Check for updates

Received: 15 October 2019 | Revised: 9 September 2020 | Accepted: 11 September 2020

DOI: 10.1111/jvs.12956

SPECIAL FEATURE: DISPERSAL AND ESTABLISHMENT

Journal of Vegetation Science IAVS

Grazing livestock increases both vegetation and seed bank diversity in remnant and restored grasslands

Rozália E. Kapás | Jan Plue | Adam Kimberley | Sara A.O. Cousins

Department of Physical Geography, Stockholm University, Stockholm, Sweden

Correspondence
Rozália E. Kapás, Department of Physical Geography, Stockholm University, 10691, Stockholm, Sweden.
Email: rozalia.kapas@natgeo.su.se

Funding information
The Foundation for Baltic and East European Studies and Oscar and Lili Lamm Memorial Foundation.

Co-ordinating Editor: Péter Török

This article is a part of the Special Feature Plant dispersal and establishment as drivers of vegetation dynamics and resilience, edited by Péter Török, James M. Bullock, Borja Jiménez-Alfaro and Judit Sonkoly.

Abstract

Questions: Restoring grasslands is of great importance to biodiversity conservation to counteract widespread, ongoing losses of plant species diversity. Using source populations in remnant habitats and increasing functional connectivity mediated by grazing animals within and between habitats can benefit grassland restoration efforts. Here we investigate how grazing contributes to vegetation and seed bank diversity and composition in remnant and restored grassland communities in fragmented landscapes.

Location: Stockholm archipelago, Sweden.

Methods: We investigated the effects of the presence or absence of grazing animals as potential elements of functional connectivity on grassland species composition in both the vegetation layer and in the seed bank. Species inventory and seed bank sampling were carried out in 2 m × 2 m plots in remnant grassland habitats and adjacent restored grasslands on former arable fields.

Results: Species composition varied between remnant and restored grasslands, with management-dependent species more common in remnant grasslands. Remnant habitats with active grazing management contained a higher number of species in both the vegetation and seed bank compared to restored grasslands, but grazing reduced dispersal limitation from higher distance to source populations for specialist species. Where grazing was absent fewer plant species occurred in both the vegetation and in the seed bank.

Conclusion: Our results show that grazing livestock play a key role in facilitating both spatial and temporal dispersal in fragmented grasslands. This results in increased species diversity in the vegetation and the seed bank of grazed grasslands compared to those maintained by mowing only. Functional connectivity provided by grazing management increases the possibility for species establishment from both the belowground seed bank and the surrounding landscape, thus increasing the resilience of plant communities against disturbances or climatic changes.

KEYWORDS
biodiversity, functional connectivity, grazing, restoration, seed bank, seeds, semi-natural grasslands, spatial dispersal

© 2020 International Association for Vegetation Science

1 | INTRODUCTION

Grassland habitats continue to decrease globally at alarming rates (Watson et al., 2016). Large amounts of semi-natural grassland in Europe have already been lost (Pärtel et al., 1999; Adriaens et al., 2006). This habitat loss, combined with increased isolation of remaining grasslands, severely threatens the exceptional plant diversity of grassland communities, particularly in northern Europe (Wilson et al., 2012). In Sweden, up to 96% of semi-natural grassland has been converted into either forest or arable fields during the last 150 years (Cousins and Eriksson, 2008; Cousins et al., 2015). Species loss is mostly driven by the abandonment of long-term, low-intensity grassland management on which many typical grassland species depend (Eriksson et al., 2002; Aavik et al., 2008).

Conservation of grassland habitats is of the utmost importance because they are often a major source of plant diversity in arable field or forest-dominated landscapes (Cousins and Lindborg, 2008). Hence, grassland restoration is a key goal for conservationists, with the aim of reducing the detrimental effects of habitat loss and isolation. In Europe, and Sweden in particular, many marginal or less productive arable fields have been converted into grassland over the past 50 years. Following the initial sowing of a simple seed mixture, these grasslands are used for producing fodder for livestock and for livestock grazing. These species-poor grasslands on former arable fields, though often overlooked, offer an opportunity for the restoration of species-rich grassland at landscape scales.

Effective restoration requires species to be able to reach grasslands via dispersal, and successfully establish upon arrival (i.e. plant functional connectivity (Auffret et al., 2017)). Spatial dispersal can occur either passively from nearby populations, or via dispersal vectors moving within the landscape. For instance, plant species may be transferred over longer distances by domestic livestock, enabling the colonisation of isolated, unoccupied grasslands (Couvreur et al., 2004; Albert et al., 2015). Such animal-mediated (zoochorous) dispersal (Fischer et al., 1996; Albert et al., 2015) can enhance spatial connectivity for most grassland species, irrespective of whether their seeds have specific trait adaptations facilitating animal dispersal (Couvreur et al., 2004; Auffret et al., 2012). Connectivity between grassland sites is therefore important for the development of biodiversity in restored grasslands, but dispersal also plays a large role in maintaining biodiversity in older and remnant grassland habitats, with the loss of functional links (i.e. abandonment of grazing management) resulting in the loss of species diversity (Auffret et al., 2012). Consequently, the introduction of grazing is often included in management plans, with animals moved between species-rich and species-poor habitats (Mitlacher et al., 2002; Wit and Schwabe, 2010).

Spatial dispersal need not result in immediate establishment in the grassland vegetation. Seeds that reach restored sites via successful dispersal events (either animal-mediated or otherwise) may begin to accumulate in the seed bank once they arrive, even where local conditions for establishment may not immediately be favourable (Plue and Cousins, 2013). As local conditions improve, these seeds may support delayed colonisation at some point in the future, even if the spatial connection is lost (Plue and Cousins, 2013). Grazing animals can facilitate subsequent species establishment from the soil seed bank in both remnant and restored grasslands by creating physical disturbances (Saatkamp et al., 2014). Grubbing, trampling or wallowing creates vital microsites in which dispersed seeds can germinate and possibly establish (Eriksson and Ehrlén, 1992; Pakeman and Small, 2005). These activities can also help seeds to integrate into the soil and to build up a persistent seed bank (Faust et al., 2011; Klaus et al., 2018). Grazing therefore leads to higher species diversity in both the vegetation and the seed bank communities, which enhances the resilience of grasslands. A persistent and diverse seed bank can act as a reservoir and delay extinction or accelerate species establishment after changes in the environment (Vandvik and Goldberg, 2006; Lindborg, 2007; Auffret et al., 2017).

Hence, the presence of grazing animals can influence grassland plant diversity via multiple dispersal mechanisms (Vandvik and Goldberg, 2006; Alexander et al., 2012; Auffret et al., 2015). However, grazing can also influence species diversity directly at the local scale, by inhibiting the growth of competitive species and favouring stress-tolerant or grazing-adapted plants (Pykälä, 2003; Rook et al., 2004). The regular disturbance provided by the presence of grazing animals can therefore also shape plant communities independent of dispersal, in both restored and remnant grasslands, by removing biomass through their selective diet (Rook et al., 2004).

Understanding how management such as grazing affects plant communities can bring forward crucial guidelines enhancing the efficacy of grassland restoration in fragmented landscapes. Here, we investigate the effect of grazing on vegetation and seed bank diversity in remnant semi-natural and adjacent restored grasslands on former arable fields. We investigated the importance of species' seed bank persistence and species' ability to use various dispersal mechanisms (including zoochory) in grazed and non-grazed sites. By comparing the response (i.e. species richness and composition) of both vegetation and seed bank communities to the presence of grazing, we aim to determine how the importance of spatial dispersal and establishment potential vary for different species groups, and under different management conditions. We focus on how different species groups establish in restored grassland such as grassland specialist. These species are often regarded as indicator species of a well-managed semi-natural grassland and predominantly favour grazing. Thus, their occurrence is a conservation goal in restored grasslands. With this approach, we can provide an insight into the filtering effects of dispersal and the establishment of these species on grassland plant community assembly under grazing management, and hence, into the potential contribution of nearby habitat remnants and the soil seed bank to biodiversity recovery in restored grasslands in the Stockholm archipelago.

We hypothesised that grazing is associated with higher species richness in vegetation and seed bank communities, with positive effects of grazing on both spatial dispersal and subsequent establishment, helping grassland specialist species to colonise restored grasslands.

