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Environmental factors driving seed bank diversity in alkali grasslands

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ABSTRACT

For an effective conservation and management in grasslands it is essential to understand mechanisms sustaining biodiversity. To gain knowledge is especially crucial in stressed grasslands harbouring a unique flora and fauna, like alkali grasslands. Aboveground vegetation, seed bank and environmental factors were studied in three stands of the following alkali grassland types: (i) *Artemisia* dry alkali grasslands at highest elevations; (ii) *Puccinellia* high and (iii) *Puccinellia* low grasslands at medium to low elevations, and (iv) *Juncus* wet alkali grasslands at the lowest elevations. We tested the following hypotheses: (i) Seed bank species diversity and density are the highest in the most stressed grassland types, where regeneration by seeds could have a major importance in sustaining vegetation diversity. (ii) Seed bank density of hygrophytes increases with decreasing elevation, because the cover of hygrophytes in the vegetation increases with decreasing elevation. The mean seed bank density ranged from 30,104 up to 51,410 seeds/m^2, which is higher than in most dry grasslands. Both the lowest seed bank density and diversity were detected in the most stressed *Puccinellia* high grasslands; *Spergularia salina* was the only abundant seed bank species (possessing at least 1000 seeds/m^2). These results not supported our first hypothesis. We detected the highest seed densities of almost all hygrophyte species in the lowest-elevated *Juncus* grasslands. But, we did not find a significant monotonous correlation between elevation and the overall hygrophyte seed bank density; because most of the hygrophyte species were missing from the seed bank at the medium-elevated, but most saline *Puccinellia* grasslands. Thus, our results only partly supported the second hypothesis. In total we detected more species in the seed bank than in the aboveground vegetation which emphasises that seed bank plays an important role in sustaining the diversity of alkali grasslands. However, characteristic graminoids possessed no considerable seed bank, except for *Juncus compressus* (up to 38,619 seeds/m^2). We can conclude that persistence and establishment of most alkali grassland species are not supported by the local persistent seed bank.

1. Introduction

Conservation of grassland biodiversity is an urgent task nowadays, because grasslands are in decline and they contribute with a significant part to the biodiversity of Europe, harbouring a very diverse flora and fauna of conservation interest at different spatial scales (Donath et al., 2007; Kovács-Hostyánszky et al., 2011; Reitalu et al., 2013; Wilson et al., 2012). To achieve an effective conservation and management of grassland biodiversity it is essential to understand mechanisms and ecosystem functions sustaining natural grassland communities from local up to landscape scales (Lindborg et al., 2008; Drobnik et al., 2011; Zeiter et al., 2013). Understanding ecosystem functions responsible for grassland biodiversity also supports the planning and implementation of grassland restoration actions (Török et al., 2011a; Prach et al., 2013). To gain knowledge is especially crucial in environmentally stressed grasslands which harbour a unique flora and fauna like alkali grasslands (Kelemen et al., 2013).

There are contrasting views regarding the role of persistent seed bank in sustaining biodiversity of stressed communities. Several authors argue that in stressful conditions instead of sexual reproduction a higher investment in clonal spread is necessary, which suggest that seed bank may plays a subordinate role in these communities (Chang et al., 2001; Bossuyt and Honnay, 2008). However, the seed bank can have a crucial importance in vegetation dynamics in stressed communities as found by others (see Fenner and Thompson, 2005). Persistent seed bank allows species to bridge temporally unsuitable habitat conditions for germination

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and establishment (Bossuyt and Honnay, 2008). The role of persistent seed bank was found to be especially important in salt-affected communities, where highly saline conditions generally hamper seed germination, resulting in the formation of persistent seed bank (Ungar, 1991). Soil salinity is found to be one of the major factors determining seed germination either osmotically or through a specific ion-effect in salt-affected communities (Egan and Ungar, 2000).

Several authors studied the seed bank of salt-affected communities in relation with aboveground vegetation, soil salinity or water regime. The majority of these studies focused on inland (e.g. Badger and Ungar, 1994; Egan and Ungar, 2000) or coastal salt marshes (Chang et al., 2001; Shumway and Bertness, 1992), seashore meadows (Jutila, 1998), Mediterranean salt grasslands (Maranón, 1998) or salt deserts (Khan, 1993), but such studies in inland alkali grassland's seed bank are still lacking.

Inland alkali grasslands are of special interest of the Natura 2000 network, and are included as "Pannonic salt steppes and salt marshes (1530)". Pannonic alkali grasslands are one of the best preserved grassland habitats in Europe typical for the Pannonian biogeographical region (Török et al., 2011b). They harbour several plants and animal species listed in Annex I and Annex II like *Cirsium brachycephalum* or *Gortyna borelii lunata*. Alkali grasslands are present at sites with moderate to high salt content and high springtime groundwater levels in continental climate. Alkali grasslands are traditionally used as extensively managed pastures, because their poor soil quality and fluctuating water regime makes them unsuitable for intensive agriculture and forestry (Molnár and Borhidi, 2003; Török et al., 2011a). The plant life in alkali grasslands is influenced by several stress factors like the (i) high osmotic pressure, (ii) ion-toxicity, (iii) unbalanced and fluctuating ion concentrations, (iv) unfavourable soil structure, (v) suboptimal soil pH and (vi) nutrient deficiency (Füzy et al., 2010). Elevation and soil pH were found to be important factors affecting vegetation composition in salt-affected communities (Davy et al., 2011; Wanner et al., 2014). In line with the uneven pattern of these stressors, different types of alkali grasslands are situated along an elevation gradient where even a few centimetre difference in elevation results in the formation of a different aboveground vegetation composition (Kelemen et al., 2013).

