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REGULAR ARTICLE

Effects of substrate type, moisture and its interactions on soil seed survival of three *Rumex* species

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Abstract
Background and Aims Seed bank persistence plays a highly relevant role for population dynamics. The impact of interacting environmental factors on seed longevity has only scarcely been investigated. We aimed to analyse the effects of varied soil substrate type and moisture on soil seed survival.
Methods Seeds of three *Rumex* species native to different habitats were buried in pots placed in open-air basins. The factors substrate (sand, loam, mud), water table depth (WTD; high, intermediate, low), time, and their interactions were investigated. Viability was tested after 6, 12, and 18 months.
Results Seeds of *R. acetosella* (dry habitat) were short-term persistent with highest survival in low WTD on sand. Survival in *R. acetosa* (moist habitat) was very strongly reduced after 6 months with highest survival under wet conditions. *R. maritimus* (wet habitat) had overall long-term seed survival, where 'substrate type' had the strongest impact. Significant interactions of 'substrate type' and WTD were detected.
Conclusions Seed bank longevity is not a fixed species trait, but varies with environmental factors. Soil moisture, substrate type and their interactions have different effects on the studied species. Persistence-classifications ought to consider the impact of environmental factors.

Keywords Soil seed bank persistence · *Rumex acetosella* · Soil substrate · Water table depth · Seed burial · Longevity index

Abbreviations

WTD	Water table depth
DFT	Diurnally fluctuated temperature
CAL	Calcium lactate
GLMM	Generalized linear mixed model

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. 2011a, b). Long term establishment of species regenerating from seeds may even depend strongly on soil seed survival under specific habitat conditions (Poschlod et al. 2013). 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

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classification and also introduced the Longevity Index to classify seed persistence in the soil. Neither the Longevity Index of a species nor their seed bank classifications are entirely consistent traits (Thompson et al. 1997; Kleyer et al. 2008). It appears that both the origin of the examined seeds as well as study conditions have impacts on longevity and seed bank survival. Most species, even some which are commonly classified to the transient soil seed bank type, 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 could well be due to seeds responding differently in their longevity to different environmental conditions. On the other hand, this might be caused by methodological reasons. 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). The seedling emergence method is an indirect method which exposes soil samples which were collected mainly at one single date to 'favorable' conditions for germination in order to identify and count seedlings. This method is prone to influences from various factors, namely dormancy (Thompson and Fenner 2000), the low seed production often found in rare species (Thompson and Grime, 1979), vertical distribution of seeds (Bekker et al. 1998b), successional stage (Espinar et al. 2005; Erfanzadeh et al. 2010) and also timing of sampling (Saatkamp et al. 2009). Seed burial experiments may have their own methodological constrains. Survival could depend on the depth seeds were buried (Saatkamp et al. 2011a), but also on soil physical, chemical and biological conditions varying along a successional sere. These factors can impact on germination results, the interpretation of which can lead to differences in classifications of seed bank types. 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 differently impacted upon 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).

Seed longevity in the soil may be affected by different parameters. First, there may be effects of species-specific attributes. 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. 2011a, b). Secondly, environmental conditions may strongly affect the longevity of the soil seed bank. Soil seed longevity is associated with soil microbial activity (Schafer and Kotanen 2003; O'Hanlon-Manners and Kotanen 2006; Wagner and Mitschunas 2008; Dalling et al. 2011; Mordecai 2012) 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;). 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 (Schafer & Kotanen 2003, Long et al. 2009). Therefore, the results of previous studies cannot explain the interaction between environmental factors on seed longevity.

We studied the impacts of soil type and moisture on soil seed survival of three *Rumex* species with different seed bank classifications 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). Soil seed bank persistence data of all of *Rumex acetosa*, *R. acetosella,* and *R. maritimus* contained entries for transient, short-term and long-term persistent in the different data bases. We aimed at an evaluation of changes in species seed longevity when soil conditions are varied from 'similar' to 'dissimilar' as compared to the species native habitat and therefore varied soil moisture and soil types/properties in a factorial design. We hypothesize that inconsistent seed bank categories assigned to certain species are in part due to variable habitat conditions at different sampling sites. We also hypothesize that soil seed longevity of a species is highest under conditions most closely resembling conditions in its

natural habitat. Thus, for our example of three *Rumex* species, we asked the following questions:

> Can differences in soil properties and soil moisture conditions explain why *Rumex* species are assigned to a variety of 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 (*R. acetosa, R. acetosella, R. 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 Table 1).