2 | METHODS

2.1 | Study area

The Stockholm archipelago in the Baltic Sea, Sweden, consists of approximately 29,000 islands, ranging from small islets and skerries to larger, permanently inhabited islands (Figure 1). After the Weichselian deglaciation, the area was submerged, with the present-day archipelago beginning to rise above sea level ca. 3,000 years ago and continuing to rise at a rate of 4 mm per year due to isostatic rebound. The study area covers ca. 96 km^2 of the central part of the archipelago and consists of 27 islands (geographical centre: 59°24′17″ N, 18°43′05 E), ranging from 0.15 km^2 to 5.3 km^2 in size.

Since the 1600s, small-scale agriculture and rotational grazing have been the main shaping factors for the archipelago landscape (Aggemyr and Cousins, 2012) and several farms have been situated in the study area in the same place since then. Agricultural activities in the archipelago have been naturally limited by lack of cultivable soils. The low-intensity management practices on the arable fields (e.g. no artificial fertilisers, no deep ploughing), coupled with abandonment of less productive arable fields at the beginning of the 1930s have resulted in a mosaic of ancient and recovering grassland communities on former arable fields. These grasslands are embedded in a sea–forest matrix and today´s landscape is dominated by forest (72%). Historical records show that present-day forests were either significantly more open or absent in the 18th and the 19th centuries as forests were both a primary source of timber and fuel and used for extensive grazing.

In our study, we focus on the mosaic of ancient grassland remnants and the recovering grassland communities on former arable fields. Initially, upon abandonment, the arable fields were sown in by managers of the land with a grassland seed mixture to develop into grassland, which still serves an agricultural purpose via follow-up grazing and/or mowing management. Therefore, these recovering grasslands themselves can be considered as restored grasslands on former arable fields, some of which to this day remain subject to rotational livestock grazing; others are mown to produce winter fodder, while still other grasslands are subject to both. This means that both grazed and non-grazed grassland types receive regular disturbance throughout the year. As such, the primary differences between grazed and non-grazed sites are the additional soil disturbance and potential functional connectivity

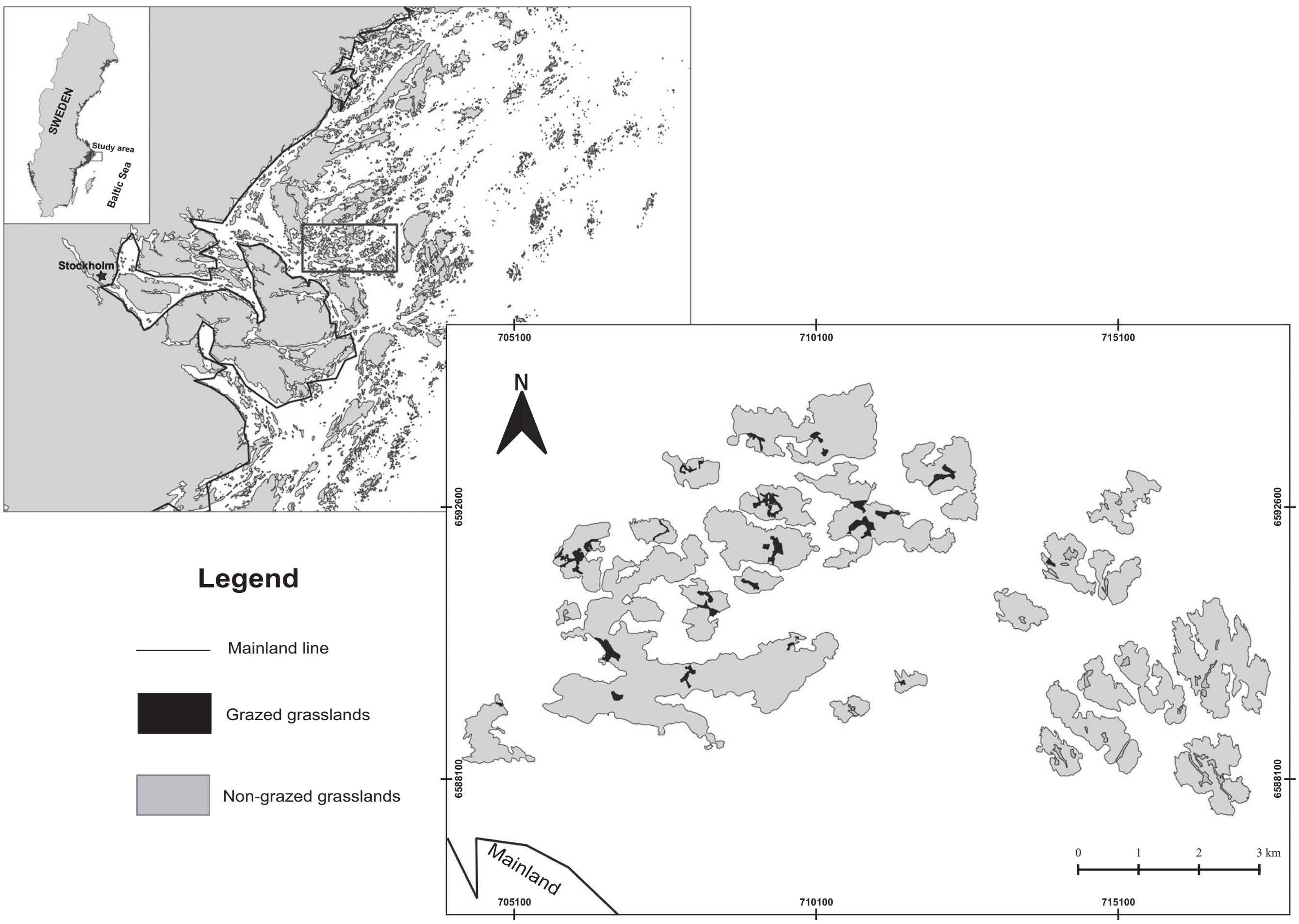

FIGURE 1 Map of the study area in the central part of Stockholm archipelago. The 53 studied grassland areas are indicated with polygons. Black polygons (27) denote grazed grasslands while darker grey (26) polygons are grasslands without grazing management (i.e. mown grasslands). The black line was used for measuring the distance to the mainland among the islands

FIGURE 2 A conceptual profile of the system (a) with a corresponding photo of one of the 53 studied grassland (b). Sampling plots were placed as pairs, one at the edge of remnant grassland and one in the centre of the restored grassland. Grazing animals could move freely between plots and other habitat patches in the surrounding [Colour figure can be viewed at wileyonlinelibrary.com]

caused by the presence of livestock. The restored grasslands on former arable fields are often surrounded by ancient grassland remnants (Figure 2). The ancient grassland remnants are situated on thin soils on bedrock or moraine, whereas the former arable fields have deep (tillable) soils, which often creates a sharp microtopographic edge between ancient grassland remnants and the former arable fields. The recovering grasslands where grazing takes place are generally part of a rotational grazing system. This requires livestock transportation by boat among the various islands, for instance between the home islands and grazing islands during summer. Grazing animals (sheep or cattle) roam freely across entire islands or within enclosures which always comprise a mosaic of grasslands (remnant and restored) and forests.

The combination of ancient grassland remnants adjacent to restored grassland on islands with or without rotational grazing management provides an excellent study system to investigate effects of grassland restoration and management on species diversity including species dispersal and establishment. Prior to fieldwork, suitable grasslands were selected in ArcMap 10.5.1 (ESRI, 2017) using satellite imagery and historical maps. During site selection, we focused on balancing the design of the study to incorporate grasslands found on former arable fields both with and without rotational grazing. From this point forward, we will refer to the grasslands on former arable fields as restored grassland, even if restoration was passive and not a goal per se.

2.2 | Data collection

Species inventories were carried out by using 2 m × 2 m plots in a paired design, to be able to establish if species were recolonising restored grasslands from adjacent ancient grassland communities, hereafter referred to as remnant grassland. This meant that one plot was located in the remnant grassland close to the border of the restored grasslands, and a second plot approximately in the centre of the restored grassland (i.e. in the former arable field site). In 13 large grasslands, more than one pair of plots was established. A total of 140 plots were inventoried in 27 grazed and 26 non-grazed grasslands.

