

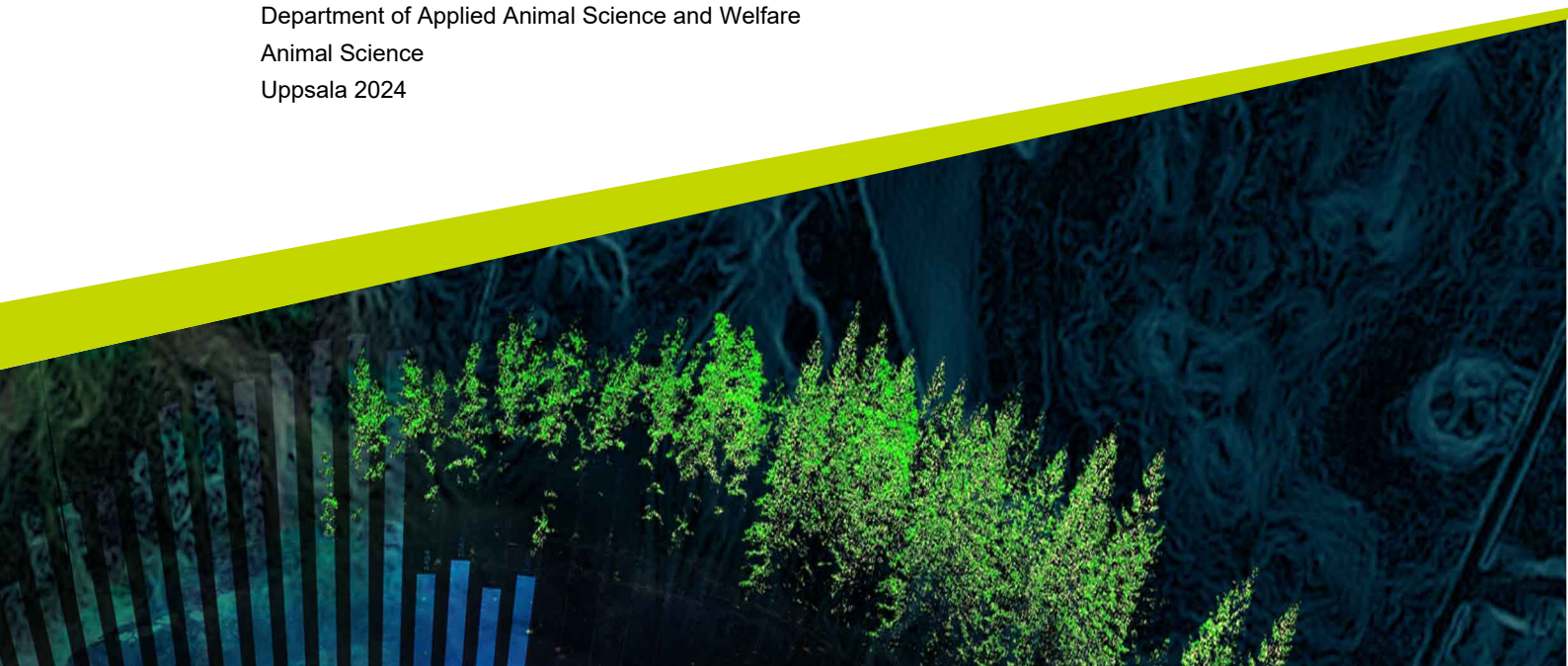


# Impact of horses on year around grazing without supplementary feeding on pastoral herbaceous plants

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Independent project • 30 credits  
Swedish University of Agricultural Sciences, SLU  
Department of Applied Animal Science and Welfare  
Animal Science  
Uppsala 2024



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## Abstract

European grasslands and the biodiversity therein are lost as a result of changes in land use, which has led to the necessity for the development of effective restoration strategies. This study investigates the impact of reintroducing a Swedish national breed of horses (Gotland Russ) on herbaceous plant diversity in abandoned agricultural landscapes in southeast Sweden. Twelve horses were introduced into three 10-13 hectare enclosures in a three-year (2014-2016) rewilding experiment. Plant species richness, evenness, and diversity were investigated in both grazed and ungrazed areas. The results indicated that horse grazing significantly increased herbaceous plant species diversity and richness, with higher Shannon and Simpson diversity indices in grazed areas. In addition, the abundance of white clover (*Trifolium repens*), a signal species beneficial to pollinators, increased significantly in grazed areas. These findings emphasise the necessity of integrating large herbivore grazing into ecological restoration practices. In light of the recently enacted EU restoration law, which aims to restore 20% of Europe's degraded ecosystems by 2030, this research provides critical insights into scalable restoration methods. The implementation of rewilding strategies that include large herbivores could enhance the resilience and biodiversity of European grasslands and forests, thereby aligning with the EU's restoration goals.

*Keywords:* biodiversity, diversity index, Equus, grassland, grazing impact, restoration, rewilding.

# Table of contents

<b>List of tables .....</b>	<b>5</b>
<b>List of figures.....</b>	<b>6</b>
<b>Introduction .....</b>	<b>7</b>
<b>Material and Methods.....</b>	<b>10</b>
Study area.....	10
Experimental design.....	11
Experimental herbivore .....	11
Vegetation surveys.....	12
Diversity Indices .....	12
Statistical Analysis.....	13
<b>Results .....</b>	<b>14</b>
Plant diversity and evenness .....	14
Plant species richness.....	14
Impact on indicator plant species.....	15
<b>Discussion .....</b>	<b>19</b>
<b>References .....</b>	<b>22</b>
<b>Popular science summary.....</b>	<b>32</b>
<b>Acknowledgements.....</b>	<b>33</b>
<b>Appendix 1 .....</b>	<b>34</b>
<b>Appendix 2 .....</b>	<b>36</b>

## List of tables

Table 1. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant species richness fitted to a GLMM. $\beta$ = model regression coefficient estimate. SE = standard error. N = 378 observations for the flowering plant species richness model. ....	15
Table 2. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of <i>Trifolium medium</i> fitted to a GLMM. $\beta$ = model regression coefficient estimate. SE = standard error. N =29 observations for the abundance model. ....	16
Table 3. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of <i>Trifolium repens</i> fitted to a GLMM. $\beta$ = model regression coefficient estimate. SE = standard error. N =216 observations for the abundance model. ....	17
Table 4. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of <i>Trifolium pratense</i> fitted to a GLMM. $\beta$ = model regression coefficient estimate. SE = standard error. N =16 observations for the abundance model. ....	18

## List of figures

Figure 1. Map of the study area. Location and experimental design at Krusenberg estate in southeastern Sweden. ....	10
Figure 2. Vegetation survey design. Seven paired grazed and un-grazed inventory plots on grassland were surveyed (black circles indicate the inventory plots).....	12
Figure 3. a) The Shannon-Winner diversity index, b) Simpson's diversity index and c) Pielou's evenness index comparison between treatments (grazed vs. un-grazed) within the three years experimental time. Black line represents the mean value, black dot represents the outliers. (Detailed comparison within each enclosure can be found in Appendix 1.) Significant symbol: $p \leq 0.001$ : "****"; $p \leq 0.01$ : "***"; $p \leq 0.05$ : "**"; $p > 0.05$ : "ns". ....	14
Figure 4. The interaction effect of plant species richness with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE. ....	15
Figure 5. The interaction effect of <i>Trifolium medium</i> abundance with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE. ....	16
Figure 6. The interaction effect of <i>Trifolium repens</i> abundance with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE. ....	17
Figure 7. a) two treatments (grazed and un-grazed) effect on <i>Trifolium pratense</i> abundance, b) time effect on <i>T. pratense</i> abundance. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE.....	18

