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Role of the Soil Seed Bank during Succession in a Subalpine Meadow on the Tibetan Plateau

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Abstract

We studied the seed bank of abandoned fields in four successional stages (habitat A: 1 year; habitat B: 10 years; habitat C: 20 years; and habitat D: mature meadow) in eastern Tibetan Plateau. The seed density in seed banks decreased with successional age, but species richness and diversity increased, and the highest species diversity and species richness appeared in habitat C. Similarity between the seed bank and vegetation decreased gradually with succession in the whole. The vegetation is more similar to the seed bank in the shallow layer than to the seed bank in the deeper soil, which shows that the vegetation contributes less to the seed bank as soil depth increases. The seed bank was mainly composed of first successional species during the whole successional range. Species of the later successional stages contributed little to the seed bank. Most of species from early successional stage produced longer-lived seeds, which stayed viable in the soil for a long period (more than 20 years). Hypotheses about changes in seed bank during succession, predicting decreasing species richness and seed diversity, were not confirmed. The hypothesis that density of buried seeds declined during succession was confirmed. We conclude that seed bank plays an important role on vegetation in the early succession stage. In the later succession stages, seed bank's role becomes weaker and weaker, and which likely relies on vegetative reproduction and dispersal.

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Introduction

Many species have long-lived seeds (Thompson et al., 1997), and it is likely that the soil seed banks of successional plant communities comprise a mixture of present and previous vegetation. Thus, they represent a record of site history, allowing insights on past vegetation dynamics (Thompson, 2000). Target species that are lost from the vegetation may be reestablished from the seed bank and, hence, play an important role in ecological restoration (van der Valk and Pederson, 1989; Bakker et al., 1996). It is important to know whether restoration requires only the previously accumulated seed bank to permit re-establishment of the plant communities, or whether succession depends upon input via seed dispersal (Bekker et al., 2000) or vegetative growth.

Buried seed banks are important for arctic and alpine vegetation recovery after disturbance (Freedman et al., 1982; Gartner et al., 1983; Ebersole, 1989). However, the roles of soil seed banks in vegetation structure are still relatively unknown (Freedman et al., 1982; Leck and Simpson, 1995). On one hand, the restoration of plant covers on eroding subalpine and alpine soils could be initiated by species with persistent seed banks (Klug-pümpel and Scharfetter-Lehr, 2008). On the other hand, due to the low floristic similarity between the standing vegetation and the seed bank (Chambers, 1993; Kiirikki, 1993; Jensen, 1998; Holmes and Cowling, 1997; Morgan, 1998; Arroyo et al., 1999), clonal growth is considered the major factor in maintaining the vegetation structure in subarctic and arctic habitats. Moreover, persistent seed banks have a minor role compared to clonal growth in the regulation of vegetation structure (Welling et al., 2004). Diemer and Prock (1993) concluded that plants in cold habitats rely more heavily on vegetative growth than on reproduction by

seed as a means of persistence; Wearne and Morgan (2006) also found that the regeneration of the studied flora in the subalpine vegetation cannot rely on the soil seed bank.

The relationship between seed banks and successional series has been studied in old fields and forests (Symonides, 1986; Roberts and Vankat, 1991), heathland (Hester et al., 1991), and alpine and subalpine grassland (Welling et al., 2004; Wearne and Morgan, 2006). However, the majority of these studies focus on long-term succession where there are large differences in plant communities between the stages (as in a dune succession from bare sand to forest) and lack detailed information of the relationship between the vegetation and the seed bank (Bekker et al., 2000). The relationship between changes in the vegetation and associated changes in seed bank composition has been of particular interest to plant ecologists (Wagner et al., 2006). So far, no studies of seed banks from Tibetan Plateau exist.

We wanted to test several hypotheses proposed for seed bank characteristics under succession, and these hypotheses have never been tested in subalpine and alpine areas. Species richness and species diversity in the seed bank decreases during succession as has been proposed both for grasslands (Donelan and Thompson, 1980) and old fields (Symonides, 1986; Roberts and Vankat, 1991). The density of buried seeds declines during succession (Thompson, 1978; Symonides, 1986; Roberts and Vankat, 1991). Based mainly on results from studies of agricultural ecosystems, Thompson (2000) formulated a paradigm of “declining seed numbers and diversity and decreasing similarity between seed bank and vegetation as succession proceeds.”

Our aims were to answer the following research questions: (1) How does total seed density and species composition of the seed

bank change along a successional stage? (2) How does composition of the viable seed bank change with successional stage and diverge from the vegetation? (3) What is the role of seed banks for species composition of the vegetation during succession?

Material and Methods

STUDY SITE

The study was conducted in Hezuo (34°55'N, 102°53'E), Gansu Province, China, on the eastern Tibetan Plateau, with an elevation of 2900–3000 m above sea level (a.s.l.). The precipitation is 531.6 mm per year, from 2.4 mm in January to 110.3 mm in July; the average temperature is 2.4 °C, from –9.9 °C in January to 12.8 °C in July. The vegetation is dominated by *Poa* sp., *Agrostis hugoniana*, *Festuca ovina*, *Elymus nutans*, *Stipa aliena*, *Kobresia humilis*, *Gentiana macrophylla*, *Aster flaccidus*, and *Ligularia virgaurea*. The germination experiment was conducted in Hezuo, with an elevation of 2900 m a.s.l. The average temperature there is 2.0 °C, and precipitation is 557.8 mm.

