REGULAR ARTICLE

Effects of disturbance intensity on seasonal dynamics of alpine meadow soil seed banks on the Tibetan Plateau

Miaojun Ma · Xianhui Zhou · Guozhen Du

Abstract

Background and Aims Information on soil seed bank processes is crucial for understanding vegetation dynamics. Despite the documented importance of soil seed banks in many ecosystems, their role is not fully understood in some sensitive habitats, such as the alpine meadows of the Tibetan Plateau.

Methods We studied the seasonal dynamics of the germinable soil seed bank under four disturbance intensities in an alpine meadow on the Tibetan Plateau as well as seed size distribution relative to disturbance intensity. Composition of the seed bank was compared with that of the standing vegetation.

Results Density of buried seeds increased with disturbance intensity, but species richness and species diversity decreased. Seed density and species richness of the seed bank varied seasonally in all layers (0–2, 2–7, 7–12 cm) and the whole (0–12 cm). The species composition of seed bank was not significantly influenced by season. There was no trend in seed size distribution as disturbance increased. Seasonal seed bank turnover rates increased with increase in disturbance. The result of the NMDS showed that species composition of seed bank and vegetation exhibited a fairly uniform pattern in each season.

Conclusions Although as a whole the species composition of the vegetation and seed bank showed a relatively low degree of similarity in each season, similarity was highest in the most disturbed habitat. There was no alteration in species composition of seed bank regardless of disturbance intensity, but seed density decreased as disturbance increased. Disturbances in alpine plant communities might increase persistence of regeneration niches. Regeneration from the seed bank together with vegetative reproduction contributed to aboveground vegetation in highly disturbed habitats. Clonal species played an important role in regeneration of vegetation in slightly disturbed areas, where there was little contribution of ruderals from soil seed banks.

Keywords Alpine meadow · Grazing disturbance · Seasonal dynamics · Soil seed bank · Tibetan Plateau · Vegetation

Abbreviations
NMDS   Nonmetric multidimensional scaling

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Introduction

So far, much research has been conducted on disturbance effects on plant communities (e.g. Pickett and White 1985; Huston and Smith 1987). However, effects on soil seed banks have received little attention

(Bueno et al. 2011) although seed banks strongly influence the resilience (van Andel and Grootjans 2005) and restorability (Norbert and Annette 2004; Klimkowska et al. 2009) of plant communities. Because buried seeds form a reservoir of individuals capable of replacing vegetation following disturbance, information on soil seed bank processes is crucial for evaluating how to manage species with seed banks for conservation (Satterthwaite et al. 2007) and understanding vegetation dynamics, particularly in ecosystems experiencing frequent disturbance (Scott et al. 2010) such as alpine meadows. If seed density and species richness in the soil seed bank are sufficient and if seeds of target species have survived in the soil, successful restoration might be possible in degraded habitat (Jalili et al. 2003). Despite the documented importance of soil seed banks in many ecosystems, their role is not fully understood in some sensitive habitats (Funes et al. 2003), such as the alpine meadows of the Tibetan Plateau (Ma et al. 2010a, b).

Recent studies have shown that intensive grazing in the high Arctic tundra deplete seed banks (Cooper 2006). Bueno et al. (2011) also found lower quantities of seeds in disturbed alpine grasslands. The highest number of species was found at intermediate disturbance intensities, and it decreased drastically with high disturbance intensity (Levassor et al. 1990). Decreases in seed density may be related to reduced seed set due to livestock eating plant reproductive parts (Cooper and Wookey 2003), high mowing frequency (Mitlacher et al. 2002), or heavy grazing intensity (Sternberg et al. 2003). In fact, high grazing pressure reduced the investment in sexual reproduction in many reindeer forage species (Bråthen and Junttila 2006). Despite these results, other investigators have predicted that the density of buried seeds would decline with decreasing intensity of disturbance (e.g. Matus et al. 2005; Michaela and Wolfgang 2009). Kalamees et al (2012) found high seed densities in the soil seed banks of grazed sites. Species subjected to frequent disturbance tended to expend a large portion of their resources on the development of survival mechanisms. Thus, large seed banks would occur in communities subject to recurrent, large-scale disturbances; where the life spans of most species are shorter than the average disturbance interval and recruitment is confined to the immediate post-disturbance period (Pierce and Cowling 1991). In addition, large seed banks are created by potentially limiting the number of propagules that actually contribute to the existing population, i.e. seeds do not germinate. Our first of three hypotheses is that the Tibetan alpine meadow seed bank density increases with increasing disturbance intensity.

There is conflicting evidence, however, concerning the potential of soil seed banks to contribute to grassland restoration. The role of seeds in the recovery process from large disturbances in alpine grasslands might be only secondary (Bossuyt and Honnay 2008). This is likely because grassland plant species do not produce many seeds (Bossuyt and Honnay 2008) and seeds are dispersed over a relatively short-distance (Körner 1999). Moreover, this may also be related to a lack of suitable niches in a dense vegetation canopy in less disturbed habitats, preventing seed germination and /or establishment. However, an alternative proposal has been made. Moore (1980) suggested a high similarity between the vegetation and the seed bank for frequently disturbed communities. Some studies show that with increasing disturbance intensity the difference between the seed bank and the aboveground vegetation increases (e.g., Michaela and Wolfgang 2009). The ability of a plant community to regenerate from the soil seed bank dramatically decreases with decreasing disturbance intensity (e.g. Michaela and Wolfgang 2009), because disturbance-adapted species are characterized by high seed accumulation capacity and high seed bank persistence. In this study, we examined the similarity in species composition of the seed bank and standing vegetation along a disturbance gradient. Our second hypothesis was that similarity of seed bank and vegetation increases with increasing disturbance intensity and most likely that soil seed banks together with the clonal perennials contribute to aboveground vegetation in seriously disturbed habitats.

