

**The significance of connectivity by livestock  
for restoration and conservation of calcareous grasslands**



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## SUMMARY

Connecting remnants of calcareous grasslands (*Festuco-Brometea*) by (rotational) grazing livestock is considered to be an important tool for the restoration and conservation of these ancient and species-rich habitats. The planning of conservation measures is, however, difficult due to the lack of information on the specific potential that typical calcareous grassland grazers (e.g., cattle, goats and sheep) may provide for the spatial dispersal of diaspores between isolated and fragmented habitat patches. Moreover, information on the long-term success of livestock-based restoration measures is hardly available. Consequently, the thesis at hand aims to fill these gaps.

**Chapter 1** provides a general introduction to the history of calcareous grasslands, briefly summarizing the development and the traditional management of these nutrient-poor, species-rich habitats. Furthermore, current problems regarding the continuity of calcareous grassland are addressed and the significance of spatial connectivity as well as the implementation of restoration measures aiming to increase the cover area of calcareous grasslands are emphasised.

**Chapter 2** deals with investigating the epi- and endozoochorous dispersal capability of the historically three most important calcareous grassland grazers – cattle, goats and sheep.

Epizoochorous dispersal was shown to be best realized with sheep. The analysis of endozoochorous dispersal showed that cattle were superior in terms of dispersed species richness, in particular for Red List species, indicating the significance of rotational cattle grazing for maintaining species-rich calcareous grassland networks alongside rotational grazing with sheep. The importance of rotational sheep grazing was further explored in the next two chapters.

Former calcareous grasslands, which had been abandoned and overgrown or afforested in the past, were restored by clearance in the early 1990s

and were subsequently managed by extensive sheep grazing. Since restoration was initiated, the vegetation composition has been continuously monitored over a period of 25 years at seven different study sites with different initial vegetation conditions located in the Swabian Alb. In **Chapter 3**, the floristic changes of these restored grasslands were analysed in a comparison with adjacent ancient grasslands. The vegetation composition of the restored grasslands distinctly developed towards the reference *Festuco-Brometea* grasslands but after 25 years differences in terms of nutrient content, rare species and habitat characteristic species were still detected between both grassland categories. Overall, the restoration of former calcareous grasslands by clearance and subsequent grazing proved to be a successful measure, but the reestablishment of the floristic composition was not yet completed after 25 years.

Since functional plant traits were suggested as a more appropriate tool for tracking changes in the vegetation composition, the functional composition was investigated in **Chapter 4** for one of the clear-cut study sites from Chapter 3 ('Haarberg-Wasserberg'). Covering the observation period of 25 years, ecological meaningful functional trait attributes were compared between restored and reference grasslands and the development of plant functional groups was investigated.

Most functional traits converged quickly towards reference values. Dispersal- and establishment-related traits recovered within the first years after restoration, while persistence-related traits showed differences for more than 20 years after initial restoration. Differences that could be detected after 25 years were mostly related to the composition of plant species.

Moreover, three functional groups were identified which were characterised by traits that correspond to the classification into competitiveness, stress-



tolerators and ruderals according to Grime's CSR-strategy. After 15 years, the plant functional group of ruderal species could no longer be found, while the group of competitive species became less important containing mostly woody shrub and tree species. Stress tolerators dominated the restored and the reference grassland after 20 years showing no differences between both grassland categories, which indicated that the functional composition of the restored vegetation recovered over a period of less than 25 years.

**Chapter 5** shortly summarises the results of the previous chapters and evaluates, based on these results, the potential of grazing livestock for connecting calcareous grasslands and their importance for habitat restoration. Finally, the general advantage of zoochorous dispersal vectors for conservation measures is addressed and recommendations for the implementation of restoration measures concerning calcareous grasslands are given.

**CHAPTER 1**  
**GENERAL INTRODUCTION**



## THE HISTORIC DEVELOPMENT AND CURRENT SITUATION OF CALCAREOUS GRASSLANDS

Nutrient-poor, species-rich calcareous grasslands are an ancient man-made habitat which had their peak expansion between the period of the Roman Empire and the middle of the 19<sup>th</sup> century (Poschlod, 2017; Poschlod and Baumann, 2010). Moreover, there is evidence that these grasslands were already present during the Neolithic period, at least locally (Dutoit et al., 2009; Poschlod and Baumann, 2010; Robin et al., 2018).

Calcareous grasslands were traditionally managed by extensive grazing or occasionally by mowing (Poschlod, 2017; Poschlod and WallisDeVries, 2002). In this context, rotational grazing by flocks of sheep and goats, but also cattle, was reported for former times (Baumann et al., 2005; Poschlod, 2017). Before the invention of mineral fertiliser, the dung of the animals was a valuable resource and therefore livestock, in particular sheep, grazed the calcareous grasslands during the days and were flocked onto arable fields during the night for defecation (Poschlod, 2017). This led to the development of a specific plant species composition which is adapted to the constant nutrient impoverishment and pressure caused by the traditional low intensive grazing management (Karlík and Poschlod, 2009; Poschlod and WallisDeVries, 2002). Additionally, many typical calcareous grassland species rely on livestock to act as mobile vector for the dispersal of their diaspores (Fischer et al., 1996; Poschlod et al., 1998).

Calcareous grasslands rank among the most species-rich habitats worldwide and are considered as the most species-rich habitat types in Central Europe (WallisDeVries et al., 2002; Willems, 1990), harbouring many vascular plants that are endemic to Europe (Hobohm and Bruchmann, 2009).

During the past 150 years changes in traditional land-use practices have altered the European landscape tremendously (Poschlod, 2017;

Poschlod and WallisDeVries, 2002) leading to a severe decline in number and size of calcareous grasslands across Europe. For the county of Dorset, United Kingdom, Hooftman & Bullock (2012) reported for instance an 89% decline in managed calcareous grassland area between 1930 and 2000. For a Belgian hotspot region, the area loss amounted to more than 90% since the beginning of the 20<sup>th</sup> century (Adriaens et al., 2006; Bisteau and Mahy, 2005) while Cousins et al. (2015) reported a decline of 96% of the calcareous grassland area for a Swedish landscape during the same period and Pärtel et al. (1999) found a decline of 70% for an Estonian hotspot region since the 18<sup>th</sup> century.

A similar extent for the loss of calcareous grassland was also found for a German hotspot region in the Swabian Alb, Baden-Württemberg, which represents one of the two study areas that are included in this thesis. Here, a decline of at least 75% of the calcareous grassland area was observed for the period between 1827 and 2016 (Figure 1). Similar numbers were also recorded for other regions of Baden-Württemberg (Mailänder et al., 2004; Poschlod, 2017).

For the second study region of this thesis, which is located in the Franconian Alb in the municipality of 'Kallmünz', Bavaria, the expansion of calcareous grasslands was studied for the period between 1830-40 and 1990 (Baumann et al., 2005). Here, a comparable small area loss of 31% was found. It is, however, likely that there has been a further loss of calcareous grassland area over the past three decades, but there are no recent investigations on this matter.

Concerning the location of 'Haarberg-Wasserberg', the strongest driver for the loss in calcareous grassland area was the establishment of forest, most likely in the form of afforestations. Shrub encroachment due to successional process following abandonment and the conversion into arable fields were, however, also frequently recorded as reason for the decline.

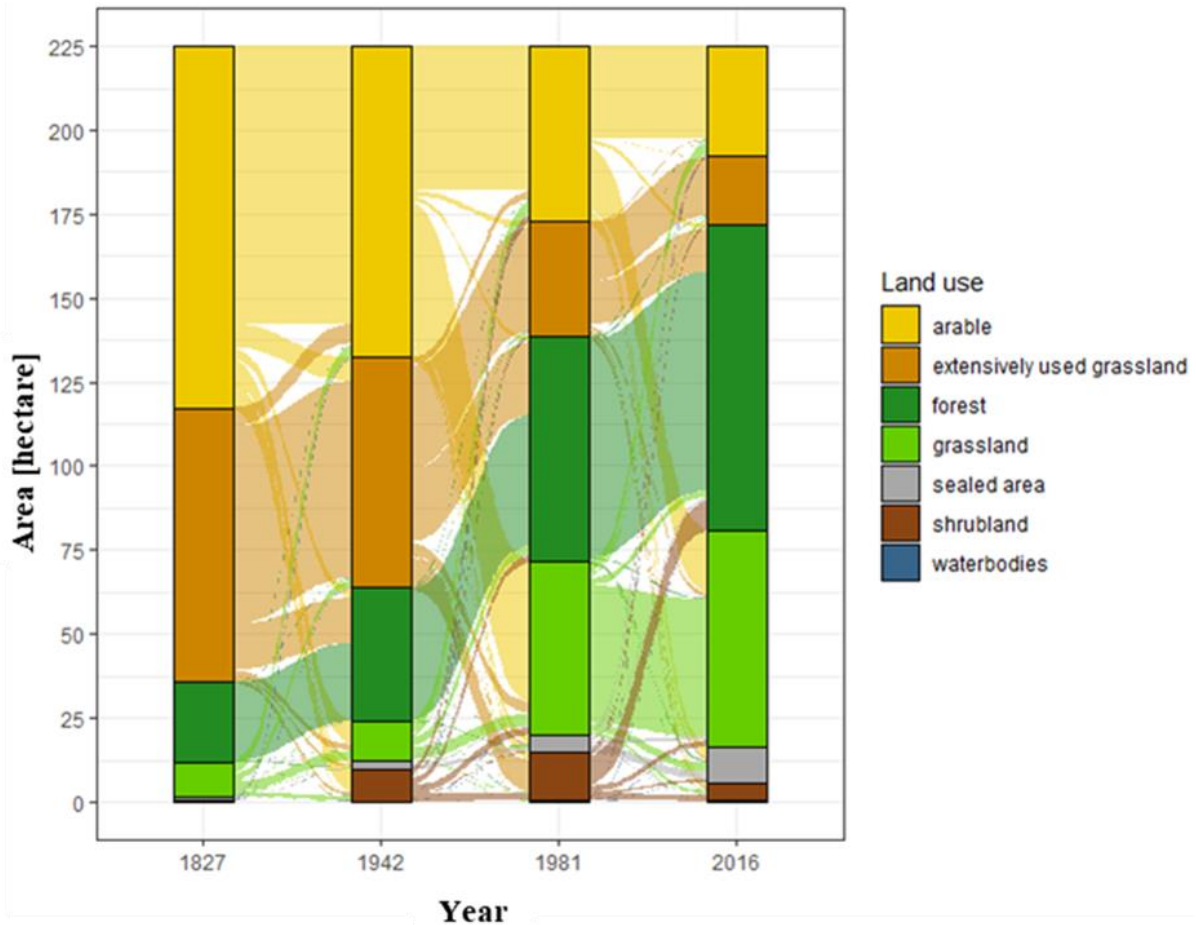


Figure 1: Land-use changes between 1827 and 2016 in an area of 225 ha surrounding the nature reserve 'Haarberg-Wasserberg' in the Swabian Alb, Baden-Württemberg, Germany. Differences in the respective land-use distributions between the four survey dates are illustrated in the flow chart. The extraction on land-use information was done in ArcGIS 10.3.1 and was based on cadastre maps from 1827 (Landesamt für Geoinformation und Landentwicklung Baden-Württemberg (LGL)), topographic maps from 1942 (Reichsamt für Landesaufnahme), 1981 and 2016 (Landesvermessungsamt Baden-Württemberg). Additionally, aerial imagery from the years 1988 and 2017 were used.

For the region in the surroundings of 'Kallmünz', afforestation and abandonment were mentioned as main drivers for the area loss (Poschlod et al., 2016).

On the European scale, the rapid loss and fragmentation of calcareous grasslands can be led back to the intensification of the traditional land-use practices during the 20<sup>th</sup> century. Consequently, former grasslands have either been ameliorated, were afforested or abandoned (Kimberley et al., 2021; Poschlod et al., 2005; Ridding et al., 2015; WallisDeVries et al., 2002).

As a result, a minor loss of calcareous grassland area has already been recorded towards the end of the 19<sup>th</sup> century. During this period, prices for sheep wool dropped since this good was increasingly shipped from overseas accordingly leading to a first decline in sheep husbandry. With the invention of mineral fertiliser in the 20<sup>th</sup> century, the agricultural usage of marginal yield sites like calcareous grasslands became highly uneconomical which marked the beginning of a particularly strong area loss (Poschlod, 2017; Poschlod and WallisDeVries, 2002).

Due to the tremendous decline in calcareous grassland area over the past 150 years, the habitat is protected by European law since the 1990s under the Natura 2000 Habitat Directives (Habitat Code: 6210, European Commission 1992). Consequently, remaining calcareous grasslands are a main issue in restoration and conservation ecology (Poschlod et al., 2005; WallisDeVries et al., 2002). The conservation of the typical high biodiversity is thereby a particular important topic in European conservation strategies (Kahmen and Poschlod, 2008). Several projects aiming to investigate the success of restoration and conservation measures in calcareous grasslands have therefore been initiated since the 1990s (Poschlod et al., 2005).

However, abandonment or maintenance with an unsuitable management is an ongoing problem since more and more shepherds quit herding sheep due to the long and hard workdays, the poor financial compensation or the poorly maintained infrastructure (i.e., vaccines for sheep illnesses, sheepshearers, poor connecting routes between pastures). The list of problems shepherds face nowadays are ongoing. The above mentioned problems were frequently raised in interviews with nine shepherds in the Western Eifel in the winter of 2017/18 (Munck et al., 2018). Due to these reasons, there is an constant decline in practising shepherds and sheep in Germany (BLE 2020). Those shepherds, who still herd sheep, no longer earn their money by selling the wool and meat, but by grazing the grasslands that are of high conservation value while being (poorly) compensated by the European Union (Strijker, 2005).

The fact that more and more shepherds are quitting is highly problematic for the continued existence of the calcareous grasslands, as more and more species-rich habitats can no longer be appropriately maintained. Extensive grazing has been stressed to be crucial for the maintenance of the high species and genetic diversity in calcareous grasslands while migrating sheep have

been proposed as important vectors for the dispersal of seeds between calcareous grasslands (Adriaens et al., 2006; Fischer et al., 1996; Lehman et al., 2020; Manzano and Malo, 2006; Rico et al., 2012; Tälle et al., 2016). The area loss and the increasing fragmentation in turn hamper the dispersal of plant species.

Therefore, studies on the services that livestock provide for the connectivity of habitats and thus for the maintenance of biodiversity are important in order to acknowledge and promote the profession of shepherds as well as of farmers that practice rotational livestock grazing from a political point of view.

### **THE IMPORTANCE OF CONNECTIVITY AND HABITAT RESTORATION**

For the successful restoration and conservation of remaining calcareous grasslands with their high biodiversity, it is crucial to understand the connectivity between habitats (Plue and Cousins, 2018; Poschlod et al., 1998) which includes the pollen transfer by insects as well as the dispersal of diaspores of plant species (Auffret et al., 2015). Thereby, Brückmann et al. (2010) or Steffan-Dewenter and Tschardtke (1999) could, for instance, demonstrate that habitat fragmentation leads to a decrease in pollinator efficiency and thus, to reduced exchange of pollen between populations. Contrary, a positive influence of seed dispersal with sheep (Fischer et al., 1996) or in general, with livestock (Kimberley et al., 2021) was found for species richness as well as for the genetic diversity (Rico et al., 2014) in calcareous grassland habitats.

The potential of livestock to act as mobile corridor and to connect remnants of calcareous grasslands by diaspore dispersal is, however, still poorly understood, even though it has been stressed that the alternations in the historic land-use and hence, changes in the connectivity between habitats, have a severe impact on the dispersal of plant species in the present day landscapes (Hooftman and

Bullock, 2012; Luoto et al., 2003b; Poschlod and Bonn, 1998). This thesis therefore focused on investigating the importance of connectivity via livestock, which includes the dispersal in the fur or intestinal tract of animals (Poschlod et al., 2003).

Moreover, many studies found that the species richness of remaining habitats are endangered by the occurrence of an extinction debt (Auffret et al., 2018; Diekmann et al., 2019; Helm et al., 2006; Piqueray et al., 2011a), meaning the delayed extinction of plant species due to the fragmentation as species are less likely immigrate from nearby populations to prevent local extinctions (Damschen et al., 2019; Hooftman et al., 2016). In this context, the restoration of former calcareous grassland habitats as well as the expansion of existing habitats might be crucial to prevent a further loss of biodiversity (Eriksson et al., 2002).