Many parts of the subalpine meadow in Hezuo had been used for agriculture before the 1960s. Afterwards the government has aimed to return the intensively used agricultural meadows to species-rich meadow over the succeeding decades. Similar to other studies (Kalamees and Zobel, 1998; Bekker et al., 2000), we chose a chronosequence in these habitats representing a successional series. Seed bank sampling and assessments of vegetation composition were carried out at four different successional habitats.

Habitat A was abandoned from agriculture only 1 year. Grazing was prohibited. Vegetation cover is low, only 30–40%. Dominant species are the ruderals: *Artemisia hedinii*, *Aconitum gymmandrum*, *Plantago asiatica*, and *Potentilla anserine*.

Habitat B was abandoned from agriculture for nearly 10 years, and the differences with habitat A are now quite significant. The proportion of ruderal species is less and that of graminoids is higher compared with habitat A. Vegetation cover is 80–98%. The meadow has been lightly grazed by livestock (e.g. yak and Tibetan sheep) since abandonment, and disturbance was low. Dominant species are *Elephantopus mollis*, *Elymus dahuricus*, *Roegneria nutans*, and *Medicago ruthenica*.

Habitat C was abandoned from agriculture for approximately 20 years. There was no significant difference with mature meadow after 20 years restoration. Vegetation cover is 100%. The meadow has been lightly grazed by domestic animals for 20 years. Dominant species are *Kobresia humilis*, *Artemisia tangutica*, and *Elymus dahuricus*.

Habitat D is a mature meadow (a typical subalpine meadow). The meadow has never been used for agricultural activity. The vegetation is very species rich where it is covered with sedges and graminoids. Vegetation cover is 100%. The differences with habitat C are small. The mature meadows are generally low degree of grazing by Tibetan sheep and yak, and disturbance was low. Dominant species are *Thalictrum alpinum*, *Kobresia humilis*, *Scirpus pumilus*, and *Stipa capillata*.

Detailed repeated studies on seed bank development in a single area are very rare (Leck and Leck, 1998; Willems and Bik, 1998; Falinska, 1999). However, the availability of a chronosequence gave us a good opportunity to study species dynamics in the seed bank in more detail (Bekker et al., 2000). The four study habitats for vegetation and seed bank sampling were at similar elevations. Distances between sites range from 500 to 1000 m. The species composition of vegetation among the four habitats is very different due to different time of field abandonment. Selection of

these habitats was designed to maximize representation of different types of grassland communities as well as maintaining interspersions; both vegetation and environmental factors were homogeneous within each habitat (Acosta et al., 1992). Av, Bv, Cv, and Dv represent vegetation in habitat A, B, C, and D, respectively, and As, Bs, Cs, and Ds represent the seed bank.

SOIL COLLECTION AND PROCESSING

Ten randomly selected plots (10 m × 10 m) were established in each habitat, and soil samples were collected from these plots in July 2005. The soil seed bank was sampled by the concentration method (ter Heerdt et al., 1996). There in each of 10 subplots (0.4 m × 1 m), randomly distributed in each plot, 10 cylindrical soil cores (3.6 cm diameter) were taken (Kalamees and Zobel, 2002). The soil cores were separated into three fractions: the shallow soil layer (0–2 cm deep), the mid layer (2–7 cm deep), and the deepest layer (7–12 cm deep). Ten cores at each depth were pooled per subplot. Overall, there were 30 samples in each plot (10 samples in every layer), and 300 soil samples at each habitat. Thus, the area sampled at each habitat was 1.02 m², and a total bulk of soil samples of 0.151 m³.

MAINTENANCE OF SEED TRAYS

Soil samples were placed on a table in front of a north-facing window for 10 days of direct exposure, then the samples were sieved through sieves (mesh width 0.2 mm) in order to remove plant fragments and stones (ter Heerdt et al., 1996; Funes et al., 1999). Visual inspection of the coarse particles retained by the wide mesh sieve indicated that no large seeds were retained together with these particles. The direct germination method of Thompson and Grime (1979) was used to access the readily germinable seed species composition (Gross, 1990; Rothrock et al., 1993). The seedling emergence method usually detects more than 90% of species present in soil samples of grassland systems (ter Heerdt et al., 1996). Samples were then put in germination trays on a layer of sterilized sand (140 °C for 24 h). The samples were spread evenly in sterilized sand in a plastic tray (width 30 cm). Depth of the soil layer in the germination trays was less than 2 cm. Control trays with only sterilized sand was set alongside the field samples to detect contamination by wind dispersal seeds. The soils were watered regularly. Emerging seedlings were identified and removed or replanted for later identification, to maintain a low density in the germination trays and to allow the germination of other seeds. Soil samples were carefully turned over following cessation of the initial flush of germinates in order to facilitate the emergence of new seedlings. After the first germination period (August to October), the experiment was stratified for 5 months; the second germination period lasted 7 months. Sampling was stopped when no more seedlings occurred for several consecutive weeks, and then sifting and careful inspection found that no seeds remained.