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 setup consisted of a total of 216 pots (5 l) 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, 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 1 cm brim at the top) with either of three soil substrates (sand, mud, loam), which were collected in the respective habitats of the examined *Rumex*-species (Table 1). Soils were not sterilized in order to retain their (micro-) biological characteristics. In each pot 25 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). Seeds were contained in small sewn nylon bags (5×6 cm, one bag per species, see Saatkamp et al. 2011a, b) 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. The 'high WTD'-treatment can be considered to

Table 1 Habitat descriptions of localities (all in Bavaria, Germany), where seeds and soil substrates were collected

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

produce anaerobic conditions for the buried seeds (Oomes et al. 1996). The experiment was set up in autumn 2009, when freshly ripened seeds of all species were available. The time steps of seed retrieval were i) April 15th 2010 (6 months after the onset of the experiment), ii) October 15th 2010 (12 month) and iii) 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 fluctuating temperature (DFT) (day/night cycle 14 h/10 h; temperature 22 °C/14 °C) in both light/dark alternation and in constantly dark conditions which have been shown to be suitable germination conditions for all species. Germination of seeds was recorded during 45 days. Seeds were considered germinated when the radicle had protruded at least 1 mm. After 45 days viability of non-germinated seeds was checked by a Tetrazolium test. Seeds were assessed as viable when both, embryo and endosperm were coloured red (ISTA, 1996). For testing seeds after burial treatments, all seeds were sterilized in their nylon bags for two minutes with sodium-hypochlorite (5 %) and were removed from the bags to petri dishes for germination tests. All obviously dead (empty or mouldy seeds) were removed. For all potentially viable seeds first the germinability was tested using the DFT regime at light/dark alternation as described for the fresh seeds. After 45 days, all non-germinated seeds were then stratified for six weeks at 4 °C and afterwards moved to conditions as described above to examine germination over a time period of another 45 days. Finally, for all remaining non-germinated seeds the Tetrazolium test was applied. The stratification treatment was not necessary in the case of *R. acetosa* and *R. maritimus*. Seeds of both species were always non-dormant.

Soil analysis

Soil moisture in pots was recorded regularly (20 times in regular intervals, n=5) during the vegetation period 2010 by use of a moisture meter (Theta Probe ML2x, Delta-T Devices Ltd, UK). Soil chemistry was analyzed when first samples were exhumed in 15 April 2010. The dried and sieved samples were extracted using the CAL method (calcium lactate) and analyzed for phosphorus contents (Thermo Spectronic UV1), as well as for potassium, sodium and magnesium contents using an AAS (Solaar Atomic Absorption Spectrometer Thermo Elemental).

Conductivity (of water extracted samples) and pH (both of water extracted samples and $CaCl_2$ extracted samples) were measured with the WTW Multi 340i Set (WTW GMBH, Weilheim, Germany) using the probe WTW Tetracon 325 for conductivity and WTW Sentix 41–3 for pH. 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, Tables 5 and 6) were analyzed with factorial ANOVAs in a generalized linear mixed model (GLMM) 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).

Results

Germination of untreated seeds

Rumex species had different germination patterns (Table 2). Seeds of all species had high viability (92–99 %). Both *R. maritimus* and *R. acetosella* germinated only in lighted conditions (99 % germination as compared to 0 % germination in dark conditions and 37 % germination as compared to 3 % germination in dark conditions, respectively). By contrast, *R. acetosa* germinated in darkness as well as light treatment (97 % germination as compared to 80 % germination in dark conditions).

Table 2 Overview of seed germination characteristics 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[i]	Seed mass (mg)[ii]	Seed bank types[ii]	Longevity index[i]
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

i (according to Kleyer et al., 2008); ii (according to Thompson et al., 1997; Kleyer et al., 2008)

Soil analysis

The three substrates used in this experiment had different physicochemical properties and overall moisture contents. The used sand and loam differed distinctly concerning nutrient contents, since the loam contained roughly by an order of magnitude more nitrogen, phosphorus, potassium and magnesium (Table 3). The nutrient content of the muddy substrate lay between those of the sand and the loam, with the exception of N-contents and C/N-ratio, which were similar between the loam and the mud substrate. Concerning pH, the muddy substrate was more acidic (pH around 4), while both the used sand and loam were close to neutral or very slightly acidic. Soil moisture varied considerably with substrate and WTD (Table 4). On average the sand substrate had the lowest water contents in each respective WTD, followed by loam and the mud substrate. Likewise, the minimum moisture content (after 25 consecutive dry summer days) was lowest in sand followed by loam and the mud substrate, and the max. water contents brought similar results. Concerning the overall effects of WTD, the step in water contents from the low to the intermediate WTD was about 100 to 200 % increase depending on substrate and about 12 to 29 % increase from intermediate to high WTD. Also, due to the effect of irregular precipitation, soils in low WTD had more pronounced variation in soil moisture than the other soil substrates (Table 4).