# Introduction

Global biodiversity is diminishing (Tschamntke et al. 2005; Butchart et al. 2010; Kehoe et al. 2017). Since the 1970s, there has been a marked increase in the impact of human activity on the planet due to increased population and consumption, with species extinction and ecosystem degradation accelerating at an unprecedented rate (Díaz et al. 2019). The first species that disappear as a result of these factors are large vertebrates (Donlan et al. 2006), primarily as they have been exposed to overhunting (Jerozolinski & Peres 2003). Changes in land use caused most of the landscape biodiversity to decrease with the expansion of farmlands and an increased use of pesticides and fertilisers which had negative impacts on the extinction of birds (Green et al. 2005) and insects (Geiger et al. 2010). Such biodiversity loss affects the functioning of natural ecosystems with detrimental effects also on human well-being (Xu et al. 2021). This is among the biggest environmental challenges that humanity is now facing (Ceballos et al. 2015).

In Sweden, the primary threats to biodiversity in agricultural ecosystems are attributed to intensification and abandonment (SCBD 2014). During the mid-20th century, the changes in land use (i.e., agricultural abandonment, reduced semi-natural pastures and meadows) and overgrowth that took place in the agricultural landscape threatened the rural landscape biodiversity (Cousins et al. 2015). Notably, approximately half of Sweden's red-listed species occur in farming landscapes and rely on it for survival (Sandström et al. 2015). This negative shift in land use dynamics adversely affected most species within these ecosystems (SCBD 2014).

There are two contrasting suggestions for the management of abandoned land: (i) a more active stance, which entitles the implementation of measures to control negative environmental impacts, or (ii) a passive approach, which involves allowing the natural process to continue (Holl & Aide 2011). However, there is no consensus on how to manage abandoned land, while with widespread land abandonment, degradation of landscape and biodiversity, alternatives for abandoned land management should be advocated to reduce the negative effects on biodiversity (Lasanta et al. 2015).

In order to tackle current biodiversity declines, new alternative methods are needed. Trophic rewilding (*sensu* Svenning et al. 2016) is gaining momentum as a restoration approach. It is defined as a restoration strategy that supports self-regulating biodiverse ecosystems by introducing keystone species to restore top-down trophic interactions and related trophic cascades (Svenning et al. 2016).

Due to the worldwide extinction of large herbivores (weight 45–999 kg (Malhi et al. 2016)12/06/2024 08:52:00) in the late Quaternary, the functional diversity has been lost, negatively impacting the ecosystems (Smith et al. 2019; Malhi et al. 2022). The reintroduction of large herbivores could restore ecosystem functions and have positive effects on biodiversity. Large herbivore grazers have been shown to have positive effects on grassland biodiversity (Tälle et al. 2016), and play a crucial role in ecosystems (Pringle et al. 2023). In addition, they play a more significant function by influencing the structure and functioning of ecosystems (Bakker et al. 2016), than only the immediate effects of grazing and browsing via creating a physical disturbance or role as seed-dispersers (Navarro et al. 2015). For instance, large herbivores have been shown to inhibit some plant species' growth, which favoured the growth of other plant species however, livestock cannot functionally replace wild communities of large herbivores (Pringle et al. 2023). Recent research has shown that large herbivores' dietary preferences affect different plant growth forms with negative impacts on graminoids and positive impacts on forbs (Lundgren et al. 2024).

Horses, historically roamed as wild species for centuries, but the last Tarpan horse (*Equus ferus ferus*) went extinct at the start of the last century (Kavar & Dovč 2008), while the Przewalski's horse (*Equus ferus przewalskii*) became extinct in the wild during the 1960s due to factors like hunting, competition with livestock and climate change (van Dierendonck & de Vries 1996). Horses have been domesticated for thousands of years, and used in agriculture and transportation (Bendrey 2012). In ecosystems, horses act as grazers, influencing plant community dynamics through foraging and trampling (Mutillod et al. 2024), and as natural fertilizers (Valdés-Correcher et al. 2019) and plant dispersers (Campbell & Gibson 2001). Unlike ruminants, horses can ensure their diet quality in limited conditions; they can eat plants that are closer to the ground which ensures their survival and makes them good candidate species for wood-pasture restoration via rewilding (Menard et al. 2002; Fleurance et al. 2016). However, overgrazing of horses can also result in the formation of bare patches with unpalatable grass (Crofts 1999). Wild horses may also benefit plant species richness, evenness and heterogeneity more than domestic ruminants (Mutillod et al. 2024). Restoring extinct large wild herbivore communities could therefore potentially benefit biodiversity and ecosystem functions (Garrido et al. 2019, 2021).



Recent research has shown that large herbivores were fundamental components of former European landscapes, playing key roles in ecosystems and maintaining open landscape structures (Pearce et al. 2023). These grassland-dominated landscapes were maintained by highly functionally diverse herbivore communities (Davoli et al. 2024). However, since the late Quaternary human expansion has been associated with megafaunal declines (Svenning et al. 2024), and that defaunation process (Dirzo et al. 2014) has produced significant ecological shifts in different biomes (Barnosky et al. 2016) with cascading effects on plant community composition, vegetation structure and fire regimes (Gill 2014). Therefore, reintroducing an ecologically functional substitute of extinct wild horses could tackle current biodiversity declines and restore threatened grassland ecosystems. Horses are key candidate species for rewilding due to their great plasticity of suitable climates and habitats they can live in, their important ecological functions as grazers as well as the significant knowledge of their ecology and management (Naundrup & Svenning 2015).

To assess the potential effects of re-introducing a functional substitute of an extinct wild horse in the restoration of wood-pastures, a three-year rewilding experiment was conducted in 2014 in southeast Sweden. Here, twelve one-year-old Gotland Russ (*Equus ferus caballus*) were introduced into three enclosures (sized ca.10-13 hectares) to free-range without supplementary feeding. The hypothesis was that the reintroduction of horses would positively influence biodiversity and boost plant species richness, while additionally impacting indicator species positively by grazing, nutrient redistribution and trampling.

# Material and Methods

## Study area

The experiment was conducted between May 2014 and September 2016 on a property of the Swedish University of Agricultural Sciences (SLU) in Krusenberg, Uppsala, Sweden (59°44'8" N, 17°38'58" E) (Figure 1). The experimental design was approved by the Uppsala Animal Welfare Ethics Committee (protocol C28/14). The mean temperature in the study area during the experimental period from April to October was  $12.6 \pm 4.5$  °C ( $\pm$ SD) and the mean precipitation was  $51 \pm 33$  ( $\pm$ SD) mm. From November to March the mean temperature reached  $1.1 \pm 2.7$  °C ( $\pm$ SD) and the mean precipitation averaged  $51 \pm 18$  ( $\pm$ SD) mm (*Temperatur* | SMHI n.d.). The study area consisted of forest and pastures, with wooded-dominated areas interspersed with grassland (Garrido et al. 2021).