Further evidence on the importance of habitat size and connectivity has been given by Evju and Sverdrup-Thygeson (2016), who were able to show that large sized grasslands and grasslands, which were situated in closer proximity to each other, were characterised by a higher species richness. A positive effect of increasing the size of isolated calcareous grassland fragments was also detected for the occurrence of important pollinators (e.g. bees, Klaus et al. 2021; orthopterans, König & Krauss 2019).

This thesis was therefore dedicated to investigate the potential of connectivity between fragmented calcareous grassland habitats provided by grazing livestock as well as the evaluation of a restoration measure aiming to increase the size of calcareous grassland habitats. Information on both topics may be crucial for guiding future restoration and conservation measures in the best possible way.

## THESIS OUTLINE

Restoration and conservation of species-rich and nutrient-poor calcareous grasslands has been a

main research topic throughout Europe for the past decades (Poschlod et al., 2005). Some major findings of previous studies implied that extensive grazing by livestock is not only important for the maintenance of the high biodiversity but also for promoting the connectivity by the dispersal of plant species in the fur or the intestinal tract of migrating animals (Adriaens et al., 2006; Fischer et al., 1996; Manzano and Malo, 2006; Rico et al., 2012; Tälle et al., 2016). However, detailed information on this topic is still missing.

The first topic of this thesis was therefore the validation of the potential capability of the historically most important grazers, sheep as well as cattle and goat (Baumann et al., 2005; Poschlod and WallisDeVries, 2002), to act as mobile vector between remnants of calcareous grasslands. Thus, **Chapter 2** was dedicated to the investigation of the endo- and epizoochorous seed dispersal capability of typical calcareous grassland species with these three livestock types.

The restoration of former calcareous grasslands was the second topic of this thesis. Thereby, **Chapter 3** dealt with the floristic analysis and **Chapter 4** used a functional approach to evaluate restoration success on seven former calcareous grasslands, restored by clearance and subsequently managed by rotational sheep grazing. Until now, restoration success of more than one study location has only been estimated based on short-term observations or chronosequence studies (e.g. Barbaro et al., 2001; Bistea and Mahy, 2005; Piqueray et al., 2011b). The long-term observations of this study enabled the investigation of several study locations on continuously recorded vegetation data. Using the here presented approach, the influence of site-specific habitat conditions on the restoration success can be excluded (Helsen et al., 2013). Moreover, it is stressed that long-term observations are prudent for accurately detecting general trends (Cusser et al., 2021).

To conclude this thesis, **Chapter 5** discussed the significance of rotational livestock grazing and the

long-term success of animal-assisted restoration measures, based on the results of Chapter 2 to 4, and assessed their value for restoration and conservation of calcareous grassland habitats.

## CHAPTER 2

# CATTLE AND NOT ONLY GOATS AND SHEEP MAY SERVE AS IMPORTANT MOBILE CORRIDORS CONNECTING CALCAREOUS GRASSLANDS - A COMPARATIVE STUDY ON THE ZOOCHOROUS DISPERSAL CAPABILITY

### ABSTRACT

Land use changes have profoundly altered the connectivity of calcareous grasslands by migratory livestock during recent decades. Specific information on the individual dispersal capability of typical livestock grazers for calcareous grassland plant species is therefore urgently needed, but is so far, largely missing. This study aims to fill this gap by comparing the three most common calcareous grassland grazers (cattle, goats and sheep) in their endo- and epizoochorous dispersal capability. In addition, we investigate which plant functional traits have an influence on the dispersal probability.

The study region is located in the Franconian Alb, Germany, where, in addition to grazing with sheep and goats, rotational grazing with cattle was practised until the middle of the 20th century. Individuals of each livestock type grazed on the same calcareous grassland for a period of four days twice a year in 2018 and 2019. Dung samples and seeds adhering to the fur were collected at fixed time intervals to obtain information on transported seed densities and plant species composition. Functional characteristics of transported plant species were additionally considered.

In terms of epizoochory, most frequently transported species were characterised by seed appendices and originated from the *Poaceae* family. Sheep with long, curly hair transported significantly more seeds and plant species than cattle and goats with short, straight hair.

Regarding endozoochory, we found that feeding preference, low seed mass and low canopy height (only in cattle) positively influenced dispersal likelihood while seed supply and seed persistence had no significant impact. Most frequently transported families were *Poaceae*, *Fabaceae* and *Rubiaceae*. Thereby, cattle not only dispersed the highest proportion of species but also of Red List species.

We strongly recommend not only the deployment of sheep and goat but also of cattle grazing for restoration and conservation management in calcareous grassland habitats as all three animal types have the potential to act as important mobile vector to enhance connectivity between grasslands.

### KEYWORDS

Binomial model, biodiversity, endozoochory, epizoochory, grazing, functional traits, seed dispersal



## INTRODUCTION

Seed dispersal, an important life-history trait of plant species, is a key factor for explaining species diversity and has long been acknowledged as an important aspect in restoration and conservation ecology of plant communities (Bakker et al., 1996b; Bistea and Mahy, 2005; Poschlod et al., 1998; Poschlod and Bonn, 1998). Through the dispersal of seeds, fragmented habitat patches can be connected (Auffret et al., 2012; Rico et al., 2014) as well as restored habitats with a donor site (Poschlod et al. 1998, Chapter 3). This connectivity is important for the persistence (Kimberley et al., 2021; Luoto et al., 2003a) and establishment of plant metapopulations (Hanski, 1999; Kapás et al., 2020; Poschlod, 1996). Seed dispersal becomes even more relevant when plant species cannot rely on regeneration from a long-term persistent seed bank (Ozinga et al., 2005). In this case spatial seed dispersal, particularly long-distance dispersal, becomes a crucial bottle-neck in restoration and conservation ecology (Pywell et al., 2002; Trakhtenbrot et al., 2005). Animal-mediated seed dispersal is hereby considered the most important long-distance vector in this context, potentially capable of dispersing seeds up to several hundred kilometres (Manzano and Malo, 2006; Pakeman, 2001; Thomson et al., 2011). Two dispersal vectors are most commonly distinguished: Epizoochory, where seeds are transported by attaching to body parts of the animals (e.g., fur, hooves) and endozoochory, where seeds are ingested and pass through the animals' gut before being defecated.

Animal-mediated seed dispersal also plays an important role in calcareous grasslands (*Festuco-Brometea*), a habitat type, which is nowadays a major priority of restoration and conservation ecology in many parts of Europe. These grazed grassland systems are characterised by a high biodiversity and harbour many species that have adapted to grazing (Poschlod and WallisDeVries, 2002; Willems, 1990). Due to severe land-use

changes there was a rapid loss of these valuable habitats during recent decades throughout Europe (Cousins et al., 2015; Hooftman and Bullock, 2012; Poschlod, 2017). At least in Germany, this development was accompanied by a rapid decline in the use of typical calcareous grassland grazers like goats and sheep for managing these grasslands. Moreover, cattle were increasingly kept in stables (Baumann et al., 2005; Poschlod, 2017). Since then, high-performance animals have been bred (especially concerning cattle breeds) that are unsuitable for grazing calcareous grasslands due to the low fodder quality (Poschlod and WallisDeVries, 2002) which additionally led to the endangerment of old extensively grazing breeds (Taberlet et al., 2008). Today, connectivity in southern German calcareous grasslands only relies on sheep grazing (Poschlod and WallisDeVries, 2002). Together with increasing habitat fragmentation, these developments led to a severe decrease in the connectivity between these grassland systems (Poschlod and Bonn, 1998).

Nevertheless, since the 1990ies, European calcareous grasslands are protected by law (92/43/EEC, European Commission 1992) and conservation but also restoration of the habitat is a major goal. As roughly only one third of the typical calcareous grasslands species are assumed to have at least a short-term persistent seed bank (Bekker et al., 1998b; Mitlacher et al., 2002; Poschlod et al., 1998; Von Blanckenhagen and Poschlod, 2005), many authors have suggested that the high species diversity will only be maintained if the remaining and restored habitats are spatially connected via grazing animals (Couvreur et al., 2004; Fischer et al., 1996; Kimberley et al., 2021; Luoto et al., 2003a; Rico et al., 2014). In this context, information on the endo- (via dung) and epizoochorous (via fur) seed dispersal capability of the three most important grazers (cattle, goat and sheep, (Poschlod, 2017; Poschlod et al., 2008)) is crucial for understanding processes that can positively influence future



Figure 2: Cattle herding on calcareous grasslands in the surroundings of Kallmünz, Bavaria, Germany. The picture was taken approximately around the year 1915 on the plateau of a grassland named 'Eicherberg' (Photo: Archiv Verlag Lassleben)

restoration and conservation projects aiming to maintain or re-establish these valuable habitats. Yet, most studies so far only focussed on one dispersal mode or one animal type (Auffret et al., 2012; Cosyns et al., 2005a; Kuiters and Huiskes, 2010; Mitlacher et al., 2002; Mouissie et al., 2005b; Wessels et al., 2008) or were conducted in other habitat types (near-coastal dry grasslands, Benthien et al. 2016). Other studies rather concentrated on experimental simulations of the zoochorous seed dispersal capabilities (Couvreur et al., 2005; Manzano et al., 2005; Milotić and Hoffmann, 2016; Mouissie et al., 2005a; Römermann et al., 2005; Wang et al., 2017). However, these studies only allow for assumptions regarding the potential seed dispersal efficiency of the respective livestock but did not

enable a direct comparison regarding the potential seed dispersal of cattle, goat and sheep. In this paper, we therefore present an experiment under field conditions, which allows a direct comparison of the zoochorous dispersal capability of three different livestock species. Therefore, we selected ancient extensively grazing breeds of cattle, goats and sheep, nowadays mainly kept and bred for landscape management, and gave them access to the same combination of calcareous grassland plant species. We then collected dung samples and seeds adhering to the fur at standardised time intervals. We hypothesised that the three livestock species differ in their epi- and endozoochorous dispersal capability in terms of seed abundance, and that these differences are mediated by



are annually grazed by the same semi-free ranging flock of sheep in early summer (between late May and early July). The sheep stay on the grassland for the for the duration of approximately two weeks, where they are allowed to graze during the day and are fenced in during the night.

### **LIVESTOCK**

We selected three different species of herbivores: Cattle, goats and sheep, which were the most common calcareous grassland grazers in the study region until the past century (Baumann et al., 2005; Poschlod et al., 2008). For the experiment, we used extensive grazing breeds for all three livestock types (Figure 4): One female German Red cattle and her calf (*Bos taurus* L.), two Thuringian goats (*Capra aegagrus hircus* L.) and two (2018) and three (2019) Coburger Fox sheep (*Ovis aries* L.). All animals were provided by the same local farmer family and are animals breeds, which are nowadays used for extensive grassland management. Cattle and goats had both very short and straight hair with a general mean length of 0.5 and 1.7 cm, respectively, while sheep had curly hair with a varying mean length of at least 4 cm (July 2018) to 8 cm (August 2019).

### **EXPERIMENTAL DESIGN**

The experiment was repeated four times in July and August of 2018 and 2019 during the main seed shedding period (Table 1).

For the experiment, an area of approximately 0.7 ha was fenced in, in 2018 on the calcareous grassland at the study location 'Mailerberg' and in 2019 at the study location 'Castle Kallmünz' (Figure 3). The fenced plot was further divided into three sections (1 x 0.4 ha for cattle and 2 x 0.15 ha for sheep and goat, respectively). Goats and sheep were each put in one of the smaller 0.15 ha sections and cattle in the larger 0.4 ha section to compensate for the higher fodder requirement. All animals had access to the same combination of plant species.

Directly before the animals were allowed to graze the fenced grasslands, vegetation surveys were conducted. Therefore, ten 1x1 m plots were randomly established within the 0.7 ha grassland. The abundances of all occurring plant species were noted in each plot according to a three-scale scheme (R = rare, 1-2 individuals; F = frequent, between 3 and 10 individuals, A = abundant, more than 10 individuals) as well as the mean canopy height and the phenological stage. A distinction was made between vegetative, flowering, seed producing and seed shedding individuals and the most advanced phenological stage was noted for each species. Finally, all additional plant species, which were not recorded in the ten plots but occurred in the study site, were noted. Following the vegetation surveys, one animal of each livestock type was cleaned from all seeds adhering to its fur and then let to graze the fenced grassland sections for the next 96 h (= four days).

After the first 24h, the droppings of all animals were removed from the fenced grassland to prevent collecting samples that contained seeds from the grassland where the animals had grazed previously. After 48h, 72h and 96h, dung of the three different grazers were carefully collected to avoid contamination from seed from the standing vegetation. Also, seeds adhered to the fur of one goat, one sheep and the adult cattle were collected after 48h and 96h (two samplings per trial).

Longer grazing periods were not feasible due to material constraints (e.g. material for fencing), time restrictions of the supporting farmer family and animal welfare, as they had to be separated from their flock. During the third trial (July 2019), the three sheep were able to escape their enclosure between 24h and 48h. Therefore, no seeds could be collected from their fur after 48h. As the surrounding vegetation was largely similar to the study site, we continued to collect dung samples over the following two days and collected seeds from the fur of the sheep after 72h instead of 48h. The fur of the sheep was again cleaned of all seeds after the escape.

The dung samples were immediately processed following Stender et al. (1997). Firstly, samples were air dried for two weeks and the dry weight of the dung was determined for each animal type for each 24h period. Subsequently, the dried dung was spread on sterilized potting soil in an open greenhouse under natural conditions and watered regularly. Reference containers, containing only the sterile potting soil substrate were set up to record species that arrived from external sources. *Taraxacum officinale* agg. and *Stellaria media* were the only two species present in the control container and were excluded from the analysis. Over a period of one year (including a winter period where the samples were exposed to the respective temperatures and snow cover), all seedlings emerging from the dung samples were identified, recorded and subsequently removed.

Individuals that could not be identified to the species level were grown in a separate pot until identification was possible. All *Poa* species, besides *Poa angustifolia*, were hereby assigned to *Poa* ssp. Seedlings were identified with the help of plant identification literature (Eggenberg and Möhl, 2013; Jäger et al., 2017). The nomenclature followed Jäger et al. (2017). After one year, dung samples were discarded as no new seedlings were detected.

Seeds collected from the fur of the animals were identified using a binocular microscope, seed identification literature (Brouwer and Stählin, 1975; Cappers et al., 2012) and a seed herbarium of the working group as well as a self-created seed herbarium, which was built up of seeds of the species occurring at the study sites.



Figure 4: Herbivores used in the experiment (from left to right): German Red cattle and calf (Photo: P. Poschlod), two Thuringian goats (Photo: P. Krickl), one of the three Coburger Fox sheep (Photo: P. Krickl).

Table 1: Overview of the locations, the year and month as well as the exact date on which dung and fur samples were collected for all four trials.

\*sheep escaped on the 3<sup>rd</sup> of August and seed were collected only after 72h.

Trial	Location	Year	Month	Dung collecting dates			Fur collecting dates	
				48h	72h	96h	48h	96h
1	Mailenberg	2018	July	15 <sup>th</sup>	16 <sup>th</sup>	17 <sup>th</sup>	15 <sup>th</sup>	17 <sup>th</sup>
2	Mailenberg	2018	August	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>	8 <sup>th</sup>	10 <sup>th</sup>
3	Castle Kallmünz	2019	July	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	4 <sup>rd</sup> *	5 <sup>th</sup>
4	Castle Kallmünz	2019	August	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>

## STATISTICAL ANALYSES

### *Epizoochory*

The seeds attached to the fur of the animals were counted for each animal type per sampling date (after 48h and 96h). Subsequently, a Kruskal-Wallis test followed by pairwise Wilcoxon-tests was used to test for differences in seed numbers between the three animal types while a paired Wilcoxon-test was used to test for differences between years, months and sampling dates. All analyses were conducted in R (R Core Team, 2020) using R base.

For each plant species, we estimated mean abundance and mean canopy height based on the standing vegetation and extracted information on seed appendages, seed mass and seed number per ramet from the LEDA traitbase (Kleyer et al., 2008). Previous studies have identified these traits as important proxies for estimating seed attachment and retention rates (Adriaens et al., 2007; Fischer et al., 1996; Römermann et al., 2005).