Effects of soil substrate and WTD on soil seed longevity

The multi-way ANOVA results reveal that all main factors ('species', 'WTD', 'soil type', 'duration of burial') and their interactions had significant effects

Table 3 Chemical properties of the three used soil substrates: sand, loam, muddy substrate. Soil was sampled from the experiment 6 months after its onset when first samples were exhumed (means±SE for n=3). BDL=below detection limit

Chemical and physical properties	Sand			Loam			Muddy substrate		
WTD	Low	Inter-mediate	High	Low	Inter-mediate	High	Low	Inter-mediate	High
pH in 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 in $CaCl_2$	5.7±0.0	5.6±0.0	5.7±0.0	5.9±0.1	6.1±0.1	6.4±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	90.0±42.5	26.3±9.2	64.0±33.8	493.0±159.3	482.0±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
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	/	/	/	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 4 Soil moisture (m^3 m^{-3}, means ± SE for n=8) of the three soil substrates: sand, loam, muddy substrate. Measurements were carried out 20 times in regular intervals during the vegetation period 2010

	Sand			Loam			Muddy substrate		
	Low	Inter-mediate	High	Low	Inter-mediate	High	Low	Inter-mediate	High
Min	0.03±0.01	0.35±0.01	0.38±0.00	0.07±0.01	0.38±0.01	0.46±0.01	0.09±0.03	0.43±0.01	0.52±0.01
Max	0.23±0.02	0.47±0.01	0.54±0.01	0.32±0.01	0.51±0.01	0.63±0.01	0.36±0.02	0.65±0.03	0.79±0.04
Average	0.13±0.01	0.40±0.01	0.45±0.01	0.20±0.02	0.43±0.01	0.54±0.01	0.24±0.02	0.51±0.01	0.66±0.02

on soil seed longevity (Table 5). According to their high F-values, factors that most strongly influenced seed longevity were 'species' (F=3855.86), 'duration of burial' (F=254.20) and 'species × duration of burial' (F=107.97), as well as 'species × WTD' (F=79.82, Table 5). Although 'soil type' had a significant effect on viability, its effect was less pronounced than that of other factors.

The above multi-way ANOVA with 'species' as a factor merely gives general information, while the following ANOVA analysis are carried out for the three *Rumex*-species individually (Table 6). Seed viability values varied strongly between species and, as an overall assessment, were reduced along the moisture gradient from dry to wet conditions (Fig. 1). Viability of *R. acetosella* seeds was strongly affected by 'duration of burial' (F=289.63, Table 6) and 'WTD' (F=122.52, Table 6). Seed viability was slightly reduced along the moisture gradient after 6 months (Fig.1a), but showed a clear and significant decline especially in wet loam and wet mud after 12 and after 18 months of burial (Fig. 1b, c). Dry mud substrate sustained the highest proportion of viable *R. acetosella* seeds (Fig. 1c). In *R. acetosa* 'duration of burial' (F=80.35, Table 6) and 'WTD' (F=27.75, Table 6) and their interactions had strong effects on soil seed viability. Seeds retrieved after 6 months of burial showed viability significantly increasing with soil moisture (Fig. 1d). Over the entire course of the experiment, viability strongly decreased. While after 12 months viable seeds were still found (Fig. 1e), there were nearly no viable seeds left after 18 months in all conditions (Fig. 1f). Time of burial (F=80.35, Table 6), moisture (F=27.75, Table 6) and their interactions had the main effect on soil seed viability. In the case of *R. maritimus,* 'duration of burial' was not significant (Table 6) and seed viability was generally higher than 80 % in all treatments and time steps (Fig. 1g–i). Only 'soil type' (F=15.32, Table 6) and interaction of 'soil type' x 'WTD' (F=3.57, Table 6) had a significant effect on seed survival, which mainly shows up in muddy substrate in the high water level treatment, where viability as compared with the other treatments is reduced (Fig. 1g–i).

