protecting roots from Al is unlikely.

Al as an environmental filter in acidic soils

It was thus far recognized that Al is one important factor among others that vary with soil pH and account for species distribution. Especially for agricultural systems it is known as one of the strongest factors restricting the cultivability of certain crop species on acidic soils (e.g. Kochian et al., 2004; Haling et al., 2010; Zheng, 2010). What is new is that (i) this applies to wild species (66% of the variation in species EIV explained by Al toxicity) and (ii) Al tolerance permits or prevents root development, which is a direct prerequisite for survival. To a high extent and especially on neutral to acidic soils it defines which species can still occur at a certain soil-pH and which species cannot. With respect to the model of environmental filters (Woodward, 1987; Weiher and Keddy, 2001 and others), we therefore suggest that on acidic mineral soils Al acts as an environmental filter. Only species with high physiological tolerance to Al can maintain root growth and manage to persist through the seedling stage at the respective site. As with other non-resource stresses, it can be argued that for a plant to be able to establish at a certain site, either Al-concentration has to be low or the plant's tolerance has to be high. It is only when these conditions are given that resource availabilities and competition for resources come into play (compare Diaz et al., 1999).

The presented findings are important for plant ecologists, since they are still struggling with the mechanisms of how plant species manage to coexist, despite the fact that they all require the same set of basic resources (e.g. Silvertown, 2004). With respect to plant distribution at the acid end of the soil-pH gradient, the non-resource stress Al plays an important role by allowing only tolerant species to develop roots and to survive. Conversely, low seedling survival in non-tolerant species will have implications for all life stages in a population including the seed bank, and consequently this will impact on species occurrence in the long run (Grubb, 1977).

While Al-toxicity and differences in species tolerance explain well survival on neutral to strongly acidic soils it does not explain species' occurrence along the calcareous part of the soil-pH gradient. These lie beyond the scope of this study. A tradeoff between the ability to tolerate factors on calcareous soils and those on acidic soils is obvious (Tyler, 2003), since most species are rather specialized in their pH-requirement (Ellenberg et al.,

1991). Calcifuge species are thought to be unable to efficiently utilize the forms of different nutrients prevailing in calcareous soils and to suffer from Iron-, Phosphorus- and Zink-deficiency as well as from Calcium-toxicity (Gigon, 1987; Lee, 1998; Zohlen and Tyler, 2000; Marschner, 2002; Lambers et al., 2008).

Re-evaluation of existing data

The re-analysis of literature data from English grassland species (Grime and Hodgson, 1969) was carried out in order to analyze a second data set for a correlation between Ellenberg R-value and Al-tolerance. The high regression coefficient found here corroborates our finding that a species' occurrence along a pH-gradient strongly depends on its Al-tolerance (Fig. 6). Though methods differed to ours, the principal outcome is the same: variation in Al-tolerance is largely explained by the occurrence along the pH-gradient ($R^2 = 0.82$). Grime and Hodgson (1969) used hydroponic culture while we used the filter paper method. The filter paper method is arguably more comparable to conditions in natural soils (Tamas et al., 2006), e.g. because exuded organic acids remain in the system and close to the root, and because roots are allowed to direct new lateral roots away from the solute. This explains why Grime and Hodgson (1969) found comparatively low concentrations to impair root growth. Still, methodological differences aside, both data sets have in common their strong explanatory power and the shape of the fitted curve (Figs. 5 and 6). The re-analysis of literature data therefore strengthens our point that Al-tolerance is one of the strongest factors determining species occurrence along the pH-gradient, especially in acidic soils, and is suitable to support the re-evaluation of Ellenberg's R-indicator values.

Re-evaluating Ellenberg R-values

EIVs have been used extensively in different fields of applied vegetation ecology, but they are also often criticized for subjectivity and circularity (Diekmann, 2003). Judging from our results, Al-tolerance of species might have a potential to improve conclusions drawn from Ellenberg R-values by offering additional measurable ED95 values. It has to be stressed that EIVs represent assessments of ecological optima (Ellenberg et al., 1991),

while Al-tolerance values rather indicate upper limits of what a species can tolerate. The steps that would need to be carried out to come to an applicable system would be to first prove that the correlation of Al-tolerance and EIV holds for diverse other habitats as well. We propose the use of our filter paper based system, because it is a low-cost and straightforward method. The experimental effort might be even reduced by measuring root biomass instead of ARG and LRHZ. The next step would be to determine ED95 values for all desired species individually. This task would be very laborious but possibly worthwhile in the long run, since in addition to EIV-based analysis of habitats it would allow accessing the importance of Al-toxicity. However, it should be not forgotten that the occurrence of a species along a pH gradient depends also on other factors such as the form of available nitrogen, Fe availability or physical properties of the soil (see Introduction).

Conclusions

Al-tolerance and survival along the pH-gradient in grasslands are closely coupled and finely tuned. Tolerated Al-concentrations correspond to the concentrations the respective species are exposed to under natural conditions. This offers a mechanistic explanation for species distribution along the pH gradient. Al-tolerance itself appears to be defined by the radicle's ability to maintain at least a minimum of growth and of root hairs to sustain the establishing seedling. In acidic sandy soils Al toxicity is so strong that it appears to acts as an environmental filter that allows only the Al-tolerant species to occur. Furthermore, the confirmation of the usefulness of EIV by the experimental validation shows that there are objectively measurable traits available to underpin and re-evaluate subjectively derived indicator values. At the same time, and this may be even more important, it demonstrates that the power of expert judgements of ecological requirements of plants, in this case based on Ellenberg's careful observation in the field, should not be underestimated.

Chapter 3

Soil moisture but not soil type limit soil seed survival - a comparative study in three *Rumex* species

Abstract

Seed persistence has an important role in restoration ecology and population dynamics, but mechanisms of soil seed longevity are still under investigation. Environmental factors as well as species specific factors influence soil seed survival of certain species. We tested seed survival of three *Rumex* species in different moisture levels and soil types. Two water table depth adjusted with rainwater inside water basin and one treatment outside the water basin were intended to simulate wet, intermediate, and dry soil conditions, respectively. Seed Germination patterns and seed viability of the examined *Rumex* species were tested both before the onset of the experiment (untreated seeds) and after burial for 6, 12, or 18 months, respectively. Seed germination and soil chemical properties were also measured. We found that soil moisture is, after species-specific attributes, arguably the second strongest factor in the context of seed longevity in the soil. Environmental factors may also explain variation of soil seed persistence of a certain species in data bases. Soil moisture acts as a limiting factor for species from dry habitats, but it does increase soil seed survival for species from wetter habitats. Soil moisture and it's interaction with other environmental factors should be considered in the term of seed survival in different habitats.

Introduction

Persistence of seeds in the soil is an important parameter in the life history of plants driving population dynamics and also "a tool" for restoring populations and habitats (Bakker et al., 1996; Bossuyt and Honnay., 2009; Saatkamp et al., 2011b). Long term establishment of species regenerating from seeds may even depend strongly on soil seed survival under specific habitat conditions (Poschlod et al., 2013). In addition, for successful management of weedy species, seed bank dynamics should be considered.

However, the understanding of the mechanisms of seed longevity in the soil is still limited.

Seeds can survive in the soil seed bank up to several decades or even hundreds of years. Thompson et al., (1997) developed a method for soil seed bank classification and also introduced the Longevity Index to classify seed persistence in the soil. Despite the fact that species have a different Longevity Index (Kleyer et al., 2008), soil seed bank databases give different soil seed bank types even within a certain species (Thompson et al., 1997). Most species, even some which are commonly classified as transient soil seed bank types, can be persistent in the soil under certain circumstances (Thompson et al., 1997; Saatkamp et al., 2009). Different classifications for one and the same species could potentially be due to at least two non-exclusive reasons. On the one hand, this might be caused by methodological reasons (outlined below). On the other hand, this could well be due to seeds responding differently in their longevity to different environmental conditions. As far as the methodological reasons are concerned, differences between "seed persistence" assessed by the so called 'seedling emergence method' and "seed survival" assessed with methods of seed burial are the main source of variations in species seed bank types (Saatkamp et al., 2009). Seedling emergence method is an indirect method which exposes soil samples to 'favourable' conditions for germination in order to identify and count seedlings. Also, in this method level of dormancy (Thompson et al., 2003), low seed production mainly for rare species (Thompson and Grime, 1979), vertical distribution and succession (Bekker et al., 1998b; Espinar et al., 2005; Erfanzadeh et al., 2010) and also soil seed bank sampling time (Saatkamp et al., 2009) can create different seed bank types for certain species. As far as responses to different habitat conditions are concerned, soil seed banks in plant communities with different environmental conditions have been shown to have specific species compositions (Bekker et al. 1998a, Poschlod et al., 2013). This might suggest that different species' seed survival is affected differently impacted by certain soil conditions. Yet, functional explanations on how such conditions affect not only vegetation but also soil seed bank persistence are still lacking. Therefore, it is necessary to demonstrate and understand the role of environmental factors in soil seed survival (Saatkamp et al., 2009).

Effect of Species- specific attributes and environmental factors

Seed longevity in the soil may be affected by different parameters. First, there may be species – specific attributes effects. Seed traits such as size or seed coat thickness and also seed germination traits may influence the longevity of the soil seed bank. These traits maybe variable within one species as well (Thompson et al., 1993; Bekker et al., 1998b; Thompson et al., 2003; Gardarin et al., 2010; Saatkamp et al., 2011b). Second, environmental conditions may strongly affect the longevity of the soil seed bank. Soil seed longevity is associated with soil microbial activity (Leishman et al., 2001; Schafer and Kotanen, 2003; O'Hanlon-Manners and Kotanen, 2006; Wagner and Mitschunas, 2008; Dalling et al., 2011) and at the same time with soil properties (Long et al., 2009; Pakeman et al., 2012), soil nutrients (Bekker et al., 1998c; Davis, 2007), soil temperature (Akinola et al., 1998) and soil moisture and hypoxia (Murdoch and Ellis, 2000; Voesenek and Blom, 1992; Bekker et al., 1998d; Nicol et al., 2003 ; Webb et al., 2006). The influence of single environmental factors on seed longevity has been examined separately already several times, not only in the field, but also under controlled greenhouse conditions. However, nearly no experiment has studied a combination of environmental factors. Therefore, the results of previous studies cannot explain the interaction between environmental factors on seed longevity. Furthermore, so far no study exists that examines the effect of standardized moisture and hypoxia condition in seed burial experiments.

Among environmental factors, soil moisture and also soil physical and chemical properties can strongly define habitat conditions. Therefore, soil seed survival of three *Rumex* species with different assessments of seed bank classification according to the Soil Seed Bank Database of the Northwest European Flora (Thompson et al., 1997), BioPop (Poschlod et al., 2003) and LEDA (Kleyer et al., 2008) were studied. Soil seed bank persistence data of *Rumex acetosa* and *R. acetosella* and *R. maritimus* contained entries for transient, short-term and long-term persistent. We aimed at evaluating changes of seed longevity in their own habitat and under different environmental conditions to understand the role of soil moisture and soil types in soil seed bank longevity patterns. Thus, we asked the following questions:

Can soil and moisture conditions explain why *Rumex* species have different seed bank categories in respective data bases?

How strongly is the longevity of soil seed banks affected by soil moisture and soil types?

Are species occurring in dry habitats more sensitive to water logging conditions than species from wet habitats, and vice versa?

Material and Methods

Experimental strategy and choice of species

A pot experiment with different soil types and different moisture levels was used to bury seeds of three *Rumex*-species native to different habitats. Seed viability was tested 6, 12, and 18 months after burial to test for species differences in reactions to soil type and moisture.

In this study three *Rumex* species (*Rumex acetosa, Rumex acetosella, and Rumex maritimus*) with narrow habitat ranges concerning soil type and moisture were chosen. *R. maritimus* is a common species for wet and muddy soils of mudflat communities in amphibious habitats such as river banks and ponds (Oberdorfer, 2001). *R. acetosella* occurs on dry and sandy soils of *Corynephorus canescens*- or *Armeria elongata*- grassland communities in grazed (inland) dune habitats. *R. acetosa* grows on mesic and loamy soils in *Arrhentherum elatius*- or *Alopecurus pratensis*-meadows. Ripe fruits of each species were collected in the respective communities at three different localities in Bavaria in summer 2009 (see Tab.7).

Table 7 Habitat descriptions of localities, where seeds and soils were collected.

Species	Locality	Plant community (Union)	Soil type	Soil moisture
Rumex acetosella	Siegenburg	Corynephorion	Sand	Dry
Rumex acetosa	Regensburg	Arrhenatherion	Loam	Moderately moist
Rumex maritimus	Charlottenhof	Bidention	Mud	Wet

Experimental setup

This study was set up as an outdoor experiment in the facilities of the Botanical Garden of Regensburg (DE) that provides rain-water-filled basins (148 cm* 128 cm) that allow the adjustment of a constant water level. The set up consisted of a total of 216 pots (5 liter) representing 8 replicates x 3 soil types x 3 water levels x 3 time steps for seed excavation. Each pot contained seeds of the three study species (see below for details). A block design was set up, where each of eight water basins represented one block containing one replicate of each treatment. Different water table depths (WTD), and consequently different soil moistures (compare Tab. 9), were established by placing the pots on metal grids which were adjusted in different depth below the water surface, i.e. pots were placed either on a deep or a shallow grid or on the walkway directly adjacent the respective basin. On each of a block's different water levels pots with different soil types were distributed in a modified latin-square pattern. All pots were placed adjacent to other pots or to the walls of the water basins so that direct sunlight could reach the soil surface but not the faces of the pots. We used 5 L plastic pots (18.6×18.6×20 cm l/w/h) that contained a weed block fabric at the bottom as well as 2.5 cm of sand to facilitate water flow. Pots were filled (leaving 1cm brim at the top) with either of three soil types (sand, mud, loam), which were collected in the respective habitats of the examined *Rumex*-species (Tab.7). Soils were not sterilized in order to retain their (micro-) biological characteristics. In each pot seeds of all three *Rumex*-species were buried at 5 cm depth, where ambient light and fluctuating temperature have been found to have no impact on germination (Van Assche et al., 2002). In detail, each pot received 25 seeds per species. Seeds were contained in small sewn nylon bags (5×6 cm, one bag per species, compare Saatkamp et al., 2011b) produced of 0.2 mm nylon mesh fabric (Bückmann GmbH, Mönchengladbach, DE).

WTD were adjusted with rainwater to either 1 cm above the seed position ('high WTD'), or to 10 cm below seed position ('intermediate WTD'), or to entirely drained ('low WTD' treatment, positioned outside the water basins and were intended to simulate wet, intermediate, and dry soil conditions, respectively. Oomes et al., (1997) showed that water level 5 cm below the seed position creates anaerobic conditions. Therefore, high WTD in our study indicates also anaerobic conditions. The experiment was set up after seed ripening of all species in autumn 2009. The time steps of seed retrieval took place on

April 15th 2010 (six months after the onset of the experiment), October 15th 2010 (12 month) and April 15th 2011 (18 month).

Germination tests

Germination patterns and seed viability of the examined *Rumex* species were tested both before the onset of the experiment (untreated seeds) and after burial for 6, 12, or 18 months, respectively. For testing the untreated seeds, 25 seeds were placed on two 90-mm-diameter filter paper discs (Sartorius 3 hw) in petri dishes (n=8 per species). After filter papers were saturated with deionized water, dishes were placed in a climate chamber. Germination was tested in diurnally fluctuated temperature (DFT) (day/night cycle 14 h/10 h; temperature 22°C/14°C) in both light/dark alteration and in constantly dark conditions which have been shown as suitable germination conditions for all species. Germinated seeds were counted during 45 days. For the identification of germination, a seed was considered to have germinated, if the radicle had protruded at least 1 mm. After 45 days viability of non-germinated seed was checked with a Tetrazolium test. Seeds were assessed as viable when both, embryo and endosperm were coloured red (ISTA, 1996). For testing seeds after burial treatments, they were sterilized in their nylon bags for two minutes with sodium-hypochlorite (5 %) and were removed from the bags to petri dishes for germination test. Methods were identical to those applied to seeds before burial (see above), except for no constant dark treatment was included. In addition, we ran a test to account for possible physiological dormancy. Therefore, imbibed non-germinated seeds were stratified for 6 weeks at 4°C and germinated seeds were counted in similar climate chamber during 45 days afterwards.

