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How best to quantify soil seed banks in arid rangelands of the Nama Karoo?

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Abstract Sampling design and three sample treatments prior the application of the seedling emergence method were tested in order to find the best method for seed bank quantification in arid Nama Karoo rangelands. I analyzed species composition and seed densities by contrasting undercanopy and open-matrix samples from two soil depths and by comparing the effects of cold-, heat-, and no stratification on germination rates of species in a greenhouse setting. The soil seed bank showed minimal similarity to the standing vegetation, with only 20 plant species germinated. Spatial distribution of seeds was highly heterogeneous. Nearly 90% of germinated seeds were located in 0- to 4-cm compared to >4- to 8-cm soil depth. Undercanopy seed banks contained significantly more species and seeds than open-matrix seed banks. Neither the number nor the diversity of seeds germinated differed significantly among the three treatments. Cold stratification tended to detect more species and seeds only at >4- to 8-cm soil depth. The results highlight the importance of spatial heterogeneity in the accurate evaluation of soil seed banks in the arid Nama Karoo and the importance of considering seasonal variability in the availability of readily germinable seeds. Data also suggest that sample pretreatment in germination trials may give little return for cost and effort, which emphasizes that it is more important to choose the sampling design most likely to give a representative number of seed bank species. Further studies are needed to analyze seed bank dynamics and species-specific germination requirements to promote recruitment of plant taxa underrepresented in the seed bank.

Keywords Seedling emergence method · Namibia · Sampling strategy · Seed density · Species composition · Stratification

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Introduction

Over the last century, a considerable amount of research has focused on the examination of soil seed banks and quantification techniques in various countries and vegetation types (Roberts 1981). Applied vegetation management has increased the demand for standardized information in plant ecology on ecological attributes of plants, such as seed banks and seed germination requirements (Thompson et al. 1997). Recent years have seen an increase in the number of seed bank studies from (semi)arid regions of Africa examining seed bank richness and composition, and

seed densities against the background of rangeland degradation (e.g., Jones and Esler 2004; Snyman 2004; Solomon et al. 2006; Kassahun et al. 2009). Such studies can improve our understanding of local response of spatiotemporal seed bank patterns to ecosystem disturbance and provide valuable information for the quantification of seed banks in different (semi)arid regions, ecosystems, and plant communities of Africa. However, currently, no seed bank information is available for Namibia, whose rangelands are similarly affected by degradation due to frequent droughts (Msangi 2004) and livestock pressure (Klintenberg and Seely 2004). Despite the ecological usefulness of seed bank studies to better understand vegetation dynamics in rangelands, there is a general lack of basic knowledge on seed bank characteristics and species-specific germination requirements in the arid Nama Karoo. Therefore, studies are needed in the basic conditions necessary for accurate estimates of species composition and seed densities. Based on such studies, future assessments could facilitate more refined investigations of soil seed banks in response to environmental conditions.

Requirements of seed bank studies differ depending on the aim of the study, i.e., whether to determine species composition, seed densities, or both with acceptable precision (Fenner and Thompson 2005). Environmental factors that impact spatial patterns in seed distribution, phenological patterns, and germination triggers have to be considered in the choice of sampling design and for seed bank quantification. It is well known that sampling strategies and seed quantification methods influence results of soil seed bank studies, as discussed by, e.g., Major and Pyott (1966) and Roberts (1981). This is particularly true for (semi)arid ecosystems, whose often patchy vegetation and variable climate account for much spatiotemporal variation in seed banks (Kemp 1989). Patch-scale heterogeneity is a determining factor in the spatial patterning of soil seed banks with physical barriers acting as seed traps (Caballero et al. 2008; Reichman 1984). Several seed adaptations have evolved that buffer the environmental heterogeneity of microsites and irregular rainfall patterns, such as those that cope with the uncertainty of favorable conditions for germination and establishment (Venable and Brown 1988). Therefore, in ecosystems that lack baseline soil seed bank data, it is recommended that prior to application, the adequacy of a chosen sampling design be tested for its efficacy (Page et al. 2006) and that analysis methods be compared in order to select the one most accurate in detecting the species composition and density of seeds in seed banks (Gross 1990).

The present study addresses both the adequacy of sampling design and the effectiveness of sample stratification treatments prior the application of the seedling emergence method. I used emergence methods rather than extraction methods (which are usually criticized for their time-intensive nature) for two reasons. Firstly, direct seedling emergence (Thompson and Grime 1979) is a standard method for soil seed bank quantification that is commonly applied in (semi)arid regions worldwide (e.g., Kinucan and Smeins 1992; Kinloch and Friedel 2005; Kassahun et al. 2009). This method provides reliable results, especially when applied in combination with pretreatments such as cold stratification (Gross 1990; Thompson et al. 1997; Funes et al. 2003), although only the readily germinable fraction of seeds is detected. Second, emergence methods have the advantage that no technical equipment for seed extraction and no skills regarding seed identification are required. The latter is an important point, as it also qualifies land managers to assess seed banks for evaluations of rangeland conditions. Therefore, for reasons of practicality, methods should ideally require only a minimum of equipment, should be easy to apply, and should provide reliable data.

