Seed bank composition of open and overgrown calcareous grassland soils—a case study from Southern Belgium

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Abstract

The success of calcareous grassland recreation following abandonment depends to a large extent on the composition of the soil seed bank. We studied the species richness and composition of the seed bank along a chronosequence from well-developed calcareous grassland to scrub and forest vegetation, which had developed on calcareous grassland over the last 225 years. The seed bank density was highest in the calcareous grassland soils (930 seeds/m²). However, the seed bank was mainly composed of rather common species of nutrient poor grassland, which were poorly represented in the surface vegetation. There were no significant differences in soil seed bank density and species composition between the calcareous grassland and scrub vegetation that was less than 15 years old, largely because several grassland species had persisted in the scrub vegetation and were therefore able to replenish the soil seed bank. In contrast seed density and species richness declined significantly after 40 years of grassland abandonment. Indeed, forest soils had the lowest seed densities (214 seeds/m²) with only a few grassland species represented. This reflects the lack of grassland species in the field layer of the forest, and, therefore, the lack of seed production and seed bank replenishment. It is clear that recreation of calcareous grassland on long abandoned sites cannot rely on germination of target species from the seed bank alone. Even in the calcareous grassland soil, seeds of target species are not abundant as the majority produces transient seeds, which decay rapidly if they do not germinate immediately. Successful grassland recreation on such sites therefore may require seeds of target species to be introduced artificially (e.g. as seed mixtures, green hay, etc.). Alternatively, reinstatement of traditional practices, such as grazing or mowing, will increase the natural dispersal potential of these species, allowing population reestablishment in the long term.

Keywords: Festuco-Brometea; Grassland recreation; Seed bank; Seed longevity; Succession; Traditional management

1. Introduction

Calcareous grasslands represent some of the most species rich communities in Western Europe, containing a large diversity of higher plant species, mosses, lichens and invertebrates. However, the maintenance of this high diversity is dependent on grazing or mowing, which halts or deflects secondary succession away from scrub or high forest. For centuries, calcareous grasslands were traditionally used as extensive pasture, mainly for sheep production, although after 1900, and particularly from 1940 onwards, livestock grazing declined throughout Europe leading to the abandonment of low intensity grasslands over large areas (Poschlod et al., 1998; Willems and Bik, 1998). As a consequence large tracts of grassland reverted to scrub vegetation dominated by Crataegus monogyna, Juniperus communis, Prunus spinosa or Buxus sempervirens and further towards secondary forest of mainly Quercus robur and Fraxinus excelsior (Poschlod et al., 1998; Rosén and van der Maarel, 2000). In addition large areas were also planted with alien conifers (Maccherini and De Dominicis, 2003), ploughed up for agriculture or destroyed by urban development (Fuller, 1987). As a result of these land use changes the total area of calcareous grassland in Europe declined dramatically resulting in the loss of populations of typical calcareous grassland plant species (e.g. Pulsatilla vulgaris; Wells, 1968) many of which are now Red Listed (Stöcklin and Fischer, 1999; Willems, 2001).

One of the mechanisms which typically affect the viability of plant populations isolated in habitat fragments is a reduced seed exchange between populations. This lack of seed exchange can severely restrict gene-flow in some species thereby preventing populations from being ‘rescued’ from extinction by colonizing...
individuals from other fragments (Brown and Kodric-Brown, 1977). Furthermore poor seed exchange will reduce the chance of re-colonization of empty fragments after extinction (Hanski, 1999). Two processes in particular are likely to reduce seed exchange between grassland remnants. Firstly, the conversion of large areas of grassland to intensive agriculture means that the remnants become more spatially isolated within a ‘sea’ of hostile land-use (Thompson, 1994). Consequently, the probability of successful seed dispersal between the fragments by anemoo- or endozoochory is much reduced. Secondly, as a result of increased spatial isolation and reduced grassland size, remnant grassland fragments become much more difficult to graze by cattle and sheep, both of which are known to carry a significant amount of seeds of several plant species in their fur (Poschlod et al., 1998; Couvreur et al., 2004) thereby promoting seed dispersal between grassland fragments. Since sheep are no longer exchanged regularly between the grasslands, seed dispersal possibilities between grassland fragments become even more limited.

