Keywords
Abandonment; Grassland management; Grazing; Limestone grassland; Optimal sampling time; Seed density; Seed mass; Semi-natural grassland; Species richness; Traditional management

Nomenclature
Tutin et al. (1964–1980)

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Abstract

Questions: What is the overall restoration potential of the persistent soil seed bank of abandoned alvar grassland sites? To what degree does the share of characteristic alvar grassland species in the soil seed bank change during secondary succession from traditionally managed pasture towards stages overgrown by Corylus avellana and Juniperus communis?

Location: Northern Estonia.

Methods: The species composition of above-ground vegetation and the persistent soil seed bank were investigated in successional alvar grasslands. Particular attention was given to typical grassland species in the persistent soil seed bank. Soil seed bank density was also recorded. General linear models and non-metric multidimensional scaling were used to assess successional trends and the restoration potential of the soil seed bank.

Results: The cessation of traditional management has led to a considerable decline in above-ground plant species richness. The composition of above-ground vegetation in different successional stages was considerably more variable than that of the seed bank. We detected large and species-rich persistent soil seed banks in all our study sites. About 70% of species in the seed banks of overgrown sites could be classified as typical grassland species. The frequency of typical alvar grassland species, such as Arabis hirsuta, Arenaria serpyllifolia, Cerastium fontanum, Helianthemum nummularium, Poa angustifolia, Potentilla crantzii, Sedum acre and Veronica spicata in the seed bank of long-term abandoned (25 and 50 yr) sites was unexpectedly high.

Conclusions: Abandoned semi-natural grassland sites in northern Estonia still contain a relatively large grassland community species pool, including a large and effective soil seed bank. The soil seed bank could thus play a more important role in the restoration management of abandoned dry grassland communities than could be concluded from previous studies.

Introduction

Semi-natural calcareous grasslands in Europe have a history, dating back several thousand years. Such habitats have generally arisen due to long-term grazing or mowing, and in most instances they need continuous management in order to persist (Rosén 1982; Gibson et al. 1987; Pärtel et al. 1999a). European semi-natural dry grasslands on shallow calcareous soils are notable for their very high small-scale plant species richness, which is almost unrivalled across terrestrial ecosystems (Eriksson et al. 2002; Poschlod & WallisDeVries 2002; Jacquemyn et al. 2003).

Alvars are a particular type of semi-natural grassland that is found on thin calcareous soils. Their distribution is very limited: typical alvar grasslands are found on the large islands of the Baltic Sea (Laasimer 1965; Pärtel et al. 1999a); large alvar grasslands are also distributed along the northern and western coast of mainland Estonia, and smaller areas are found in Västergötland in mainland Sweden (Rosén 1982; Milberg & Hansson 1994). Dry
calcareous grassland vegetation that is very similar in nature to alvar grassland is nonetheless present at other sites in Europe (Gibson & Brown 1991; WallisDeVries et al. 2002; Dengler & Löbel 2006) and elsewhere (Schaefer & Larson 1997; Cantero et al. 1999; Stark et al. 2003; Tomlinson et al. 2008) where low-intensity management intersects with suitable climatic conditions and thin soils on lime-rich bedrock.

Until the middle of the 20th century, alvar grasslands were widespread in Estonia, where they were used extensively as pasture for sheep, horses and cattle (Laasimer 1965; Laasimer 1981; Pärtel et al. 1999b). However, in recent decades dramatic changes in land use have occurred, and following the cessation of traditional management, only a small fraction of these grasslands survive today. Most existing alvar grasslands are no longer used for grazing and are therefore becoming increasingly overgrown by shrubs and trees (typically Scots pine, Pinus sylvestris; juniper, Juniperus communis; and hazel, Corylus avellana; Pärtel et al. 1999a). The increasingly uncertain status of alvars combined with their significance as a characteristic element of traditional landscapes and a uniquely species-rich European habitat makes them a priority for nature conservation. This is reflected in the corresponding legislation, where alvars are designated in Annex I of the EC Habitats Directive (92/43 EEC) as a priority habitat (Nordic alvar and precambrian calcareous flatrocks).

