Seed banks trigger ecological resilience in subalpine meadows abandoned after arable farming on the Tibetan Plateau

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Abstract. Although long-term agricultural activity frequently decreases biodiversity, it remains unclear whether such biodiversity losses are readily reversible. There is no doubt that the important ecological function of seed bank is ecological memory, but few researchers have explored the role of seed banks in grassland ecosystem resilience and threshold theory. We used a space-for-time subrogation method, i.e., a natural meadow (never farmed but used for moderate gazing) and meadows farmed for 30 yr and then abandoned for 1, 10, and 20 yr, to determine if the biodiversity/ecosystem of subalpine meadows could be reversed to the natural vegetation state and to investigate the role of soil seed banks in grassland ecosystem restoration and resilience. After 20 yr of natural regeneration, aboveground vegetation composition and properties had recovered to the natural meadow state, suggesting that critical thresholds were not crossed. Seed bank composition and structure exhibited almost no change after agricultural disturbance for decades. The persistent seed bank had the highest contribution to vegetation regeneration in the 1-yr abandoned field, which had the highest seed density. Similarity between the seed bank and aboveground vegetation and seed density decreased with years since abandonment. Since the seed bank still reflected the desired state, the system had inherent resilience and had not crossed the transition threshold. Thus, high-diversity persistent seed banks are an important indicator of high resilience of this ecosystem. High similarity between the seed bank and vegetation in early-abandoned fields may indicate that ecological resilience is triggered and be a warning signal that interventions are needed to avoid a state transition. In applying alternative stable state theory to ecological restoration, much attention should be given to the soil seed bank.

Key words: alternative stable state; ecological warning; ecosystem resilience; restoration; soil seed bank; threshold.

INTRODUCTION

Abandoned agricultural fields may have low biodiversity (Peco et al. 2012) and habitat patchiness, and invasive species, litter accumulation, and wildfires may become important problematic issues in the reestablishment of the original plant community (Rey Benayas et al. 2007). In some cases, an old-field site can remain in a persistent state of degradation for decades, and recovery to the natural or historical state is unlikely or at best very slow (Whisenant 1999, Cramer and Hobbs 2007). Restoration of abandoned fields to the natural state is important for biodiversity conservation, but the recovery to the pre-agriculture state creates significant scientific and ecological challenges (Cramer et al. 2008).

Two control models have been proposed to explain the threshold dynamics of ecosystem changes in response to perturbation: driver and feedback. The driver control model predicts that ecosystem characteristics or functions, such as biodiversity and production, decline with high disturbance. However, they gradually recover to the original state from seeds or remnant plants once the driver (disturbance) is relaxed (Bestelmeyer et al. 2011). The feedback control model predicts that anthropogenic perturbation (such as agricultural activities) reduces the resilience of a high-diversity stable-state plant community so much that the system crosses a threshold point and enters a self-reinforcing (feedback controlled) low-diversity stable state (Scheffer et al. 2001, Suding et al. 2004, Suding and Hobbs 2009, D’Odorico et al. 2012), thereby reducing ecosystem characteristics or functions even without the driver.

Following disturbance, the soil seed bank can play a critical role in determining the species composition of

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the future plant community (Warr et al. 1993) and the
degree to which the ecosystem can be restored (Thompson et al. 1997, Grime 2001). Friesewyk and Zedler (2006) proposed three major seed bank dimensions with which to assess habitat degradation and ecosystem resilience: seed density, species and guild richness, and floristic quality. Assessing changes in species composition of the seed bank is crucial for estimating the restoration potential of native communities (Gioria and Osborne 2009). However, almost no research has focused on the role of seed bank in ecosystem resilience over time, but two theories have been developed with regard to the soil seed bank and plant community reassembly in abandoned fields: alternative stable state theory (Scheffer et al. 2001, Suding et al. 2004, Suding and Hobbs 2009) and framework of plant community assembly theory (Cramer et al. 2008).

Alternative stable state theory predicts that an ecosystem has more than one stable state for a particular set of environmental conditions, and changes in environmental conditions lead to very little change in function or species composition of the community until a threshold is crossed. However, after a threshold is cross sudden changes in composition or function may occur (Scheffer et al. 2001, Suding et al. 2004, Suding and Hobbs 2009, Downing et al. 2012).