First, all vascular species and the total plant cover (%) were recorded within each plot (nomenclature: Mossberg and Stenberg, 2003). Then, the seed bank was sampled by randomly collecting 50 core samples within the same plot using a soil core sampler (3.5 cm diameter and 5 cm deep; cf. Plue and Hermy, 2012). The vegetation, moss and litter layers were discarded to remove transient seeds. The soil cores were bulked into one sample per plot and transported to the greenhouse for the germination trials. Samples were concentrated following the method of Ter Heerdt et al. (1996). The concentrated samples were spread on trays of steam-sterilised potting soil. The trays were placed in the greenhouse under a 16 h day and 8 h night regime and watered until the trial was ended after 20 weeks. Emerged seedlings were counted, identified and removed. Unidentified seedlings were transferred to pots and grown until identification. Control trays, containing only potting soil, were placed among the samples and no contamination was detected in the trays.

2.3 | Plant and seed traits

To investigate the importance of spatial seed dispersal and temporal seed dispersal (seed dispersal in time via soil seed banks) in controlling plant community assembly on restored grasslands, and potential filtering effects of grazing, we extracted traits on spatial dispersal strategy and seed bank type from the LEDA database for all of the recorded plant species (Appendix S1; Kleyer et al., 2008). Dispersal traits were divided into two main categories: species favouring assisted dispersal (species dispersed by grazing animals over long distances; i.e. species listed as either endo-, epi- or dyscochor in the LEDA trait database (Kleyer et al., 2008)) vs. species which only rely upon unassisted dispersal (all other species). Species without specialised dispersal strategy (eight species) were excluded from the analysis. Seed bank type categories were short-term persistent (between one and five years) and long-term persistent (>5 years). All recorded plant species were categorised into two categories based on their dependence on grassland management (Ekstam and Forshed, 1997). The specialist group includes species which disappear rapidly once grassland management ceases. All other species, which have no clear preference for grassland management, were categorised into a separate group called non-specialist. This group contains all the species from (sub-)groups of generalist, ruderal and forest edge species and species without categories (Appendix S1). We focus only on these two groups, as we expect grassland specialist species to be more sensitive to grazing management, and investigating the patterns in grassland specialist species compared to other grassland species will help determine directly how grazing management affects the presence of these species both in the vegetation and the seed bank.

2.4 | Landscape data

To differentiate effects on the grassland plant communities of grazing management from those of spatial landscape context, we calculated the following landscape characteristics for each plot: island area, grassland area, distance to the mainland and distance between each pair of remnant and restored plots within a grassland. All calculations were performed in ArcMap 10.5.1 (ESRI, 2017).

We compared the landscape characteristics with Wilcoxon signed-rank tests. Grazed islands tend to be larger than non-grazed islands (median of 94 ha [grazed] vs. 67 ha [non-grazed]; Wilcoxon signed-rank test: $W = 1792$, $p = 0.007$). Also, non-grazed grasslands were smaller than the grazed grasslands (median 11 vs. 16 ha; Wilcoxon signed-rank test: $W = 1692$, $p = 0.006$; Appendix S2A). Grazed grasslands were closer to the mainland than non-grazed grasslands (median of 6.1 km [grazed] vs. 9.4 km [non-grazed]; Wilcoxon signed-rank test: $W = 4{,}386$, $p < 0.001$). The median distance between paired plots was 26.6 m in grazed grasslands, and 35.2 m in non-grazed grasslands (Wilcoxon signed-rank test: $W = 3{,}008$, $p = 0.018$). However, these variables did not have any effect in the final models and were dropped during the model selection (result not shown).

2.5 | Statistical analysis

To investigate differences in plant community composition between remnant and restored grasslands in both the vegetation and seed bank, a non-metric multidimensional scaling ordination (NMDS) based on the Sørensen dissimilarity matrix was first performed on the presence/absence-based species × site matrix of the vegetation (206 species × 70 sites) and seed bank (147 species × 70 sites).

We assessed the impact of grazing and grassland origin on species composition in both the vegetation and seed bank (using Sørensen distances) with a permutational multivariate analysis of variance (PERMANOVA).

In order to test whether grazing management homogenises plant community composition in remnant and restored grasslands, we performed an analysis of multivariate homogeneity of group dispersions (centred around the median, *betadisper*; Oksanen et al., 2019) on both the vegetation and seed bank matrices.

Species richness (*S*) was calculated for both vegetation and seed bank plant communities and for each trait group (total species richness, grassland specialists, non-specialists, species richness grouped per dispersal mechanism and grouped per seed bank persistence type). Prior to modelling, all response variables were tested for the presence of spatial autocorrelation. We constructed three Generalised Least Squares (GLS) models for each response variable. Each GLS model was a GLS null model to which one of the three most common spatial covariance structures was added as a model term, either Gaussian, exponential or spherical. Each GLS model was compared to its GLS null model counterpart via a likelihood ratio test. Where significant at $p < 0.05$, that particular spatial covariance structure would explain some of the variation in the response variable under scrutiny, confirming the presence of spatial autocorrelation. The likelihood ratio test indicated a significant improvement in the model for only one response variable, namely the non-specialist species richness. In all the other cases, none of the likelihood ratio tests proved significant; therefore, this series of tests statistically suggests that there is no spatial correlation among the tested response variables, eliminating the need to correct for space during further modelling steps. In addition, we also tested the potential influence of grassland identity on response variables due to the paired sampling design. We performed a GLS model together with a Linear Mixed-Effects model (LME) where the random factor was grassland identity (where the plots were paired) and compared the results with the same likelihood ratio test (Zuur et al., 2009). Grassland identity as a random effect improved the model for non-specialist species richness, unassisted dispersal in vegetation, and in the seed bank species with short-term persistence seed bank type. Hence, later LME model was fitted for these response variables to account for the important paired structure of the data, whereas for the non-specialist species richness we fitted LME model with an exponential covariance structure to account for paired structure and the spatial autocorrelation among the sampled plots.

Full factorial generalised mixed models (Poisson-error or quasi-Poisson distribution) or linear mixed models with the random effect (grassland identity and/or covariance structure) models were created using management type (grazing present or absent), grassland origin (remnant or restored) and landscape characteristics (island area, distance to the mainland, distance between plots) to explain the patterns of species richness in both the vegetation and seed bank. We combined management type and grassland origin into one single factor (with four levels; grazed remnant, non-grazed remnant, grazed restored and non-grazed restored). The interaction between combined management type/grassland origin and distance between plots was included to investigate how effect of the increased distance between plots is influenced by the presence of grazing management. Distance between plots was expected to have a stronger effect in non-grazed grasslands as the zoochorous dispersal is limited in these areas. We detected over-dispersion for two variables (total species richness and specialist species richness) and applied a quasi-Poisson distribution for correcting the standard errors in these models (Zuur et al., 2009). For the simplest model supported by the data, non-significant predictor variables were dropped ($p \geq 0.05$) in a step-wise manner in order of the descending *p*-values until only significant effects remained. After modelling, potential collinearity among the remaining predictor variables was tested using variance inflation factors (VIFs). Collinearity was absent among the explanatory variables and landscape characteristics used, with all VIFs below 2. Hence, all variables could safely be included in subsequent models (Zuur et al., 2010). Vegetation cover was tested using the same methods as described above between the grazed and non-grazed grasslands and origin of the grasslands.

All statistical analyses were done in R version 3.5.3 (R Core Team, 2019), using the *vegan* (functions: *metaMDS*, *vegdist*, *ordiellipse*, *adonis2*) (Oksanen et al., 2019) and *nlme* packages (functions: *gls*, *lme*) (Pinheiro et al., 2020) and R base (functions: ANOVA, *glm*).

3 | RESULTS

In total 246 plant species were found, 107 of which occurred in both the vegetation and seed bank communities (Appendix S1). We recorded 206 plant species in the vegetation, while the seed bank contained 147 species among the recorded 37,722 seedlings. The remnant grassland contained 296 (± 18.6 SE) seedlings per plot, whereas from the restored grasslands 186 (± 21.0 SE) seedlings emerged per plot. We found 57 grassland specialist species and *Agrostis stolonifera*, *Hypericum maculatum* and *Hypericum perforatum* were the most abundant species among the emerged seedlings.