In addition, in continuously grazed sites, the average seed mass of species present in seed bank samples is generally smaller than that of less disturbed sites (Kalamees et al. 2012). In perennial grasslands, gap colonizers are characterized by having relatively rapid vegetative spread (Kotanen 1997) and a relatively small seed mass (Kalamees and Zobel 2002). Eriksson and Eriksson (1997) have also shown that gaps are relatively more important for the regeneration of grassland species with smaller seeds. Thirdly, we hypothesized that soils in seriously disturbed alpine meadow habitats would contain seeds with smaller mean mass than those in non-disturbed habitats.

Material and methods

Study sites

The study was conducted in an alpine meadow in Maqu, Gansu, China on the eastern Tibetan Plateau (N35°58′, E101°53′, 3500 m. a.s.l.). The average annual temperature is 1.2 °C, ranging from −10 °C in January to 11.7 °C in July. Precipitation is 620 mm per year (over the last 35 years) and is mainly distributed during the short, cool summer. The area has 2580 h of sunshine and more than 270 frost days per year. The soil type is alpine meadow, and parent materials are from a variety of sources including glacial, alluvial, residual, and residual slope deposits (Chen and Wang 1999). The community type is mainly alpine meadow and is dominated by many monocotyledons, primarily Poaceae and Cyperaceae and by various dicotyledons, such as Ranunculaceae, Polygonaceae, Saxifragaceae, Asteraceae, Scrophulariaceae, Gentianaceae, and Fabaceae (Ma et al. 2010a, b).

Seed bank sampling and assessments of vegetation composition were carried out in four meadow habitats. These habitats, referred to as C, F, L, and S, represent a gradient of increasing grazing disturbance intensity (Table 1) and were 500–1000 m apart. C-ve, F-ve, L-ve, S-ve represent vegetation in habitats C, F, L, and S, respectively, and C-sb, F-sb, L-sb, and S-sb represent the soil seed bank. Germination studies of seeds in soil samples were conducted in the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University (Hezuo Branch Station) (N34°55′, E102°53′), Gansu, China, located on the eastern Tibetan plateau at an elevation of 2900 m above sea level. The average temperature there is 2.0 °C and precipitation is 557.8 mm.

Soil seed bank sampling

Soil samples were collected three times: in May 2005 and May 2006, before seed germination in the field, and in July 2005 after the spring germination flush but before dispersal of the current season seeds. Ten randomly selected replicate sites (2 m×2 m in May, 2005; 20 m×20 m in July, 2005 and May, 2006) were established in each habitat, each with the same slope and exposure. In each of 10 plots (0.4 m×1 m in May, 2005; 5 m×5 m in July, 2005 and May, 2006), randomly distributed in each site, 10 cylindrical soil cores (3.6 cm diameter) were taken randomly. The soil cores were separated into three layers, shallow (0–2 cm deep), mid (2–7 cm deep), and deep (7–12 cm deep). For each plot, the ten cores from each depth were pooled. Overall, there were 30 samples from each site (10 samples for each layer), and 300 soil samples for each habitat type.

Treatment of soil samples and maintenance of seed trays

The soil samples were stored for 15 days until they could be processed. The seed bank was sampled by the concentration method followed by seedling emergence as described by Ter Heerdt et al (1996) and Ma et al. (2009, 2011, 2012). That is, samples were sieved, after which they were washed through a coarse (4 mm mesh) and then a fine (0.2 mm mesh) sieve to remove debris, root fragments, and coarse and fine soil material. Visual inspection of the coarse particles retained by the wide mesh sieve revealed that no large seeds were also removed. The concentrated samples were spread evenly on a layer of sterile sand (sterilized at 140 °C for 24 h) in sterile plastic germination trays (width 30 cm). Depth of the soil layer was less than 2 cm. Thirty control trays with only sterilized sand were set alongside the experimental trays and used to determine if there were any airborne seeds or contaminants from other samples. Trays were watered regularly. Emerging seedlings were removed, and those that could not be identified immediately were grown separately until identification was possible. Soil samples were carefully turned over following cessation of the initial germination flush to facilitate the emergence of new seedlings. After 5 months when no more seedlings emerged for several consecutive weeks, sampling was stopped. Subsequent sifting and careful inspection found that no seeds remained.

Seed mass

Seeds of 138 of the 155 species found in the seed bank were collected in Maqu from August to October in 2005 at the start of natural dispersal. Seeds of a given species were pooled, mixed well, and three subsamples of 100 seeds selected. Each subsample was placed into an envelope. Seed mass was defined as the mass of the

Table 1 Short descriptions of the habitat types investigated. All habitats have same slope, exposure, altitude, annual mean temperature (1.2 °C) and annual mean precipitation (620 mm)