Soil analysis

Soil moisture was recorded regularly (20 times in regular intervals, n= 5) during the vegetation period 2010 with moisture meter (Theta Probe ML2x, Delta-T Devices Ltd, UK). Soil chemistry was analyzed when first samples were exhumed in 15 April 2010.

pH and conductivity were measured on spot with the help of a Multikit, WTW Multi 340i Set (WTW GMBH, Weilheim, Germany) using the probe WTW Tetracon 325 (for conductivity) and WTW Sentix 41-3 (for pH). Mg, Na, K were measured by an atomic absorption spectrometer SOLAAR M5 [ThermoElemental, Franklin, MA]). Phosphate was measured with Spectronic UV1 Spectrophotometer (Thermo Spectronic, Rochester, NY). N and C were measured with a C/N analyzer Vario EL (Elementar Analysentechnik GmbH, Germany).

Statistics

Effects of 'soil type', 'WTD', 'duration of burial' and 'species' on seed viability (whole data set and split by *Rumex*-species, Tabs. 11 and 12) were analyzed with factorial ANOVAs in generalized linear mixed model (GLMM). GLMM was carried out using SAS statistical software (SAS, Cary North Carolina, USA). Data were checked for ANOVA assumptions (homogeneity of variance checked by Bartlett's test, normality checked by Kolmogorov-Smirnov-test). No deviations from homogeneity of variance assumptions were detected. The assumption of normality was not fulfilled, which is unproblematic because of the large enough sample size (Kleinbaum et al., 2007; Fitzmaurice et al., 2004).

Viability data for each species after six, twelve and eighteen month after burial were analyzed with a non-parametric test separately. Differences in seed viability were compared with Kruskal-Wallis tests combined with multiple Mann-Whitney U-tests with Bonferroni correction (Figs. 7-9). Non-parametric test were used because normality and homogeneity of variance assumptions were not fulfilled in these cases.

Results

Germination of untreated seeds and seed bank characteristics

Rumex species had different germination patterns. Germination rate of *R. acetosa* and *R. acetosella* seeds after collection was higher when seeds were incubated in the presence of light as compared to darkness. In contrast, *R. acetosa* germinated in darkness as well as light treatment. Concerning the longevity index according to the seed bank database of the Northwest European flora (Kleyer et al., 2008), it is rather low in *R. acetosa* whereas it is higher in the other species (Tab. 8).

Table 8 Overview of seed germination traits and seed traits of the study species prior to burial treatments. Seed bank types are 1: transient, 2: short term persistent and 3: long term persistent. Germination values are mean percentages ± SE.

Seed germination				Seed Traits and soil seed bank types			
Species	(22/14) Light	(22/14) Dark	Viability	Seed shape Index[1]	Seed mass[1]	Seed bank Types[2]	Longevity Index[1]
R. acetosella	37 ± 2.1	3 ±1.9	92 ± 0.5	0.03	0.37	1,2,3	0.51
R. acetosa	97 ± 0.4	80 ±0.7	97 ± 0.4	0.06	0.55	1,2,3	0.14
R. maritimus	99 ± 0.2	0	99 ± 0.2	0.05	0.44	1,2,3	0.58

1 (according to Kleyer et al., 2008); 2 (according to Thompson et al., 1997; Kleyer et al., 2008)

Soil analysis

The three soils used in this experiment had different physical and chemical properties and soil moisture contents as well. There was no distinct chemical difference between loamy and muddy soils. Both contained more nutrients, including potassium, magnesium, sodium, and phosphate, but muddy soil had higher conductivity and lower pH. By contrast, sandy soil contained less nutrients than the other soil types (Tab. 9). Measurement of soil moisture(m^3 m^3) during study showed clear difference between soil types along a WTD. The sandy soil had the lowest and the muddy soil had the highest soil moisture. Soils in low WTD had much higher soil moisture variation compared to others (Tab. 10)

Table 9 Chemical properties of the three used soil types: sand, loam, mud. Soil was sampled from the experiment 6 month after its onset when first samples were exhumed. BDL= below detection limit. C /N was not calculated because no available data for C and N.

Chemical and physical properties	Sand			Loam			Mud		
WTD	Low	Inter-mediate	High	Low	Inter-mediate	High	Low	Inter-mediate	High
pH with water	7.2 ± 0.2	6.2 ± 0.1	6.0 ± 0.0	6.1 ± 0.1	6.6 ± 0.1	6.7 ± 0.0	4.2 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
pH with $cacl_2$	5.7 ± 0.0	5.6 ± 0.0	5.7 ± 0.0	5.9 ± 0.1	6.1 ± 0.1	6.36 ± 0.0	4.2 ± 0.3	3.9 ± 0.1	3.9 ± 0.1
Conductivity (dS m^{-1})	4.7 ± 2.0	7.7 ± 3.6	5.7 ± 2.4	90 ± 42.5	26.3 ± 9.2	64 ± 33.8	493 ± 159.3	482 ± 324.0	244.7 ± 187.4
Phosphate (mg kg^{-1})	BDL	BDL	BDL	10.4 ± 0.8	12.3 ± 7.8	5.8 ± 0.6	2.6 ± 0.8	16.9 ± 10.8	2.2 ± 2.1
K^+ (mg kg^{-1})	21.2 ± 1.5	20.5 ± 0.5	20.8 ± 0.5	186.8 ± 13.8	172.5 ± 8.8	184.2 ± 14.4	43.3 ± 3.6	47.9 ± 1.4	51.1 ± 5.1
Na^+ (mg kg^{-1})	0.1 ± 0.0	0.07 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.10 ± 0.0	0.1 ± 0.0	0.1 ± 0.01
Mg^{+2} (mg kg^{-1})	9.2 ± 0.5	10.0 ± 1.2	9.6 ± 0.5	51.0 ± 0.0	52.5 ± 1.1	51.7 ± 0.4	12.2 ± 1.0	13.7 ± 0.6	14.0 ± 1.5
C (mg kg^{-1})	BDL	BDL	BDL	5.0 ± 0.3	4.7 ± 0.2	5.2 ± 0.3	3.4 ± 1.0	4.6 ± 0.8	3.7 ± 0.7
N (mg kg^{-1})	BDL	BDL	BDL	0.4 ± 0.0	0.3 ± 0.02	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
C/N	BDL	BDL	BDL	13.9 ± 0.9	15.5 ± 0.1	14.5 ± 0.3	11.9 ± 0.3	12.2 ± 0.4	11.5 ± 0.1

Table 10 Soil moisture (m^3 m^3) of the three soil types: sand, loam, mud. Soil was measured 20 times in regular intervals during the vegetation period 2010.

	Sand			Loam			Mud		
	dry	moist	wet	dry	Moist	wet	dry	moist	wet
Min	0.03 ± 0.01	0.35± 0.01	0.38 ± 0.00	0.07 ± 0.00	0.38 ± 0.00	0.46 ± 0.00	0.09 ± 0.02	0.43± 0.01	0.52± 0.01
Max	0.23 ± 0.02	0.47 ± 0.01	0.54 ± 0.00	0.32 ± 0.01	0.51± 0.01	0.63 ± 0.01	0.36 ± 0.02	0.65 ± 0.02	0.79 ± 0.04
Average	0.13 ± 0.01	0.40 ± 0.00	0.45 ± 0.00	0.20 ± 0.02	0.43 ± 0.00	0.54 ± 0.01	0.24 ± 0.02	0.51± 0.01	0.66 ± 0.02

Effects of soil and WTD

The multi-way ANOVA results reveal that all main factors ('species', 'WTD', 'soil type', 'duration of burial') and their interactions had significant effects on soil seed longevity. According to their high F-values, factors that significantly influenced seed longevity were 'species', 'duration of burial' and 'species × duration of burial' and 'species × WTD'. Although 'soil type' had a significant effect on viability, the effect was less important in comparison to other factors (Tab. 11). 'Species' is the main factor affecting seed survival (F = 3855.86, Tab. 11) followed by 'duration of burial' (F = 254.20, Tab. 11) and 'species × duration of burial' (F= 107.97, Tab. 11).

Table 11 Results of multi-way ANOVA for effects of soil type, WTD, duration of burial and species on seed viability.

Factors	*d. f*	*F value*	*P value*
Species	2	3855.86	**< 0.001**
WTD	2	34.58	**< 0.001**
Soil Type	2	13.46	**< 0.001**
Duration of Burial	2	254.20	**< 0.001**
Species × WTD	4	79.82	**< 0.001**
Species × Soil Type	4	9.72	**< 0.001**
Species × Duration of Burial	4	107.97	**< 0.001**
WTD × Soil Type	4	16.87	**< 0.001**
WTD × Duration of Burial	4	26.99	**< 0.001**
Soil Type × Duration of Burial	4	5.91	**< 0.001**
Species × WTD × Soil Type	8	7.74	**< 0.001**
Species × WTD × Duration of Burial	8	9.76	**< 0.001**
Species × Soil Type × Duration of Burial	8	6.31	**< 0.001**
WTD × Soil Type × Duration of Burial	8	5.46	**< 0.001**
Species × WTD × Soil × Duration of Burial	16	4.21	**< 0.001**

Since the multifactorial ANOVA with ‘species’ as a factor merely gives information about species–specific aspects of seed survival and also on differences of species seed longevity but not about how the factors ‘WTD’ and ‘soil type’ differ between species, subsequent multi-way ANOVA analysis on individual *Rumex*-species were carried out to address the influence of environmental factors on the species level (Tab. 12). Seed viability values varied strongly between species and, as an overall assessment, were reduced along the moisture gradient from dry to wet conditions (Fig. 7, Fig. 8 and Fig. 9). Viability of *R. acetosella* seeds was strongly affected by ‘duration of burial’ (F= 289.63, Tab. 12) and ‘WTD’ (F = 122.52, Tab. 12). Seed viability was slightly reduced along the moisture gradient after 6 months (Fig. 7a), but showed a clear and significant decline especially in wet loam and wet mud after 12 and after 18 months of burial (Fig. 7b, c). Dry muddy soil maintained the highest proportion of viable seed (Fig. 7c). In *R. acetosa* ‘duration of burial’ (F= 80.35, Tab. 12) and ‘WTD’ (F= 27.75, Tab. 12) and their interactions had strong effect on soil seed viability. After six month of burial *R. acetosa* showed a significant increase in seed viability (Fig. 8a) with soil moisture. However, viability strongly decreased with duration of burial. Whereas after twelve months viable seeds were still found (Fig. 8b), there were nearly no viable seeds left after eighteen

months in all conditions (Fig. 8c). Time of burial (F= 80.35, Tab. 12) and moisture (F= 27.75, Tab. 12) and their interactions had the main effect on soil seed viability. In the case of *R. maritimus*, 'durationof burial' was not significant (Tab. 12) and seed viability was generally higher than 80% in all treatments and time steps (Fig. 9), which shows that seeds were highly persistent under different soil and moisture conditions. Only 'soil type' (F = 15.32, Tab. 12) and interaction of 'soil type' x 'WTD' (F = 3.57, Tab. 12) had a significant effect on seed survival, which mainly shows up in muddy soil in the high water level treatment, where viability as compared with the other treatments is reduced (Fig. 9).

Table 12 Results from multi-way ANOVA performed individually for the three *Rumex* species for effects of soil type, WTD, duration of burial and species on seed viability. (Bold values indicate statistical significance).

Species	Factors	*d.f.*	*F value*	*P value*
R. maritimus	WTD	2	1.75	0.177
	Soil Type	2	15.32	**< 0.001**
	Duration of Burial	2	0.71	0.494
	WTD × Soil Type	4	3.57	**< 0.001**
	WTD × Duration of Burial	4	0.08	0.988
	Soil Type × Duration of Burial	4	0.36	0.838
	WTD × Soil Type × Duration of Burial	8	0.92	0.505
R. acetosella	WTD	2	122.52	**< 0.001**
	Soil Type	2	12.04	**< 0.001**
	Duration of Burial	2	289.63	**< 0.001**
	WTD × Soil Type	4	19.61	**< 0.001**
	WTD × Duration of Burial	4	19.73	**< 0.001**
	Soil Type × Duration of Burial	4	12.46	**< 0.001**
	WTD × Soil × Duration of Burial	8	8.29	**< 0.001**
R. acetosa	WTD	2	27.75	**< 0.001**
	Soil Type	2	1.66	0.193
	Duration of Burial	2	80.35	**< 0.001**
	WTD × Soil Type	4	1.56	0.186
	WTD × Duration of Burial	4	28.45	**< 0.001**
	Soil Type× Duration of Burial	4	0.89	0.474
	WTD × Soil Type × Duration of Burial	8	1.53	0.150

Figure 7 Viability values of *R. acetosella* seeds. Values are means ± SE. Different letters denote significant (P < 0.05) differences between means (Kruskal-Wallis combined with Mann-Whitney U-test with Bonferroni correction. (A, 6 month after burial; B, 12 month after burial; C, 18 month after burial and ns indicate non significant differences in Kruskal wallis test).

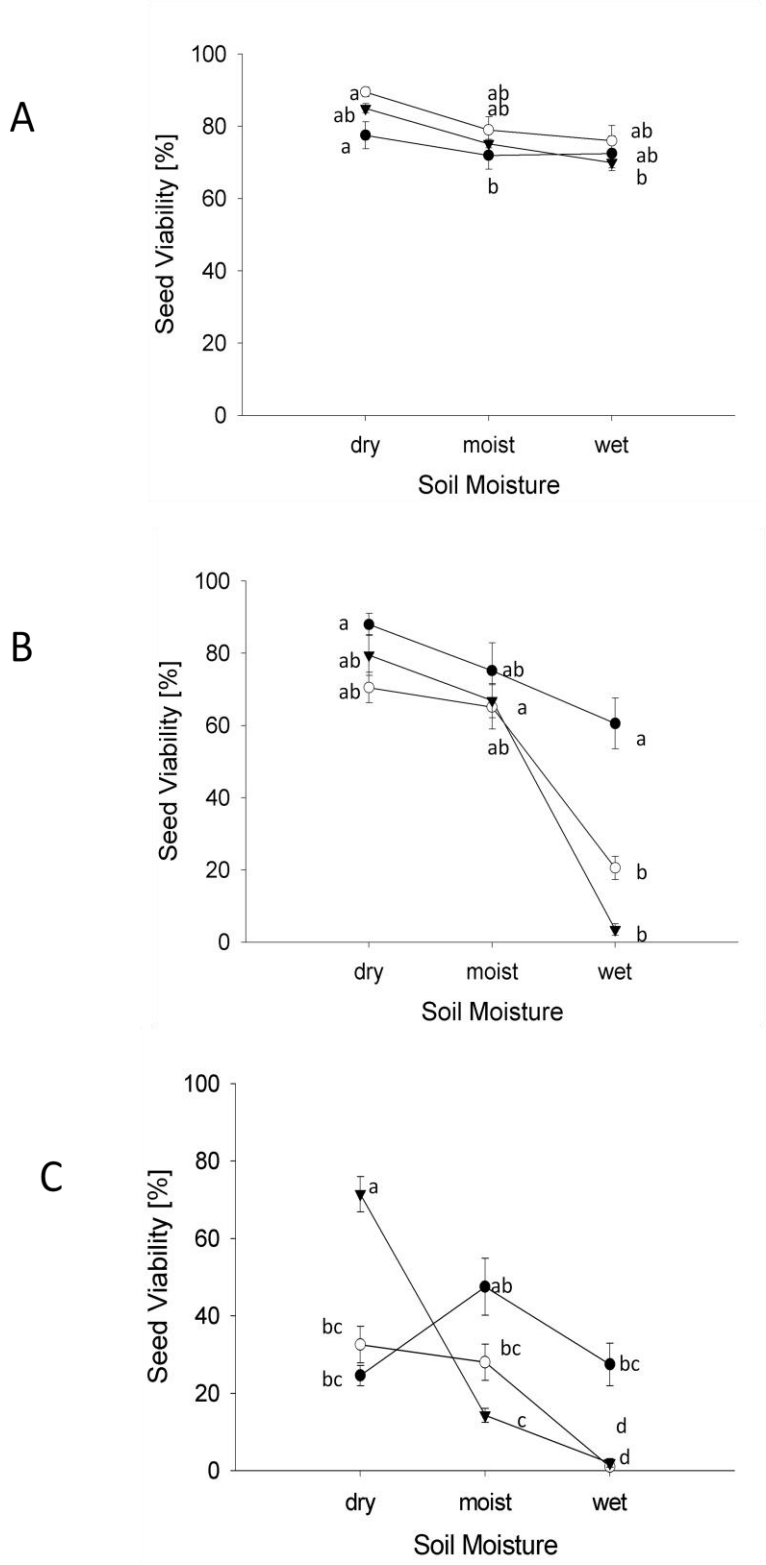


Figure 8 Viability values of *R. acetosa* seeds. Values are means ± SE. Different letters denote significant (P < 0.05) differences between means (Kruskal-Wallis combined with Mann-Whitney U-test with Bonferroni correction. A, 6 month after burial; B, 12 month after burial; C, 18 month after burial and ns indicate non significant differences in Kruskal wallis test).