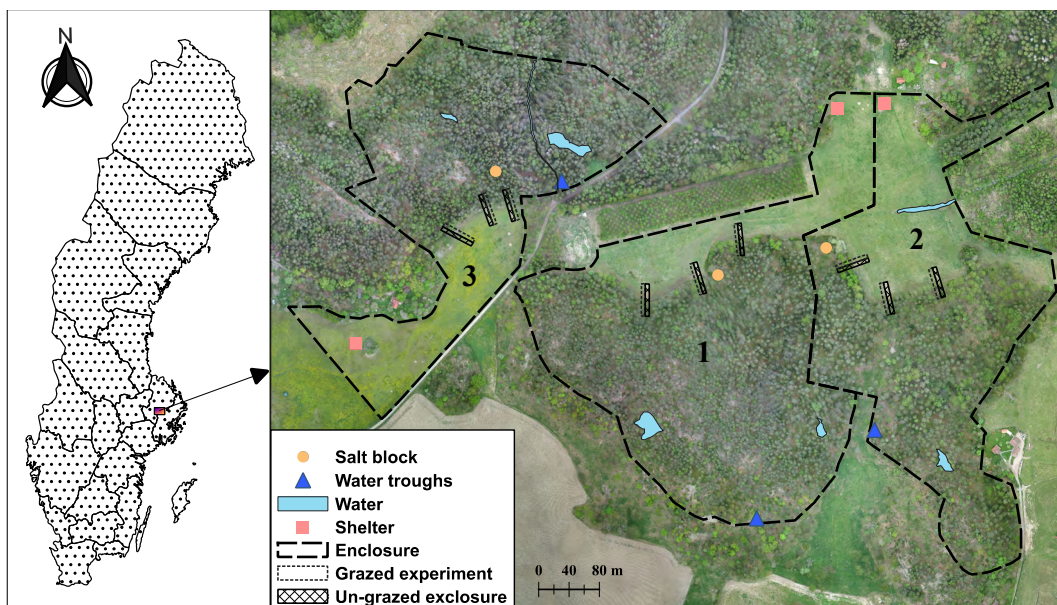


Figure 1. Map of the study area. Location and experimental design at Krusenberg estate in southeastern Sweden.

## Experimental design

Three enclosures of similar sizes (10, 11 and 13 ha) were established within the study area. The enclosures were composed of approximately one-third grassland and two-thirds forest (Ringmark et al. 2019). In each enclosure, three rectangular-shaped exclosures (size 42.5 x 5 m, located 20 meters into the forest and 22.5 meters into the grassland) were set up centred over the edge zone between the grassland and the forest in May 2014. An experimental (grazed) area of equal size was delineated parallel to each exclosure (Figure 1). Both enclosures and exclosures were fenced to prevent other herbivores from interfering with experiments, while the exclosures excluded herbivory and mimicked abandonment. The experimental area was abandoned for 10 years prior to the experiment, with no pasture cultivation nor grazing (from 2004 to 2014) (Ryberg, pers.comm.), although enclosures 1 and 2 were occasionally grazed by cattle, and enclosure 3 was used for forage production.

## Experimental herbivore

Twelve one-year-old Gotland Russ stallions (average body weight  $185 \pm 21$  kg from the study start) were used as experimental herbivores. The Gotland horses were randomly allocated into the three enclosures in groups of four at the start of the experiment in May 2014. Average grazing pressure was estimated at 0.35 horse/ha (Ringmark et al. 2019). To replicate the ecosystem functions of wild herbivores, the horses were kept throughout the year without receiving any supplemental feed; each enclosure was expected to meet the energy requirements of three to four 250 kg horses based on prior estimates of grassland productivity (Ringmark et al. 2019). As the experiment used a crossover design, during each growing season in 2015 and 2016, the groups of horses were alternated between the three enclosures. In each enclosure, a 16 m<sup>2</sup> shelter was placed, and a salt block with trace minerals was provided. Water was available *ad libitum* in automatic water troughs during summer, spring, and autumn. However, during winter, when temperatures were below 0°C, water was provided manually once a day in plastic troughs. Natural water supply was also available in the enclosures the whole year around.

A total of four horses were temporarily removed from the experiment due to low body condition during the winter of 2014, and one Gotland Russ was excluded from the study due to injury in January 2016 (Tydén et al. 2019).

## Vegetation surveys

Vegetation surveys were conducted in seven paired inventoried plots on grassland within each enclosure, paralleled with outside plots on the grazed area (0.25 m<sup>2</sup>, 0.282 m radius) spaced 2.5 m apart (Figure 2). Vegetation surveys were performed in July and September 2014 and in May, July, and September 2015 and 2016.

Plots were measured with lines and tapes, and marked with black plastic needles hammered into the ground to facilitate successive inventories of the same area. All plants were identified at the species level and their abundance (percentage of coverage) was noted. The abundance of shrubs and trees was not included in the total vegetation cover. Only inventory data from July each year was utilised in this study. Two different observers conducted vegetation surveys, in 2014 and 2015, the surveys were conducted by one individual, while in 2016, they were performed by another observer.

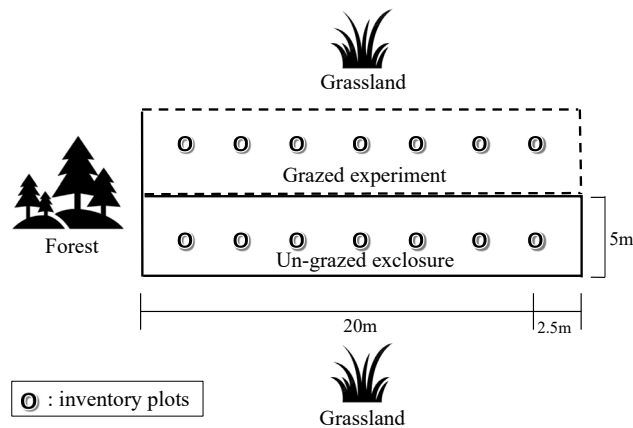


Figure 2. Vegetation survey design. Seven paired grazed and un-grazed inventory plots on grassland were surveyed (black circles indicate the inventory plots).

## Diversity Indices

The Shannon–Wiener and Simpson diversity indices are the two most commonly used indices for measuring diversity (McGarigal 1995). Shannon–Wiener’s diversity index is more sensitive to the richness and presence of rare species, in contrast, Simpson’s diversity index is relatively less sensitive to richness and focuses more on the common or dominant species (McGarigal 1995; Nagendra 2002), with a higher value indicating more diverse. Species richness is defined as the number of distinct plant species present within a defined area (Ostfeld & Keesing 2024). Species evenness is defined as a measure of the relative abundances of species within a given community (Ostfeld & Keesing 2024). Pielou’s evenness index  $J'$  is a reliable measure of “relative evenness” because it is independent of

richness (Jost 2010). The value of  $J'$  ranges from 0 to 1, with a higher value indicating more even distributions in species abundance (Zhang et al. 2012).

## Statistical Analysis

All statistical analyses and plotting were performed using the R program version 4.3.2 (R Core Team 2024).