According to the framework of plant community assembly theory (Cramer et al. 2008), when the environment is disturbed by agriculture, two thresholds (biotic and abiotic) may be exceeded. Depending on the degree that the biotic and abiotic thresholds are crossed, there are three trajectories of plant community assembly in abandoned fields. (1) When the biotic and abiotic thresholds are not crossed, abandoned fields can recover to the original natural vegetation state unassisted within decades along a broadly repeatable successional trajectory. (2) When the biotic threshold is crossed, plant community assembly has a low likelihood of recovery to the historical or natural vegetation state. Further, there is an increased possibility of the appearance of novel species and the development of a novel plant community. (3) As the biotic and abiotic threshold is crossed, soil structure, nutrient levels, and hydrology may be negatively altered for plant growth. Hence, abandoned fields show little recovery to the historical or natural vegetation state because nutrient and water cycles are permanently altered, and the vegetation is highly fragmented. The vegetation may become dominated by exotic species and thus be in an alternative stable state. However, there are no data of sufficient duration to determine whether such diversity and composition changes are readily reversible between high- and low-diversity stable states or whether ecosystems go through a critical threshold point (biotic and abiotic threshold) as they go from a high- to a low-diversity (alternative) stable state (van Nes and Scheffer 2004, Scheffer 2009).

Using the basic ideas in the alternative stable and the framework of plant community assembly theories, we developed a conceptual model of aboveground vegetation and soil seed bank changes after the cessation of the agricultural disturbance (Fig. 1). Theoretically, after the ecosystem experiences long-term disturbance (such as agricultural activities), the aboveground vegetation is totally changed (Fig. 1a). There are three possible scenarios with regard to the role of the seed bank in vegetation recovery (Fig. 1b, d, f), once the disturbance stops (Fig. 1c, e, g).

First, composition and structure of the seed bank are the same as it was prior to long-term agricultural disturbance (Fig. 1b). In this situation, the seed bank still reflects the desired state, and it could trigger ecosystem resilience, resulting in recovery of the original/natural state of the aboveground plant community by unassisted natural successional processes (Fig. 1c). That is, the seed bank provides high ecosystem resilience. Second, under long-term agricultural disturbance, many (but not all) of the species in the original vegetation have disappeared from the seed bank, and some species that have been introduced into the disturbed site are now present in the seed bank. Hence, seed bank composition and structure have changed to an alternative state (Fig. 1d). In this situation, aboveground plant community could be recovered to the original/natural state after a transient/intermediate state because the seed bank still has seeds of native key species (Hopfensperger 2007, Bossuyt and Honnay 2008; Fig. 1e). Third, during long-term agricultural disturbance few or none of the species in the original vegetation remain in the seed bank (Fig. 1f). In this situation, the plant community cannot recover to the original state (without input of seeds from other places) and the system would transit to an alternative stable state (Fig. 1g).

Further, we predicted that if a plant community is destroyed by agricultural disturbance but the seed bank still reflects the desired state, then the system has inherent resilience and may not have crossed the transition threshold. On the other hand, if both aboveground vegetation and seed bank have been lost, the plant community cannot be recovered without seed/plant input from other sources. That is, the system no longer has resilience and cannot be recovered. We further predict that a seed bank has ecological resilience and is an important part of ecosystem resilience. Thus, the similarity between the seed bank and aboveground vegetation could be an important indicator or warning signal of ecosystem resilience.

To test out predictions, we investigated the soil seed bank, aboveground vegetation, and soil characteristics of a natural subalpine meadow and of meadows that had been used for arable farming for 30 yr and then abandoned for various periods of time. Specifically, we asked three questions. (1) Can the biodiversity/ecosystem functions of a natural subalpine meadow be restored when agricultural exploitation is stopped? (2) Can an abandoned field be restored to a meadow by seed bank-triggered-ecosystem resilience? (3) Can seed banks serve as an important indicator of ecosystem resilience or lack thereof? and (4) How do soil physical, chemical and biotic characteristics change during succession?
MATERIALS AND METHODS

Study site

Our study was conducted in the subalpine zone on the northeastern part of Tibetan Plateau, Gannan (34°55′ N, 102°53′ E), Gansu Province, China, with an elevation of 2,900 m above sea level. At the study site, the average annual temperature is 2.4°C, ranging from −9.9°C in January to 12.8°C in July, and the average annual precipitation is 531.6 mm, ranging from 2.4 mm in January to 110.3 mm in July. The soil type is classified as alpine meadow soil. The aboveground vegetation is typical of species-rich subalpine meadows, which are dominated by perennial grasses (*Elymus nutans*, *Stipa aliena*, *Agrostis hugoniana*, and *Festuca ovina*), sedges (*Kobresia humilis*), and forbs (*Gentiana macrophylla*, *Aster flaccidus*, and *Ligularia virgaurea*; Ma et al. 2013, Zhang et al. 2015).