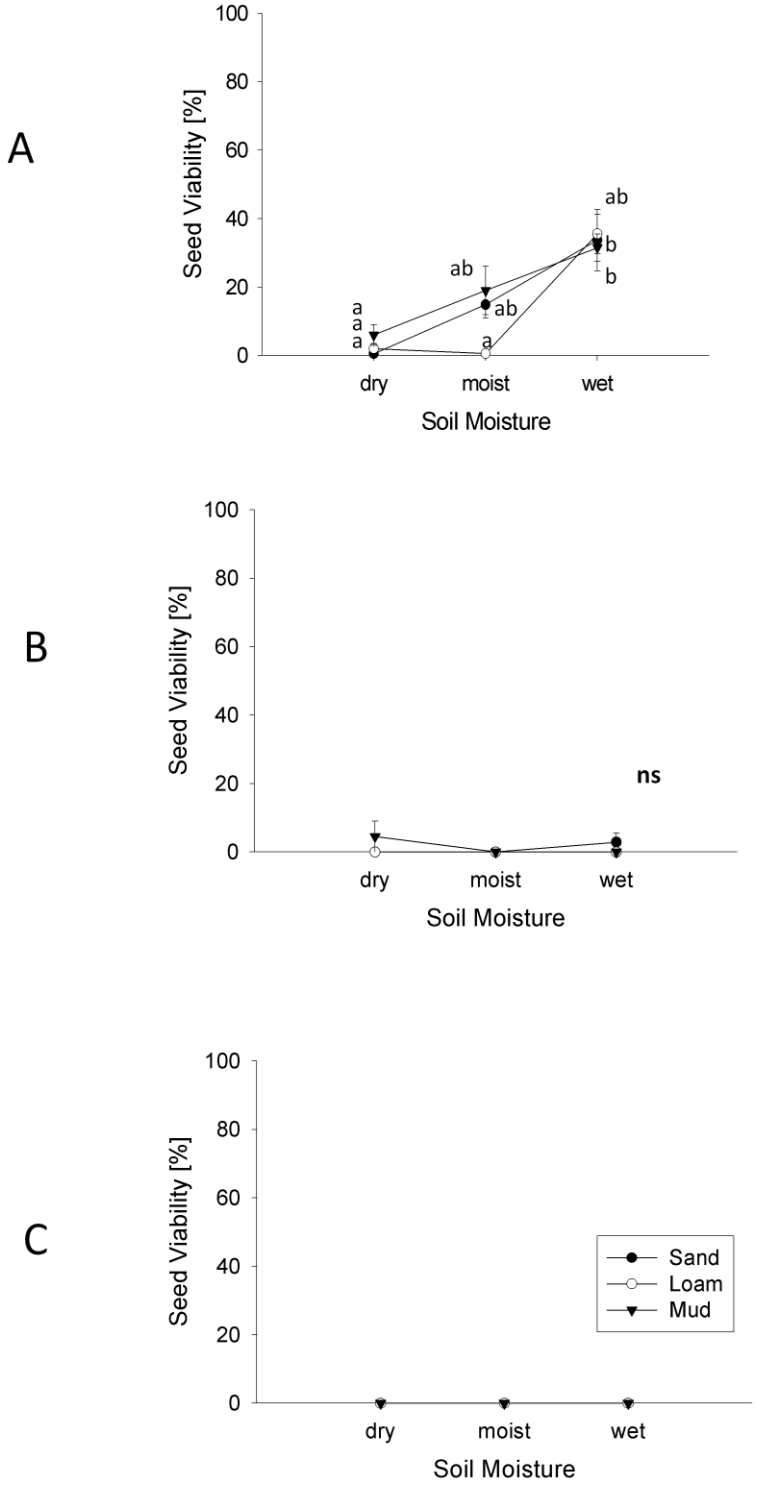
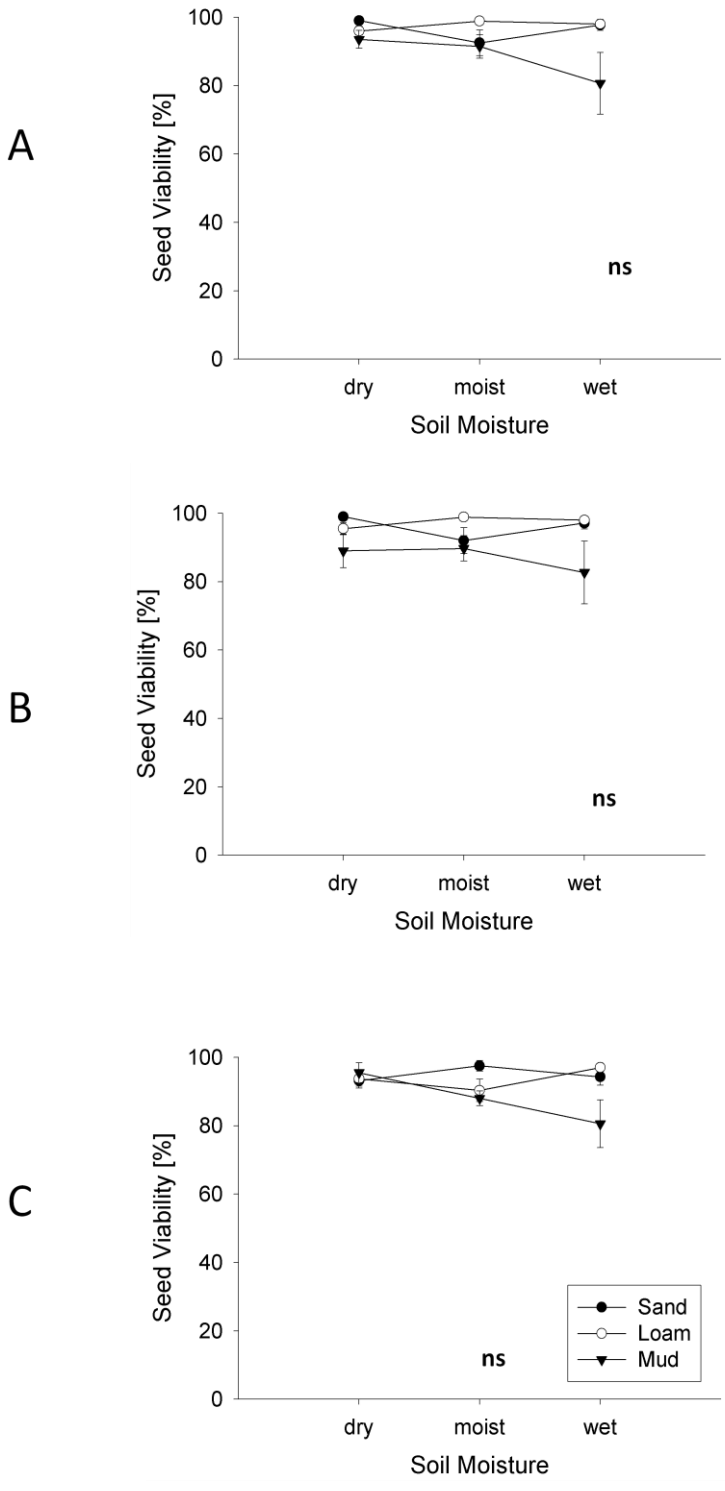


Figure 9 Viability values of *R. maritimus* seeds. Values are means ± SE (A, 6 month after burial; B, 12 month after burial; C, 18 month after burial and ns indicate non significant differences in Kruskal wallis test).



Discussion

Methodological concerns of soil seed bank persistence classifications

Rumex species have different Longevity Indices showing species specific soil seed persistence variation (Kleyer et al., 2008). However, even within each individual species, seed bank persistence is not a fixed trait, but varies strongly from transient to long term persistent in different studies (Tab. 8; Thompson et al., 1997). Considering our own results, *Rumex* species have different soil seed longevities: *R. acetosa* can only survive a comparatively short while, mostly only some months (Fig. 8). By contrast, in the case of *R. maritimus*, soil seed survival could be very high, up to several years, resulting in a long-term persistent seed bank. For a third species, *R. acetosella* we found an intermediate longevity with seeds surviving one year or, depending on soil conditions, even more (Fig. 7, Tab. 12). However, both *R. acetosella* and *R. maritimus* have the same Longevity Index.

Different non-exclusive explanations are discussed as sources of soil seed bank variation in data bases. On the one hand, there are methodological explanations with the date of soil seed bank sampling, the soil volume collected and the recommended time appyling the seedling emergence method (Thompson and Grime, 1979; Bekker et al., 1998b; Thompson and Fenner, 2000; Saatkamp et al., 2009), on the other hand there are environmental factors. To study the effect of environmental factors with the seedling emergence method, it is not clear how the influence of habitat conditions and effects of methodological problems on soil seed persistence can be separated (see Ter Heerdt et al., 1999; Bernhardt et al., 2008). Only burial experiments allow to find out the role of environmental parameters in soil seed survival without methodological problems. As indicated in the results, environmental parameters directly affecting soil seed survival, particularly in *R. acetosa* and *R. acetosella* (Fig. 7c; Fig. 8a).

Furthermore, environmental factors can also play a role in interpretation of soil seed bank types with seedling emergence method. For instance, the interaction of environmental factors and soil seed bank sampling time can explain variation of soil seed persistence classification of transient species. The results of *R. acetosa* indicate that a considerable amount of its seeds may survive until early summer of next year in moist and wet habitat conditions (Fig. 8a). However, the recommended time for soil seed bank

sampling is the end of winter to early spring (Bakker et al., 1996). As a consequence, seed persistence of this species mostly in moist and wet habitats has to be classified according to the scheme of Thompson et al. (1997) as short term persistent seed bank, which is in this case simply wrong. Therefore, edaphic conditions and especially soil moisture should be taken into account when species seed bank classification. Especially if this classification is based merely on a few references reporting species. Otherwise, it is predictable that this species creates entirely different seed banks in other habitats.

Environmental factors and/or species-specific attributes affecting soil seed survival

Not only environmental factors (WTD and soil types) but also species-specific attributes may affect soil seed bank persistence. Different factors can lead to seed longevity variation among species. This is, however, in our study only the case for germination but not for seed morphological attributes. Species reaction to light can explain soil seed longevity (Grime, 1989; Milberg et al., 2000; Saatkamp et al., 2011b). The fact that *R. acetosa* germinates in darkness (Tab. 8) is one explanation of its short soil seed longevity compared to the other two species. Seed shape index and seed mass were found to be approximately similar between species (Tab. 8) and are therefore unlikely explanations for differences in soil seed longevity. Concerning our results WTD is, after species-specific attributes, arguably the second strongest factor (after "species") in the context of seed longevity in the soil ($F = 79.82$, Tab. 11). In addition, a number of soil properties intensely cross-interact with WTD (Tab. 12), supporting the thesis of a high importance of WTD. Therefore, the fact that WTD and soil type affect soil seed survival suggests that under natural conditions both species-specific attributes and environmental factors define soil seed longevity.

Habitat moisture as limiting soil seed survival and species occurrence

One of the central questions in plant and vegetation ecology is why a species occurs where it occurs. Since the soil seed bank and its persistence are important life cycle stages, which may buffer a population's extinction, we may ask the question if soil seed bank persistence is the highest under those soil conditions where a species has its main occurrence (Dalling et al., 2011). For species with a persistent soil seed bank non-

sensitive to environmental factors (such as *R. maritimus* in this study) high seed survival in all treatments demonstrates that seed survival cannot be a limiting factor for species occurrence. habitat conditions cannot play an important role in seeds survival. Thus, other factors like germination, establishment of seedling from fresh seeds, or further ecological parameters may play a major role for the occurrence of non-sensitive species. For sensitive species (*R. acetosella* and *R. acetosa*), however, our results suggest that seeds of species from dry habitats cannot survive in wet habitats and likewise species from wet habitats cannot survive in dry habitats. *R. acetosella* occurs in dry habitats and *R. acetosa* mainly in moderately moist habitats. But these species showed a different soil seed survival reaction along a gradient in WTD. High WTD acts as a limiting factor for *R. acetosella*, but it does increase soil seed survival for *R. acetosa*. This result is consistent with studies that show increasing soil moisture limit the seed survival of species from dry habitat (Blaney and Kotanen, 2001; Schafer and Kotanen, 2003). A possible explanation is that in water-saturated soils (high WTD), fungal activity is inhibited and cannot play a role (Griffin, 1972). But, influence of anoxic condition on seed metabolic processes may cause high seed mortality in extremely wet conditions (Bekker et al., 1998d). In contrast, *R. acetosa*, which occurs in wetter habitats than *R. acetosella* showed the higher seed survival in moist soils and also in anoxic conditions. These results is also consistent with studies that show species of wet habitats survive better due to adaptations that allow their basic metabolism to proceed under anoxic conditions (Skoglund and Hytteborn., 1990; Bekker et al., 1998d; Murdoch and Ellis, 2000; Oomes et al., 1997; Poschlod et al., 1996). The main reason for seed depletion in anoxic conditions have not yet been studied in detail, but some factors like presence of antagonistic, nonpathogenic bacteria (Anderson et al., 1980), or seed chemical defense (Dalling et al., 2011) can increase the seed survival of species from wet habitats. Therefore, seed survival limitation under certain environmental conditions suggest at least the occurrence of certain species since their population viability is limited due to low soil seed bank persistence.

Interaction of environmental factors as habitat condition

The interaction of different habitat conditions with soil seed survival is not yet fully understood. On the one hand, seed survivals in the field with different habitat conditions

are not directly interpretable in terms of the underlying factors causing this variation (O'Hanlon-Manners and Kotanen, 2006; Pakeman et al., 2012; Voesenek and Blom, 1992; Davis et al., 2005). On the other hand, expect of few studies (Long et al., 2009; Blaney and Kotanen, 2001; Schafer and Kotanen, 2003; Mordecai, 2012), other studies with focus on single environmental factor in controlled conditions also can not indicate all habitat conditions (Bekker et al., 1998c; Bekker et al., 1998d; Nicol et al., 2003; Webb et al., 2006; Davis, 2007). Therefore, we suggest cross-interaction of principle environmental factors may simulate habitat conditions than single factors or field survival assessment. Concerning our results (Tab. 12), influence of soil properties in constant moisture (Long et al., 2009) and cross interaction of soil moisture and fungi (Blaney and Kotanen, 2001; Schafer and Kotanen, 2003; Mordecai, 2012), soil moisture and its interaction with other environmental factors have a major role on soil seed longevity because effect of both soil properties and soil fungi strongly depend on soil moisture content.

To conclude, Habitat conditions are, after species-specific attributes, arguably the second strongest factor in the context of seed longevity in the soil. Species seed survival could be explained with habitat moisture. Species from dry habitats are sensitive to adding water and species from wetter habitat also have the less survival in dryer conditions. Soil seed survival can be a limiting factor for species occurrence in certain habitats.

Chapter 4

Germination ecology and local assembly of dry sandy grasslands

Abstract

Dry sandy grasslands create hazard conditions with low moisture content and high soil surface temperatures. Species have developed several mechanisms to avoid these risky conditions in germination and establishment stages. We tested germination ecology of 30 dry sandy grasslands in different light and temperatures conditions. In order to figure out the seed germination traits, four indices including germination speed, indice for light and indices for temperature were calculated for each species (T_{50}, ΔG_{DFT}, LTG and ΔG_{light}). Dormancy patterns of species were determined by testing germination of fresh seeds and after three months dry storage, in the following. In addition, 6 weeks stratification was applied for all species and two species (*Cytisus scoparius, Trifolium arvense*) were scarified. Except for seed coat thickness, seed persistence and seed traits were determined from available databases. We found that species with underdeveloped embryo are underrepresented and most of species are non dormant and physiological dormant. Sandy grassland species germinate faster compare to the mean values of grasslands. According to the results, species scores in PCA axis 1 highly correlated to their longevity indices indicating seed germination traits and seed traits can explain seed persistence patterns in dry sandy grassland species

Introduction

Dry sandy grasslands belong to the most species rich grasslands and were once widespread in central Europe on fluvial sand deposits and inland sand dunes (Poschlod et al., 2009). Low moisture content of the sandy soils and high soil surface temperatures create harsh conditions for plants in these grasslands (Jeckel, 1984; Jentsch, 2001). These hazardous conditions may also affect species regeneration and establishment. The transition from seed to seedling is a high risk period in the life cycle (Harper, 1977).