In this study, data on vertical and horizontal seed bank distributions are analyzed from two southern Namibian pastures, and cool and hot temperature sample stratifications are tested in their ability to promote germination compared to untreated samples. The study sites were located in a region with a highly spatiotemporally variable climate, where widespread rangeland degradation has occurred. The aim was to identify a suitable method for quantifying soil seed banks in the arid Nama Karoo of Namibia by means of seedling emergence methods. Results are presented and interpreted with the goal of providing baseline data that may be useful in future assessments of local soil seed banks. Furthermore, the current

investigation is the first report on Namibian soil seed banks.

Materials and methods

Study site

The study was conducted at two neighboring biodiversity monitoring sites of the BIOTA Southern Africa project located about 20 km north of Keetmanshoop, southern Namibia (26°24.0717′ S, 18°1.2905′ E, 1,100 m above sea level). One site is situated on the lightly grazed governmental research farm Gellap-Ost and the second on the Nabaos communal rangeland, heavily grazed by goats. The study area is located in the Nama Karoo Biome. The climate is arid, averaging 150 mm of precipitation per year with a coefficient of variation of 70–80% (Mendelsohn et al. 2002). The rainy season is in summer, peaking in February and April. Mean annual temperature is approximately 22°C with maximum temperatures during the germination season regularly exceeding 35°C. Soil units in the study sites are loamy–sandy-textured regosols and cambisols, covered by fine to coarse gravel.

Data were collected from level sites in an open shrub–grass matrix with scattered occurrences of small-sized bare patches. All sampling sites were similar regarding geology, soil type, and topography and were homogeneous in vegetation structure. The pastures included differed in aboveground plant species composition as to the replacement of perennial grasses by annuals on the communal site. I was interested in the quantification of the seed bank, in general, rather than the analysis of site effects. Thus, grazing impacts on the environment and seed bank were not studied.

Sampling strategy

Seed bank sampling took place in April 2006 when germination was completed and after natural present-year seed shed was almost finished. Three 20 × 50-m plots were sampled on each farm. Within each plot, soil samples were taken from six sampling points within the open matrix and under the canopies of six shrubs. Sampling points were positioned using an easily replicated design. For open-matrix samples, two imaginary 50-m transects were laid out running parallel approximately 5 m north and 5 m south from the centerline of the plot (Fig. 1). At the start of each transect, an over-the-shoulder blind throw of a stone in the direction of the transect determined the position of the first soil sample. From that point, the stone was again thrown in this fashion to determine the next sampling point and likewise a third time to the end of the transect. Although this method is not a strictly random sampling design, it provides a suitable degree of randomness in sampling and was chosen as an easily replicated

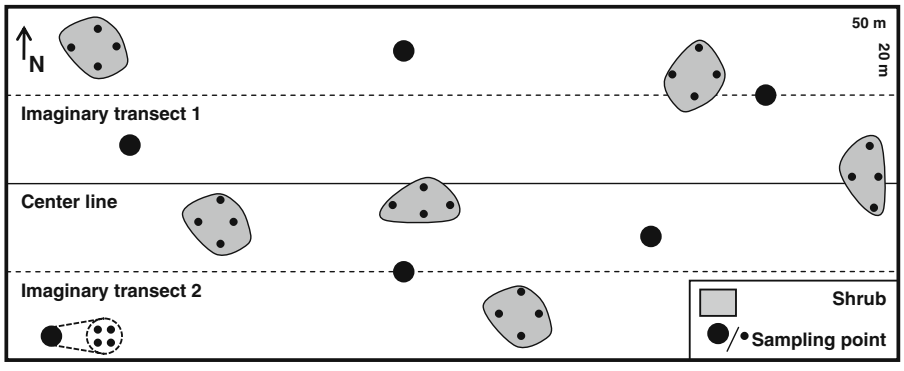

Fig. 1 Schematic representation of one out of six 20 × 50-m plots indicating open-matrix and undercanopy sampling points. At each of the six sampling points within the open matrix, four replicate soil cores were pooled for 0- to 4- and >4- to 8-cm soil depths. Under the canopies of six shrubs, one soil core was taken at each cardinal point, and all four samples were pooled per soil depth