Over recent decades calcareous grasslands (Festuco-Brometalia) have received increased protection throughout Europe (Romao, 1997). This has led to policy initiatives in a number of countries intended to maintain and enhance the quality of the remaining grasslands as well as restore or recreate grasslands in areas where they were formerly present. The latter have included the reinstatement of grazing management on nature conservation sites following the removal of scrub and forest vegetation on overgrown sites (Rosén and van der Maarel, 2000). Recreation success on these sites depends on the availability of seeds of target species (Bakker et al., 1996a; Bakker and Berendse, 1999; Poschlod et al., 1998). Populations of target species can establish through seed dispersal from other grassland fragments or through germination of seeds present in the soil seed bank. Since spontaneous seed dispersal between fragments is unlikely to occur on spatially isolated fragments, recreation success will be mainly determined by the species composition of the seeds present in the soil. In stable grasslands, re-colonization of larger gaps is to a large extent based on germination from the soil seed bank (Kalamees and Zobel, 2002). Therefore, the importance of the seed bank will be even greater on sites where recreation management such as scrub or forest clearance causes large-scale disturbances. Knowledge of the seed bank composition is hence a crucial factor in the recreation process.

Several studies investigated the composition of the seed bank in abandoned calcareous grassland subject to spontaneous succession (Dutoit and Alard, 1995; Milberg, 1995; Bakker et al., 1996b; Davies and Waite, 1998; Pärtel et al., 1998; Willems and Bik, 1998; Rosén and van der Maarel, 2000). Most studies found a significant decline in overall species richness and seed density and in the number of target species during the course of succession thereby limiting the probability of successful recreation. Almost all calcareous grassland species produce short-lived (transient) seeds that stay viable in the soil for a limited time period. However, some studies found no significant differences in total seed density or species richness of the seed bank between grassland and forest (Dutoit and Allard, 1995; Milberg, 1995; Maccherini and De Dominicis, 2003). In these studies, it is likely that the open nature of the forest allowed the development of rich field layer vegetation that continuously replenished the seed bank.

In this study, we compared the species composition and seed density of the soil seed bank in a range of calcareous grassland, scrubland and forest, which had developed on former calcareous grassland in the Viroin valley in southern Belgium. The forests included both conifer plantations (Pinus sylvestris and Pinus nigra) and secondary deciduous forests up to 225 years old. Since, the field layer vegetation in these dense forests was virtually absent, we assumed that most seeds present in the soil were produced in former successional stages. Our aims were to answer the following research questions:

- How does total seed density and species composition of the soil seed bank change along a successional gradient with increasing time since grassland abandonment?
- How long do seeds of calcareous grassland species remain viable following abandonment?
- What is the probability of recreation success on former forest sites?

2. Material and methods

2.1. Study region

The study region is the Viroin Valley in southern Belgium. Geologically the region is dominated by calcareous hard sediments of Devonian and Carboniferous age. These consist of alternating soft schist stones in the valleys with harder calcareous rocks on the tops of hills (Goossens, 1984). The habitats which have developed on the shallow calcareous soils on these hills can, therefore, be considered as ‘natural islands’ in a landscape matrix of soils on schist weathering material. As elsewhere in Europe, the abandonment of grazing management since 1900 led to the disappearance of large areas of grassland in the region, which either underwent spontaneous succession towards B. sempervirens scrub or Q. robur forest. In addition many grasslands were transformed into conifer plantations (P. nigra or P. sylvestris). As a consequence the area of calcareous grassland declined and isolation between fragments increased. Phytosociologically the calcareous grasslands of the study region belong to the Festuco-Brometalia which has its main distribution in the Atlantic, central European and sub-Mediterranean region (Royer, 1991).

2.2. Data collection

Within the study area five existing calcareous grasslands were sampled to provide reference plant communities and compared to three sites, which had recently been abandoned (scrub vegetation less than 15 years old) and to seven forest sites abandoned between 40 and 225 years ago. The year of abandonment was deduced from seven historical land use maps, dating between 1775 and 2003 and distinguishing
between grassland, forest, arable land and built area. The forest sites included two conifer plantations, three deciduous forest sites and two mixed forests (planted conifers in combination with natural regeneration of deciduous forest species).

