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Effects of past fragmentation and habitat loss and current management methods on the changes in vascular plant communities.
– An evaluation of extinction debt in semi-natural grasslands in Sweden

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Abstract

Habitat loss and fragmentation are believed to be two of the main reasons for high extinction rates of species, resulting in decreased biodiversity. According to the island biogeography theory, the species richness in a patch, here a semi-natural grassland, is dependent on the landscape composition, and therefore changes in the landscape composition will result in changes in the species richness of the grassland. However, this change in species richness may be delayed for several years, causing an extinction debt. The aim of this study was to examine the change of species richness of vascular plants in Swedish semi-natural grasslands between 2007 and 2020 and investigate if there is evidence of an extinction debt and evaluate what factors causes changes in the plant community. Data of species richness and occurrence for 40 semi-natural grasslands, as well as data of landscape changes in area and connectivity between the 1950:s and the 2000:s for these grasslands, were analysed. This study found that changes in species richness in semi-natural grasslands were affected by the changes in connectivity of the landscape. However, the effect depended on the degree of specialisation of the species to semi-natural grassland. Between 2007 and 2020, the species richness of semi-natural grasslands specialist decreased, while the species richness of non-specialist species increased. This resulted in a mean increase of overall species richness between 2007 and 2020. Observed immigration of new non-specialist species appears to suggest that, not only the connectivity, but also the habitat types in the matrix surrounding the semi-natural grasslands may substantially influence the species composition in the grassland; this is in contrast to what is predicted by the original theory of island biogeography. Species that were classified as specialist were more vulnerable to ceased management, such as grazing, than to area and connectivity decrease. This was likely because the ceased management increased the competition for light. The results also indicated that re-established management of abandoned grasslands may increase specialist species richness, highlighting the need for management actions taken in order to reverse extinction debt.

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1. Introduction

1.1 Biodiversity in semi-natural grasslands

Species are going extinct at record high rates and some experts express concerns about a new mass extinction (Pimm *et al.* 2014). One of the main reasons of this is habitat loss, especially in habitats with a high biodiversity (Brooks *et al.* 2002).

A large part of the biodiversity in Sweden is found in the agricultural landscape. About half of all the vascular plants in the country can be found in agricultural habitats and approximately the same proportion goes for mammals (Bernes 2011). Among insects such as butterflies, bees and bumblebees there are many species that exclusively live in agricultural habitats (Bernes 2011). However, a large part of the diversity in the agricultural landscape is concentrated to remnant patches where the land is still managed with traditional methods (Eriksson 2021).

Semi-natural grasslands are one of the most species-rich agricultural habitats (Bernes 2011). This type of habitat has a long history of grazing or mowing, management methods where the nutrients is continuously removed from the habitat, resulting in a nutrient-poor environment. The nutrient-poor environment is believed to be one of the main factors contributing to the high species diversity in semi-natural grasslands (Hansson & Fogelfors 2000). Species growing in this habitat are adapted to the low nutrient conditions. If the habitat becomes too nutrient rich more competitive species can take over, causing the resident species to decline in number and disappear (Römermann *et al.* 2009). Grazing and mowing also keeps the landscape open. If management ceases, species will need to compete for the sunlight, resulting in the extinction of less competitive species. The management of semi-natural grasslands therefore benefit species that require a lot of sunlight and prevent more competitive species to take over, resulting in a higher biodiversity. A study done by Pykälä et al. (2005) found that species richness in semi-natural grasslands decreases with time after ceased management (Pykälä *et al.* 2005). Plant species commonly found in semi-natural grasslands often show one or more adaptations to grazing and/or mowing (Römermann *et al.* 2009). An example of this would be low, creeping plants that are not affected by mowing, plants with thorns and spines that keep grazers away and plants that fruit early before the grazing starts (Bernes 2011).

The traditional methods of grazing and mowing are not considered effective by todays standard and a lot of the semi-natural grasslands have been converted into more productive and cost-effective arable land (Hansson & Fogelfors 2000). The total area of semi-natural grasslands in Sweden decreased by 96% during the 20th century (Cousins *et al.* 2015). Another large contribution to this decrease is the conversion of grasslands for forestry (Cousins *et al.* 2015). The few remaining semi-natural grasslands are often abandoned and as a result become more nutrient rich and overgrown, creating a new type of habitat. A study by Hansson and Fogelfors (2000) found that semi-natural grasslands of wooded meadow type developed a full canopy of deciduous forest in 15 years after management ceased. Abandoned grasslands as well as conversion of grasslands for other types of land use results in small remaining patches of biodiversity with decreased connectivity (Rosqvist 2005). It is of interest for nature conservation to preserve these grasslands by maintaining regular disturbances such as grazing (Römermann *et al.* 2009).

1.2 Island biogeography

One of the primary threats to biodiversity is fragmentation of the landscape (Rosqvist 2005). Fragmentation is a process where a habitat is divided into smaller patches with a matrix of another habitat type surrounding the remaining patches (Fahrig 2003). It is common that the surrounding matrix is unhabitable for species living in the original habitat which can make it harder for them to migrate between the remaining patches (Saunders *et al.* 1991). Examples of fragmentation is when a road is built through a grassland, splitting the habitat, or when parts of a forest is cut down in order use the land for agriculture. Another process often coupled with fragmentation is habitat loss, which is when the total area of the habitat type decreases (Fahrig 2003). The aftermath of the fragmentation and habitat loss processes depends on factors such as area of the remaining patches, time since isolation of patches and the amount of connectivity between patches (Saunders *et al.* 1991).

According to the island biogeography theory, the species richness in a patch surrounded by a matrix of another habitat has a positive relationship with the area and a positive relationship with the connectivity (MacArthur & Wilson 1967). There are two main ecological processes that create the species-area relationship (Rybicki & Hanski 2013). The first is that habitat heterogeneity increases with increasing habitat area. This allows for a lager diversity of species to inhabit the habitat (Rybicki & Hanski 2013). The second process is that a larger habitat area increases the probability of population survival, and therefore also the survival probability of individual species is higher (Rybicki & Hanski 2013). Larger habitats often support larger populations, which tends to be more stable and less vulnerable for stochastic events, resulting in a decreased probability of extinction (MacArthur & Wilson 1967, Hanski 1994, Rösch *et al.* 2013). Among several studies that have tested this, a study performed in semi-natural grasslands in Sweden by Vessby et al. (2002) found that species richness of vascular plants, as well as the species richness of birds, showed a positive relationship with the area of the grassland.

The positive relationship between species richness and connectivity can be explained by the dispersal distance of the species. Connectivity facilitates the spread of species populations and improves their genetic exchange. A poor connectivity can lead to extinction of populations due to inbreeding and loss of genetic diversity (Hejkal *et al.* 2017). A smaller habitat that only supports smaller populations lead to a higher extinction risk, especially for species with poor dispersal ability as isolated patches experience local extinction that may not easily be reversed by re-colonization of the same species from a close-by habitat (Cousins *et al.* 2007).