method by anyone for future seed bank sampling in the Nama Karoo. At each sampling point, four replicate soil cores (5.5 cm in diameter × 8 cm deep; total area sampled 96 cm^2) were taken and divided into two depth classes (0–4 and >4–8 cm). Material from each depth was pooled per sampling point (total volume 380 cm^3). For under-canopy samples, six shrubs with two diameters of minimum 80 cm were selected. Due to the low density of shrubs within the 1,000-m^2 plots, selection could not follow any randomized design. If less than six shrubs of the designated size were present within the plot, the nearest shrub(s) to the plot was/were selected from the immediate surroundings. Under shrubs, one soil core was taken at a point along each cardinal direction halfway between shrub center and canopy edge, and the four samples were pooled per shrub and depth (Fig. 1). If litter was present, it was included in the soil sample. Samples were stored under low humidity at ambient temperature in the dark for 3 months to allow for afterripening of the year's freshly matured seeds.

The seed bank sampling plots corresponded to six previously established 1,000-m^2 biodiversity monitoring plots of the BIOTA Southern Africa project (Jürgens 1998). For each of these plots, data on aboveground species composition were already available. The species inventory of each plot was recorded in March 2006 at the peak of the growing season and compared with the seed bank composition of the respective plot. Nomenclature follows Germishuizen and Meyer (2003).

Sample treatments and glasshouse germination

Within 1 week after sampling, the soil material was sieved (2-mm mesh width) to reduce the sample by removing coarse plant fragments and stones. The retained material was screened for any seeds, and any seeds found were returned into the sample. At the University of Hamburg, each sample was stirred and then apportioned into three subsamples. Each subsample was spread out in two 9 × 9-cm plastic trays to a depth of about 8 mm (depending on the sample volume after sieving) on a steam-sterilized sand–peat mix in 9 × 9-cm plastic trays.

Three sample treatments (TRE) were applied prior the application of the seedling emergence method: untreated (U), cold-stratified (CS), and heat-stratified (HS), the latter two in order to help break dormancy. The CS treatment was a dry stratification method, in which the trays were chilled in a refrigerator at 5°C for 2 weeks, similar to the method of Funes et al. (2003). For HS, trays were put in a ventilated drying chamber at 50°C for 2 weeks to replicate any possible effect of high summer temperatures at the topsoil level in the natural habitat. Afterward, trays were placed in a random arrangement in the greenhouse and kept moist under controlled conditions, with a diurnal cycle of 14-h light at 30°C and 10-h darkness at 17°C. These light/temperature conditions are close to the optimum for germination previously reported for species from arid Southern Africa (compare Veenendaal and Ernst 1991). In addition, as a control against contamination, 54 trays containing only the steam-sterilized sand–peat mix were evenly distributed among the sample trays in the greenhouse.

The number of emerged seedlings was counted daily for 8 weeks until the germination rate approached zero. Representative seedlings of each species were transplanted in separate trays and grown to a stage when they were identifiable to species.

Data analyses

Statistical analyses were conducted using generalized linear models. Poisson regressions with log link function and correction for overdispersion (Quinn and Keough 2002) were used to analyze microsite and TRE effects on species numbers and seed densities for data from 0- to 4-cm soil depth. In the lower soil depth, very few seedlings emerged, and data contained a high percentage of zero values (76% of subsamples). According to Cabral et al. (2007), there are limitations of correction for overdispersion using data showing such an extreme distribution, and logistic regression is then appropriate. Therefore, TRE effects on data from >4- to 8-cm depth were analyzed separately with counts transformed into presence–absence data. All analyses were carried out using SPSS 15.0.1 (SPSS Inc. 2007).

For an indication of the number of undiscovered seed bank species in relation to the sample size, a rarefaction curve (Mao Tau expected richness function) was constructed, and the first-order jackknife estimate of species richness was calculated for all samples inclusive of all treatments from 0- to 4-cm soil depth, following Page et al. (2006) using EstimateS 7.5 (Colwell 2005). Following this, the Lomolino and Michaelis–Menten functions, which are appropriate for species sampling curves (compare Dengler 2009), were fitted to the rarefaction curve and the associated Jackknife estimates, and the models were compared regarding the best estimate of species richness. Curve fitting was performed with nonlinear regression using STATISTICA 8.0 (StatSoft Inc. 2007) and default values of the program.

To compare the standing vegetation (data from BIOTA Southern Africa) with seed bank species composition, the Sørenson index of similarity was calculated (Hopfensperger 2007) based on total species counts per 1,000-m^2 plot and species counts from all soil samples (0- to 8-cm soil depth) per plot.