From the perspective of nature conservation, the key issues linked to alvar grasslands are preservation of remaining areas and restoration of overgrown former grasslands. Following the cessation of management, steep declines in above-ground plant species richness generally occur in parallel with the increase of scrub cover (Rejmánek & Rosén 1988). However, the soil seed bank may represent an important refuge for species disappearing from the vegetation after cessation of grazing. Knowledge of seed bank species composition and density is thus essential for successful habitat restoration and management (Hutchings & Booth 1996; Davies & Waite 1998; Bakker & Berendse 1999). If the density of persistent seeds of typical grassland species in the soil seed bank is sufficient, successful grassland restoration might still be possible.

There is conflicting evidence concerning the potential of soil seed banks to contribute to grassland restoration. The majority of previous studies have concluded that soil seed banks in temperate semi-natural grassland tend to be relatively small because characteristic grassland species do not possess seeds that persist in the soil (in Europe: Davies & Waite 1998; Kalamees & Zobel 1998; Rosén & van der Maarel 2000; Kalamees & Zobel 2002; Bisteu & Mahy 2005; Bossuyt et al. 2006; Valkó et al. 2011; in North America: Stark et al. 2003). Consequently, the significance of the soil seed bank for the restoration of calcareous grassland communities has been assumed by these authors to be low. However, other studies have found equivalent seed bank densities and species richness levels in open grassland and overgrown sites (Milberg 1995; Maccherini & De Dominics 2003).

Restoration of semi-natural grassland vegetation is generally implemented in one of two very different circumstances. When re-creation of semi-natural grasslands takes place on former arable land, characteristic species may have been absent for decades, and it is necessary both to restore initial habitat conditions and to facilitate the arrival of characteristic species (Walker et al. 2004; Klimeš et al. 2010). By contrast, in overgrown grasslands, restoration actions mainly consist of removing shrubby vegetation and resuming an appropriate management regime. This approach is usually relevant to the restoration of alvar grasslands, because the general unsuitability of shallow soil areas has limited the historical conversion of alvars to arable land. Indeed, the dry and shallow soils of Estonian alvar grasslands have very rarely been used as crop or hay fields and the area of this habitat has decreased mostly due to abandonment (Pärtel et al. 1999b). Depending on the composition and viability of the soil seed bank, overgrown alvar sites could have potential for successful vegetation restoration and could consequently play an important role in the preservation of Estonian semi-natural grassland, as previously noted by Kalamees & Zobel (1997).

The objectives of the current research were to describe the dynamics in size and species composition of the persistent soil seed bank during secondary succession from traditionally managed pasture to sites overgrown by C. avellana and J. communis after the cessation of grazing. In particular, we aimed (1) to describe the representation of characteristic alvar grassland species in the soil seed bank of different successional stages, and (2) to evaluate the overall restoration potential of the soil seed bank in overgrown sites.

Methods
Study site
The study site was located at Haavakannu (59° 28’N, 25° 17’E), about 30 km east of Tallinn, in northern Estonia. The soil of the study areas is shallow (5–10 cm) rendzic leptosol (Reintam 1995), lying on a slightly weathered Ordovician limestone. The Haavakannu site has a long documented management history – it was already open pasture in 1803 (Germann 1805). In 1923, Gustav Vilberg surveyed alvar vegetation in northern Estonia and described several grasslands including alvar plant communities in pasture at Haavakannu village (Vilberg 1927). Nowadays most of these sites have been seriously altered or destroyed due to abandonment or
urban development. The alvar grasslands at Haavakannu provide an excellent site to study the vegetation and soil seed bank dynamics during secondary succession because various areas there have been abandoned for different lengths of time. The process of abandonment started soon after World War II, with some places now critically overgrown. Most sheep and cattle grazing at Haavakannu ceased around the beginning of the 1990s. However, some small areas are still continuously grazed by cattle, providing a valuable source of reference. The gradual expansion of C. avellana and J. communis shrubs following abandonment is observable from aerial photographs taken in 1952, 1973 and 2000. Images from 1952 indicate that the area as a whole was almost free of shrubs. By 1973 shrubs had covered almost half of the area, while at the time of the study the open grassland area amounted to approximately 20%.