Experimental design

Extensively managed farmlands are common in the species-rich subalpine meadows of the northeastern Tibetan Plateau, and many sites in this area have experienced extensive agricultural exploitation (plowed and farmed)
since the 1950s, especially for production of highland barley (Hordeum vulgare var. nudum). The farming activities have caused meadow degeneration and a serious loss of meadow biodiversity and ecosystem function (Chu et al. 2007, Li et al. 2009, Zhang et al. 2015). Thus, the local government has decided to return those intensively used agricultural fields to species-rich meadows, and farming of some meadows was stopped in the 1980s, 1990s, and 2000s. The abandoned fields were fenced and grazing was forbidden (Li et al. 2009, Ma et al. 2013, Zhang et al. 2015). Thus, fenced sites have been protected from agricultural activities for 1, 10, and 20 yr. Also, some never-farmed meadows (control or natural meadows) have been fenced to prevent overgrazing. Natural meadows generally are subjected to a low degree of grazing by herbivores, such as yak and Tibetan sheep.

For our research, we used the space-for-time subrogation method. That is, we used fields that had been abandoned for 1, 10, and 20 yr and a non-disturbed site in our study. We determined the extent to which cessation of agricultural activities led to recovery of the degraded sub-alpine meadows to the natural meadow state, or whether the system had irreversibly shifted from a high-diversity to a low-diversity/degraded state. Moreover, we also tested the role of soil seed banks in ecosystem resilience.

The 1-, 10- and 20-yr-old abandoned fields and the never-farmed meadow were at least 120 ha in area, were located within an area of 10 km², had the same facing aspect, orientation, and position on the slope. Ten randomly selected replicate sites (20 × 20 m) were established in each abandoned field and the natural meadow. Each of the 10 sites was an independent spatial replicate for the each abandoned field or natural meadow. Data on the aboveground plant community and soil seed bank were collected in each site. Also, soil samples were collected in each site with which to determine the physical, chemical and biotic characteristics of the soil.

Sampling method

**Aboveground vegetation sampling.**—Vegetation was sampled in July 2005 and 2006, during the peak of the summer growing season, using 50 × 50 cm quadrats placed within each of the three abandoned fields and natural meadow where soil samples had been collected. Ten quadrats (one in each of the 10, 20 × 20 m sites established in each plant community) were sampled in each plant community in both 2005 and 2006. Previously, it had been shown that 10, 50 × 50 cm quadrats gave an adequate sample of the meadow vegetation (Yang et al. 2012, Zhang et al. 2015). We sampled the vegetation in 2 yr because soil seed bank samples were collected in 2 yr. In each quadrat, we recorded the number of ramets of each species, species richness, and cover of each species within each quadrat. Cover of each species was recorded as cover per unit ground area (0.25 m²). Therefore, total cover could exceed 100% due to the overlap of plant canopies. Because most of the grasses and sedges are clonal species, we used both abundance and cover to quantify their occurrence.

**Soil seed bank sampling and treatment.**—To differentiate the persistent fraction of the seed banks and to capture both the transient and the persistent seed bank (assuming that seeds of species with a persistent seed bank remain in the soil for >1 yr, Thompson et al. 1997), we collected soil samples two times. Samples were collected in July 2005 (after field seed germination but before the production of new seeds, when only the persistent fraction of the seed bank should be present in the soil) and in April 2006 (before field seed germination, i.e., transient seed bank; Funes et al. 2003).

Ten sites (20 m × 20 m) were randomly selected in each abandoned field (1-, 10- and 20-yr) and the natural meadow (control), and 10 plots (0.4 m × 1 m) were randomly selected in each site. Ten cylindrical soil cores (3.6 cm diameter) were collected randomly in each plot. The depth of each soil core was 12 cm. The 10 cores were pooled into a soil sample for each plot. Overall, there were 10 soil samples from each site (1 sample × 10 plots), and 100 soil samples (1 sample × 10 plots × 10 sites) for each abandoned field and natural meadow, and 400 soil samples in all (1 sample × 10 plots × 10 sites × 4 meadows (3 abandoned fields and 1 natural meadow)). Thus, the sampling area at each meadow/field was 1.02 m² (3.14 × 0.018 m² × 10 soil cores × 10 plots × 10 sites), and the total volume of soil sampled at each meadow/field was 0.122 m³ (3.14 × 0.018 m² × 0.12 m × 10 soil cores × 10 plots × 10 sites).