The seed bank experiment was carried out in September 2003. At each site, 10 1 × 1 m quadrats were selected at random and the soil was sampled 10 times from each quadrant with a 2.5 cm diameter soil auger. Due to the shallow nature of the soils samples were only taken to a depth of 5 cm. All 10 samples were then bulked from each quadrant, returned to the laboratory and processed using the methodology described by ter Heerdt et al. (1996). Each bulked sample was washed through a coarse (4 mm) and a fine (0.2 mm) sieve in order to remove both coarse and fine soil material, roots and vegetative parts. These concentrated samples were then spread out in a thin layer (2 mm) on 15 × 15 cm trays filled with sterilized potting soil. In addition, a thin layer (1 cm) of expanded clay granules was placed on the bottom of each tray in order to prevent waterlogging. The trays were placed on shelves in a greenhouse with a light regime of 8 h darkness and 16 h light and kept moist by capillarity. The light was provided by a combination of neon tubes, imitating full sunlight conditions. The temperature was not controlled and varied between 14–25°C. Five control trays filled with sterilized potting soil were also placed randomly on the shelves to test for contamination. No seedlings germinated in the control trays.

Seedlings were identified as soon as possible after germination, counted and removed or, if they could not be identified immediately, transplanted in pots to allow further growth. After 8 weeks, when no further seedlings emerged, the trays were placed in a refrigerated room at a temperature of 2°C for three weeks. After this period, they were returned to the shelves for another 8 weeks. Only a few seedlings germinated after the chilling period.

For the five grassland sites vegetation data were also collected during the summer of 2003. In each site a variable number (3–7) of randomly positioned 1 × 1 m quadrats was sampled (26 in total). In each quadrat, the cover of all species was estimated using 10% classes, except for species with a cover lower than 10%, which were recorded as 1, 3 or 5%. Nomenclature for vascular plants follows De Langhe et al. (1988).

2.3. Data analysis

For each site the total number of species found in the seed bank and the average seed density was calculated. Since, the distribution of these data was not normal, a non-parametric statistical test was used (Siegel and Castellan, 1988). Values were correlated with time since grassland abandonment using a Spearman rank correlation coefficient. Differences between grassland, scrub and forest were tested using the Kruskal–Wallis test with multiple comparisons. For species with at least five seedlings, individual seed density was also correlated with time since grassland abandonment using a Spearman rank correlation coefficient. Differences in individual seed density

between grassland, scrub and forest were also tested with the Kruskal–Wallis test.

Species which emerged from the seed bank were divided into five ecological groupings (i.e. species of calcareous grasslands, species of nutrient rich grasslands on acid to neutral soil, species of nutrient poor grasslands on acid to neutral soil, species of forest, edges and clearings and species of arable land and pioneer habitat) based on Cosijn et al. (1994); De Langhe et al. (1988). For each seed bank sample, the absolute seed density, the number of species and the relative contribution to seed density of each ecological grouping was calculated. Similarly, for each vegetation quadrat the relative contribution of each ecological grouping to overall abundance was also determined. To avoid problems with pseudoreplication the obtained values were then averaged over all quadrats of a site resulting in 15 values for the seed bank and five for the grassland vegetation.

The values for the seed bank were then correlated with time since grassland abandonment using a Spearman rank correlation coefficient. Differences in the relative contribution of each ecological grouping between grassland vegetation, grassland seed bank, scrub seed bank and forest seed bank were tested with a Kruskal–Wallis test with multiple comparisons.

For each vegetation quadrat and seed bank sample, the average seed longevity index, weighted by cover or seed density, was also calculated, based on the database of Thompson et al. (1997). The seed longevity index ranges between 0 and 1 and is a measure for the probability that a seed remains persistent in the soil (Bekker et al., 1998). Similarly, we also calculated the weighted average Ellenberg indicator values for light, moisture, pH and nitrogen for each vegetation quadrat and seed bank sample (Ellenberg et al., 1991). The obtained values were also averaged for each site.