Two replicate study areas were selected and four successional stages were compared in each: continuously managed pasture and three stages of abandonment – abandoned for about 10, 25 and 50 yr. Detailed information about land-use history and approximate abandonment dates was derived from aerial photographs from 1952, 1973, 2000 and from local people. The distance between replicate areas was ~500 m. The distance between sampling sites, relatively uniform areas of about 10 m × 10 m representing a chronosequence of successional stages, within a replicate study area was ~30 m.

On extant open grassland areas the vegetation is species-rich and related to the Filipendula vulgaris–Trifolium montanum community (according to Laasimer 1965). Sites that have been abandoned for 10 yr are still open, and the vegetation is species-rich. In areas abandoned about 25 yr ago, some small grassland patches have survived. In these areas, the vegetation is still relatively species-rich, but late successional species (e.g. Hepatica nobilis, Alchemilla glaucescens, Aegopodium podagraria, etc.) are more frequent than in true grassland, and sparse shrub layer is formed by J. communis and C. avellana. In the sites abandoned 50 yr ago the J. communis and C. avellana are spread rather continuously in the bush layer and Pica abies and P. sylvestris are found in the tree layer.

Vegetation and soil seed bank sampling

The established vegetation was recorded at the end of Jun 2005 in ten randomly located 50 cm × 50 cm plots at each of the eight sampling sites (four successional stages in two replicate study areas), where all vascular plant species were recorded using a 1–5 abundance scale. We also used historical vegetation records for 1923 from our study site that were published in Vilberg (1927). These data serve as an excellent reference for approximating the original species composition and species abundances of traditionally managed alvar grassland.

The seed bank was sampled at the beginning of Jun 2005. This timing ensured that samples reflected the persistent seed bank, because seeds produced in the previous growing season should already have germinated, while the season’s seed rain should not yet have started. In each of the eight sampling sites, a relatively homogeneous area of vegetation was selected. At each site ten soil samples were taken (10 cm × 10 cm, ~5-cm deep, or to the bedrock when the soil was shallower). Following the concentration method (ter Heerdt et al. 1996), the seedling emergence method was used to quantify viable seed content of the soil samples. Samples were washed over a fine sieve (mesh size of 0.2 mm), spread out in trays as a thin layer over a 1:1 mixture of pure sand and fertilized peat, and set to germinate in an unheated greenhouse. Ten control trays filled with pure substrate were placed randomly between the trays in order to monitor airborne seed contamination. The soil in trays was kept constantly moist. During the whole growing period emerging seedlings were identified as soon as possible, recorded and removed. Seeds in the samples were allowed to germinate for 4 mo. No attempt was made to assess the number of ungerminated seeds potentially remaining in the samples.

Data analysis

Numbers of seeds in soil samples were extrapolated to express seed density m−2, and all variables were natural log-transformed prior to statistical analyses. Seed densities from seed bank samples were converted into five abundance classes (1 = 1–5 seeds; 2 = 6–10; 3 = 11–35; 4 = 35–50; 5 = >50) for comparing seed bank and established vegetation. The percentage cover of vascular plant species of the historical vegetation descriptions (1 m × 1 m plots from Vilberg 1927) were converted to a 1–5 abundance scale (1 = <5; 2 = 5–24; 3 = 25–49; 4 = 50–74; 5 = >75). Weighted (by abundance) average Ellenberg indicator values (Ellenberg et al. 1992) for light, moisture, pH and nitrogen (N) were calculated over all species found in each of the vegetation plots (both, historical and contemporary plots) and soil seed bank samples.

We distinguished typical alvar grassland species from the total species pool recorded during this study as species that have been classified in vegetation surveys as being characteristic of open, thin soil calcareous grassland communities (Vilberg 1927; Laasimer 1965; Krall et al. 1980; Pärtel et al. 1999a).

The effect of successional stage on various parameters of the vegetation and seed bank was analysed using general linear models (GLM). The factor ‘study area’ (two levels denoting the two replicate areas) was also
included in the model. Given the small number of levels and given that the two studied areas were the only available patches of alvar vegetation in the locality, ‘study area’ was treated as a fixed factor in the model. Because differences between replicate areas were not of particular interest for the present study, significance values for area will not be presented. The following dependent variables were considered: total species richness and grassland species richness in both the established vegetation and in the soil seed bank; and total seed density and the seed density of grassland species in the soil seed bank. The seeds of Juncus spp. were not included in seed density analysis.