Habitats (Fresh biomass)	Dominant species	Description
Control meadow (C) (834.4±49.6 gm^{-2})	*Kobresia graminifolia, Taraxacum mongolicum, Elymus dahuricus, Scirpus distigmaticus,* and *Gueldenstaedtia multiflora*	Disturbance intensity was low and it is the typical mature alpine meadow in the eastern Tibetan Plateau. This habitat was mild grazed by livestock (e.g., yak and Tibetan sheep). This functioned as the control site for this study.
Fenced meadow (F) (1299.2±83.5 gm^{-2})	*Kobresia graminifolia, Poa poophagorum, Poa pratensis, Stipa aliena,* and *Elymus dahuricus*	Fenced in October 1999, this area was subsequently grazed by livestock (e.g., yak and Tibetan sheep) only during winter. This low grazing intensity only removed dead biomass; live biomass was not affected. It is dominated by graminoids that were taller than meadows L and S.
Largely disturbed meadow (L) (642.8±52.7 gm^{-2})	*Kobresia graminifolia, Elymus dahuricus, Potentilla fragarioides, Taraxacum mongolicum,* and *Kobresia humilis*	Disturbance intensity was higher than site C and less than site S. The vegetation was slightly degraded in some areas because it was overgrazed by livestock (e.g., yak and Tibetan sheep). The proportion of ruderal species was less and graminoids higher compared than site S.
Seriously disturbed meadow (S) (508.0±15.3 gm^{-2})	*Potentilla anserina, Plantago asiatica, Ajania tenuifolia, Elymus dahuricus, Taraxacum mongolicum,* and *Poa pratensis*	Exposure to long-term overgrazing and trampling by livestock (e.g. yak and Tibetan sheep) resulted in loss of vegetation. Where vegetation was low and sparse ruderal species dominated. In other places there were gaps created by Tibetan Pika (*Ochotona curzoniae*), marmot (*Marmota himalayana*), and livestock trampling (Yak and Tibetan sheep). *Potentilla anserina* colonizes this site.

embryo and endosperm, plus the seed coat; dispersal structures were not included. The average mass of the three subsamples was used as the seed mass variable.

Above-ground vegetation sampling

Vegetation sampling was performed during the peak of the summer growing season (July, 2005 and 2006). On the basis of species-area curve on the eastern alpine meadow of Tibetan plateau, we calculated that the minimum sampling area was 50×50 cm. Thus, we used 50 cm×50 cm quadrats placed randomly within each of the four habitats to collect soil seed bank samples. In 2005, five quadrats were used in each habitat, and in 2006 10 quadrats were used in each habitat. 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). Clonal species were examined by presence of ramets or tillers and species with sexual reproduction were examined by presence of seedlings (Harper 1977).

Data analysis

To estimate seasonal variation in the seed bank, seed bank depletion (e.g. Funes et al. 2003) and accumulation were calculated as:

$$(\text{No. seeds per plot in May, } 05 - \text{No. seeds per plot in July, } 05)/\text{No. seeds per plot in May, } 05. \quad (1)$$

$$(\text{No. seeds per plot in May, } 06 - \text{No. seeds per plot in July, } 05)/\text{No. seeds per plot in May, } 06. \quad (2)$$

Species richness in aboveground vegetation was analysed using a two-factor repeated measures ANOVA (factors disturbance level and sampling time) to test for general effects and their interactions and using post-hoc tests to reveal the factor-level differences. Seed bank density and species richness in soil seed bank were analysed using three-factor repeated-measures ANOVA (factors disturbance level, sampling time, and soil depth)

to test for general effects and their interactions and using post-hoc tests to reveal the factor-level differences. The differences of proportion of life form in vegetation and soil seed bank, seed mass, seed bank depletion and accumulation in different disturbance intensities were compared by ANOVA and Tukey range test. To meet the requirement of variance homogeneity, the data were log-transformed prior to analysis. All ANOVA tests were conducted with a SPSS 13.0 program.

Species composition similarity among different vegetation and seed banks in three seasons (May, July, 2005; May, 2006) was analyzed 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). Similarity between the seed bank and the vegetation within each of the four habitats was tested using a NMDS, and data for each season were analyzed separately. Ordination was made using the R-program for Windows version 2.0.7, applying package VEGAN by Jari Oksanen. NMDS is considered the best method for graphical representation of floristic relationships (Clarke 1993). We calculated similarity matrices using the Bray Curtis coefficient. All ordinations were based on frequency data.

Results

Aboveground vegetation

We recorded 79 species, belonging to 21 families in 2005, and 15.4 % of the species were annuals, 2.6 % biennials, and 82.1 % perennial herbs. In 2006, 62 species were recorded, belonging to 17 families, and 9.7 % of the species were annuals, 4.8 % biennials, and 85.5 % perennial herbs. The proportion of perennial species decreased with disturbance intensity (Fig. 1). Species richness per quadrat differed significantly along a disturbance gradient both in 2005 and 2006 (Table 2, Fig. 2), with the lowest species richness in the most disturbed habitat, S. In contrast, species richness per quadrat did not differ significantly between two seasons (Table 2). There was no interaction between disturbance level and season.