VEGETATION SAMPLING

The vegetation sampling was performed in summer (July 2005), during the peak of the growing season. The vegetation was recorded by randomly placing 10 quadrats of 50 cm × 50 cm within each of the habitats where the soil seed bank samples were taken, 40 quadrats in four habitats altogether. We recorded the presence and cover of all species within each quadrat. Cover was estimated using the Braun-Blanquet scale (Westhoff and Van Der Maarel, 1978).

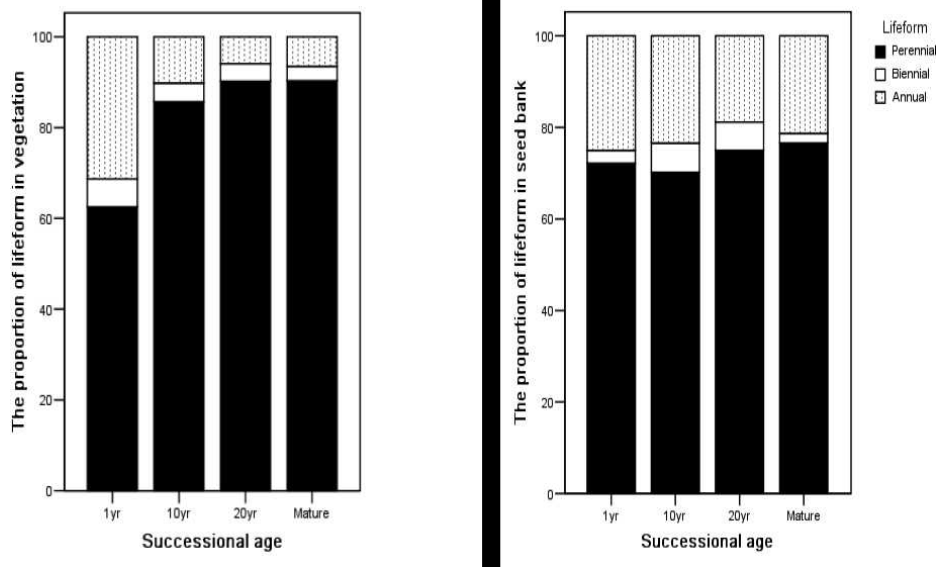


FIGURE 1. The proportion of life forms at different successional ages in vegetation and seed bank.

DATA ANALYSES

We used the Sorensen coefficient (Warr et al., 1993; Peco et al., 1998) to evaluate the similarity between the presence of species in the germinable seed bank and in the vegetation of each community. The differences of the species richness and mean number of seeds among different habitats and different layers were compared by the one-way analysis of variance (ANOVA) and Tukey range test. To meet the requirement of variance homogeneity, the mean number of seeds data was log-transformed prior to analysis. We calculated diversity using the Shannon index (Westhoff and Van Der Maarel, 1978). Species richness and species diversity were compared among four habitats by means of a one-way analysis of variance with Tukey range test. All analysis of variance was conducted with the program SPSS 13.0.

The species composition similarity between the seed banks and the vegetation among four habitats was tested using a nonmetric multidimensional scaling (NMDS), a nonparametric ordination technique that represents a similarity matrix in a multidimensional space and preserves the ordering of relationships among the original items (Legendre and Legendre, 1998). Ordination was made using the R-program for Windows version 2.0.7 (Ihaka and Gentleman, 1996), applying package VEGAN by Oksanen. Both R and VEGAN are available for free at <http://cran.r-project.org/>. NMDS makes no assumptions about the data (Faith et al., 1987) and is considered the best method for graphical representation of floristic relationships (Clarke, 1993). The resulting NMDS axis values are produced such that sites with similar species composition lie close to one another in ordination space. NMDS assumes only monotonicity with respect to ecological distance and thus is a relatively robust ordination technique compared to linear methods (e.g., principal components) (Fasham, 1977). We calculated similarity matrices using the Bray Curtis coefficient, which has been found to be robust with respect to ecological distance (Faith et al., 1987). All ordinations were based on presence/absence data.

Results

PLANT COMMUNITIES

In vegetation, we recorded a total of 101 species, belonging to 26 families. Of these species 14.9% were annual, 5% were

biennial, and 80.1% were perennial herbs. The proportion of perennial species increased with successional stages, but it was much the same between habitat C and D (Fig. 1). The total number of species was poorest at the youngest habitat (habitat A), but increased along successional stages (Table 1). The species richness per quadrat in four study habitats differed significantly ($F = 108.758, P < 0.001$). Habitat D was the richest in species with 34.6 species, with approximately three times more species than in habitat A (12.4). Nine species occurred in all four habitat types: *Plantago asiatica*, *Roegneria nutans*, *Potentilla anserina*, *Medicago ruthenica*, *Lancea tibetica*, *Elymus dahuricus*, *Taraxacum mongolicum*, *Anaphalis lacteal*, and *Artemisia desertorum*.

SEED BANK

The visual inspection of the samples after germination revealed very few ungerminated seeds, which indicated that we have an accurate estimation of the number of species in our seed bank samples. No seedlings were recorded in the control trays, indicating negligible airborne seed contamination. There are three species that could only be identified to genus level (*Viola* sp., *Carex* sp., and *Stipa* sp.). After two germination periods, a total of 18,464 seedlings of 64 species, belonging to 21 families, germinated from the soil samples. Of these species, 23.4% were annuals, 4.7% were biennial, and 71.9% were perennial herbs. The range of variation of life forms at different successional ages was small, and the proportion of annual species increased in the seed bank relative to vegetation (Fig. 1).

