Vegetation establishment after restoration measures in calcareous sandy grasslands

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ABSTRACT

Calcareous sandy grasslands are among the most threatened habitats in Europe, primarily because of abandonment, acidification, eutrophication and fragmentation. Much of the biodiversity found in calcareous sandy grasslands depend on the availability of calcareous sand in the topsoil layer. As this sand is naturally depleted of $CaCO_3$ heavy disturbances such as erosion or use of military vehicles is essential to preserve high species richness and avoiding degeneration of the habitat. For calcareous sandy grasslands different methods have proven successful in restoration and management, such as grazing, liming, topsoil removal and soil perturbation. The aim with this study was to test the hypothesis that the restoration methods topsoil removal and soil perturbation can restore calcareous sandy grasslands. An inventory of the vegetation composition and collection of soil samples were carried out in eight locations in eastern Scania, all treated with topsoil removal or soil perturbation. The treatments resulted in significantly higher pH and partly lower levels of nutrients compared to control plots. Species richness and species diversity were higher in control plots, whereas number and proportion of target species was higher in plots treated with topsoil removal. Since the main restoration target is the specialist species, the rather drastic treatment of topsoil removal and soil perturbation described in this study could be considered a success due to the increase of target species and thereby altering the habitat towards calcareous sandy grasslands. The treatment reduces plant-available N, increases pH and creates disturbance. From this study it seems that high pH might be the most important factor to establish target species in calcareous calcareous sandy grasslands in southeast Sweden. This study strengthens the perspective that restoration of sandy calcareous grasslands is a complicated long-term process, which generally includes restoration of both biotic and abiotic conditions.

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INTRODUCTION

Xeric sand calcareous grasslands are one of the most threatened habitats in Europe. The habitat is considered a priority habitat by Natura 2000 under the Habitat Directive and by Naturvårdsverket (Natura 2000 code 6120, Naturvårdsverket 2011). As with many other ecosystems in Europe the habitat is threatened by changing land use such as abandonment and agricultural intensification, leading to acidification and eutrophication (Bakker & Berendse 1999, Poschlod et al. 2005, Olsson & Ödman 2011). Furthermore, most of the remaining areas are strongly fragmented, often to areas so small that it poses an additional threat to the rarest and most specialised species (Kiehl & Pfadenhauer 2007, Olsson & Ödman 2011).

Calcareous sandy grasslands depend on the availability of calcareous-rich sand in the topsoil layer. However this sand is naturally depleted of $CaCO_3$ and therefore the habitat depends on regular soil disturbance to bring up new calcareous-rich sand to the surface (Naturvårdsverket 2011, Olsson 1994). Soil disturbance affects the abiotic properties of the soil, but it also have a number of effects on plant traits, physiological attributes and life-cycle length (Schnoor & Olsson 2010). Disturbance also decreases competitive pressure (Schnoor & Olsson 2010). Therefore many of the threatened characteristic plant species depends on regular soil disturbance for many reasons (Olsson & Ödman 2011). Disturbances such as grazing, mowing, rabbits or wind and water erosion is consequently essential to preserve high species richness and endangered plant species (Schnoor & Olsson 2010, Olsson & Ödman 2012). Otherwise the habitat will degenerate due to lowered pH and concentration of humus (Olsson 1994) and become other habitats such as grey dunes or inland dune grasslands (Naturvårdsverket 2011). In some cases grazing may not be enough to bring up calcareous-rich sand to the surface and further disturbance such as military training or natural land erosion in slopes is needed (Naturvårdsverket 2011).

The threats against the calcareous sandy grasslands remain and conservation and restoration measures are needed (Naturvårdsverket 2011, Olsson 2009). It is important to reduce the fragmentation by expanding the existing areas and enhance the connectivity, for example by restoring degraded nearby sites (Eichberg et al. 2010, Smits et al. 2008). Restoration of habitats after abandonment or intensively managed agricultural systems can be difficult and does not always restore species richness and composition. Studies show that sites with a short history of agricultural intensification have the greatest potential for restoration and have a high conservation value (Bakker & Berendse 1999). The choice between different management treatments have been shown to have major impact on the success of restoring habitats (Poschlod et al. 2005). For calcareous sandy grasslands different methods have proven successful in restoration and management, such as grazing, liming, topsoil removal, soil perturbation and monitoring the vegetation, levels of nutrients and pH (Buisson et al. 2006, Kiehl & Pfadenhauer 2007, Olsson 2009, Stevens et al. 2011).

Aims

The aim was to test the hypothesis that topsoil removal and soil perturbation are good methods to restore calcareous sandy grasslands, thereby serving the Natura 2000 goals for xeric sand calcareous grasslands. This will be done by:

- Investigate the vegetation composition of restored sites and to measure the presence of characteristic species in these sites. If the succession is successful, the results should show a larger presence of characteristic species in treated plots than control plots and thereby indicate an improvement of the habitat due to restoration measures.
- Compare soil characteristics, such as levels of phosphorus, nitrogen and pH, between treated plots and non-treated control plots. Increased pH and reduced nutrient availability will be considered as improved habitat quality.

Background

Calcareous sandy grasslands are mainly located in the steppe regions of south Russia, close to the Black Sea (Mattiasson 1974). The Swedish areas are an isolated northwestern outpost, and therefore hold a high scientific and conservation value (Mattiasson 1974). In Sweden, calcareous sandy grasslands occur fragmentary in the eastern part of Scania and in some small areas on Öland and is locally called sand steppe (Tyler 2003, Andersson 1950, Olsson 1994, Sjörs 1967). The habitat exhibits a unique composition of rare species under Swedish conditions (Naturvårdsverket 2011, Olsson 2009), with approximately 70 red-listed species of animals, plants and bryophytes (Tyler 2003, Olofsson 2006). The total area of xeric sand calcareous grasslands in Sweden is difficult to determine, but is probably less than 50 ha (Mattiasson 1974, Olsson 1994, Tyler 2003). Most of the areas in Scania are situated in the municipalities of Kristianstad, Simrishamn and Tommelilla (Tyler 2003, Andersson 1950). Calcareous sandy grasslands are restricted to inland dunes and glaciofluvial deposits (Mattiasson 1974, Olsson & Ödman 2011), and exist as small fragments remaining from an elderly agricultural landscape where farming was only possible in combination with intermediate periods of fallow (Naturvårdsverket 2011). Xeric sand calcareous grasslands occur on warm sun-exposed sites with well-drained, calcareous nutrient-poor sandy soil (Mattiasson 1974, Olsson 1994, Olsson & Ödman 2011). The vegetation cover is discontinuous and low, and the perennial grass *Koeleria glauca* is the key indicator species (Olsson 1994, Olsson & Ödman 2011). Other common plant species are *Dianthus arenarius* ssp. *arenarius* (endemic to the Baltic region), *Anthericum liliago*, *Phelum arenarium*, *Alyssum alyssoides*, *Satureja acinos*, *Androcaseae septentrionalis*, *Festuca polesica* and *Silene conica* (Olsson 1994, Tyler 2003, Naturvårdsverket 2011, Olsson & Ödman 2011). Bush- and tree cover should not exceed 10 % (Naturvårdsverket 2011).