**Treatment of samples and maintenance of seed trays**

All the soil samples were placed on a table in front of a north-facing window for 15 d. Then each sample was sieved to remove root fragments and coarse debris, after which it was sieved through a coarse and then a fine sieve. The seedling emergence method, which usually detects more than 90% of the species present in the soil seed bank of grasslands, was used to examine species richness and seed density in the seed bank (ter Heerdt et al. 1996, Ma et al. 2018). This work was conducted at the Research Station of Alpine Meadow and Wetland Ecosystems at Lanzhou University, Gansu Province, China (34°55' N, 102°53' E, 2,900 m above sea level).

The soil samples were evenly spread on a layer of sterile sand (depth 15 cm), which was sterilized at 140°C for 24 h, in sterile plastic germination trays (30 cm diameter × 30 cm height). Thirty control trays with only sterilized sand were set alongside the germination trays to determine the presence of contamination by wind-dispersed seeds. All trays including germination and control trays were placed outside on the ground, and water was added several times each day to keep the soil moist. All trays were monitored daily and, after seedlings could be identified, they were removed to keep densities low. Seedlings that could not be identified were
grown separately until they could be identified. After 5 months, when no additional seedlings had emerged for several consecutive weeks, the soil was sifted and carefully inspected; no seeds were found.

**Analyses of soil environmental properties**

Soil samples were collected in August 2006 from random locations within each site, where aboveground plant community quadrats and soil seed bank samples were collected.

Three cores (3.6 cm diameter) to a depth of 40 cm were collected in each field and the natural meadow and subsequently divided into two soil depths (0–20 cm and >20–40 cm). The soil from each field (or the meadow) was composted to generate a single soil sample. We collected eight mixed soil samples (four upper layers and four lower layers samples) from each abandoned field. Overall, 64 (4 meadows × 2 soil depths × 8 samples = 64) soil samples were used to analyze soil characteristics.

After removing stones and plant material, each soil sample was divided into two parts. One part was air-dried and used for soil physical and chemical analyses. The second part was sieved through a 2-mm sieve, adjusted to 50% of water holding capacity and then incubated for 2 weeks at 25°C to permit uniform rewetting and stabilization for soil microbial biomass carbon and nitrogen analysis.

For this study, soil moisture content (SMC), bulk density (BD), soil pH, total nitrogen (TN), soil organic carbon (SOC), and soil organic matter (SOM) were analyzed. SMC was determined by the oven-drying method. Soil BD was calculated by dividing the mass of the oven-dry soil (105°C) by the core volume (3.6 cm diameter × 20 cm depth) with a mean of three replicates per field. Soil pH was measured using a pH meter with a glass electrode (soil:KCl ratio 1:2.5). TN was determined using the Kjeldahl method (Institute of Soil Science, Academia Sinica 1978), and SOC was determined using the dichromate oxidation method (Kalembasa and Jenkinson 1973). SOM was measured using the K2Cr2O7 method (Agriculture Chemistry Council, Soil Science Society of China 1983).

**Analysis of soil microbial biomass**

Microbial biomass carbon (MBC) and nitrogen (MBN) of the soil samples were determined by the chloroform fumigation–extraction method (Brookes et al. 1985). Three portions of soil were fumigated at 25°C for 24 h with CHCl3 prior to extraction by K2SO4. Then, the fumigated and non-fumigated samples were extracted with 100 mL of 0.5 mol/L K2SO4 by horizontal shaking for 1 h at 200 rpm and then filtered. Dichromate oxidation was used to measure the contents of K2SO4-extracted carbon from the CHCl3-treated and nontreated soils. Kjeldahl digestion method was used to measure the contents of K2SO4-extracted nitrogen from the CHCl3-treated and nontreated soils. MBC and MBN were calculated by dividing the differences of total extractable carbon and nitrogen between fumigated and non-fumigated samples by the conversion factors 0.38 and 0.45 for biomass carbon and nitrogen, respectively.

**Statistical analyses**

The differences in species richness, abundance, cover, and functional groups (grasses, sedges, and forbs) in aboveground vegetation of the four plant communities were compared using one-way analysis of variance (ANOVA) and the Tukey test. Pielou evenness and Shannon-Weiner index of aboveground plant communities and seed banks were compared using one-way ANOVA and the Tukey test; data for each year were analyzed separately. Seed density and species richness of the seed bank of the successional stages were compared using ANOVA and the Tukey test. Prior to analysis, data were examined for normality and homogeneity of variance, and data were log-transformed. Sørensen similarity indexes between seed bank and aboveground plant community change along the successional gradient were analyzed by one-way ANOVA and the Tukey test. All ANOVA tests were performed with SPSS 23.0 (SPSS, Chicago, Illinois, USA).