Table 5 Results of multi-way ANOVA for effects of 'species', 'water table depth' (WTD), 'soil type', and 'duration of burial' on seed viability. Total variation in seed viability explained by this model was 95 %

Factors	df	*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**

Discussion

Species-specific attributes and environmental factors affecting soil seed survival

The aim of this study was to explore the impact of environmental factors (WTD, soil type, and their interactions) on survival of three different species. Clearly, before the impact of environmental factors can be discussed, we need to first consider in how far the

Table 6 Results from multi-way ANOVA performed individually for the three *Rumex* species for effects of 'water table depth' (WTD), 'soil type', and 'duration of burial' on seed viability. (Bold values indicate statistical significance)

Species	Variation explained by the model (R^2)	Factors	*df*	*F-value*	*P-value*
R. acetosella	0.87	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	0.67	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
R. maritimus	0.24	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

examined species have certain seed and germination traits that might affect soil seed bank persistence. Clear differences could be found for germination properties, but not for other seed attributes (see Table 2). Especially species reactions to light can explain soil seed longevity (Grime 1989; Saatkamp et al. 2011a), and the fact that *R. acetosa* germinates in darkness (Table 2) and therefore, also germinated in the soil is an 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 (Table 2). Hence, these traits have, in this particular case, no explanatory power for the species soil seed longevity, although on a more general basis they are claimed by Bekker et al. (1998b) to be important traits to understand soil seed bank longevity.

Considering our results on the impact of environmental factors, WTD is, along with species-specific attributes, a strong factor impacting on seed longevity in the soil (F=79.82, Table 5). In addition, 'Soil type' had significant effects and intensely cross-interacted with WTD (Tables 5 and 6), supporting the thesis of a high importance of WTD. This confirms that under experimental and likely under natural conditions not only species-specific attributes but also environmental factors define soil seed longevity.

Habitat-specific soil moisture limiting 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 may buffer against a population's extinction, we may ask the question whether a species' seed bank persistence is highest in soil conditions a species encounters in its main natural habitat (Dalling et al. 2011). The answer to this appears to be twofold and to depend on species. In the case of 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 is not a limiting factor for the species'