First, differences in plant species diversity and evenness between treatments (grazed and un-grazed) and time during the study period were tested with Shannon-Winner diversity index, Simpson's diversity index and Pielou's evenness index (used the diversity function in package "vegan"(Oksanen et al. 2022)). Wilcoxon signed-rank test was used to assess whether there were any significant differences among grazed and un-grazed conditions through time within each of the calculated biodiversity indices (differences were considered significant at  $p < 0.05$ ). Trees and shrubs were excluded from the analysis since their abundance was not included in the total plant coverage.

Second, I tested how plant species richness was affected by horse grazing and time by fitting a Generalized Linear Mixed Effects Model (GLMM) with Poisson distribution (log-link) including an interaction between treatment and time as well as a random effect for plots within enclosures and exclosures (used the function *glmer* in package "lme4" (Bates et al. 2015)).

Third, the grazing impact on the abundance of three *Trifolium* species, used as indicator species, was assessed using GLMM with Poisson distribution (log-link) fitted (package "lme4" (Bates et al. 2015)), with the interaction between treatment, time and their interacting effect as predictors. This model also included a random effect term (plots within exclosures and enclosures).

# Results

A total of 95 different species of flowering plants were recorded in the inventoried plots in July during the three-year experiment (the list of table can be found in Appendix 2).

## Plant diversity and evenness

Shannon's diversity index was significantly higher in grazed areas (2014:  $p=0.028$ ; 2015 and 2016:  $p<0.01$ ) (see Figure 3 a). Simpson's diversity index was also found to be significantly higher in grazed conditions compared to the un-grazed areas (2014:  $p=0.04$ ; 2015:  $p<0.01$ ; 2016:  $p=0.034$ ) (Figure 3 b). The plant species evenness was higher in the grazed area in 2015 ( $p=0.02$ ), however, there were no significant differences between grazed and un-grazed areas in 2014 and 2016 (Figure 3 c).

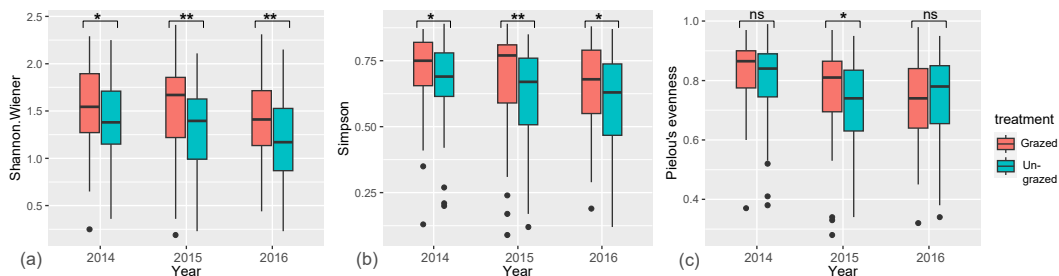


Figure 3. a) The Shannon-Wiener diversity index, b) Simpson's diversity index and c) Pielou's evenness index comparison between treatments (grazed vs. un-grazed) within the three years experimental time. Black line represents the mean value, black dot represents the outliers. (Detailed comparison within each enclosure can be found in Appendix 1.) Significant symbol:  $p\leq 0.001$ : "\*\*\*";  $p\leq 0.01$ : "\*\*";  $p\leq 0.05$ : "\*";  $p> 0.05$ : "ns".

## Plant species richness

In general, plant species richness was higher (although not significant) in grazed compared to un-grazed areas during the study period (see Table 1, Figure 4). Such difference in the mean number of plant species significantly increased with time and treatment (Table 1, Figure 4).

Table 1. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant species richness fitted to a GLMM.  $\beta$  = model regression coefficient estimate. SE = standard error.  $N = 378$  observations for the flowering plant species richness model.

GLMM plant species richness				
Main effects	$\beta$	SE	z value	p-value
Intercept	1.8	0.11	16.4	<0.001
2015	0.24	0.07	3.4	<0.001
2016	0.16	0.69	2.27	<0.05
Un-grazed	-0.12	0.07	-1.67	0.1
Interaction effects				
2015: un-grazed	-0.05	0.09	-0.52	0.6
2016: un-grazed	-0.23	0.1	-2.24	<0.05

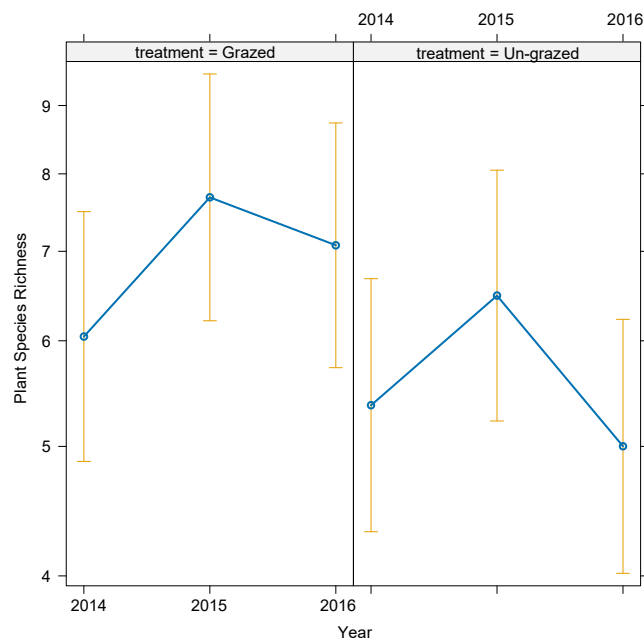


Figure 4. The interaction effect of plant species richness with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE.

## Impact on indicator plant species

The abundance of zigzag clover (*Trifolium medium*) exhibited an increase in un-grazed conditions (in 2015,  $p < 0.05$ ), although it was not significant in 2016. In

contrast, the opposite effect was observed in grazed areas over time (Table 2, Figure 5). Nonetheless, zigzag clover abundance was generally higher in un-grazed areas.

Table 2. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of *Trifolium medium* fitted to a GLMM.  $\beta$  = model regression coefficient estimate. SE = standard error. N = 29 observations for the abundance model.

GLMM plant abundance of <i>Trifolium medium</i>				
Main effects	$\beta$	SE	z value	p-value
Intercept	2.34	0.29	7.95	<0.001
2015	-0.74	0.22	-3.37	<0.001
2016	-0.19	0.22	-0.87	0.39
Un-grazed	0.56	0.15	3.6	<0.001
Interaction effects				
2015: un-grazed	0.64	0.26	2.47	<0.05
2016: un-grazed	0.47	0.26	1.82	0.07

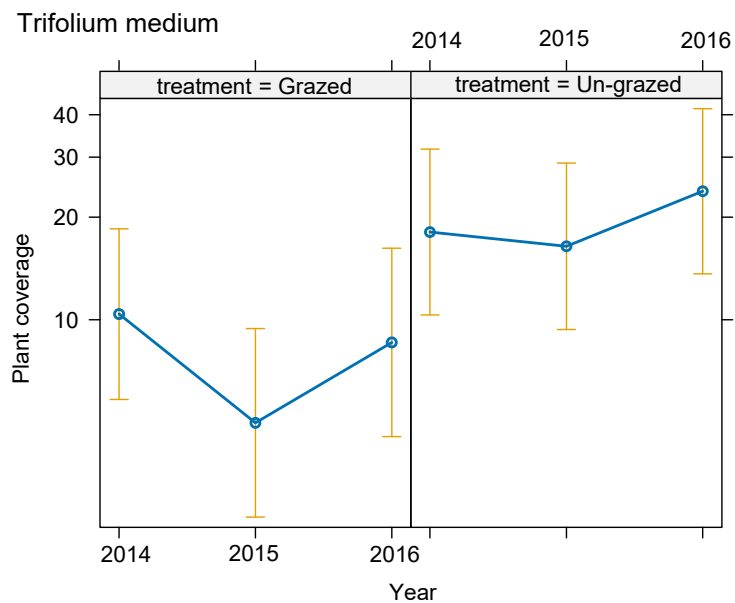


Figure 5. The interaction effect of *Trifolium medium* abundance with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE.