Variability in species composition in established vegetation and in the soil seed bank was estimated as the average Bray–Curtis dissimilarity (Bray & Curtis 1957) among plots within study sites. Bray–Curtis dissimilarity is a widely used semi-metric dissimilarity coefficient in ecological data analysis, it represents dissimilarity among sampling units represented by abundance counts (Clarke 1993). GLM analysis was used to test the effect of successional stage on the dissimilarity measure.

Seed mass data were obtained from the databases BIOFLOR (Klotz et al. 2002) and Seed Information Database (SID; Royal Botanic Gardens, Kew, UK 2008). A weighted average seed mass was calculated for each vegetation plot (both historical and contemporary plots) and soil seed bank sample over all recorded species.

Changes in weighted average seed mass of species that were found in the soil seed bank samples and in above-ground vegetation during secondary succession were analysed using GLM. Statistically significant differences between successional stages were assessed using Tukey’s multiple comparisons test. A probability level of $P < 0.05$ was considered statistically significant. All statistical analyses were performed in Statistica 7.0 (StatSoft Inc., Tulsa, OK, US).

Non-metric multidimensional scaling (NMS) was used to plot the vegetation and seed bank data in multi-dimensional space. Historical vegetation records were used jointly with the modern data in order to approximate the original species composition and species abundances in a traditionally managed alvar grassland community. NMS is a non-parametric ordination method that performs well on data from arbitrary, discrete, non-normal or otherwise unorthodox scales (Kenkel & Orlo´ci 1986; McCune & Grace 2002). All ordinations were run using PC-ORD 5.0 (MJM Software Design, Gleneden Beach, OR, US) in the ‘auto-pilot’ mode, which uses random starting configurations and assesses dimensionality by minimizing stress. In all instances the number of iterations was 500. Bray–Curtis dissimilarity was used as the dissimilarity measure for each initial matrix.

Following ordination of the seed bank data, the sample scores of NMS axes were correlated (Spearman rank correlation) with successional stage, weighted average Ellenberg indicator values (Ellenberg et al. 1992) for light, moisture, pH and N, as well as weighted average seed mass, calculated for each of the seed bank samples. The average number of species and number of seeds in seed bank samples were also correlated with NMS axes to evaluate whether and in what way the soil seed bank communities are related to these variables.

Results

Altogether 101 species were recorded in the established vegetation and in the soil seed bank. Among the 65 species detected in the seed bank, 20 did not occur in the vegetation, while among the 81 species recorded in the established vegetation, 36 were not detected in the seed bank. The seed bank and the established vegetation had 45 species in common. A complete list of species recorded in this study is presented in Supporting Information, Appendix S1.

Above-ground vegetation

Successional stage had a significant effect on all studied parameters of the established vegetation – on the total species richness ($F_{3, 72} = 52.50, P < 0.001$), on the grassland species richness ($F_{3, 72} = 120.78, P < 0.001$) and on the variability of species composition (Bray–Curtis dissimilarity; $F_{3, 150} = 23.30, P < 0.001$).

Mean species richness was significantly lower in 25- and 50-yr abandoned sites compared with grazed sites. Mean typical grassland species richness was significantly lower in 25-yr abandoned sites compared with grazed and 10-yr abandoned sites, and significantly even lower in 50-yr abandoned sites (Tukey’s multiple comparisons test; Fig. 1b). Variability in species composition (mean Bray–Curtis dissimilarity within successional stage) was highest in 50-yr overgrown sites, but we found no significant differences between grazed, 10- and 25-yr abandoned sites (Tukey’s multiple comparisons test; see Fig. 3).

