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Effects of degradation and species composition on soil seed density in the alpine grasslands, China (Postprint)

Authors: LI Chunming

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Abstract

Grassland degradation can alter the structure and function of ecosystem and soil seed bank. Therefore, estimating the role of soil seed bank in vegetation regeneration of degraded grasslands is crucial. We selected grasslands with three levels of degradation, namely non-degraded (ND), mildly degraded (MD), and heavily degraded (HD) to analyze the effect of grassland degradation on soil seed bank, as well as the role of soil seed bank on vegetation regeneration of the alpine grasslands, China. Soil samples from each level were collected in May, before seedling emergence, in August, after completion of transient seed bank germination, and in December, after seed dispersal, to determine the seed density and species composition through germination experiment. Result showed that a total of 35 plant species was identified, including 15 species observed in both soil seed bank and above-ground vegetation. A total of 19, 15, and 14 species of soil seed bank were identified in December, May, and August, respectively. The most abundant species in soil seed bank were Compositae (5 species), followed by Poaceae (4 species), and Cyperaceae (3 species). Degradation level has no significant impact on species richness and Shannon- Wiener index of soil seed bank. In addition, sampling month and grassland degradation affected soil seed bank density, in which December>May>August, and ND>MD>HD, indicating that density of transient seed bank was greater than persistent seed bank. Soil seed bank density of surface layer (0–5 cm) accounting for 42%–72% of the total density, which was significantly higher than that of deep layer (5–10 cm). Similarity of species composition between vegetation and soil seed bank was low, and it increased with degradation level (ranged from 0.14 to 0.69). We concluded that grassland degradation affects soil seed bank density more than species diversity, and soil seed bank contributed slightly to vegetation regeneration of degraded alpine grassland. Therefore, it is unlikely that degraded alpine meadow can be restored solely through soil seed bank.

Full Text

Preamble

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Effects of degradation and species composition on soil seed density in the alpine grasslands, China

LI Chunming[1]*, MA Jiahui[1], LI Liangyu[1], HUANG Junlin[1], LU Jinhua[1], HUANG Mei[2], Allan DEGEN[3], SHANG Zhanhuan[2]

[1]Life Science and Engineering College of Northwest Minzu University, Lanzhou 730124, China
[2]State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou 730000, China
[3]Desert Animal Adaptations and Husbandry, Wyler Department of Dryland Agriculture, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Beer Sheva 8410500, Israel

Abstract

Grassland degradation can alter the structure and function of ecosystems and soil seed banks. Therefore, estimating the role of soil seed banks in vegetation regeneration of degraded grasslands is crucial. We selected grasslands with three levels of degradation—non-degraded (ND), mildly degraded (MD), and heavily degraded (HD)—to analyze the effects of grassland degradation on soil seed banks and the role of soil seed banks in vegetation regeneration of alpine grasslands in China. Soil samples from each degradation level were collected in May (before seedling emergence), in August (after completion of transient seed bank germination), and in December (after seed dispersal) to determine seed density and species composition through germination experiments. Results showed that a total of 35 plant species were identified, including 15 species observed in both the soil seed bank and above-ground vegetation. A total of 19, 15, and 14 soil seed bank species were identified in December, May, and August, respectively. The most abundant species in the soil seed bank belonged to Compositae (5 species), followed by Poaceae (4 species) and Cyperaceae (3 species). Degradation level had no significant impact on species richness or the Shannon-Wiener index of the soil seed bank. However, both sampling month and grassland degradation affected soil seed bank density, following the patterns December > May > August and ND > MD > HD, respectively, indicating that transient seed bank density was greater than persistent seed bank density. Soil seed bank density in the surface layer (0–5 cm) accounted for 42%–72% of the total density, which was significantly higher than that in the deeper layer (5–10 cm). Similarity of species composition between vegetation and soil seed bank was low, but increased with degradation level (ranging from 0.14 to 0.69).

We concluded that grassland degradation affects soil seed bank density more than species diversity, and that soil seed banks contributed only slightly to vegetation regeneration in degraded alpine grassland. Therefore, it is unlikely that degraded alpine meadows can be restored solely through soil seed banks.