Nonmetric multidimensional scaling (NMDS) was used to evaluate species composition similarity among abandoned fields aboveground vegetation and seed banks in 2 yr, and species composition similarity between seed bank and aboveground vegetation in 2 yr. All ordinations and Bray Curtis dissimilarity indices were based on relative abundance data. NMDS was conducted using the R program for Windows, using package vegan (Oksanen et al. 2007) in the R 3.1.3 (R Development Core Team, 2015).

**RESULTS**

**Aboveground plant community changes with succession**

A total of 95 species, belonging to 20 families was recorded during 2 yr of sampling the vegetation. Total species richness, abundance, and cover of the plant community increased significantly with years since abandonment (Fig. 2a–c). Specifically, species richness, abundance, and cover of the three functional groups (grass, sedge, and forbs) differed significantly among the years but, overall, they showed an increase with years since abandonment (Fig. 2a–c). There was no significant difference for cover between natural meadow and 20-yr abandoned meadow for each function group and total. There was almost no grass in the 1-yr abandoned field, and it increases significantly from the 10-yr field to the natural meadow. Further, there was no sedge in the 1- and 10-yr fields, and it increased significantly from the 20-yr field to the natural meadow (Fig. 2a–c).

Both the Pielou evenness index and the Shannon-Weiner diversity index of plant community differed
significantly among the years, and they showed an obvious increased trend with years since abandonment (Fig. 2d, e).

With time since disturbance stopped, species composition of the plant communities in both 2005 and 2006 showed a clear restoration trajectory with species composition becoming increasingly similar to that of the natural meadow (Fig. 3a, b). Finally, the 20-yr abandoned field clustered together with the nature meadow.

**Soil seed bank changes with succession**

A total of 38,887 seedlings, belonging to 113 species and 26 families, was identified in the soil samples collected in 2005 (841.72 ± 56.50 in 1-yr, 632.10 ± 33.49 in 10-yr, 215.43 ± 14.36 in 20-yr field, and 132.08 ± 6.24 seeds/m² in natural meadow) and 2006 (1830.41 ± 106.21 in 1-yr, 1296.36 ± 77.59 in 10-yr, 525.17 ± 48.04 in 20-yr field, and 376.62 ± 19.60 seeds/m² in natural meadow). Species richness, seed density, Pielou evenness index, and Shannon-Weiner diversity index both in 2005 (persistent seed bank) and 2006 (transient + persistent seed bank) differed significantly among the different abandoned fields (Fig. 4a–d). Species richness, Pielou evenness index, and Shannon-Weiner diversity index increased, but seed density decreased with time since fields were abandoned. Specifically, the highest species richness was for the 20-yr field, while the lowest richness.
FIG. 3. Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of seed banks and vegetation among different years since abandonment (1 yr, abandoned 1 yr; 10 yr, abandoned 10 yr; 20 yr, abandoned 20 yr) and NM, natural meadow in subalpine meadows on the Tibetan Plateau. Soil seed banks in two seasons (a, July 2005; b, April 2006), vegetation in two years (c, July of 2005; d, July of 2006), and the similarity between seed bank and vegetation (e, two seasons seed banks and 2006 vegetation) were showed in different panels, respectively. All the ordination was based on relative abundance data. Different marks represent different vegetation and seed bank types. The location of ordination points within each diagram indicates the degree of similarity between each one.
was in 1-yr field in both 2005 and 2006 (Fig. 4a). The highest seed density was in the 1-yr and the lowest in the natural meadow in both 2005 and 2006 (Fig. 4b). The highest Pielou evenness and Shannon-Weiner indexes were for the natural meadow and the lowest for the 1-yr field in both 2005 and 2006 (Fig. 4c, d).

Compared to the aboveground plant community (more divergence), changes in species composition of the seed bank were more convergent among different years since abandonment with almost no difference between the 20-yr field and natural meadow (Fig. 3c, d). The Sørensen similarity indexes of soil seed banks among four meadows (three abandoned fields and a natural meadow) were very high, and all values are higher than 70% (Table 1). A comparison between the seed bank in 2005 (Fig. 3c: persistent seed bank) and 2006 (Fig. 3d, transient + persistent seed bank) revealed that the seed bank in 2005 was more diverse than in 2006.