The two NMDS ordinations showed that species composition is different between restored grasslands and remnant grasslands in both vegetation and seed bank (vegetation: stress = 0.21; seed bank: stress = 0.27; Figure 3). Communities on grassland remnants and restored grasslands partially overlapped, suggesting that communities share a considerable number of grassland species. The PERMANOVA analyses indicated that the species composition varied significantly in vegetation and seed bank due to the grassland origin (vegetation: *F*-value = 28.580, *p*-value < 0.001; seed bank: *F*-value = 17.779, *p*-value < 0.001) and presence of grazing (vegetation: *F*-value = 9.931, *p*-value < 0.001; seed bank: *F*-value = 6.568, *p*-value < 0.001). The multivariate homogeneity test showed that the variation between remnant and restored grassland where grazing was present was low in both vegetation ($p = 0.948$) and seed bank ($p = 0.767$). The vegetation cover was significantly higher in restored grasslands compared to remnant grasslands, while grazing reduced the vegetation cover (Appendix S2B).

Total species richness in the vegetation was higher in remnant grassland plots than in restored grassland plots, both with and without grazing present (Figure 4a, Table 1). This appears to be driven by an increase in the number of grassland specialists in grassland remnants in both vegetation and seed bank (Figure 5). Furthermore, grazing had a stronger effect on the specialist species group in remnant grasslands than in restored grasslands. Total species richness in the seed bank showed similar patterns as in the established plant communities (Figure 4b, Table 2).

In the vegetation, species trait groups varied considerably with grazing management type (grazing present or absent) and between remnant and restored grasslands (Figure 5a, Table 1). In general, non-specialist species (species not dependent upon grazing management) dominated both grazed and non-grazed grasslands. However, more grassland specialist species were present when remnant and restored grasslands were grazed compared to ungrazed grasslands. Furthermore, there was a negative effect of the increasing distance between plots on specialist species richness, but this effect was less negative in grazed grasslands, indicating that the distance between plots has less influence where grazing was present (Table 1). The number of non-specialists was higher in remnant grasslands where grazing was not active anymore (Figure 5a). Remnant grasslands held more specialists than restored grasslands irrespective of grazing presence.

The seed bank showed similar patterns regarding grazing management (Figure 5b, Table 2) in that grazing had a positive effect on the number of grassland specialists recorded in the seed bank, both in remnant and restored grasslands, being higher in the former. However, contrary to the effects seen in the vegetation, grazing increased the number of non-specialist species in restored grasslands and within the distances between the two grassland origins (i.e. remnant and restored).

Both grazed and ungrazed remnant grasslands contained greater numbers of both assisted and unassisted dispersers compared to

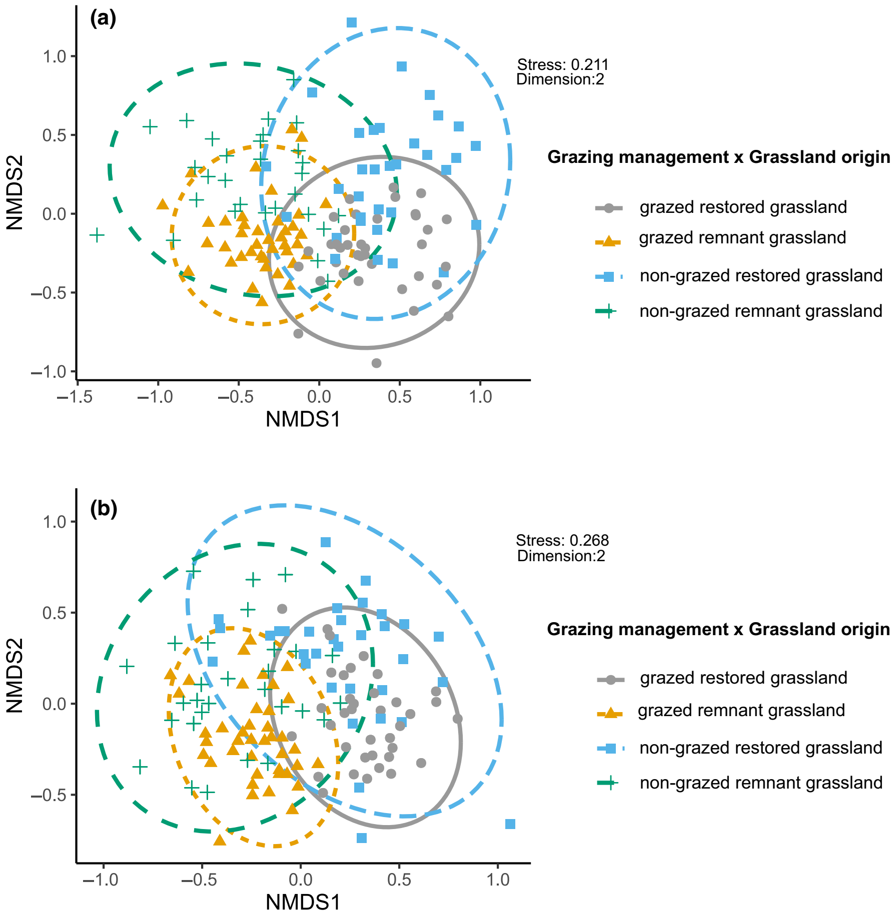

FIGURE 3 Two NMDS ordinations of 140 plots including vegetation (a) and seed bank (b) with Sørensen dissimilarity matrix based on 53 grassland sites in the Stockholm archipelago. Dashed lines (ellipses) represent the effect of the interaction between the presence/absence of grazing and grassland origin on the community composition. The ellipses around the clusters of predictors are based on the 95% confidence interval [Colour figure can be viewed at wileyonlinelibrary.com]

restored counterparts, whether being grazed or not (Figure 6a, Table 1). Even though the richness of species withassisted dispersal mechanism was lower than that of species with unassisted dispersal mechanism in both remnant and restored grasslands, active grazing resulted in a more pronounced increase in species number, especially in the remnant grasslands.

The number of species with a short-term-persistent seed bank dominated plant communities in restored and remnant grasslands, with a clear positive and additive effect of grazing presence (Figure 6b, Table 1). Despite there being fewer species with a long-term-persistent seed bank among plant communities, grazing did increase the number of such species in both remnant and restored grassland. Increased distances between paired plots led to a decrease of the number of species with long-term-persistent seeds in non-grazed grasslands. In the seed bank, the seed persistence type showed different patterns relative to species occurring in the vegetation (Figure 6c, Table 2). Fewer short-term persistent species were found in the seed bank compared to the vegetation. The number of short-term-persistent species increased with grazing and was highest in the remnant grasslands. This was true for the long-term-persistent species too. In grasslands with grazing, more species were found with long-term-persistent seeds than in grasslands without grazing. During model selection, the landscape biogeographic characteristics (distance to the main island, island area) showed no significant effect ($p \geq 0.05$) on any of the different response variables; thus, they were dropped (data not shown).

4 | DISCUSSION

In this study, we show that grazing contributes to both vegetation and seed bank diversity in restored and remnant grassland habitats. Our results show that the establishment of grassland specialists in species-poorer restored former arable fields increases with the presence of grazing animals. This response of specialist species to grazing management and to the distances between remnant and restored grasslands suggests that some species are able to disperse into restored grasslands with the help of grazing animals. Furthermore, a positive effect of grazing also in remnant grassland sites suggests that the effects of grazing on species establishment are not simply due to enhanced spatial dispersal alone.