Keywords: degradation; transient soil seed bank; persistent soil seed bank; seed density; vegetation regeneration

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1 Introduction

Over the past 50 years, human activities such as overgrazing have led to widespread grassland degradation (Shang and Long, 2007; Harris, 2010). In the Qilian Mountains, the total area of degraded grassland is 6.37×10^{6} hm^2, accounting for 75% of the total natural grassland area, with mildly degraded grassland covering 4.31×10^{6} hm^2 (51%) (Li et al., 2018). Grassland degradation causes rapid loss of plant species diversity and alters species composition in vegetation communities (Hopfensperger et al., 2009). The soil seed bank is a key factor in explaining vegetation community assembly and regeneration (Silva et al., 2013) and serves as a good indicator of grassland management and restoration (Shang et al., 2013). Similarity in species composition between soil seed banks and vegetation is related to vegetation state, disturbance history, and succession (Shang et al., 2016), providing valuable information about community resilience to disturbance, drivers of succession, and potential for restoring community diversity (Hopfensperger, 2007). Previous studies reported that soil seed bank density decreases with increasing degradation (Li et al., 2012), reducing fourfold in degraded grasslands compared to non-degraded grasslands (Gonzalez and Ghermandi, 2021). Vegetation and soil seed banks exhibited similar trends during succession across different degraded grasslands (Shang et al., 2016).

To better restore and sustainably manage degraded grasslands in the Qilian Mountains, it is necessary to clarify the mechanisms by which alpine grassland degradation affects soil seed banks. Based on seed dormancy and germination characteristics, researchers classify soil seed banks into two components: transient seed banks (persisting for less than 1 year) and persistent seed banks (persisting for more than 1 year) (Thompson and Grime, 1979; Bekker et al., 1998). Seeds that germinate immediately after dispersal are considered transient seed bank Type I, while seeds that survive the winter until the following year are considered transient seed bank Type II (Thompson and Grime, 1979). Seed survival and viability in soil depend on physiological factors such as germination, dormancy, and viability, as well as environmental factors including precipitation, humidity, temperature, light, seed predators, and pathogens (Hoyle et al., 2013; Santos et al., 2013; An et al., 2020). Sampling soil seed banks at different time points allows identification of transient and persistent components (Snyman, 2004). Species appearing only in May and December but not in August have transient seed banks, while species present in all sampling periods have persistent seed banks (Thompson and Grime, 1979; Ma et al., 2010). In areas with low vegetation cover, transient seed banks can provide seeds to support regrowth (Thompson and Grime, 1979), while persistent seed banks serve as crucial resources for restoring vegetation after unpredictable or frequent disturbances (Chambers, 1993; Kiss et al., 2018). According to Kalamees et al. (2012), persistent seed banks are more critical than transient seed banks for ecosystem recovery and management.

Vegetation coverage and forage proportion were used as the basis for classifying degraded grasslands (Ma et al., 2002). More knowledge of the spatial-temporal dynamics of soil seed banks is needed to inform grassland restoration strategies and vegetation regeneration. We determined soil seed bank density and species richness in non-degraded (ND), mildly degraded (MD), and heavily degraded (HD) grasslands. Three research questions were proposed: (1) Are there changes in soil seed banks along the degradation gradient of alpine grasslands? (2) What are the direct and indirect effects of grassland degradation on transient and persistent seed banks? (3) What is the potential contribution of transient and persistent seed banks to grassland regeneration?

2.1 Study Area

The study was conducted in alpine grasslands of the Qilian Mountains in Gansu Province, China (37°11 -37°14 N, 102°40 -102°47 E; 2710–3080 m a.s.l.), on the edge of the Qinghai-Tibetan Plateau. The study area consists of alpine meadows with mountain chernozem soils. The annual mean air temperature is 0.1°C, averaging -18.3°C in January and 12.7°C in July. Mean annual precipitation is 416 mm, concentrated from July to September; annual evapotranspiration is 1592 mm (3.8 times precipitation); and annual sunshine is 2600 h.