In the present study we provide a detailed analysis of seed bank composition of four types of alkali grasslands in relation with aboveground vegetation and some crucial environmental parameters (elevation, salinity, soil water content, soil organic matter and soil water capacity). We aimed at the analysis of environmental parameters in relation to the species composition and diversity of aboveground vegetation and soil seed bank in the studied alkali grassland types. We specifically tested the following hypotheses: (i) Seed bank species diversity and density are the highest in the most stressed grassland types where regeneration by seeds could have a major importance in sustaining vegetation diversity (Hopfensperger, 2007). (ii) Seed bank density of hygrophytes increases with decreasing elevation, because the cover of hygrophytes in the vegetation increases with decreasing elevation (Jutila, 2002). Our ultimate goal was to analyze the effects of environmental parameters on the species composition and density of the soil seed bank.

2. Materials and methods

2.1. Study area

Our study area is located in a mosaic alkali landscape in Nagy-Szik, Hortobágy, near the town Balmazújváros in East-Hungary (47° 35′ N and 20° 30′ E). The region is characterized by a moderately continental climate. The mean annual temperature is 9.5 C, while the mean annual precipitation is 550 mm with high among-year variations. The vegetation of the region is characterized by alkali grasslands, with scattered alkali marshes on the lowest and loess grassland patches on the highest elevations. The whole study area is traditionally managed by moderate cattle and sheep grazing. Meadow solonetz soils with a salt accumulation zone in deeper soil layers are typical in the study area. The selection of studied grassland types was based on Kelemen et al. (2013) study; we selected the most widespread alkali grassland types along an elevation gradient. The difference between highest and lowest elevated plot was only 30 cm. We studied the aboveground vegetation, seed bank and environmental factors in each of the three stands of a given of alkali grassland type: (i) *Artemisia* dry alkali grasslands at highest elevations; (ii) *Puccinellia* high and (iii) *Puccinellia* low grasslands at medium to low elevations, and (iv) *Juncus* wet alkali grasslands at the lowest elevations.

2.2. Sampling setup

2.2.1. Analysis of environmental variables

In three stands of each grassland type we designated five 1 m × 1 m permanent plots in early spring, 2009. We measured the elevation (a.s.l.) in the centre of each plot with 1–3 cm accuracy (TOPCON GRS-1). We collected five soil cores (4-cm in diameter, 10-cm in depth) with a soil corer in late April 2010 for soil analysis. To determine soil moisture, we collected wet soil in plastic bags. The weight of the collected wet soil was measured with a tare balance immediately after transportation into laboratory. Soil samples were then air-dried at room temperature. The weight of air-dried samples was measured with 0.01 g accuracy. Soil water content was calculated from the differences between the weight of wet and air-dried samples. Soil solution was prepared for pH and conductivity measurements. From the wet soil 6.0 g samples were put into plastic beakers and filled with 50 ml deionised water.

The pH was measured using a digital type Testo 206 (Testo AG, Germany). Salinity was expressed by soil electrical conductivity (EC_a), which was measured with Soil Test EC & Temp HI98331, Mauritius. Soil organic matter (SOM) was assessed with loss on ignition (LOI) method. Air-dried samples (0.5 g) were dried at 105 °C overnight. Then, samples were weighted and then combusted at 550 °C for 5 h in a muffle furnace (Nabertherm L5/C6, Germany). After combustion, samples were cooled in desiccators and weighted again with an analytical balance (type SARTORIUS 6MBH Germany). Soil organic matter (SOM) was calculated using the following equation: $LOI_{550} = ((DW_{105} - DW_{550})/DW_{105})*100$; where "$LOI_{550}$" means the soil organic matter, "DW_{105}" the soil dry weight at 105 °C, and "DW_{550}" the soil dry weight at 550 °C (MSZ-15296:1999). Soil water capacity was determined using Arany-type plasticity index (P_A). In a mortar 100 g air-dried soil was put and it was mixed with deionised water until a homogeneous paste was formed. After the deionised water was added drop-wise till the upper limit of plasticity was realized by the so-called thread proof (MSZ-08 0205:1978). Arany-type plasticity index was calculated by the following equation: $P_A = 100 * V/M$, where "V" is the amount of deionised water used, while "M" is the weight of the soil. The measured soil water capacity can be assigned to the following physical soil categories: $P_A < 25$ coarse sand, $P_A = 25–30$ sand, $P_A = 31–37$ sandy loam, $P_A = 38–42$ loam, $P_A = 43–50$ clay loam, $P_A = 51–60$ clay and $P_A > 60$ heavy clay soils.

2.2.2. Vegetation and seed bank sampling

The percentage cover of vascular plants was recorded in each plot in June 2009. Soil seed bank was analysed with the seedling emergence method. Three soil cores (4-cm in diameter and 10-cm in depth; 126 cm^3/core) per plot were drilled after snowmelt