MacArthur and Wilson (1967) argued that the number of species in a habitat is an equilibrium between the number of species colonizing the habitat (immigration rate) and number of species leaving the habitat (extinction rate) (Figure 1). If these parameters change, the number of species present in the habitat will change. If we assume that the extinction rate depends on the habitat area and that the immigration rate depends on the connectivity, it follows that changes in either habitat area or connectivity will result in the species richness reaching a new equilibrium. A decrease in habitat area will cause the extinction rate to rise, resulting in a steeper extinction curve (Figure 1). A steeper extinction rate curve results in a new equilibrium with fewer species in the habitat. In the same way, if the connectivity of the patch decreases the immigration rate will decrease, resulting in a flatter immigration rate curve. This causes the equilibrium to move further to the left, which means less species present in the habitat.

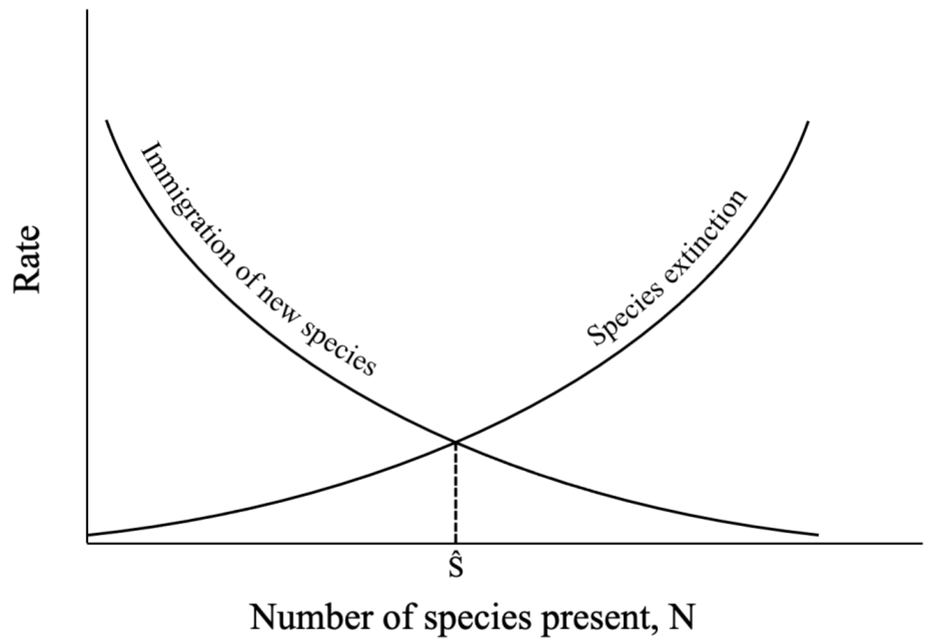

Figure 1. Equilibrium model of the species richness. The equilibrium number of species is reached at the intersection of the extinction rate curve and the immigration rate curve. (After MacArthur and Wilson, 1967)

1.3 A delayed equilibrium

After a change in the environment, such as decreased area or connectivity, there is a time lag before the species richness will respond to the new conditions and reach the new equilibrium (Ovaskainen & Hanski 2002). A population's survival depends on the relationship between the mortality rate, the natality and the immigration rate. If the mortality rate is higher than the natality and immigration rate combined, the population will decrease in numbers and eventually go extinct (if the rates remain constant) (Jackson & Sax 2010). How long this will take depends on the generation time of the species and on the difference between the mortality rate and the combined natality and immigration rate combined (Jackson & Sax 2010). This entails that populations of species that generally have a long lifespan (such as trees) can persevere in a changed environment for decades, even if the change in environment has made reproduction and immigration impossible. Because immigration rate also affects the survival of the population, populations living in isolated habitat often go extinct sooner than those living in less isolated habitats after a change in the environment. This is because newly immigrated individuals can compensate for deceased individuals in the patch (Kuussaari *et al.* 2009, Hylander & Ehrlén 2013). Eventually, when the last individuals of a population die and are not replaced by new recruits, the whole population goes extinct. The number of species that will eventually go extinct as a result of the change in environment are referred to as an extinction debt. This means that after a change in the landscape composition, even though few changes can be observed immediately, in a longer time scale there will be species that disappear and populations that decrease in size (Hylander & Ehrlén 2013). For long-lived species the time lag to extinction can be several hundred years.

Changes in the landscape composition can also bring new species. The time it takes for new species to establish depends on several factors such as dispersal success, survival and reproduction success, both short and long term (Jackson & Sax 2010). If one of these steps fails or is delayed, the arrival of new species may be delayed or absent. The success rate of dispersal is determined by the connectivity in the landscape and the dispersibility of the species (Jackson & Sax 2010). It takes time for the number of species in a habitat to reach a new equilibrium due to the delayed establishment of new species and the extinction debt. This often results in a species composition that better reflects the historical environment than the current one (Bommarco *et al.* 2014). The idea of a delayed extinction present after a change in the landscape has been confirmed by several studies. Helm *et al.* (2005) found that the number of habitat specialists present in Estonian calcareous grasslands was explained by the grasslands' area and connectivity 70 years ago and not by the current area and connectivity. Evidence of an extinction debt has also been found in semi-natural grasslands in Sweden where changes in connectivity is believed to have contributed to a delayed extinction (Lindborg & Eriksson 2004). A study done by Bommarco *et al.* surveyed 45 semi-natural grasslands and found that the species richness of vascular plants was better explained by the connectivity in the landscape 50 years ago rather than the current connectivity. Since the area and connectivity of the grasslands has decreased during the last 50 years, they argued that the results indicated a delay in plant species extinctions, an extinction debt (Bommarco *et al.* 2014). Another study by Otsu *et al.* (2017) found that there was a time lag between habitat loss and extinction of species that were grassland specialist. They also found that the impact of habitat loss on both extinction rates and colonization rates of specialist species can be reduced by management.

1.4 Climate change

Another influence on plant community composition is the climate (Auffret & Thomas 2019). Climate change can alter the abiotic conditions of a habitat making it harder or impossible for some species to live there. Several studies have shown that species distributions tend to move further north and/or to higher altitudes as a result of a warming climate (Parmesan & Yohe 2003, Parmesan 2007, Dirnböck *et al.* 2011, Auffret & Thomas 2019). As the distribution of species moves further north, an extinction debt is produced at the trailing edge while new colonisations occur at the leading edge (Figueiredo *et al.* 2019). This is believed to be the reason to the decrease in species with northerly distribution limits, they simply can't move much further north (Tyler *et al.* 2018). A study of the Swedish flora found that species that are associated with warmer and wetter conditions make up an increasingly larger proportion of species in plant communities as a result of a shift in climate since the early-mid 20th century (Auffret & Thomas 2019).