Three grasslands representing different degradation levels were selected: ND, MD, and HD (Table 1). The dominant plant species in ND and MD were *Poa pratensis* L., *Oxytropis ochrocephala* Bunge, and *Polygonum vivipara* (L.) Gray, while HD was dominated by *P. vivipara* and *Potentilla multicaulis* Bge. (Table 1). Soil pH in the study area ranged from 7.0 to 8.2, and soil organic carbon (SOC) content ranged from 10% to 16%.

2.2 Vegetation Survey and Soil Measurement

Plant communities were surveyed in August 2020 when above-ground biomass peaked. A 1 m × 1 m quadrat was randomly selected in each grassland to determine species composition, richness, and frequency. Above-ground biomass was collected at ground level, dried at 110°C for 0.5 h, then dried at 65°C to constant weight to determine dry matter biomass. To characterize the soil environment, three soil samples (500 g each) were collected from the top 0–10 cm in each quadrat, totaling 9 samples (3 grasslands × 3 replicates). Soil pH was measured using a pH meter (Sartorius PB-10, Goettingen, Germany) in a 2.5:1.0 water:soil solution. Soil water content was determined by drying soil in a forced-air oven at 105°C for 48 h. SOC was measured by H_2SO_4-$K_2Cr_2O_7$ oxidation method (Kalembasa and Jenkinson, 1973). Soil total nitrogen (TN) was determined by micro-Kjeldahl method with H_2SO_4 digestion followed by steam distillation (FIAstar 5000 Analyzer, Hillerød, Denmark). Soil available nitrogen (AN) was extracted with 2.0-M KCl solution (Jones et al., 2004), and soil available phosphorus (AP) was extracted with 0.5-M $NaHCO_3$ solution and determined spectrophotometrically (Bao, 2000).

2.3 Sampling and Germination of Soil Seed Bank

Soil seed banks were sampled three times in 2020: 10 May (before seedling emergence, containing both transient and persistent seed banks), 8 August (after seed germination, containing only persistent seed bank), and 5 December (after seed dispersal, containing both transient and persistent seed banks) (Guo et al., 2023). This approach allowed estimation of both transient and persistent soil seed banks (Thompson and Grime, 1979). Sixteen random soil cores (10 cm × 10 cm) were collected each month in ND, MD, and HD grasslands. Each soil core was divided into three depths: shallow (0–5 cm), mid (5–10 cm), and deep (10–15 cm), totaling 432 samples (16 replicates × 3 grasslands × 3 depths × 3 times). Soil samples were packed in cloth bags, transported to the laboratory for air drying, and sieved through a 2-mm mesh to remove debris.

Species composition and richness of soil seed banks were determined by the seedling emergence method (ter Heerdt et al., 1996). Each soil sample was spread on a plastic tray containing 2–3 cm of sterilized vermiculite (150°C) and placed in a greenhouse with controlled temperature between 10°C and 25°C (Ma et al., 2010). Soil was watered thoroughly initially and kept moist by watering every 2–3 d. Seedling numbers and species were recorded regularly, and identified seedlings were removed. Difficult-to-identify seedlings were transplanted to another tray for further development (Niknam et al., 2018; Chu et al., 2019). If no new seedlings appeared for 3 consecutive weeks, the soil was turned over and watered. The experiment stopped if no new seedlings appeared after another 3 weeks (Ma et al., 2010). The entire process lasted 6 months.