Results

No seedlings emerged in the control trays, but nine seedlings of known greenhouse weeds were detected in sample trays over time. Seedlings of the Namibian seed bank started to emerge 3 days after the first irrigation event. Within the first 2 weeks, 71% of the total observed seedlings had already germinated. The general germination behavior was similar among treatments, resulting in no suppressed or delayed germination (Fig. 2). In total, 635 seedlings (66,167 seeds per square meter) of 20 plant taxa were recorded from the germinable seed bank (Table 1). Of these, three forbs only (*Amaranthus praetermissus*, *Helichrysum candolleanum*, and *Trianthema parvifolia*) accounted for 74.5% of the germinated seed bank.

The average Sørenson index calculated was 32.3% indicating a low similarity between the seed bank and vegetation composition at the six sampling plots. Just three species present in the seed bank (*Eragrostis biflora*, *Hermannia fruticulosa*, and *Myxopappus hereroensis*) were absent in the standing vegetation. In contrast, 50 species recorded in the vegetation did not occur in the germinable seed bank (Table 1).

This discrepancy was also found by comparing the extrapolated species richness of the seed bank at 0- to 4-cm soil depth. Fitting the Lomolino function to the rarefaction curve described the species–sample relationship best ($R^2 = 99.9$), giving an extrapolated richness of 32.5 species (Table 2). Thus, 72 samples covering a total surface area sampled of 6,912 cm^2 underestimated the expected species richness by 39% based on the 20 species detected in the germinable seed bank.

The vertical and horizontal distribution of seeds showed marked differences between the sampled soil depths and microsites. Undercanopy seed banks were more species-rich than open-matrix seed banks (mean ± standard error [SE], 3.3 ± 0.3 vs. 1.2 ± 0.1, Poisson regression: Wald χ^2 47.9, $p < 0.001$) and contained on average five times the number of seeds per square meter (mean ± SE, 1,395 ± 230 vs. 292 ± 45, Poisson

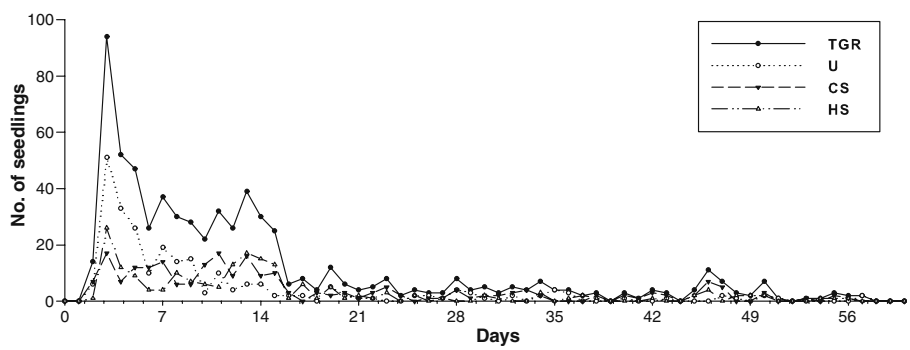

Fig. 2 Germination rate in days after first irrigation event ($t = 0$) by the seedling emergence methods. *TGR* total germination rate, *U* untreated, *CS* cold-stratified, *HS* heat-stratified

Table 1 Plant species recorded in the standing vegetation and emerged plant species in the greenhouse from all soil samples (0- to 8-cm soil depth, $n = 144$)