In contrast, 101 species were recorded in the vegetation, of which 47 species were also found in the seed bank. Seventeen species that germinated from the seed bank were not present in the vegetation. More seeds (43.9%) germinated from the 2–7 cm layer, and 25.4% from the 0–2 cm layer. The most abundant species in the seed bank were *Artemisia hedinii*, *Artemisia desertorum*, and *Plantago asiatica*, which together made up 87.7% of seedlings recorded. The density of these three species decreased with time since grassland abandonment. Obviously, the late successional species showed an increase in the number of seeds during succession. However, most of the early successional species still were dominant species in seed bank throughout the entire gradient. There were 27 species, which made up 75% of species

TABLE 1

Total and mean number (\pm SE) of species from vegetation ($n = 10$) and of species and seeds in the seed bank ($n = 10$) at three depths in four successional habitats (sample area = 1017 cm² for 0–2, 2–7, and 7–12 cm). Different letters indicate significant differences ($p < 0.05$), indicated by different superscripts assessed by a Tukey-test after one way ANOVA. The numbers of seeds in the seed bank were log-normally transformed.

	Successional age (years)				<i>p</i>
	1	10	20	Mature	
Vegetation					
Total number of species	32	49	51	62	
Species richness per quadrat	12.4 \pm 1.0 ^c	20.4 \pm 0.8 ^b	33.5 \pm 1.0 ^a	34.6 \pm 1.3 ^a	<0.001
Seed bank					
Total number of species per layer	19/26/28	29/33/31	23/40/29	26/37/26	
Total number of species, 0–12 cm	36	46	48	47	
Species richness, 0–2 cm	5.8 \pm 0.7 ^b	10.1 \pm 0.6 ^a	10.5 \pm 0.5 ^a	8.7 \pm 0.7 ^a	<0.001
Species richness, 2–7 cm	7.5 \pm 0.7 ^c	11.4 \pm 0.3 ^b	14.4 \pm 1.0 ^a	12.1 \pm 0.8 ^{ab}	<0.001
Species richness, 7–12 cm	9.0 \pm 0.5 ^{bc}	10.0 \pm 0.6 ^{ab}	12.0 \pm 0.8 ^a	9.2 \pm 0.9 ^{bc}	<0.05
Species richness, 0–12 cm	13.9 \pm 0.9 ^c	17.6 \pm 0.9 ^b	21.5 \pm 1.2 ^a	18.8 \pm 0.7 ^{ab}	<0.001
Mean number of seeds, 0–2 cm	1.9 \pm 0.04 ^b	2.4 \pm 0.05 ^a	1.8 \pm 0.04 ^{bc}	1.6 \pm 0.04 ^d	<0.001
Mean number of seeds, 2–7 cm	2.6 \pm 0.03 ^a	2.4 \pm 0.03 ^b	2.0 \pm 0.03 ^c	1.7 \pm 0.04 ^d	<0.001
Mean number of seeds, 7–12 cm	2.5 \pm 0.04 ^a	2.1 \pm 0.03 ^b	1.7 \pm 0.03 ^c	1.6 \pm 0.05 ^d	<0.001
Mean number of seeds, 0–12 cm	2.9 \pm 0.03 ^a	2.8 \pm 0.02 ^b	2.3 \pm 0.03 ^c	2.1 \pm 0.02 ^d	<0.001

recorded in habitat A, that were unvaried in all successional stages (from habitat A to D).

The mean number of seeds per plot differed significantly in each separate layer (0–2, 2–7, 7–12 cm) and in total (0–12 cm) (Table 1). When only the shallow layer was considered, the highest number of seeds was found in habitat B. In the case of the middle layer, lowest layer, and in total, habitat A had highest number of seeds, and it decreased along successional stages (Table 1). Seed density ranged from an average of 8417 m⁻² in habitat A to 1321 m⁻² in habitat D. Seed density per plot in each separate layer and in total of the seed bank showed an obvious decrease with increasing successional age (Figs. 2a–2d). The largest total number of species originating from habitat C (48), is near to habitat B (46) and habitat C (47), but there are only 36 species in habitat A (Table 1). The species richness per plot in each separate layer and in total differed significantly, and they all increased with increasing successional age (Table 1). The species diversity index per plot differed significantly among the four habitats ($F = 239.665$, $P < 0.001$). It was highest in habitat C (2.18 ± 0.4) and lowest in habitat A (0.77 ± 0.5). The species diversity index showed an obvious increase with increasing successional age (Fig. 3). The highest species diversity and species richness appeared in habitat C whether considering each separate layer or the total.

SIMILARITY OF VEGETATION AND SEED BANK

Similarity between the seed bank and vegetation decreased gradually with successional stage in the whole (0–12 cm) (50–38.5%); it was also decreased in the 7–12 cm layer, but no trend was shown in the 0–2 cm or 2–7 cm layers (Fig. 4). For the shallow layer, there were higher similarities between the present vegetation and the seed bank, and the similarity decreased with increasing soil depth except for the 1 yr habitat.