Therefore, depending on their habitat, species have developed different mechanisms in germination strategies, dormancy and seed persistence and also in seedling growth to have a successful establishment and minimize the risk of this transition (Meyer et al., 1997; Fenner and Thompson., 2005, Poschlod et al., 2013). Furthermore, if species cannot successfully develop mechanisms to adopt to these conditions, then habitat conditions may act as limiting factors during germination stage and seedling establishment (Poschlod et al., 2013). Aiming at mechanisms underlying species adaptation, we target studying the germination ecology of dry sandy grassland species to evaluate in how far it can be named a principal factor for their occurrence in such a specific habitat.

Dormancy pattern in dry sandy grasslands and ecological correlates

Dormancy reduces the risk of seedling mortality due to environmental hazard mainly through drought affecting germination time until suitable conditions, spreading seed germination over several seasons (Grubb, 1977; Baskin and Baskin, 1998). Dormancy is one of the primary mechanisms that promote coexistence in plant communities, as conceptualized by bet-hedging and storage effect (Warner and Chesson, 1985; Venable, 2007). Several studies have described dormancy types and how it is related to the distribution of species (Baskin and Baskin, 1998; Baskin and Baskin, 2003) and their environment (Jurado et al., 2003; Jurado and Flores, 2005), however only on a global scale. The role of seed dormancy in ecological filtering and local assembly processes was only considered in a few studies (Kos et al., 2012). To classify dormancy, different types have been suggested beside being non dormant (ND), such as morphological dormancy (MD), morphophysiological dormancy (MPD), physiological dormancy (PD), physical dormancy (PY) and combinational dormancy (PD + PY)(Baskin and Baskin, 1998). Concerning ecological aspect, there are two main groups among different dormancy classes: species with under developed embryo indicate MD and MPD, which embryo first needs to grow to the certain size to germinate. This growth needs imbibitions with considerable remaining soil moisture. In another group embryo is fully developed and it can germinate in suitable condition (Baskin and Baskin, 2004). Therefore, we expect in dry sandy soil which moisture cannot be remained for long time (Scheffer et al., 2002) species with morphological or morphophysiological is underrepresented. Highest soil moisture occurs after snow melting (von Müller, 1956). Therefore, species with physiological dormancy might be overrepresented.

Germination speed

Furthermore, species can tolerate or adapt to fast drying soils by having high germination speed (Gutterman, 1993). Species germinate faster in extreme habitat than those in less extreme habitats (Grime et al., 1981) and also in coarse soil texture than those in fine soils texture (Kos and Poschlod, 2010). Therefore, we expect that species in dry sandy grasslands also have a faster germination speed than the average of grassland species.

Role of light and temperature as gap and depth detection mechanism

Species show different reaction to light and diurnally fluctuated temperature (DFT). On the one hand, some studies have shown that germination became less dependent on light and DFT with increasing seed size (Milberg et al., 2000; Saatkamp et al., 2011b). These mechanisms allow species with small seed size to create persistent seed banks (Bekker et al., 1998b; Moles et al., 2000; Thompson et al., 2001; Peco et al., 2003; Schwienbacher et al., 2010; Zhao et al., 2011). On the other hand, mainly light prevent germination of seeds with small size in Mediterranean climate and also in coastal sand dunes (Thanos et al., 1991; Yu et al., 2007), preventing germination on fast drying soil surfaces (Leishman and Westoby, 1998; Bell et al., 1995). In this respect seed size positively correlates to seed persistence (Leishman and Westoby, 1998; Yu et al., 2007). Therefore, the mechanism of species to react these conditions considering light and DFT is not obvious.

In addition, the role of DFT in seed longevity has been described in several studies as a gap and depth detection mechanism (compare Saatkamp et al., 2011a,b). However, negative role of germination in low temperatures in seed persistence has not been considered so far. Several studies have been described the role of cold stratification on seed germination, dormancy breaking and seedling emergence (Baskin and Baskin, 1998; Allen and Meyer, 1998), but functional role of germination in low temperatures in seed persistence as a seed germination traits is not clear.

However, nearly no experiments have studied germination ecology of dry sandy grassland species concerning germination and seed traits. Therefore, the results of previous studies cannot explain the effects of sandy grasslands environment on seed ecological traits clearly. Therefore, the germination ecology of thirty species from dry sandy grasslands was tested. We aimed at evaluating changes of seed germination in

different light and temperature conditions to understand the role of light and also temperature in germination ecology and soil seed bank longevity patterns. Thus, we asked the following questions:

Does the proportion of dormancy types reflect the relatively harsh and dry conditions of dry sandy grasslands during summer and autumn?

Does germination speed reflect the low water capacity and fast desiccation of sandy soils?

Does the germination pattern at DFT reflect the low competitively of most dry sandy grassland species?

Do germination and seed trait patterns reflect soil seed bank persistence?

Material and Methods

Study system

Dry sandy grasslands occur throughout Central Europe and Southern Germany where sand was deposited, mostly during and after the last ice age (Bork et al., 1998; Bateman and Godby, 2004). Dry sandy grasslands are typically characterized by periodic drought, soils of low fertility, harsh microclimatic conditions and high disturbance (reviewed by Jentsch and Beyschlag, 2003; Jeckel, 1984; Ödman et al., 2012). Sandy grasslands host many rare and endangered plant species (Jentsch, 2001). Changing land use during the last century caused a decline of former sandy grassland to around or even less than 1 % in southern Germany (Poschlod et al., 2009).

Study species

We tested germination of different species from sandy grassland. Thirty species were selected that represent typical and very common species of dry sandy grasslands according to the phytosociological classification of South German vegetation (Korneck, 1978). Ripe fruits of each species were collected in the respective communities at different localities in Bavaria in summer 2010 (see Tab. 13).

Table 13 Overview of study species with respective locations of seed collections.

Species	family	Origin of seeds
Aira caryophyllea L.	*Poaceae*	Zenzing (Regentalhänge, Bavaria)
Alyssum montanum ssp. gmelinii (Jord. & Fourr.) Hegi & Em. Schmid	*Brassicaceae*	Kitzingen (Lower Franconia, Bavaria)
Androsace septentrionalis L.	*Primulaceae*	Kitzingen (Lower Franconia, Bavaria)
Arabidopsis thaliana (L.) Heynh.	*Brassicaceae*	Hallstadt at Bamberg (Lower Franconia, Bavaria)
Arenaria serpyllifolia L.	*Caryophyllaceae*	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Armeria maritima ssp. elongata (Hoffm.)Koch	*Plumbaginaceae*	Nürnberg (Central Franconia, Bavaria)
Calluna vulgaris (L.)Hull	*Ericaceae*	Siegenburg (Upper Palatinate, Bavaria)
Cerastium arvense L.	*Caryophyllaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Corynephorus canescens (L.) P. B.	*Poaceae*	Siegenburg (Upper Palatinate, Bavaria)
Cytisus scoparius (L.)LK.	*Fabaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Deschampsia flexuosa (L.)Trin.	*Poaceae*	Siegenburg (Upper Palatinate, Bavaria)
Dianthus deltoids L.	*Caryophyllaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Erigeron acris L.	*Asteraceae*	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Erophila verna (L.) Chevall.	*Brassicaceae*	Bamberg (Upper Franconia, Bavaria)
Filago arvensis L.	*Asteraceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Filago minima (SM.)Pers.	*Asteraceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Helichrysum arenarium (L.) Moench	*Asteraceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Hieracium pilosella L.	*Asteraceae*	Siegenburg (Upper Palatinate, Bavaria)
Holosteum umbellatum L.	*Caryophyllaceae*	Bamberg (Upper Franconia, Bavaria)
Hypochoeris radicata L.	*Asteraceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Jasione montana L.	*Campanulaceae*	Kirchheim/Ries (Swabia, Bavaria)
Koeleria glauca (Spr.) DC.	*Poaceae*	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Onosma arenaria Waldst. & Kit.,	*Boraginaceae*	Mainzer Sande (Mainz, Rhineland- Palatinate)
Potentilla argentea L.	*Rosaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Rumex acetosella L.	*Polygonaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Scleranthus annuus L.	*Caryophyllaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Sedum acre L.	*Crassulaceae*	Kornburg, Nuremberg (Central Franconia, Bavaria)
Spergula morisonii Boreau	*Caryophyllaceae*	Bamberg (Upper Franconia, Bavaria)
Teesdalia nudicaulis (L.)R. Br	*Brassicaceae*	Bamberg (Upper Franconia, Bavaria)
Trifolium arvense L.	*Fabaceae*	Ramsberg (Middle Franconia, Bavaria)

Seed traits

Seed mass, seed shape and seed coat thickness were determined for each species (Tab. 14). Seed mass and seed shape index were determined according to Biopop data bases (Poschlod et al., 2003). The dimensionless Seed shape values varied between 0 for spherical seeds and increase up to 0.2 for elongated or flattened seeds (Thompson et al, 1993). To measure seed coat thickness seeds were exposed to X-rays at radiation intensity 18 kV for 10 s (Faxitron MX-20 cabinet X-ray system). In an image five different dimensions of 10 seeds were measured with ImageJ software (Abramoff et al., 2004). soil

seed bank persistence was first determined applying the longevity index (LI)(Kleyer et al., 2008). In the following, the LI values were evaluated using three new soil seed bank studies in dry sandy grasslands to classify the species in two groups, transient and persistent, respectively. To reduce classification errors, species mainly with LI value o or equal to 0 were selected as transient and species with high LI (< 0.3) were selected as persistent.

Seed dormancy and germination patterns

In order to determine dormancy patterns, germination of fresh seeds and after three months dry storage in the following was determined. In addition, germination was tested after 6 weeks stratification. Two species (*Cytisus scoparius, Trifolium arvense*) were scarified as they were supposed to have physical dormancy condition (Tabs. 15, 16).

In details, germination patterns and viability of species were tested. Therefore, in petri dishes, 25 seeds (n= 8) were germinated on two 90-mm-diameter filter paper discs (Sartorius 3 hw). After filter papers were saturated with deionized water, dishes were placed in a climate chamber. Germination was tested at diurnally fluctuating temperatures (DFT) (day/night cycle 14 h/10 h; temperature 22°C/14°C) and also at constant temperatures (22°C) in both light/dark alternation and in constantly dark conditions. Germinated seeds were counted during 45 days. For the identification of germination, a seed was considered to have germinated if the radicle had protruded at least 1 mm. These conditions were applied for fresh seeds, after 3 months after ripening and also for stratification. Seeds stratification was applied with 6 week storage in 4°C.

Seed germination traits

In order to figure out the seed germination traits, four indices were calculated for each species. T_{50} indicates germination speed, two indices show temperatures influences ($\Delta G_{DFT:}$ germination in diurnal fluctuating temperatures and LTG: low temperature germination) and an indice for light ($\Delta G_{light)}$ shows the effect of light. Germination speed was determined as days until 50% germination (T_{50}) (n=8) (Coolbear, 1984; Saatkamp et al., 2011b). In addition, to compare germination speed to other grassland habitat in central Europe, T_{50} average of 86 species (including 29 grasses, 48 forbs and 8 shrubs) from in

British grasslands were calculated (Grime et al., 1981). Similar to our study, germination after three month dry storage were selected to calculate T_{50}. Species with very low germination rate were excluded to avoid error.

Index for species relative light germination was also calculated according to Saatkamp et al., (2011b):

$$\Delta G_{light} = [(G_{light} - G_{dark})/ (G_{light} + G_{dark})] \times 100$$

To calculate the index, germinated seeds at light (G_{light}) and germinated seeds at darkness (G_{dark}) were counted. To release dormancy and reach highest germination percentages, germinated seeds after stratification (according to Milberg et al, 2000) were counted under fluctuating temperature regime (day/night cycle 14 h/10 h; temperature 22°C/14°C) at light and darkness. All species had positive values ($\Delta G_{light} > 0$; Tab. 17). To validate the relation of light and seed size we used the independent data set from literature (Beier, 1991; n= 38) for dry calcareous grassland as similar evolutionary habitats and re-analysed the data by calculating light index and correlating the results to seed size.

Species were also classified according to their relative temperature index according to Saatkamp et al., (2011b):

$$\Delta G_{DFT} = [(G_{fluct.} - G_{const.})/ (G_{fluct.} + G_{const.})] \times 100$$

Positive values show higher germination at DFTs than at constant temperatures ($\Delta G_{DFT} > 0$, n= 23) and negative values indicate higher germination at constant temperatures compared to fluctuated temperatures ($\Delta G_{DFT} < 0$, n= 5; Tab. 17). For calculation, germination at fluctuated temperatures ($G_{fluct.}$) and germination at constant temperatures ($G_{const.}$) were determined. Germination percentage after 3 month dry storage were counted in darkness at DFTs of 22°C(14h) and 14°C (10h) and also under constant 22°C, except for *Cerastium arvense, Holosteum umbellatum* and *Onosma arenaria,* which germination was tested after stratification because these species were physiologically dormant and had no germination in any treatment. We didn't calculate ΔG_{DFT} for all species after stratifications similarly like ΔG_{light} because of low germination of most species in darkness. We also calculated germination percentage at low temperatures (LTG) for species to show

which species can germinate at 4°C during stratification period. Some species had no germination at these temperatures (n= 10). Other species had mainly very high germination rates during stratification. *Cytisus scoparius* and *Trifolium arvense* were excluded from analysis according to their low seed germination rates.

Statistics

To find out what is the mechanism of seeds to avoid harsh soil surface conditions in sandy soils, relation between light indices and seed size were tested with linear regression using R statistical software (R Foundation for Statistical Computing 2009). GLM were also applied to find out the relation between dormancy, germination speed and seed persistence with seed traits which showed no special relations (Appendix 1). To detect general specialization trends across the sandy grassland's germination ecology, we organized the data into a single 7 trait × 23 species matrix (five species excluded by software during analysis in order to have missing values). We submitted the matrix to a Principal Component Analysis (PCA) based on the correlation matrix of variables (Jongman et al., 1995; Diaz et al., 2004). Then the three highest eigenvector for each axes were selected to show main seed traits and seed germination traits which explain the variation of each axis. To evaluate whether the patterns of specialization detected on the basis of seed traits and seed germination traits to seed persistence, we correlated the score of species along PCA axes 1 against their longevity indices with spearman test. Because dormancy is a categorical data, therefore correlation test is not sufficient. We compared the dormant and non dormant species according to species scores along PCA axes 2.

Results:

Variation among seed traits

Species had variable seed mass from 0.01 g for *Filago minima* to 7 g for *Onosma arenaria*. Seed coat thickness varied from 9.3 µm for *Spergula morisonii* to 154.1 µm for *Onosma arenaria*. Seed shape index varied from 0.02 to 0.15 indicating a round seed for Alyssum *montanum* to elongated seeds for *Hypochaeris radicata* (Table 14).

Table 14 Seed traits for study species.

Species	Seed mass(mg)	Seed shape Index	Seed coat thickness (µm)
Aira caryophylea	0.18	0.12	-
Alyssum montanum	0.60	0.02	-
Androsace septentrionalis	0.17	0.05	-
Arabidopsis thaliana	0.02	0.05	11.64
Arenaria serpyllifolia	0.05	0.05	20.02
Armeria maritima	0.88	0.11	10.66
Calluna vulgaris	0.04	0.05	11.92
Cerastium arvense	0.21	0.04	27.60
Corynephorus canescens	0.12	0.13	12.34
Cytisus scoparius	7.78	0.09	77.74
Deschampsia flexuosa	0.43	0.11	14.04
Dianthus deltoids	0.25	0.09	24.12
Erigeron acris	0.14	0.15	15.68
Erophila verna	0.02	0.08	13.66
Filago arvensis	0.01	0.10	10.72
Filago minima	0.03	0.11	14.72
Helichrysum arenarium	0.05	0.14	10.08
Hieracium pilosella	0.10	0.13	25.76
Holosteum umbellatum	0.08	0.05	9.44
Hypochoeris radicata	0.05	0.15	20.38
Jasione montana	0.02	0.12	17.51
Koeleria glauca	0.10	0.12	10.08
Onosma arenaria	7.00	0.05	154.13
Potentilla argentea	0.09	0.06	66.34
Rumex acetosella	0.36	0.03	49.98
Scleranthus annuus	1.40	0.08	30.13
Sedum acre	0.04	0.09	19.75
Spergula morisonii	0.16	0.15	9.32
Teesdalia nudicaulis	0.27	0.05	72.40
Trifolium arvense	0.39	0.02	41.42

Dormancy types

Species had four different dormancy types. Sixteen species had the highest germination rate during two weeks after collecting seeds. They can be classified as non dormant. Nine species did not germinate after collection but only three months after seed collection (Tab. 15, 16). They can be classified as non-deep physiological dormant. *Cerastium arvense* and *Onosma arenaria* had the highest germination rates only after cold stratification which corresponds to deep physiological dormancy. *Cytisus scoparius* and *Trifolium arvense* germinated only after scarification. The seeds are physically dormant. No morphological

and morpho-physiological dormancy were found among the studied species (according to Baskin and Baskin, 1998; Fig. 10, Tab. 15, 16).