Depending on the vegetation cover, composition of bryophytes and number of annual species, calcareous sandy grasslands can be divided into three stages; initial stage, optimal stage and degenerative stage (Mattiasson 1974, Olsson 1994, Naturvårdsverket 2011). From a conservation point of view the intial- and optimal stage is the most desirable (Mattiasson 1974). The vegetation in the initial stage is sparse and moderate, including large areas with no vegetation at all (Mattiasson 1974). The optimal stage is characterized by a species rich and often colorful vegetation cover, not exciding 50 % coverage (Mattiasson 1974). The degeneration stage is easy to identify with a closed/nearly closed vegetation cover, dominated by lichens (Mattiasson 1974). Today most of the calcareous sandy grasslands in Sweden are degenerated and in need of rather drastic restoration measures (Naturvårdsverket 2011, Olsson & Ödman 2011, Olsson 1994).

The main reasons for this are acidification, eutrophication, fragmentation, overgrowth and reforestation (Naturvårdsverket 2011, Olsson & Ödman 2011). Typical species for degenerated calcareous sandy grasslands are *Arrhenaterum elatius* in the case of eutrophication and *Corenophorus canescence* in the case of acidification (Mattiasson 1974, Mårtensson & Olsson 2010).

Restoring the soil chemistry is a prerequisite for specialist plant species to be favored. Since many potential restoration sites are located on formerly arable fields with high nutrient content, the lowering of available soil nutrients is usually necessary (Eichberg et al. 2010). Removal of nutrients is important in restoration of species-rich vegetation because most target plant species have low abilities to compete for light in nutrient-rich substrates (Roem & Berendse 2000, Smits et al. 2008, Eichberg et al. 2010). Mowing and removal of the hay is one way to reduce nutrient levels without removal of the upper soil layer and thereby conserving the present vegetation (Smits et al. 2008). However, this method is slow, and has proven successful in reducing levels of nitrogen in less than ten years, but does not seem to lower the levels of phosphorus (Smits et al. 2008).

Soil pH has a wide and complex effect on all processes and organisms in the soil, such as solubility of toxic metals and nutrient availability (Olsson et al. 2009, Stevens et al. 2011). Therefore a shift in pH can result in changes of species composition and richness (Johnston et al. 1986, Houdjik et al. 1993, Stevens et al. 2011). Reduction of pH can be caused by many factors, such as acids in precipitation, loss of cations through ion exchange, plant uptake and nitrification (Johnston et al. 1986, Stevens et al. 2011). Sandy grasslands in calcareous areas in Sweden show an exceedingly high variation in soil pH over short distances, with either alkaline or strongly acidic pH (Olsson et al. 2009). Intermediate pH in sandy soils seems to be rare, probably due to the very low cation buffering capacity of sandy soils once the lime has been depleted (Johnston et al. 1986, Olsson et al. 2009). These differences in pH results in a high diversity within the habitat (Olsson et al. 2009). It seems that the highest species richness in calcareous sandy grasslands is found within pH level 5-8.5 and that a wide range of pH within the habitat is optimal (Roem & Berendse 2000, Critchley et al. 2002, Mårtensson & Olsson 2010, Olsson et al. 2009). However, low levels of pH due to lack of disturbance and depletion of lime results in degeneration of the habitat (Johnston et al. 1986, Olsson et al. 2009). Often the $CaCO_3$ is depleted to a depth of 30 cm or more, requiring rather drastic restoration measures to restore optimal conditions (Olsson & Ödman 2011, Olsson et al. 2009).

Topsoil removal and soil perturbation is done in order to restore a lime-rich, nutrient-poor habitat and induce vegetation succession towards calcareous sandy grasslands (Stevens et al. 2011, Kiehl & Pfadenhauer 2007, Buisson et al. 2006, Olsson & Ödman 2011). However, removal of the topsoil can result in very low levels of nitrogen, causing succession to be slow (Roem & Berendse 2000, Olsson 2009). Liming can be a good complement to topsoil removal and soil perturbation (Olsson 2009). By spreading lime-rich sand around mechanically restored areas, a larger area with high heterogeneity is created (Olsson 2009). However, even after restoring appropriate soil conditions, establishment of target species is not always successful due to impoverished seed banks and limited dispersal range (Bakker & Berendse 1999, Kiehl & Pfadenhauer 2007). Transferring of hay or other sources of seed has proven successful (Kiehl & Pfadenhauer 2007, Eichberg et al. 2010). It is also important to point out that the well-studied soil chemistry of the optimal stage of calcareous sandy grasslands may not reflect the conditions required for the regeneration/initial stage since the niches may differ (Olsson & Ödman 2011). Therefore it can take some time for specific species or vegetation types to establish and the timing of disturbance events is especially important when the target species has a short-term seed bank (Olsson & Ödman 2012).

Management measures such as grazing and mowing becomes increasingly important over time after restoration in order to prevent restored areas from degenerating (Eichberg et al. 2010, Kiehl & Pfadenhauer 2007). Initially after restoration the grazing need to be cautious since plants growing in sand are loosely rooted (Olsson 2009). Mosses and fungi are benefited from relatively heavy grazing, whereas some plant species benefit from more gentle grazing (Olsson 2009). Grazing by horses has proven successful since horses also create disturbance by kicking in the ground (Olsson 2009).