1.5 Aim of the study

The aim of this study was to analyse if changes in plant species richness in semi-natural grasslands between 2007 and 2020 can be explained by changes in area and connectivity of the grasslands between the 1950:s and the 2000:s, and thereby testing if there is an extinction debt present. This was done by analysing data of plant species collected in 40 semi-natural grasslands in 2007 and 2020 respectively. These were the same grasslands where Bommarco *et al*. (2014) found indications of an extinction debt. Based on the theoretical foundation of island biogeography theory and extinction debt, as described above, and on the previous results from the study by Bommarco et al., it was expected that patches with a larger decrease in area and connectivity will have a greater decrease in species richness. The present study also analysed the significance of grazing on the change in species richness. It was expected that ceased grazing will affect the species richness in the habitat negativity, especially with a decrease in species adapted to grazing. Lastly, the effects of global warming on species survival were analysed. As species with more northern distributions has been proven to be more vulnerable to global warming, it was anticipated that those species will have decreased the most.

2. Methods

2.1 Obtained data

Species- and landscape data was obtained from previous surveys done in 2007 and 2020. The data from the survey performed in 2007 had previously been published in a study by Bommarco et al. (2011).

The species data had been collected through an inventory of vascular plant communities in 40 semi-natural grasslands in 2007 and in 2020. Of these 40 semi-natural grasslands, 22 was located in Östergötland and 18 in Uppland. The sites were selected in order to get a gradient in both the area of the focal grasslands and in the proportion of semi-natural grasslands in the surrounding landscape. Ten randomly placed plots with an area of $1m^2$ were surveyed in each grassland. The plots were not placed in the exact same spot in each grassland in 2007 and in 2020. Additional species, not present in the ten plots, were recorded by walking though the semi-natural grassland in a standardized pace. It was also noted which grasslands were grazed at the time of the inventory. The surveying was done from late June to mid-august each year.

The obtained landscape data for each grassland, comprised of estimates of grassland area in the early 2000:s, as well as estimates of the historical area of the grasslands from the 1950:s. Measurements of the area of surrounding grasslands in a 2 km radius from the grassland studied were also obtained. The collected data had been estimated using aerial photographs taken 1952 to 1960and 1999 to 2005.

2.2 Calculations and classifications made from the obtained data

It was found that differences in inventory techniques among regions and person performing the inventory each year, when it came to recording additional species by walking through the grasslands, resulted in wildly different number of recorded species. It was therefore decided that, to obtain reliable results, this study will restrict itself to the analysis of the data recorded in the ten plots in each grassland as this method was more standardized and therefore display less variation among the different people doing the inventories.

From the retrieved data, differences in the number of species in each grassland between 2007 and 2020 was calculated, as well as the change in area of the grassland and change in connectivity from the 1950:s to the 2000:s. The calculated data was used as measurements of difference in species richness, difference in area and difference in connectivity. The difference in species occurrence was defined and calculated as the difference in how many grasslands each species was recorded in between 2007 and 2020.

To analyse if species adapted to semi-natural grasslands was more prone to extinction than species that were not adapted to semi-natural grasslands, each plant species was classified as being either a semi-natural grassland specialist or a non-semi-natural grassland specialist. This classification was based on data from Tyler *et al.* (2021). Species that "demands repeated/continuous grazing/mowing", are "strongly favoured by grazing/mowing and disappears within a few years if management ceases" and species that are "strongly favoured by regular grazing/mowing, but endures some years without management" were classified as semi-natural grassland specialists. Species that did not need grazing in order to survive or do not endure grazing were classified as non-semi-natural grassland specialists. Eleven species couldn't be classified to be a semi-natural grasslands specialist or not because of a lack of data.

In order to test if the consequences of climate change affects the plant community composition in semi-natural grasslands, an estimate of the mean annual temperature for the distribution of each species was retrieved from Auffret & Thomas (2019). The mean annual temperature for each species distribution will here on be referred to as species temperature index. Nine species lacked values for species temperature index.

2.3 Statistical analysis

The compiled data was analysed in R-studio (RStudio Team 2021). For all statistical tests, a 95% confidence interval was used as the significance level. The difference in species richness, species richness of specialist species and species richness of non-specialist species in each grassland between 2007 and 2020 was analysed using a t-test. Two-sample Wilcoxon tests were used to confirm that the change in grassland area as well as change in connectivity was not associated with the presence of grazers.

To test if the change in species richness could be explained by the changes in area, connectivity or the management methods a Generalised Linear Model (GLM) was applied. After evaluation of several different GLMs, the standard linear model with normal residual distribution was chosen as the GLM used in the analyses in this study. A GLM tests if the response variable depends on one or several of the predictors included in the model. Here the response variable is the difference in species richness between 2007 and 2020 for each grassland. The numerical predictors used in the model were the difference in area of the grassland and the difference in connectivity. The categorical predictors used in the model were the presence of grazers in 2007 and 2020, each factor with two levels, present and absent. The GLM used included interaction between the difference in area and the difference in connectivity as well as interaction between the presence of grazers in 2007 and the presence of grazers in 2020. The residuals were checked for normality using a QQ-plot.

Similarly, a GLM was used to test if the difference in species richness of specialist species between 2007 and 2020 can be explained by the same four predictors; difference in area, difference in connectivity, presence of grazers in 2007 and presence of grazers in 2020. The model used included interaction between difference in area and difference in connectivity as well as interaction between presence of grazers in 2007 and presence of grazers in 2020. Using the same predictors as previously mentioned, the difference in species richness of non-specialist species between 2007 and 2020 was analysed. The residuals of both models were checked for normality using a QQ-plot.

The presence of a relationship between the difference in species occurrence between 2007 and 2020 the species' characteristics, was tested using a GLM. Here the difference in species occurrence was set as the response variable. The species temperature index and the classification of specialists were used as predictors. The last one being categorical with two levels, semi-natural grassland specialist and non-semi-natural grassland specialist. In this analysis the seventeen species lacking data for mean temperature of species distribution and/or data for classification of specialists were excluded. The residuals of this model were checked for normality using a QQ-plot.

3. Results

3.1 Observed differences in species richness, grassland area and connectivity

In total, 285 different species of vascular plants were recorded among the different grasslands and years. In 2007, a total of 197 species was found across all 40 grasslands (Table 1). In 2020, 234 species were found in total. The mean species richness per grassland had significantly ($t = -3.18$, $df = 76.4$, $p = 0.002$) increased between 2007 (52) and 2020 (59). Taking grassland specialization into account, the mean number of species, classified as specialists per grasslands had decreased; it was 16 in 2007 and 14 in 2020, this was border-line significant ($t = 1.918$, $df = 77.941$, $p = 0.059$). In contrast, the mean number of non-specialist species per grassland had significantly increased, 36 in 2007 and 44 in 2020 ($t = -5.304$, $df = 75.617$, $p < 0.001$).