Table 15 dormancy classification according to germination treatments

	Germination treatment				Dormancy Types
	No dormancy in fresh matured seed	Loss of dormancy after ripening	Loss of dormancy after cold stratification	Loss of dormancy after scarification	
1	Yes	-	-	-	Non dormant
2	No	Yes	-	-	Non deep physiological dormancy
3	No	No	Yes	-	Deep physiological dormancy
4	No	No	No	Yes	Physical dormancy

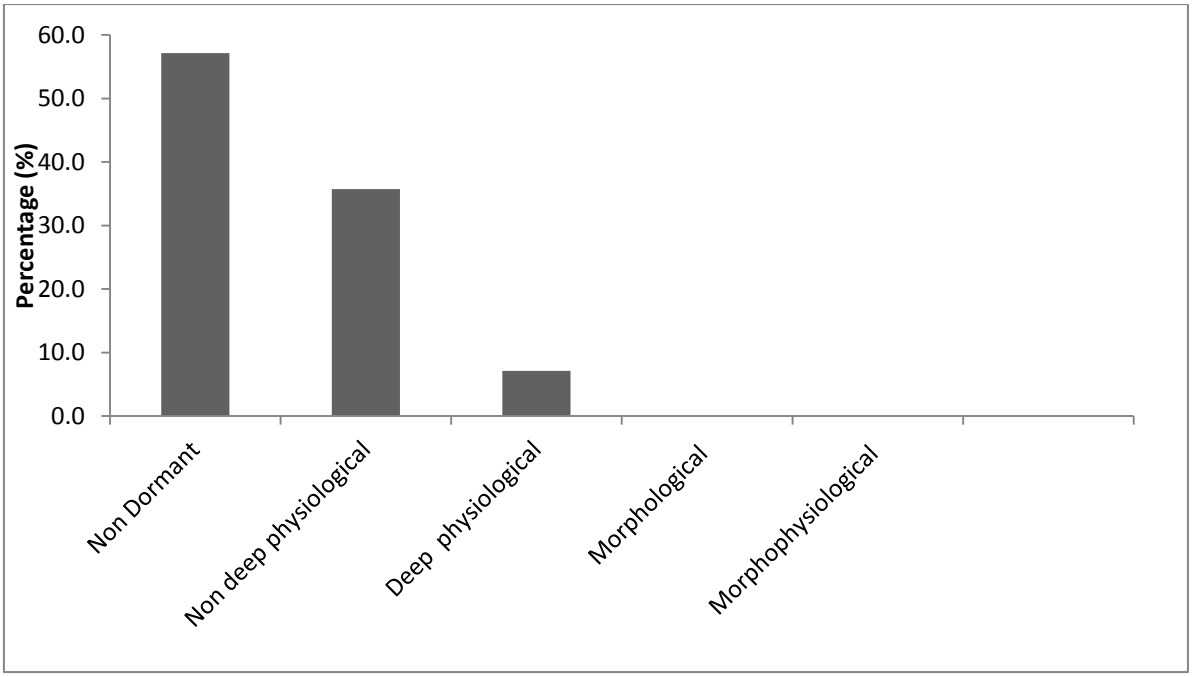


Figure 10 Percentage of dormancy types among study species. Physiological dormancy types are the main dormancy types and there is no dormancy type belongs to morphological and morphophysiological dormancy.

Table 16 Germination of study species and dormancy types. F-F-L indicate fresh harvested-fluctuated temperatures- light, A-F-L indicate After ripening- fluctuated temperatures- light, S-F-L indicate stratification- fluctuated temperature- light and Sc-F-L indicate scarification- fluctuated temperature – light. In dormancy types: ND indicate non dormant. NPD indicate non deep physiological dormancy, DPD indicate deep physiological dormancy and PD indicate Physical dormancy.

Species	F-F-L	A-F-L	S-F-L	Sc-F-L	Dormancy Types
Aira caryophylea	**81.5 ± 4.5**	99.5 ± 0.5	100 ± 0.0	-	ND
Alyssum montanum	**95.0 ± 1.8**	99.5 ± 0.5	84.5 ± 4.0	-	ND
Androsace septentrionalis	**84.0± 2.3**	84.5 ± 4.7	46 ± 3.1	-	ND
Arabidopsis thaliana	11.0 ± 2.2	**80 ± 1.7**	80 ± 3.1	-	NDP
Arenaria serpyllifolia	**89.5 ± 2.9**	84.85 ± 2.4	90.98 ± 2.5	-	ND
Armeria maritima	29.5 ± 5.2	**77.9 ± 4.5**	68.5 ± 6.2	-	NDP
Calluna vulgaris	**80.5 ± 6.2**	57.6 ± 4.3	45.6 ± 4.1	-	ND
Cerastium arvense	3.5 ± 0.5	0.0 ± 0.0	**23.5 ± 2.7**	-	DPD
Corynephorus canescens	**52.5 ± 4.9**	43 ± 3.4	68.5 ± 6.1	-	ND
Cytisus scoparius	4 .0±1.5	16 ± 1.9	14 ± 2.9	**75 ± 5.4**	PD
Deschampsia flexuosa	3.5 ± 0.9	**20.5 ± 2.4**	13.6 ± 2.0	-	NDP
Dianthus deltoids	**99.5 ± 0.5**	99.5 ± 1.2	94 ± 1.7	-	ND
Erigeron acris	-	84 ± 2.4	64 ± 7.2	-	ND
Erophila verna	2.0 ±1.1	**80.01± 3.6**	72.43± 4.6	-	NDP
Filago arvensis	**99.5 ± 0.5**	100 ± 0.0	71.09± 5.1	-	ND
Filago minima	**98.5 ± 1.1**	88.5 ± 4.7	38.4 ± 6.6	-	ND
Helichrysum arenarium	**37.5 ± 3.2**	29.5 ± 3.8	30 ± 4.7	-	ND
Hieracium pilosella	**80.0 ± 6.0**	90 ± 1.9	89 ± 2.7	-	ND
Holosteum umbellatum	0.5± 0.5	0 ± 0.0	**54 ± 4.8**	-	NPD[1]
Hypochoeris radicata	**52.0 ± 5.5**	63.5 ± 4.5	48.8 ± 7.1	-	ND
Jasione montana	**100 ± 0.0**	96.5 ± 1.9	66.5 ± 5.8	-	ND
Koeleria glauca	**66.5 ± 4.9**	67 ± 2.4	43 ± 3.1	-	ND
Onosma arenaria	0.0 ± 0.0	1 ± 0.7	**46.5 ± 3.9**	-	DPD
Potentilla argentea	24.5 ± 3.7	**95 ± 1.3**	29.6 ± 5.2	-	NDP
Rumex acetosella	2 .0± 1.1	36.5 ± 2.1	17.5 ± 2.5	-	NDP
Scleranthus annuus	19.5 ± 1.4	**35 ± 3.8**	36 ± 2.5	-	NDP
Sedum acre	19.0± 3.3	**92 ± 2.1**	-	-	NDP
Spergula morisonii	0.0 ± 0.0	**40.5 ± 4.4**	14 ± 1.9	-	NDP
Teesdalia nudicaulis	**94.5 ± 3.1**	99 ± 0.7	83.83 ± 3.2	-	ND
Trifolium arvense	7.5 ± 2.7	3 ± 1.6	2 ± 1.3	**84 ± 4.2**	PD

1 because these species germinate completely during stratification (Tab. 17, LTG) therefore it should be classified as non deep physiological dormancy (Baskin and Baskin, 1998)

Seed germination traits

Results of germination tests showed remarkable differences in germination speed (Tab. 17). Germination speed varied from 1.6 day for *Alyssum montanum* up to 18.9d for *Calluna vulgaris* (Mean= 5.1 ± 0.6). To compare germination speed of our habitat to other grasslands we reanalyzed data available from literature (compare Grime et al., 1981). These data showed grasslands have slower germination speed (Mean= 11.7 ± 1.0) than sandy grasslands in our study.

Species had different light requirements. All species had a better germination in the presence of light than darkness. Some species like *Calluna vulgaris*, *Erophila verna*, *Potentilla argentea*, *Filago minima* and *Helichrysum arenarium* only germinated at light. ΔG_{light} and seed mass had a significant negative relation ($R^2 = 0.41$; $p < 0.001$) which show small sized seeds are more dependent on the light compare with those with large sized seeds. To validate the correlation of ΔG_{light} with seed mass in our dataset we reanalyzed data available from literature (compare Beier, 1991). These data showed the same fit between ΔG_{light} and Seed mass. Relation was as strong as dry sandy grasslands ($R^2 = 0.28$; $p < 0.001$; Fig. 11).

Species also had different temperature requirements. Four species including *Aira caryophyllea*, *Sedum acre*, *Erigeron acris* and *Hypochaeris radiata* germinated better at constant than at fluctuating temperatures. On the other side, species such as *Erophila verna* or *Spergula morisonii* germinated only at fluctuating temperatures. Concerning germination in low temperatures, nine species including *Aira caryophyllea*, *Armeria maritime*, *Alyssum montanum*, *Corynephorus canescens*, *Erophila verna*, *Filago arvensis*, *Hieracium pilosella*, *Holosteum umbellatum*, *Teesdalia nudicaulis* had considerable germination during cold stratification. The remaining species had no germination or low germination during stratification.

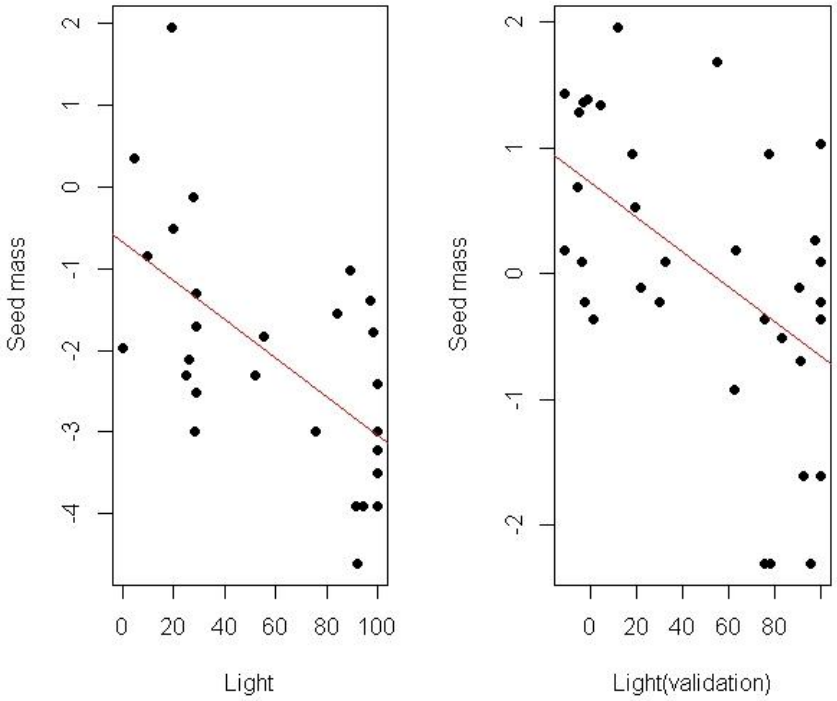


Figure 11 Regression for ΔGlight and seed mass. Displayed small size seed have higher light requirements. The linear regression with (n= 28; R^2 = 0.41; P = 0.01) for sandy grassland in left side. The right side show dry calcareous grassland reanalysed after Beier (1991). The linear regression with (n= 38; R^2 = 0.21; P = 0.01).

Seed persistence and Germination patterns

Fifteen species were persistent and the remaining species had transient seed bank (Tab. 17). To find out which species create soil seed bank and also can seed traits and seed germination traits explain dormancy and seed persistence the PCA test were applied.

Table 17 relative germination rates for fluctuating temperatures (Δ_{GDFT}), light requirement (ΔG_{light}), germination speed (T_{50}) and low temperature germination of the 28 studied species. P indicates persistent and T indicates transient seed bank types.

Species	ΔG_{DFT}	ΔG_{Light}	T_{50}	LTG	Seed persistence
Aira caryophyllea	-8.87	29.03	4.03	100 ± 0.00	P
Alyssum montanum	6.70	19.63	1.64	82.8 ± 3.9	T
Androsace septentrionalis	33.33	97.85	5.87	7.1 ± 0.5	P
Arabidopsis thaliana	0	93.94	4.94	79.5 ± 3.1	P
Arenaria serpyllifolia	15.58	75.38	3.51	18.4 ± 0.5	P
Armeria maritima	27.09	27.74	7.88	56.5 ± 5.1	T
Calluna vulgaris	0	100	18.90	0.0 ± 0.0	P
Cerastium arvense	60.00	84.31	4.20	0.7 ± 0.1	P
Corynephorus canescens	7.91	26.27	9.15	68.5 ± 6.1	P
Deschampsia flexuosa	8.47	9.68	8.89	13.6 ± 2.0	T
Dianthus deltoids	6.12	96.86	4.21	0.5 ± 0.0	P
Erigeron acris	-0.99	0	4.25	0.0 ± 0.0	T
Erophila verna	100.00	100	3.23	68.1 ± 4.3	P
Filago arvensis	7.55	92	1.78	67.2 ± 4.8	P
Filago minima	88.24	100	3.66	0.0 ± 0.0	T
Helichrysum arenarium	11.59	100	5.76	0.0 ± 0.0	T
Hieracium pilosella	7.10	25.06	4.19	73.6 ± 2.2	T
Holosteum umbellatum	13.20	28.64	7.34	100.0 ± 0.0	T
Hypochoeris radicata	-3.35	28.42	2.61	0.0 ± 0.0	T
Jasione montana	2.86	91.37	4.04	0.0± 0.0	T
Koeleria glauca	3.70	52.21	5.13	24.0 ± 1.7	T
Onosma arenaria	72.60	19.23	3.69	0.0 ± 0.0	NA
Potentilla argentea	0	100	4.17	0.0 ± 0.0	P
Rumex acetosella	55.56	89.19	4.60	0.3 ±0.0	P
Scleranthus annuus	15.89	4.65	4.04	28.1 ± 0.0	T
Sedum acre	-34.78	-	3.93	-	P
Spergula morisonii	100.00	55.38	4.40	14.0 ± 1.9	P
Teesdalia nudicaulis	74.25	28.91	4.07	82.2 ±3.2	P

Considering the PCA results, the first PCA axis was identified as an axis for seed persistence, accounting for ca. 27.70 % of the total variance. PCA axis 2 accounted for a further ca. 22.58 % of the total variance and appeared to be a dormancy related axis. The PCA identified certain seed traits of particular importance in explaining total variation among species: light, LTG and seed mass had the highest loadings on PCA axis 1, and seed coat thickness, seed mass and seed shape scored high on PCA axis 2 (Tab. 18; Tab.

19). Species scores in PCA axis 1 highly correlated to their longevity indices (R^2= 58, P= 0.006).