Seed bank

Altogether 8221 seeds belonging to 66 species were determined in the persistent soil seed bank. The most abundant species in the seed bank were Veronica chamaedrys (23.2% of all recorded seeds), Veronica spicata (11.6%) and Arenaria serpyllifolia (11.1%). The overall mean seed density in the soil was 11 302 seeds m$^{-2}$ (ranging from 3705 seeds m$^{-2}$ in 50-yr abandoned sites to 20 145 seeds m$^{-2}$ in grazed
No seedlings were found in the control trays.

The share of characteristic alvar grassland species in the persistent soil seed bank of all our study sites, both grazed and abandoned, was surprisingly high - 80.2% of all species detected in the soil seed bank could be classified as typical alvar grassland species, 76.9% in grazed, 82.2% in 10-yr abandoned, 82.6% in 25-yr abandoned and 71.1% in 50-yr abandoned sites. *Arabis hirsuta*, *A. serpyllifolia*, *Cerastium fontanum*, *Helianthemum nummularium*, *Poa angustifolia*, *Potentilla crantzii*, *Sedum acre* and *V. spicata* were those typical grassland species that were abundantly represented in the seed bank of overgrown sites. The frequency of typical grassland species in the seed bank of long-term abandoned (25 and 50 yr) sites was relatively high (Appendix S1). The frequency of alien species or species not characteristic to alvar grassland communities was relatively low in the soil of all sites. There were only 31 seeds of various *Juncus* species found during the study, and these originated mainly from the soil of grazed sites. The overall mean seed density of typical alvar grassland species in the soil was 9491 seeds·m⁻² (ranging from 3610 seeds·m⁻² in 50-yr abandoned sites to 16 575 seeds·m⁻² in grazed sites).

Successional stage had a significant effect on all studied parameters of the soil seed bank – on total species richness (GLM; $F_{3, 72} = 4.15, P = 0.009$), on grassland species richness ($F_{3, 72} = 5.11, P = 0.003$), on seed density of all species ($F_{3, 72} = 33.18, P < 0.001$), on seed density of grassland species ($F_{3, 72} = 23.65, P < 0.001$) and on variability of species composition (Bray–Curtis dissimilarity; $F_{3, 350} = 18.97, P < 0.001$).

We found no significant differences in total species richness between grazed, 10- and 25-yr abandoned sites, but in 50-yr overgrown sites this parameter was lower than in 10-yr abandoned sites. Mean typical grassland species richness followed a similar pattern as total species richness – there were no differences between grazed, 10- and 25-yr abandoned sites and between grazed and 50-yr abandoned sites, but in 50-yr abandoned sites richness was significantly lower compared with 10- and 25-yr abandoned sites (Tukey’s multiple comparisons test; Fig. 1a).

Mean seed density of all species was significantly higher in grazed sites and lower in 50-yr abandoned sites. Mean typical grassland species seed density was significantly lower in 25-yr abandoned sites than in 10-yr abandoned and grazed sites, and significantly even lower in 50-yr abandoned sites (Tukey’s multiple comparisons test; Fig. 2). Variability in species composition (mean Bray–Curtis dissimilarity within successional stage) was significantly higher in 50-yr abandoned sites compared with other successional stages. In 25-yr abandoned sites variability was higher than in grazed sites, and there were no
that in the seed bank (sional stages (Table 1).

sites were significantly heavier than those in other succes-

the seed mass of species found in grazed, 10- and 25-yr

P 0.001). There were no significant
differences between the seed mass of species found in the

and 50-yr abandoned sites. There were no significant
differences in weighted mean seed mass found in the

variance in species composition and species abundances. Axes 1, 2 and 3 explained 17.0%, 32.5% and 28.8% of the variance, respectively. Stress and instability for the final solution were 16.71 and 0.0021, respectively. Vegetation changes due to overgrowing succession in alvar grassland are expressed fairly clearly in the ordination plot. The sequence of stages from grazed sites to 50-yr abandoned sites is correctly ordered along Axis 2, while historical vegetation plots are located in the vicinity of the modern vegetation plots from grazed sites. The seed bank samples and vegetation plots segregated along Axis 3. Seed bank samples exhibited a fairly uniform pattern compared with that of vegetation plots. The two replicate study sites with similar management history generally overlapped to a large degree, with the exception of the modern grazed sites, which diverged along Axis 3.