The mean decrease in area of the grasslands between the 1950:s and the 2000:s was -1.4 ha and the mean decrease in connectivity was -42.8 ha. No significant difference was found between the difference in area of the grasslands with grazers present in 2007 and grasslands without grazers in 2007 ($W = 115$, $p = 1$). Furthermore, no significant difference was found between the difference in area of the grasslands that were grazed 2020 and the grasslands without grazers in 2020 ($W = 145$, $p = 0.671$). Lastly, no significant difference could be found for change in connectivity between grazed and un-grazed grasslands, neither in 2007 ($W = 104$, $p = 0.701$) nor in 2020 ($W = 185$, $p = 0.455$).

Table 1. The area and connectivity for each grassland in the 1950:s and 2000:s as well as the presence of grazers and species richness in 2007 and 2020 for each grassland. The grasslands are named as they were in the study by Bommarco et al. (2011). In the present day study the names for each grassland can be disregarded.

Semi-natural grassland	Area 1950:s (ha)	Area 2000:s (ha)	Connectivity 1950:s (ha)	Connectivity 2000:s (ha)	Grazers 2007	Grazers 2020	Species richness 2007	Species richness 2020
A02	4.41	3.88	47.37	20.95	present	present	36	50
A03	3.86	3.53	32.84	13.34	present	present	57	76
A04	6.92	4.84	51.22	14.09	present	present	36	63
A05	5.36	4.77	44.10	28.18	present	present	40	61
A06	11.62	10.81	34.32	16.91	present	present	51	61
A07	5.32	4.19	32.34	21.22	present	present	47	60
A08	8.07	3.97	23.35	11.16	present	present	51	40
A09	3.94	3.82	71.17	48.84	absent	present	40	48
A10	5.61	4.35	58.20	26.39	absent	absent	65	49
A11	4.38	4.25	87.94	44.33	absent	present	42	55
A12	8.15	7.62	106.65	76.52	present	present	60	71
A13	12.03	11.96	101.76	50.53	present	present	44	46
A15	3.6	3.55	81.41	43.44	present	present	44	50
M01	3.64	1.88	69.37	33.33	present	absent	52	64
M02	4.43	4.27	50.22	24.83	present	present	41	56
M03	6.85	6.09	88.64	55.71	present	present	45	47
M04	6.16	4.88	54.25	27.74	present	absent	33	52
M05	10.42	6.92	73.47	44.19	absent	present	47	56
M07	5.07	5.07	140.57	95.66	present	present	56	59
M08	2.68	2.68	64.43	46.01	present	absent	45	44
M09	1.93	1.91	183.98	122.54	absent	present	49	72
M10	2.12	2.11	85.27	49.84	present	present	48	46
M12	6.92	3.18	102.32	48.33	present	present	63	73
M13	7.12	4.12	225.96	125.37	present	present	65	56
M14	5.35	4.55	183.03	126.35	present	absent	57	64
M15	1.98	1.85	260.73	185.42	present	absent	53	60
F01	2.02	1.86	105.02	19.59	present	present	47	59
F02	3.65	1.29	86.92	24.23	present	absent	64	61
F03	21.91	3.96	93.05	24.56	present	present	65	77
F04	3.49	3.37	110.97	45.95	present	present	61	64
F05	4.44	4.44	41.38	12.88	present	present	54	62
F06	3.63	3.01	59.90	27.20	absent	absent	37	52
F07	2.28	2.25	79.52	39.31	present	absent	55	59
F08	3.05	2.85	67.54	36.00	absent	present	52	62
F09	4.65	4.65	200.56	75.27	present	absent	82	75
F10	3.5	3.5	51.50	23.71	present	absent	60	60
F11	11.15	5.3	96.96	62.12	present	present	54	56
F12	12.12	11.73	104.08	75.30	present	present	37	53
F13	5.37	4.53	119.15	75.72	present	present	60	69
F15	4.65	4.65	140.24	89.69	present	present	66	54

3.2 Relation between overall species richness and area, connectivity and grazing predictors

While no relationship between the change in area of the grassland and the change in species richness for each grassland could be found between 2007 and 2020, there was a suggestive, border-line significant, relationship between the difference in connectivity between the 1950:s and the 2000:s and the difference in species richness, such that grasslands with less decrease in connectivity had a larger increase in species (Table 2, Figure 2). Similarly, there was a suggestive, but non-significant, positive relationship between the presence of grazers in 2020 and the difference in species richness in each grassland (Table 2), such that grazed sites often gained more species than ungrazed sites (Figure 3), while presence of grazers in 2007 had no significant influence on the change in species richness in the grasslands (Table 2).

There was no significant relationship between the interaction between area and connectivity and the difference in species richness, nor between the interaction between the presence of grazers in 2007 and 2020 and the difference in species richness (Table 2).

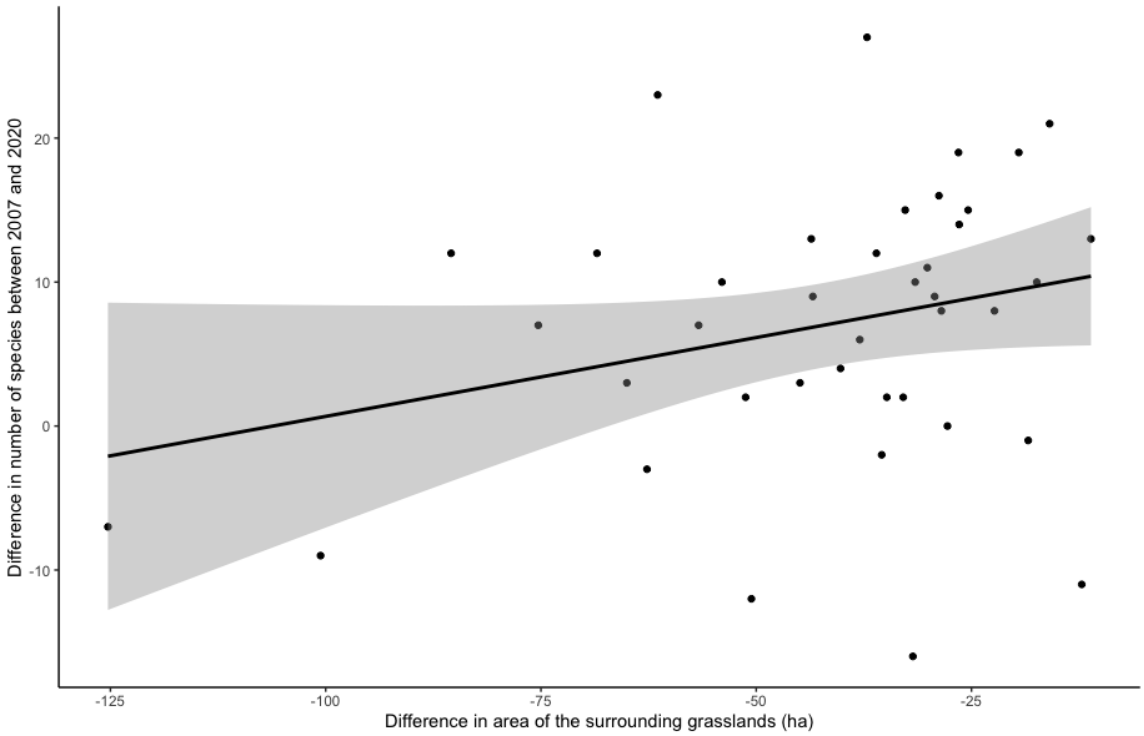

Figure 2. The positive relationship between the difference in species richness between 2007 and 2020 and the difference in connectivity between the 1950:s and the 2000:s. The grey area shows the 95% confidence interval for the regression line.