The plant communities in habitat S from 2005 to 2006 were dominated by clonal species, such as *Potentilla anserine*, *Elymus dahuricus*, and *Poa*
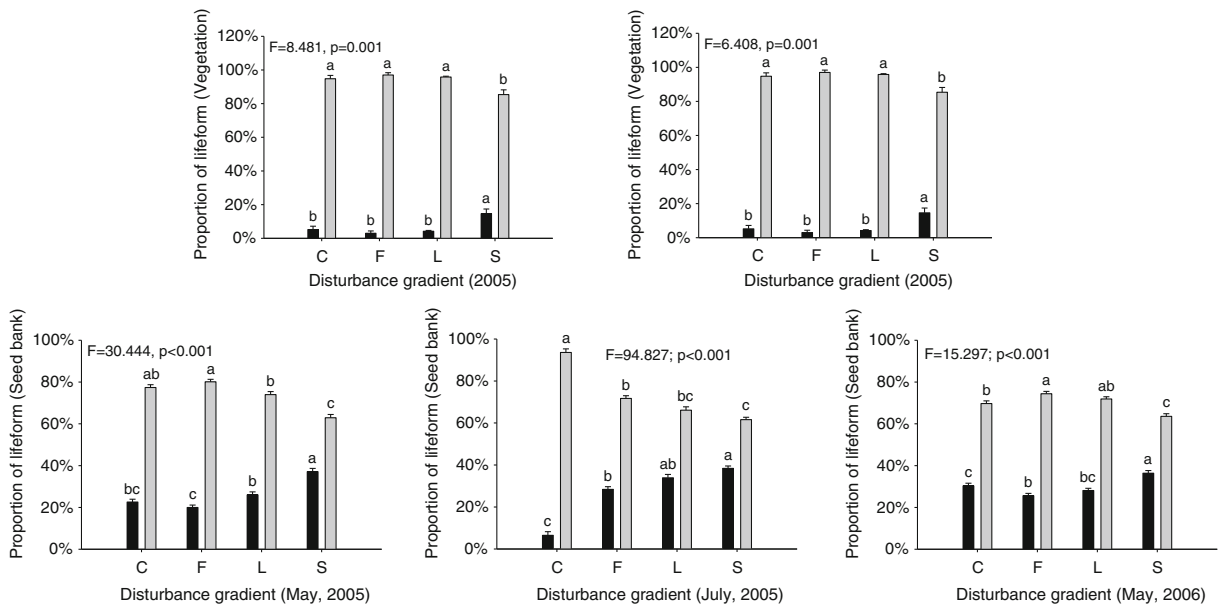

Fig. 1 The proportion of life forms changes (per quadrat, mean± SE, n=10) in vegetation and seed bank in different seasons along a disturbance gradient in the Tibetan Plateau. *Letters* indicate significant differences (ANOVA, Tukey range test) in proportion of lifeform among disturbance types within one date. *Black bar*: annual and biennial species, *gray bar*: perennial species. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C< F<L<S)

Table 2 Repeated-measures ANOVA for the effect of disturbance level and season on species richness in aboveground vegetation (two-factor), and the effect of disturbance level, season and soil depth on seed density and species richness in soil seed bank (three-factor). Seed density comparisons were carried out in log transformed data. Differences shown in bold are statistically significant

Variable	df	Seed density		Species richness	
		F	P	F	P
Aboveground vegetation					
Disturbance level	3			43.789	**0.0001**
Season	1			3.443	0.069
Disturbance level × season	3			1.445	0.240
Error	52				
Soil seed bank					
Disturbance	3	36.615	**0.0001**	4.655	**0.003**
Season	2	47.598	**0.0001**	65.597	**0.0001**
Soil depth	2	125.486	**0.0001**	22.297	**0.0001**
Disturbance × season	6	3.229	**0.004**	5.197	**0.0001**
Disturbance × soil depth	6	2.749	**0.013**	4.047	**0.001**
Season × soil depth	4	25.046	**0.0001**	19.882	**0.0001**
Disturbance × season × soil depth	12	1.108	0.352	1.614	0.086
Error	324				

pratensis. Plant cover of *Potentilla anserine* was 52.0 ±22.8 % in 2005 and 46.1±12.6 % in 2006. The plant communities in habitat F from 2005 to 2006 were dominated by clonal species, such as *Elymus dahuricus*, *Koeleria cristata*, *Kobresia graminifolia*, *Poa poophagorum*, *Kobresia capillifolia*, and *Stipa aliena*. For example, plant cover of these clonal species was 56.0±4.4 % in 2005 and 59.8±3.5 % in 2006. The

dominate clonal species in habitat S from 2005 to 2006 were *Kobresia graminifolia*, *Elymus dahuricus*, *Poa poophagorum*, *Stipa aliena*, *Roegneria nutans*, *Kobresia capillifolia* with their cover being 63.9± 6.0 % in 2005 and 47.9±2.8 % in 2006.

The results of NMDS showed that the first Dim separated habitat S-ve from other habitats in both 2005 and 2006, whereas C-ve, F-ve, and L-ve overlapped with each other along Dim 1 (Fig. 3).
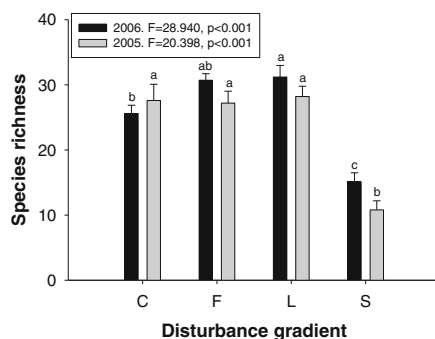
Soil seed bank

Visual inspection of soil samples after germination had ceased revealed very few nongerminated seeds, indicating an accurate estimation of the seed bank. No seedlings were recorded in the control trays, indicating that there were no airborne seed contaminants. Overall, 155 species emerged from the three seasonal sets of seed bank samples. Three species could only be identified to the family level (Compositae sp. Gramineae sp. and Polygonaceae sp.) and two only to genus (*Saussurea* sp. and *Pedicularis* sp.).