The average values for the seed bank were correlated with time since grassland abandonment using a Spearman rank correlation coefficient. Differences between the average seed longevity and indicator values between grassland vegetation and seed bank of grassland, scrub and forest were tested with a Kruskal–Wallis test with multiple comparisons.

SPSS 11.0 was used for all statistical analysis (SPSS, 2001).

3. Results

A total of 419 seedlings of 42 plant species germinated from the soil samples, corresponding to a seed density of 559 seeds/m² over all sites. Of these, 45 seedlings died before they could be identified and only 87 seedlings germinated after the chilling period. The most abundant species in the seed bank were Hypericum perforatum (33% of the seedlings), Potentilla verna (11%), Helianthenum nummularium (6%) and Arenaria serpyllifolia (5%) (Table 1). In contrast 102 species were recorded in the surface grassland quadrats, of which 31 were also found in the seed bank. Therefore, 11 species that germinated from the soil samples were not present in the vegetation quadrats.

Both total seed density and number of species significantly decreased with increasing time since abandonment
Seed density ranged from an average of 930 seeds/m² in grassland soils to over 760 seeds/m² in scrub vegetation to 214 seeds/m² in forest. The density of six species significantly decreased with time since grassland abandonment (Cerastium fontanum, Galium verum, H. nummularium, H. perforatum, P. verna, Urtica dioica) and only the density of Fragaria vesca increased, although this result was only marginally significant (Table 1). Five species had significantly different seed densities between the vegetation types, three of them having the highest seed density in the grassland (C. fontanum, H. nummularium and P. verna) whereas H. perforatum and U. dioica had the highest seed density in the scrub vegetation. In addition, there was a significant decline in the number of seeds of species with age of abandonment for species associated with calcareous grassland (Rs = 0.82, p < 0.001), nutrient rich grasslands (Rs = 0.60, p = 0.019) and nutrient poor grasslands (Rs = 0.73, p = 0.002). Total seed density and seed density for these three ecological groupings were also significantly different between grassland and forest sites (Fig. 2).

The average seed longevity or indicator values of the seed bank did not change significantly with time since grassland abandonment (Table 2), but the seed longevity index of grassland vegetation was always significantly lower than the one of the seed bank. The indicator value for pH was lower and the indicator value for nitrogen was higher for the forest soil seed bank than for the grassland vegetation. For the seed bank of grassland and scrubs, all indicator values were similar to those for grassland vegetation. The proportion of calcareous grassland species in the seed bank decreased with time since abandonment while the proportion of forest species increased (Table 2). Correspondingly, the proportion of calcareous grassland and of forest species was significantly different between the forest soil seed bank and the grassland vegetation.

Only species with more than five seedlings emerging are given. The correlation between seed density and time since abandonment is given and significant differences between the seed densities in each of the vegetation types are indicated with different letters (Kruskal–Wallis test). (*), 0.05 < p < 0.1; *, 0.01 < p < 0.05; **, 0.001 < p < 0.01; ***, p < 0.001.

Fig. 1. Seed density (a) and species richness (b) in the soil seed bank as a function of time since abandonment.

Table 1
The frequency and cover of species in the surface vegetation (n=26), and average seed bank density (seeds/m²) in the soil of grassland, scrub and forest sites