Species	FoO (%)			NoG (seeds per square meter)			Total NoG (seeds per square meter)
	U	CS	HS	U	CS	HS	
Seed bank							
Forbs							
Amaranthus praetermissus	14	15	14	6,148	4,064	4,585	14,796
Helichrysum candolleanum	29	31	13	9,065	7,711	4,897	21,674
Indigastrum argyroides	6	9	6	1,459	2,292	938	4,689
Kohautia caespitosa	3	6	2	521	834	313	1,667
Limeum argute-carinatum	0	0	1	0	0	104	104
Lotononis platycarpa	1	3	1	104	417	208	729
Microcharis disjuncta	0	2	0	0	313	0	313
Mollugo cerviana	0	0	2	0	0	313	313
Myxopappus hereroensis[a]	2	4	0	417	625	0	1,042
Tephrosia dregeana	0	1	0	0	104	0	104
Trianthema parvifolia	9	7	10	4,585	3,334	4,897	12,817
Tripteris microcarpa	1	1	1	208	104	104	417
Dwarf shrubs							
Hermannia fruticulosa[a″]	0	1	0	0	104	0	104
Grasses							
Aristida adscensionis	0	3	0	0	417	0	417
Enneapogon cenchroides	0	1	1	0	208	417	625
Eragrostis biflora[a]	1	1	1	104	208	208	521
Eragrostis sp.	0	0	1	0	0	104	104
Setaria verticillata	1	4	2	313	521	1,042	1,876
Stipagrostis spp.[b]	5	6	6	938	1,146	1,250	3,334
Others							
Unidentified taxon	0	1	2	0	208	313	521
Total no. of species	11	17	15				20
Total no. of germinants				23,862	22,611	19,694	66,167
Standing vegetation only							
Forbs							
Aizoanthemum dinteri, *Citrullus lanatus*, *Codon royenii*, *Corallocarpus dissectus*, *Dicoma capensis*, *Dicoma schinzii*, *Euphorbia glanduligera*, *Forsskaolea viridis*, *Geigeria pectidea*, *Gisekia africana*, *Limeum rhombifolium*, *Lophiocarpus polystachyus*, *Monsonia umbellata*, *Pergularia daemia*, *Portulaca oleracea*, *Sesamum triphyllum*, *Tribulus pterophorus*, *Tribulus terrestris*, *Trichodesma africanum*							
Dwarf shrubs							
Aptosimum spinescens, *Blepharis obmitrata*, *Hermannia modesta*, *Hoffmannseggia lactea*, *Indigofera pechuelii*, *Kissenia capensis*, *Limeum aethiopicum*, *Monechma genistifolium*, *Peliostomum leucorrhizum*, *Ptycholobium biflorum*, *Solanum dinteri*							
Shrubs / Trees							
Acacia mellifera, *Acacia nebrownii*, *Asparagus* sp., *Boscia foetida*, *Cadaba aphylla*, *Calicorema capitata*, *Catophractes alexandri*, *Hibiscus elliottiae*, *Lycium* sp., *Maerua schinzii*, *Microloma incanum*, *Phaeoptilum spinosum*, *Rhigozum trichotomum*, *Sisyndite spartea*, *Tetragonia schenckii*							
Grasses							
Enneapogon desvauxii, *Leucophrys mesocoma*, *Melinis repens*, *Schmidtia kalahariensis*, *Stipagrostis hirtigluma*							

Frequency distribution as the percentage of the total sample set, and number of germinants subdivided by treatments

FoO frequency of occurrence, *NoG* number of germinants, *U* untreated, *CS* cold-stratified, *HS* heat-stratified

[a]Species only found in the seed bank

[b]*Stipagrostis* spp. includes *S. hochstetteriana* and *S. uniplumis,* which hardly can be differentiated in early seedling stages

Table 2 Species richness estimates for two functions fitted to species-sample curves constructed from the number of species of seedlings germinated from samples of 0- to 4-cm soil depth ($n = 72$)

Model	Rarefaction curve		Jackknife estimate	
	Estimate	R^2	Estimate	R^2
Lomolino	32.5	99.9	31.7	99.8
Michaelis–Menten	23.7	99.0	28.7	99.5

regression: Wald χ^2 26.5, $p < 0.001$; Fig. 3). There was a general decline in mean species numbers and mean seed densities with increasing soil depth (Fig. 4). About 87% of germinable seeds detected were found in the top 4 cm of soil. Within this layer, all 20 plant taxa recorded from the germinable seed bank were present, whereas samples from >4- to 8-cm soil depth yielded only 14 plant taxa.

The probability of a seed germinating was not significantly affected by the treatments (Fig. 4). For the whole sample set, most species were detected by means of CS (17 species), followed by HS (15 species), while U revealed 11 species only. In contrast, most germination events were recorded without pretreatment (229 seedlings [23,862 seeds per square meter]), followed by CS (215 seedlings [22,611 seeds per square meter]), and HS (191 seedlings [19,694 seeds per square meter]; Table 1). However, for samples from 0- to 4-cm soil depth, TRE had no overall significant effect either on species numbers (Poisson regression: Wald χ^2 2.3, $p = 0.318$) or on seed densities (Poisson regression: Wald χ^2 1.3, $p = 0.524$) detected per sample.

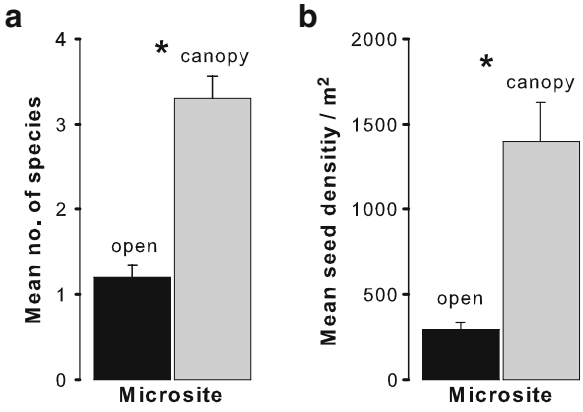

Fig. 3 Mean number of species (±SE, $n = 36$ samples) (**a**) and mean number of seeds per square meter (±SE, $n = 36$ samples) (**b**) of the germinable soil seed bank in 0- to 4-cm soil depth per microsite. *Asterisks* indicate significant differences ($p < 0.001$)