Altogether 17531 seedlings from 87 species emerged from the May 2005 soil samples, and 19.6 % of them were annuals, 5.7 % biennials, and 74.7 % perennial herbs. In the July, 2005 samples, 14195 seedlings from

Fig. 2 Species richness (per quadrat, mean±SE, n=10) changes in vegetation along a disturbance gradient in the Tibetan Plateau. *Letters* indicate significant differences (ANOVA, Tukey range test) in species richness among disturbance types within a date. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C<F<L<S)

Fig. 3 Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of seed banks and vegetation along a disturbance gradient, and seed banks and vegetation in different seasons in alpine meadows of the Tibetan Plateau. (Stress value= 0.10 in May, 2005; Stress value=0.11 in July, 2005 and May, 2006; Stress value= 0.15 in vegetation change; Stress value=0.17 in seed bank change). Ordination was based on species frequency data. Different marks represent different vegetation and seed bank types. There were 10 quadrats in each seed bank, 5 plots for the vegetation in 2005, and 10 plots for the vegetation in 2006. The location of ordination points within each diagram indicates the degree of similarity between each one. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C<F<L<S)
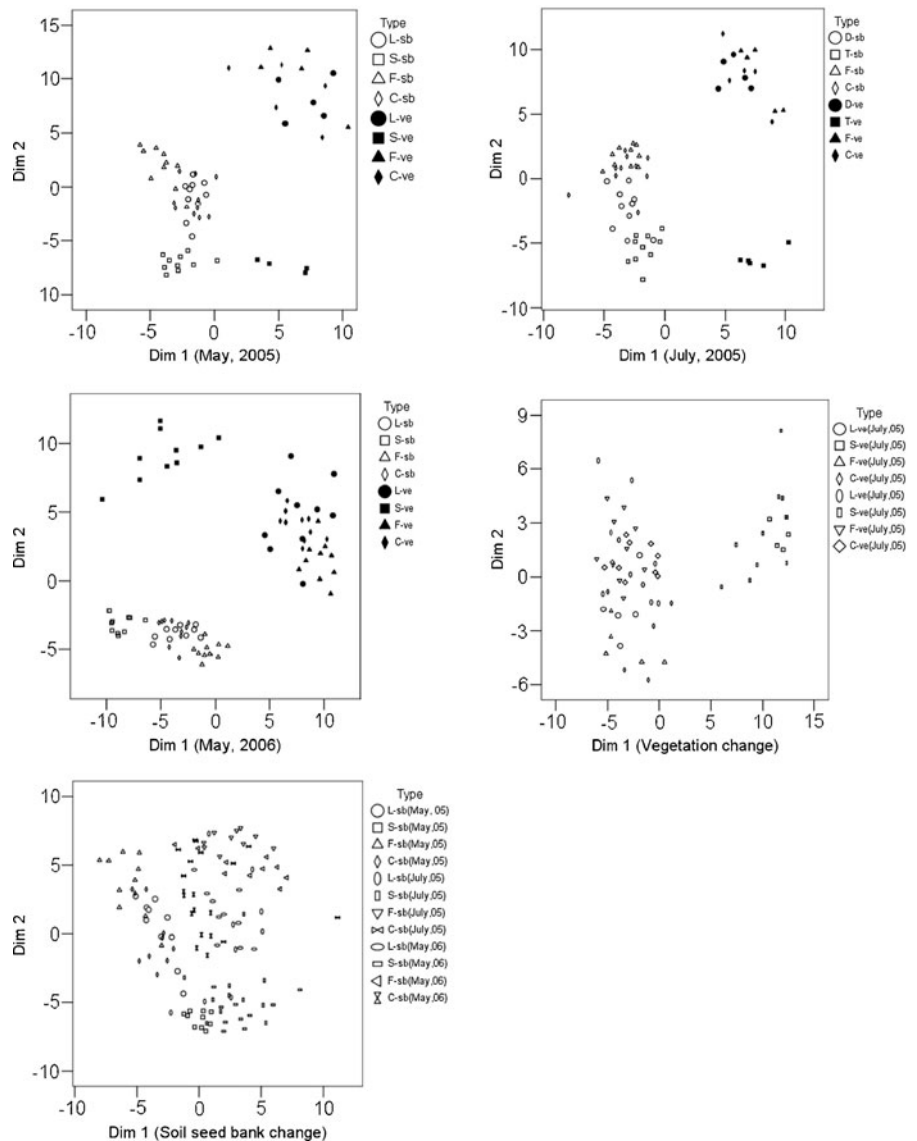
130 species were recorded with 26.4 % of them being annuals, 5.4 % biennials, and 68.2 % perennial herbs. A total of 24098 seedlings from 112 species were recorded from May 2006 samples, and 26.6 % of them were annuals, 3.7 % biennials, and 69.7 % perennial herbs. The proportion of perennial species in the seed bank showed a decreasing trend with disturbance gradient in each season (Fig. 1).

Seed density and species richness change

Mean seed density and species richness per plot differed significantly among the four habitats,

different seasons and soil depths (Table 2, Fig. 4). Both for seed density and species richness, there was a significant interaction between disturbance level—season, disturbance level—soil depth, and season—soil depth (Table 2). Three way interactions were not detected (Table 2).