In the NMDS ordination (Fig. 5), the first axis clearly separated Cv and Dv groups from other groups, the second axis separated Bv, Cv, and Dv groups from Av and seed bank groups. Group Av clustered together with the seed bank groups. The ordination revealed a separation in groups according to the successional stages in vegetation. The vegetation groups were close to each other gradually from Av to Dv. However, the seed bank

groups did not show a clear separation along either axis; only group As had a little difference with other seed bank groups.

Discussion

VEGETATION CHANGES

The result of NMDS showed that vegetation was separated by the two axes (Fig. 5). It indicated that during the succession, the vegetation showed a clear successional trend, the species composition had significant difference among four habitats, but these differences gradually got smaller with succession; at last, habitat C was close to habitat D. The successional trend in the vegetation shows that the habitat A is dominated by *Artemisia hedinii*, *Aconitum gymnantrum*, *Plantago asiatica*, and *Potentilla anserine*. These pioneer species are gradually replaced by *Elephantopus mollis*, *Elymus dahuricus*, *Roegneria nutans*, and *Trigonella ruthenica* in the second successional stage in habitat B, and are subsequently replaced by *Kobresia humilis*, *Artemisia tangutica*, and *Elymus dahuricus* in the third successional stage (habitat C). Ultimately, vegetation is dominated by *Thalictrum alpinum*, *Kobresia humilis*, *Scirpus pumilus*, and *Stipa* sp. in habitat D.

SEED BANK CHANGES

The seed density we measured (1303–8302 viable seeds m⁻²) was larger compared with some other subalpine meadows, alpine meadows, arctic meadows, and high altitude meadows (e.g., 99–1109 m⁻² viable seeds m⁻², Welling et al., 2004; 0–3367 viable seeds m⁻², McGraw and Vavrek, 1989; 2416 viable seeds m⁻², McGraw et al., 1991; 3203–4647 viable seedlings m⁻², Chambers, 1993; 0–4080 viable seeds m⁻², Molau and Larsson, 2000). In cold climates the diversity of both seed predators and pathogenic fungi is low (McGraw and Vavrek, 1989) and low temperatures are also associated with low embryonic metabolic rates and slow consumption of seed reserves, favoring greater seed longevity (Villiers, 1973; Murdoch and Ellis, 1992).

The result of NMDS showed that the species composition of the seed bank is almost unvaried among the successional stages; only habitat A appeared a little difference from the other three