The separate NMS ordination restricted to seed bank samples alone (Fig. 5) revealed more details concerning the pattern of species composition in below-ground seed communities. The three-dimensional NMS solution, with a final stress of 17.98 and final instability of 0.0008 explained 76.3% (27.0%, 23.8% and 25.5% on axes 1, 2 and 3) of the variance in species composition and abundances in the soil seed bank. The samples from grazed sites and 50-yr abandoned sites were separate from the samples from all other sites and from each other. However, the majority of samples showed no clear pattern of differentiation according to their origin, indicating that the seed banks of slightly overgrown areas (i.e. 10- or 25-yr abandoned) did not differ greatly in terms of species composition. The NMS axes were significantly correlated with successional stage (Axis 3, Spearman’s r = -0.578, P < 0.001), with soil seed density (Axis 1, r = -0.299, P < 0.05, Axis 3, r = 0.533, P < 0.001), with species richness (Axis 1, r = -0.397, P < 0.001) and with weighted mean seed mass (Axis 3, r = -0.317, P < 0.05). Weighted average Ellenberg indicator value for light was correlated

Non-metric multidimensional scaling ordination

All available data, including both modern and historical vegetation plots and seed bank samples, were plotted in NMS ordination space to provide a general overview of variability in species composition and species abundances in successional alvar grassland communities in our study areas (Fig. 4). The final NMS solution was three-di-

This difference indicates that the seed banks of slightly overgrown areas (i.e. 10- or 25-yr abandoned) did not differ greatly in terms of species composition. The NMS axes were significantly correlated with successional stage (Axis 3, Spearman’s r = -0.578, P < 0.001), with soil seed density (Axis 1, r = -0.299, P < 0.05, Axis 3, r = 0.533, P < 0.001), with species richness (Axis 1, r = -0.397, P < 0.001) and with weighted mean seed mass (Axis 3, r = -0.317, P < 0.05). Weighted average Ellenberg indicator value for light was correlated

**Table 1.** Weighted average seed mass (mg) of species in vegetation plots and seed bank samples in different successional stages. Different letters indicate statistically significant differences (Tukey’s multiple comparisons test, applied separately on vegetation and seed bank data).

<table>
<thead>
<tr>
<th></th>
<th>Grazed</th>
<th>10-yr abandoned</th>
<th>25-yr abandoned</th>
<th>50-yr abandoned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetation</td>
<td>1.964 ± 0.100a</td>
<td>2.061 ± 0.107a</td>
<td>1.953 ± 0.096a</td>
<td>2.684 ± 0.194a</td>
</tr>
<tr>
<td>Seed bank</td>
<td>0.445 ± 0.036a</td>
<td>0.863 ± 0.097v</td>
<td>0.663 ± 0.065v</td>
<td>0.845 ± 0.115v</td>
</tr>
</tbody>
</table>

**Fig. 3.** Bray–Curtis dissimilarity between sampling plots in the same successional stage. Measures are calculated for soil seed bank and established vegetation plots for each of the four different successional stages. Different letters accompanying bars denote significant differences between mean values using Tukey’s multiple comparisons test at P < 0.05 following GLM analysis, applied separately to vegetation and seed bank data. Error bars represent 95% confidence intervals for the means.

Seed size

There were significant differences between weighted average seed masses of species that were in the established vegetation in different successional stages (F1, 152 = 5.84, P = 0.001). There were no significant differences between the seed mass of species found in grazed, 10- and 25-yr abandoned sites, but those in the soil of 50-yr abandoned sites were significantly heavier than those in other successional stages (Table 1).