The highest density was recorded in the most disturbed site (S), while the lowest density was recorded for the control site (C), and increasing from habitat C to habitat S regardless of season, separate layer, or all layers combined (Table 2, Fig. 4). The highest seed densities were found in surface samples (0–2 cm), and decreased with depth (Fig. 4). The species richness per
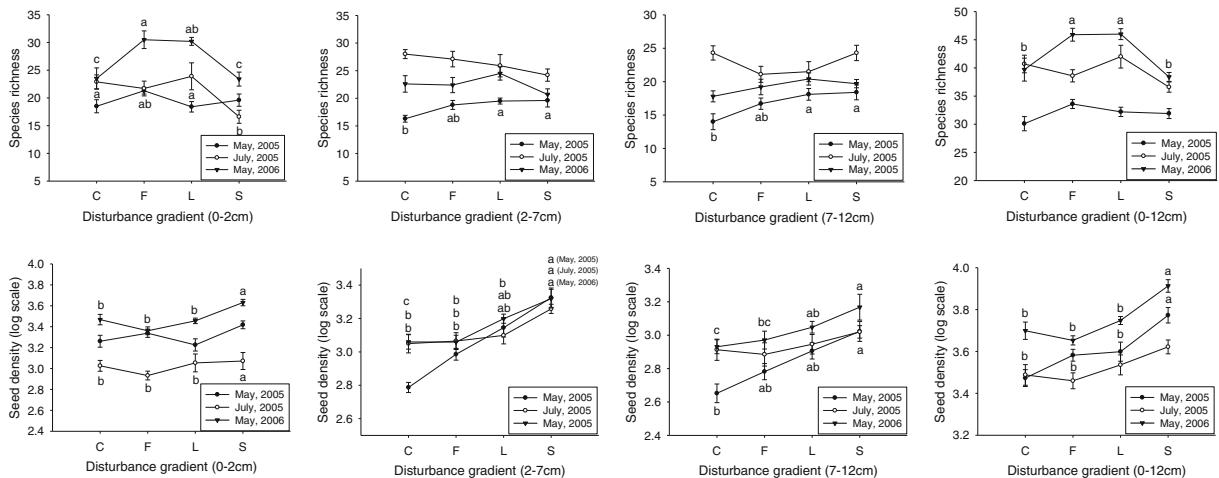
Fig. 4 Mean species richness (per plot) (top two rows) and seed density m^{-2} (±SE) (bottom two rows) (±SE) at four soil depths classes along a disturbance gradient of Tibetan Plateau. *Letters* indicate significant differences (ANOVA, Tukey range test) among disturbance types within a date. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C<F<L<S)

plot did not differ significantly with layer or total for all layers combined along the disturbance gradient, and it showed an increase trend in 2–7 cm and 0–12 cm with increase in disturbance; the lowest soil depth (7–12 cm) showed no trend (Table 2, Fig. 4). The Shannon-Wiener Index per plot differed significantly among the four habitats (Fig. 5) and was highest in habitat F and lowest in habitat S. The species diversity index decreased with an increase in disturbance intensity.
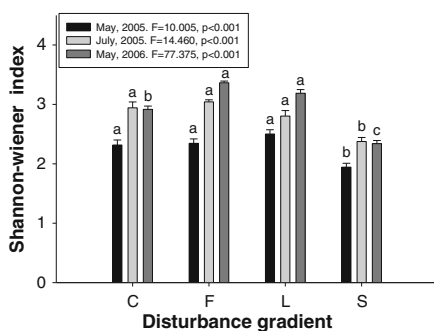

Fig. 5 The Shannon-wiener index per plot (n=10) found in soil seed bank along a disturbance gradient. *Different letters* indicate significant differences, indicated by different superscripts assessed by a Tukey-test after one way ANOVA on differences between disturbance types for a respective sampling time. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C<F<L<S)

Seasonal seed bank dynamics

Both seed bank density and species richness differed significantly in each depth layer and total for all layers among seasons, except seed density in the mid-layer (2–7 cm) (Table 3). The NMDS results showed that the seed bank communities were similar to each other with disturbance intensity in each season and among different seasons (Fig. 3).

Depletion and accumulation

Seasonal depletion in seed bank density differed significantly along the disturbance gradient (F=8.268, p<0.001), increasing with disturbance; however, accumulation did not differ (F=1.178, p>0.05). The highest accumulation (46.9±16.2 %) and depletion (27.9±15.2 %) occurred in habitat S (Fig. 6).

Seed mass

For the 138 species examined, mass per seed ranged from 0.0018 mg (*Juncus effusus*) to 4.5354 mg (*Cynoglossum zeylanicum*). Seed mass did not differ significantly along the disturbance gradient in each season (F=0.374, p>0.05 in May, 2005; F=0.026, p>0.05 in July, 2005; F=

Table 3 Results of ANOVA of seed density and species richness change in each separated layer (0–2, 2–7, 7–12 cm) and total (0–12 cm) among different seasons

Soil depths	Seed density		Species richness	
	F	P	F	P
0–2 cm	53.385	**0.0001**	24.356	**0.0001**
2–7 cm	0.982	0.377	39.533	**0.0001**
7–12 cm	6.606	**0.002**	29.049	**0.0001**
0–12 cm	22.946	**0.0001**	56.379	**0.0001**

0.593, p>0.05 in May, 2006). There was also no trend in seed size distribution with disturbance or with soil depth.