Logistic regression revealed no overall relationship between TRE and the occurrence of a germination event for samples from >4- to 8-cm soil depth (Wald-χ^2 3.17, $p = 0.205$). Neither CS (Wald χ^2 0.32, $p = 0.574$) nor HS (Wald χ^2 1.5, $p = 0.221$) was significantly different from U. Nevertheless, regression coefficients (B) and odds ratios [Exp(B)] indicated that CS tended to increase the probability of a germination event [Exp(B) = 1.2, $B = 0.2$], while HS tended to have the opposite effect [Exp(B) = 0.6, B = -0.5]. This applies to both the mean number of species and the mean seed densities in >4- to 8-cm soil depth as shown in Fig. 4.

No clear species-specific germination pattern in response to a certain TRE was observed. Comparing the three TREs, only slight differences were detected in the frequency of single species occurrences (Table 1). Most obvious, germination of *H. candolleanum* was much smaller if heat-stratified, while germination of *T. parvifolia* was decreased if cold-stratified. Although some species were detected by only one treatment, these had a low frequency in general, with only a few germinable seeds present. Moreover, frequency distributions and number of germinants indicated an aggregated distribution of seeds (Table 1).

Discussion

Seed bank spatial structure

The overall quantification of the seed bank revealed a sharp decline in species numbers and seed densities with increasing soil depth and by contrasting undercanopy with open-matrix seed banks. Seed numbers per sample varied most extremely in samples from 0- to 4-cm soil depth, ranging from zero to nine in the open matrix and zero to 49 under shrubs. Such distribution patterns have been found in various studies (e.g., Reichman 1984; López 2003; Li 2008) and are

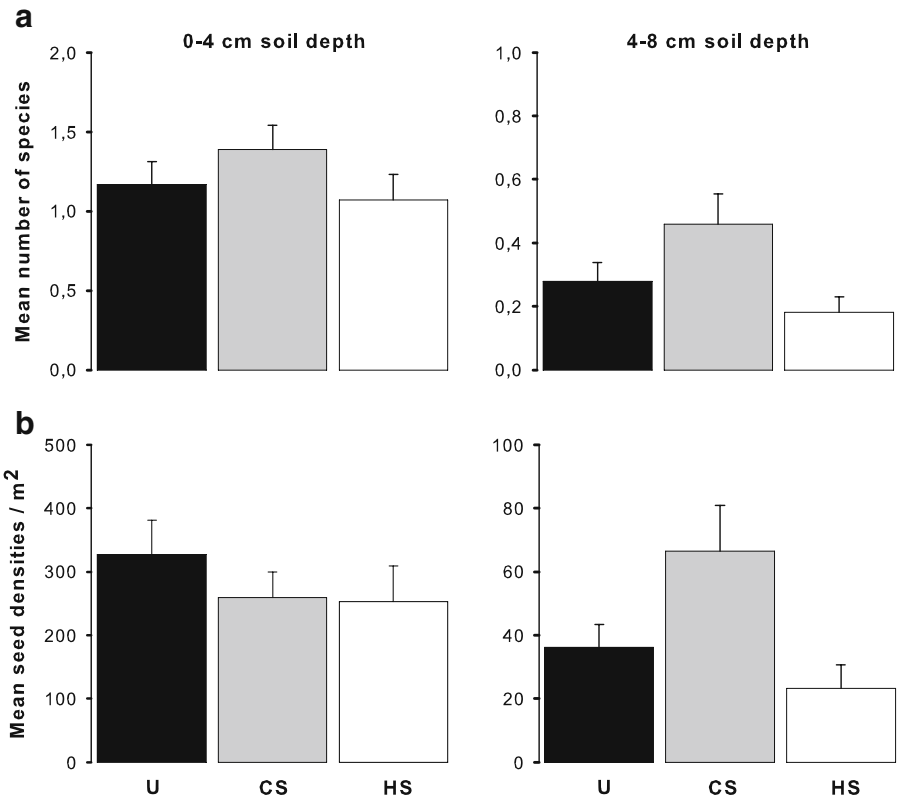

Fig. 4 Mean number (±SE) of species per sample (**a**) and mean number (±SE) of seeds per square meter (**b**) that emerged from the seed bank and effect of different treatments by sampling depth. *U* untreated, *CS* cold-stratified, *HS* heat-stratified. Samples per treatment: $n = 72$

typical for arid environment seed banks (Kemp 1989; Flores and Jurado 2003). The mean seed density per square meter found under shrubs in 0- to 4-cm soil depth corresponds to the mean 1,371 seeds per square meter reported by Jones and Esler (2004) for closed-canopy microsites in more humid (mean 341 mm annual rainfall) Nama Karoo rangelands in the Eastern Cape, South Africa. By contrast, comparing open-canopy sites from both studies (only "plains" considered), the Namibian seed bank contained per square meter on average about 175 seeds less than the South African sites sampled in April.