2.4 Statistical Analyses

Species richness of soil seed banks was calculated as the number of species at each degradation level. Soil seed bank density was calculated as the number of germinating seeds per square meter of soil. To assess the impact of grassland degradation on species composition of vegetation and soil seed banks, plants were divided into four functional groups: sedges, forbs, grasses, and legumes

(Wang et al., 2020). Two-way analysis of variance (ANOVA) tested differences in species richness and density of soil seed banks among degradation levels and sampling months (SPSS v.24.0 software, Chicago, IL, USA). One-way ANOVA assessed the effects of degradation level and soil depth on density, Shannon-Wiener, Simpson, and Pielou diversity indices of soil seed banks, followed by multiple comparisons. Non-metric multidimensional scaling (NMDS), based on Bray-Curtis distance using the meta-MDS function in the Vegan package with R v.4.0 software (Oksanen et al., 2020), tested differences in species composition between above-ground vegetation and soil seed banks among degradation levels (An et al., 2020). The 'adonis' function tested differences between species composition of vegetation and different soil seed banks in degraded grasslands (Ochoa-Hueso et al., 2018). The Sørensen index was used to determine similarity of species composition between soil seed banks and above-ground vegetation at each degradation level (Reiné et al., 2004).

3 Results

A total of 38 species belonging to 16 families and 30 genera were identified in above-ground vegetation and soil seed banks across all sites. Specifically, 27 species were identified in ND, 21 species in MD, and 21 species in HD (Table S1).

3.1 Soil Seed Bank

There were 2017 seedlings from 19 species, 17 genera, and 11 families. The greatest number of species occurred in December (19), followed by May (15) and August (14) (Fig. S1), indicating that species richness was greater in the transient seed bank than in the persistent seed bank. All species present in the seed bank in May and August were also present in December, while three species (*Agropyron cristatum* (L.) Gaertn, *Potentilla bifurca* L., and *Gentiana squarrosa* Ledeb.) appeared only in December (Fig. S1 and Table S1). Sampling month significantly affected species richness and Shannon-Wiener, Simpson, and Pielou diversity indices ($P < 0.001$), while degradation level and the interaction between sampling month and degradation level had no effect (Fig. 1 [Figure 1: see original paper]). Both sampling month and degradation level significantly affected soil seed bank density (0–15 cm) ($P < 0.001$), though their interaction did not (Fig. 2 [Figure 2: see original paper]). Soil seed densities in ND and MD were greater in December than in May and August, with no difference between May and August. Soil seed bank density decreased with increasing degradation regardless of sampling month, with density greater in ND than in MD and HD (Fig. 2). Additionally, soil seed bank density decreased with soil depth, with greater density in the surface layer (0–5 cm) than in the bottom layer (10–15 cm) (Fig. S2).

The proportion of seeds in the surface layer relative to total seed density was 0.53–0.71 in ND, 0.42–0.54 in MD, and 0.47–0.61 in HD. The proportion of forbs in the soil seed bank increased with degradation level, while sedges had the highest proportion in ND (Fig. 3 [Figure 3: see original paper]). The highest number of seeds for forbs was recorded for *Ranunculus tanguticus* (Maxim.) Ovcz., for sedges was *Kobresia filifolia* (Turcz.) C. B. Clarke, for grasses was *Koeleria macrantha* (Ledeb.) Schult., and for legumes was *Medicago ruthenica* (L.) Trautv. (Table S1). The dominant Poaceae species were *P. pratensis* and *K. macrantha* in ND and MD, while *E. nutans* was dominant in HD. In HD, *M. ruthenica* (Fabaceae) was observed in May and August but not in December. Similarity of species composition in soil seed banks between ND and MD was high at all sampling months, ranging from 0.33 to 0.67, while similarity between ND and HD was only 0.22–0.44. Similarity of species composition across different sampling months ranged from 0.50 to 0.87 (Table 2). Strong separation occurred between ND and HD, indicating that grassland degradation substantially impacted species composition of soil seed banks. However, there was no clear change among different sampling months, indicating that temporal dynamics of species composition in soil seed banks were small (Fig. 4 [Figure 4: see original paper]).