A significant increase in the abundance of white clover (*Trifolium repens*) was observed in grazed areas while declining over time in un-grazed conditions (Table



3, Figure 6). White clover abundance was generally higher in grazed conditions (Table 3, Figure 6).

Table 3. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of *Trifolium repens* fitted to a GLMM.  $\beta$  = model regression coefficient estimate. SE = standard error. N =216 observations for the abundance model.

<b>GLMM plant abundance of <i>Trifolium repens</i></b>				
<b>Main effects</b>	$\beta$	SE	z value	p-value
Intercept	2.2	0.12	18.6	<0.001
2015	0.15	0.06	2.4	<0.05
2016	0.44	0.06	7.32	<0.001
Un-grazed	-0.4	0.08	-5.12	<0.001
<b>Interaction effects</b>				
2015: un-grazed	-1.63	0.18	-9.35	<0.001
2016: un-grazed	-2.02	0.38	-5.21	<0.001

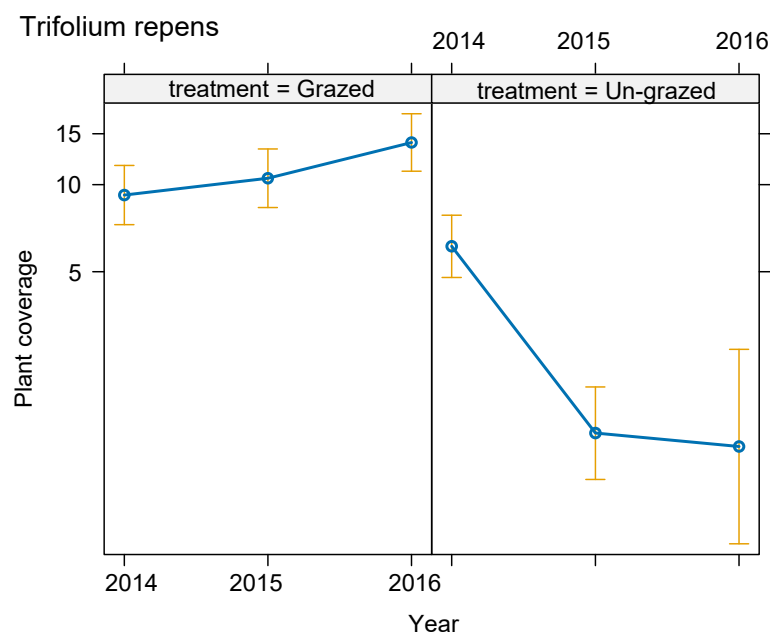


Figure 6. The interaction effect of *Trifolium repens* abundance with time and treatment. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE.

The abundance of *Trifolium pratense* (red clover) exhibited a decline over time (Figure 7b), with un-grazed areas showing higher abundance than grazed areas (Table 4, Figure 7a).

Table 4. Results regarding the impact of experimental treatment (grazed and un-grazed) and time on plant abundance of *Trifolium pratense* fitted to a GLMM.  $\beta$  = model regression coefficient estimate. SE = standard error.  $N = 16$  observations for the abundance model.

GLMM plant abundance of <i>Trifolium pratense</i>									
in relation to treatment					in relation to time				
Main effects	$\beta$	SE	z value	p-value	Main effects	$\beta$	SE	z value	p-value
Intercept	0.48	0.37	1.3	0.19	Intercept	2.77	0.28	9.94	<0.001
Un-grazed	1.53	0.38	4.02	<0.001	2015	-2.1	0.48	-4.32	<0.001
					2016	-2.4	0.34	-6.94	<0.001

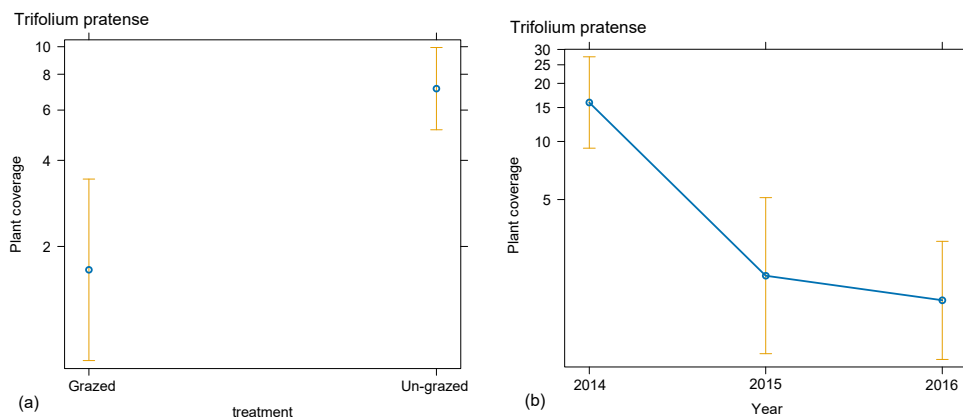


Figure 7. a) two treatments (grazed and un-grazed) effect on *Trifolium pratense* abundance, b) time effect on *T. pratense* abundance. The yellow bar in the graph represents the standard error (SE), the blue spot indicates the Mean; the short line extending upwards is Mean+SE and the short line extending downwards represents Mean-SE.

## Discussion

This study showed that the diversity of flowering herbaceous plants could be enhanced by reintroducing domesticated large herbivore grazers, exemplified by the horses, as an ecologically functional replacement for extinct wild taxa. Shannon's and Simpson's diversity indexes both exhibited notable responses to grazing showing significantly higher scores in grazed compared to the un-grazed areas. This suggests that grazing positively influenced the overall diversity of flowering herbaceous plants. Loucougaray et al. (2004) reported higher values for species diversity for horse grazing on grasslands in western France, while Lyseng et al. (2018) found that cattle grazing did not affect the plant diversity in northern temperate grasslands in Canada, whereas Ali Shtayeh et al. (2010) showed the opposite effect on semi-arid grassland under sheep and goat herd grazing in Jordan. In the present study, Shannon diversity index, which reflects both richness and evenness, was significantly higher in grazed areas, indicating a more even distribution of species abundances. Similar results were obtained by Marion et al. (2010) and Li et al. (2021), who found that grazing by horses, cattle and sheep contributed to increases in Shannon diversity scores in France and China, respectively. The higher values of Simpson's diversity index suggest a reduction in competitive-dominant species, with a more equitable distribution of species abundances induced by grazing which may result in an increased plant diversity as previously reported for the same rewilding experiment (Garrido et al. 2019). Plant evenness, however, was notably higher in grazed conditions in 2015, while such a difference dissipated in 2016. The lack of a significant difference in 2016 in evenness scores between grazed and un-grazed areas may be explained by the horses making short patches while selecting the palatable species, thus reducing the cover of plant species and the evenness distribution of the species (Ali Shtayeh et al. 2010). Another potential explanation might be due to an observer effect which does not accurately estimate plant abundance (Morrison 2016) as surveys were performed by different people during the study period.