METHODS

Research area

An inventory of the vegetation composition and collection of soil samples were carried out in eight locations, in the areas of Everöd and Ripa, located in the municipality of Kristianstad in eastern Scania (Table 1).

Table 1. Research areas and experimental design. Top soil removal was performed as the experimental treatment 2006 at the Everöd site and soil perturbation in 2011 at the Ripa site.

Area	Topsoil removal/soil perturbation	Liming	Control
Everöd	3 plots	0 plots	3 plots
Ripa	5 plots	5 plots	5 plots

West of the village of Åhus, a large area of calcareous sandy grasslands and fields are called Ripa and Horna sandar. The Everöd area consists of calcareous sandy grasslands around Kristianstad airport. The area holds important areas of the threatened xeric sand calcareous grassland (Natura 2000 code 6120), locally called sand steppe. The habitat in the area is mostly degenerated, but in particular around Everöd, smaller patches of species rich sand steppe remains (Olsson & Ödman 2012). The sandy soil is a result of redeposited wave washed sand during the end of the last ice-age (Olsson & Ödman 2012, Kindström 2009). The sandy soil in the area is more or less calcareous due to the lime-rich underlying bedrock (Mattiasson 1974, Kindström 2009).

These areas have been farmed by humans since the Bronze Age, but because of the dry, nutrient poor soil, farming was only possible in combination with intermediate periods of fallow and grazing by animals (Olofsson 2006, Naturvårdsverket 2011). This special way of farming has continued into modern times and created an elderly, more open landscape, with a unique composition of vegetation and wildlife, including many rare plants, insects and birds (Olofsson 2006, Kindström 2009). The modernization of agriculture in the sixties and increased forestation, to try to reduce drifting sand, has made the landscape less open than it used to be (Olofsson 2006). However, this area is still in many ways a good representation of how the Kristianstad plain used to look (Olofsson 2006). Today agriculture is the dominating land use, with vegetables, potatoes and sugar beets being the dominant crops (Kindström 2009). Large parts are pastures and some parts are pine plantation (Kindström 2009).

Experimental design

At all eight sites non-treated control plots (10 x 10 m) were established 1 m away from the treated areas (Fig. 1). Topsoil was removed using an excavator and soil perturbation was done using a backhoe loader (Appendix A, Fig. 6). Adjacent to each soil perturbation plot in Ripa, an area of ca 100 m^2 was treated by spreading of lime-rich sand (Fig. 1). Specific information about each location can be found in Table 2.

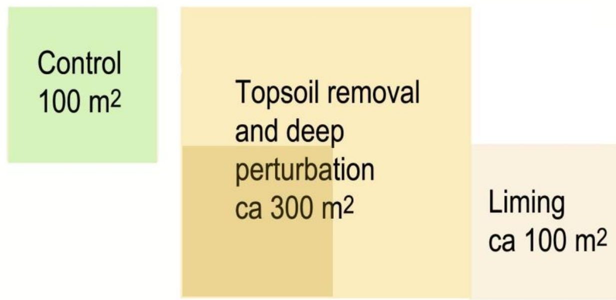

Fig.1 Outline of experimental design in Ripa. The control plot was situated 1 m away from the topsoil removal plot and always on the opposite side from the limed plot. A 100 m^2 plot was established within each treated plot for vegetation analysis in order to have equal sized plots for each of the three treatments. The same method was used in Everöd, but without the limed areas.

Everöd 1-2

Everöd 1 was situated within the Everöd airport area and in close proximity to areas of slightly degenerated sand steppe. Everöd 2 was situated 100 m away, in a pasture just outside the airport area. The vegetation was Fennoscadian lowland species-rich, dry to mesic grassland (Natura 2000 code 6270). Everöd 1 was managed by mowing and harrowing. Everöd 2 was a former pine plantation, managed by occasional low-intensity grazing (Appendix A, Fig. 7a).

Everöd 3

This site was situated 3 km SSE of Everöd 1 and 2. The vegetation was Fennoscadian lowland species-rich, dry to mesic grassland (Natura 2000 code 6270), adjacent to areas with sand steppe. The area was not being grazed, but subject to occasional disturbance by bicycles and motor cycles (Appendix A, Fig. 7b).

Ripa 1-3

Ripa 1-3 were located on the Ripa sandar pastures, ca 2 km W of the village Ripa and the vegetation were semi-natural dry grasslands and scrubland facies on calcareous substrates (Natura 2000 code 6210). All three locations at Ripa sandar were no longer being cultivated and were grazed by cattle (Appendix A, Fig. 8a and b).

Ripa 4

This site was situated on the Älleköpinge pasture, ca 2 km N of the village Ripa. Here the vegetation was a semi-natural dry grasslands and scrubland facies on calcareous substrates (Natura 2000 code 6210), with some elements of xeric sand calcareous grasslands (Natura 2000 code 6120). Ripa 4 was no longer being cultivated and was grazed by cattle (Appendix A, Fig. 9a).

Ripa 5
This site was located in a pasture ca 6 km NNE of the village Ripa. The vegetation was semi-natural dry grasslands and scrubland facies on calcareous substrates (Natura 2000 code 6210), with some elements of xeric sand calcareous grasslands (Natura 2000 code 6120). Ripa 5 was no longer being cultivated and was grazed by cattle. The area was at the time of the inventory intended for nature preservation, education and recreation (Kindström 2009) (Appendix A, Fig. 9b).

Table 2. Information about location and treatments for each of the eight experimental sites. At Everöd 3 both topsoil removal and soil perturbation was performed as experimental treatment.