3.2 Above-Ground Vegetation and Soil Measurements

A total of 35 species, 15 families, and 29 genera were surveyed in vegetation: 24 species, 11 families, and 20 genera in ND; 18 species, 8 families, and 14 genera in MD; and 19 species, 9 families, and 19 genera in HD (Table 3). There were 4 common species across all degradation levels, 6 common species between ND and MD, 4 between MD and HD, and 8 between HD and ND (Fig. S1). Perennial species accounted for 82%–84% of all vegetation species. The largest families were Asteraceae and Fabaceae (6 species each), followed by Poaceae (3 species). *E. nutans*, *P. pratensis*, and *K. macrantha* (Poaceae) were dominant in ND; *P. pratensis* and *K. macrantha* were dominant in MD; while *Anaphalis lacteal* Maxim. and *Taraxacum mongolicum* Hand.-Mazz. (Asteraceae) were dominant in HD (Table S1).

The proportion of grasses was greater in MD than in ND and HD, whereas proportions of forbs and legumes were greater in HD than in ND and MD (Fig. 2). Above-ground biomass was greater in ND (64.8 ± 5.35 g/m^2) and MD (47.67 ± 9.53 g/m^2) than in HD (5.23 ± 4.32 g/m^2) (Fig. S3; $P < 0.050$), decreasing with increasing degradation. Species richness and Shannon-Wiener and Simpson diversity indices of vegetation were greatest in ND, followed by HD and MD (Fig. S4). The Sørensen index was 0.49 between ND and MD, 0.56 between ND and HD, and 0.39 between MD and HD (Table 2). Soil pH increased, while soil moisture, SOC, AN, AP, and TN decreased with increasing grassland degradation level (Fig. S5; $P < 0.050$).

3.3 Relationship Between Soil Seed Bank and Vegetation

A total of 16 species were observed in both above-ground vegetation and soil seed banks (*Kobresia capillifolia* (Decne.) C. B. Clarke, *K. macrantha*, *Artemisia frigida* Willd., and *P. vivipara* were dominant), while only 3 species (*K. filifolia*, *R. tanguticus*, and *Allium ramosum* L.) were observed in soil seed banks across all sampling months (May, August, and December) (Fig. S1; Table S1). Similarity in species composition between vegetation and soil seed banks ranged from 0.15 to 0.69. Based on degradation level and sampling month, HD displayed the highest similarity index (0.43–0.69), followed by ND, then MD. The similarity index was greater in August (0.33–0.55) than in May and December, indicating that the persistent seed bank was more similar to vegetation species than the transient seed bank (Table 2).

4.1 Effect of Sampling Month on Soil Seed Bank

Sampling month, vegetation type, and grassland degradation level affect temporal dynamics of soil seed banks (Chu et al., 2019; He et al., 2021). This study revealed differences in seed density across sampling months, with the greatest density in December after seed dispersal, followed by a slow decline. Soil seed banks increase after seed maturation in the following year, while decreasing due to animal consumption and fungal infections (Dalling et al., 2011). Sampling month impacted species richness and Shannon-Wiener and Simpson diversity indices of soil seed banks. Several reasons explain these effects: (1) study sites were grazed only in summer, with livestock consuming forage selectively and dispersing seeds through epizoochory (wool) and endozoochory (dung) (Cosyns et al., 2005; Wang and Hou, 2021); (2) invertebrates such as ants and small mammals like rodents transport and cache seeds, affecting soil seed bank species composition (Zhao et al., 2020); and (3) physiological seed characteristics vary, with some germinating shortly after autumn dispersal, some requiring overwintering to germinate in spring, and others needing even longer periods (Thompson and Grime, 1979).

In contrast to previous findings that transient seed bank density remains stable along degradation gradients (He et al., 2021), this study found that transient seed bank density decreased with increasing degradation level. This difference may be attributed to variations in vegetation types and dominant species. Soil seed bank density was greatest in December, followed by May and August, indicating that transient seed bank density exceeded persistent seed bank density. Species composition across different sampling months displayed minimal change, suggesting that species compositions of transient and persistent seed banks remain relatively stable between years.