**Soil biotic and abiotic factors change with succession**

Soil pH, SOC, SOM, TN, and C:N differed significantly among the four plant communities both in the first (0–20 cm) and the second (>20–40 cm) soil layers (Table 1). The highest SMC was in the natural meadow and the lowest in the 20-yr field. The lowest soil pH was in the natural meadow, but there was no difference among the abandoned fields in either the upper or lower soil layers. The highest SOC was in the natural meadow and the lowest in 1-yr field, and SOC showed an increase trend with year since abandonment only in the first layer. The highest SOM and TN were in the natural meadow and the lowest in the 20-yr field, and they decreased in the 1- to 20-yr fields meadow and increased in the natural meadow. Both MBC and MBN differed significantly only in 0–20 cm soil layer. The highest MBC and MBN were in the natural meadow and the lowest in the 10-yr field (Table 1).

**Role of seed bank in aboveground plant community regeneration with increasing time since abandonment**

Similarities between the seed bank and the aboveground vegetation (both in 2005 and 2006) differed significantly among years since abandonment and gradually decreased with time. The 1-yr field had the highest and the natural meadow the lowest similarity between seed bank and aboveground vegetation (Fig. 5).

After putting aboveground vegetation and soil seed bank together in NMDS (Fig. 3e, f), we found that the aboveground plant community showed a clear difference among the abandoned fields, and species composition gradually became similar to that of the natural meadow as time since abandonment increased. The seed bank for the four communities almost clustered together in 2005 (persistent seed bank) but not in 2006 (transient + persistent seed bank). In 2005, the seed bank of the four communities clustered together with the 1-yr field (Fig. 3e),

![Fig. 4. Species richness, seed density, Pielou evenness and Shannon-Weiner index of the soil seed bank with years since abandonment.](image-url)
Table 1. Soil physical and chemical variables (mean ± SE) in the upper (0–20 cm) and lower (20–40 cm) soil depths among different abandoned meadows.

<table>
<thead>
<tr>
<th>Variables and soil depth (cm)</th>
<th>Abandoned years</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 yr</td>
</tr>
<tr>
<td>SMC</td>
<td>0.14 ± 0.01ab</td>
</tr>
<tr>
<td>BD</td>
<td>1.32 ± 0.03</td>
</tr>
<tr>
<td>pH</td>
<td>8.34 ± 0.04a</td>
</tr>
<tr>
<td></td>
<td>8.40 ± 0.02a</td>
</tr>
<tr>
<td>SOC</td>
<td>73.69 ± 10.24c</td>
</tr>
<tr>
<td></td>
<td>77.91 ± 5.85b</td>
</tr>
<tr>
<td>SOM</td>
<td>40.32 ± 1.88b</td>
</tr>
<tr>
<td></td>
<td>45.08 ± 2.05a</td>
</tr>
<tr>
<td>TN</td>
<td>1.94 ± 0.06b</td>
</tr>
<tr>
<td></td>
<td>2.03 ± 0.01a</td>
</tr>
<tr>
<td>C:N</td>
<td>20.84 ± 1.56b</td>
</tr>
<tr>
<td></td>
<td>22.23 ± 1.08b</td>
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<tr>
<td>MBC</td>
<td>268.37 ± 19.71b</td>
</tr>
<tr>
<td></td>
<td>107.31 ± 10.79</td>
</tr>
<tr>
<td>MBN</td>
<td>27.73 ± 1.07bc</td>
</tr>
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<td></td>
<td>22.56 ± 7.81</td>
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Notes: 1 yr, abandoned 1 yr; 10 yr, abandoned 10 yr; 20 yr, abandoned 20 yr; NM, natural meadow. SMC, soil moisture content; BD, bulk density; SOC, soil organic carbon; SOM, soil organic matter; TN, total nitrogen; C:N, carbon:nitrogen ratio; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. Values in boldface type are the lowest values for each soil variable. Different letters after means indicate significant differences (P ≤ 0.05, ANOVA, Tukey range test) of mean values.

while in 2006 only the 1-yr seed bank was very similar to that of the 1-yr field vegetation (Fig. 3f). The distance between seed bank and aboveground plant community increased with years since abandonment (Fig. 3e).

**Discussion**

*Can the biodiversity/ecosystem could be reversible to the natural state after a 30-yr perturbation?*