Area	Year of treatment	Size	RT90 Coordinates	Liming	Topsoil depth	Perturbation depth
Everöd 1	2006	700 m^2	X: 6201754 Y: 1392804	No	0.5 m	No
Everöd 2	2006	182 m^2	X: 6201865 Y: 1392825	No	0.5 m	No
Everöd 3	2006	208 m^2	X: 6198852 Y: 1393859	No	0.3 m	0.3 m
Ripa 1	2011	380 m^2	X: 6199212 Y: 1400489	Yes	No	0.4 m
Ripa 2	2011	380 m^2	X: 6199197 Y: 1400559	Yes	No	0.4 m
Ripa 3	2011	280 m^2	X: 6199103 Y: 1400665	Yes	No	0.4 m
Ripa 4	2011	272 m^2	x: 6200904 y: 1401682	Yes	No	0.4 m
Ripa 5	2011	340 m^2	x: 6200912 y: 1402858	Yes	No	0.4 m

Vegetation analyses

Vegetation inventory was carried out in the end of May and the first half of June 2012. All plant and bryophytes species present in each plot was recorded. Bryophytes were determined only to the genus level to simplify the inventory. Additionally the total number of individuals for all target species was recorded in all treated plots (Table 6). Which species to be considered as target species was determined in accordance with earlier studies made by Olsson (1994), Tyler (2003) and Naturvårdsverket (2011).

To make an inventory of the vegetation composition, squares (size 0.25 m^2), divided in 25 smaller squares, were placed 10 times in a systematic order along lines across each plot (Appendix A, Fig. 10). The presence/absence of each species in the small squares was recorded, thereby getting a frequency of 0-25 for each species. In this inventory a 100 m^2 plot was established within the treated plots in order to equal the size of the control and limed plots (Fig. 1).

Soil sampling and analyses

The topsoil (0-10 cm) in all sites, five subsamples per plot, was sampled in late May and early June 2012. Samples were collected using a soil corer (∅ 3 cm). The samples were stored at -20° C until analysis.

To measure pH, 10 g of soil was dissolved in 50 ml distilled water on a rotator for 2 h. After 24 h sedimentation the pH was measured electrometrically in the supernatants.
Measuring extractable phosphate-P was done using Bray 1 extraction (Olsson & Ödman 2011), followed by flow injection analysis (FIA).

The exchangeable nitrate-N and ammonium-N were determined by extraction of 20 g soil with 100 ml 0.1 M $BaCl_2$ for 2 h on a rotator, followed by flow injection analysis (FIA).

Statistical analyses

The vegetation characteristics and soil chemical properties of Ripa 1-5 were analyzed with a one-way ANOVA, followed by Tukey's posthoc test to reveal differences between the three types of treatment. For the Everöd sites a paired t-test was used. For these tests the IBM SPSS Statistics 20 software was used.

Species richness was calculated on two scales; as average number of species per 0.25 m^2, which equals the size of the inventory squares, and as number of species per plot.

Species diversity was also calculated on two scales, per 0.25 m^2 and per plot (= in ten 0.25 m^2 squares), using the Shannon-Wiener index.

Relations between soil chemical properties and vegetation characteristics were tested using linear regression, using IBM SPSS Statistics 20 software.

The correlation between pH and the concentration of NH_4^+ was tested using the Pearson test, using IBM SPSS Statistics 20 software.

The vegetation composition of Ripa and Everöd was analyzed with correspondence analysis, CA, using CANOCO 4.5 software.

RESULTS

Soil chemistry

Topsoil removal and soil perturbation plots had higher pH than control plots (Tables 3 and 4). The limed plots also had higher pH than control plots (Table 4), but did not differ from soil perturbation plots.

The concentration of NH_4^+ and NO_3^- differed between areas and treatment (Table 3 and 4). In Everöd there were no significant difference between treated and control plots, for neither NH_4^+ nor NO_3^-. In Ripa the soil perturbation treated plots had significantly lower levels of NH_4^+ than both control and limed plots, but showed no significant difference in the levels of NO_3^-. The limed plots had higher levels of NO_3^- than control plots, but did not differ from soil perturbation plots.

No significant difference in the amount of extractable P could be found between treated and control plots using the Bray 1 method, neither in Everöd nor Ripa (Tables 3 and 4).

Table 3. Mean values ± SE (n = 3) of vegetation characteristics, nutrient levels and pH for Everöd, as well as p-values from paired t-test.

		Everöd		
		Control	Topsoil removal	P
Large scale	Species richness (no/plot)	30±3.1	29±0.58	0.742
	Target species (no/plot)	4.0±1.0	7±1.2	0.286
	Proportion target species (no/plot)	0.13±0.02	0.24±0.04	0.241
	SW index (no/plot)	2.7±0.18	2.4±0.18	<0.01
Small scale	Species richness (no/0.25 m^2)	9.8±1.4	5.2±0.51	<0.05
	Target species (no/0.25 m^2)	0.20±0.15	0.93±0.13	<0.05
	Proportion target species (no/0.25 m^2)	0.02±0.01	0.19±0.04	<0.05
	SW index (no/0.25 m^2)	2.3±0.20	1.9±0.16	<0.05
Soil chemistry	pH	7.0±0.09	8.8±0.10	<0.05
	$NH4^+$ (µg/g)	3.2 +-1.2	0.55±0.15	0.129
	$NO3^-$ (µg/g)	0.31±0.08	0.25±0.08	0.314
	P (µg/g)	44±19	13±4.2	0.297

Table 4. Mean values ± SE (n = 5) of vegetation characteristics, nutrient levels and pH for Ripa, as well as p-values from one-way ANOVA. Different letters indicate significant differences between treatments.

			Ripa	n = 5	
		Control	Limed	Soil perturbation	p
Large scale	Species richness (no/plot)	24±1.5	23±1.0	27±2.4	0.275
	Target species (no/plot)	1.8±0.58	1.8±0.58	2.2±0.58	0.857
	Proportion target species (no/plot)	0.07±0.02	0.08±0.03	0.08±0.02	0.986
	SW (no/plot)	2.7±0.08[a]	2.6±0.08[ab]	2.5±0.03[b]	<0.05
Small scale	Species richness (no/0.25 m^2)	11±0.51[a]	8.6±0.44[b]	4.4±0.47[c]	<0.001
	Target species (no/0.25 m^2)	0.38±0.11	0.10±0.03	0.22±0.07	0.071
	Proportion target species (no/0.25 m^2)	0.04±0.01	0.01±0.00	0.05±0.02	0.072
	SW (no/0.25 m2)	2.2±0.06	2.2±0.08	2.3±0.03	0.379
Soil chemistry	pH	6.5±0.28[a]	7.7±0.10[b]	8.3±0.21[b]	<0.001
	$NH4^+$ (µg/g)	3.1±0.67[a]	4.2±0.28[a]	0.80±0.13[b]	<0.001
	$NO3^-$ (µg/g)	0.21±0.09[a]	0.63±0.11[b]	0.41±0.03[ab]	<0.01
	P (µg/g)	37±7.1	25±7.4	29±18	0.781

Vegetation characteristics

When comparing species richness, number of target species and the proportion of target species as an average of the 10 sample squares for each plot (0.25 m^2 scale), some differences due to treatment could be detected (Tables 3 and 4). Species richness (average number of species per 0.25 m^2) was higher in control plots than in plots treated with topsoil removal or soil perturbation, both in Everöd and Ripa. Limed plots had higher species richness than topsoil removal plots and lower species richness than control plots.