Species richness of plants was also higher in grazed compared to un-grazed conditions. Positive effects of grazing on plant species richness have been shown for sheep grazing (Stewart & Pullin 2008; Škornik et al. 2010), semi-feral horses and cattle grazing (Bonavent et al. 2023), horse grazing (Öckinger et al. 2006; Köhler et al. 2016; Mutillod et al. 2024) as well as cattle grazing (Beck et al. 2015). Positive impacts of grazing have also been reported by Marion et al. (2010) and

(Dvorský et al. 2022). However, negative effects of grazing on grassland plant diversity have also been observed (Zhang et al. 2023). The positive effects of grazing may be modulated by habitat productivity and herbivore size (Bakker et al. 2006). Such positive effects on biodiversity modulated by habitat productivity have also been reported in a rewilding context (van Klink et al. 2020). Herbivore grazing positively affects plant diversity and richness by limiting light competition (Huisman & Olff 1998) and increasing sunlight availability for short plants or seeds to grow (Jutila & Grace 2002). Moreover, herbivores may also create open patches by rooting, wallowing and trampling which may benefit arthropod diversity (van Klink & de Vries 2018). Additionally, horses as selective grazers, show strong preferences for grass or grass-like plants (Cosyns et al. 2001; Allen et al. 2013; Martinson et al. 2016; Zielke et al. 2019), thus limiting the use of flowering plants which may positively impact pasture diversity, increase forb cover (Beck et al. 2015) and pollinator diversity (Öckinger et al. 2006). The results of the present study are therefore in line with current research. This is not surprising as large herbivore grazers have been an important component of grassland and wood-pasture ecosystems for millennia (Pearce et al. 2023; Davoli et al. 2024). Thus, the reintroduction of a functional replacement species of an extinct wild large herbivore may have positive effects on biodiversity and the restoration of ancient wood-pasture landscapes (Garrido et al. 2019, 2021, 2022).

Indicator species can be used to determine the overall diversity or environmental conditions from the abundance or richness of species, using only a small number of species as an alternative to the large number of species needed to investigate diversity (Tälle et al. 2023). Among indicator plant species, the genus *Trifolium* is of high interest for grassland conservation due to the ability to fix nitrogen and improve soil health (Hill et al. 2021). *Trifolium* species can improve soil fertility through nitrogen fixation which may contribute to promoting other plants' vigour (Lane et al. 2000; McKenna et al. 2018), further the un-digested or deposited nitrogen consumed by animals could return as fertilizer for other plants in the pasture (Hancock & Harmon n.d.), resulting in increasing landscape diversity and plant species richness.

In this study, *T. repens* increased in grazed areas but declined in un-grazed conditions. *T. medium* showed increased abundance in un-grazed areas over time, and *T. pratense* declined over time, with higher abundance observed in un-grazed areas. These differential responses highlight the importance of considering individual species' dynamics when assessing the ecological impacts of grazing (Liu et al. 2015). Grazing can affect species composition and distribution, potentially altering competitive interactions and resource availability within the plant community (Kohyani et al. 2011; Li et al. 2022). The three *Trifolium* species are all perennial species. *T. medium* is a long-lived perennial which is thought to be the

most persistent clover species (Egan et al. 2021), and *T. pratense* is a short-lived perennial, normally 2-3 years, that might decrease in abundance in sub-optimal conditions (Hyslop et al. 1999). *T. repens* is a competitive species, which adapts to harsh climates and soil conditions better than other species of clover (Sawicka et al. 2023). Recent research suggested that an increase in *T. repens* abundance favoured the abundance and diversity of wild bees (Beye et al. 2022), which might explain the results reported by Garrido et al. (2019), where they found that butterfly and bumblebee species richness and feeding activities were higher in grazed areas. *Trifolium* species might be therefore important food resources for pollinating insects (Pywell et al. 2005). *T. pratense* is also one of the most favoured legumes for bumblebee species for pollen and nectar foraging in summertime (Kleijn & Raemakers 2008), and it is considered to provide late-season flowering resources to mitigate the loss of pollinators (Rundlöf et al. 2014). Therefore, using *Trifolium* species as indicator taxon to elucidate potential rewilding effects on biodiversity may facilitate biodiversity-oriented assessments of grasslands in future restoration projects.

This study demonstrates the positive impact of rewilding with horses on the diversity of flowering herbaceous plants. These findings are consistent with previous research showing that horse grazing is beneficial for plant diversity and pollinator richness. The different *Trifolium* species responded variably to grazing which suggests that they may be useful as indicator species for biodiversity assessments in rewilding projects. Given that 80% of EU habitats are degraded, restoration is crucial. The European Commission has adopted a Nature Restoration Law (Directorate-General for Environment 2022), which aims to restore European ecosystems. Rewilding could therefore be a valuable addition to EU restoration efforts in forests, wetlands and grassland ecosystems which could help increase and ensure a sustained recovery of biodiversity and restore ecosystem function and resilience.

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## Popular science summary

The global biodiversity crisis is accelerating, with species extinction rates increasing due to human activities such as habitat destruction, pollution, and climate change. One promising approach to addressing this issue is trophic rewilding, which involves the reintroduction of keystone species to restore natural ecosystems. A recent study conducted in southeast Sweden sought to examine this concept by reintroducing Gotland Russ horses, a hardy breed, to determine their potential to help restore and enhance biodiversity in abandoned agricultural landscapes.

The three-year experiment, conducted from 2014 to 2016, aimed to investigate whether these horses could have a positive impact on plant diversity and ecosystem health. Twelve young horses were introduced into three 10-hectare enclosures, where they grazed freely without supplemental feed. This study investigated plant species richness, diversity, and evenness in both grazed and un-grazed areas within these enclosures, and found that areas grazed by horses had significantly higher plant species diversity and richness compared to un-grazed areas.

Additionally, certain plant species thrived under grazing conditions. For example, the abundance of white clover (*Trifolium repens*) increased in grazed areas, highlighting the beneficial effects of horse grazing on certain plant species. Horses help to maintain open landscapes by foraging and trampling, reducing plant dominance, and allowing a greater diversity of plants to flourish.

This study highlights the importance of large herbivores in maintaining ecosystems health. Historically, wild horses and other large grazers played a crucial role in shaping grassland and forest ecosystems across Europe. Their reintroduction can help restore these landscapes to their former biodiversity-rich states.

In conclusion, the rewilding of ecosystems with large herbivores, such as the Gotland Russ, represents a viable strategy for combating biodiversity loss. By promoting plant diversity and ecosystem balance, rewilding can help create healthier and more resilient environments. Given the ongoing environmental challenges we face, innovative approaches such as this are essential for the conservation of our planet.