| Frequency in grassland vegetation | Cover in grassland vegetation | Correlation seed density vs. time since abandonment | Seed density
|---------------------------------|-------------------------------|-----------------------------------------------|-------------|
| Anthyllis vulneraria            | 0.35                          | 1.1 Ns                                        | Grassland  
| Arenaria serpyllifolia          | 0.08                          | 1 −0.47(*)                                    | Scrub       
| Betula pendula                  | 0                            | −Ns                                           | Forest      
| Brachypodium pinnatum           | 0.96                          | 33.6 −0.47(*)                                 |            
| Cerastium fontanum              | 0.08                          | 1.1 −0.60*                                    |            
| Fragaria vesca                  | 0                            | −Ns                                           |            
| Galium verum                    | 0.27                          | 2.1 −0.55*                                    |            
| Helianthenum nummularium        | 1                             | 7.3 −0.57*                                    |            
| Hypericum perforatum            | 0.73                          | 1 −0.71**                                     |            
| Lotus corniculatus              | 0.73                          | 1.6 −Ns                                       |            
| Medicago lupulina               | 0.12                          | 6.0 −Ns                                       |            
| Potentilla reptans              | 0                             | −Ns                                           |            
| Potentilla verna                | 0.88                          | 8.1 −0.66**                                   |            
| Scabiosa columbaria             | 0.62                          | 2.4 −Ns                                       |            
| Senecio jacobea                 | 0.04                          | 1 −Ns                                         |            
| Teucrium chamaedrys             | 1                             | 3.1 −Ns                                       |            
| Thymus pulegioides              | 0.15                          | 1.3 −0.47(*)                                  |            
| Urtica dioica                   | 0                             | −0.55*                                        |            

4. Discussion

4.1. Density and composition of the seed bank under calcareous grassland vegetation

The average seed density found in the calcareous grassland soils (930 seeds/m²) in this study is relatively low in comparison to other studies (Akinola et al., 1998: 2549 seeds/m², Kalamees and Zobel, 2002: 2362 seeds/m², but see Willems and Bik, 1998: 770 seeds/m²). This can be partly explained by the limited soil depth that was sampled although one would have expected that the rocky substrate encountered in this study might act as a barrier preventing seeds from moving deeper into the soil and, therefore, causing them to accumulate at a shallow (<5 cm) depth. However, also in deeper soils, only a low proportion of the seeds move deeper than 5 cm into the soil and seed density strongly declines deeper than 5 cm (Bekker et al., 1998). Alternatively, low seed density may be due to low seed production as a result of high grazing pressure, or to a high degree of seed predation on the sites.

Species with the highest seed bank densities in the grassland sites were rather common, non-target species typical of nutrient poor grassland, such as *H. perforatum* or *A. serpyllifolia*, although these species are not abundantly present in the vegetation. This result is not surprising. Species of nutrient poor grasslands or heathlands generally produce longer-lived seeds, which stay viable in the soil for a long period (Pywell et al., 2000).
et al., 2002; Walker et al., 2002; Bossuyt and Hermy, 2003). The low abundance of these species in the grassland vegetation, therefore, suggests that only a few individuals would have been needed to produce numerous persistent seeds that accumulated in the soil seed bank.

In contrast, species with high indicator values for pH (i.e. typical calcareous grassland species) had a rather low density in the seed bank but a very high cover value in the vegetation (86% of the total cover). Moreover, seeds of 71 species present in the calcareous grassland vegetation were not detected in the soil. This poor representation of typical calcareous grassland species has been found in a wide range of other studies (Willems, 1995; Bakker et al., 1996b; Davies and Waite, 1998; Pärtel et al., 1998; Rosén and van der Maarel, 2000). Undoubtedly, this is partly due to the inherent sampling limitations of seed bank studies. Since, sample processing is time consuming, the number of samples taken is always rather small in comparison with the area covered. However, the area sampled in this study (0.005 m²/1 m² plot with 10 plots per site) is comparable to other studies (Bossuyt and Hermy, 2004), so we can assume that the absence of seeds of many calcareous grassland species is a reflection of the short-lived, transient nature of the seed banks of these species.

4.2. Changes in seed bank density and composition after grassland abandonment

In this study, there was no decline in seed density or species richness under the scrub vegetation and the species composition of the seed bank was rather similar to that of the calcareous grassland. This means that calcareous grassland species and/or their seeds remain persistent under the developing scrub vegetation for at least 15 years whilst less light-demanding or competitive species, such as *U. dioica*, only gradually establish and replenish the soil seed bank. Milberg (1995) and Bakker et al. (1996a) found no significant differences in seed density and species richness between grassland and spontaneously established forest or scrub 18 years after grazing abandonment, although the latter authors found significantly fewer species and seed densities after 55 years succession towards scrub vegetation. Also Maccherini and De Dominicis (2003) found no decline in species richness for 25 years old coniferous plantations on calcareous grassland. Dutoit and Allard (1995) found the highest seed densities in grassland abandoned 40 years ago. It is most likely that the amount of light that reaches the soil in the scrub or forest vegetation is responsible for these findings. In this case, a higher number of species, continuously replenishing the seed bank, will be able to survive and set seed under the scrub or tree vegetation. This situation is comparable to forest edges, which have a higher seed density than the forest interior where less species are able to grow or at least to set seed due to light stress (De Vlaeminck et al., 2005). Here also, grassland species can, due to the open canopy cover of the scrub vegetation, persist up to 15 years after grazing abandonment, which is reflected in the soil seed bank composition.