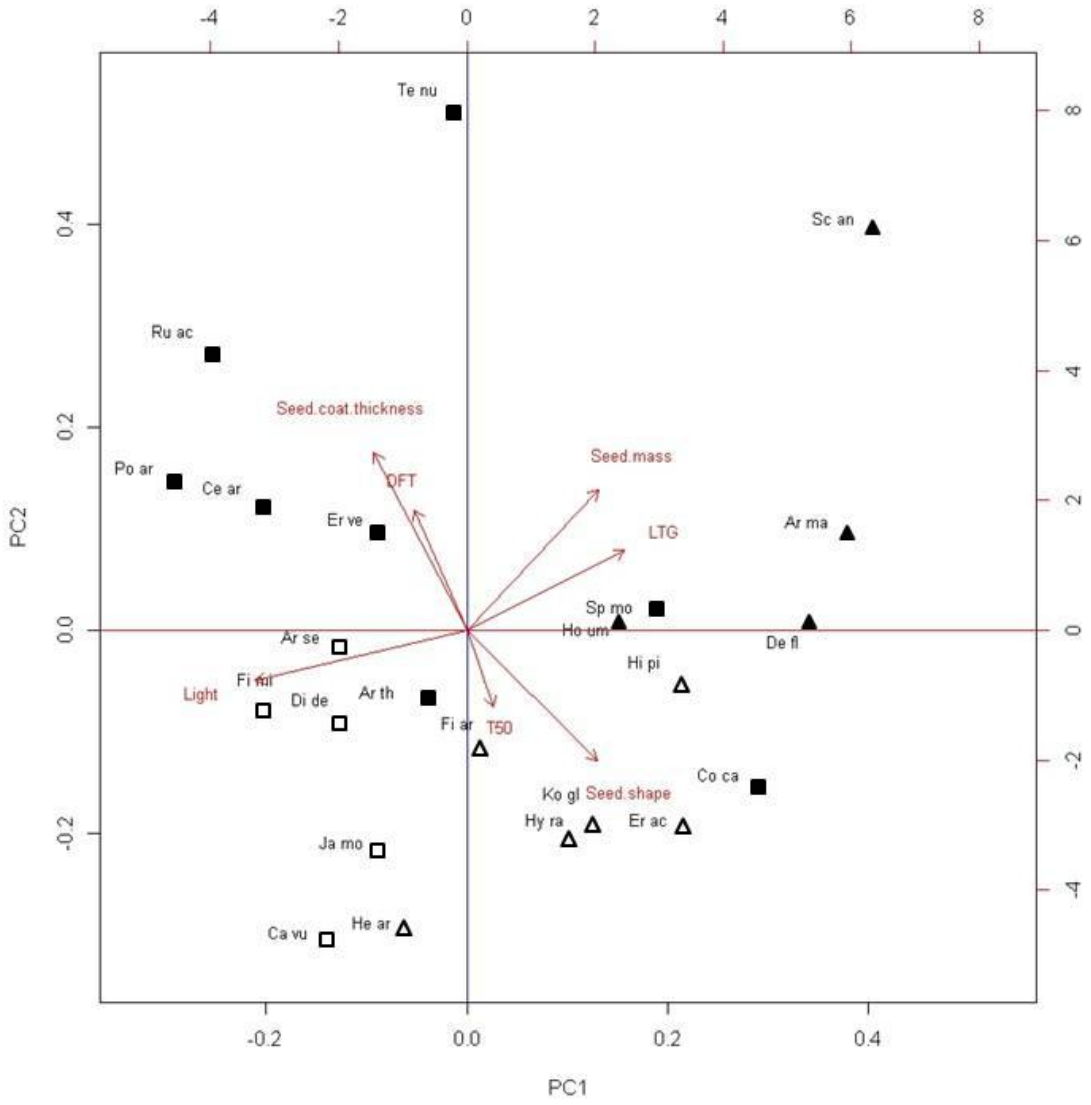


Figure 12 Principal components analysis (PCA) performed including the seed traits and seed germination traits. Arrows represent the increasing values of compounds. Symbols indicate four groups: Open triangles indicate transient- non dormant species and closed triangles indicate transient- dormant species. Open squares indicate persistent- non dormant and closed squares indicate persistent –dormant species. The first PCA axis was identified as an axis for seed persistence, accounting for ca. 27.70 % of the total variance. PCA axis 2 accounted for a further ca. 22.58 % of the total variance and appeared to be a dormancy related axis.

Table 18 Eigen vector scores of seed traits in three main PCA axes, obtained from a matrix of 7 traits × 23 species. Bold indicate the three highest eigenvector scores for each PCA axes. Values in parenthesis indicate variance accounted for by each axis.

Traits	*PCA 1 (27.50%)*	*PCA 2 (22.58 %)*	*PCA 3 (17.73 %)*
T_{50}	0.078	-0.245	0.674
Light	**-0.623**	-0.161	-0.061
DFT	-0.153	0.385	-0.492
LTG	**0.462**	0.255	-0.132
Seed mass	**0.385**	**0.449**	0.260
Seed shape Index	0.381	**0.416**	-0.441
Seed coat thickness	-0.272	**0.570**	0.135

Table 19 Soil seed bank persistence and parameters affecting (+ and ++) the Seed longevity.

	Seed persistence	
	Dormant	**Non dormant**
T_{50}	-	+
Light	+	++
DFT	++	+
LTG	-	-
Seed mass	+	-
Seed shape Index	-	-
Seed Coat thickness	++	-

Discussion

We found that species with different germination mechanisms adapt to environmental conditions in dry sandy grasslands. The seed germination, as well as seed persistence is among the most vital processes during plant establishment. This, in turn, is one of the most crucial stages in a plant's life cycle (Grubb, 1977).

Low soil moisture limit occurrence of species with underdeveloped embryo

Most of species in dry sandy grassland are non dormant or physiological dormant and no species were found with morphological and morphophysiological dormancy (N= 130, unpublished data), thus supporting our first hypothesis that sandy soils limit species with underdeveloped embryo. The ecological interpretation of this pattern is that low soil moisture remains limit germination of underdeveloped embryo because of moisture requirement for imbibitions. These results are consistent with studies which indicated limitation of under developed embryo species with increasing sand content (Kos et al, 2012; Baskin and Baskin, 1998). In other side, non dormant and physiological dormancy allow species occur over several seasons. These mechanisms allow species to germinate in favorite conditions of soil moisture and temperatures

Species germinate fast

High germination speed among species in this habitat mainly for small size seeds allow seeds to avoid these hazards and reach to deeper layers (Tab. 17). Dry sandy grasslands species can germinate faster compare to other habitats. Although there are no studies to compare the germination speed of different habitats with details. But concerning our results and comparison to British flora (Grime et al., 1981) sandy grassland species germinate considerably faster that average grasslands. Soil surface hazard is not a limiting factor for large seeds and these species can germinate and produce powerful seedling.

Light and temperature act as gap and depth detection mechanisms

Concerning our results, negative relation of light to seed size shows seeds have depth detection mechanisms which allow small sized seed germinate only in high available light (Fig. 11). This mechanism has a trade off with seed persistence allow small size species to create persistent seed banks and wait until high amount of light availability during disturbance which is very common in sandy habitats. Small and rounded seeds are buried more easily and incorporated more quickly into the soil than large and flattened or elongated seeds (Bekker et al., 1998b; Moles et al., 2000; Thompson et al., 2001; Peco et al., 2003; Schwienbacher et al., 2010; Zhao et al., 2011). Sandy grassland species function

as light and DFT is similarly like other central Europe habitats than other sandy habitats in coastal lands and Mediterranean habitats which don't germinate in light availability (Thanos et al., 1991; Yu et al., 2007). They argue that in coastal sand dunes photoinhibation help small size seeds to avoid germination in harsh soil surface conditions. Therefore, considering reaction of seeds to light, dry sandy grasslands soil is not a limiting factor in this aspect and seed highly germinate in presence of light. As explanation, climate conditions and land use history of sandy grassland shows that this habitat managed similarly like other grassland (Poschlod et al., 2009). Therefore, these habitats can also have mainly the same mechanism like other dry grasslands in central Europe than coastal sand dune in Mediterranean regions.

Seed traits and seed germination traits explain soil seed persistence

Seed persistence is a mechanism of the species to tolerate harsh conditions (Venable, 2007). However, the functional role of soil seed bank is still a challenging topic in seed ecological research. Concerning our results, seed traits and seed germination traits (mainly reaction to light and temperatures) form species seed persistence (Fig. 12). Two groups of species create the persistent soil seed bank: In one side, small size seeds which are mainly non dormant can create soil seed bank. These species only germinate in high availability of light and are less sensitive to DFT. They also had no germination in low temperatures. The ecological interpretation is that these factors play a role as depth detection mechanism to avoid too deep germination in soil. This mechanism allows small size seeds to be persistent in the soil and wait until favorable conditions for germination like light presence. In addition, species with thick seed coat which are dormant react better to DFT and less to light. The underlying cause of the patterns would be thick seed coat may not only increase longevity with preventing the seed and embryo against microbial activity and temperature fluctuation (Mohamed-Yasseen et al., 2004; Gardarin et al., 2010), but concerning our results also act as mechanism which seeds avoid germination in the deep soils and only germinate in the soil surface with high DFT. In other side, two groups of species create transient soil seed bank: elongated seeds which are non dormant germinate highly in darkness and constant temperature lead to transient soil bank. In addition, some dormant seeds with high seed mass which germinate in cold temperature and darkness create transient soil seed bank. Concerning seed dormancy, species with higher seed mass or thicker seed coat are more dormant than those with small size seeds (Fig. 12).

These results is consistent to other studies in central Europe which show small and round seeds and also species less sensitive to light and DFT can mainly create persistent seed bank than those with large seed size and elongated seeds (Bekker et al., 1998; Saatkamp et al., 2011b). Some studies ignored the role of light and introduced the DFT as a main driver in depth detection mechanism (Saatkamp et al., 2011a). But concerning our results light influence seed longevity of small size seeds in sandy grasslands (Fig. 11), which is consistent with studies that show the relation of light and seed size (Milberg et al 2000). As an explanation, in fine soil texture light cannot penetrate and is less important than DFT but in sandy soils light may go deeper and has more influence than other habitats (Ciani et al., 2005; Benvenuti, 1995; Saatkapm et al., 2011).

Furthermore, considering the role of temperature in seed longevity, LTG was not mentioned so far. DFT may increase the persistence, however LTG can increase soil seed bank depletion in other side. In most of the germination studies, seeds after stratification will move to climate chamber with optimum temperature to reach maximum germination and evaluate the effect of stratification in dormancy breaking. Counting geminated seeds during stratification or in the end of stratification can be used as a seed germination trait showing seed bank depletion during winter. Seed persistence was determined using LI and our own measurement. Therefore we cannot define exact soil seed bank classification. Whereas most of species had extreme low (i.e 0) or high LA values (i.e values > 0.3) indicating acceptable transient and persistence classes. We expect to find same pattern also with burial study and with more data about depth detection mechanism. Sandy grasslands allow particular plants species coexist which were adapted to these conditions. Species showed a germination adaptation during regeneration and establishment period to tolerate this condition. In general, species with creating seed bank for small size seeds, faster germination speed and having physiological dormancy may tolerate this habitat conditions.

Chapter 5

Seed traits explain ex situ seed longevity

Abstract

Ex situ seed preservation has an important role in species conservation and re-establishment of new populations of rare species. Mechanisms of seed longevity variation in dry storage conditions are not yet fully understood. Different factors, including mainly physiological characteristics, determine seed longevity, but some seed traits also can explain some seed survival variations. Furthermore, it is possible that soil seed longevity and ex situ seed longevity follows same patterns and seed traits explain these patterns. Therefore, we tested seed longevity of 18 species from sandy grasslands under ex-situ conditions by ageing them with LiCl solution. Time to lose 50 % germination (P_{50}) was used as a seed longevity trait to compare seed ageing of species. Our results showed that seed traits significantly correlated to P_{50}. Species with thick seed coat are more long lived seeds. In contrast, germination traits have no significant relation to dry storage seed longevity compare to soil seed longevities. These results show seed traits could be used as traits to predict seed ex situ longevity.

Introduction

Ex situ conservation of seed germplasm is one of the challenges of the Global Strategy for Plant Conservation (Sharrock and Jones, 2009). It is, however, not only important for rare species conservation but also “a tool” for preserving genetic diversity of plants (Khoury et al., 2010). Successful re-establishment of extinct populations or establishing new populations of rare species may therefore depend on preserved seeds in gene banks (Godefroid et al., 2011). Despite large efforts worldwide (Guerrant et al., 2004), the understanding of the mechanisms of seed longevity in the air- dry storage is still limited.

Seeds can survive as seed germplasm storage up to several decades according to a standardized rapid ageing protocol (Ellis and Roberts, 1980). This protocol (Hay et al.,

2008; Newton et al., 2009; Probert et al., 2009) was developed to better estimate seed longevity in ex situ conditions. Time to lose 50 % germination (P_{50}) was suggested to compare seed ageing revealing seed survival variability from a day to several decades. Seed survival depends mainly on its physiology but may be also correlated to certain seed traits and the environment the seeds came from.

Storage moisture and temperature conditions affect seed longevity during storage (Ellis and Roberts, 1980, Pritchard, 2004). Seeds of species from cool, wet climates are more short lived in ex situ than species from dry, warm climate (Probert et al., 2009). Seeds from alpine species are more short lived than those of lowland species (Mondoni et al., 2011). However, except from biochemical/physiological traits such as seed protein, carbohydrate and oil content (Pritchard and Dickie, 2003; Horbowicz and Obendrof, 1994; Probert et al., 2009), the level of physiological maturity and environment conditions duing seeds development (Hay and Probert, 1995; Smith et al., 2003; Kochanek et al., 2009; Kochanek et al., 2010) and being endospermic or non-endospermic (Probert et al., 2009) no other seed (ecological) traits were correlated with seed longevity in dry storage. In contrast, other seed (ecological) traits may strongly affect soil seed bank longevity (Poschlod et al., 2013, Saatkamp et al., 2013). Although there are few studies which claim a correlation of ex-situ seed longevity to soil seed longevity (Bekker et al., 2003; Long et al., 2008; Schoeman et al., 2010) one might expect that the same traits correlated to soil seed bank longevity should be relevant for ex-situ seed longevity. Seed mass and seed shape (Bekker et al., 1998; Thompson et al., 1993), also seed coat thickness (Gardarin et al., 2010), seed germination traits (Grime, 1989; Milberg et al., 2000 Saatkamp et al., 2011b) seed dormancy (Baskin and Baskin, 2006; Thompson et al., 2003) can explain some soil seed persistence variation among different species.

One cause that these different longevity patterns were not found in the above mentioned studies is the fact that the studied species had their origin in different biomes and/or habitats. This becomes obvious from the fact that considerable variations in seed lots longevity from the same species could be shown during accelerated ageing (Kochanek et al., 2009; Schoeman et al., 2010) although other studies reported similarity between seed ageing for different collections within a species and even genus (Walters et al., 2005; Hay et al., 2006; Probert et al., 2009).

To overcome this problem and to eliminate environmental effects on seed longevity, species occurring within the same habitat may be studied. Since environmental parameters are more or less constant for species of the same habitat, most of seed variation should be explained by seed traits.

Until now, there is no study available on seed or other plant traits and seed longevity under ex-situ conditions for species from the same habitat. Furthermore, no study is existing where the effect of seed germination traits in seed ageing experiment was examined so far. Therefore, we examined seed longevity of eighteen species of different life history types from dry sandy grassland. We aimed at evaluating changes of seed longevity to understand the role of seed anatomical/morphological and seed germination traits in seed longevity patterns under ex-situ conditions. Thus, we asked the following question:

Is soil seed longevity and ex situ seed longevity related to each other?

Can seed traits and seed germination traits explain why species have different seed bank longevity in ex situ conditions?

Material and Methods

Study system

Dry sandy grasslands occur throughout Central Europe and Southern Germany where sand was deposited, mostly during and after the last ice age (Bork et al., 1998; Bateman and Godby, 2004). Sandy grassland host many rare and endangered plant species (Jentsch et al., 2009). Changing land use during the last century caused a decline of former sandy grassland to less than 1 % in southern Germany (Poschlod et al., 2009). Besides in-situ conservation plans for this habitat, ex-situ conservation of species also support the future conservation plan success.

Study species

We tested seed longevity of different species from sandy grassland under ex-situ conditions by ageing them with LiCl solution. 18 species were selected that represent typical and very common species of dry sandy grasslands according to the

phytosociological classification of South German vegetation (Korneck, 1978). Ripe fruits of each species were collected in the respective communities at different localities in Bavaria in summer 2010 (see Tab. 20).

Table 20 Overview of study species with respective locations of seed collections.

Species	Origin of seeds
Aira caryophyllea L.	Zenzing (Regentalhänge, Bavaria)
Arenaria serpyllifolia L.	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Corynephorus canescens (L.) P. B.	Siegenburg (Upper Palatinate, Bavaria)
Dianthus deltoids L.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Erigeron acris L.	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Erophila verna (L.) Chevall.	Bamberg (Upper Franconia, Bavaria)
Filago minima (SM.)Pers.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Helichrysum arenarium (L.) Moench	Kornburg, Nuremberg (Central Franconia, Bavaria)
Hieracium pilosella L.	Siegenburg (Upper Palatinate, Bavaria)
Hypochoeris radicata L.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Jasione montana L.	Kirchheim/Ries (Swabia, Bavaria)
Koeleria glauca (Spr.) DC.	Sandhausen (Karlsruhe, Baden-Wuerttemberg)
Onosma arenaria Waldst. & Kit.,	Mainzer Sande (Mainz, Rhineland- Palatinate)
Potentilla argentea L.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Scleranthus annuus L.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Sedum acre L.	Kornburg, Nuremberg (Central Franconia, Bavaria)
Spergula morisonii Boreau	Bamberg (Upper Franconia, Bavaria)
Teesdalia nudicaulis (L.)R. Br	Bamberg (Upper Franconia, Bavaria)
Trifolium arvense L.	Ramsberg (Middle Franconia, Bavaria)

Germination traits, seed trait and dormancy

Methods for measurement dormancy, germination traits and seed traits were explained in chapter 4.