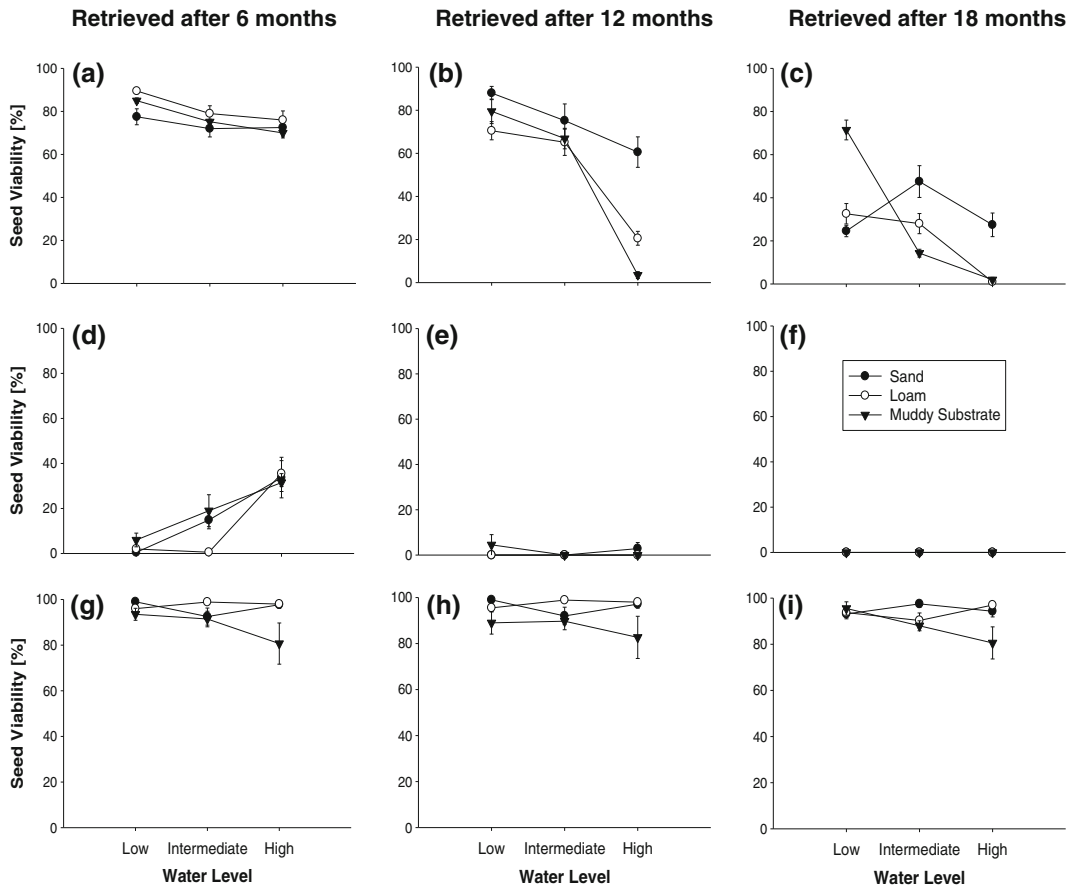

Fig. 1 Seed viability values of three *Rumex* species (means ± SE, *n*=8) in different combinations of soil substrate x water level. *Upper row* (panels **a**–**c**): *R. acetosella*; *middle row* (panels **d**–**f**): *R. acetosa*; *lower row* (panels **g**–**i**): *R. maritimus*. Seeds were retrieved from soil after different duration of burial. Within rows, reading from *left* to *right*, duration of burial was 6 months (winter season), (panels **a**, **d**, **g**), 12 months (panel **b**, **e**, **h**), and 18 (panels **c**, **f**, **i**). The extend to which the underlying multi-way-ANOVAs on the included factors explained total variation in seed viability was species dependent and can be found in Table 6

occurrence. In such cases habitat conditions cannot play a pivotal role in seed survival. Thus, other factors like germination, establishment of seedlings from fresh seeds, or further ecological parameters also limiting the niche of the adult plant may play more important roles for the occurrence of non-sensitive species. On the other hand, in the case of species sensitive to environmental factors (*R. acetosella* and *R. acetosa*), the outcome is different. High WTD acts as a limiting factor for the survival of seeds of *R. acetosella*, but it does increase soil seed survival for *R. acetosa*. This result suggests that seeds of species from dry habitats can only scarcely persist in wet soils and likewise species from wet habitats can only scarcely persist in dry soils. This interpretation is consistent with studies showing that seed survival of species from dry habitats is diminished at higher soil moisture. Seeds susceptible to high soil moisture are thought to suffer from deleterious fungi and bacteria that are favored under these conditions (Blaney and Kotanen 2001; Schafer and Kotanen 2003; Dalling et al. 2011). In even wetter soils, conditions become anoxic so that microbial activity is inhibited and cannot have the effects described above (Griffin 1972). It is assumed that for certain species anoxic conditions disrupt metabolic processes and thus cause high seed mortality (Bekker et al. 1998d). This appears to be the case for the drought-adapted *R. acetosella*. By contrast, *R. acetosa*, which occurs in wetter habitats than *R. acetosella,* showed higher seed survival in moist soils and also in the presumably anoxic conditions of high WTDs. Seeds of species from wet habitats may better tolerate anoxic conditions (Skoglund and Hytteborn 1990; Murdoch and Ellis

2000; Poschlod et al. 1996; Oomes et al. 1996), which can be attributed to adaptations allowing their basic metabolism to endure (Bekker et al. 1998d). Conversely, such species may be more susceptible to high oxygen levels in dry soils (Hendry 1993; Bahin et al. 2011) or simply to drought (Schütz 2000).

Interaction of environmental factors

The interaction of different habitat conditions with soil seed survival is not yet fully understood. This is partly due to experimental restrictions as already mentioned in the introduction. Results from field observations are often hard to interpret, because various environmental factors depend on each other and are hard to vary individually (Voesenek and Blom 1992; O'Hanlon-Manners and Kotanen 2006; Davis et al. 2005; Pakeman et al. 2012). While 'substrate type' was found to have no effect in a study by Long et al. (2009), soil type did have considerable effects in our study (significant for both *R. acetosella* and *R. maritimus*). In *R. maritimus*, 'Soil type' interacted with WTD, and seed survival in muddy substrate x high WTD was reduced in comparison to any other 'WTD x substrate type' combination (see also Fig. 3). While this is in contrast to our hypothesis that seeds can survive better under those conditions where the species occurs, it still demonstrates that interactions of environmental factors need to be included in the study of seed bank longevities.