Although the scrub vegetation had a high seed bank density and species richness, these values strongly decreased with increasing time since abandonment. For example, the seed density of forest sites abandoned 40 years ago was well below the seed density in the grassland sites, and further decreased with increased forest age. This is likely to be the result from a combination of seed decay and a lack of input of new seeds. The field layer vegetation in the forests is very scarce, so that almost no seeds are currently produced. Soils in deciduous forest seem to contain a larger amount of seeds than soils in coniferous forest of the same age, which may also be due to a higher light level at the soil. However, because of the low number (4) of equally aged sites in this study it is impossible to prove this statistically. Besides the sharp decrease in density, there was also a shift in species composition of the seed bank. The seed bank became less similar to the vegetation or the seed bank of calcareous grassland with time. The proportion of seeds of calcareous grassland species decreased with time since abandonment, while the proportion of seeds of species of forest, edges and clearing increased. Correspondingly, the forest seed bank had a higher relative density of species with a low indicator value for pH and a high indicator value for nitrogen.

Seeds of calcareous grassland species seem hence to decline more rapidly than seeds of other species, while seeds of forest species, mainly *Betula pendula*, *F. vesca* and *Hyperium montanum*, became incorporated in the soil, although at very low densities (Bakker et al., 1996a). Most forest species indeed do not produce a large amount of persistent seeds (Bossuyt and Hermy, 2001; Bossuyt and Hermy, 2003). Some calcareous grassland species (e.g. *Carex caryophylla*, *H. nummularium*, *Lotus corniculatus*, *Medicago lupulina* and *P. verna*) were still present in the forest soil, but at lower densities than in the grassland seed bank. Seeds of *Carex flacca* and *Scabiosa columbaria* were only found in the soil of forest planted less than 40 years ago, and were absent in older forests. *L. corniculatus* (Milberg, 1995), *C. caryophylla* and *M. lupulina* (Pärtel et al., 1996; Poschlod et al., 1998) are also known to produce long-term, persistent seeds.

4.3. Implications for calcareous grassland recreation

It is clear from the results that recreation of species rich calcareous grassland after cutting of the forest trees cannot rely on germination from the seed bank alone. There were very few seeds of a few calcareous grassland species present in all sites, and only at very low densities. Taking into account the isolation of the sites, natural dispersal and colonisation from other grassland fragments is very unlikely unless traditional management practices, which might transport propagules between sites, are reinstated or restored (e.g. grazing and mowing). Therefore, when such grasslands are to be restored, other possibilities will need to be considered, including the artificial introduction of species as seed mixtures, plug-plants, green hay or turf transplants (Rosén and van der Maarel, 2000; Walker et al., 2004) combined with the re-introduction of traditional practices, such as sheep grazing and mowing, which
transport propagules between grassland fragments (Poschlod et al., 1998).

4.4. Conclusions

The results of this study indicate that:

1. The seed bank in the soil of calcareous grasslands was largely composed of common species of nutrient poor grasslands. Typical calcareous grassland species, abundantly present in the vegetation, were poorly represented as seeds in the soil.

2. Species composition of the seed bank under grassland that was abandoned 15 years ago was similar to the species composition in the soil of grassland. In contrast, seed density in the soil and similarity in species composition with the seed bank under grassland strongly decreased after 40 years of grassland abandonment, indicating that only few calcareous grassland species produce long term persistent seeds.

3. This means that recreation of calcareous grassland on sites abandoned more than 15 years ago cannot rely on germination from the seed bank.

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