Controlled aging

Millennium Seed Bank Project standard protocol for studying 'comparative seed longevity' was used for controlled ageing (Newton et al., 2009; Probert et al., 2009). In autumn 2010, glasses including 50 dry seeds per species were placed, without their lids, at 20 °C over a non-saturated LiCl solution (385 g L^{-1}; 47% relative humidity (RH)) inside a sealed 300 × 400 × 102 mm enclosure box (Ensto Cubo O ABS[ASYNTEK

Electrotechnik GmbH, Egesheim, Germany]). After 14 days of equilibration in the dark, one sample of each species was removed for a germination test and the remaining samples were transferred to a second electrical enclosure box for ageing experiment, at 40 °C over a non-saturated solution of lithium chloride (300 g L-1 ; 47% relative humidity(RH)). To maintain 60% RH, a sample of the LiCl solution from inside the box was measured regularly by use of a calibrated hygrometer (Hygropalm Aw1[Rotronic GmbH, Ettlingen, Germany]) and RH solution adjusted with addition of water. Seed sampled retrieved and germination were checked as mentioned for the germination experiments.

Seed germination test

For testing seeds after ageing treatments, the first samples were retrieved in autumn 2010. In the following days, other samples were retrieved after 2, 5, 10, 20, 30, 50, 75, 100, 125, 150 days and seeds of each glass were moved to the petri dishes. In petri dishes, 50 seeds were germinated on two 90-mm-diameter filter paper discs (Sartorius 3 hw). After filter papers were saturated with deionized water, dishes were placed in a climate chamber (day/night cycle 14 h/10 h; temperature 22°C/14°C) since the other germination experiments showed maximum germination rate at this treatment. In addition, we ran a test to account for possible physiological dormancy. Therefore, imbibed non-germinated seeds were stratified during 6 weeks at 4°C and germinated seeds were counted in similar climate chamber during 45 days afterwards. After 45 days viability of non-germinated seed was checked with a Tetrazolium test. Seeds were assessed as viable when both, embryo and endosperm were coloured red (ISTA, 1996).

Statistic

To compare species ageing, P_{50} (the time for viability to fall to 50%) were determined for each species. Values were calculated using a probit analysis in R statistical software (drc add-on package; Ritz and Streibig, 2005; R Foundation for Statistical Computing 2009). For graphical presentation species were divided in two rapidly ageing species (n= 9) and slowly ageing species (n= 9) according to the median value of 20.43 d. To find the relation between traits and P_{50} GLM were applied.

Results

Variation in p_{50} among species

In the ageing assays, species differed clearly in their seed longevities. Table 21 illustrates the contrasted patterns revealed by ageing experiments. P_{50} ranged between 6.2 d for *Erigeron acris* to 82.2 d for *Scleranthus perennis.* Although species were selected from same habitat, there were clear differences in P_{50} between short lived and long lived species with mean estimates for P_{50} of 14.9 d and 56.7 d, respectively.

Seed traits and P_{50}

Species also had variable seed mass from 0.01 g for *Filago minima* to 7 g for *Onosma arenaria*. There is a significant relation between seed mass and P_{50} (R- squared = 0.43, P= 0.002, Fig. 13). Seed shape Index also had a significant correlation to P_{50} (R- squared = 0.55, P= 0.0004, Fig. 13). Species with high seed shape index had longer lived seeds than species with lower. In addition, seed coat thickness correlated with P_{50} as well (R- squared = 0.47, P= 0.002, Fig. 13). Species with thicker seed coat had higher seed longevity(Tab. 21).

Table 21 P_{50} and Seed traits and soil seed bank longevity index for study species.

Species	P_{50}(d)	Longevity Index[1]	Seed mass(mg)[2]	Seed shape Index[2]	Seed coat thickness (µm)
Aira caryophyllea	43.1 ± 1.3	0.3	0.18	0.12	-
Arenaria serpyllifolia	32.9 ± 2.2	0.4	0.05	0.05	20.02
Corynephorus canescens	13.4 ± 0.8	0.4	0.12	0.13	12.34
Dianthus deltoids	19.1 ± 1.0	0.17	0.25	0.09	24.1
Erigeron acris	6.2 ± 1.0	0	0.14	0.15	15.68
Erophila verna	28.6 ± 2.5	0.32	0.02	0.08	13.62
Filago minima	14.2 ± 0.7	0	0.01	0.11	14.66
Hieracium pilosella	19.5 ± 1.0	0.08	0.1	0.13	25.76
Hypochoeris radicata	16.5 ± 1.6	0.09	0.05	0.15	20.38
Jasione montana	21.3 ± 1.2	0.03	0.02	0.12	17.48
Koeleria glauca	10.7 ± 0.7	0	0.10	0.12	10.08
Onosma arenaria	82.2 ± 7.5	-	7.00	0.05	154.1
Potentilla argentea	65.5 ± 2.2	0.4	0.09	0.06	66.34
Scleranthus annuus	63.8 ± 7.5	0.03	1.47	0.05	30.03
Sedum acre	27.8 ± 1.7	0.2	0.04	0.09	19.71
Spergula morisonii	16.5 ± 0.9	0.17	0.16	0.15	9.30
Teesdalia nudicaulis	17.8 ± 0.8	0.38	0.27	0.05	72.42

Trifolium arvense	55.3 ± 3.1	0.39	0.39	0.02	41.42

1 (according to Kleyer *et al.,* 2008); 2 (according to Jackel *et al.* 2006);

Seed germination traits and P_{50}

Results of germination test showed remarkable differences in germination speed by T_{50} (Tab. 22). Species speed varied from 1.7 day for rapidly germinating species like *Trifolium arvense* up to 15.3d for slowly germinating species like *Scleranthus perennis*. Species also had different light and temperature requirements. All species had a better germination in presented of light than constant darkness. *Potentilla argentea* only germinated in light. Species also had different temperature requirements. Some species such as *Sedum acre* germinated better under constant than fluctuating temperatures. In contrast, some species such as *Scleranthus perennis* or *Spergula*

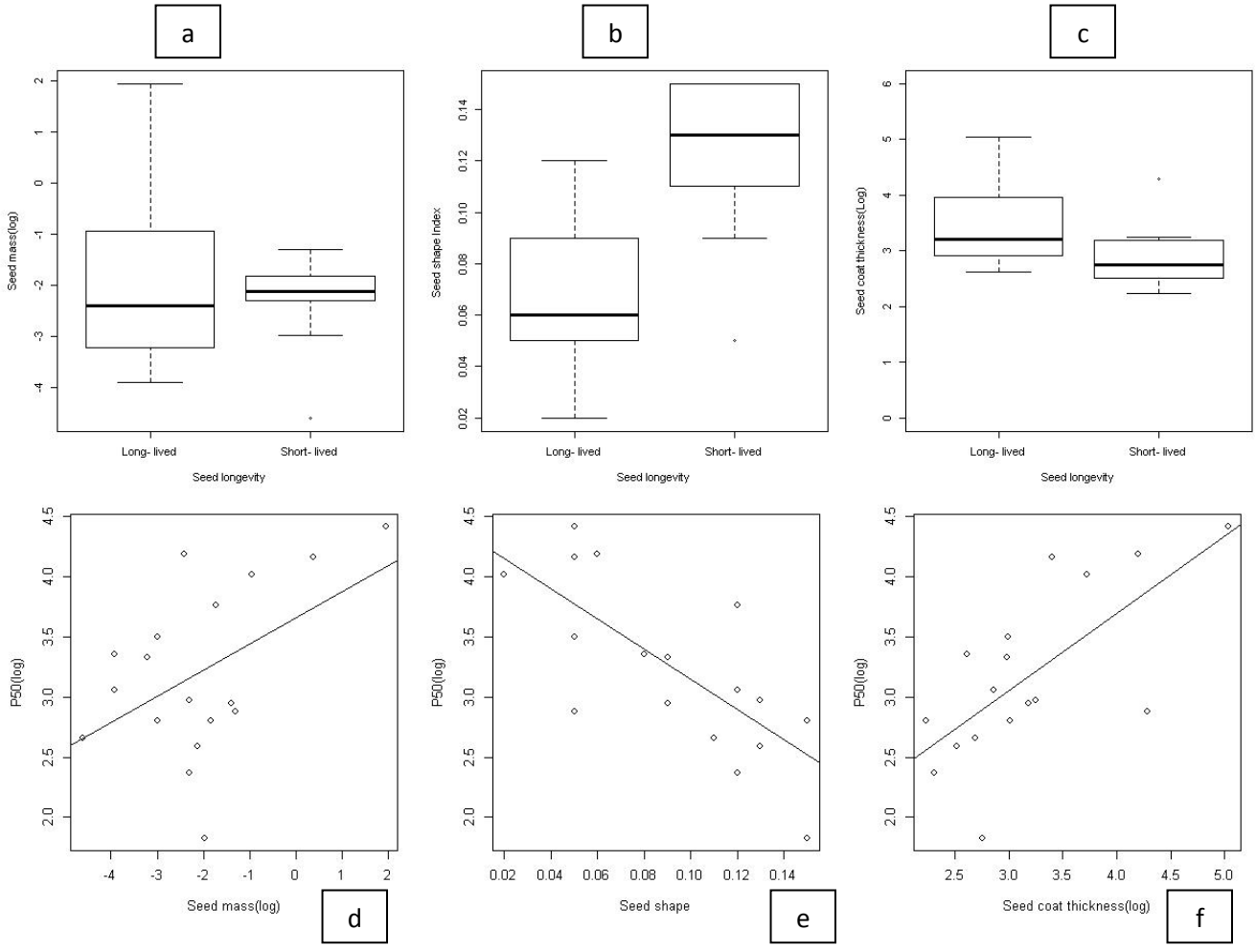


Figure 13 P_{50}(d) and seed traits- (Seed mass, seed coat thickness and P_{50} were log transformed). P_{50} significantly correlated to seed mass(R- squared = 0.43, P= 0.002), Seed shape Index (R- squared = 0.55, P= 0.0004) and seed coat thickness (R- squared = 0.47, P= 0.002).

morisonii have only germinated under fluctuating temperatures. No significant relation was found between seed ageing and germination speed (R-squared = 0.07, P=0.32, Fig. 14), ΔG_{light} (R-squared = 0.04, P=0.39, Fig. 14) and ΔG_{DFT} (R-squared = 0.16, P= 0.1, Fig. 14). There is a significant effect of dormancy on seed longevity (R-squared = 0.50, P=0.02).According to species seed dormancy classification, most of the short lived species are non dormant (92 %) compared to long lived species (22 %). Dormant seeds can survive longer than non dormant seeds (Fig. 14).

Table 22 relative germination rates for fluctuating temperatures (ΔG_{DFT}) and light requirement (ΔG_{light}) of the 18 studied species. ND indicate non dormant and D indicate dormant seeds.

Species	ΔG_{DFT}	ΔG_{Light}	T_{50}	Dormancy Types
Aira caryophyllea	-8.9	14.4	4.0	ND
Arenaria serpyllifolia	15.6	20.5	3.5	ND
Corynephorus canescens	7.9	6.8	9.2	ND
Dianthus deltoids	6.1	58.6	4.2	ND
Erigeron acris	-0.1	5.7	4.3	ND
Erophila verna	100.0	95.1	3.2	D
Filago minima	88.2	72.7	3.7	ND
Hieracium pilosella	7.1	4.1	4.2	ND
Hypochoeris radicata	-3.4	11.4	2.6	ND
Jasione montana	2.9	82.9	4.0	ND
Koeleria glauca	3.7	8.9	5.1	ND
Onosma arenaria	72.6	18.7	3.7	D
Potentilla argentea	-	100.0	4.2	D
Scleranthus annuus	100.0	33.3	4.0	D
Sedum acre	-34.8	84.9	3.9	D
Spergula morisonii	100.0	70.5	4.4	D
Teesdalia nudicaulis	74.3	70.4	4.1	D
Trifolium arvense	-	-	-	D

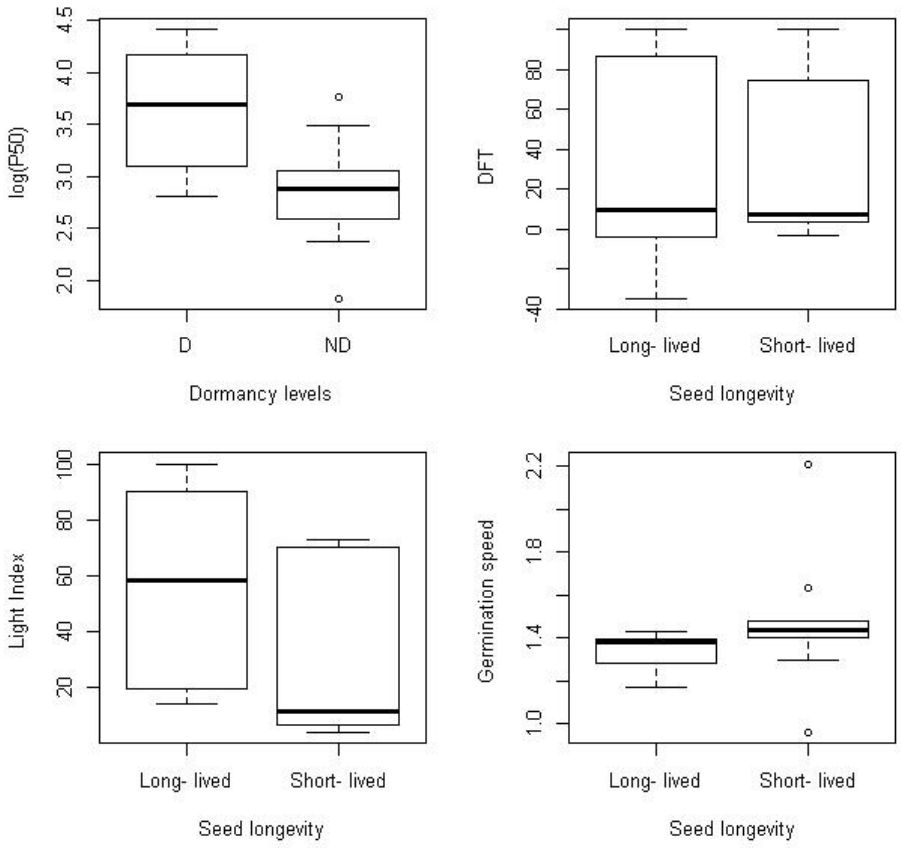


Figure 14 Both P_{50} (d) and seed germination traits. Both T_{50} and P_{50} was log transformed. ND indicate non dormant and D indicate dormant seeds. D indicate dormant and ND non dormant. DFT show temperature index

All traits and P_{50}

The GLM results reveal that among main factors only seed shape had significant effects on the seed longevity ($F = 12.4$, $P < 0.0001$, Tab. 23).

Table 23 Results of GLM for effect of seed mass, seed shape index, T_{50}, Δ_{DFT}, Δ_{Light} and dormancy types. Bold letters indicate significance. Seed coat thickness was excluded in model due to auto correlations.

Factors	*Estimate*	*SD*	*T value*	*P value*
Seed mass	0.124	0.151	0.822	0.430
Seed shape Index	-9.065	4.675	-1.939	***0.081***
T_{50}	-0.081	0.104	-0.781	0.452
Δ_{DFT}	-0.002	0.003	-0.732	0.480
Δ_{Light}	0.001	0.007	0.189	0.854
Dormancy types	0.241	0.509	0.474	0.645

Ageing and Soil seed bank

There is a significant relation between longevity index (LI) and P_{50} (R-squared = 0.41, P=0.005, Fig. 15).

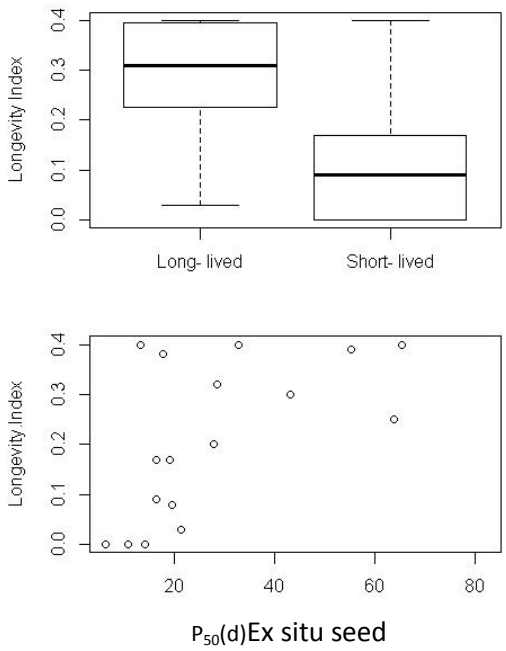


Figure 15 P_{50}(d) and soil seed bank longevity index (R-squared = 0.41, P=0.005).