In Everöd both the number of target species and the proportion of target species were higher in treated plots than in control plots when analyzed with one-way ANOVA, with an average of 19 % target species in treated plots and only 2 % in control plots (Table 3). The number of target species and proportion of target species did not differ significantly in Ripa, where all plots had only a few percent target species (Table 4).

For the species richness, number of target species and the proportion of target species for each individual plot, there were no significant differences due to treatment, neither at Everöd nor Ripa (Tables 3 and 4). Species richness and number of target species was somewhat higher in Everöd than in Ripa, both for treated and control plots. Target species found in each treatment is shown in Table 5. The total number of individuals of some target species in each topsoil removal plot was also counted (Table 6). The number of target species was much higher at the Everöd sites than the Ripa sites.

Species diversity was higher in control plots than in treated plots in Everöd on both scales. Treated plots in Everöd were the only plots with an average SW index below 2 (Table 3). In Ripa there was no significant difference in species diversity between treatments on the 0.25 m^2 scale, however on the plot scale the SW index was significantly lower in soil perturbation treated plots than in control plots (Table 4). Limed plots did not differ from either soil perturbation plots or control plots at neither scale (Table 4).

Table 5. Target species (Olsson 1994, Tyler 2003, Naturvårdsverket 2011, Gärdenfors 2010), and the number of plots they were found in.

	Everöd	n = 3	**Ripa**	n = 5	
Species	Control	Top soil removal	Control	Limed	Soil perturbation
Alyssum alyssoides (VU)	0	0	0	3	2
Androsace septentrionalis	3	3	1	0	4
Anthyllis vulneraria	0	1	1	0	0
Camelina microcarpa (VU)	0	0	0	1	1
Carex ericetorum	0	1	0	0	0
Dianthus arenarius (EN)	0	1	0	0	0
Festuca polesica	0	1	0	0	0
Helichrysum arenarium	2	2	3	2	1
Koeleria glauca (EN)	1	3	1	1	1
Pulsatilla pratensis	3	3	0	0	0
Satureja acinos	1	0	0	0	0
Silene conica	1	2	1	2	3
Silene nutans	1	2	0	0	0

Table 6. Mean number of individuals of target species ± SE in topsoil removal treated plots in Ripa and Everöd (Olsson 1994, Tyler 2003, Naturvårdsverket 2011, Gärdenfors 2010).

Species	**Everöd** n = 3	**Ripa** n = 5
Alyssum alyssoides (VU)	0	1.6±0.68
Androsace septentrionalis	155±135	16 ±10
Camelina microcarpa (VU)	0	0.20±0.20
Carex ericetorum	6.3±6.3	0
Dianthus arenarius (EN)	8.3±8.3	0
Festuca polesica	13±13	0
Helichrysum arenarium	69±35	0.60 ±0.60
Koeleria glauca (EN)	755±272	0.20±0.20
Pulsatilla pratensis	54±33	0
Silene conica	76±66	38±25
Silene nutans	4.7±3.7	0

Vegetation composition

In Everöd the CA showed a cluster of two control plots and two treated plots, at the low end of both axis 1 and 2 (Fig. 2). In the cluster the treated plots differed slightly from the control plots along both axis 1 and 2. The control plot in Everöd 3 clearly differed from the cluster along axis 1, mainly due to the species *Helictotrichon pratense*, *Arrhenatherum elatius*, *Ranunculus acris* and *Berteroa incana*, who all scored high on axis 1. The treated plot in Everöd 1 clearly differed on axis 2, here mainly due to the species *Androsace septentrionalis*, *Helichrysum arenarium*, *Corynephorus canescens* and *Erodium cicutarium*.

In Ripa most of the plots showed little variation along axis 1 (Fig. 3). The limed plot and control plot in Ripa 4 differ from the cluster with low scores on axis 1 and very high scores on axis 2. The soil perturbation plot in Ripa 4 holds an intermediate positon, in between the other Ripa 4 plots and the cluster. The control plot in Ripa 5 holds high scores on both axis 1 and 2, with the limed plot in an intermediate positon. Species with high scores on axis 1 were *Arrhenatherum elatius*, and *Stellaria graminea*. Species with high scores on axis 2 were *Anthyllis vulneraria*, *Hieracium* spp., *Oenothera biennis*, *Jasione Montana*, *Scleranthus perennis* and the bryophytes *Peltigera canina* and *Syntrichia* spp. Species that scored high on both axis 1 and 2 were *Koeleria glauca*, *Thymus serpyllum*, and the bryophyte *Hypnum cupressiforme*.

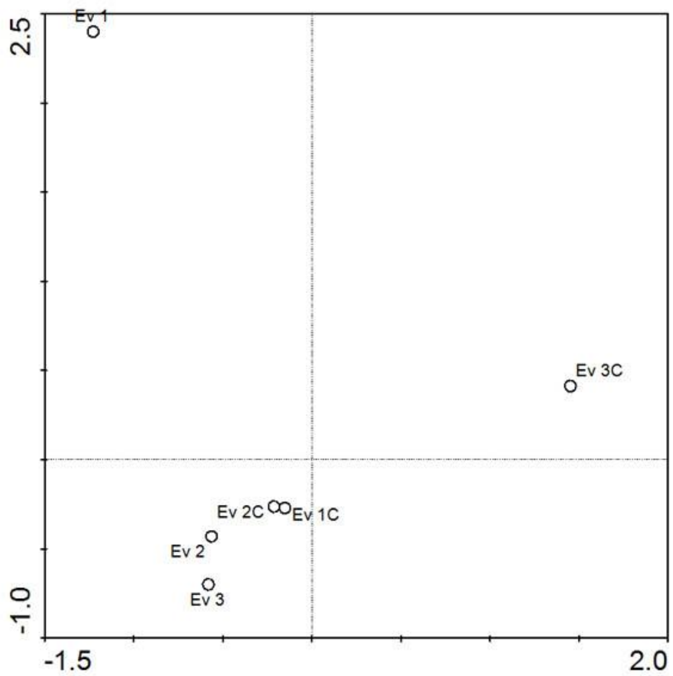

Figure 2. CA for Everöd sites. CA 1 (35%), CA 2 (31%).