Disturbance by agricultural activities may reduce the resilience of a high-diversity stable state, increasing the likelihood that the system will cross a critical threshold into a low-diversity self-reinforcing stable state (van Nes and Scheffer 2004, Downing et al. 2012, Török et al. 2018). Some research has shown how the recovery of abandoned fields may be possible (Meiners et al. 2002, Hermy and Verheyen 2007). However, other research has found that the exclusion of agricultural activities may not lead to the recovery of natural grassland vegetation (Whisenant 1999, Cramer and Hobbs 2007). Hence, researchers need to explore the new theories about abandoned field restoration to understand the dynamics of change/recovery in abandoned fields, such as frameworks of alternative states. If the biotic threshold is crossed, recovery would require vegetation manipulation. If the abiotic threshold is crossed, recovery would require modification of the physical environment (Cramer et al. 2008).
We found that species diversity, abundance, typical functional groups (grasses and sedges), key species, and species composition of abandoned fields recovered 20 yr after cessation of agricultural disturbance. That is, the low-diversity state was not stable, and thus a shift to an alternative stable state did not occur in the old fields in our study site. Our findings can be explained by the following mechanisms. First, although most biotic (SMBC and SMBN) and abiotic soil factors differed significantly among the different-aged abandoned fields, the lowest biotic and abiotic factors (except SOC) were not found in the 1-yr field. Hence, there were no nutrient limitations for recovery of the abandoned fields, and the abiotic threshold was not crossed in our system after long-term agricultural disturbance. Second, based on the model of Cramer et al. (2008) and on our conceptual model (Fig. 1), the biotic threshold can be divided into aboveground (vegetation) and belowground biotic (seed bank) parts. Although the aboveground biotic legacy was destroyed by the long-term agricultural activities, there was almost no change in the belowground biotic legacy (Fig. 3c, d). Our study system generally exhibited relatively rich seed banks resources (high richness and density). Specially, the persistent seed banks remained almost unvaried in the abandoned fields (Fig. 3e), with almost 70% of the seed bank the same as in the nondisturbed natural meadow (Table 2). Therefore, the composition and structure of the plant community in abandoned fields approach that of the natural meadow after 20 yr of recovery.

We found that successful recovery of key species of grasses and sedges exhibited a time lag during the restoration. Other research also has revealed a time lag for the recolonization of desired species and the seed bank community is intact, abandoned fields can be restored to meadows even if the aboveground plant community has been destroyed by long-term agricultural disturbance. Therefore, the regime shifts between alternative stable states and a novel successional trajectory did not occur in our study because of the strong role of the soil seed bank in ecosystem resilience. High species diversity of the soil seed bank provides much “rebuild capital” or “ecological memory” for restoration of abandoned fields. We concluded that if the abiotic environment is suitable for the recolonization of desired species and the seed bank community is intact, abandoned fields can be restored to meadows even if the aboveground plant community has been destroyed by long-term agricultural disturbance.

### If abandoned field could be reversed by seed bank triggered ecosystem resilience?

Two stable states (desired and degraded) were proposed in the alternative stable state model for restoration of ecosystems (Suding et al. 2004, Cramer et al. 2008). Generally, the transition from desired to degraded state is based solely on the composition of the aboveground vegetation (Dakos et al. 2012). However, if the seed bank still has seeds of the target or native species for the desired state, then the ecosystem has inherent resilience and may not have crossed the transition threshold. In our study, we found almost no change in the seed bank composition after long-term agricultural disturbance; this result is consistent with results from other research in which successional stage species produced many long-lived seeds, resulting in persistent seed banks for all of the successional stages (Kuikki 1993, Török et al. 2009).

Our results support the first scenario in the conceptual model (Fig. 1). We conclude that abandoned fields can recover by seed-bank-triggered ecosystem resilience. The following mechanisms support our conclusion. First, species composition of seed banks mostly remained unvaried between historical and altered states, i.e., early successional stage species were still present in the seed bank. In particular, we found target restoration species of grasses (e.g., Poa pratensis, Elymus dahuricus, and Roegneria nutans) and sedges (e.g., Kobresia humilis) in the seed bank of each year after abandonment. Thus, the loss of target/key species of grasses and sedges was still reversible and an exclusion of agricultural perturbation leads to restoration of the degraded vegetation.

### Table 2. Sørensen similarity index among the soil seed banks of persistent soil seed bank (July 2005) with an increase in years since abandonment.

<table>
<thead>
<tr>
<th></th>
<th>1 yr (%)</th>
<th>10 yr (%)</th>
<th>20 yr (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 yr</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 yr</td>
<td>70.73</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 yr</td>
<td>73.81</td>
<td>80.85</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>72.29</td>
<td>73.12</td>
<td>75.79</td>
<td>100</td>
</tr>
</tbody>
</table>