4.2 Effects of Grassland Degradation on Above-Ground Vegetation and Soil Seed Bank

Above-ground biomass decreased gradually with increasing grassland degradation, likely due to depleted soil nutrients. This was supported by decreases in soil SOC, TN, AN, and AP contents with increasing degradation (Fig. S5). Plant species richness and Shannon-Wiener and Simpson diversity indices were greater in HD than in MD, likely due to increased richness and abundance of forbs in HD, particularly Asteraceae (*Saussurea alpina* (L.) DC, *A. lacteal*, and *T. mongolicum*). Soil physical-chemical properties relate not only to seed input but also to seed survival. Ma et al. (2017) reported that soil seed bank density was negatively correlated with pH but positively correlated with soil organic matter and water content. Similar results emerged in this study. However, seed density, species richness, and Shannon-Wiener diversity index of soil seed banks were lower in HD than in ND, indicating that grassland degradation detrimentally impacted soil seed bank density and diversity. Seed density of annual species (*E. nutans*, *K. macrantha*, and *Plantago depressa* Willd.) varied significantly with grassland degradation, while perennial species (*S. alpina*, *R. tanguticus*, and *K. filifolia*) were not affected.

Persistent seed bank density exceeding 200 seeds/m^2 at 10–15 cm depth in ND and MD in August indicated that seeds reached that depth through animal activities and could withstand surface disturbance (Shang et al., 2013). Transient seed bank density in HD at 10–15 cm soil depth in May accounted for 26.7% of total seed density, attributable to forb dominance. Forbs have small seeds that minimize predation by rapid burial (Gonzalez and Ghermandi, 2008). In this study, the proportion of forbs in soil seed banks and vegetation increased with degradation, accounting for 0.55–0.68 of the total in HD (Fig. 3). Two explanations are possible: (1) forbs have competitive advantages in HD with low soil moisture and poor nutrients because they produce deep roots that utilize nutrients from deep soil layers (Shang et al., 2015); and (2) grazing livestock selects grasses, benefiting forb establishment and growth and ultimately increasing forb seed numbers (Zhang et al., 2020). For example, *T. mongolicum*, *Stellera chamaejasme* L., and *P. depressa* were present only in HD. Forbs in HD may have provided a protective environment for sedges, enabling some sedges to thrive. The wide distribution of forbs in HD may have reduced grazing pressure on surrounding plants by releasing unpleasant scents that deter livestock (Zhang et al., 2020).

4.3 Relationship Between Above-Ground Vegetation and Soil Seed Bank

Previous studies found low similarity between vegetation and soil seed banks in alpine regions (Ma et al., 2010), with similarity decreasing with grassland degradation (He et al., 2021). Low similarity in this study indicated that degraded alpine meadows relied more on vegetation colonization capacity than on soil seed banks. Several explanations exist. First, seed yield was low. In this study, 5 annual species (*P. pratensis*, *A. lactea*, *G. squarrosa*, *P. depressa*, and *Silene conoidea* L.) and 30 perennial species were identified in vegetation, with plant communities dominated by perennials. Dominant perennials *Potentilla chinensis* Ser. and *P. vivipara* relied on sexual reproduction, contributing little to soil seed banks (Ma et al., 2010). Additionally, the relatively short growing season and low air temperature on the Qinghai-Tibetan Plateau limited seed development, leading to greater reliance on clonal regeneration than seedling recruitment (Jaganathan et al., 2015; Fernandez-Pascual et al., 2021). However, vegetation in HD was dominated by forbs with low livestock palatability. Most seeds produced by forbs such as *Aster alpinus* L. and *A. lacteal* contributed to soil seed banks, resulting in high similarity of forb species between soil seed banks and vegetation in HD.

Second, seed germination rates were low. Most alpine seeds have dormancy requirements (Jaganathan et al., 2015; Fernandez-Pascual et al., 2021), and greenhouse temperature and humidity changes were insufficient for some dormant seeds to germinate quickly. Seeds from Gentianaceae and Apiaceae undergo dormancy and require long cold periods before germination (Liu et al., 2011), which may explain why *Gentiana straminea* Maxim. and *Cicuta virosa* L. were observed in soil seed banks. Some seeds in soil seed banks contacted Ascomycetes, increasing seed mortality and loss (Wagner and Mitschunas, 2008). Therefore, direct germination methods may underestimate soil seed bank density and diversity, and consequently underestimate similarity between soil seed banks and vegetation.