Weighted average seed mass calculated on the basis of vegetation plots was on average three times higher than that in the seed bank (F1, 152 = 440.89, P < 0.001). Seed mass in established vegetation was significantly higher in 50-yr abandoned sites compared with other sites (F3, 72 = 5.84, P < 0.001; Table 1). There were also significant differences in weighted mean seed mass found in the soil seed bank between different successional stages (F3, 72 = 9.06, P < 0.001). Seeds of species in the soil of grazed sites were significantly lighter than those in the 10- and 50-yr abandoned sites. There were no significant differences between the seed mass of species found in the three abandoned sites (Table 1).
Discussion

The cessation of traditional management at Haavakannu has led to a considerable decline in above-ground plant species richness, but only in those sites that have been abandoned for a long period of time. There were no differences between overall species richness and typical grassland species richness in grazed and 10-yr abandoned sites. The species richness of typical grassland plants was significantly reduced in 25-yr abandoned sites and a dramatic decline was evident in the 50-yr abandoned sites, where few grassland species remained in the above-ground vegetation. A decline in species richness following the cessation of traditional grassland management (i.e. grazing or mowing) has been widely observed and is largely associated with increasing competition for light in the higher swards of abandoned grasslands (Bobbink & Willems 1987; Mitchley & Willems 1995; Davies & Waite 1998; Poschlod & WallisDeVries 2002; Jacquemyn et al. 2011).

The seed bank in the studied grassland successional stages was characterized by unexpectedly high seed densities and levels of species richness. Numerous previous studies of European semi-natural grasslands have reported fairly small and species-poor soil seed banks, containing a negligible share of characteristic grassland species (Akinola et al. 1998; Davies & Waite 1998; Kalamees & Zobel 1998, 2002; Rosén & van der Maarel 2000; Bisteau & Mahy 2005; Bossuyt et al. 2006; Valkó et al. 2011). It seems that several of these studies did not make a clear distinction between the temporary and persistent fractions of the seed bank and, as a consequence of their timing, sampled the transient seed bank from the previous or the same year. As highlighted by Baskin & Baskin (1998), only those seeds that survive until the second germination season should be classified as persistent seeds in the soil seed bank. Consequently, in order to specifically assess the persistent seed bank, sampling should take place in late spring or early summer, after the occurrence of the spring germination flush and before seeds produced in the current season are dispersed. Conversely, sampling outside this period may overestimate the number and species richness of seeds in the soil seed bank. The fact that our sampling was targeted at the persistent fraction of the seed bank makes the contrast with previous findings even more remarkable.

Average seed bank density in our study was 20 145 seeds m⁻² in grazed grassland, 9925 seeds m⁻² in sites abandoned 10 yr ago (which still contained open grassland), 11 435 seeds m⁻² in 25-yr abandoned and 3703 seeds m⁻² in 50-yr abandoned grassland. Typical grassland species seed densities were 16 575, 9575, 8205 and 3610 seeds m⁻² in the respective stages. The majority of previous studies have reported noticeably fewer seeds in the soil seed bank, such that seed densities reported elsewhere

with Axis 1 \((r = -0.300, P < 0.05)\) and Axis 3 \((r = -0.368, P < 0.001)\) and value for N was correlated with Axis 1 \((r = 0.330, P < 0.05)\).
from open, managed grasslands are generally similar to those from our 50-yr overgrown grassland. For example, Willems & Bik (1998) reported 4000 seeds m$^{-2}$ in mown sites and 770 seeds m$^{-2}$ in unmanaged sites (samples were taken in Sep), Bisteteau & Mahy (2005) 4645 seeds m$^{-2}$ in grazed and 1508 seeds m$^{-2}$ in unmanaged sites (in Mar), Valkó et al. (2011) 3806 seeds m$^{-2}$ in mown and 6008 seeds m$^{-2}$ in unmanaged sites (immediately after snow-melt) and Jacquemyn et al. (2011) 3972 seeds m$^{-2}$ in grazed and 1630 seeds m$^{-2}$ in unmanaged sites (in Nov). The seed densities found by Stark et al. (2003) in southern Ontario, Canada – 250–700 seeds m$^{-2}$ – were even lower than those reported for similar studies in Europe. Somewhat higher densities were recorded by Bakker et al. (1996), who reported 13 416 seeds m$^{-2}$ in grazed and 5064–10 008 seeds m$^{-2}$ in unmanaged alvar sites (samples were taken in Apr) in Öland, Sweden.