Methodological concerns of soil seed bank persistence classifications

Soil seed bank persistence is not a fixed trait, even within each individual species, but varies strongly from transient to long-term persistent in different studies (Table 2; 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. 2). By contrast, in the case of *R. maritimus*, soil seed survival can last very long, over 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. 1, Table 6). However, both *R. acetosella* and *R. maritimus* have been assigned to the same Longevity Index according to Kleyer et al. (2008).

Therefore, edaphic conditions and especially soil moisture should be taken into account when classifying species seed banks, especially if this classification is based merely on a few references reporting on the species. For Central Europe, for instance, this could be done by using vegetation relevées from the soil seed bank sampling sites and calculating the average Ellenberg Indicator Value for soil moisture. Otherwise, divergence from such classification would need to be expected due to the impacts of diverse environmental factors. One example from our study is *R. acetosella*. Judging merely from results from the treatment 'high WTD' x 'muddy substrate', this species might be classified as 'short-term', as seed viability after 12 months was reduced from initial 92 % to less than 5 %. In another treatment, though, 'low WTD' × 'muddy substrate', seed survival after 18 months was still at about 75 %, and classification would be rather 'long-term'. With these considerations in mind we suggest that future classifications of seed longevity indices ought to evolve from assigning a species to a single class to assignments to a range of classes that takes environmental impacts into account.

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References

Akinola MO, Thompson K, Hillier SH (1998) Development of soil seed banks beneath synthesized meadow communities after seven years of climate manipulations. Seed Sci Res 8:493–500

Bahin E, Bailly C, Sotta B, Kranner I, Corbineau F, Leymarie J (2011) Crosstalk between reactive oxygen species and hormonal signalling pathways regulates grain dormancy in barley. Plant Cell and Environ 34:980–993

Bakker J, Poschlod P, Strykstra R, Bekker R, Thompson K (1996) Seed banks and seed dispersal: important topics in restoration ecology. Acta Bot Neer 45:461–490

Bekker R, Schaminée J, Bakker J, Thompson K (1998a) Seed bank characteristics of Dutch plant communities. Acta Bot Neer 47:15–26

Bekker RM, Bakker JP, Grandin U, Kalamees R, Milberg P, Poschlod P, Thompson K, Willems JH (1998b) Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Funct Ecol 12:834–842

Bekker RM, Knevel IC, Tallowin JBR, Troost EML, Bakker JP (1998c) Soil nutrient input effects on seed longevity: a burial experiment with fen meadow species. Funct Ecol 12:673–682

Bekker RM, Oomes MJM, Bakker JP (1998d) The impact of groundwater level on soil seed bank survival. Seed Sci Res 8:399–404

Blaney C, Kotanen P (2001) Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. J Appl Ecol 38:1104–1113

Bossuyt B, Honnay O (2009) Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. J Veg Sci 19:875–884

Dalling JW, Davis AS, Schutte BJ, Elizabeth Arnold A (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. J Ecol 99:89–95

Davis AS (2007) Nitrogen fertilizer and crop residue effects on seed mortality and germination of eight annual weed species. Weed Sci 55:123–128

Davis AS, Cardina J, Forcella F, Johnson GA, Kegode G, Lindquist JL, Luschei EC, Renner KA, Sprague CL, Williams MM (2005) Environmental factors affecting seed persistence of annual weeds across the US corn belt. Weed Sci 53:860–868

Erfanzadeh R, Hendrickx F, Maelfait JP, Hoffmann M (2010) The effect of successional stage and salinity on the vertical distribution of seeds in salt marsh soils. Flora 205:442–448

Espinar JL, Thompson K, García LV (2005) Timing of seed dispersal generates a bimodal seed bank depth distribution. Am J Bot 92:1759–1763

Gardarin A, Dürr C, Mannino MR, Busset H, Colbach N (2010) Seed mortality in the soil is related to seed coat thickness. Seed Sci Res 20:243–248

Griffin DM (1972) Ecology of soil fungi. Chapman & Hall, London

Grime JP (1989) Seed banks in ecological perspective. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. London Academic Press, London

ISTA International Seed Testing Association (1996) International rules for seed testing. Seed Sci Research 24