### *Endozoochory*

#### *Seed number, seed densities and species richness*

The total number of viable seeds that were found in the dung samples were counted for each animal type and each observation day. We only included seeds from plant species that were recorded during the vegetation surveys on the study sites. We did

this to correct for the introduction of new species from the previously grazed grassland via the intestinal tract of the animals, as these species would bias the comparison of the potential dispersal capability, especially in terms of species richness. For each sampling date and for each animal type, seed densities (seeds per 100 g dry dung) and total plant species richness were calculated. Subsequently, we determined mean seed densities and mean species richness in the dung of each animal type over all four trials.

In a next step, we tested for differences in seed densities and species richness between years and months using paired Wilcoxon-tests, and between the three animal types using a Kruskal-Wallis-test followed by pairwise Wilcoxon-tests. Differences in seed numbers between sampling dates were tested with a Kruskal-Wallis-test.

#### *Species composition and similarity with vegetation*

First, we compared plant species occurrences in the dung samples between animals and calculated the proportion of plant species and families that germinated most frequently in the dung samples. Based on species presence in the dung samples in comparison to the grassland vegetation, we then calculated the proportion of plant species dispersed per animal type during each trial. We restricted our calculations to those plant species that were recorded as seed producing or seed shedding during the vegetation surveys.



*Red List and habitat characteristic species*

Species affiliations to the *Festuco-Brometea* vegetation type (Oberdorfer, 2001) and Red List status for Bavaria (Scheurer & Ahlmer 2003) were extracted for all plant species. Species were classified as “Red List species” from “endangered” onwards. We calculated the proportion of habitat-characteristic and Red List species for the vegetation (excluding species that were only recorded as vegetative or flowering) as well as for each animal type for each sampling date and tested for differences using Kruskal-Wallis tests. All analyses were conducted in R (R Core Team, 2020) and figures were produced using the package “ggplot2” (Valero-Mora, 2010) and “ggpubr” (Alboukadel Kassambara, 2020).

*Seedling abundance explained by plant functional traits*

To estimate which plant characteristics best explained the occurrence of seedlings in the dung samples, generalized multiple linear regression models (GLMs) were run. All species that had potentially been dispersed via the dung (species that occurred as seed producing or seed shedding in the vegetation) were included. For model fitting, we used the amount of seed per plant species found in the dung samples as response variable. As explanatory variables, we used five traits that have been shown to be important in explaining endozoochorous seed dispersal in

previous studies: canopy height, animal feeding preferences, seed bank, seed mass and seed supply (Albert et al., 2015a, 2015b; Auffret et al., 2012; Bruun and Poschlod, 2006; Cosyns et al., 2005b; Gilhaus et al., 2017; Kuiters and Huiskes, 2010; Mouissie et al., 2005b). Canopy height, seed mass and seed supply were averaged and scaled prior the model analyses. Seed supply was calculated as the mean number of fruiting flower stems counted per 1x1 m plot during the vegetation surveys multiplied by the mean number of seeds per ramet, following Mouissie et al. (2005b). All traits were extracted from the LEDA traitbase (Kleyer et al., 2008), except for the animals feeding preference, which was extracted from the BIOPOP database (Poschlod et al., 2003) and the mean number of fruiting flower stems, which originated from the vegetation surveys (Table 2).

Missing trait values were inserted by taking the mean of all extracted records for the respective variable. Canopy height, seed mass and seed supply were scaled prior the model analyses. We constructed GLM models using ‘negative binomial’ as family which uses as default link-function a log transformation (function ‘glm\_nb’, MASS-package, Ripley et al. 2019). Negative binomial was chosen, since we used overdispersed count data as response variable. Models residuals were checked using the “DHARMA” package (Hartig, 2020).

Table 2: Overview on plant traits included in the GLM calculations with respective attributed and data source (LEDA (Kleyer et al., 2008); BIOPOP (Poschlod et al., 2003)).

Parameter	Attributes	Values	Source
Canopy Height	[m]	numeric	LEDA
Feeding preference	avoided, poisonous	0	BIOPOP
	eaten, preferred food	1	
Seed bank	transient	0	LEDA
	short- or long-term persistent	1	
Seed mass	[mg]	numeric	LEDA
Seed supply	seed number per ramet	numeric	LEDA
	$\times$ mean number of fruiting stems	numeric	Vegetation surveys



## RESULTS

*EPIZOOCHORY*

In the fur of the three animals, we found 293 seeds during the eight sampling dates (two each trial). We could identify 290 seeds at least to the genus level (Table 3) while the three seeds, that could not be identified, were further discarded from the analyses. Eight species were distinguished, representing 9% of the seed shedding or seed producing plant species at the study locations. The most common plant families were *Poaceae* (5 species, 94.8% of all seeds), followed by *Rosaceae* (1 species, 3.5% of all seeds) while *Lamiaceae* and *Rubiaceae* were found in 1% and 0.7% of all cases (1 species each). Most commonly transported species were *Bromus erectus* (63.5%) and *Festuca ovina agg.* (21.4%). A significant difference in seed quantities was found between early (48h) and late (96h) sampling

dates (Wilcoxon,  $p=0.012$ ), but not among years or months.

All detected species, except *Salvia pratensis*, had seed appendices (Table 3) while the two most frequently detected species, *Bromus erectus* and *Festuca ovina agg.*, were also classified as abundant in the vegetation. All other plant characteristics (canopy height, seed mass and seed number) showed inconsistent patterns.

Of the 290 detected seeds, 31 (11% of all seeds), 3 (1%) and 256 (88%) were found on cattle, goat and sheep fur, respectively. Here, the seeds found on sheep were significantly higher ( $p<0.001$ ; Kruskal-Wallis-test) than cattle and goats, which among them were not different. Per sampling date (two times each trial, eight in total), cattle transported a mean of  $3.9 \pm 8.2$  (standard deviation), goat of  $0.4 \pm 0.7$  and sheep of  $31 \pm 21$  seeds. The highest species number was found for sheep (all eight species), while cattle and goat each transported two plant species.

Table 3: Number of seeds found in the fur of cattle, goat and sheep allocated to the respective plant species. Abundance in vegetation was estimated in vegetation surveys and characteristic plant traits were extracted from the LEDA traitbase (Kleyer et al. 2008). Abbreviations: Arr ela - Arrhenatherum elatius, Bra pin - Brachypodium pinnatum, Bro ere - Bromus erectus, Fes ovi - Festuca ovina agg., Gal ssp. - Galium ssp., Geu urb - Geum urbanum, Hel ssp. - Helictotrichon ssp., Sal pra - Salvia pratensis; \* Abundance (mean occurrence of plant species in the vegetation surveys): rare = in only 1 plot out of 10 per trial, frequent = in 2 to 5 plots, abundant = in more than 5 plots; \*\* rough = rough seed surface

	Arr ela	Bra pin	Bro ere	Fes ovi	Gal ssp.	Geu urb	Hel ssp.	Sal pra
<b>Seeds cattle</b>	-	-	30	-	-	-	1	-
<b>Seeds goat</b>	-	-	2	1	-	-	-	-
<b>Seeds sheep</b>	22	2	152	61	3	10	4	2
<b>Abundance in vegetation*</b>	Frequent	Rare	Abundant	Abundant	Frequent	Rare	Frequent	Frequent
<b>Canopy height [cm]</b>	> 80	41-60	61-80	21-40	21-40	21-40	41-60	21-40
<b>Seed appendices</b>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No, rough**
<b>Seed mass [mg]</b>	> 2	> 2	> 2	< 2	< 2	= 2	= 2	> 2
<b>Seed number per ramet</b>	< 100	< 100	< 100	> 100	< 100	> 100	> 100	> 100

**ENDOZOCHORY***Seed number, seed densities and species richness of the dung samples*

In the 36 dung samples, in total 13 290 viable seeds emerged with no differences between years for seed density (Figure 5) and mean species richness (Figure 5) in the dung samples. However, when testing for differences between months, significantly higher seed densities (Wilcoxon,  $p \leq 0.001$ , Figure 5) and species richness ( $p = 0.01$ , Figure 5) were found in August than in July. We found no differences in seed densities between the three sampling dates (48h, 72h and 96h) per trial. Overall, 12 389, 296 and 605 seeds originated from cattle, goat and sheep dung, respectively

(Table 4). Mean seed density (seeds per 100 g dry dung) per sampling date was  $16.3 \pm 11.2$  (standard deviation) for cattle,  $6.9 \pm 6.5$  for goats and  $13.2 \pm 11.9$  for sheep. Cattle transported in total 52 different plant species in the dung, goats 27 and sheep 24. Mean species richness per sampling date was  $24.3 \pm 5.2$  for cattle,  $6.3 \pm 4.2$  for goats and  $6.6 \pm 4.9$  for sheep samples. No fully significant differences were detected for seed densities between cattle, goats and sheep (Kruskal-Wallis,  $p = 0.076$ , Figure 5) while species richness was significantly higher in cattle dung (Kruskal-Wallis,  $p \leq 0.001$ , Figure 5).

Table 4: Total amount of seeds (seed number) and seeds per 100 g dry dung (seed density) recorded for cattle, goats and sheep during the three sampling dates per trail (1: July 2018, 2: August 2018, 3: July 2019, 4: August 2019).

Trial	Animal	Seed number			Seed density		
		24h-48h	48-72h	72-96h	24h-48h	48-72h	72-96h
1	Cattle	2034	831	817	30.6	12.3	11.4
2		2275	959	895	42.4	15.4	12.2
3		229	292	200	4.1	5.8	4.2
4		1100	1696	1061	16.7	22.0	18.7
1	Goat	4	5	16	2.7	6.5	19.0
2		39	26	29	10.0	4.3	4.7
3		3	5	3	0.8	0.9	0.5
4		37	100	29	5.6	19.7	8.2
1	Sheep	3	1	9	2.4	0.6	11.3
2		109	40	27	28.5	13.7	7.5
3		2	16	8	7.7	3.1	1.2
4		91	185	114	17.1	34.9	29.4

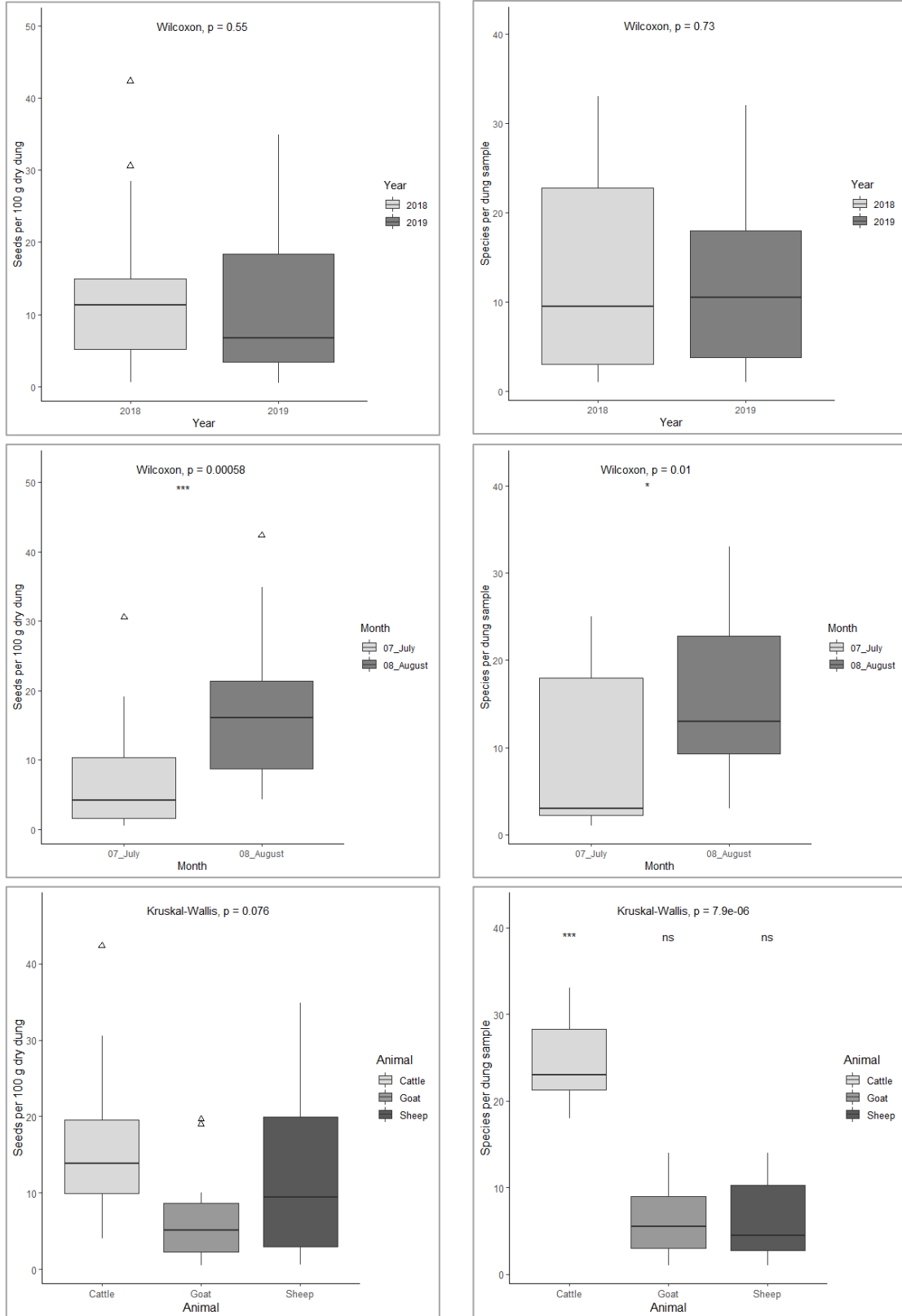


Figure 5: Differences in seeds per 100 g dry dung (Seed densities; left side) and species richness in the dung samples (right side) between years (above), months (middle) and cattle, goats and sheep (below). Conducted tests with respective p-values and, if necessary, the results of post-hoc pairwise Wilcoxon-Tests are illustrated. Signif. codes:  $p > 0.05$  ns,  $p \leq 0.05$  \*,  $p < 0.01$  \*\*,  $p < 0.001$  \*\*\*

*Species composition and similarity with vegetation*

Overall, we found 52 species (out of 90 in our vegetation surveys) belonging to 17 different plant families, which germinated in the dung samples. Of these species, 22 were detected only in cattle dung, while no species were found exclusively in goat or sheep dung samples.

The most abundant species were *Galium verum* (18% of all viable seeds), *Poa* ssp. (16.8%), *Festuca ovina* (7%), *Galium mollugo* (6.6%), *Luzula campestris* (6.5%), *Poa angustifolia* (5.8%), *Medicago lupulina* (5.4%) and *Trifolium pratense* (5.5%). Seeds originated mostly from three different plant families: *Poaceae* (33.6% of all viable seeds), *Rubiaceae* (25.1%) and *Fabaceae* (24.1%), which represented the most dominant families in the dung of all three animal types.

When comparing the species, germinated in the dung samples, with those species, recorded as seed producing and seed shedding in the grazed vegetation, we found that cattle transported at least one third of the species in the vegetation in July 2019 and more than half during the other three trials. Goats and sheep, contrary, both transported approximately 9% of the seed shedding or seed producing species in July and 27% in August in 2018 as well as in 2019.

*Proportion of habitat specialists and Red List species*

Thirty-six species (41 % of all species) that were present in the vegetation classified as typical calcareous grassland species. Of these species, 24, 12 and 8 were found in the dung of cattle, goats and sheep, respectively, while 12 species were missing in the dung samples. Species that germinated only in the cattle dung were *Brachypodium pinnatum*, *Briza media*, *Carex caryophylla*, *Centaurea jacea*, *Dianthus carthusianorum*, *Gentiana cruciata*,

*Helictotrichon pratense*, *Hieracium pilosella*, *Prunella grandiflora*, *Sanguisorba minor* and *Trifolium montanum*.

Overall, no significant differences between the vegetation and the dung samples of the three animal types could be found with regard to the relative proportion of seeds from habitat characteristic species of the *Festuco-Brometea* class (Figure 6).

Approximately about one third of the species occurring in the grazed vegetation was listed on the Red List for Bavaria (28 species). The mean proportion of Red List species was higher in the vegetation than in any of the three types of dung samples (Kruskal-Wallis,  $p=0.018$ , Figure 6). In the dung of cattle, goats and sheep, 14, 5 and 6 Red List species were found, respectively, transporting half of the Red List species, which occurred in the vegetation. Red List species that were only found in cattle dung were *Alyssum montanum*, *Dianthus carthusianorum*, *Gentiana cruciata*, *Helianthemum nummularium*, *Helictotrichon pratense*, *Prunella grandiflora* and *Trifolium montanum*.