Few studies are available from dry calcareous grasslands where the permanent soil seed bank has been appropriately assessed. In Västergötland, southwest Sweden, on isolated limestone grassland, Milberg & Hansson (1994) found approximately 10 060 seeds m$^{-2}$ in the permanent seed bank both in grazed and ungrazed plots. Kalamees & Zobel (1997) reported 2345 seeds m$^{-2}$ in the permanent seed bank of a calcareous grassland in western Estonia and Davies & Waite (1998) about 5896 seeds m$^{-2}$ in calcareous grassland in the UK. It is immediately noticeable from the cases reported above that the highest seed densities have been reported from alvar grasslands in Estonia and Sweden.

We found that species richness and seed density in the seed bank decreased along a gradient of management history from original managed grassland to closed overgrown community. The same trend has generally been reported in similar studies (Donelan & Thompson 1980; Bakker et al. 1996; Davies & Waite 1998; Willems 2001; Jacquemyn et al. 2011), although a different pattern was reported from old chalk grassland in France (Dutoit & Alard 1995), where seed densities were higher in grassland abandoned 40 yr ago (5044 seeds m$^{-2}$) than in open grassland (1800 seeds m$^{-2}$).

There is an inverse relationship between seed size and seed longevity among the herbaceous grassland plants in northwest Europe (Thompson et al. 1993; Bekker et al. 1998; Hodkinson et al. 1998) and central Argentina (Funes et al. 1999). Species that produce small and light seeds tend to exhibit the greatest rates of seed bank persistence, while larger seed size increases the probability of seedling emergence under dense shade or deep burial (Leishman et al. 2000). Species found in the seed bank possessed on average three times smaller seeds than those in the vegetation. In continuously grazed sites the average seed mass of species present in seed bank samples was for the most part smaller than that in overgrown sites. This result is consistent with the high seed densities recorded in the soil seed bank of our grazed study sites. Species in the vegetation of 50-yr abandoned sites had significantly heavier seeds than those recorded in more open grassland sites. This result also follows the accepted generalization that species that prefer shaded, mesic and late-successional habitats tend to have larger seeds than those from open, arid, or early-successional habitats (Salisbury 1942; Gross 1984; Leishman et al. 2000).

Numerous studies have concluded that the soil seed bank has negligible potential to restore European seminatural grasslands (Bakker et al. 1996, 2002; Bekker et al. 1997; Davies & Waite 1998; Willems & Bik 1998; Bisteteau & Mahy 2005; Klimeš et al. 2010). While we found a significant decline in species richness and soil seed bank density during secondary succession, the seeds of several typical grassland species were still abundant in the soil of overgrown sites. The overall high species richness and high seed densities of typical grassland species in the soil seed bank of the overgrown grasslands studied here suggest that the soil seed bank can potentially play a more important role in the restoration of abandoned alvar grasslands than could be concluded from previous studies. The restoration of species-rich calcareous grassland communities can be successful in landscapes where the local species pool of appropriate species is still present. Consequently, suitable restoration management of Estonian overgrown alvar grasslands should consist of the removal of shrubs and trees and resumption of a grazing regime.

In areas under intensive agricultural land use the community species pool is likely to be critically impoverished, and the lack of typical grassland species can be an insurmountable problem in restoration practices. Several studies have addressed grassland restoration on ex-arable lands in agricultural landscapes (Graham & Hutchings 1988; Hutchings & Booth 1996; Pywell et al. 2002; Fagan et al. 2008). However it is hard to interpret the findings of these studies due to the high numbers of seeds from ruderal species present in an agricultural landscape and the sampling regimes employed in these studies (most samples were taken in autumn, winter or early spring, so the balance of species composition and frequency relations are likely to be heavily influenced by the transient seed bank).

Due to their dry and shallow soils, Estonian alvars have never been subjected to intensive agricultural practices. Abandoned alvar grasslands that have not entirely vanished may therefore retain a large species pool of previous open grassland species in the seed bank and a relatively small share of ruderal species. Hence, the restoration prospects of species-rich alvar grasslands are likely to be higher
compared to other types of grassland abandoned for similar time periods.

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References


**Supporting Information**

Additional supporting information may be found in the online version of this article:

**Appendix S1.** Frequencies ($n = 20$) of plant species in the above-ground vegetation and in the soil seed bank in different successional stages at Haavakannu. Species typical of dry alvar grassland communities are indicated with an asterisk.

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