Relationship of soil seed bank and vegetation

In general, species composition of seed bank and vegetation exhibited a fairly uniform pattern in each season (Fig. 3). In vegetation communities, S-ve was different from the other three communities that clustered together. The major vegetation changes in habitat S were not reflected in the seed bank. The seed bank communities overlapped with each other, except S-sb that only has a little difference with other seed bank groups. Among the four seed bank and four vegetation communities, C-ve, L-ve, and F-ve were the most different along dim 1 and 2 axes; however, community S-sb was very close to community S-ve relative to the other seed bank communities and their corresponding vegetation communities. Aboveground plant community of habitat S strongly reflected its seed bank composition (Fig. 3).
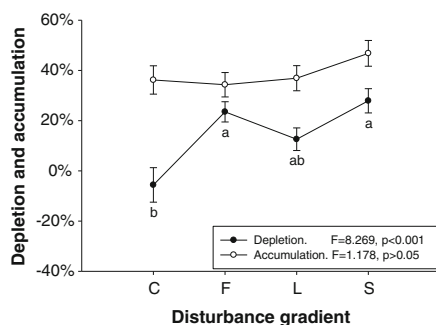

Fig. 6 Seed bank depletion (May, 2005 to July, 2005) and accumulation (July, 2005 to May, 2006) (mean±SE, n=10) in alpine meadow along a disturbance gradient in Tibetan Plateau. *Letters* indicate significant differences (ANOVA, Tukey range test) in depletion among disturbance types within a date. Abbreviations of sites names: C: Control meadow site, F: Fenced site, L: Largely disturbed site, S: Seriously disturbed site (disturbance C<F<L<S)

Discussion

Soil seed bank changes with disturbance intensity and season

Many alpine areas are known to be seed limited in the soil seed bank (e.g. Turnbull et al. 2000; Eskelinen and Virtanen 2005). However, we found a relatively high number of seeds in the seed bank in the investigated alpine meadows (Fig. 4). The seed densities (3069–6105 m^{-2} in May, 2005, 2991–4297 m^{-2} in July, 2005, and 4563–8355 m^{-2} in May, 2006) are similar to those from our previous Tibetan Plateau studies (Ma et al. 2010a, b), and larger than other alpine meadow, arctic meadows and high-altitude meadows (e.g. Funes et al. 2003; Bossuyt et al. 2007; Bueno et al. 2011). Large amounts of seeds occur at such sites because cold climates could contribute to the maintenance of many seeds in the soil (Cavieres and Arroyo 2000; Ma et al. 2010a, b).

Density of seeds in the soil increased with disturbance intensity, a result consistent with our first hypothesis. This may be due to the interaction of several factors. There is selection for persistence-related traits (k-strategy) under less disturbed conditions (e.g., Diaz et al. 2004) and a strong selection for reproductive traits (r-strategy, Grime 1979) under disturbed conditions. The high relative contribution of ruderal strategists in the seed banks can be seen as a function of their rapid growth, high seed production, and seed bank persistence, which are adaptations to disturbed habitats (Thompson et al. 1997; Fenner and Thompson 2005; Wellstein et al. 2007). We found that the proportions of annuals and biennials (often r-strategists) in the vegetation increased with disturbance, and they were relatively high in the most disturbed habitat (S) (Fig. 1). Hence, we think competitive strategies in habitats C and F are gradually replaced by ruderal strategies with increasing disturbance intensity.

In some cases, species from disturbed sites invest predominantly in generative propagation and produce large amounts of seeds (e.g. Bakker and Berendse 1999). Increased disturbance may enhance seed production at the community level (Klimkowska et al. 2009). Thus, ruderal species generally make a higher contribution to the seed bank because of high seed production. Our results also indicate that disturbance favours high seed production (e.g. Grime 2002).

The dominant seed bank species in disturbed habitat S were *Plantago asiatica*, *Artemisia desertorum*, and *Poa annua*, which together made up 70.7 % of the seedlings recorded in May, 2005, 55.2 % in July, 2005, and 56.1 % in May, 2006. Therefore, seed density to a very large extent depends on the distribution and seed production of these species. However, they occurred only sporadically in the aboveground vegetation, indicating that their seeds have persistent seed banks. There is selection for more persistent seeds under conditions of disturbance (Thompson et al. 1998). This is supported by our data, which show that density of buried seeds increased as disturbance increased.

Overall, species richness and diversity showed a decreased trend along the disturbance gradient (Figs. 4 and 5). We know that seed bank size is determined by the balance between seed input and output (Fenner 1985), and that floristic composition is determined by the current species composition and by vegetation history (Grandin and Rydin 1998). Our results also showed that species richness of the vegetation decreased significantly along the disturbance gradient (Fig. 2). Species richness, however, showed no clear trend in the deepest soil depth (7–12 cm) with increased disturbance. This was due to a larger number of long-lived seeds. The species composition of this layer, made up of persistent seed bank species, is not influenced by seed input to the surface layers. Although the aboveground vegetation changed with the disturbance intensity, the persistent seed bank, varied little.

The greatest seed bank accumulation and depletion occurred in the most disturbed habitat (S), and each increased with increase in disturbance (Fig. 6). Thus, similarity between soil seed bank and vegetation increased along the disturbance gradient, and seed bank turnover rates increased with an increase in disturbance.

The result of NMDS showed that the seed bank groups were close to each other with disturbance intensity in each season (Fig. 3. May, 2005; July, 2005;

May, 2006) and among seasons (Fig. 3. Soil seed bank change). Although seed bank density and species richness had an obvious seasonal change (Table 3), there was no difference in seed bank species composition under different disturbance intensities and among different seasons (Fig. 3).