*Plant traits correlated with occurrences in dung samples*

The animal's feeding preference for the plant species had a significant positive effect on seedling occurrence in the dung samples of all three animal types (GLMs, Table 5 A-C). On the other hand, species that produce heavier seeds were less likely to germinate in the dung samples. For cattle, species with a taller canopy height had significantly lower seed densities in the dung (Table 5 A) while for sheep and goats, the GLMs showed no correlation with seed densities. Moreover, the GLMs showed for all three animal types no correlation between seed densities and seed supply as well as seed persistence.

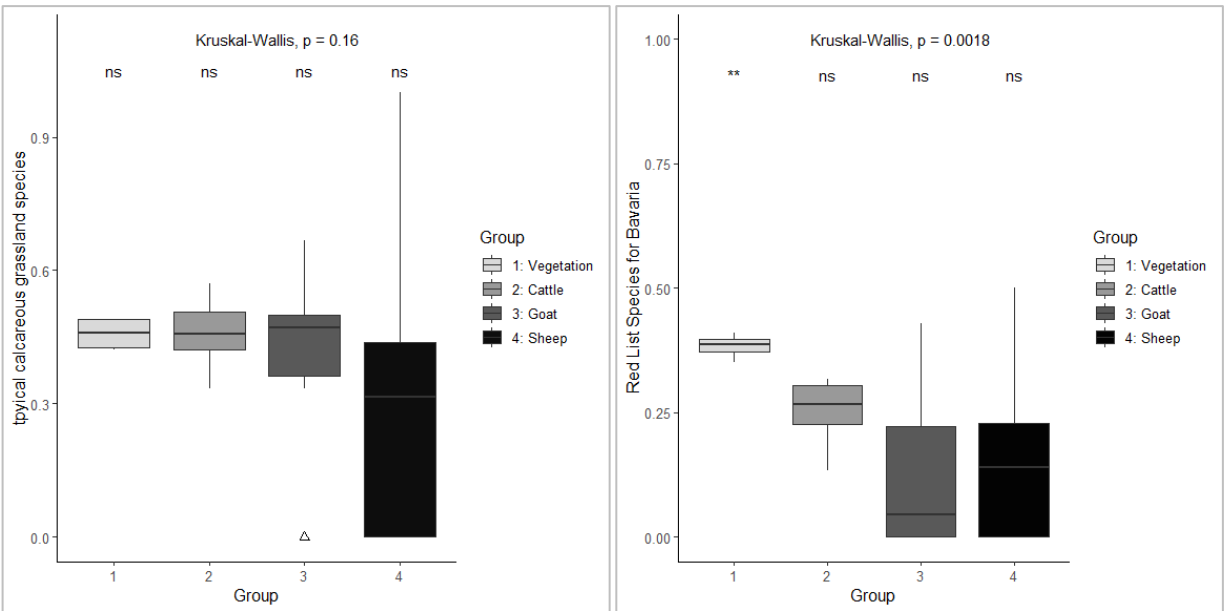


Figure 6: Mean proportions of typical calcareous grassland species (left side) and Red List species (Bavaria, right side) in the calcareous grassland vegetation (Group 1) and in the dung samples of cattle, goats and sheep (Group 2, 3 and 4, respectively) for the different sampling dates.

CHAPTER 2

Table 5 A-C: Results of the generalized linear models explaining seed densities of the individual plant species in the dung samples of A) cattle, B) goats and C) sheep. For each explaining variable the estimate, standard error (SE), z-value and p-value are given. Variables having a significant impact are illustrated in bold. For each of the three binomial models the respective Null deviance, residual deviance, theta, the standard error of theta (SE.t) and the 2xlog-likelihood are given. Information on the explanatory variables can be found in the Material and Method section. Signif. codes: p>0.05 ns, p<0.05 \*, p<0.01 \*\*, p<0.001 \*\*\*  
a) <0 -> positive effect on response variable, 0< -> negative effect on response variable

<b>A) Cattle</b>		<b>Estimate <sup>a)</sup></b>	<b>SE</b>	<b>z-value</b>	<b>p-value</b>	
	<b>(Intercept)</b>	<b>2.554</b>	<b>0.545</b>	<b>4.684</b>	<b>&lt;0.001</b>	<b>***</b>
<b>Response variable</b>	<b>Explanatory variable</b>					
Seeds per species in cattle dung	<b>Canopy height</b>	<b>-0.596</b>	<b>0.302</b>	<b>-1.977</b>	<b>0.048</b>	<b>*</b>
	<b>Preference (ref: not eaten) eaten</b>	<b>2.598</b>	<b>0.611</b>	<b>4.25</b>	<b>&lt;0.001</b>	<b>***</b>
	Seed bank (ref: transient) persistent	-0.647	0.609	-1.062	0.288	
	<b>Seed mass</b>	<b>-1.453</b>	<b>0.355</b>	<b>-4.096</b>	<b>&lt;0.001</b>	<b>***</b>
	Seed supply	0.078	0.28	0.277	0.782	
Null deviance: 110.3 on 89 degrees of freedom Residual deviance: 89.6 on 84 degrees of freedom Theta: 0.146 SE.t: 0.023 2 x log-likelihood = -680.998						
<b>B) Goat</b>		<b>Estimate <sup>a)</sup></b>	<b>SE</b>	<b>z-value</b>	<b>p-value</b>	
	<b>(Intercept)</b>	<b>-0.102</b>	<b>0.633</b>	<b>-0.161</b>	<b>0.872</b>	
<b>Response variable</b>	<b>Explanatory variable</b>					
Seeds per species in goat dung	Canopy height	-0.107	0.342	-0.313	0.755	
	<b>Preference (ref: not eaten) eaten</b>	<b>1.772</b>	<b>0.703</b>	<b>2.522</b>	<b>0.012</b>	<b>*</b>
	Seed bank (ref: transient) persistent	-1.071	0.689	-1.553	0.120	
	<b>Seed mass</b>	<b>-1.120</b>	<b>0.490</b>	<b>-2.287</b>	<b>0.022</b>	<b>*</b>
	Seed supply	0.450	0.306	1.472	0.141	
Null deviance: 64.2 on 89 degrees of freedom Residual deviance: 55.4 on 84 degrees of freedom Theta: 0.125 SE.t: 0.030 2 x log-likelihood = -276.703						
<b>C) Sheep</b>		<b>Estimate <sup>a)</sup></b>	<b>SE</b>	<b>z-value</b>	<b>p-value</b>	
	<b>(Intercept)</b>	<b>-0.859</b>	<b>0.758</b>	<b>-1.133</b>	<b>0.257</b>	
<b>Response variable</b>	<b>Explanatory variable</b>					
Seeds per species in sheep dung	Canopy height	0.235	0.393	0.598	0.550	
	<b>Preference (ref: not eaten) eaten</b>	<b>3.056</b>	<b>0.821</b>	<b>3.72</b>	<b>&lt;0.001</b>	<b>***</b>
	Seed persistence (ref: transient) persistent	-0.951	0.788	-1.206	0.228	
	<b>Seed mass</b>	<b>-1.883</b>	<b>0.721</b>	<b>-2.612</b>	<b>0.009</b>	<b>**</b>
	Seed supply	0.087	0.351	0.248	0.804	
Null deviance: 64.4 on 89 degrees of freedom Residual deviance: 49.6 on 84 degrees of freedom Theta: 0.095 SE.t: 0.023 2 x log-likelihood = -283.04						

## DISCUSSION

In our study we found strong evidence that all three animal types are potentially able to transport typical calcareous grassland species in their fur (epizoochory) as well as in their digestive tract (endozoochory). Sheep performed best in terms of epizoochory while cattle showed the best results for endozoochory, confirming our hypothesis that these animal types differ in their capability to disperse seeds according to the two investigated dispersal modes. Whereas the seed dispersal capacity of livestock is well known (Auffret et al., 2012; Mouissie et al., 2005b, 2005a; Römermann et al., 2005) this study is the first to evaluate specific differences in individual zoochorous dispersal capability between gazing livestock types for a specific and strongly endangered vegetation type, namely calcareous grasslands.

### ***EPIZOOCHORY IN RELATION TO SEED DENSITIES, SPECIES RICHNESS AND PLANT CHARACTERISTICS***

In terms of epizoochorous dispersal, we found that sheep not only transported significantly more seeds than cattle and goats, but also four times as many different plant species. Benthien et al. (2016) also detected higher seed densities and species richness in sheep than in goat fur. This effect can most likely be attributed to differences in the hair structure between animal types, as it has already been shown that the long and curly hair of sheep is better suited for seed transportation than the short and straight hair of cattle and goat (Couvreur et al., 2005; De Pablos and Peco, 2007; Mouissie et al., 2005a; Römermann et al., 2005). Therefore, this outcome is consistent with previous findings.

However, the amount of epizoochorously transported seeds was in our experiment rather low. Studies conducted in other semi-natural grassland habitats showed comparably higher seed densities and species richness in the fur of cattle (Couvreur et al., 2004), goats (Benthien et al., 2016) and sheep (Benthien et al., 2016; Fischer et

al., 1996). This may be due to the fact that the standing vegetation was severely trampled during the course of our study as the animals were herded onto comparable small grassland patches. This in turn reduced the mean canopy height, an important factor for explaining seed attachment rates (Adriaens et al., 2007; Fischer et al., 1996; Stender et al., 1997). Moreover, after four days, most of the standing vegetation, with the exception of some *Poaceae* shoots, had disappeared, as the animals had grazed the fenced grassland patched intensively. As a result, there were gradually fewer seeds available for fur attachment which was further supported by the finding that significantly fewer seeds were found in the fur of the animals at the second sampling date. Furthermore, as we collected seeds for the first time only after two days, vegetation trampling and grazing could have already influenced mean canopy height and seed availability and thus reduced seed attachment rates. These effects could explain the low seed densities compared to other studies where animals were able to graze larger grassland areas without significantly reducing the available seed pool.

We found that cattle and goat only transported *Gramineae* species while sheep additionally transported some herbaceous plant species, e.g., *Salvia pratensis*. *Poaceae* is a frequently transported plant family (Benthien et al., 2016; Fischer et al., 1996; Stender et al., 1997), yet the recorded species richness was, in comparison with these studies, comparably lower. This might be related to methodical issues as only seeds that adhered to the fur of the animals over a period of two days were observed. In the studies above mentioned, seeds had more time to accumulate in the fur, as the aim was to evaluate which plant species were potentially transported and not to compare different animal types. Therefore, the fur of the animals was not cleaned from seeds in advance. However, experimental studies have shown that for most plant species, the retention time of at least single seeds can potentially exceed the two-day period (Fischer et al., 1996; Manzano

and Malo, 2006). Hence, the higher species richness in these studies could be related to prolonged seed retention times of individual seeds. Additional evidence is provided by Fischer et al. (1996), who found a larger number of plant species in the sheep fur represented by only one or two seeds. Moreover, we could observe that sheep but also goat avoided feeding on *Poaceae* species as long as possible and rather concentrated on herbaceous species, effectively reducing the available seed pool for these species while seeds of highly abundant species like *Bromus erectus* or *Festuca ovina* agg. were still available due to being avoided. The observation of a reduced seed availability, based on the feeding preferences of the animals, can potentially explain why seeds of graminoid species were found more frequently in contrast to seeds of herbaceous species. In summary, it can be assumed that, at least for sheep fur, the overall species richness would have increased over the vegetation period, especially if the total area for grazing, and thus plant species abundances, would have been increased.

Studies investigating epizoochorous seed dispersal have generally concluded that seeds are retained for longer periods in the fur when they have a smaller seed mass (<2 mg) and are equipped with one or more appendices (Albert et al., 2015a; De Pablos and Peco, 2007; Fischer et al., 1996; Römermann et al., 2005; Stender et al., 1997) or at least had no smooth seed surface (Mouissie et al., 2005a). In accordance with these findings, all our retrieved species had seed appendices or at least a rough seed surface but we found no indication for the importance of seed weight, since the species (*Bromus erectus*) most commonly transported by all three animals had comparable heavy seeds. Plant height has also been found to be a factor positively influencing epizoochorous seed dispersal (Adriaens et al., 2007; Fischer et al., 1996; Thomson et al., 2011). Nevertheless, in our study mean canopy height of the second most frequently dispersed species (*Festuca ovina* agg.) was comparable low (<40

cm). However, as already discussed, mean plant height might have been reduced due to trampling and grazing and hence, has been of minor importance for seed attachment. In contrast, the abundance of plant species seemed to be important as the two species with highest proportion of seeds in the fur were also abundant in the vegetation. Similar findings were also reported by Fischer et al. (1996) or Adriaens et al. (2007), although in both cases plant height was more important than species frequency. However, our small sample size has limited informative value on the characteristics of species that benefit from epizoochorous dispersal. Therefore, an experiment with larger grassland patches for grazing, more individuals of each animal type and smaller intervals for seed collection would probably provide more detailed information on the epizoochorous dispersal capability of typical calcareous grassland plant species.

#### **IMPLICATIONS OF EPIZOOCHOROUS SEED DISPERSAL**

Although the capability for epizoochorous seed dispersal has most likely been underestimated, we could show that sheep in particular, and to a lesser extent cattle and goats, are able to transport at least some calcareous grassland species. Epizoochorous seed dispersal has already proven to be difficult to track, as seed attachment and seed retention times are rather stochastic events (Bullock et al., 2011; Nathan et al., 2008) and realised dispersal is additionally influenced by animal behaviour (Liehrmann et al., 2018; Wessels et al., 2008), leading to the assumption that the total number of transported seeds was probably much higher considering the long period between sampling times discussed above. However, for long-distance dispersal between fragmented habitats seeds that adhered to the fur for a short period have little relevance for habitat connectivity.

Another insight our study revealed is that intensive grazing resulted in reduced seed availability,



especially of forbs. Nevertheless, the intensive grazing of calcareous grasslands is a recommended and common practice in Germany. Since we rarely detected seeds without specific adaptations for epizoochorous dispersal in fur of the animals after grazing for four days, it can be presumed that long-distance dispersal is more relevant for species that have a high seed retention potential (e.g. *Poaceae* species, Adriaens et al. 2007) and species that grow along migration routes or at the edge of species-rich grasslands. This puts emphasis on the importance of transhumant migrating flocks of animals for long-distance seed dispersal, which is nowadays hardly practiced anymore (Manzano and Malo, 2006; Poschlod and WallisDeVries, 2002). Most animals are rather transported by vehicles to overcome increasing distances between fragmented grasslands or to avoid hostile landscapes (e.g., highways or large areas of agricultural landscapes). We therefore stress that epizoochorously dispersed calcareous grassland species are threatened by the loss of traditional practices (Kimberley et al., 2021). Moreover, the loss of diverse and species-rich migration routes might additionally endanger the dispersal of seeds adapted to epizoochorous dispersal.

#### **ENDOZOCHORY IN RELATION TO SEED DENSITIES, SPECIES RICHNESS AND PLANT CHARACTERISTICS**

In terms of endozoochorous dispersal, we found no differences in seed densities per unit of dry dung between the three animal types. However, we found that cattle transported on average four times as many species in their dung than goat and sheep. A similar result was found by Mitlacher et al. (2002) when comparing cattle and sheep in a grazed dry grassland system in Sweden. A comparison with other studies indicates that the endozoochorous dispersal capability for each animal type could be habitat specific. For instance, Mouissie et al. (2005b) reported differences in seed densities rather than species richness for

cattle and sheep in a wet grassland system and Benthien et al. (2016) found higher seed densities for goats but higher species richness for sheep in a coastal dry grassland. Moreover, when comparing seed densities with the above-mentioned studies, we also found comparable low seed numbers in our study. Cattle in particular were shown to potentially transport higher amount of seeds in the dung (Mitlacher et al., 2002; Mouissie et al., 2005b) but greater seed densities have also been reported for goats (Benthien et al., 2016) and sheep (Benthien et al., 2016; Kuiters and Huiskes, 2010; Mitlacher et al., 2002; Mouissie et al., 2005b). However, when considering species composition, these studies found that non-target plant species like *Urtica dioica* (Benthien et al., 2016; Kuiters and Huiskes, 2010) or *Juncus* ssp. (Gilhaus et al., 2017; Mitlacher et al., 2002; Mouissie et al., 2005b) were present in comparatively high abundances in the dung samples and thus contributing to higher mean seed densities, whereas these two plant species were not detected in our study. The fact that *Poaceae* and *Fabaceae* in particular, but also *Rubiaceae* species, were frequently transported in the dung of all three animals was nevertheless also shown in these studies. Moreover, experimental studies confirmed high viability rates for especially *Fabaceae* and *Poaceae* species after having passed through the animal intestinal tracts (Malo and Suárez, 1995; Mancilla-Leytón and Martín Vicente, 2011; Wang et al., 2017).