Discussion

Species have different seed longevity

In this study, we have shown that seed traits can explain some variation in seed longevity under ex-situ conditions. Species have different P_{50} showing species seed longevity variation (Probert et al., 2009). Considering our own results, sandy grassland species have different seed longevities (Tab. 21): Some species can only survive a comparatively short while, mostly only some days such as *Erigeron acris*. By contrast, seed survival of some species can be over up to 80 days such as for *Scleranthus perennis*. The mechanisms that bring about such species differences are thus far unknown.

Seed traits explain ex situ seed longevity

Different non-exclusive explanations are discussed as sources of seed longevity variation in ex situ conditions. On the one hand, there are environmental factors mainly air moisture and temperature (Ellis and Roberts, 1980; Probert et al., 2009), on the other hand there are species- specific attributes. To study the effect of environmental factors with different air moisture and temperature, it is not clear how the influence of environmental factors and effects of species- specific attributes on seed longevity can be separated. Only with comparative ageing experiment we can find out the role of species-specific attributes in seed longevity where temperature and moisture are constant. Therefore here we explained the influence of species-specific factors in seed longevity variations. As indicated in the results, Seed traits themselves may affect seed longevity or are at least correlated with.

The fact that seed traits affect seed longevity suggests that in the ex situ conditions species-specific attributes can define seed longevity. This result is consistent with studies that show small (and rounded) seeds have longer seed persistence in soil seed banks (Bekker et al., 1998; Thompson et al. 1993). Gardarin et al., (2010) also showed that species with thick seed coat have a longer soil seed persistence. Seed germination traits have an influence in soil seed persistence (Grime, 1989; Milberg et al., 2000 Saatkamp et al., 2011b), but no clear affect in seed longevity in ex situ conditions. As an explanation, seeds have seed germination traits as an mechanisms to react to variable soil conditions, light avalilability and tempreture flactuations (Saatkamp et al., 2013). However, in dry storage which environmental conditions are constant seed germination play not any roles.

Relation between seed traits and seed ageing can be used as a functional traits for explaination of diffrenet seed longevity in dry storage. Positive correlation between soil seed bank and seed longevity in ex situ conditions (Fig. 15) shows that seed longevity in the soil can be estimated with accelarated ageing test. But due to ecological complexity of soil conditions, rely only on accelrated ageing result with simple and constant environmental conditions can fail to estimate seed longevity. seed ageing classifications may be useful for main classification of species according to their longevity.

Chapter 6

Conclusions and perspective

Sandy grassland and seed ecology

Several studies have shown different seed ecological patterns in local habitats and worldwide, the mechanisms underlying these patterns and also tradeoffs between seed ecological traits (Baskin and Baskin, 1998; Thompson and Fenner, 2000; Wang and Smith, 2002; Vander Wall and Longland, 2004; Poschlod et al., 2013; Ch. 2-5). Considering the important role of seed ecology in ecosystem understanding, managing and restoring (Bakker et al., 1996; Willems and Bik, 2009), it is necessary to interpret the seed ecological studies in terms of environmental conditions of certain habitats (Fig. 16).

Habitat physical heterogeneities, disturbance and seeds mechanisms

Different environmental triggers like precipitation and temperatures can start the seed ecological cycle in the habitat (Thompson, 1969; Thompson et al., 1977; Baskin and Baskin, 1998), which could be modified by habitat conditions. Sandy grasslands are a heterogeneous habitat including both open soil gaps and vegetation patches. Open gaps cause fast drying soil surface and high temperature fluctuations (Jeckel, 1984; Jentsch, 2001), which vegetation patches would intercept water and modify soil moisture and temperatures. These conditions strongly influence seed bank, dormancy and germination (Bewley and Black, 1994; Probert, 2000; Ludwig et al., 2005). Species with different mechanisms like those creating persistent seed banks for small sized seeds, faster germination speed, gap detection mechanisms and the development of physiological dormancy may tolerate sandy grasslands habitat conditions.

(Ch. 4). In addition to this, also successional stages (Jentsch and Beyschlag, 2003; Beyschlag et al., 2008) and management treatment like grazing and military activity strongly may influence sandy grassland heterogeneity (Jentsch et al., 2002; Jentsch et al., 2009; Tschöpe and Tielbörger, 2010; Faust et al., 2011a; Faust et al., 2011b; Ödman et al., 2012).

Habitat biological conditions and seed reserves fill up and depletion

Species can build up stores in the soil, the soil seed bank with both dormant and non-dormant seeds. These stored seeds from previous seasons play an important role in vegetation dynamics and restoration (Bakker et al., 1996; Bossuyt and Honnay, 2009). The mechanisms of species to build up storages has been explained as storage and bet hedging mechanisms (Warner and Chesson, 1985; Facelli et al, 2005; Venable, 2007; ch. 4). In addition, environmental factors may strongly affect seed dormancy and soil seed bank. In the case of seed persistence, soil moisture was identified as the strongest factor (Chp. 3). Seed bank depletion may happen through microbial activity, and even here soil moisture plays the major role (Mordecai, 2012). Post-dispersal predation is a second factor in seed bank depletion (Wang and Smith, 2002; Vander Wall and Longland, 2004; Wagner and Mitschunas, 2008). If the amounts of moisture, nutrients, light and temperatures are favorable to initiate germination (these amounts are referred to as a *critical germination threshold*, Baskin and Baskin, 1998), there may be an addition to seed production (Ludwig and Tongway, 2000). Species have different requirements concerning light and temperatures (Ch. 4) which understanding these germination requirement benefit researchers for species establishment. If species succeed in establishing and reproducing after germination, then produced seeds can fill up the soil seed bank. In addition, seeds that lead to established plant may increase diversity and density of

vegetation patches and modify microbial and chemical habitat conditions which again influence seed bank and germination (Beyschlag et al., 2008).

Implications for restoration and management

In grassland restoration projects, different techniques are applied depending on the costs and questions (Török et al., 2011). In order to successfully implement restoration and conservation projects several factors like habitat conditions and seed availability should be considered (Eriksson and Ehrlén, 1992; Roelofs, 1996; Falk et al., 1996; Matus et al., 2003; Münzbergová and Herben, 2005; Bakker et al., 2006; Clark et al., 2007). Considering the results of the thesis at hand, a number of seed ecological aspects could be applied. First, understanding the mechanisms of seed adaptation to habitat heterogeneities, disturbance and chemical environmental factors can help restoration planners to predict species response to considered habitat modifications and also introduce the suitable species (Falk et al., 1996). For instance, response of species to high available aluminum in acidic soil (Ch. 2; Olsson et al., 2009; Hydbom et al., 2012), germination mechanisms in gap detection (Ch. 4) and species reaction to soil moisture variation (Ch. 3) can show which species are sensitive to certain environmental conditions. Second, understanding the dormancy and seed persistence of species in habitat (Ch. 4; Matus et al., 2005; Bossuyt et al., 2007) and also patterns and mechanisms behind it (Ch. 3-4) can help planners to evaluate the seed reserves conditions. Finally, seed ecophysiological studies can provide information to select species that can successfully establish in target habitats. Such information can also be used for interpretation of restoration failures like scarce species establishment in low pH soils (Ch. 2)

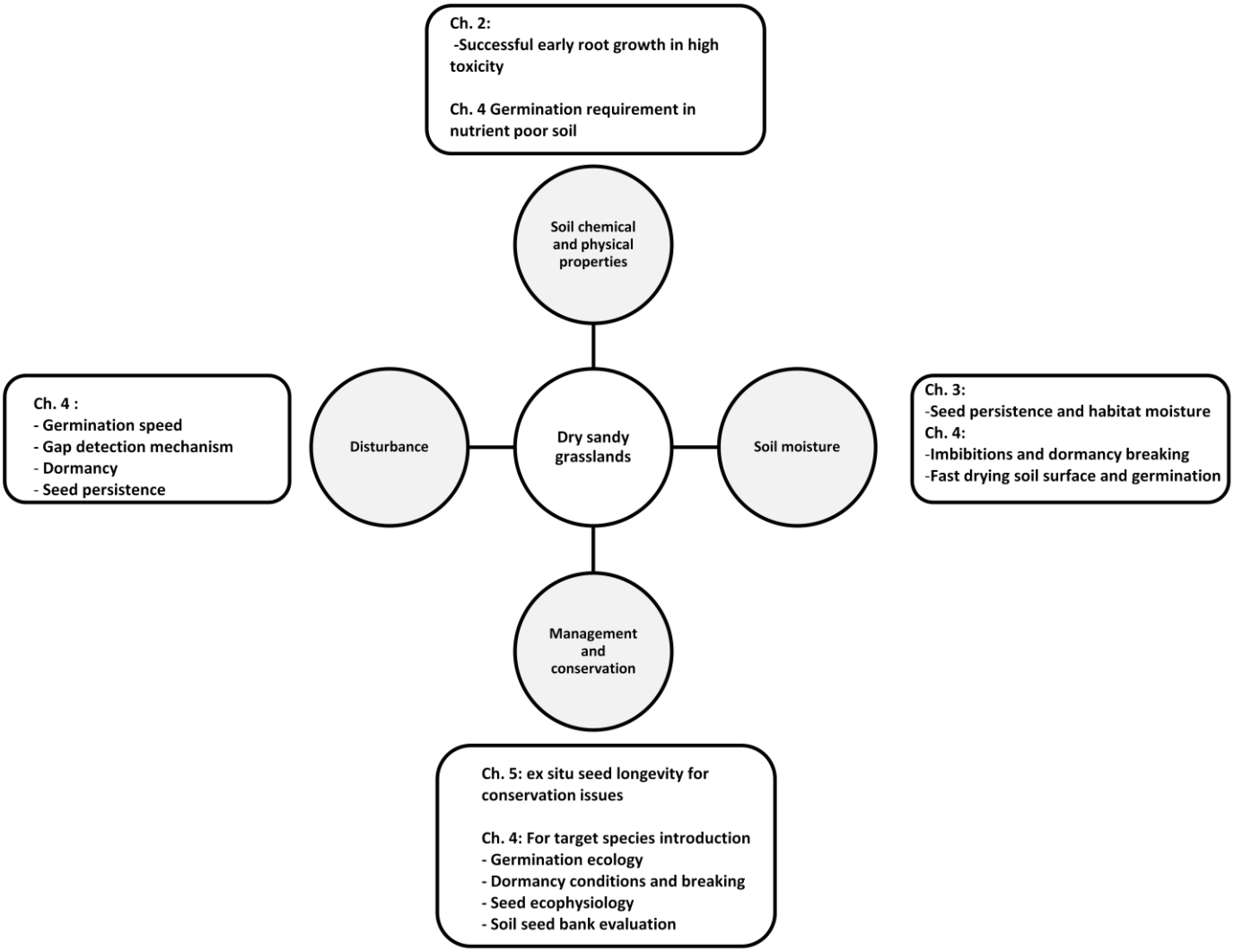


Figure 16 Driving environmental factors in dry sandy grasslands (Grey background) and related seed ecological traits. These traits indicate the role of seed ecological traits in local assembly of dry sandy grasslands. Seed traits can act as limiting factor for occurrence of species or strongly influence on species coexistence inside the habitats.

Seed ecological protocol for linking seed ecology and community assembly

Why certain aspects of seed ecology were not yet considered as functional traits in ecological research? First, there is not enough data on seed ecological traits (Poschlod et al., 2013). Furthermore, because of the lack of comprehensive protocols, even available data are not

satisfactorily comparable. Available databases like LEDA, SID, Biopop (Kleyer et al., 2008; Liu et al., 2008; Jackel et al., 2006) prepared comparable data for seed morphological traits, seed persistence and seed dispersal, but seed germination traits and dormancy are still descriptive and not comparable. Seed ecological traits in the data base developed by Poschlod et al., (2003) also are not yet incorporated in a worldwide protocol. This limitation not only is evident in data bases but also in comparative studies which studied seed ecology of different species in certain habitats (Grime et al., 1981; Bell et al., 1993; Commander et al., 2009; Schwienbacher et al., 2011). These studies produce valuable data for understanding patterns, mechanisms, and they give a general overview but have no *indices* for seed germination traits that can be transferred to other habitats and be used for community assembly and comparisons.

Determination of fast and easy measurable methods like methods for aluminum toxicity (ED50, ED95; Ch. 2), P_{50} (Ch. 5; Probert et al., 2009), seed germination traits for light and temperatures and germination speed (ΔG_{light}, ΔG_{DFT}, LTG; Ch. 4), or optimum temperature for germination ($T_{min5,}$ Rosbakh unpublished data) can create comparative seed germination data bases. These traits could be used for community analysis and for searching assembly rules in a certain habitat and on a global scale as well. Although each research project mainly needs special indices depending on question and precisions, it is necessary to find agreements among seed ecologist about seed ecological traits to create worldwide seed ecological data bases. Also the complex aspects of seed ecology like dormancy and persistence could be better explained with simple seed ecological traits (Saatkamp et al., 2011; ch. 4). Different abiotic or biotic conditions can filter occurrence of species in dry sandy grasslands, here we showed how early root growth trait can explain the occurrence of species along a pH gradients inside the

sandy grasslands. Application of other seed ecological traits along environmental gradients such as altitude gradients, moisture gradients, grazing gradients is promising.

Perspective

Results from the thesis at hand described a number of aspects of seed ecology in dry sandy grasslands. However, some further details could also be studied to continue our research and to improve our knowledge of dry sandy grasslands. In chapter 2, we tested ecological filtering of acidic soil simulated with aluminium toxicity. It would be also interesting to analyse seed ecology of species occurring in high pH values and study the filtering effect of calcareous soil for acidic soil species. There are also not yet any comprehensive seed germination studies concerning reproduction strategies along pH gradient. In order to find out the mechanisms of species adaptation, vegetation traits along pH gradients could be tested as well. It is necessary to know how seeds persist in the soil along pH gradients mainly under high toxicity conditions. In chapter 3, we tested interactions of soil moisture and types, but studies on interaction of soil types and microbial activities in seed longevities would also be necessary to find out which role microbial activities play in different soil types and nutrient conditions. In addition, we need to apply these interactions analyses also on other seed ecological traits like dormancy and seed production to interpret the role of environmental factors in seed ecological patterns. In chapter 4, we tested the germination ecology of sandy grasslands, but some other ecological questions like reaction of species to PEG for simulation of drought resistance and also germination along temperatures gradients to find out minimum germination temperatures is necessary to figure out the mechanisms of species adaptation in dry sandy grasslands. In chapter 5, we showed the relation of seed traits and seed germination traits to seed longevities in dry soils, but still information on chemical composition of seeds such as oil and protein

content for most of species is missing. In addition, with measuring more species, the patterns found in this study could be validated or generalized.

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Appendix

		LI		
Factors	***Estimate***	***SD***	***T value***	***P value***
T50	0.49	0.46	1.06	0.28
Light	0.07	0.04	1.59	0.11
Dormancy	-0.39	1.93	-0.20	0.83
Seed mass	1.18	2.41	0.49	0.62
Seed shape Index	3.52	22.2	0.16	0.87
Seed coat thickness	0.17	0.15	1.16	0.24

Table 24 Result of general linear model with binomial distribution for LI (longevity index) and seed traits

		Dormancy		
Factors	***Estimate***	***SD***	***T value***	***P value***
Flowering end	0.69	0.97	0.71	0.47
Light	0.15	0.12	1.22	0.22
T50	-0.23	0.24	-0.98	0.23
Seed mass	14.50	9.72	1.50	0.13
Seed shape Index	-59.08	49.62	-1.04	0.23
Seed coat thickness	-0.05	0.05	-1.13	0.25

Table 25 Result of general linear model with binomial distribution for Dormancy and seed traits

		T_{50}		
Factors	***Estimate***	***SD***	***T value***	***P value***
Persistence	1.90	1.95	0.97	0.35
Light	-0.02	0.03	-0.40	0.65
Dormancy	-1.94	2.14	-0.90	0.38
Seed mass	0.65	3.24	0.20	0.84
Seed shape Index	-39.85	27.6	-1.44	0.17
Seed coat thickness	-0.11	0.05	-2.00	***0.06***

Table 26 Result of general linear model for T50 for Dormancy and seed traits

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