These results strongly suggest that sampling below 4-cm soil depth is not useful in the environment studied, as the majority of germinable seeds detected were located in the upper soil layer (including any litter layer). Furthermore, shrubs act as effective seed traps, providing a diverse understory seed pool. Therefore, in order to capture a significant part of arid Nama Karoo seed banks, samples should be taken from microsites differing in their ability to trap seeds.

Seed bank composition

Both rangelands sampled have a relatively long history of consistent grazing regimes for a minimum of 30 years. Thus, the current seed bank composition is likely to reflect not only present grazing impacts on the established vegetation but also grazing history. The comparison of the aboveground and belowground species compositions indicated an overall weak relationship between these irrespective of rangeland condition. This is comparable with similarity values given by Solomon et al. (2006) for other African semiarid rangelands with various grazing intensities. However, according to Hopfensperger (2007), this is rather an exception, and there is a general trend in arid-region grasslands to have a high floristic similarity between seed bank and vegetation. In this study, about 60% of the aboveground species were not found. The germinable seed bank was dominated by small-seeded species of which a few, i.e., *A. praetermissus*, *H. candolleanum*, and *T. parvifolia*, were overrepresented. A similar

pattern was also found in North American deserts (Guo et al. 1999) and is often observed in relation to grazing favoring an abundance of annuals that produce numerous seeds (Navie et al. 1996; Snyman 2004). In contrast, the seed bank lacked large-seeded species. In particular, woody species common in the standing vegetation (e.g., *Boscia foetida*, *Calicorema capitata*, and *Phaeoptilum spinosum*) were absent. Both the aggregated distribution of seeds found and the absence of larger-seeded species detected in this study indicate species-specific seed distribution patterns, such as short dispersal distances or directed dispersal. The latter often results in spatially disproportionate deposition of seeds (Nathan and Muller-Landau 2000). Postdispersal removal of seeds by rodents, which are numerous on the study sites (Hoffmann and Zeller 2005), is widespread in (semi)arid ecosystems (Hulme 1998) and can considerably affect seed contents in samples (Nelson and Chew 1977; Reichman 1979; Price and Joyner 1997). This could explain the underrepresentation of many woody species in the seed bank, whose larger seeds are attractive to rodents.

This study unfortunately did not consider temporal seed bank dynamics that may have resulted in a higher observed seed bank–vegetation similarity. The time of sample collection in relation to phenological patterns can influence results (Baskin and Baskin 2001). Sampling took place in April immediately after seed shed of most species. Thus, the persistent seed bank of the previous year and a portion of the current year's transient fraction should have been captured. It is possible that due to incomplete seed rain leading up to the period of sampling, some species with a transient seed bank were still absent from the seed bank or present in low quantities only. Precipitation falls infrequently and is highly localized in the region. Drought years without seed set are common. Thus, a high degree of temporal variability both seasonally and interannually is to be expected in the seed bank. Indeed, Jones and Esler (2004) reported from another portion of the Nama Karoo seedling numbers twice as high in samples from spring (October) compared to autumn (April). These also differed in species composition, with some species present only in one season.

Sampling design

The outcome of seed bank analyses depends greatly on the sampling design (Roberts 1981), sample size, and sample quantity (Bigwood and Inouye 1988; Page et al. 2006). Therefore, the low number of observed species compared to extrapolated richness and, for arid grasslands, atypically low (sensu Hopfensperger 2007) floristic similarity between seed bank and standing vegetation suggest an inadequate sampling design underestimating the seed bank composition.

In relation to other seed bank studies in semiarid regions, the total surface area sampled (6,912 cm^2) was relatively high (see Page et al. 2006), and different microsites with varying seed-trapping capabilities were considered. However, spatial patterns in seed distribution also include small-scale seed bank gradients in density and composition that are beyond the simple comparison between undercanopy and open-matrix seed banks (Caballero et al. 2008). Therefore, a larger number of samples should be taken, considering also the smaller-scale spatial patterning of seed deposition (compare Reichman 1984) in order to capture the actual species diversity in the seed bank.