*Note:* 1 yr, abandoned 1 yr; 10 yr, abandoned 10 yr; 20 yr, abandoned 20 yr; control, natural meadow.
However, in our study, the highest species richness and seed density were found in the 1-yr abandoned field. This state has selected for plant traits that are adapted to high reproduction ability and thus rapid colonization following agricultural disturbance. Most of these species in the seed bank of the 1-yr field were annuals with a short generation time, high seed production, and high colonization ability. These species having a high intrinsic rate ($r$ strategy) of increase will recover quickly from agricultural disturbances in this state before the threshold point. Meanwhile, the presence of species germinated from the seed bank can contribute to the biotic legacy of cultivation and also can improve the long-term maintenance of an abiotic legacy (Cramer et al. 2008). Hence, native species could be regenerated spontaneously from the seed bank, and seed bank accelerates succession of degraded vegetation. Third, the highest similarity between the seed bank and aboveground vegetation was also found in the 1-yr abandoned field and it also exhibited the lowest plant community cover, with bare soil areas (Ma et al. 2013). This situation provides plenty of regeneration niches for seed bank species and promotes a high contribution of the seed bank to aboveground plant community regeneration and degraded vegetation restoration. The system was restored after 20 yr and the soil seed bank triggered ecosystem resilience.

We can conclude that high seed bank diversity and density could increase ecosystem resilience because a diverse seed bank has an increased likelihood of including particularly resilient species. Based on the framework of plant community assembly in abandoned fields (Cramer et al. 2008) and our conceptual model (Fig. 1), if the aboveground biotic part (vegetation) is lost but the seed bank still reflects aboveground vegetation or remains unvaried for all the successional stages, the ecosystem has a high natural resilience. Thus, the system could be restored by seed-bank-triggered ecosystem resilience. Our study highlights the role of belowground biotic legacy (soil seed bank) in systems that appear to be an important role in aboveground plant community regeneration and degraded vegetation restoration. The system was restored after 20 yr and the soil seed bank triggered ecosystem resilience.

Could the seed bank be an ecological warning?

There is no doubt that the important ecological functions of seed banks include “buffering capacity” and “warning indicators,” but these ideas have not received much research attention. Bhattachan et al. (2014) indicated that seed bank depletion can serve as an indicator that a system is reaching a tipping point toward alternative stable states. In our study, we asked if the similarity between seed bank and vegetation could predict ecosystem recovery, thus highlighting their use as early-warning indicators.

In our study, the soil seed bank was not changed by cultivation disturbance. If the intensity and scale of disturbance (cultivation) increase further, the belowground biotic (seed bank) and abiotic threshold could be crossed and the consequences may be a reduction in the belowground biotic (seed bank), limitation of seed dispersal to the site, and an increase in invasive species. The soil seed bank will be gradually impoverished and the abiotic environment threshold eventually crossed. Under this circumstance, the similarity between seed bank and aboveground vegetation would decrease if the threshold is crossed.

We found that similarity decreased with years since abandonment. Further, species composition both in seed bank and aboveground vegetation are almost the same in the 1-yr abandoned field. Thus, the seed bank played an important role in aboveground plant community regeneration in the 1-yr abandoned field, but its role decreased with years since abandonment. Generally, plants from stable habitats have low persistence of seeds in the soil (Thompson et al. 1998). Moreover, dense stands (cover $>$100%, Fig. 2c) of perennials species with clonal reproduction, such as grasses and sedges, prevent seed regeneration from the seed bank in the 20-yr abandoned field and the natural meadow. Hence, similarity between seed bank and aboveground vegetation could be an important ecological indicator of ecosystem resilience, and thus is very useful in assessing the stages of ecosystem degradation. Further, the similarity between the seed banks and aboveground vegetation indicates the level of effort required to restore the system to the historical or natural vegetation states.

If any other disturbance postponed or stopped the restoration of the vegetation from the soil seed bank, the natural resilience provided by the seed bank would be destroyed. Hence, if seed bank resources were depleted and fragmented, it would likely slow or stop subalpine meadow restoration. In the case of further degradation, expensive and time-consuming restoration measures such as seed addition would be needed to restore the vegetation (Zhang et al. 2013).

CONCLUSIONS

The low-diversity state of the 1-yr abandoned field was not stable, but it did not cross the threshold point and enter an alternative state. The 1-yr abandoned field could be reversed to the typical natural meadow since the belowground seed bank community was present even after the aboveground plant community had been destroyed by agricultural perturbation for 30 yr. We think the conclusions about alternative stable states need to carefully consider all aspects of the plant community above and belowground. Our study highlights the role of soil seed banks in ecosystem restoration and resilience and for developing effective preservation and
restoration strategies. The similarity between the seed bank and aboveground vegetation provides an early-warning signal for the onset of grassland degradation. The detection of change in similarity between seed bank and aboveground vegetation would be a cost-effective method of determining when management actions are needed to prevent degradation.

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LITERATURE CITED


**DATA AVAILABILITY**

Data available from Figshare: https://doi.org/10.6084/m9.figshare.8229143.v2