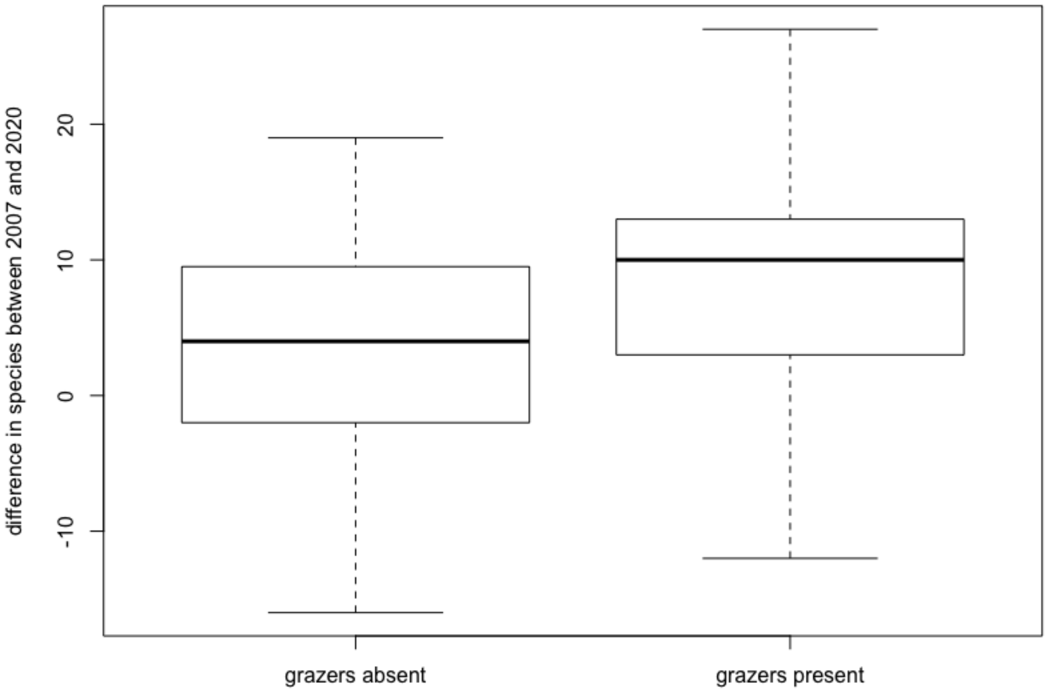

Figure 3. Grasslands that were grazed in 2020 gained more species than the grasslands that were not grazed.

3.3 Relation between species diversity of specialist/non-specialist species and area, connectivity and grazing predictors

Focusing firstly on semi-natural grassland specialists, there was a fairly strong, significant positive relationship between the presence of grazers in 2020 and the difference in species richness of specialist species (Table 2); the absence of grazers resulted in more species lost (Figure 4). In addition, a negative, borderline statistically significant, relationship was found between the interaction between the presence of grazers in 2007 and 2020 and the difference in specialist species richness (Table 2) (Figure 5). No significant relationship was found in terms of difference in species richness of specialist species for neither difference in area, difference in connectivity, presence of grazers in 2007 nor the interaction between the difference in area and connectivity (Table 2).

Non-specialist species richness increased significantly with the decrease of area, as well as with the difference in connectivity (Table 2). The number of specialist species increased with a lesser decrease in area and connectivity. Moreover, the interaction between the difference in area and the difference in connectivity had a positive effect on the difference in species richness of non-specialist species (Table 2, Figure 6). The presence of grazers appeared to have no effect on the species richness of non-specialist species, neither in 2007, 2020, nor as the interaction between the two (Table 2).

Table 2. Result from the different generalized linear models with (a) difference in species richness between 2007 and 2020 as the response variable, (b) difference in species richness of specialist species between 2007 and 2020 as the response variable, (c) difference in species richness of non-specialist species between 2007 and 2020 as the response variable. Statistically significant p-values in bold. Connectivity was measured as the area of surrounding grasslands in a 2 km radius. The difference in area and difference in area of the surrounding grasslands was calculated between the 1950:s and the 2000:s.

Predictor	t	df	p
(a)			
Difference in area (ha)	1.088	39	0.285
Difference in connectivity (ha)	1.992	39	0.055
Difference in area (ha) : Difference in connectivity (ha)	1.181	39	0.246
Presence of grazers in 2007	0.933	39	0.358
Presence of grazers in 2020	2.155	39	0.091
Presence of grazers in 2007 : Presence of grazers in 2020	-1.367	39	0.181
(b)			
Difference in area (ha)	-1.130	39	0.267
Difference in connectivity (ha)	0.546	39	0.589
Difference in area (ha) : Difference in connectivity (ha)	-1.143	39	0.261
Presence of grazers in 2007	1.100	39	0.279
Presence of grazers in 2020	2.155	39	**0.039**
Presence of grazers in 2007 : Presence of grazers in 2020	-1.884	39	0.068
(c)			
Difference in area (ha)	-2.053	39	**0.0481**
Difference in a connectivity (ha)	2.382	39	**0.0231**
Difference in area (ha) : Difference in connectivity (ha)	2.184	39	**0.036**
Presence of grazers in 2007	0.671	39	0.507
Presence of grazers in 2020	1.197	39	0.240
Presence of grazers in 2007 : Presence of grazers in 2020	-0.840	39	0.407