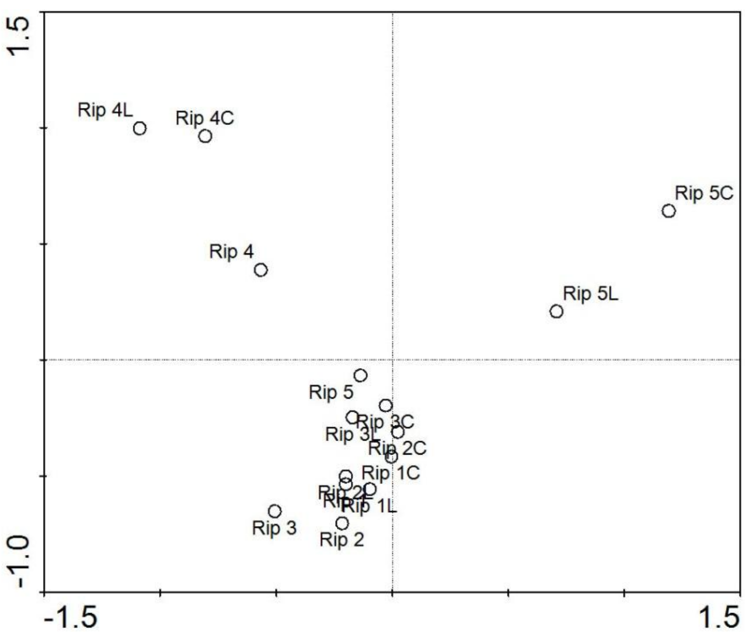

Figure 3. CA for Ripa sites. CA 1 (22%), CA 2 (18%).

Relations between soil chemistry and vegetation characteristics

When investigating relationships between soil chemical properties and the vegetation using linear regression, no relationships were found on the plot scale. However, on the 0.25 m^2 scale, a relationship was found between pH and proportion of target species (Fig. 4). The proportion of target species increase as the pH increases ($p = 0.026$, $r^2 = 0.235$, $n = 21$).

There was a negative relation between the concentration of NH_4^+ and proportion of target species on the 0.25 m^2 scale ($p = 0.018$, $r^2 = 0.275$, $n = 20$), which can be seen in Fig. 5.

Since the results from the linear regression seemed to mirror each other, the correlation between pH and the concentration of NH_4^+ was tested using the Pearson test. There was a correlation between pH and the concentration of NH_4^+ ($p = 0.01$, $r^2 = 0.450$, $n = 20$), showing that high levels of pH correlates with low concentration of NH_4^+ and vice versa.

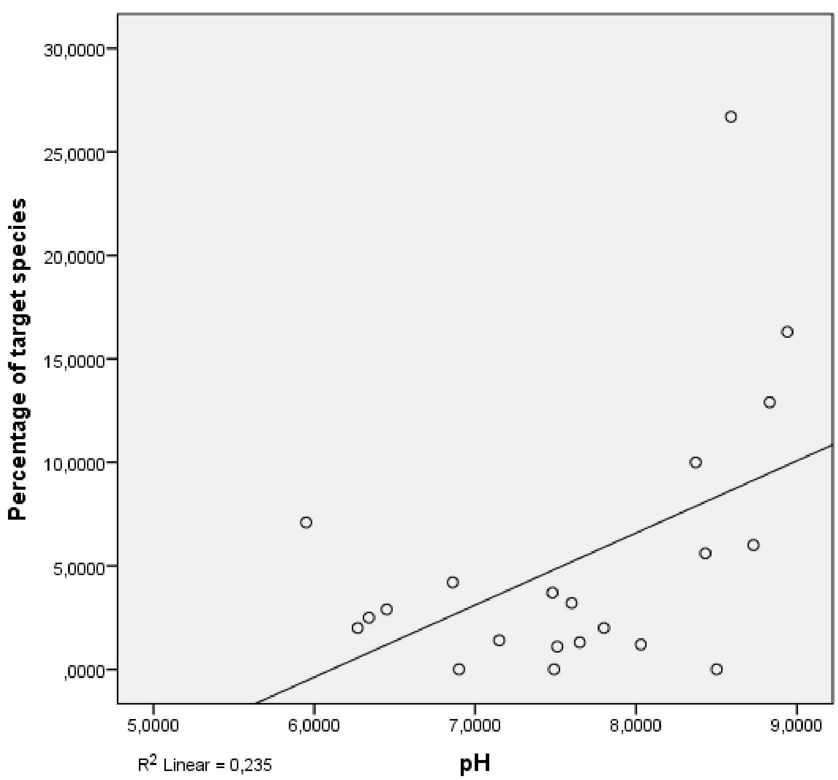

Figure 4. Relationship between pH and proportion of target species . The relationship includes all 15 sites in Ripa and all 6 sites in Everöd, n = 21.