Kleinbaum DG, Kupper LL, Muller KE (2007) Applied regression analysis and other multivariable methods. Duxbury Press, California

Kleyer M, Bekker R, Knevel I, Bakker J, Thompson K, Sonnenschein M, Poschlod P, Van Groenendael J, Klimeš L, Klimešová J, Klotz SRGM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel A-K, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B (2008) The LEDA Traitbase: a database of life–history traits of the Northwest European flora. J Ecol 96:1266–1274

Hendry GAF (1993) Oxygen, free radical processes and seed longevity. Seed Sci Res 3:141–153

Long RL, Steadman KJ, Panetta FD, Adkins SW (2009) Soil type does not affect seed ageing when soil water potential and temperature are controlled. Plant Soil 320:131–140

Mordecai EA (2012) Soil moisture and fungi affect seed survival in California grassland annual plants. PLoS ONE 7:1–8

Murdoch AJ, Ellis RH (2000) Dormancy, viability and longevity. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities. C.A.B..I International, Wallingford

O'Hanlon-Manners D, Kotanen P (2006) Losses of seeds of temperate trees to soil fungi: effects of habitat and host ecology. Plant Ecol 187:49–58

Oberdorfer E (2001) Pflanzensoziologische Exkursionsflora. Eugen Ulmer, Stuttgart

Oomes M, Olff H, Altena H (1996) Effects of vegetation management and raising the water table on nutrient dynamics and vegetation change in a wet grassland. J Appl Ecol 33:576–588

Pakeman RJ, Small JL, Torvell L (2012) Edaphic factors influence the longevity of seeds in the soil Plant Ecol 213:1–9

Poschlod P, Abedi M, Bartelheimer M, Drobnik J, Rosbakh S, Saatkamp A (2013) Seed ecology and assembly rules in plant communities. In: van der Maarel E, Franklin J (eds) Vegetation Ecology. 2nd ed., Wiley-Blackwell, Chichester

Poschlod P, Bonn S, Bauer U (1996) Ökologie und Management periodisch abgelassener und trockenfallender kleinerer Stehgewässer im schwäbischen und oberschwäbischen Voralpengebiet. Veröffentlichungen Projekt Angewandte Ökologie 17:287–501

Poschlod P, Kleyer M, Jackel AK, Dannemann A, Tackenberg O (2003) BIOPOP – a database of plant traits and internet application for nature conservation. Folia Geobot Phytotax 38:263–271

Saatkamp A, Affre L, Baumberger T, Dumas PJ, Gasmi A, Gachet S, Arène F (2011a) Soil depth detection by seeds and diurnally fluctuating temperatures: different dynamics in 10 annual plants. Plant Soil 349:331–340

Saatkamp A, Affre L, Dutoit T, Poschlod P (2009) The seed bank longevity index revisited: limited reliability evident from a burial experiment and database analyses. Annals Botany 104:715–724

Saatkamp A, Affre L, Dutoit T, Poschlod P (2011b) Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. Annals Botany 107:415–426

Schafer M, Kotanen PM (2003) The influence of soil moisture on losses of buried seeds to fungi. Acta Oecol 24:255–263

Schütz W (2000) Ecology of seed dormancy and germination in sedges (*Carex*). Per Plant Ecol Evol Syst 3:67–89

Skoglund J, Hytteborn H (1990) Viable seeds in deposits of the former lakes Kvismaren and Hornborgasjön, Sweden. Aquat Bot 37:271–290

Thompson K, Bakker JP, Bekker RM (1997) The soil seed banks of North West Europe: methodology, density and longevity. Cambridge University Press, Cambridge

Thompson K, Band S, Hodgson J (1993) Seed size and shape predict persistence in soil. Funct Ecol 7:236–241

Thompson K, Ceriani RM, Bakker JP, Bekker RM (2003) Are seed dormancy and persistence in soil related? Seed Sci Res 13:97–100

Thompson K, Fenner M (2000) The functional ecology of soil seed banks. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities. CABI, Wallingford

Thompson K, Grime J (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J Ecol 67:893–921

Van Assche J, Van Nerum D, Darius P (2002) The comparative germination ecology of nine Rumex species. Plant Ecol 159:131–142

Voesenek L, Blom C (1992) Germination and emergence of Rumex in river flood-plains. I. Timing of germination and seedbank characteristics. Act Bot Neer 41:319–329

Wagner M, Mitschunas N (2008) Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. Basic Appl Ecol 9:191–203