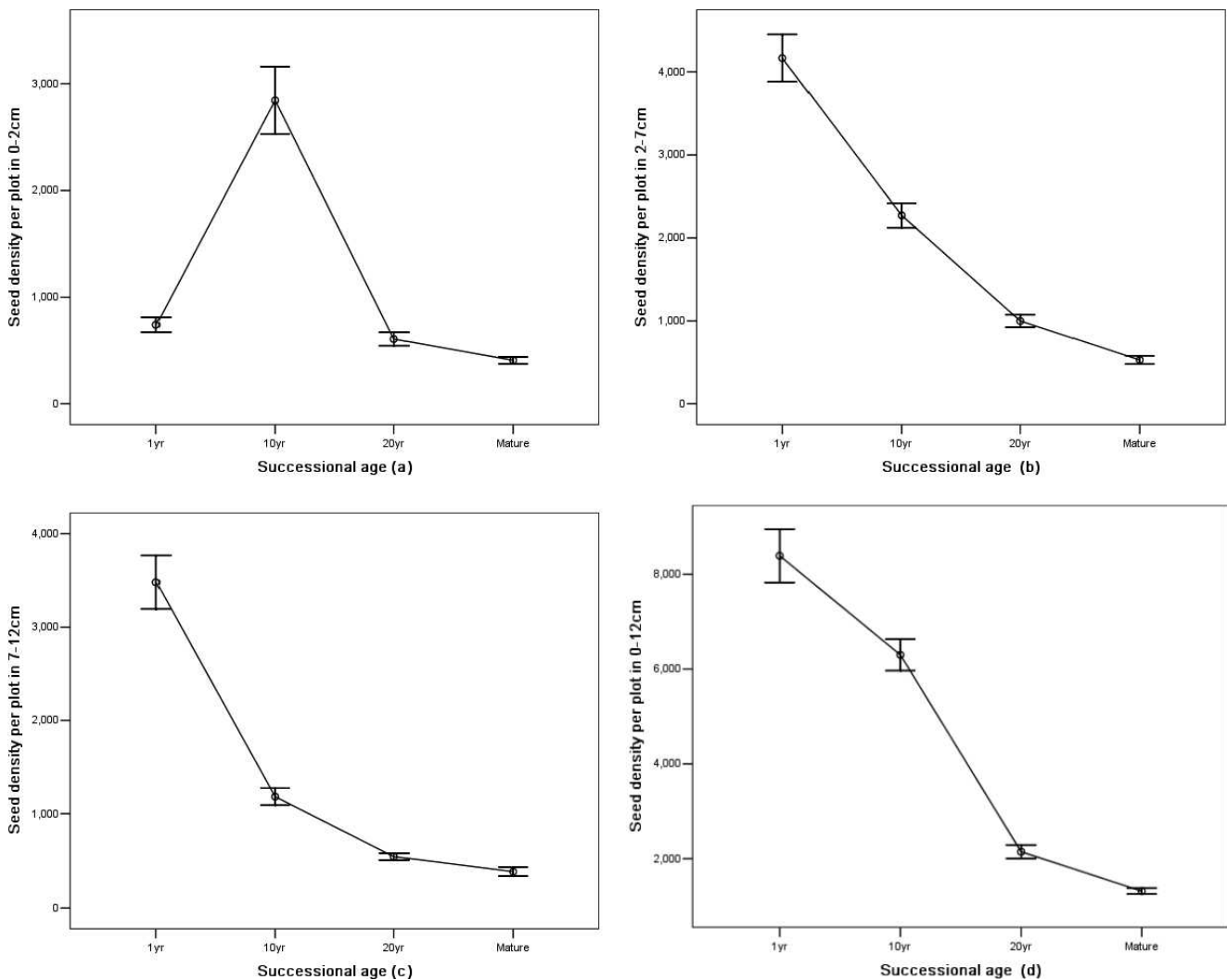


FIGURE 2. The seed density per plot ($n = 10$) (m^{-2}) found at each separate soil layer (a, b, c) and in total (d) along the different successional ages.

stages, and there were nearly no difference among habitats B, C, and D (Fig. 5). Since seed bank size is the product of the balance between seed input and output (Fenner, 1985), the high seed density in the first successional stages can be attributed to the high seed input by most pioneer species from vegetation (*Artemisia hedini*, *Artemisia desertorum*, and *Plantago asiatica*). The result showed that most of the early successional species still were dominant species in the seed bank throughout all successional stages, and the late successional species (*Roegneria nutans*, *Potentilla fragarioides*, *Potentilla anserine*, etc.) showed an increase in the number of seeds, but the quantity of them is very low, so there is little contribution to the seed bank. In the same way, investigating the seed bank of abandoned fields in southern Finland, Kiiirikki (1993) found that, even after a period of abandonment of 21 years, the seed bank was still dominated by common species from the early stage of succession. Soil seed banks that were similarly dominated by a few species have also been shown in a high subalpine site in the Oregon Cascade Mountains (Ingersoll and Wilson, 1993).

The even depth distribution of seeds in the present study was interesting because most studies report that the majority of seeds are in the topsoil in grasslands (Symonides, 1986; Rice, 1989; Kiiirikki, 1993; Bakker et al 1996). This was not supported by our study. The highest seed densities were found in 2–7 cm soil layer;

the upper 2 cm of the soil had a relative lower density in our study. The reasons could be: First, perhaps because we sampled the persistent seed bank and the surface seeds that are likely to have better condition of germination than deeper layer were no longer present (Ray and Steeves., 1983), which might cause a faster depletion of seeds in the upper soil layer. Second, most of the numerous, small, often long-lived and dormant seeds produced by early successional species do not germinate (Symonides, 1986). These seeds will become buried because of physical forces and soil formation and remain dormant in the deeper soils, where germination is not triggered (Matlack and Good, 1990). The seeds in the shallow layer of habitat A were only approximately one-sixth that of the middle layer. The reasons are likely to be the high levels of disturbance associated with the arable soils, which caused intense selection for species with a persistent seed bank (Thompson, 1978; Grime, 1979); and the soil was much less compact in habitat A, and consequently more seeds have been buried actively. Seeds in the third layer remained very low over the whole successional range, which is probably because of burial difficulties and the transient or short-term persistent character of most occurring species, a similar situation as in salt marshes (Wolters and Bakker, 2002). The deeper soil layer is not expected to contribute significantly to species replacement in the vegetation.

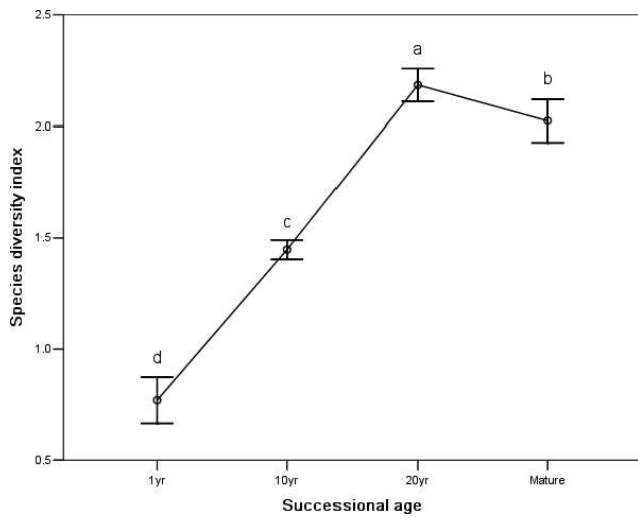


FIGURE 3. The species diversity index per plot ($n = 10$) found at different successional ages. Different letters indicate significant differences ($p < 0.001$), indicated by different superscripts assessed by a Tukey-test after one way ANOVA.

DO SPECIES RICHNESS AND SPECIES DIVERSITY DECREASE DURING SUCCESSION?