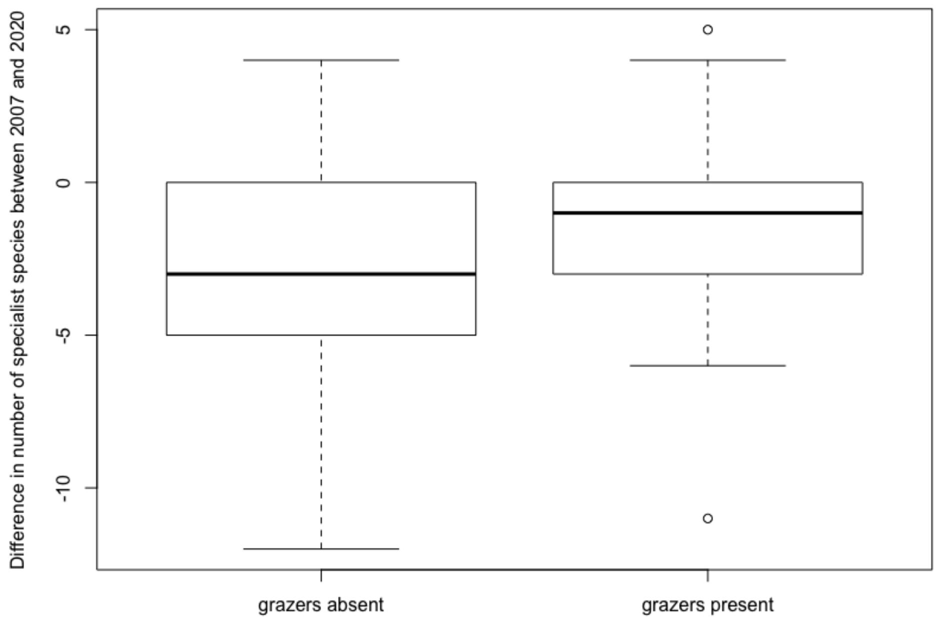

Figure 4. Grasslands that were grazed in 2020 lost fewer specialist species than those that were not grazed.

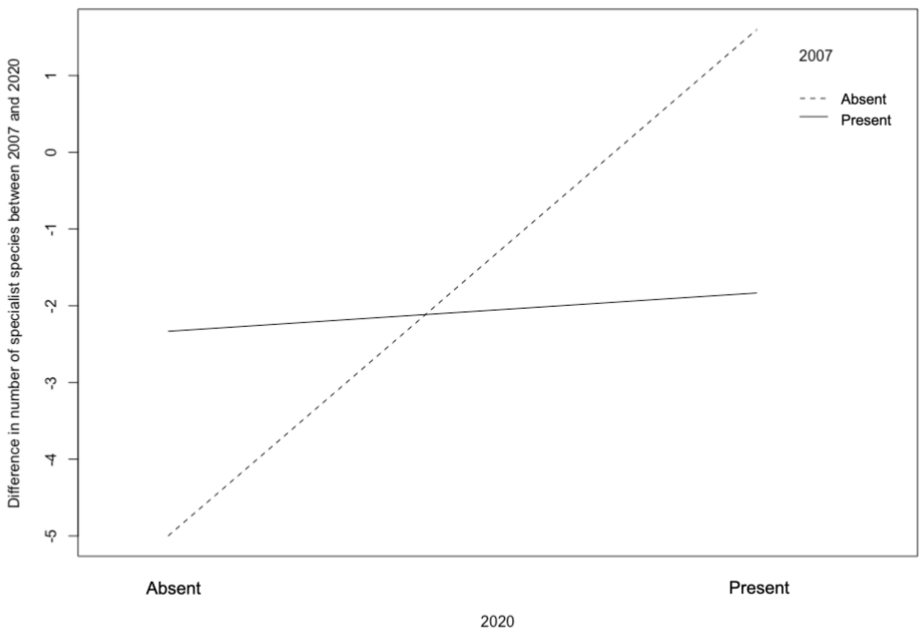

Figure 5. Grasslands that were not grazed in 2007 and grazed in 2020 experienced an increase in number of species. Species that were not grazed in 2007 and 2020 had the largest decrease in species. Grasslands that were grazed in 2007 showed little difference whether or not they were grazed in 2020 or not.

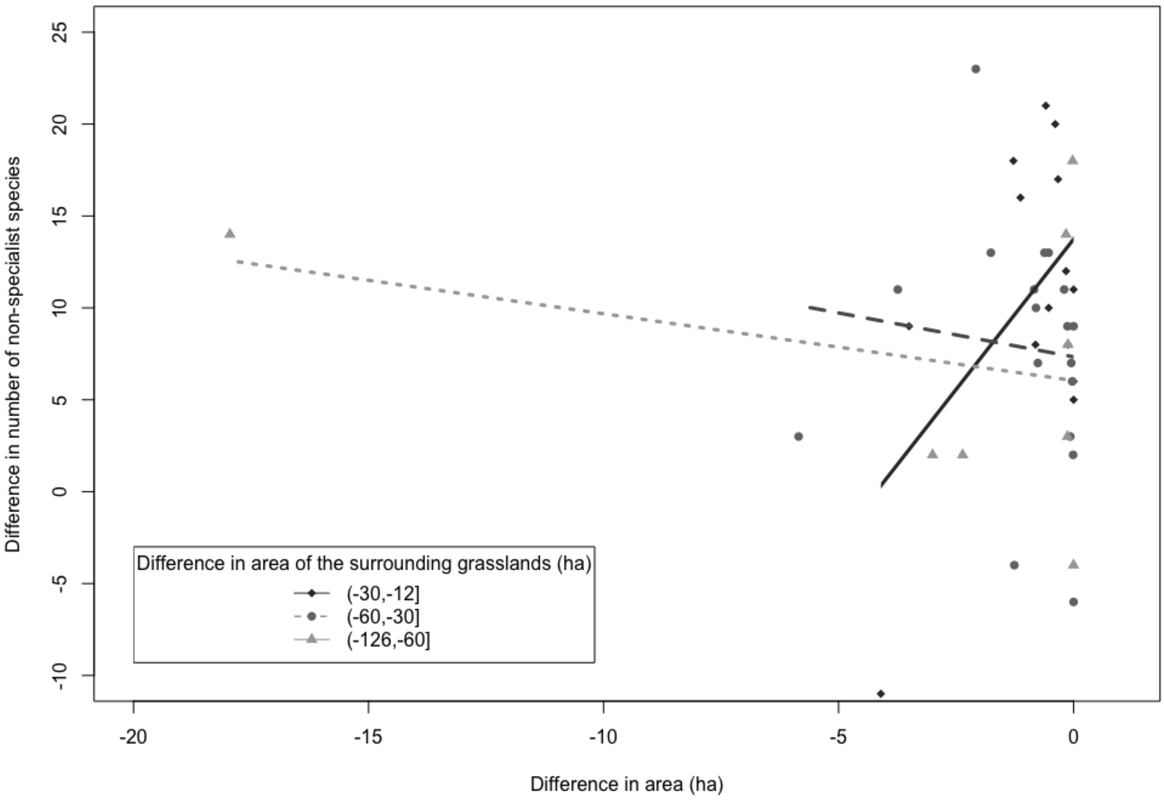

Figure 6. The difference in species richness of non-specialist species is affected by the interaction between the difference in area and the difference in connectivity. Grasslands with a smaller decrease in connectivity (between -30 and -12 ha) increased in species richness of non-specialist species when the loss of area was smaller. Grassland with a larger decrease in connectivity (between -126 and -30 ha) decreased in species richness of non-specialist species when the loss of area was smaller. The lines in the plot are the regression lines for the analyses stratified by connectivity classes.