Nearby remnant grassland habitats can contribute to the colonisation of former arable fields by serving as a source for species establishment (Cousins and Lindborg, 2008). Although species adapted for dispersal via animal vectors occurred more frequently in remnant grasslands with grazing compared to those without grazing, similar patterns were also seen for species with an unassisted dispersal mechanism. Indeed, grazing animals increased all species in remnant grasslands and in restored sites independent of dispersal strategy (Table 1, Figure 6a). One explanation might be that propagules of non-zoochorous species can also be dispersed by livestock (Couvreur et al., 2004; Rico et al., 2014) and will establish in the vegetation and accumulate in the seed bank. On the other hand, most

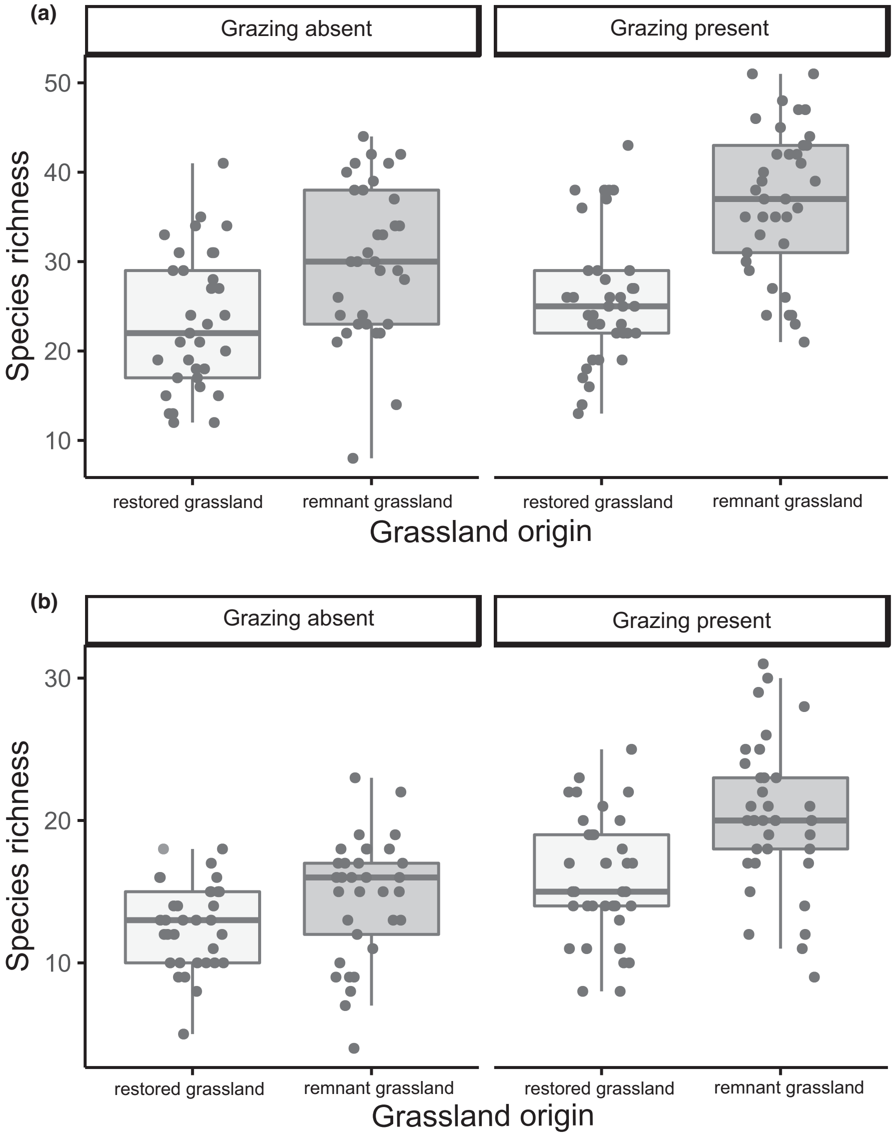

FIGURE 4 Box-plots of species richness in the vegetation (a) and in the seed bank (b) with the effect of grazing and the two grassland origin (remnant vs. restored grassland). Levels of significance can be found in Tables 1 and 2. Dots represent the species richness of the sampled plots (140)

grassland species are only capable of dispersing short distances and many rely on clonal spreading rather than propagule production (Thompson et al., 1997; Bossuyt and Honnay, 2008). This is also supported by our findings, as the number of species without specific biotic vectors dominated in the grasslands and the specialist species richness was limited by the distance between grassland origins. The latter mechanism implies that grassland species are dispersal-limited but that they are strongly dependent on grazing animals improving local conditions, holding competitive species in check and facilitating re-establishment (Tölgyesi et al., 2019).

Increasing distance between restored and remnant grasslands was associated with a lower species richness in the restored site (Table 1). However, this effect was only weak, which may be because there is little dispersal limitation within the restored grassland sites at these scales (i.e. they are in relatively close proximity to remnant populations in many cases). The positive effect of grazing is primarily due to its promotion of species establishment and enabling a build-up of a diverse seed bank. Distance to the grassland origins may not be the only possible factor controlling the level of dispersal limitation present in grasslands. Other landscape characteristics such as the total amount and proximity of other remnant grassland in the landscape or grazing management practices (e.g. stock density and rotation schedule among the islands) likely also influence the possibility of species reaching remnant and restored sites. Hence, the presence of grazing animals may add an important spatial dispersal effect in sites where the landscape species pool is relatively poor.

The restored grasslands are situated on former arable fields that were abandoned as crop fields several decades ago due to low economic profitability. In the archipelago, it was never possible to use heavy machinery on the farms, and thus, soils are not compacted and intensive fertilisation was not used. Hence, these former arable fields are likely to be highly suitable for grassland restoration, as otherwise typical restoration challenges such as high soil nutrient levels often hindering the re-establishment of grassland specialists are of limited concern (Smits et al., 2008). However, even in these relatively suitable conditions for restoration, establishment of grassland communities in restored sites only appears to occur slowly even after several years or decades of grassland management (Waldén and Lindborg, 2016)

TABLE 1 Results of the generalized linear models (GLM) or linear mixed models (LME†) testing for effects of grassland origin, grazing, distance between the paired plots on overall species richness, species groups (specialist, non-specialist), seed bank persistence (short-term/long-term) and dispersal mechanism (assisted/unassisted) in the vegetation. Values are parameter estimates from the models, *p*-values indicated with asterisks

	Overall species richness	Species groups		Dispersal mechanism		Seed bank persistence	
		Specialist	Non-specialist†	Assisted	Unassisted†	Short-term	Long-term
Intercept	3.238***	2.219***	16.561***	1.881***	17.509***	2.700***	2.178***
Distance between plots	−0.002	−0.007*	−0.001	0.0002	-0.044	−0.001	-0.005**
Non-grazed remnant × distance between plots	0.0004	0.007	−0.043	0.0003	-0.008	−0.001	0.005
Grazed restored × distance between plots	0.003	0.009**	0.021	0.002	0.063	0.002	0.006*
Grazed remnant × distance between plots	0.003	0.009**	−0.011	−0.0001	0.064	0.001	0.006**
Non-grazed remnant	0.240*	0.213	4.289**	0.401**	4.715**	0.346***	0.060
Grazed restored	−0.042	0.046	−1.779	0.189	−1.135	0.0001	−0.033
Grazed remnant	0.346***	0.583***	3.084	0.753***	4.228*	0.395***	0.335**
Number of species	206	71	135	65	125	136	46

Significance values: ***, p ≤ 0.001, **, 0.01 ≤ p< 0.001, *, 0.05 ≤ p <0.01.