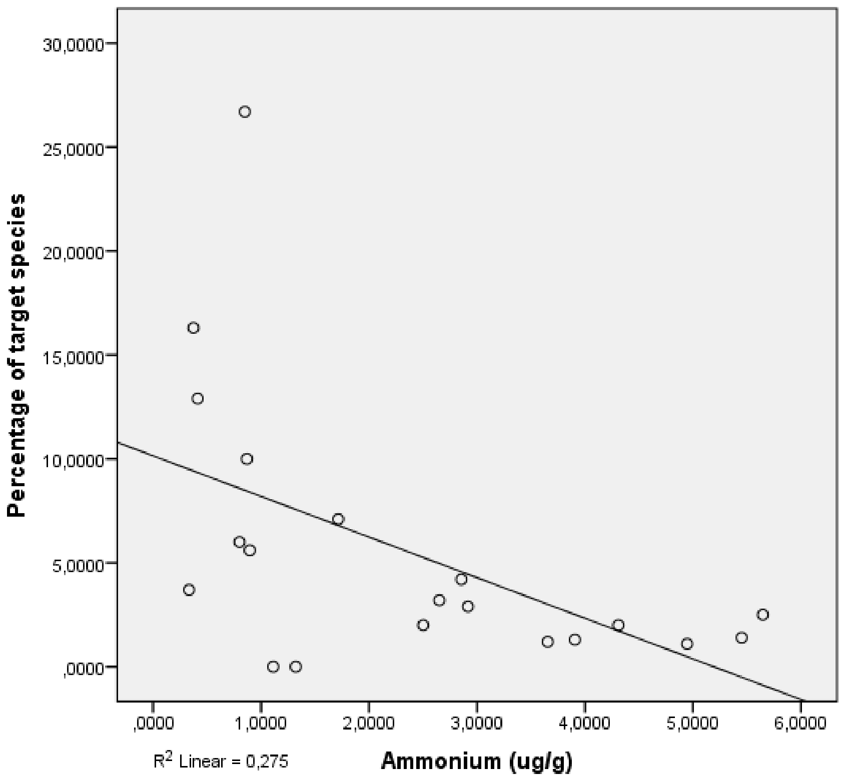

Figure 5. Relationship between NH_4^+ and proportion of target species. The relationship represent 14 sites in Ripa (limed plot in Ripa 4 excluded because missing value for NH_4^+) and all 6 sites in Everöd, n = 20.

DISCUSSION

Topsoil removal is an internationally accepted method to restore grasslands and increase biodiversity by reducing acidification, reducing levels of nutrients and causing positive disturbance (Buisson et al. 2006, Kiehl & Pfadenhauer 2007, Eichber et al. 2010).

The higher species richness and species diversity in control plots, compared to topsoil and soil perturbation treated plots, is in accordance with an earlier study conducted in the same area (Olsson 2012). This implicates that the succession is rather slow in this nutrient stressed habitat (Roem & Berendse 2000, Olsson 2009, Olsson 2012). Furthermore, studies show that species richness and number of target species is highest between soil pH 6 and 8 (Roem & Berendse 2000, Critchley et al. 2002, Mårtensson & Olsson 2010, Olsson et al. 2009), and all plots had a pH of 6 or higher. However, the main restoration target is the specialist species and they were very few in the control areas. Disturbance has a profound effect on both abiotic and biotic properties. The importance of disturbance is in line with the fact that many of the sand steppes that remains today are located on military training grounds and on former sandpits, where disturbance is substantial (Schnoor & Olsson 2010, Olsson & Ödman 2011). To maintain species from the intial stages, it is important that the disturbance is continuous (Mårtensson & Olsson 2010, Schnoor & Olsson 2010).

The fact that no significant differences could be found regarding number of target species and proportion of target species on the large scale is not that surprising. The habitats chosen for this experiment all had elements similar to calcareous sandy grasslands, especially the Everöd sites, and all control plots had some target species represented. However, when comparing at the 0.25 m^2 scale, a different picture emerged.

The rather drastic treatment of topsoil removal and soil perturbation described in this study could be considered a success due to the increase of target species and thereby altering the habitat towards xeric sand calcareous grasslands. This was visible in the older plots at Everöd, were the proportion of target species was as high as 19 % in topsoil removal plots, compared to 2 % in control plots. The reason that the soil perturbation plots in Ripa did not differ significantly from control plots may be due to the early succession, heavy grazing and trampling and the fact that the control plots in Ripa had almost the double proportion of target species compared to Everöd at the 0.25 m^2 scale. Furthermore, the much higher number of target species in treated plots in Everöd compared to Ripa (Table 6), shows that improvement of the habitat increases over time due to this treatment. It also seems likely that the heavier grazing at the Ripa sites slowed down the regeneration and that grazing and trampling during the earliest initial phase should be limited to achieve optimal result. This is supported by other studies, especially if the restored areas are small, and even rabbit overgrazing has been observed (Faust et al. 2011, Olsson & Ödman 2011). However, grazing becomes more and more important over time to maintain successful management (Eichber et al. 2010, Olsson & Ödman 2011).

The increase in target species proves the success of topsoil removal and soil perturbation as treatments to restore calcareous sandy grassland habitats. Even though the species richness and diversity did not increase at this scale, the increase in target species is a potential to enhance species richness and diversity at a landscape scale. The improvement towards sand steppe could also be seen visually, where the treated plots showed a high heterogeneity with patchy, very varying vegetation, whereas the control plots had more or less the same vegetation evenly distributed throughout the plot, with no bare sand.

The positive results of topsoil removal and soil perturbation are likely due to the restoration of abiotic conditions more resembling to the xeric sand calcareous grassland habitat, especially pH. Topsoil removal and soil perturbation had significantly increased pH in both Everöd and Ripa to around pH 8.5, and thereby created a very suitable habitat for calcicole species (Bakker & Berendse 1999, Olsson et al. 2009). In this study 23.5 % of the increase in proportion of target species is explained in relationship to increase in pH (Fig. 5). This is in accordance with other studies illustrating a relationship between increasing pH and target species in calcareous sandy grasslands (Houdjik et al. 1993, Roem & Berendse 2000, Olsson et al. 2009). Higher levels of pH are especially important to species with a seedling establishment at high pH, such as *Koeleria glauca* (Olsson & Ödman 2012). On the other hand, *Corynephorus canescens* has proven to be quite insensitive to high pH in its germination and establishment phase and in addition it is a rapid colonizer of bare sand (Olsson et al. 2009). Even though *Corynephorus canescens* in many cases is a wanted target species, in these habitats it is an indicator of acidification and thereby a degeneration towards gray dunes or inland dune grasslands (Mattiasson 1974, Olsson & Ödman 2011, Naturvårdsverket 2011).