It was shown that the endozoochorous dispersal success can be estimated by using different plant characteristics (Albert et al., 2015a; Bruun and Poschlod, 2006; Kuiters and Huiskes, 2010) and accordingly we detected significant correlations. Two characteristics were important for all herbivores, namely feeding preference for the plant species and seed mass. A positive correlation with the feeding preference was also observed, for example, by Gilhaus et al. (2017) for cattle. Moreover, the value accumulates plant species known to be ingested by livestock. Contrarily, this

suggests that avoided or even poisonous species are dispersal limited via endozoochory. Accordingly, we found no seedlings of species such as *Allium oleraceum*, *Euphorbia cyparissias* or *Sedum* ssp., which belong to the latter category. The observation that heavier seed are limited in their endozoochorous dispersal was also made by Auffret et al. (2012) for cattle and sheep or Kuiters and Huiskes (2010) for sheep. These authors linked the higher frequency of small seeds to higher seed availabilities. That species with high seed production rates are generally more likely to invest in small seeds is a well explored phenomenon (Harper et al., 1970; Jakobsson and Eriksson, 2000; Leishman, 2001). However, we found that seed supply had no influence on seed densities in the dung. Roughly two thirds of the plant species that were present in the vegetation, including most *Fabaceae* and *Poaceae* species, which were the most abundant plant families in the dung samples, had a low seed production rate (<100 seeds per ramet, Kleyer et al., 2008). Therefore, we conclude that the correlation with the low seed number was most likely due to the specific calcareous grassland species composition as most habitat-characteristic species were characterised by rather low seed production rates. It has been previously observed that cattle are generally less selective grazers (Pykälä, 2000; Rook et al., 2004) as they graze larger bits of vegetation, which they pull out with their tongues close to the ground. Goat and sheep on the other hand are more selective and can graze individual plant species. Accordingly, we found that the amount of endozoochorously dispersed seeds in cattle dung increased with decreasing mean canopy height while we could not find that correlation for goats and sheep.

It has been suggested that persistent seeds are better adapted to survive passage through the intestinal tract (Bruun and Poschlod, 2006; Kuiters and Huiskes, 2010) and are therefore found more frequently in the dung of livestock. However, we found only limited indications that

persistent seeds are transported more frequently. This again is presumably depending on the species specific vegetation composition of calcareous grasslands, as most of the observed species produce transient rather than persistent seeds (Bekker et al., 1998b; Kleyer et al., 2008).

In contrast to the results regarding epizoochory, we found no evidence of lower seed densities in the dung over the observation period per trial. However, mean seed retention times are known to range from 24h to 48h for goats (Mancilla-Leytón and Martín Vicente, 2011) and sheep (Cosyns et al., 2005b; Wang et al., 2017) and between 24h to 70h for cattle (Cosyns et al., 2005b). Therefore, the effect of lower seed availability due to trampling and grazing had no time to take effect in our samples as mean seed retention times in the intestinal tract can amount to several days. On the other side, at least for the period between 24h and 48h, emerging seedlings were more likely to originate from seeds transported in the intestinal tract from the previous grazing site. Therefore, we deleted the few seeds originating from species that occurred in the previous but not in the study location for our analyses. This still allowed for an assessment of potential zoochory dispersal of typical calcareous grassland species, but seed densities were probably slightly underestimated during the first days of observation.

#### **IMPLICATIONS OF ENDOZOOCHOROUS SEED DISPERSAL**

We could show that all animal types were able to transport a great proportion of typical calcareous grassland species although cattle transported more species overall, and in relation to body size also more seeds (Rook et al., 2004). Therefore, endozoochorous dispersal by all three animals holds great potential for the implementation in restoration and conservation ecology. However, it is thereby important to take the retention times of the seeds in the gut of the animal types into account.

Red List species were shown to be restricted in their potential endozoochorous dispersal, as they were significantly underrepresented in the dung samples. It was not possible to clarify whether these species were not detected because they were not adapted to survive the intestinal passage, were avoided as fodder species or were not ingested by the animals due to their low abundances in the vegetation.

We detected a significant difference in seed number and species richness between samples taken in July and August. We therefore conclude that the highest proportion of typical calcareous grassland species is transported towards the end of the vegetation period (Auffret et al., 2012; Kuiters and Huiskes, 2010; Mouissie et al., 2005b), making this an important period for (endo-) zoochorous dispersal.

#### **COMPARISON OF ZOOCHOROUS DISPERSAL CAPABILITY**

Based on the mean seed number per day, one sheep could potentially transport a minimum of 946 seeds in its fur during the two months (61 days) of the experiment, while cattle and goat transported extrapolated 119 and 12 seeds, respectively. We generally discovered very low epizoochorous seed transportation rates especially for cattle and goat, while sheep transported at least 9% of the local vegetation in the fur. We nevertheless assume that our study design was not ideal for estimating epizoochorous dispersal and that we highly underestimated the rate of transported seed. However, these numbers represent seeds that can potentially be dispersed over long-distances and that can therefore still play an important role in assuring habitat connectivity. In addition, at least one habitat-characteristic species (*Salvia pratensis*) was found in the fur of sheep which did not germinate in any dung sample and which was not found to be endozoochorously dispersed in other studies (Kleyer et al., 2008). For endozoochorous dispersal much higher transportation rates of

62 977, 1 500 and 3 075 seeds (extrapolated for the two months period based on mean seed densities per day) were found for cattle, goat and sheep, respectively. Depending on the retention time in the intestinal tract and the duration of grazing, these seeds can make a particular important contribution to the dispersal of species between calcareous grasslands. It was additionally shown that cattle transported more than half of the seed-shedding or seed-producing vegetation in their dung, whereas sheep and goats only transported about a quarter, supporting the relevance of cattle grazing.

Other studies provided strong indications that epi- and endozoochorous seed dispersal might be complementary to each other, especially when using different animal types (Albert et al., 2015a; Treitler et al., 2017). In the present study, endozoochorously transported seeds by goats and sheep were only a subset from species transported by cattle while species that were epizoochorously dispersed by cattle and goats were a subset of species dispersed by sheep. In contrast, we detected species that were only epizoochorously dispersed by sheep or endozoochorously by cattle. In the case of the calcareous grassland vegetation, the theory that the zoochorous dispersal modes are complementary to each other could therefore be supported, particularly if more than one animal type is present.

This finding also hints that species richness may be threatened by the abandonment of a mixed grazing system. In earlier times, mixed grazing systems were widespread and not only calcareous grasslands (Baumann et al., 2005; Poschlod and WallisDeVries, 2002), but other semi-natural grasslands like for example alpine pastures were grazed by a combination of different livestock (sheep, goat, cattle, horse; Ellenberg and Leuschner, 2010; Englmaier, 1978, Jaritz, 2014). From our study we can conclude that mixed grazing systems could make a positive contribution to the dispersal of habitat-characteristic plant species between the remnants

of the calcareous grasslands and thus, this finding most likely also applies to other grazed systems. Moreover, we detected Red List species (*Alyssum montanum*, *Dianthus carthusianorum* and *Gentiana cruciata*), which germinated only in cattle dung. In addition, we found no information in the LEDA traitbase (Kleyer et al., 2008) to indicate that these species have ever been confirmed to be dispersed endozoochorously by goats or sheep or epizoochorously by any livestock in other studies. This finding emphasises the importance of cattle grazing for the potential dispersal of some rare habitat-specific plant species.

## CONCLUSION

The historically most important calcareous grassland grazers, cattle, goat and sheep, all proved to be important dispersal vectors for a large proportion of habitat characteristic plant species. Generally, it can be concluded that endozoochory, best realised by cattle, is a more reliable dispersal vector than epizoochory. Contrary, sheep seems to be the best epizoochorous dispersal vector, even though seed attachment and retention rates are more random and therefore less predictable.

Based on these findings, we concluded that not only the dispersal of plant species in the fur of sheep (Fischer et al., 1996), but also in the dung of cattle and, to a lesser extent, of goats and sheep is important for the restoration and conservation of calcareous grasslands which are connected by migrating livestock. Although the success of endozoochorous dispersal depends on the timing when the seeds are released from the intestinal tract of the animals, we recommend the reintroduction of cattle grazing for the conservation of species richness (including Red List species). Despite the fact, that in former times cattle grazing of calcareous grasslands used to be more common than sheep grazing, at least at a local scale (Baumann et al., 2005), it is largely ignored or even rejected by many conservation

authorities. An additional factor, even though not specifically addressed in our study but nevertheless observed, is that goats are able of reducing the shrub cover by damaging them (Mancilla-Leytón et al., 2014) and hence, may counter shrub encroachment. It is therefore recommended that goats should always be part of a migrating sheep flock. This used to be a common traditional practice in Central Europe and is still practiced today in the Mediterranean region (Evans, 1940).

However, more research in the context of epi- and endozoochorous dispersal needs to be done. For instance, we detected less Red List species in the dung of the animals than in the vegetation, suggesting that these species are not sufficiently dispersed. Therefore, we need a better understanding which dispersal processes allowed these species to migrate (Poschlod and Bonn, 1998).

**CHAPTER 3**

**HOW FAST CAN AFFORESTED AND OVERGROWN CALCAREOUS GRASSLANDS BE RESTORED? - A FLORISTIC ASSESSMENT AFTER 25 YEARS!**

**ABSTRACT**

Nutrient-poor calcareous grasslands, ancient man-made and extremely species-rich habitats, have declined rapidly throughout Europe during the past centuries due to drastic land-use changes. Nowadays, fragmentation and small habitat size threaten the conservation of their high biodiversity. So far, data on the long-term success of conducted restoration measures is limited. However, as the current UN decade is dedicated to Ecosystem Restoration, information on long-term success of implemented measures becomes highly relevant.

We provide a rare example for a continuous long-term study in which vegetation development was followed over 25 years on permanent plots. Seven study sites were installed on lost calcareous grassland areas, which had been abandoned and overgrown or afforested in the past. These sites were cleared in the early 1990s and grazing was subsequently reintroduced as connecting corridor to adjacent traditionally managed grasslands.

Our main question was whether it is possible to restore calcareous grasslands through clear-cutting and reintroducing of sheep grazing. We were particularly interested if significant differences in species composition and informative restoration indicators still occur between reference and restoration sites after 25 years.

Our results indicate a continuous floristic development of restored grasslands towards reference grasslands. A similar pattern was found for most restoration indicators. Nevertheless, even after 25 years, some significant differences between restored and reference grasslands were detectable, especially in terms of rare and habitat-characteristic species as well as in species indicating increased nutrient availability.

In summary, the hypothesis that the restoration of overgrown or afforested species-rich calcareous grassland is possible was strongly supported, although the recovery of species richness seems to be an ongoing process even after 25 years. Based on this long-term study, we can nevertheless recommend clearance of former calcareous grasslands and reintroduction of sheep grazing as restoration measure. This approach can provide an important tool for future ecosystem restoration projects in grazed calcareous grasslands.

**KEYWORDS**

clearance, ecosystem restoration, *Festuca-Brometea*, monitoring, permanent plots, time series

## INTRODUCTION

Species-rich calcareous grasslands rank amongst the most species-rich habitats in Central Europe (WallisDeVries et al., 2002; Willems, 1990). Their occurrence can be dated back at least to the Roman period, but there is evidence that these grasslands developed regionally as early as the Neolithic period (Eriksson et al., 2002; Poschlod et al., 2008; Poschlod and Baumann, 2010; Poschlod and WallisDeVries, 2002; Robin et al., 2018). Hence, these ancient habitats represent an important aspect of our cultural biodiversity heritage. Calcareous grasslands are known for their nutrient poverty due to their traditional management by grazing with sheep or cattle and subsequent penning on arable fields (Poschlod and WallisDeVries, 2002). Due to the long history of low intensity use, a great variety of species has adapted to the specific conditions characterizing this habitat. Therefore, the calcareous grassland vegetation consists of a high proportion of stress-tolerant species, which have adapted to the nutrient-poor and dry habitat conditions as well as to the environmental pressure caused by grazing (Böhmer and Bender, 2000; Grime, 1988; Poschlod and WallisDeVries, 2002).

During the past two centuries, there have been two global changes that massively threaten the existence of calcareous grasslands. First, the import of sheep wool and meat from overseas became less expensive since the middle of the 19th century and consequently, the value of products from local sheep declined. As a result, sheep husbandry became unprofitable and a tremendous decline in sheep number was recorded for Germany (Poschlod and WallisDeVries, 2002). Between 1860 and 1960, the amount of sheep declined from roughly 28 million to barely a million (Poschlod, 2017). However, grazing animals, especially transhumant flocks of sheep, are considered the most important long-distance dispersal vector for plant species in grazed grassland systems (Bonn and Poschlod, 1998; Manzano and Malo, 2006). Secondly, agricultural

practices have been modernized since the middle of the 20th century. Many marginal sites such as calcareous grasslands were abandoned, afforested or ameliorated in the course of this development (Hooftman and Bullock, 2012; WallisDeVries et al., 2002). For a comparable well preserved part of the study region for instance more than 60 % of the calcareous grassland area was lost between 1900 and 1990 (Poschlod, 2017), while in other European landscapes the proportion was even higher (Green, 1990; Hooftman and Bullock, 2012; Mailänder et al., 2004; Wolf, 1984). Another consequence of these changes is a still ongoing fragmentation of the remaining grasslands (Hooftman and Bullock, 2012) which is accompanied by a loss of traditional dispersal processes that profoundly change the connectivity between the remnant habitats (Poschlod and Bonn, 1998). This in turn can lead to a loss of biodiversity as the dispersal of propagules and pollen is constrained (Brückmann et al., 2010; Kimberley et al., 2021; Luoto et al., 2003b).

In the early 1990s, the rapid loss of these species-rich grasslands was widely acknowledged (García, 1992) and many different approaches aiming to restore and preserve remnant habitats were initiated and evaluated ever since (Poschlod et al., 2005). The overall objective of these approaches was to secure, preserve and (re)connect the remaining, restored and newly created habitats and to evaluate the success of the applied method. In this context, for example, reconversion of arable fields (Fagan et al., 2008; Öster et al., 2009b; Pywell et al., 2002; Schmid et al., 2017; Stevenson et al., 1995) or, as applied in the present study, clearance of overgrown or afforested grasslands (Bağa, 2011; Barbaro et al., 2001; Pärtel et al., 1998; Piqueray et al., 2011b) have been conducted as restoration measures. It has been stressed that the progressive fragmentation might have a time-lagged impact on biodiversity due to reduced connectivity between remnant grasslands (Helm et al., 2006; Lindborg and Eriksson, 2004a; Piqueray et al., 2011a). Therefore, the restoration of new or

formerly abandoned habitats seems to be as important as maintaining traditionally managed habitats (Eriksson et al., 2002).

However, it is often very difficult to reliably assess the long-term success of restoration measures due to the lack of continuous long-term studies carried out on permanent observation plots exists - a problem known not only for calcareous grasslands but also other ecosystems (Bakker et al., 1996; Helsen et al., 2013; Pierik et al., 2011). Most studies used indirect methods to follow vegetation development after restoration, e.g. by comparing different temporal stages of succession ("time-for-space substitutes" or chronosequences, Bakker et al., 1996; Kalamees and Zobel, 1997; Pickett, 1989) or restoration (Lindborg and Eriksson, 2004b; Piqueray et al., 2015, 2011b) or by recorded changes in vegetation composition for few years at one particular study site (Barbaro et al., 2001; Bistea and Mahy, 2005; Dzwonko and Loster, 1998). The disadvantage of these study designs is that site-specific characteristics can lead to misleading conclusions or that the long-term direction of the vegetation development might be predicted inaccurately (Helsen et al., 2013). Moreover, Cusser et al. (2021) just recently reported that study durations of more than 20 years are needed to achieve consistent results.