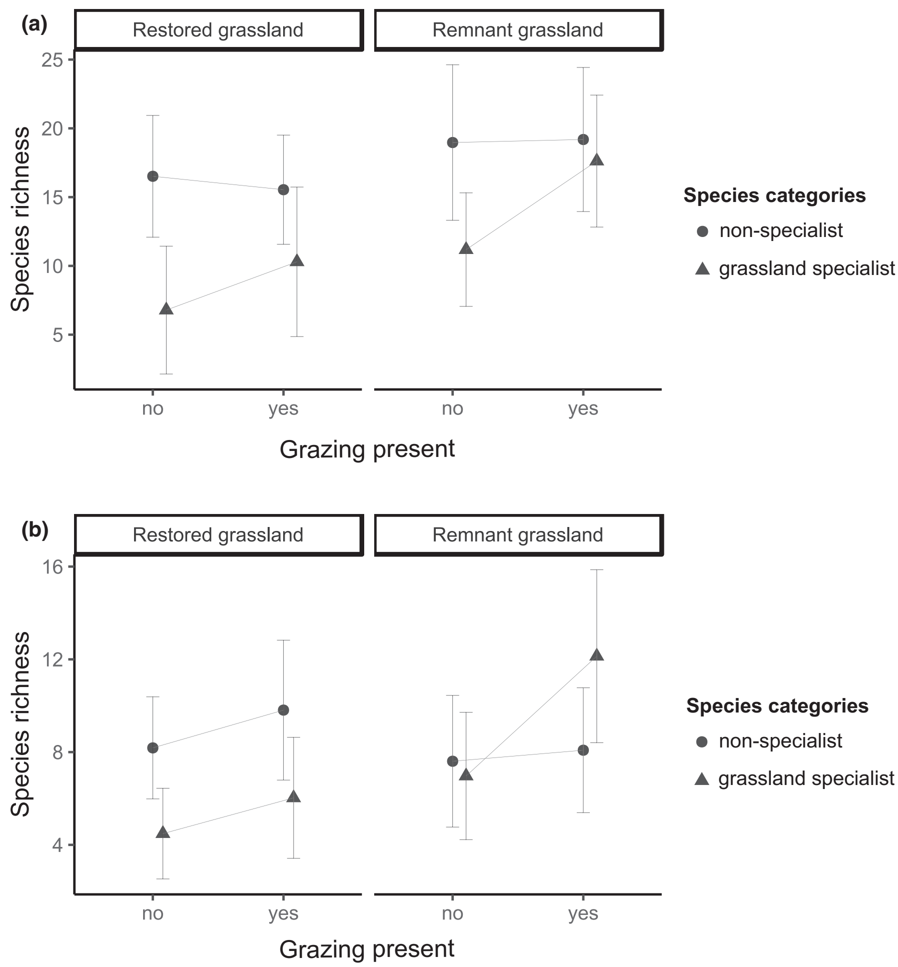

FIGURE 5 Species richness of two ecologically different species groups, non-specialist and grassland specialist, in the vegetation (a) and in the seed bank (b) in restored and remnant grasslands, both with or without grazing management. Data points are mean richness values for the groups. Error bars represent standard deviations. Levels of significance are shown in Tables 1 and 2

TABLE 2 Results of the generalized linear models (GLM) and linear mixed-effect models (LME, †) testing for the grassland origin, grazing, distance between the paired plots in terms of the species richness, species groups (specialist, Non-specialist), seed bank persistence (short-term/long-term) in the seed bank. Values are parameter estimates from the models, *p*-values indicated with asterisks

		Species groups		Seed bank persistence	
	Overall species richness	Specialist	Non-specialist	Short-term†	Long-term
Intercept	2.494***	1.503***	2.030***	5.790***	1.712***
Distance between plots	0.001	−0.0001	0.002	−0.010	0.003
Non-grazed remnant × distance between plots	−0.005*	−0.002	−0.008**	−0.009	−0.008**
Grazed restored × distance between plots	−0.0005	0.003	−0.003	0.014	−0.003
Grazed remnant × distance between plots	−0.001	0.0003	−0.002	0.003	−0.003
Non-grazed remnant	0.355***	0.507**	0.261	0.526	0.567***
Grazed restored	0.247**	0.177	0.297**	0.422	0.405**
Grazed remnant	0.518***	0.985***	0.084	2.074*	0.711***
Number of species	147	57	90	87	48

Significance values: ***, p ≤ 0.001, **, 0.01 ≤ p< 0.001, *, 0.05 ≤ p <0.01.

(Figure 3, Tables 1 and 2). For example, the presence of grazing animals continues to have a stronger effect on biodiversity in grassland remnants than in restored grasslands, because in grassland remnants the vegetation cover is less dense compared to restored grasslands (Appendix S2B). Hence, the competition for light and other resources in the vegetation between species is likely to be less intense; thus, more microsites are available for germination, recruitment and finally, establishment. The physical disturbances generated by the presence of grazing livestock may be more effective in allowing species to establish from surrounding vegetation and in activating seeds from the seed bank in grassland remnants than in restored sites (Bullock et al., 1994; Saatkamp et al., 2014). Moreover, we found that lack of grazing resulted in an increase in the dominance of non-specialist grassland species in both the restored grasslands and remnant grasslands (Figure 5, Table 1). While specialist species were a small part of the total species composition in both grasslands, their seeds did accumulate in the seed bank, especially where grazing was present and in remnant grasslands (Figure 6). These accumulated seeds may help maintain diversity until the local conditions become more favourable, and successful establishment can occur (cf. Auffret et al., 2017). The re-introduction of grazing will start to create suitable gaps in the vegetation, thus increasing the number of these species and enhancing the functional aspect of plant connectivity. In addition, as the remnant grasslands are often refuges for locally adapted specialist species (Cousins, 2006; Auffret and Cousins, 2011), grazing management offers a cost-effective way for restoration of species-poor grasslands (Pykälä, 2003; Tälle et al., 2016).

Furthermore, we found that grazing management increased the number of species in the seed bank and tended to homogenise the species composition between restored and remnant grasslands. A higher number of species, and thus an increased seed diversity in the vegetation in grazed grasslands, means more seeds which can potentially contribute to the seed bank, as long as grazing is not overly intensive so seed may set, and the disturbance and trampling activity of livestock may help these seeds to get integrated into the soil through gaps created on the surface (Jacquemyn et al., 2011; Klaus et al., 2018). Hence, long-term regular grazing may help create a positive feedback loop through which species are more able to establish initially in gaps created (either via spatial dispersal or from the soil seed bank) and are subsequently more able to regenerate from a more diverse seed bank. This may develop over relatively long timescales, possibly explaining the stronger positive effect of grazing management on both vegetation and seed bank diversity in remnant grasslands which have experienced a longer continuity of grazing management. In addition this implies that grazing animals help to build up a well-developed seed bank, which in turn provides resilience against disturbances (Plue and Cousins, 2013) or future climatic impacts (Kiss et al., 2018).

We conclude that grazing plays an important role in maintaining and increasing the biodiversity of remnant grasslands and supports the establishment of grassland species on restored sites. Although grazing has a positive effect on typical grassland specialist species richness, its influence differs depending on grassland habitat type (remnant vs. restored grassland) and where it occurs in the community (vegetation and seed bank). In the remnant grasslands, grazing has the strongest positive effect on grassland specialists relative to non-specialist species. Without the help of grazers, it is difficult for specialists to gain space in the dense vegetation dominated by non-specialist grassland species in restored grasslands, and the physical disturbances and biomass removal by grazing livestock may alter the species composition with time. Grazers thus have multiple roles in grasslands: they can serve as dispersal vectors for seeds and/or assist plant species' establishment by generating disturbances on the surface and restrict and/or favour other plant species. All roles are likely important in our study system, but the general positive effect of livestock grazing in all sites