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# Appendix 1

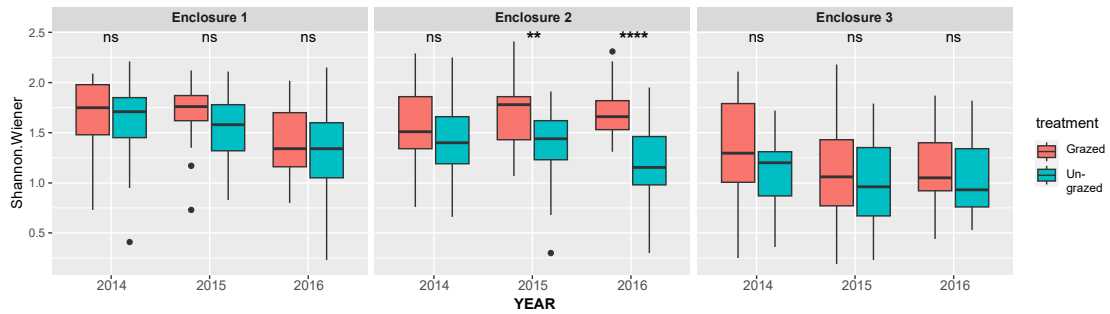


Figure S1. The Shannon-Wiener diversity index comparison among treatments in three Enclosures within the three years experimental time. Black line represents the mean value, black dot represents the outliers. Significant symbol:  $p \leq 0.0001$ : “\*\*\*\*”;  $p \leq 0.001$ : “\*\*\*”;  $p \leq 0.01$ : “\*\*”;  $p \leq 0.05$ : “\*”;  $p > 0.05$ : “ns”.

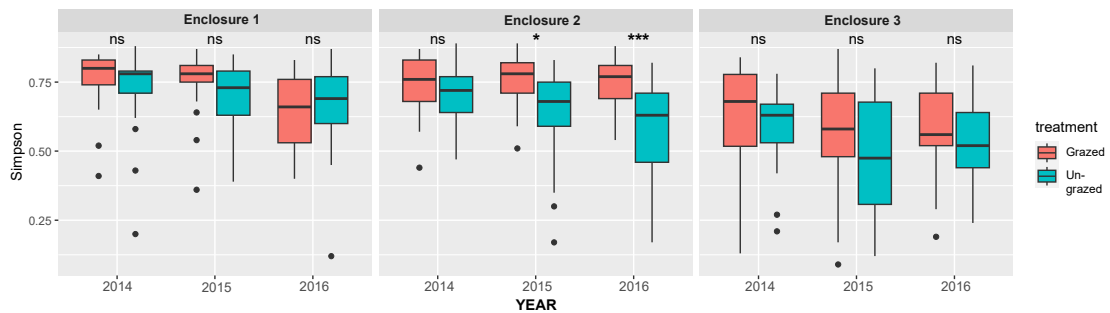
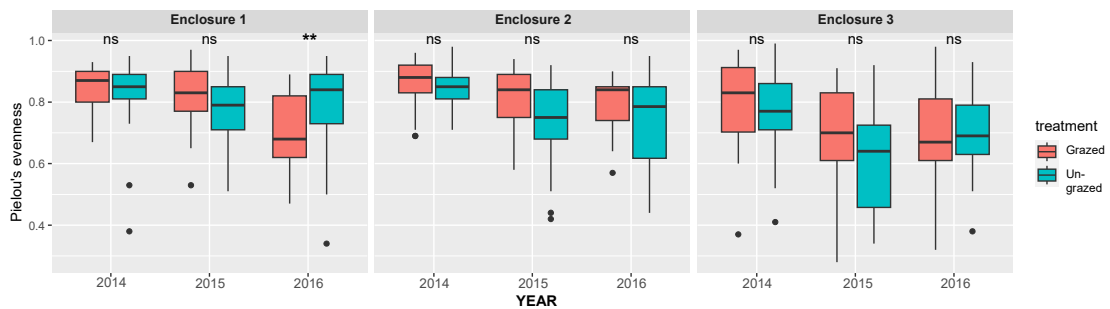


Figure S2. The Simpson's diversity comparison among treatments in three Enclosures within the three years experimental time. Black line represents the mean value, black dot represents the outliers. Significant symbol:  $p \leq 0.0001$ : “\*\*\*\*”;  $p \leq 0.001$ : “\*\*\*”;  $p \leq 0.01$ : “\*\*”;  $p \leq 0.05$ : “\*”;  $p > 0.05$ : “ns”.



*Figure S3. The plant evenness comparison among treatments in three Enclosures within the three years experimental time. Black line represents the mean value, black dot represents the outliers. Significant symbol:  $p \leq 0.0001$ : “\*\*\*\*”;  $p \leq 0.001$ : “\*\*\*”;  $p \leq 0.01$ : “\*\*”;  $p \leq 0.05$ : “\*”;  $p > 0.05$ : “ns”.*

## Appendix 2

Table S1. 95 different herbaceous species (recorded in July during the three-year experiment).

Name	Species	Functional Type	Life Cycle	Year
<i>Achillea millefolium</i>	<i>A. millefolium</i>	Forb	Perennial	2014/15/16
<i>Aegopodium podagraria</i>	<i>A. podagraria</i>	Forb	Perennial	2014/15/16
<i>Alchemilla monticola</i>	<i>A. monticola</i>	Forb	Perennial	2014
<i>Alchemilla sp</i>		Forb	Perennial	2015/16
<i>Anchusa arvensis</i>	<i>A. arvensis</i>	Forb	Annual	2016
<i>Anemone nemorosa</i>	<i>A. nemorosa</i>	Forb	Perennial	2015/16
<i>Anthriscus sylvestris</i>	<i>A. sylvestris</i>	Forb	Perennial	2014/15/16
<i>Campanula rotundifolia</i>	<i>C. rotundifolia</i>	Forb	Perennial	2016
<i>Campanula sp</i>		Forb	Perennial	2016
<i>Carex flava</i>	<i>C. flava</i>	Graminoid	Perennial	2014
<i>Carex leporina</i>	<i>C. leporina</i>	Graminoid	Perennial	2014
<i>Carex pallescens</i>	<i>C. pallescens</i>	Graminoid	Perennial	2014
<i>Carex sp</i>		Graminoid	Perennial	2015/16
<i>Centaurea jacea</i>	<i>C. jacea</i>	Forb	Perennial	2016
<i>Centaurea montana</i>	<i>C. montana</i>	Forb	Perennial	2014
<i>Cerastium arvense</i>	<i>C. arvense</i>	Forb	Perennial	2016
<i>Cerastium fontanum</i>	<i>C. fontanum</i>	Forb	Perennial	2014/15/16
<i>Cirsium arvense</i>	<i>C. arvense</i>	Forb	Perennial	2014/15/16
<i>Cirsium palustre</i>	<i>C. palustre</i>	Forb	Biennial /Perennial	2014/15/16
<i>Cirsium vulgare</i>	<i>C. vulgare</i>	Forb	Biennial	2014/15/16
<i>Cynosurus cristatus</i>	<i>C. cristatus</i>	Graminoid	Perennial	2014
<i>Epilobium sp</i>		Forb	Annual /Perennial	2016
<i>Equisetum arvense</i>	<i>E. arvense</i>	Fern	Perennial	2014/15/16
<i>Equisetum palustre</i>	<i>E. palustre</i>	Fern	Perennial	2014/15/16
<i>Equisetum pratense</i>	<i>E. pratense</i>	Fern	Perennial	2014/15/16
<i>Equisetum sylvaticum</i>	<i>E. sylvaticum</i>	Fern	Perennial	2014/15/16
<i>Festuca rubra</i>	<i>F. rubra</i>	Graminoid	Perennial	2014
<i>Filipendula ulmaria</i>	<i>F. ulmaria</i>	Forb	Perennial	2014/15/16
<i>Fragaria vesca</i>	<i>F. vesca</i>	Forb	Perennial	2014/15/16
<i>Galium album</i>	<i>G. album</i>	Forb	Perennial	2014/15/16
<i>Galium palustre</i>	<i>G. palustre</i>	Forb	Perennial	2014/15/16
<i>Galium uliginosum</i>	<i>G. uliginosum</i>	Forb	Perennial	2014/15/16
<i>Galium verum</i>	<i>G. verum</i>	Forb	Perennial	2014/15/16
<i>Geranium pratense</i>	<i>G. pratense</i>	Forb	Perennial	2016