Species diversity and species richness have been postulated to decline during succession in grasslands (Donelan and Thompson, 1980) and oldfields (Symonides, 1986; Roberts and Vankat, 1991). However, that was not confirmed in the present study and both species richness and species diversity showed increased trends during succession, a situation also reflected with depth (Table 1, Fig. 3). The floristic composition of the seed bank is partly determined by the current species composition of communities but also by the vegetation history (Grandin and Rydin, 1998). The

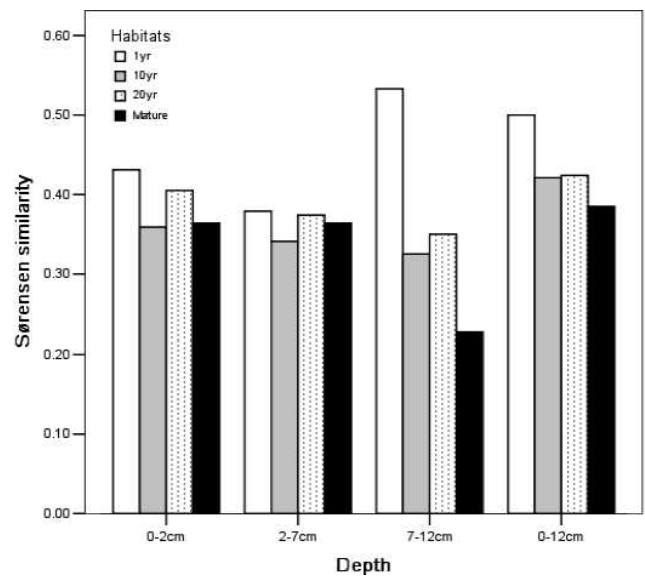


FIGURE 4. The Sørensen similarity between the seed bank and vegetation at different soil depth and four successional stages.

species richness of vegetation increased significantly with succession (Table 1). Given that some grazing occurred, moderate grazing can also increase plant diversity, as it prevents competitively superior species from gaining dominance over the weaker ones. The other reason was that most of species (27 species) from the early succession stage generally produce longer-lived seeds which stay viable in the soil in all successional stages. Species diversity and richness increased during succession which increased with the increase in vegetation, and then decreased after 20 years, which was likely due to the increasing density of clonal plants and less seed input.

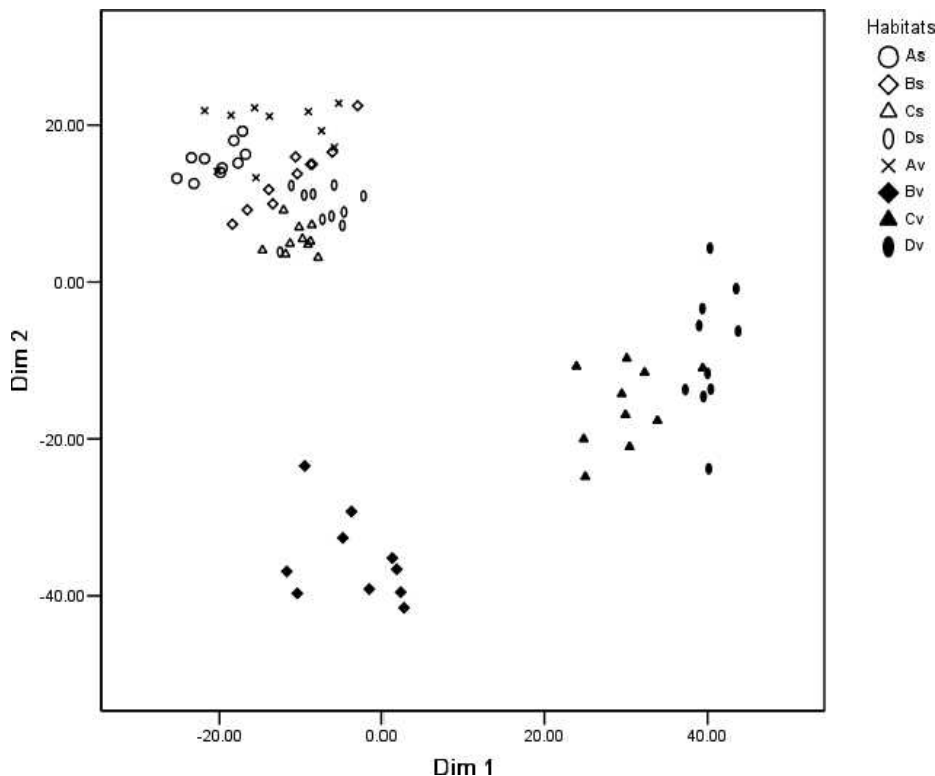


FIGURE 5. Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of seed banks and vegetation (stress value = 0.11). Ordination based on species frequency data. Successional ages ($A < B < C < D$). Av, Bv, Cv, and Dv represent vegetation in habitats A, B, C, and D; and As, Bs, Cs, and Ds represent soil seed bank. Letter codes with subscript 'v' represent vegetation from four habitats, with 10 quadrats in each habitat. Letter codes with subscript 's' represent the soil seed bank, with 10 plots from each of four habitats. The location of ordination points within each diagram indicates the degree of similarity between each one.

DOES THE DENSITY OF SEEDS DECLINE DURING SUCCESSION?