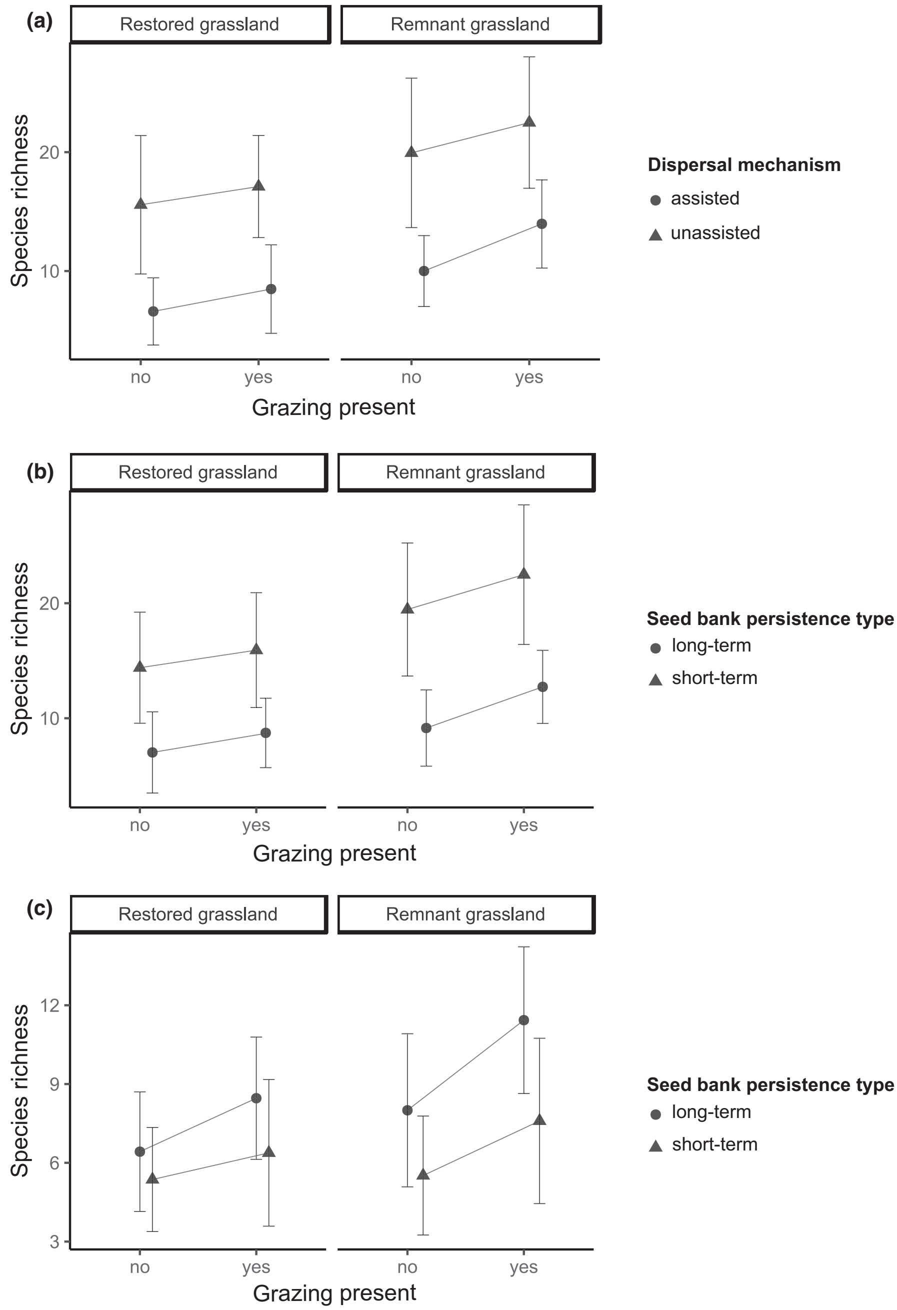

FIGURE 6 Species richness of plant species based on dispersal mechanism group (a) and seed bank type in the vegetation (b) and seed bank type in the seed bank (c) in restored and remnant grasslands, both with or without grazing management. Data points are mean species richness values for the groups. Error bars represent standard deviations. Levels of significance are shown in Tables 1 and 2

is clear. In the archipelago system, there may be some differences between restored and remnant sites, which means that some differences in species composition are inevitable regardless of management. However, it is apparent that restored sites will likely need a lot longer to recover to reflect the remnant grassland communities in the absence of grazing management. Hence, in sum, our study highlights the need for landscape-scale management (e.g. rotational grazing) to ensure the exchange of plant species among isolated habitat fragments in order to maintain plant biodiversity in fragmented landscapes.

ACKNOWLEDGEMENTS

Anne-Christine Troberg and Roel Schrauwen are thanked for their assistance in field and greenhouse work. The project was funded by the Foundation for Baltic and Eastern European Studies and the Oscar and Lili Lamm Memorial Foundation.

AUTHOR CONTRIBUTIONS

JP and SAOC conceived of the research idea, JP collected the data, REK performed the statistical analyses with contributions from JP and AK, and REK wrote the paper with significant inputs from all authors.

DATA AVAILABILITY STATEMENT

The dataset used for the analysis is available at Stockholm University´s data repository (Figshare). https://doi.org/10.17045/sthlmuni.12962963.

ORCID

Rozália E. Kapás https://orcid.org/0000-0001-7354-0575
Jan Plue https://orcid.org/0000-0002-6999-669X
Adam Kimberley https://orcid.org/0000-0002-0807-9943
Sara A.O. Cousins https://orcid.org/0000-0003-2656-2645

REFERENCES

Aavik, T., Jõgar, Ü., Liira, J., Tulva, I. and Zobel, M. (2008) Plant diversity in a calcareous wooded meadow—The significance of management continuity. *Journal of Vegetation Science*, 19(4), 475–484. https://doi.org/10.3170/2008-8-18380

Adriaens, D., Honnay, O. and Hermy, M. (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. *Biological Conservation*, 133(2), 212–224. https://doi.org/10.1016/j.biocon.2006.06.006

Aggemyr, E. and Cousins, S.A.O. (2012) Landscape structure and land use history influence changes in island plant composition after 100years. *Journal of Biogeography*, 39(9), 1645–1656. https://doi.org/10.1111/j.1365-2699.2012.02733.x

Albert, A., Mårell, A., Picard, M. and Baltzinger, C. (2015) Using basic plant traits to predict ungulate seed dispersal potential. *Ecography*, 38(5), 440–449. https://doi.org/10.1111/ecog.00709

Alexander, H.M., Foster, B.L., Ballantyne, F., Collins, C.D., Antonovics, J. and Holt, R.D. (2012) Metapopulations and metacommunities: Combining spatial and temporal perspectives in plant ecology. *Journal of Ecology*, 100(1), 88–103. https://doi.org/10.1111/j.1365-2745.2011.01917.x

Auffret, A.G. and Cousins, S.A.O. (2011) Past and present management influences the seed bank and seed rain in a rural landscape mosaic. *Journal of Applied Ecology*, 48(5), 1278–1285. https://doi.org/10.1111/j.1365-2664.2011.02019.x

Auffret, A.G., Plue, J. and Cousins, S.A.O. (2015) The spatial and temporal components of functional connectivity in fragmented landscapes. *Ambio*, 44(1), 51–59. https://doi.org/10.1007/s13280-014-0588-6

Auffret, A.G., Rico, Y., Bullock, J.M., Hooftman, D.A.P., Pakeman, R.J., Soons, M.B. *et al* (2017) Plant functional connectivity—Integrating landscape structure and effective dispersal. *Journal of Ecology*, 105, 1648–1656. https://doi.org/10.1111/1365-2745.12742

Auffret, A.G., Schmucki, R., Reimark, J. and Cousins, S.A.O. (2012) Grazing networks provide useful functional connectivity for plants in fragmented systems. *Journal of Vegetation Science*, 23(5), 970–977. https://doi.org/10.1111/j.1654-1103.2012.01413.x

Bossuyt, B. and Honnay, O. (2008) Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. *Journal of Vegetation Science*, 19(6), 875–884. https://doi.org/10.3170/2008-8-18462

Bullock, J.M., Hill, B.C., Dale, M.P. and Silvertown, J. (1994) An experimental study of the effects of sheep grazing on vegetation change in a species-poor grassland and the role of seedling recruitment into gaps. *The Journal of Applied Ecology*, 31(3), 493. https://doi.org/10.2307/2404445

Cousins, S.A.O. (2006) Plant species richness in midfield islets and road verges – The effect of landscape fragmentation. *Biological Conservation*, 127(4), 500–509. https://doi.org/10.1016/j.biocon.2005.09.009

Cousins, S.A.O., Auffret, A.G., Lindgren, J. and Tränk, L. (2015) Regional-scale land-cover change during the 20th century and its consequences for biodiversity. *Ambio*, 44, 17–27. https://doi.org/10.1007/s13280-014-0585-9

Cousins, S.A.O. and Eriksson, O. (2008) After the hotspots are gone: Land use history and grassland plant species diversity in a strongly transformed agricultural landscape. *Applied Vegetation Science*, 11(3), 365–374. https://doi.org/10.3170/2008-7-18480

Cousins, S.A.O. and Lindborg, R. (2008) Remnant grassland habitats as source communities for plant diversification in agricultural landscapes. *Biological Conservation*, 141(1), 233–240. https://doi.org/10.1016/j.biocon.2007.09.016

Couvreur, M., Christiaen, B., Verheyen, K. and Hermy, M. (2004) Large herbivores as mobile links between isolated nature reserves through adhesive seed dispersal. *Applied Vegetation Science*, 7, 229–236. https://doi.org/10.1111/j.1654-109X.2004.tb00614.x

Ekstam, U. and Forshed, N. (1997) *Om hävden upphör: Kärlväxter som indikatorarter i ängs- och hagmarker*. Stockholm: Naturvårdsverket.