With this study, we aim to overcome these disadvantages by providing a rare example for a long-term study in which the vegetation development was continuously monitored over a 25-year period on permanently installed observation plots at seven different study locations. Thereby, we recorded the vegetation composition of restored and reference grasslands, all representing different types of underlying starting vegetation, including former pine and spruce afforestations and grasslands overgrown by shrubs such as *Prunus spinosa* and *Robinia pseudoacacia* due to successional processes. The

main question we wanted to answer was whether restoring species-rich calcareous grassland after clearing and reintroducing sheep grazing is an appropriate restoration measure - with regard to the type of cleared vegetation. We were specifically interested in whether the restored grasslands had the potential to reach the high biodiversity and comparable abiotic site conditions of ancient, continuously managed grasslands over the 25-year period and whether different dispersal phases could be distinguished during this period.

For answering these questions, we analysed changes in floristic composition by applying a multivariate approach as well as an Indicator Species Analysis. We additionally used restoration indicators, namely total species richness, the proportion of characteristic Festuco-Brometea and Red List species as well as Ellenberg Indicator Values for light, moisture, nutrients and soil reaction. Since restoration is an important topic in nature conservation politics, we additionally discussed the value of our results for future restoration projects.

## MATERIAL AND METHODS

### STUDY AREA

This long-term observation study included seven different locations. An overview of the exact locations within the German federal state of Baden-Wuerttemberg is provided in Figure 7. Five of the study sites are situated with the natural area of the Gäu Plateaus and two study sites with the Swabian Alb. Information on the annual mean temperature, precipitation, altitude, the type, size and age of the cleared afforestation or succession as well as number of observed transects and plots are given for all study locations in Table 7.

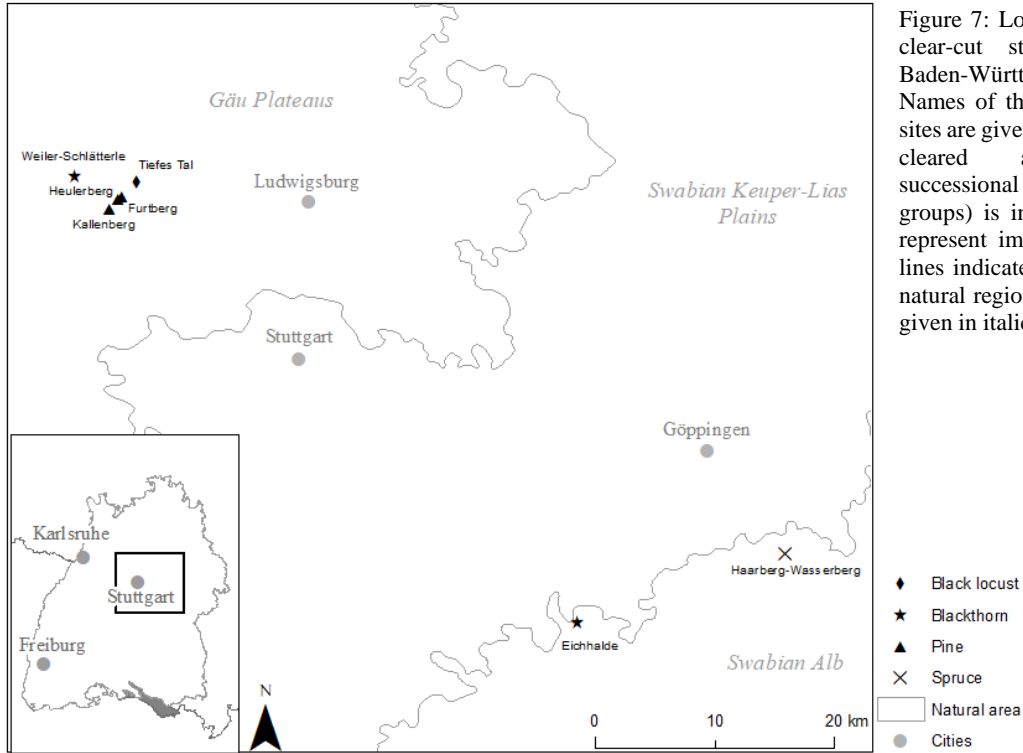


Figure 7: Location of the seven clear-cut study sites within Baden-Württemberg (Germany). Names of the individual study sites are given and the type of the cleared afforestation or successional shrubland (= groups) is indicated. Grey dots represent important cities; grey lines indicate the borders of the natural regions which names are given in italic.

### EXPERIMENTAL DESIGN

In the winter of 1991/92 or 1992/93, patches of trees or shrubs were completely removed at each location (= restored sites). In all cases, the restored areas were located in the vicinity of an ancient, continuously grazed calcareous grassland (= reference sites, *Festuco-Brometea* communities of the association *Gentiano-Koelerietum*) to ensure a connection between both categories. Rotational grazing by sheep was re-established as management to keep the clear-cut areas open on the one hand and to reintroduce the typical historic land-use and most important dispersal vector on the other hand (Fischer et al., 1996; Poschlod et al., 1996). For all study sites, permanent plots along transects were installed in restored and reference areas to find the plots in a reasonable time period but also to cover the potential habitat heterogeneity (Kent and Coker, 2012; Pfadenhauer et al., 1986). Each transect typically consists of five plots with a size of 2 x 2 m (with

the exception of one reference transect located in the study location Weiler-Schlätterle; here only 4 plots were included into the monitoring) according to the recommended size of a monitoring plot in grasslands (Bonham, 2013; Pfadenhauer et al., 1986; Traxler, 1997) resulting in an observation area of 20 m<sup>2</sup> per transect, fulfilling the minimum area for the habitat according to Van der Maarel (2005). In each transect, the individual plots were located alternately to the left and right along a common centre line and were directly adjacent to each other.

### VEGETATION DATA

Vegetation composition was monitored using the cover estimation approach according to Schmidt (1974). The continuous monitoring was initiated in the summer following restoration. On the restored sites, an additional monitoring was conducted directly before the clear-cutting. This data was not included into the analysis due to a



complete species turnover revealed by multivariate analysis. Further vegetation surveys of all permanently marked plots were carried out in 1993/94 (year 1), 1994/95 (year 2), 2001 (year 8), 2008 (year 15), 2013 (year 20) and 2018 (year 25), respectively. These recordings were always

conducted during June and July. The total observation period covered, including the vegetation surveys of 2018, an average time span of 25 years, in which both grassland categories were continuously monitored.

Table 6: Overview on restoration indicators. Species Richness, habitat specialists, Red List species and Ellenberg Indicator Values with respective attributes, abbreviation and data source.

Indicator	Attributes	Abbreviation	Data Source
Species richness	all recorded species	SR	-
Habitat specialists	characteristic <i>Festuco-Brometea</i> species	Fes.-Bro.	Oberdorfer (2001)
Red List	listed as endangered on Red List	RL	Breunig & Demuth (1999)
EIV for Light	1 (shadow plant) - 9 (light plant)	L	Ellenberg et al. (2001)
EIV for Moisture	1 (dry) - 9 (very wet)	F	Ellenberg et al. (2001)
EIV for Nutrient	1 (nutrient-poor) - 9 (nutrient-rich)	N	Ellenberg et al. (2001)
EIV for Soil Reaction	1 (acidic) - 9 (basic)	R	Ellenberg et al. (2001)

Table 7: Grouping of study sites according to former vegetation type on the cleared patches. For each study site, the age of the afforestation or succession (Aff.), the size of the restored patch, the mean annual temperature (Temp.), the mean annual precipitation (Prec.), the mean altitude and the amount of observation transects for restoration and reference are additionally given.

Group	Study site	Age of Aff.	Size of Aff.	Temp.	Prec.	Altitude	Observation transects (Reference/ Restoration)
Black locust	Tiefes Tal	30	60 m <sup>2</sup>	8 °C	700 mm	270	1/1
Blackthorn	Eichhalde	20	400 m <sup>2</sup>	8 °C	900 mm	560	1/1
	Weiler-Schlätterle	10	450 m <sup>2</sup>	8 °C	700 mm	230	1/1
Pine	Furtberg	25	150 m <sup>2</sup>	8 °C	700 mm	290	1/1
	Heulerberg	45	900 m <sup>2</sup>	8 °C	700 mm	300	1/1
	Kallenberg	35	150 m <sup>2</sup>	8 °C	700 mm	300	1/1
Spruce	Haarberg-Wasserberg	30	400 m <sup>2</sup>	7 °C	1000 mm	700	2/2

### RESTORATION INDICATORS

We selected seven different indicators suitable for evaluating the success of the restoration. These include total species richness, habitat-characteristic species, Red List species and Ellenberg Indicator Values for light, moisture, nutrients and soil reaction, which we refer to as ‘restoration indicators’ in the following. An overview of all included restoration indicators with respective attributes and data source is provided in Table 6.

The amount of recorded species was defined as total species richness. Following the phytosociological classification of Oberdorfer (2001), data on habitat characteristic species for the vegetation class of *Festuco-Brometea* was extracted. Additionally, information on the regional Red List status was collected for each species from the Red List for Baden-Wuerttemberg (Breunig and Demuth, 1999). Thereby, all species listed with any endangerment classification on the Red List (reaching from species listed on the early warning list to strongly endangered species) were included. Information on Ellenberg Indicators values for light, nutrient, moisture and soil reaction were taken from Ellenberg et al. (2001). Ellenberg Indicator Values were available for all species, except for five, which only occurred in low frequencies.

### GROUPING OF STUDY SITES

The seven study sites were divided into four groups prior to the conducted multivariate analysis (Table 7). The classification was based on the type of afforestation or shrubland, which was the “successional state of art” at the respective restoration patch prior to the clear-cutting management. Therefore, restoration patches on former “pine-” or “spruce-” afforestations and “black locust-” and “blackthorn-” successions were differentiated. These differentiations were made because both the initial conditions and the initial species inventory showed large differences between the groups. Our focus was on the

identification of general patterns in the recolonization processes and by using the group splitting, differences in the respective starting situation for vegetation recovery can thus be validated.

In the herb layer of the former pine (*Pinus sylvestris*) afforestations few typical grassland species still occurred due to the more open character of pine forests. The most common species in the grass layer was *Brachypodium pinnatum* (compare Bistea & Mahy 2005), which is known to increase in abundance when management ceases and succession begins (Oberdorfer, 2001). This group included three study sites (Furt-, Heuler- und Kallenberg), resulting in three pairs of observation areas (Table 7).

The spruce (*Picea abies*) afforestation was characterised by a dense tree layer while no species occurred in herb layer directly before the clearing (see Poschlod & Jordan 1992, Kiefer 1998). Here, only one study site was available with a total of two observation transects for reference and restored grassland, respectively (Table 7).

The successions included cleared successional stages of blackthorn (*Prunus spinosa*) and black locust (*Robinia pseudacacia*), species known for their vegetative spreading via root tillers. The herb layer of both groups included some typical grassland species prior to clearance. Two and one study sites were available for the blackthorn and the black locust, respectively (Table 7).

### STATISTICAL ANALYSES

All analyses were conducted using R v.3.6.3 (R Core Team, 2020). Packages that were used for the individual statistical analyses are mentioned in the following explanations. For all statistical analyses recorded shrub and tree species were included as cover and abundance of these species is an important indicator when evaluating the success of restoration by clearance.

To give an overview of the changes of the floristic composition during the observation period, non-metric multidimensional scaling (NMDS) using Bray-Curtis distances was applied. Calculations were based on a vegetation matrix consisting of the average cover values for each species per year and per observed transect. For calculations and plotting of the NMDS the ‘vegan’ package was used (v2.5-4, Oksanen et al. 2013). Best solution was reached after 20 iterations using three dimensions with a final stress of 0.137. Species abundance data and restoration indicator values, which were significantly associated with the NMDS axes, were added to the ordination graph. An Indicator Species Analysis according to Dufrêne & Legendre (1997) was carried out to identify species that were significantly associated with either the restored or the reference grasslands. Indicator species were hereby searched by comparing the two categories separately for each year. The calculations were based on the ‘multipatt’ function (9,999 permutations) from the ‘indicspecies’ package (v.1.7.7; De Caceres & Legendre 2009). The indicator value index, ranging from 0 to 1, was calculated and extracted for all species which were identified as significant indicator species in the analysis.

In order to detect changes in the vegetation, species richness and abundance-weighted mean community values were calculated for all restoration indicators. Species richness was calculated as the sum of species recorded for each plot per year. Calculations for habitat characteristic species, Red List species and Ellenberg Indicator Values were based on species abundance data and were computed for each plot per year and site using the ‘FD’-package (v.1.0-12, Laliberté et al. 2014). Pairwise comparison of significant differences between both categories per year were conducted by implementing the Mann-Whitney-U test of the ‘exactRankTests’ package (v.08-31, Hothorn & Hornik 2015). Differences per year were tested within each

management using a Friedman-test implemented in R-base. If significant differences were obtained, differences within categories were calculated with a post-hoc Conover test using Bonferroni-corrections (Package: ‘PMCMRplus’ (v.1.4.4), Pohlert 2020).

## RESULTS

**FLORISTIC ASSESSMENT OF RESTORATION SUCCESS BASED ON MULTIVARIATE ANALYSIS AND SPECIES INDICATOR ANALYSIS**

A multivariate approach, in this case a NMDS (3-dimensional, final stress = 0.137) indicated great floristic differences between early restoration stages and reference grasslands (Figure 8). The composition of the restored vegetation showed a distinct development towards the reference along the first axis while the reference sites showed comparable small changes over the study period of 25 years. Species like *Briza media*, *Helianthemum nummularia* or *Teucrium chamaedrys* were positively correlated with the first axis and thus with the reference sites, while many shrub species (e.g., *Crataegus monogyna*, *Prunus spinosa* or *Robinia pseudoacacia*) and some forbs such as *Galeopsis tetrahit* or *Geum urbanum* showed a correlation with the restored vegetation during the first years after clearance (Figure 9). Additionally, the multivariate analysis revealed that changes of the floristic composition at the restoration sites occurred more rapidly in the first years after restoration since differences in vegetation composition were here more pronounced.

The NMDS furthermore revealed a clear distinction between reference grasslands and the three differentiated groups of restored vegetation ( $r^2=0.2$ ;  $p\leq 0.001$ ). While the first axis described floristic differences between restoration and reference, the second axis rather reflected differences between former pine afforestations, black locust succession and blackthorn successions to former spruce afforestation. The latter thereby showed a correlation with species such as *Daucus carota* and *Gentiana germanica*. Since restoration and reference sites showed a clear distinction from each other, which was also identified as most important factor explaining

species composition in the multivariate analysis, a Species Indicator Analysis was conducted to further disentangle changes in floristic composition. This analysis revealed that floristic dissimilarities between the restored (Table 8, Appendix Table A.1) and reference (Table 9, Appendix Table A.2) grasslands were greater in the early years after restoration. Over the course of the study, a decreasing number of indicator species, which could be assigned to either of the two categories, were found.

In the first year, the analysis identified 43 characteristic species for continuously managed grasslands and 26 species for clear-cut sites, whereas after 25 years only nine and five species, respectively, were detected to be characteristic for the two categories. The amount of indicator species for the restoration sites was highest in the second year (29 species), the eighth year (28 species) and the first year (26 species) of observation. Species with a high Indicator Values like *Galeopsis tetrahit*, *Galium aparine*, *Lactuca serriola* or *Sonchus asper* were identified as characteristic species only during these years. Other highly significant Indicator species associated with the restored sites for several years included *Clematis vitalba*, *Fragaria* ssp., *Cornus sanguinea*, *Geum urbanum*, *Rubus* ssp. and *Taraxacum officinale*. Indicator species that were frequently associated with the reference sites were *Cirsium acaulon*, *Prunella grandiflora*, *Briza media*, *Festuca ovina* and *Thymus pulegioides*.

After 25 years only *Cirsium arvense*, *Clematis vitalba*, *Clinopodium vulgare*, *Deschampsia cespitosa*, *Fragaria* ssp., *Galium mollugo* and *Mentha longifolia* were identified as Indicator species for the restored sites, while for the reference sites species with a high indicator value included *Achillea millefolium*, *Cirsium acaulon*, *Potentilla erecta* or *Prunella grandiflora*.