Topsoil removal and soil perturbation in this study did not have solely positive results in decreasing levels of nutrient. The treatments was effective in lowering the concentration of ammonium and a relationship between lower concentrations of NH_4^+ and higher proportion of target species was established using linear regression (Fig. 6). This is in accordance with other studies, were topsoil removal has proven successful in lowering levels of N (Buisson et al. 2006, Kiehl & Pfadenhauer 2007,Stevens et al. 2011). Even though the concentration of NO_3^- in topsoil removal and soil perturbation treated plots did not significantly differ from the control plots, the levels were generally somewhat lower in treated areas. In particular the disturbance treatments increased the proportion of NO_3^- compared to NH4+. Thus indicating that nitrification is stimulated by the pH-increase (Roem & Berendse 2000, Stevens 2011). The relationship between decreased levels of N and increased number of target species is well studied. Decreasing levels of plant-available N is important since it reduces competition from generalist species, often fast growing perennial grasses, such as *Arrhenatherum elatius* and *Festuca rubra* (Bakker & Berendse 1999, Roem & Berendse 2000, Süss et al. 2004, Stevens et al. 2011). When levels of N are high, these species can outcompete many slow-growing or low-statured species more adapted to nutrient-poor soils (Berendse et al. 1987, Bakker & Berendse 1999, Roem & Berendse 2000).

The levels of Bray P were not significantly lower in treated plots than in control plots. High levels of P commonly results in low species richness and a species composition with more generalist species and less species characteristic of calcareous sandy grasslands (Wassen et al. 2005, Smits et al. 2008, Olsson et al. 2009). P availability is very limited by the presence of lime in the soil and therefore many calcicole specialist species prevail under P limitation (Wassen et al. 2005, Olsson et al. 2009). Therefore the reducing of P availability is especially important when restoring calcareous sandy grasslands. Measuring plant available P is difficult and a number of methods can be used (Olsson & Ödman 2012). In this study the Bray 1 method was used since it is less pH sensitive, extracts higher amounts of P and has proven useful in other related studies (Olsson & Ödman 2011, Olsson & Ödman 2012). Reducing the level of P in grasslands seems to be a challenge according to many studies evaluating different preservation treatments (Olsson & Ödman 2011, Smiths et al. 2008). This is especially problematic since high levels of P have shown to remain more than 25 years after fertilizer application stopped, compared to nitrogen, which seems to be reduced in less than 10 years (Smith et al. 2008). The level of P availability at different soil depths could be a good guideline to determine how much soil to remove for successful treatment (Olsson & Ödman 2011). The fact that levels of NO_3^- and Bray P were barely reduced may explain why some generalist species, such as *Festuca rubra* and *Festuca brevipila*, were fairly common in topsoil removal and soil perturbation plots (Roem & Berendse 2000, Wassen et al. 2005, Eichberg et al. 2010, Olsson & Ödman 2012).

Liming seems to be a good complement to improve areas around topsoil removal and soil perturbation plots to extend the area, or to improve abiotic conditions, mainly pH, in areas were topsoil removal and or soil perturbation is not wanted or possible. The method could also prove successful to lower the levels of P, since P availability is strongly limited by lime for many plant species (Tyler 1992, Olsson et al. 2009, Olsson & Ödman 2011). In this study the levels of P were generally lower in limed plots than in control plots. However the differences were not significant and further studies are needed. This method is both simpler and less costly than topsoil removal or soil perturbation, but not nearly as effective. However the treatments are still at an early stage and should be evaluated further.

Other studies have shown that topsoil removal in combination with seeding and plant transplanting has proven successful in order to make up for the diminished seed bank (Kiehl & Pfadenhauer 2007, Eichberg et al. 2010). However, the lack of transplanted plant material does not seem to cause any difficulties in the colonization of target species in this area. This is most likely due to the proximity to target vegetation, resulting in spontaneous succession. The unprompted succession of sandy grassland species has been observed both in this area, and in other places in Europe (Olsson & Ödman 2011, Rehounkova & Prach 2008). So in order to ensure colonization without plant transplanting, restoration measures should preferably be carried out in areas close to other xeric sand calcareous grasslands if possible. Topsoil removal and soil perturbation can also cause colonization of unwanted species, such as *Robinia pseudoacacia* and such risks needs to be monitored (Olsson et al. 2009, Rehounkova & Prach 2008, Olsson & Ödman 2011). However, topsoil removal will also diminish the seed banks of non-target species (Eichberg et al. 2010).

Conclusions

This study supports earlier studies showing that topsoil removal is an efficient method for restoring calcareous sandy grasslands (Kiehl & Pfadenhauer 2007, Eichber et al. 2010). The treatment reduces plant-available N, increases pH and lime-content and creates disturbance, all important factors in restoration of xeric sand calcareous grasslands (Critchley et al. 2002, Olsson & Ödman 2011). Biotic conditions such as seed dispersal and grazing are also important. From this study it seems as if high pH is the most important factor for establishment of target species, which is also in accordance with other studies (Roem & Berendse 2000, Olsson et al. 2009, Mårtensson & Olsson 2010, Critchley et al. 2002). It is also evident that these conditions prevailed 6 years after treatment. Reduced levels of plant available P remains an issue and further studies in this area are needed. The succession in these areas, especially in Ripa, is still at an early stage and further improvement of the habitat towards xeric sand calcareous grasslands is likely. However, reduced grazing in the Ripa plots would probably accelerate regeneration. This study strengthens the perspective that restoration of calcareous sandy grasslands is a complicated long-term process, which generally includes a balance of both biotic and abiotic conditions.

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APPENDIX A

Fig. 6 Backhoe loader doing soil perturbation at Ripa 1-3.

A

Fig. 7a Everöd 2, topsoil removal plot.

B

Fig 7b Everöd 3, topsoil removal plot.

A

Fig. 8a. Ripa 1 and 2, soil perturbation plot.

B

Fig 8b. Ripa 2, limed plot.

A

Fig. 9a. Ripa 4, soil perturbation and limed (background left) plots.

B

Fig. 9b. Ripa 5, soil perturbation and limed (background) plots.

Fig. 10. Inventory square divided in 25 smaller squares (total size 0.25 m^2).