<i>Geranium sylvaticum</i>	<i>G. sylvaticum</i>	Forb	Perennial	2014/15/16
<i>Geum rivale</i>	<i>G. rivale</i>	Forb	Perennial	2014/15/16
<i>Gnaphalium sylvaticum</i>	<i>O. sylvatica</i>	Forb	Perennial	2014/15/16
<i>Hypericum maculatum</i>	<i>H. maculatum</i>	Forb	Perennial	2014/15/16
<i>Hypericum perforatum</i>	<i>H. perforatum</i>	Forb	Perennial	2014/15/16
<i>Juncus conglomeratus</i>	<i>J. conglomeratus</i>	Graminoid	Perennial	2014/15/16
<i>Juncus effusus</i>	<i>J. effusus</i>	Graminoid	Perennial	2014/15/16
<i>Lathyrus palustris</i>	<i>L. palustris</i>	Forb	Perennial	2014
<i>Lathyrus pratensis</i>	<i>L. pratensis</i>	Forb	Perennial	2014/15/16
<i>Leucanthemum vulgare</i>	<i>L. vulgare</i>	Forb	Perennial	2014/15/16
<i>Lotus corniculatus</i>	<i>L. corniculatus</i>	Forb	Perennial	2015
<i>Luzula multiflora</i>	<i>L. multiflora</i>	Graminoid	Perennial	2014/15/16
<i>Luzula pilosa</i>	<i>L. pilosa</i>	Graminoid	Perennial	2015/16
<i>Melampyrum nemorosum</i>	<i>M. nemorosum</i>	Forb	Annual	2014/15/16
<i>Melampyrum pratense</i>	<i>M. pratense</i>	Forb	Annual	2014/15/16
<i>Melampyrum sylvaticum</i>	<i>M. sylvaticum</i>	Forb	Annual	2014/15
<i>Mentha arvensis</i>	<i>M. arvensis</i>	Forb	Perennial	2014/15/16
<i>Myosotis arvensis</i>	<i>M. arvensis</i>	Forb	Biennial	2014/15/16
			/Perennial	
<i>Pilosella lactucella</i>	<i>P. lactucella</i>	Forb	Perennial	2014/15/16
<i>Plantago major</i>	<i>P. major</i>	Forb	Perennial	2014/15/16
<i>Poa trivialis</i>	<i>P. trivialis</i>	Graminoid	Perennial	2014
<i>Potentilla anserina</i>	<i>P. anserina</i>	Forb	Perennial	2014/15/16
<i>Potentilla sp</i>		Forb	Annual	2016
			/Biennial	
			/Perennial	
<i>Primula veris</i>	<i>P. veris</i>	Forb	Perennial	2016
<i>Prunella vulgaris</i>	<i>P. vulgaris</i>	Forb	Perennial	2014/15/16
<i>Ranunculus acris</i>	<i>R. acris</i>	Forb	Perennial	2014/15/16
<i>Ranunculus auricomus</i>	<i>R. auricomus</i>	Forb	Perennial	2014/15/16
<i>Ranunculus repens</i>	<i>R. repens</i>	Forb	Perennial	2014/15/16
<i>Rhinanthus minor</i>	<i>R. minor</i>	Forb	Annual	2014
<i>Rhinanthus serotinus</i>	<i>R. serotinus</i>	Forb	Annual	2014/15/16
<i>Rumex acetosa</i>	<i>R. acetosa</i>	Forb	Perennial	2014/15/16
<i>Rumex acetosella</i>	<i>R. acetosella</i>	Forb	Perennial	2014
<i>Rumex crispus</i>	<i>R. crispus</i>	Forb	Perennial	2014/15
<i>Rumex longifolius</i>	<i>R. longifolius</i>	Forb	Perennial	2014
<i>Rumex sp</i>		Forb	Annual	2015/16
			/Biennial	
			/Perennial	
<i>Sagina procumbens</i>	<i>S. procumbens</i>	Forb	Perennial	2014/15
<i>Scorzonerooides autumnalis</i>	<i>S. autumnalis</i>	Forb	Perennial	2016
<i>Stellaria graminea</i>	<i>S. graminea</i>	Forb	Perennial	2014/15/16
<i>Stellaria longifolia</i>	<i>S. longifolia</i>	Forb	Perennial	2015
<i>Stellaria media</i>	<i>S. media</i>	Forb	Annual	2016
<i>Succisa pratensis</i>	<i>S. pratensis</i>	Forb	Perennial	2015

<i>Taraxacum pallidipes</i>	<i>T. pallidipes</i>	Forb	Perennial	2014/15/16
<i>Taraxacum vulgare</i>	<i>T. officinale</i>	Forb	Perennial	2015/16
<i>Tragopogon pratensis</i>	<i>T. pratensis</i>	Forb	Biennial	2014/15/16
<i>Trifolium hybridum</i>	<i>T. hybridum</i>	Forb	Perennial	14,15
<i>Trifolium medium</i>	<i>T. medium</i>	Forb	Perennial	2014/15/16
<i>Trifolium pratense</i>	<i>T. pratense</i>	Forb	Perennial	2014/15/16
<i>Trifolium repens</i>	<i>T. repens</i>	Forb	Perennial	2014/15/16
<i>Tripleurospermum maritimum</i>	<i>T. maritimum</i>	Forb	Annual /Biennial /Perennial	2016
<i>Tripleurospermum perforatum</i>	<i>T. inodorum</i>	Forb	Annual	2014
<i>Tussilago farfara</i>	<i>T. farfara</i>	Forb	Perennial	2014/15/16
<i>Urtica dioica</i>	<i>U. dioica</i>	Forb	Perennial	2015/16
<i>Veronica arvensis</i>	<i>V. arvensis</i>	Forb	Annual	2014
<i>Veronica chamaedrys</i>	<i>V. chamaedrys</i>	Forb	Perennial	2014/15/16
<i>Veronica officinalis</i>	<i>V. officinalis</i>	Forb	Perennial	2014/15/16
<i>Veronica serpyllifolia</i>	<i>V. serpyllifolia</i>	Forb	Perennial	2015/16
<i>Vicia cracca</i>	<i>V. cracca</i>	Forb	Perennial	2014/15/16
<i>Vicia sepium</i>	<i>V. sepium</i>	Forb	Perennial	2015/16
<i>Viola arvensis</i>	<i>V. arvensis</i>	Forb	Perennial	2014/15/16
<i>Viola riviniana</i>	<i>V. riviniana</i>	Forb	Perennial	2014/15/16

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