Treatment effects on germination rates

The application of different treatments to promote germination revealed no significant effects. CS only insignificantly increased the detection of species numbers and tended only to increase the probability of a germination event of seeds from >4- to 8-cm soil depth. Significant differences comparing untreated and cold-stratified samples are reported by Gross (1990) from an annually ploughed field in south-western Michigan, USA. This might be due to the fact that CS prior to germination tests is likely to affect at least a part of transient seed banks by breaking dormancy (Funes et al. 2003). However, the average minimum temperature during the coldest month (July) in south-central Namibia is 6°C with an average of 7 days of frost per year (Mendelsohn et al. 2002). Thus, supposedly, short-term low-temperature stratification may not be as effective as the natural long period of afterripening during

cooler winter months. In total, four species were detected exclusively after CS (*Aristida adscensionis*, *H. fruticulosa*, *Microcharis disjuncta*, and *Tephrosia dregeana*). Except for the dwarf shrub *H. fruticulosa*, these species were abundant in the established vegetation, with the annual grass *A. adscensionis* more common in the more heavily grazed pasture and the forb *M. disjuncta* more common in the less grazed pasture. Due to their abundance in the herbaceous layer, one would expect these species to have dense seed banks. For none of these species were, however, more than five seedlings detected indicating a low availability of readily germinable seeds at sampling date and/or time of germination trials. Therefore, it cannot be ruled out that for these species, germination is favored by low-temperature stratification.

Similarly, although no significant promotion of species-specific germination by HS was detected, this study provides no general evidence for this effect. Among shrub and herbaceous species from savannahs, physical and physiological dormancy is most common, and heat treatments often are necessary to advance seeds into a germinable condition (Baskin and Baskin 2001). Nonetheless, only three species with just one to three germinable seeds each were detected exclusively by HS. This is in line with results from Veenendaal and Ernst (1991), who exposed diaspores of Southern African savannah grasses to 50°C for 72 h that did not influence germination behavior compared to no pretreatment. It is worth mentioning that one major difference between these studies is the duration of high-temperature stratification. In summer, unshaded soil surfaces at the study sites heat up to 70°C at midday (personal observation). Such short-term pulses of high temperature can have an effect on breaking the dormancy of shallow-stored seeds (Gutterman 2000). It is known from temperate regions that stratification can change dormancy levels within short time periods (Noronha et al. 1997), and thus, the duration of heat stratification chosen (2 weeks) could also have (re)induced dormancy of some species.

Requirements for germination vary among species and are determined by the degree of dormancy (Vleeshouwers et al. 1995). This can have profound effects on results of seed bank analyses if seedling emergence methods are applied (Major and Pyott 1966). Due to the fraction of dormant seeds, the seed bank may be underestimated for some species (Roberts 1981; Gross 1990), or some species may not be detected at all. Fluctuating microclimatic conditions involving light and soil temperature are known to trigger germination response of certain species. Kos and Poschlod (2007) recently showed that germination of certain canopy-dependent plant species in a Kalahari savannah is inhibited by temperature conditions of the open vegetation matrix. Thus, such evolved mechanisms may have prevented germination of some canopy species under unshaded greenhouse conditions.

Conclusion

This study provides guidelines on how to adequately quantify soil seed banks in the arid Nama Karoo, focusing on a widespread vegetation type in southern Namibia. The methods applied provide baseline information for the design of an improved seed bank assessment technique. It was shown that sampling strategies should consider the structural heterogeneity of the environment, i.e., it should include physical barriers such as shrubs that are able to trap and accumulate seeds. However, constraints on interpretation resulting from the sampling strategy also imply the need for refined microsite sampling beyond the shrub/bare ground contrast. Furthermore, results imply that consideration should be given to seasonal constraints on the availability of readily germinable seeds. I therefore suggest that seed bank quantification aiming at the identification of total species richness in the region of the Nama Karoo studied should first analyze the spatial heterogeneity in seed distribution to gain basic knowledge of seed bank patterning. It may be appropriate to sample from October to December when both the transient and persistent seed banks are present and so that all seeds will have been subjected to at least one natural winter stratification event. By this, the direct seedling emergence method (no pretreatment) may capture seeds that are dormant in April. The results

that sample stratification may give little return for the cost and effort argue for the practicality of an assessment technique focusing only on environmental factors. This strategy would also facilitate the application of methods by land managers in the arid Nama Karoo without the need for laboratory equipment. A sampling strategy adapted in such a manner is likely to provide good results for seed bank quantification.

For an improved seed bank assessment technique for arid Nama Karoo rangelands, more information on the sample quantity is required to maximize capture of the seed bank present. There is also a need for knowledge on species-specific germination requirements or germination-promoting techniques for plant species that were absent from the seed bank but present in the vegetation.

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