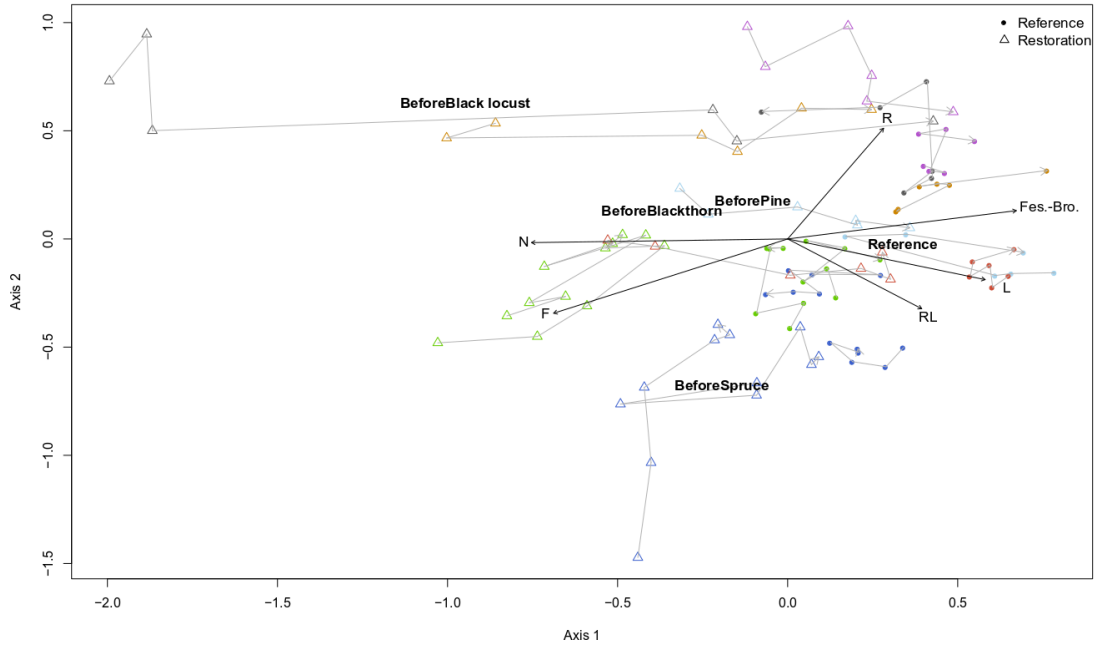


Figure 8: Non-metric dimensional scaling (NMDS) of the vegetation development over a period of 25 years of pairs of calcareous reference grasslands (•) and restored grasslands (Δ) (pairs are illustrated with the same colour, ●Δ - Eichhalde, ●Δ - Furtberg, ●Δ - Kallenberg, ●Δ - Haarberg-Wasserberg, ●Δ - Heulerberg, ●Δ - Tiefes Tal, ●Δ - Weiler-Schlätterle). Shown are the first and second axis. Arrows indicate significant ( $p < 0.05$ ) correlation of fitted indicator values (for abbreviations see Material & Methods).

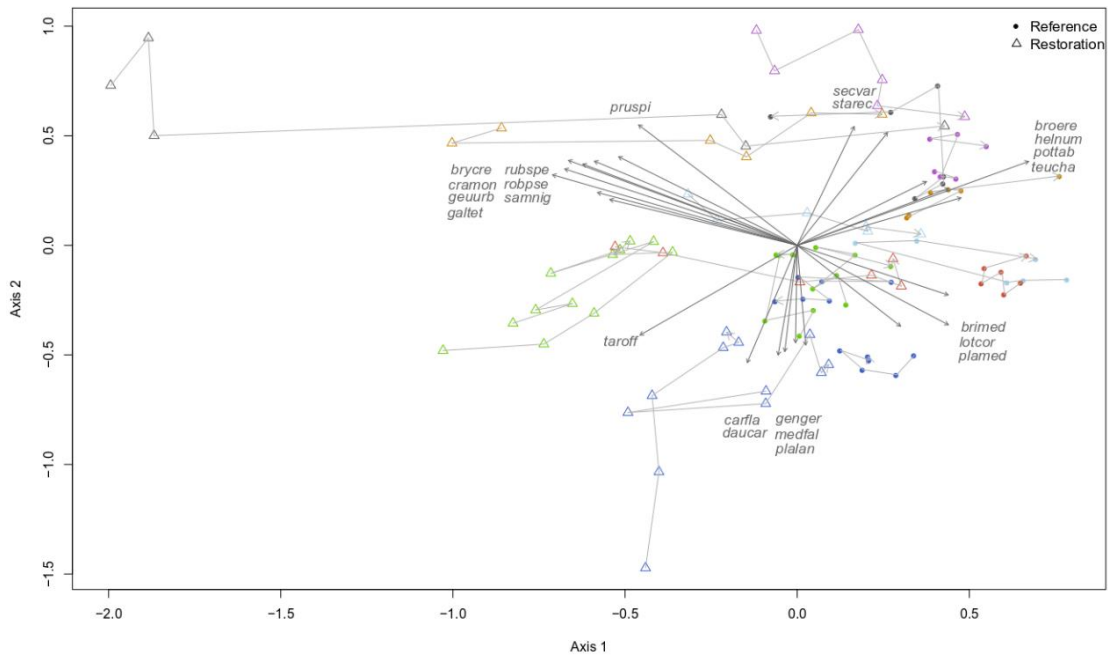


Figure 9: Non-metric dimensional scaling (NMDS) of the vegetation development over a period of 25 years of pairs of calcareous reference grasslands (•) and restored grasslands (Δ) (pairs are illustrated with the same colour, ●Δ - Eichhalde, ●Δ - Furtberg, ●Δ - Kallenberg, ●Δ - Haarberg-Wasserberg, ●Δ - Heulerberg, ●Δ - Tiefes Tal, ●Δ - Weiler-Schlätterle). Shown are the first and second axis. Arrows indicate significant ( $p \leq 0.001$ ) correlation of species abundance data. Species abbreviations: brimed – *Briza media*, broere – *Bromus erectus*, brycre – *Bryonia cretica*, carfla – *Carex flacca*, cramon – *Crataegus monogyna*, daucar – *Daucus carota*, galtet – *Galeopsis tetrahit*, geurb – *Geum urbanum*, genger – *Gentiana germanica*, helnum – *Helianthemum nummularia*, lotcor – *Lotus corniculatus*, medfal – *Medicago falcata*, plalan – *Plantago lanceolata*, plamed – *Plantago media*, pottab – *Potentilla tabernaemontani*, pruspi – *Prunus spinosa*, robpse – *Robinia pseudoacacia*, rubspe – *Rubus* spp., samnig – *Sambucus nigra*, secvar – *Securigera varia*, starec – *Stachys recta*, taroff – *Taraxacum officinale*, teucha – *Teucrium chamaedrys*.

Table 8: Result of the Species Indicator Analysis for **restored** grasslands for all observational years since restoration, based on average cover values of 45 plots (2x2m). Species are sorted by their first appearance and duration of occurrence as indicator species. Given are the total amount of indicator species per year, indicator index values and significance level (\*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001).

Year	1	2	8	15	20	25
Total amount	26	29	28	15	12	7
<i>Clematis vitalba</i>	0.83 ***	0.86 ***	0.79 ***	0.72 ***	0.68 ***	0.62 ***
<i>Fragaria ssp.</i>	0.45 **	0.52 ***	0.59 ***	0.55 ***	0.53 ***	0.45 **
<i>Cornus sanguinea</i>	0.7 ***	0.84 ***	0.75 ***	0.72 ***	0.72 ***	
<i>Erigeron canadensis</i>	0.45 **	0.54 ***	0.49 **	0.47 **	0.47 **	
<i>Geum urbanum</i>	0.63 ***	0.63 ***	0.52 ***	0.48 *	0.42 *	
<i>Rubus ssp.</i>	0.68 ***	0.71 ***	0.63 ***	0.56 ***	0.52 **	
<i>Cirsium arvense</i>	0.67 ***	0.82 ***	0.5 **		0.49 ***	0.39 *
<i>Taraxacum officinale</i>	0.76 ***	0.82 ***	0.74 ***		0.55 **	
<i>Cirsium vulgare</i>	0.65 ***	0.75 ***	0.42 **			
<i>Rubus idaeus</i>	0.39 *	0.47 **	0.37 *			
<i>Galium mollugo</i>	0.46 **	0.56 ***			0.57 **	0.61 ***
<i>Galeopsis tetrahit</i>	0.49 ***	0.56 ***				
<i>Hypericum hirsutum</i>	0.49 ***	0.49 **				
<i>Lactuca serritola</i>	0.39 *	0.58 ***				
<i>Plantago major</i>	0.39 *	0.42**				
<i>Sambucus nigra</i>	0.65 ***	0.62 ***				
<i>Sonchus asper</i>	0.76 ***	0.82 ***				
<i>Sonchus oleraceus</i>	0.45 **	0.47 **				
<i>Torilis japonica</i>	0.42 **	0.52 ***				
<i>Mentha longifolia</i>	0.39 *		0.39 *	0.42 **	0.39 *	0.45 **
<i>Cirsium palustre</i>	0.45 **			0.41 *		
<i>Angelica sylvestris</i>	0.37 *					
<i>Bryonia dioica</i>	0.39 *					
<i>Dipsacus fullonum</i>	0.47 **					
<i>Euphorbia verrucosa</i>	0.47 *					
<i>Pulicaria dysenterica</i>	0.42 **					
<i>Crataegus monogyna</i>		0.44 *	0.52 ***	0.5 *	0.52 *	
<i>Hypericum perforatum</i>		0.55 **	0.6 *	0.62 **		
<i>Phleum pratense</i>		0.37 *	0.44 **	0.38 *		
<i>Galium aparine</i>		0.49 ***	0.47 **			
<i>Arrhenatherum elatius</i>		0.39 *				
<i>Carduus nutans</i>		0.44 **				
<i>Epilobium tetragonum</i>		0.39 *				
<i>Helleborus foetidus</i>		0.38 *				
<i>Linaria vulgaris</i>		0.37 *				
<i>Ribes uva-crispa</i>		0.37 *				
<i>Deschampsia cespitosa</i>			0.47 **	0.39 *		0.4 **
<i>Senecio jacobaea</i>			0.39 *		0.37 *	
<i>Agrostis stolonifera</i>			0.45 *			
<i>Bromus sterilis</i>			0.39 *			
<i>Geranium sanguineum</i>			0.39 *			
<i>Inula britannica</i>			0.39 *			
<i>Myosotis arvensis</i>			0.45 **			
<i>Picris hieracioides</i>			0.5 *			
<i>Poa trivialis</i>			0.46 **			
<i>Prunella vulgaris</i>			0.39 *			
<i>Ranunculus repens</i>			0.37 *			
<i>Veronica arvensis</i>			0.37 *			
<i>Viola hirta</i>			0.79 ***			
<i>Clinopodium vulgare</i>				0.44 *		0.37 *
<i>Cerastium holosteoides</i>				0.5 **		
<i>Sorbus aucuparia</i>				0.42 *		

Table 9: Result of the Species Indicator Analysis for reference grasslands for all observational years since restoration, based on average cover values of 44 plots (2x2m). Species are sorted by their first appearance and duration of occurrence as indicator species. Given are the total amount of indicator species per year, indicator index values and significance level (\*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001).

Year	1	2	8	15	20	25
Total amount	43	36	21	12	14	9
<i>Cirsium acaulon</i>	0.72 ***	0.64 **	0.65 ***	0.58 **	0.59 ***	0.53 *
<i>Prunella grandiflora</i>	0.74 ***	0.76 ***	0.73 ***	0.55 *	0.64 ***	0.65 **
<i>Briza media</i>	0.91 ***	0.95 ***	0.87 ***	0.77 ***	0.75 ***	
<i>Festuca ovina</i>	0.97 ***	0.99 ***	0.96 ***	0.81 ***	0.66 **	
<i>Helianthemum nummularium</i>	0.64 ***	0.63 ***	0.65 ***	0.59 ***	0.59 **	
<i>Thymus pulegioides</i>	0.92 ***	0.9 ***	0.85 ***	0.79 ***	0.7 ***	
<i>Achillea millefolium</i>	0.87 ***	0.88 ***	0.83 ***	0.82 ***		0.8 ***
<i>Asperula cynanchica</i>	0.68 ***	0.57 **	0.55 **	0.51 **		
<i>Carex caryophylla</i>	0.71 ***	0.76 ***	0.7 ***		0.67 ***	
<i>Dianthus carthusianorum</i>	0.5 ***	0.52 ***	0.43 **		0.38 *	
<i>Anthoxanthum odoratum</i>	0.34 *	0.47 **	0.34 *			
<i>Anthyllis vulneraria</i>	0.48 **	0.5 **	0.45 **			
<i>Carlina acaulis</i>	0.34 *	0.37 *	0.37 *			
<i>Koeleria pyramidata</i>	0.89 ***	0.95 ***	0.81 ***			
<i>Plantago media</i>	0.86 ***	0.83 ***	0.77 ***			
<i>Teucrium chamaedrys</i>	0.59 **	0.58 **	0.61 **			
<i>Potentilla verna</i>	0.79 ***	0.7 ***			0.65 **	
<i>Trifolium campestre</i>	0.37 *	0.39 *				0.46 *
<i>Bromus erectus</i>	0.96 ***	0.98 ***				
<i>Centaurea jacea</i>	0.8 ***	0.74 ***				
<i>Euphrasia officinalis</i>	0.5 ***	0.52 ***				
<i>Festuca pratensis</i>	0.42 *	0.45 *				
<i>Galium verum</i>	0.89 ***	0.85 ***				
<i>Hieracium pilosella</i>	0.65 ***	0.57 **				
<i>Hippocrepis comosa</i>	0.42 *	0.45 **				
<i>Listera ovata</i>	0.34 *	0.37 *				
<i>Lotus corniculatus</i>	0.86 ***	0.91 ***				
<i>Luzula campestris</i>	0.37 *	0.4 **				
<i>Ononis repens</i>	0.4 **	0.39 *				
<i>Ranunculus bulbosus</i>	0.8 ***	0.81 ***				
<i>Rhinanthus glacialis</i>	0.5 **	0.48 *				
<i>Scabiosa columbaria</i>	0.72 ***	0.72 ***				
<i>Vicia angustifolia</i>	0.46 **	0.54 ***				
<i>Carex ormithopoda</i>	0.37 *					
<i>Carlina vulgaris</i>	0.34 *					
<i>Dactylis glomerata</i>	0.59 ***					
<i>Fagus sylvatica</i>	0.34 *					
<i>Knautia arvensis</i>	0.51 **					
<i>Phleum phleoides</i>	0.45 **					
<i>Pimpinella saxifraga</i>	0.87 ***					
<i>Sanguisorba minor</i>	0.83 ***					
<i>Trifolium repens</i>	0.49 *					
<i>Trisetum flavescens</i>	0.34 *					
<i>Potentilla erecta</i>		0.34 *	0.43 **	0.44 *	0.47 *	0.32 *
<i>Helictotrichon pratense</i>		0.42 *	0.37 *			
<i>Taraxacum laevigatum</i>		0.34 *				
<i>Centaurea scabiosa</i>			0.37 *		0.46 *	
<i>Acinos arvensis</i>			0.33 *			
<i>Trifolium pratensis</i>			0.55 **			
<i>Prunella vulgaris</i>				0.38 *	0.39 *	
<i>Vincetoxicum hirundinaria</i>				0.4 **	0.42 **	
<i>Carex flacca</i>				0.69 ***		
<i>Arrhenatherum elatius</i>					0.43 **	
<i>Hypericum maculatum</i>						0.32 *
<i>Fraxinus excelsior</i>						0.44 *
<i>Poa pratensis</i>						0.49 **
<i>Quercus robur</i>						0.53**

Table 10: Mean Species Richness and abundance weighted mean values for reference grasslands (G) and restored clear-cut sites (R) for each observation year are given. For reference grasslands 44 and for restored grassland 45 observation plots were included per year. Bold values indicate a significant difference between managements for the respective year (Mann-Whitney-U-test,  $p < 0.05$ ). The results of a Friedman-test (F.test), indicating significant differences within categories during the observation periods are given (n.s.  $p > 0.05$ ; \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ). Significant differences based on a Post-hoc-Conover test are indicated with superscript letters. Abbreviations: Fes.-Bro.: characteristic species of the *Festuca-Brometea* class, EIV: Ellenberg Indicator Value.