The hypothesis is that the density of buried seeds declines during succession (Thompson, 1978; Symonides, 1986; Roberts and Vankat, 1991). Obviously, our results also showed that during the course of succession there is an obvious decrease in seed density with time. The low seed density in the later successional stages can be attributed to a low seed input or production by most dominant species (*Elymus dahuricus*, *Roegneria nutans*, *Kobresia humilis*, *Thalictrum alpinum*, *Scirpus pumilus*, *Stipa sp.*, and *Poa pratensis*) in the vegetation. These perennial species rely almost exclusively on vegetative reproduction, and the proportion of perennial species increased with successional age (Fig. 1). The most abundant species in the seed bank were *Artemisia hedinii*, *Artemisia desertorum*, and *Plantago asiatica*, which together made up 87.7% of seedlings recorded. Seed density to a very large extent depends on the distribution and seed production of these species. These species have been found in seed banks of different successional stages, sometimes in very large numbers, and often present at great depth. Hence, early successional species produce many persistent seeds, which remain present in the soil even after the species disappear in the vegetation. Late successional species have less and less persistence in the soil; total seed density will decline with time as a result of seed senescence and decreasing seed input. Consequently, species of the first successional stages will still significantly contribute to the seed bank.

RELATIONSHIP OF ABOVEGROUND VEGETATION AND SOIL SEED BANK

In similarity with other studies (Bakker, 1989; Kjellsson, 1992; Thompson et al., 1993; Milberg, 1995; Bakker et al., 1996), we found that the present vegetation is more similar to the seed bank in the shallow layer than to the seed bank in the deeper soil, which shows that the present vegetation contributes less to the seed bank as soil depth increases (Fig. 4). Many studies have also shown that the similarity between vegetation and seed bank decreases with increasing soil depth (Leck and Simpson, 1987; Skoglund, 1990; Zhang and Maun, 1994). The similarity of the deepest layer was higher than shallow and mid layers in 1 yr habitat due to high levels of disturbance (cultivated soil).

The poor similarity in total (0–12 cm) (38.5–50%) between vegetation and the seed bank agrees with previous alpine and subalpine research (Chambers, 1993; Kiirikki, 1993; Jensen, 1998; Holmes and Cowling, 1997; Morgan, 1998; Arroyo et al., 1999). The weak similarity could be attributed to the following reasons: (1) unseasonably cold years decrease seed germination and seedling establishment (Bliss, 1985; Galen and Stanton, 1991; Stanton and Galen, 1997); (2) many species that are perennial rely almost exclusively on vegetative reproduction as mentioned above; (3) seed banks contained species of previous successional stages which produce longer-lived seeds (*Artemisia hedinii*, *Artemisia desertorum*, and *Plantago asiatica*); (4) many species (49.1%) were only present in the vegetation but absent in the seed bank (the absence of many species from the soil seed bank seems to be a common phenomenon in subalpine vegetation [Urbanska and Fattorini, 1998a, 1998b]); and (5) a great proportion of species were absent from the vegetation and whose seeds have a significant viability in the ground based on their strategies of opportunistic species (Touzard et al., 2002). A persistent seed bank in the alpine habitat would enable species to key their germination to more favorable years, while assuring maintenance of some seeds in the soil during years of poor seed production (Arroyo et al., 1999).

Thompson (2000) suggested that similarity between seed bank and vegetation decreased as succession proceeds. This assumption was supported by the present data. Some other studies also found a decrease in similarity with successional time in grassland systems (Jensen, 1998; Kalamees and Zobel, 1998; Wagner et al., 2003). Similarity between the seed bank and vegetation decreased with increasing age, providing evidence for low contribution of later successional species of the seed bank. Given that only several species in the vegetation are poorly represented in the seed bank, changes in their abundance had little influence on the seed bank in later successional stages; moreover, 75% of species recorded in habitat A remained throughout the four successional stages. Thus, the seed bank was dominated by earlier successional but perennial species. These species produced numerous long-lived seeds that stayed viable in the soil seed bank for more than 20 years.

During the course of succession, the vegetation showed a clear successional trend. However, this trend is not reflected in the seed bank, and the species composition of the seed bank is unvaried during the successional process. Only habitat A is different from the other three stages (Fig. 5). This indicated that the species composition of the vegetation has significant difference among the four successional stages, but the persistent seed bank comprised of long-living seeds, unable to break dormancy, did not undergo changes in species composition. The relationship of species composition between the seed bank and vegetation showed a significant difference in habitats B, C, D, and this difference was gradually enlarged with succession. Bekker et al (1999) and Brown (1998) reported that establishment from the seed bank is very limited in mature vegetation, despite the presence of a large seed bank, and that only a subset of the species present in the seed bank became established. The densities of the persistent seed banks were large in the subalpine vegetation of eastern Tibetan Plateau, and the low similarity between seed bank and vegetation in these habitats indicated that the seed bank plays a minor role in contribution to the regeneration of vegetation. The managers cannot rely on soil-stored seed banks for restoration. However, the above situation is not reflected in the seed bank of habitat A, which has species composition near to its vegetation. Hence, we suggest that the establishment of new species in early successional stages should be more dependent on the seed bank. To sum up, we deduce that seed bank plays an important role in vegetation regeneration in the early succession stage. However, the role of the seed bank becomes weaker and weaker in the later succession stages, and which most likely rely on vegetative reproduction and dispersal.

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