Similarity between soil seed banks and vegetation depends on vegetation type, state, species composition, and succession history (Shang et al., 2016). The greatest similarity occurred in August, suggesting that persistent seed banks play a more important role in vegetation regeneration than transient seed banks. Seeds in persistent seed banks exhibit longevity in soil, and soil environment is more important for storing persistent than transient seed banks (He et al., 2021). Similarity between soil seed banks and vegetation across sampling months was greater in HD than in ND and MD, increasing with degradation level as reported elsewhere (Luzuriaga et al., 2005; He et al., 2021). This demonstrated that soil seed bank contribution to vegetation regeneration was greater in HD than in ND and MD. The greater similarity in HD indicated that HD lost buffer capacity, while ND and MD retained some buffer capacity that could be restored through appropriate measures (Shang et al., 2016). The greater seed density of sedges and grasses in soil seed banks did not play an important role in vegetation regeneration of alpine grasslands. Restoration of HD requires long-term grazing exclusion or introduction of seeds from local plant species due to reduced sedges and grasses (Shang et al., 2013).

5 Conclusions

Effects of grassland degradation on persistent and transient seed banks were determined by direct germination methods in ND, MD, and HD grasslands. Grassland degradation reduced above-ground biomass and densities of persistent and transient seed banks but did not affect species composition or diversity of vegetation and soil seed banks. Sampling month significantly affected species richness and Shannon-Wiener and Simpson diversity indices of soil seed banks. Persistent seed banks were more important for vegetation regeneration and restoration of degraded grasslands than transient seed banks, as similarity in species composition between vegetation and persistent seed banks was greater than with

transient seed banks. Similarity was greater in HD (0.43-0.69) than in ND and MD, indicating that HD lost its buffering capacity. We concluded that restoration of HD cannot rely solely on soil seed banks, and other strategies such as grazing exclusion and/or introduction of local seeds may be necessary. Species composition and ecological niches of vegetation and soil seed banks should be considered when selecting restoration methods. After restoration is completed, long-term monitoring of vegetation, soil, and soil seed banks should continue.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions: Conceptualization: LI Chunming; Data curation: MA Jiahui, LI Liangyu, HUANG Junlin, LU Jinhua; Methodology: LI Chunming; Formal analysis: HUANG Mei; Writing—original draft preparation: HUANG Mei, LI Chunming; Writing—review and editing: Allan DEGEN, SHANG Zhanhuan; Funding acquisition: LI Chunming.

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Appendix

Fig. S1 Number of species in vegetation and soil seed bank (a); number of species in vegetation under different degradation levels (b); number of species in soil seed bank under different degradation levels (c); and number of species in vegetation under different sampling months (d). ND, non-degraded grassland; MD, mildly degraded grassland; HD, heavily degraded grassland.

Fig. S2 Soil seed density among different degradation levels and sampling months. (a) August; (b) December; (c) May. ND, non-degraded grassland; MD, mildly degraded grassland; HD, heavily degraded grassland. Different lowercase letters within the same degradation level indicate significant differences among different depths at $P < 0.05$ level. Bars are standard errors.

Fig. S3 Above-ground biomass under different degradation levels. ND, non-degraded grassland; MD, mildly degraded grassland; HD, heavily degraded grassland. Different lowercase letters indicate significant differences among different degradation levels at $P < 0.05$ level. Bars are standard errors.

Fig. S4 Species richness (a), Shannon-Wiener (b), Simpson (c), and Pielou (d) diversity indices in vegetation in non-degraded (ND), mildly degraded (MD), and heavily degraded (HD) grasslands.

Fig. S5 pH (a), and contents of soil moisture (b), SOC (soil organic carbon; c), TN (total nitrogen; d), AN (available nitrogen; e), and AP (available phosphorus; f) under different levels of grassland degradation. ND, non-degraded grassland; MD, mildly degraded grassland; HD, heavily degraded grassland. Different lowercase letters indicate significance among different degradation levels at $P < 0.05$ level.

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