3.4 Relation between specialist/non-specialist diversity and climate predictors

No, statistically significant relationship was found between the difference in species temperature index and the species occurrence (t= 0.214, df=281, p= 0.831). A suggestive positive relationship (t=1.821, df=281, p=0.070) was found between classification of grassland specialists/non-specialists and species occurrence. While this relationship was not statistically significant, there is a trend that species which were classified as semi-natural grassland specialists typically occurred in fewer grasslands in 2020 than in 2007, while species which were classified as non-semi-natural grassland specialists tended to occur in more grasslands in 2020 than in 2007. The mean difference in species occurrence was -1.8 for semi-natural grassland specialists and 0.73 for non-semi-natural grassland specialists (Figure 7). An additional t-test proved that there is a significant difference between the change in species occurrence for specialists and non-specialists (t = -3.2487, df = 88.726, p = 0.002).

The interaction between the species temperature index and the specialist/non-specialist classification had a significant positive relationship with the difference in species occurrence (t=-2.182, df=281, p=0.030). Species that were classified as specialists had a larger decrease in occurrence between 2007 and 2020 if the temperature index of the species was higher (Figure 8). The occurrence of species that were classified as non-specialists did not depend on the temperature of the distribution (t=0.227, df=218, p=0.821) (Figure 8).

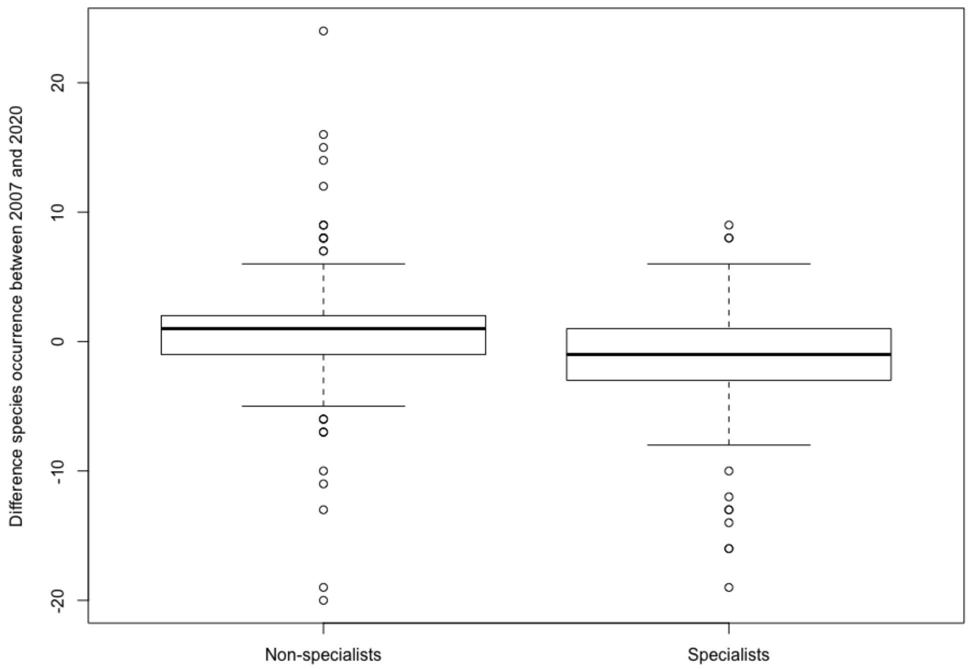

Figure 7. Difference in species occurrence between 2007 and 2020 for semi-natural grassland specialist species and non-semi-natural grassland specialist species.

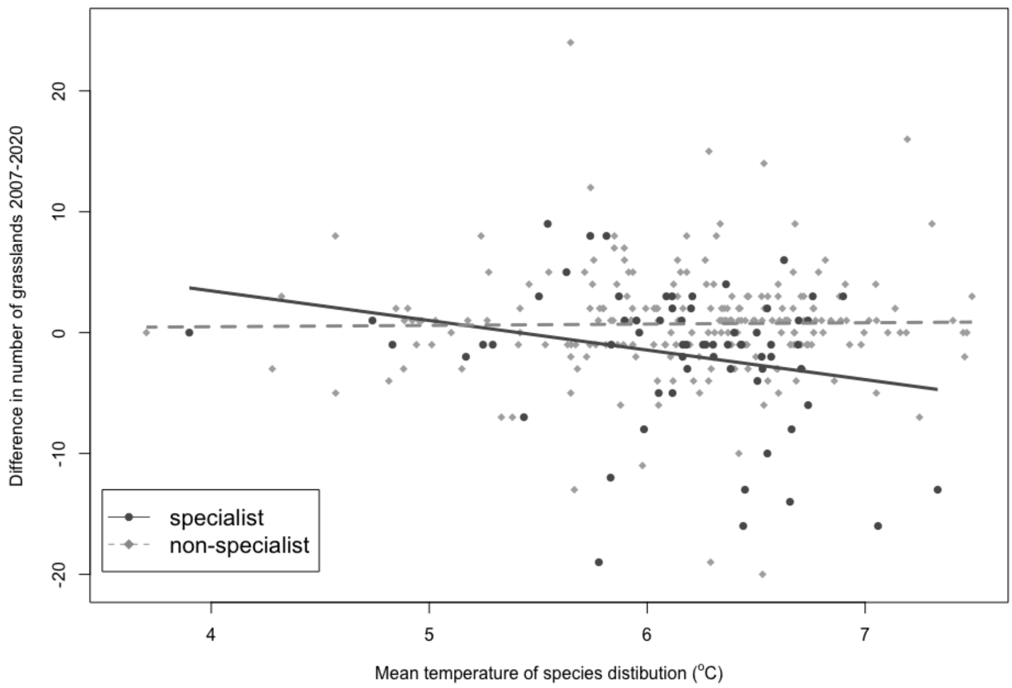

Figure 8. Species that were classed as semi-natural grassland specialist had a larger decrease in occurrence between 2007 and 2020 if the mean temperature (℃) for the species distribution was higher. Species that were classed as non-semi-natural grasslands specialists showed no larger difference in the difference in occurrence if the mean temperature of the species' distribution was higher or lower.

4. Discussion

On average, the 40 semi-natural grasslands decreased in area and connectivity between the 1950:s and 2000:s. The theory of island biogeography predicts that this would lead to a new equilibrium with fewer species in the grasslands (MacArthur & Wilson 1967). The study by Bommarco et al. (2014), using data from the same semi-natural grasslands as the present study, found that the number of species in the grasslands in 2007 was best explained by the historical connectivity, suggesting a delayed new equilibrium as a response to changes in the habitat between the 1950:s and the 2000:s. Therefore, it is expected that the number of species would continue to decrease after 2007, as a result of the loss of habitat area and connectivity that had occurred earlier.