In a previous study, we found persistence to be the most frequent seed bank strategy of Tibetan Plateau alpine meadow species (Ma et al. 2010b). In the present study, the most widely distributed and dominant species in the seed banks were *Plantago asiatica*, *Potentilla fragarioides*, *Artemisia desertorum*, *Chenopodium album*, and *Hypecoum leptocarpum*, and they accounted for 64.9 % of the seedlings recorded in May, 2005, 50.3 % in July, 2005, and 47.7 % in May, 2006. Although the transient part of seed bank changed every year, the persistent seed bank did not vary with change in the disturbance intensity. Hence, we think the seed bank was not significantly influenced by season.

Some studies have shown that small-seeded species are more dependent on disturbance for establishment than large-seeded species and that large seed size permitted seedling establishment in closed vegetation (Burke and Grime 1996). Thus, small seed size was identified as a characteristic functional trait of gap colonizers (Kalamees and Zobel 2002). In contrast, Lavorel et al. (1999) found no association between seed size and colonization. Our results support the latter opinion as we found no trend in seed size distribution with disturbance in seed bank; consequently, our third hypothesis with regard to seed size was not supported. Seed mass ranged from 0.0018 mg (*Juncus effusus*) to 4.5354 mg (*Cynoglossum zeylanicum*), but this is a smaller range of sizes than recorded in other studies (Leishman and Westoby 1998; Moles et al. 2000). The limited range of seed size in Tibetan Plateau vegetation may be due to evolutionary and ecological constraints including glaciation history, disturbance regimes, and predation pressure on larger seeds.

Role of soil seed bank

Similarity between species composition of vegetation and seed bank as a whole was low, thus agreeing with results from other alpine studies (e.g. Arroyo et al. 1999; Ma et al. 2010a, b). Indeed, this low similarity

between vegetation and seed banks seems to be a characteristic of alpine environments which have a relatively short growing season and where there is less reliance on seed banks for reproduction in favour of clonal strategies (Körner 1999; Bueno et al. 2011). The species composition of the seed bank and extant vegetation was very different from each other in C and F, the least disturbed habitats. Thus, the seed bank would play a minor role in regeneration of the vegetation of C and F, which are dominated by clonal species of Poaceae (e.g., *Elymus dahuricus*, *Poa poophagorum*, *Roegneria nutans*, *Festuca ovina*, *Stipa aliena*, and *Poa pratensis*) and Cyperaceae (e.g., *Kobresia graminifolia*, *Kobresia capillifolia*, *Kobresia humilis*, and *Scirpus pumilus*). The C and F communities of the control and fenced meadows often regenerate vegetatively forming very stable communities, and lack of similarity in these undisturbed habitats is partly due to low seed input/production by the clonal species. Also, there is little opportunity for ruderals to become established in the closed canopy of these plant communities that are dominated by clonal species.

It is probable that the role of the seed bank in alpine plant community regeneration from large disturbances is relative low. This may be due to the following reason. Firstly, many species in alpine area do not produce many seeds (Bossuyt and Honnay 2008) and have relatively short-distance seed dispersal (Körner 1999). Secondly, large disturbances may not represent a chance for colonization from seeds (Bueno et al. 2011). In addition, clonal strategies prevail among the dominant species in alpine grasslands (Körner 1999), which limits establishment from the seed bank. However, we found that the soil seed bank has the potential to play an important regeneration role in the most seriously disturbed habitat (S) (Fig. 3), and this situation was apparent in each season. This result is consistent with our second hypothesis. Similarly, others have observed a clear increase in the similarity between vegetation and soil seed bank under conditions of increasing disturbance (Bekker et al. 2000). In seriously disturbed habitat (S), cattle activities such as grazing (removing biomass) and trampling affect vegetation dynamics differently at a very fine scale (Kohler et al. 2004). Seeds at the soil surface receive more light and greater temperature fluctuation than those in the soil. The increase in light in disturbed sites increases the probabilities of seedling emergence and seedling establishment (Fenner 2000). In disturbed areas, there is more space for the establishment of

opportunistic, annual species that produce large amounts of seeds. Hence, disturbances in alpine plant communities assure the presence of regeneration niches. There is a tradeoff, however, in the maintenance of sustainable meadows and deterioration associated with overgrazing and lack of regeneration potential.

Disturbances may change the nature of community seed banks from long-term persistent to short-term persistent or transient seed banks, because of increased exposure of seeds to germination conditions (Bueno et al. 2011). In large disturbances, where the lateral expansion of dominant grasses is insufficient to recover bare ground efficiently (Kotanen 1997), soil seed banks seem to play a more determinant role in recovery (Thompson et al. 1998). Moreover, fertilization from urine and dung stimulate plant growth and reproduction; with dung there also is the possible addition of seeds from new species (e.g. Bakker and Olff 2003).

The dominant vegetation species in the seriously disturbed habitat S were *Potentilla anserine*, *Elymus dahuricus*, and *Poa pratensis*. Although these perennial species might not produce seeds in highly degraded sites, they persisted in the vegetation because they are able to spread clonally after disturbances. An especially good colonizer, *Potentilla anserina* had significantly larger plant cover in habitat S (52.0 ± 22.8 % in 2005 and 46.1 ± 12.6 % in 2006) than in the less disturbed habitats. This rhizomatous species quickly colonized trampled and mowed plots.

Our results show that soil seed banks together with the clonal growth make a contribution to aboveground vegetation in seriously disturbed alpine meadow habitats. Further, the overall high species richness and high seed bank densities in habitat S suggest that the soil seed bank can potentially play an important role in disturbed habitat restoration.

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