Eriksson, O., Cousins, S.A.O. and Bruun, H.H. (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. *Journal of Vegetation Science*, 13, 743–748. https://doi.org/10.1111/j.1654-1103.2002.tb02102.x

Eriksson, O. and Ehrlén, J. (1992) Seed and microsite limitation of recruitment in plant populations. *Oecologia*, 91(3), 360–364. https://doi.org/10.1007/BF00317624

ESRI. (2017) *ArcGIS Desktop10.5.1*. Redlands, CA: ESRI.

Faust, C., Eichberg, C., Storm, C. and Schwabe, A. (2011) Post-dispersal impact on seed fate by livestock trampling – A gap of knowledge. *Basic and Applied Ecology*, 12(3), 215–226. https://doi.org/10.1016/j.baae.2011.02.009

Fischer, S.F., Poschlod, P. and Beinlich, B. (1996) Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. *The Journal of Applied Ecology*, 33(5), 1206. https://doi.org/10.2307/2404699

Jacquemyn, H., Mechelen, C.V., Brys, R. and Honnay, O. (2011) Management effects on the vegetation and soil seed bank of calcareous grasslands: An 11-year experiment. *Biological Conservation*, 144(1), 416–422. https://doi.org/10.1016/j.biocon.2010.09.020

Kiss, R., Deák, B., Török, P., Tóthmérész, B. and Valkó, O. (2018) Grassland seed bank and community resilience in a changing climate. *Restoration Ecology*, 26(S2), S141–S150. https://doi.org/10.1111/rec.12694

Klaus, V.H., Schäfer, D., Prati, D., Busch, V., Hamer, U., Hoever, C.J. *et al* (2018) Effects of mowing, grazing and fertilization on soil seed banks in temperate grasslands in Central Europe. *Agriculture, Ecosystems & Environment*, 256, 211–217. https://doi.org/10.1016/j.agee.2017.11.008

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M. *et al* (2008) The LEDA Traitbase: A database of life-history traits of the Northwest European flora. *Journal of Ecology*, 96(6), 1266–1274. https://doi.org/10.1111/j.1365-2745.2008.01430.x

Lindborg, R. (2007) Evaluating the distribution of plant life-history traits in relation to current and historical landscape configurations. *Journal of Ecology*, 95(3), 555–564. https://doi.org/10.1111/j.1365-2745.2007.01232.x

Mitlacher, K., Poschlod, P., Rosén, E. and Bakker, J.P. (2002) Restoration of wooded meadows—A comparative analysis along a chronosequence on Öland (Sweden). *Applied Vegetation Science*, 5(1), 63–73. https://doi.org/10.1111/j.1654-109X.2002.tb00536.x

Mossberg, B. and Stenberg, L. (2003) *Den nya nordiska floran*. Sweden: Wahlström & Widstrand.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. *et al* (2019) vegan: Community Ecology Package (2.5-6) [Computer software]. https://CRAN.R-project.org/package=vegan

Pakeman, R.J. and Small, J.L. (2005) The role of the seed bank, seed rain and the timing of disturbance in gap regeneration. *Journal of Vegetation Science*, 16(1), 121–130. https://doi.org/10.1111/j.1654-1103.2005.tb02345.x

Pärtel, M., Mändla, R. and Zobel, M. (1999) Landscape history of a calcareous (alvar) grassland in Hanila, western Estonia, during the last three hundred years. *Landscape Ecology*, 14(2), 187–196. https://doi.org/10.1023/A:1008040114832

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S. and Van Willigen, B. (2020) nlme: Linear and Nonlinear Mixed Effects Models

(3.1-148) [Computer software]. https://CRAN.R-project.org/package=nlme

Plue, J. and Cousins, S.A.O. (2013) Temporal dispersal in fragmented landscapes. *Biological Conservation*, 160, 250–262. https://doi.org/10.1016/j.biocon.2013.02.010

Plue, J. and Hermy, M. (2012) Consistent seed bank spatial structure across semi-natural habitats determines plot sampling. *Journal of Vegetation Science*, 23(3), 505–516. https://doi.org/10.1111/j.1654-1103.2011.01361.x

Pykälä, J. (2003) Effects of restoration with cattle grazing on plant species composition and richness of semi-natural grasslands. *Biodiversity & Conservation*, 12(11), 2211–2226. https://doi.org/10.1023/A:1024558617080

R Core Team (2019) *R: A Language and Environment for Statistical Computing (R version 3.5.3) [Computer software]*. Vienna: R Core Team.

Rico, Y., Holderegger, R., Boehmer, H.J. and Wagner, H.H. (2014) Directed dispersal by rotational shepherding supports landscape genetic connectivity in a calcareous grassland plant. *Molecular Ecology*, 23(4), 832–842. https://doi.org/10.1111/mec.12639

Rook, A.J., Dumont, B., Isselstein, J., Osoro, K., WallisDeVries, M.F., Parente, G. *et al* (2004) Matching type of livestock to desired biodiversity outcomes in pastures – a review. *Biological Conservation*, 119(2), 137–150. https://doi.org/10.1016/j.biocon.2003.11.010

Saatkamp, A., Poschlod, P., andVenable, D.L. (2014) The functional role of soil seed banks in natural communities. In: Gallagher, R.S. (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities*, 3rd edition. Wallingford: CABI, pp. 263–295. https://doi.org/10.1079/9781780641836.0263

Smits, N., Willems, J.H. and Bobbink, R. (2008) Long-term after-effects of fertilisation on the restoration of calcareous grasslands. *Applied Vegetation Science*, 11(2), 279–286. https://doi.org/10.3170/2008-7-18417

Tälle, M., Deák, B., Poschlod, P., Valkó, O., Westerberg, L. and Milberg, P. (2016) Grazing vs. mowing: A meta-analysis of biodiversity benefits for grassland management. *Agriculture, Ecosystems & Environment*, 222, 200–212. https://doi.org/10.1016/j.agee.2016.02.008

Ter Heerdt, G.N.J., Verweij, G.L., Bekker, R.M. and Bakker, J.P. (1996) An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. *Functional Ecology*, 10(1), 144–151.

Thompson, K., Bakker, J.P. & Bekker, R.M. (1997) *The Soil Seed Banks of North West Europe: Methodology, Density and lonGevity*. Cambridge: Cambridge University Press.

Tölgyesi, C., Török, P., Kun, R., Csathó, A.I., Bátori, Z., Erdős, L. *et al* (2019) Recovery of species richness lags behind functional recovery in restored grasslands. *Land Degradation & Development*, 30(9), 1083–1094. https://doi.org/10.1002/ldr.3295

Vandvik, V. and Goldberg, D.E. (2006) Sources of diversity in a grassland metacommunity: quantifying the contribution of dispersal to species richness. *The American Naturalist*, 168(2), 157–167. https://doi.org/10.1086/505759

Waldén, E. and Lindborg, R. (2016) Long term positive effect of grassland restoration on plant diversity—success or not? *PLoS One*, 11(5), e0155836. https://doi.org/10.1371/journal.pone.0155836

Watson, J.E.M., Jones, K.R., Fuller, R.A., Marco, M.D., Segan, D.B., Butchart, S.H.M. *et al* (2016) Persistent disparities between recent rates of habitat conversion and protection and implications for future global conservation targets. *Conservation Letters*, 9(6), 413–421. https://doi.org/10.1111/conl.12295

Wilson, J.B., Peet, R.K., Dengler, J. and Pärtel, M. (2012) Plant species richness: the world records. *Journal of Vegetation Science*, 23(4), 796–802. https://doi.org/10.1111/j.1654-1103.2012.01400.x

Wit, S.W. and Schwabe, A. (2010) The fate of sheep-dispersed seeds: plant species emergence and spatial patterns. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 205(10), 656–665. https://doi.org/10.1016/j.flora.2010.04.010

Zuur, A.F., Ieno, E.N. and Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. New York: Springer.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. List of recorded species in the vegetation and seed bank and their Investigated traits throughout the 27 islands

Appendix S2. Differences in the grassland area and vegetation cover in the study system

How to cite this article: Kapás RE, Plue J, Kimberley A, Cousins SAO. Grazing livestock increases both vegetation and seed bank diversity in remnant and restored grasslands. *J Veg Sci*. 2020;31:1053–1065. https://doi.org/10.1111/jvs.12956