In the present study, no relationship was found between the difference in species richness between 2007 and 2020 and the change in grassland area between the 1950:s and the 2000:s. This was in contradiction with what was expected based on the theory of island biogeography. A positive relationship was found between the difference in species richness in grasslands between 2007 and 2020 and the change in connectivity between the 1950:s and the 2000:s (Figure 2), which was expected. As this positive relationship between the difference in species richness in grasslands and the change in connectivity only receives borderline significance, it supports the finding of a delayed equilibrium, as found by Bommarco et al. (2011). However, contrary to what was expected, the mean number of species in the grasslands increased between 2007 and 2020. This appears to contradict the existence of an extinction debt as a result of changes in landscape connectivity. Nevertheless, when stratifying the analysis by classifying species as specialists and non-specialists, in terms of semi-natural grasslands, different patterns emerges for the two groups.

One reason that the number of species increased could be that the changes in the landscape connectivity has not only resulted in extinction of some species but also enhanced the establishment of new species. This is supported by the fact that, in the present study, it was the richness of non-specialist species that increased while the richness of grassland specialists decreased. It is also supported by the result that the interaction between difference in grassland area and the difference in connectivity significantly affected the difference in the number of non-specialists. The effect of areal decrease on non-specialist species richness was larger for grasslands with low connectivity than for grasslands with high connectivity (Figure 6). This suggests that in grasslands with low connectivity decrease, a major part of the non-specialist immigration come from grasslands species. Grassland species that, although not classified as specialists, still benefit from a larger grassland area. As the connectivity decreases, an increasing part of the immigration will come from other types of habitats than grasslands. These species are likely habitat generalists or species that are adapted to other types of habitats than semi-natural grasslands, and their establishment is therefore not negatively affected by larger decreases in grassland area. This indicates that the habitats in the matrix surrounding the grassland influences the patch more than what was originally believed according the theory of island biogeography, as previously proposed by Öckinger *et al.* (2012). The decrease in area of the grasslands results in a larger proportion of the area being along the edges of the habitat. In habitat edges properties of two habitat types mix, this can result in a higher species richness of generalist and edge specialist species (Burst *et al.* 2017). This could further explain why non-specialists increased in number with a larger decrease in area of the semi-natural grassland, when the decrease in connectivity was high. The five species that had increased the most were *Ranunculus auricomus*, *Rosa dumalis, Trifolium medium, Luzula multiflora and Geum urbanum,* all classified as non-specialists. In order to confirm this hypothesis further studies are needed, especially on if there is a difference in which non-specialist species that establish in the grassland and what type of habitat the species that establish is commonly found in, when the connectivity is low, and when the connectivity is high.

In contrast, no significant effect of areal or connectivity decrease was found on grassland specialists, instead the major effect appears to come from the presence of grazing (in 2007 and 2020), as well as the interaction between these. Similarly, the overall species richness in a grassland had a positive relationship with the presence of grazers in 2020, such that grasslands that were grazed gained more species than those that were not grazed at the time of the survey (Figure 3). This supports the idea that regular disturbances such as grazing increases species richness in semi-natural grasslands (Hansson & Fogelfors 2000). As grazing keeps the habitat from overgrowing, species with high light requirements are strongly favoured by grazing (Tyler *et al.* 2018). A study by Pykälä *et al.* (2005) found that species richness of grassland species increases with solar radiation. Grasslands with grazers present in 2020 lost less specialist species than those with grazers absent (Figure 4). This result was expected as the classification of specialist and non-specialist species was based on if the species could survive without grazing. Hence, it appears that it is not the decrease in grassland area, per se, that is critical for the specialist species, but rather the relief in competition for light and resources that are provided by the grazing. The change in species richness of specialist species in a grassland was found to almost have a statistically significant relationship with the interaction between the presence of grazers in 2007 and the presence of grazers in 2020. Grasslands that were not grazed in 2007 and then grazed in 2020 showed an increase in species richness of specialists while grasslands that were not grazed both in 2007 and 2020 experienced a decrease in species richness of specialist species (Figure 5). This suggests that grazing of abandoned semi-natural grasslands is a good restoration method in order to regain species diversity (Pykälä *et al.* 2005).

The occurrence of specialist species decreased between 2007 and 2020 while the occurrence of non-specialist species increased (Figure 7). Further, species that were classified as specialists with a higher species temperature index decreased more in occurrence compared to specialist species with a lower mean temperature of its distribution (Figure 8). Species that were classified as non-specialists showed no significant difference in occurrence, depending on the mean temperatures of their distributions. The loss of specialist species is probably not a result of climate change as the mean yearly temperature in Sweden has increased during the last 20 year (Sveriges meteorologiska och hydrologiska institut. 2020), but rather a result of abandonment of semi-natural grasslands. When semi-natural grasslands are abandoned they tend to overgrow, resulting in less sunlight reaching the ground which in turn could cause the microclimate to decrease in temperature (Hansson & Fogelfors 2000, De Frenne *et al.* 2013). Specialist species that are adapted to warmer climates may be more disturbed by the overgrowth of the grassland, as this results in a decrease in temperature of the microclimate. A previous study has shown that an increase in vegetation height from 2- 5 cm in grasslands that cools the ground temperature by 3˚C caused local extinctions of a species of butterfly (Thomas 1993). However, our results do not exclude a warming macro climate being a threat to the species diversity in semi-natural grasslands, as it only favours species adapted to warmer climates. This implied increase of warm-climate-adapted species in plant communities is buffered by an increasing canopy layer (De Frenne *et al.* 2013).

Bommarco et al. (2011) argued that the number species that were classified in their study as semi-natural grassland specialists was best explained by the historical connectivity and that there was probably an extinction debt for specialist species. This was not confirmed by the present study. This could partly be because Bommarco et al. classified the specialist species based on different criteria that what was done in this study. Another reason that no relationship was found between the difference in species richness and the difference in area could be that the extinction debt is not yet paid off. The species that had not already gone extinct as a result of the landscape changes by 2007 may have had the ability to withstand the changes even longer. It would therefore be interesting to follow up this study with new surveys in about 10-20 years. An alternative reason could be that the species richness in the seminatural grasslands simply does not follow the theory of island biogeography. This could also explain why no relationship was found between the difference in area of the grassland and the difference in overall species richness in the grassland, as otherwise expected according to the theory of island biogeography.

5. Conclusion

The results of this study suggest that decreased connectivity in the landscape and ceased management may have contributed to an extinction debt of grassland specialists. The results also suggests that the habitat types in the matrix around the grassland influence the immigration of new species. However, this immigration is mainly of non-specialist grassland species. Species that are adapted to growing in semi-natural grasslands had the highest extinction risk, especially after ceased management. Specialist species adapted to warmer climates seemed to have decreased most, possibly due to cooler microclimates as a result of overgrowth of grasslands, supporting the idea that cooler microclimates can buffer the extinction debt caused by global warming. The overall effect may be a deprivation of specialist grassland communities. In order to prevent the loss of biodiversity in grasslands it is important to try to counteract the extinction debt. This study found that grazing of abandoned grassland may positively affect the number of specialist species and, thereby, reverse the extinction debt.

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