Years since clear-cutting		1	2	8	15	20	25	F. test
Species Richness	G	<b>33.1</b> <sup>a</sup>	<b>35.4</b> <sup>b</sup>	33.1 <sup>a</sup>	33.4 <sup>a</sup>	32.7 <sup>a</sup>	32 <sup>c</sup>	***
	R	<b>24.2</b> <sup>a</sup>	<b>31.2</b> <sup>b</sup>	31.9 <sup>b</sup>	32 <sup>b</sup>	31.6 <sup>b</sup>	31 <sup>b</sup>	***
Fes.-Bro	G	<b>0.71</b>	<b>0.68</b>	<b>0.68</b>	<b>0.66</b>	<b>0.66</b>	<b>0.62</b>	n.s.
	R	<b>0.24</b> <sup>a</sup>	<b>0.25</b> <sup>a</sup>	<b>0.4</b> <sup>b</sup>	<b>0.54</b> <sup>c</sup>	<b>0.52</b> <sup>c</sup>	<b>0.53</b> <sup>c</sup>	***
Red List	G	<b>0.06</b> <sup>a</sup>	<b>0.08</b> <sup>b</sup>	<b>0.05</b> <sup>c</sup>	<b>0.04</b> <sup>d</sup>	<b>0.05</b> <sup>c</sup>	<b>0.07</b> <sup>ac</sup>	**
	R	<b>0.01</b> <sup>a</sup>	<b>0.01</b> <sup>a</sup>	<b>0.01</b> <sup>b</sup>	<b>0.01</b> <sup>a</sup>	<b>0.03</b> <sup>b</sup>	<b>0.03</b> <sup>b</sup>	***
EIV Light	G	<b>7.27</b> <sup>a</sup>	<b>7.32</b> <sup>ab</sup>	<b>7.49</b> <sup>c</sup>	<b>7.5</b> <sup>d</sup>	7.37 <sup>b</sup>	7.34 <sup>b</sup>	***
	R	<b>6.96</b> <sup>a</sup>	<b>6.93</b> <sup>a</sup>	<b>6.99</b> <sup>a</sup>	<b>7.24</b> <sup>b</sup>	7.25 <sup>b</sup>	7.26 <sup>b</sup>	***
EIV Moisture	G	<b>3.50</b>	<b>3.50</b>	<b>3.49</b>	<b>3.43</b>	<b>3.44</b>	3.44	n.s.
	R	<b>4.25</b> <sup>a</sup>	<b>4.12</b> <sup>b</sup>	<b>3.86</b> <sup>c</sup>	<b>3.71</b> <sup>d</sup>	<b>3.77</b> <sup>d</sup>	3.55 <sup>e</sup>	***
EIV Nutrients	G	<b>3.04</b>	<b>2.95</b>	<b>3.03</b>	<b>3.03</b>	<b>3.07</b>	<b>3.09</b>	n.s.
	R	<b>4.93</b> <sup>a</sup>	<b>4.92</b> <sup>a</sup>	<b>4.13</b> <sup>b</sup>	<b>3.58</b> <sup>cd</sup>	<b>3.57</b> <sup>c</sup>	<b>3.41</b> <sup>d</sup>	***
EIV Soil Reaction	G	<b>7.4</b> <sup>a</sup>	<b>7.37</b> <sup>a</sup>	7.56 <sup>b</sup>	7.53 <sup>c</sup>	7.56 <sup>b</sup>	7.52 <sup>c</sup>	***
	R	<b>6.87</b> <sup>a</sup>	<b>7.17</b> <sup>b</sup>	7.49 <sup>c</sup>	7.49 <sup>c</sup>	7.47 <sup>c</sup>	7.58 <sup>d</sup>	***



### **ASSESSMENT OF RESTORATION SUCCESS BASED ON RESTORATION INDICATORS**

Besides the floristic composition seven restoration indicators were analysed (compare Table 6). During the first two years after restoration, all values obtained for these restoration indicators were significantly different between restoration and reference, while after 25 years only three values showed a significant difference (habitat characteristic species, Red List species and Ellenberg nutrient value, Table 10). For the restored grasslands the Friedman test revealed significant differences over the years for all seven indicators. The Post-hoc Conover test showed that all values changed between the first and the last year by showing a distinct development towards the values obtained for the reference sites. For the reference grasslands, only Species Richness, Red List species and Ellenberg Indicator Values for light and soil reaction showed significant differences over the years (Table 10). Here, the Post-hoc Conover test could not confirm a directed change.

Species richness was only significantly lower in the restored areas for the first and second year of observation by an average of 8.9 and 4.2 species, respectively. Contrary to this, the proportion of habitat characteristic species as well as of Red List species were both significantly lower even after 25 years (by 9 and 4 % respectively). That the reference grasslands harbour a higher proportion of *Festuco-Brometea* and Red List species was also confirmed by the NMDS (Figure 8, Appendix Table A.3). Here, habitat-characteristic species were positively correlated with the first axis and hence, the reference grasslands. Red List species showed a correlation towards the reference sites and also the study site Haarberg-Wasserberg but a negative correlation with former pine afforestation and former blackthorn or black locust succession. Ellenberg Indicator Values for soil reaction, light and moisture showed significant differences for the first two, fifteen and twenty years respectively while the nutrient value was still significantly

different in the last recording (by 0.32). Hereby, the soil reaction value and the light value were lower (by 0.53 and 0.31 in the first year, respectively) while the moisture and nutrient value were higher (by 0.75 and 1.89 in the first year, respectively) in the restored compared to the reference grasslands. The NMDS showed a similar pattern since the nutrient and the moisture value both showed a correlation with the early restoration sites. Contrary to this, the light value was positively correlated with the reference sites. The soil reaction value additionally showed a correlation towards the study sites on former pine afforestations and blackthorn and black locust successions.

### **DISCUSSION**

The analysis of the floristic composition of restored and reference grasslands based on species abundances, which were consistently recorded over a period of 25 years, showed significant differences in vegetation composition and restoration indicators during the first years after clearance. However, these differences became significantly smaller over the course of the study. The multivariate approach showed that the species compositions of the two categories overlapped after 25 years. In addition, the amount of indicator species associated with the restored grasslands decreased and restoration indicators converged over time. Thus, the successful restoration of calcareous grassland vegetation by clearance and subsequent sheep grazing was supported in this study.

### **DIFFERENCES IN FLORISTIC COMPOSITION BETWEEN RESTORATION AND REFERENCE GRASSLANDS ARE MORE IMPORTANT THAN DIFFERENCES BETWEEN THE INITIAL VEGETATION COMPOSITIONS OF THE RESTORED GRASSLANDS**

The multivariate approach showed that the vegetation development of the restored grasslands over time was the most important factor

explaining floristic differences in our data. The second most important factor was the differences in vegetation composition between former spruce afforestation and the other three groups. Only one study site (Haarberg-Wasserberg) was available for spruce afforestations which showed a clear separation from all other study sites. Species associated with Haarberg-Wasserberg were typical calcareous grassland species like *Carex flacca* or *Gentiana germanica*. In contrast, study sites on former blackthorn or black locust successions and pine afforestations were more similar in species composition and could be distinguished less clearly in the multivariate approach. This suggests a group splitting due to differences in typical species composition between study sites. In contrast to the other study sites, Haarberg-Wasserberg and the study site Eichhalde, which was placed closest in the multivariate analysis, were indeed both located on the Swabian Alb (Figure 7). Therefore, the differences between the four groups are most likely attributed to regional differences (see also Oberdorfer & Korneck 1993).

**INDICATOR SPECIES REVEALED DIFFERENT DISPERSAL PHASES DURING THE RECOLONIZATION OF THE RESTORED GRASSLANDS**

The Indicator Species Analysis provided more detailed information on vegetation recovery processes. During the first years of observation, many indicator species were detected that were significantly associated with one of the two grassland categories. Thereby, a peak of species (e.g. *Galeopsis tetrahit*, *Sonchus* ssp.), characterised by long-term persistent seeds but also by a high dispersal ability, was found in the restored grasslands during the first two years (Poschlod et al., 2003). A similar recolonization pattern was reported by Poschlod and Jordan (1992), who studied the soil seed bank and vegetation composition on a former calcareous grassland restored by clearance over a period of

two years. Thereby, the authors caught dispersed seeds in seed traps during the first years after the restoration, where they detected few seeds of calcareous grassland species but a peak of well dispersed ruderal species. In addition, woody and nutrient-favouring species (*Cornus sanguinea*, *Crataegus monogyna*, *Geum urbanum* or *Sonchus asper*) had higher occurrences on the restored grasslands, especially during the first 15 years. Similar results regarding an elevated occurrence of woody and nitrophilous species in young restorations were also found by Bistea and Mahy (2005) or by Dzwonko and Loster (1998) for five and four year old pine restorations, respectively.

In the course of the study, the number of typical calcareous grassland species (e.g. *Festuca ovina*, *Carex caryophyllea*) that correlated with the reference grasslands decreased simultaneously. Thus, it can be assumed that the frequency of typical calcareous grassland species increased in the restored grasslands. As many authors (e.g. Bistea and Mahy, 2005; Poschlod et al., 1998; Poschlod and Jordan, 1992) have already found evidence that sheep can act as crucial dispersal vector for typical calcareous grassland species, the quick arrival and establishment of habitat-characteristic species on the restored grasslands was likely attributed to the dispersal by grazing animals.

Bakker et al. (1996) and Poschlod et al. (1998) already reported that species recolonization on restored grasslands takes place in two dispersal phases - "in time" via the soil seed bank and "in space" via dispersal vectors like grazing animals - and this pattern was also observed in the present study. During the first years, many indicator species were characterised by long-lived seeds which strongly supported the concept of dispersal "in time". After eight years species with long-lived seeds but also species with high dispersal capability became less frequent in the restored sites, implying the built-up of a resilient grassland vegetation towards ruderal species. Simultaneously, the amount of typical calcareous

grassland species identified as indicator for reference grasslands decreased constantly. Therefore, it can be assumed that the second dispersal phase (“in space”) started to have an impact on vegetation composition as sheep grazing was constantly maintained over the years and thus, target species could have arrived at the restoration sites via zoochorous dispersal (Fischer et al., 1996). Epi- and endozoochorous dispersal events have already been observed for many habitat-typical species (e.g. *Bromus erectus* or *Galium verum*, Fischer et al., 1996, unpublished data). This finding was further supported in a chronosequence study by Piqueray et al. (2015), who found that epizoochorously dispersed species show a rapid increase on grasslands restored by clearance and subsequent sheep grazing.

Nevertheless, some species like *Briza media*, *Cirsium acaulon*, *Prunella grandiflora* or *Thymus pulegioides* still occurred more frequently in the reference grasslands even after 25 years. These species have, however, already been identified as mid-successional or ancient grassland indicators in other studies (Karlík and Poschlod, 2019; Piqueray et al., 2011b; Redhead et al., 2014; Schmid et al., 2017). Thus, it can be assumed that the habitat conditions (e.g. nutrient content) in the restored grassland were not (yet) suitable for the establishment of these species (Redhead et al., 2014). Contrary, indicator species that were assigned to the restored grasslands after 25 years (*Cirsium arvense*, *Galium aparine* or *Taraxacum officinale*), were classified as typical indicators for restored or young grassland in these studies.

**RESTORATION INDICATORS SHOWED SIGNIFICANT DIFFERENCES AFTER 25 YEARS BUT A CLEAR TREND TOWARDS REFERENCE SITES WAS DETECTED**

Although total species richness recovered quickly, other restoration indicators still showed significant differences between restored and reference grasslands after a period of 25 years. Assessing restoration success on basis of species

richness alone has already been proven to be an unsuitable indicator (Barbaro et al., 2001; Joshi et al., 2006; Rydgren et al., 2020; Waldén and Lindborg, 2016). Contrary, species composition, with a particular emphasis on habitat specialists, has been shown to be a better parameter for estimating restoration success (Joshi et al., 2006; Waldén and Lindborg, 2016). Based on this finding, one more meaningful restoration indicator is thus the proportion of species with main occurrence in *Festuco-Brometea* communities. Here, significant differences were still detectable, even after 25 years of observation, even though differences became significantly smaller over time. The same pattern also emerged for the nutrient value, indicating that nutrient impoverishment by grazing is still an ongoing process. Studies, dealing with the reconversion of former arable fields, already estimated that a full species recovery can take several decades up to more than a century, which they attributed to the slow depletion of nutrients in the soil but also to dispersal or establishment limitations (Fagan et al., 2008; Karlík and Poschlod, 2019; Öster et al., 2009a; Pywell et al., 2003; Redhead et al., 2014) and a similar pattern was also observed in this study. Even though nutrient impoverishment was supposed to be a less important factor in clear-cut restorations than on former arable fields, an oversupply of nutrients can still have an impact on current species composition and hence, the lack of habitat-characteristic species which are adapted to nutrient-poor conditions. Additionally, the lower abundance of Red List species in the restored grassland supported that some rare species might be dispersal or establishment limited (Piqueray et al., 2011b).

The lower mean light value during the first fifteen years, can, however, be explained when looking at the experimental setup. Restored grasslands often suffer from shrub species reproducing by root tillers (e.g. *Prunus spinosa*, *Robinia pseudoacacia*) and these species can persist and re-spread on restored grasslands for an extended

period, often posing severe problems in habitat restoration (Dzwonko and Loster, 2007; Maccherini et al., 2007). For example, the resprouting of shrub species can lead to a decline of highly light demanding species as a higher shrub cover filters for more shade tolerant species. Moreover, due to legal requirements and time and cost restrictions, often only a small part of an afforestation or shrubland was restored in this study. Therefore, restoration sites were usually directly adjacent to a forest or dense shrubland and typical shade-tolerant forest edge species can invade this patches more easily. Thus, differences in the light value during the first fifteen years – and consequently also in character species composition - might be explained due to higher shrub cover but also due to the closer vicinity to forest and shrubland edges. In later years, the constant pressure caused by grazing likely led to a gradual decline in shrub species and forest edge species, which are not adapted to grazing and thus to the built up of a more light demanding vegetation. These findings are additionally supported by the decline of shrub species for the restored grasslands in the Indicator Species Analysis.

Other restoration indicators, like the mean Ellenberg indicator value for soil reaction, recovered quickly and is most likely attributed to acidic character of litter produced by needles of coniferous trees which were decomposed during the first years of the restoration (Augusto and Ranger, 2001).

The grasslands that were in the focus of the study were low intensively used grasslands, which were managed in a traditional way for an extended period, and species composition and therefore, mean indicator value expressions of the restored grasslands were assumed to be comparatively stable. However, as we found significant differences in restoration indicators for the reference sites (species richness, Red List species, Ellenberg indicator values for light and soil reaction) that imply that external factors influence

the specifications over time. Nevertheless, important habitat characteristic attributes (Ellenberg indicator values for moisture and nutrient and proportion of habitat-characteristic species) showed no significant differences in reference grasslands over the study period. Based on these observations, we assumed the comparison of different restoration indicators between restored and reference grasslands were informative and not too strongly altered by non-management related influences.

## CONCLUSION

Our analyses of vegetation changes based on seven different study sites over a period of 25 years showed a clear trend of the vegetation composition and restoration indicators on the restored grasslands towards the nutrient-poor species-rich target community, namely calcareous grasslands. This development has already been hinted in several short-term studies (Bařa, 2011; Barbaro et al., 2001; Bistea and Mahy, 2005; Willems and Bik, 1998) and we found no indication of a reverse development during later stages in our long-term observation study. Indeed, restoration indicator values that differed even after 25 years showed a statistically significant trend towards reference values. Particular important for the successful restoration was thereby that grazing as management was re-introduced and maintained (Muller et al., 1998; Poschlod and Jordan, 1992). Grazing animals not only act as dispersal vector for typical calcareous grassland species (Poschlod et al., 1998) but also contributed to the nutrient impoverishment and to keeping the grasslands open. The latter became evident when looking at study sites that needed to be excluded from the original study (Kiefer, 1998), where management had been abandoned and which had been overgrown due to successional processes. Other studies (Barbaro et al., 2001; Szabó and Ruprecht, 2018) also reported the reoccurrence of successional processes after only few years

following restoration by clearance, but without subsequent management by grazing or mowing. Therefore, it is extremely important to promote and support the profession of being (and staying!) a shepherd in Central Europe since every year the number of active shepherds declines further, especially those who are still migrating with their flocks (BLE 2020).

Species composition is often stressed to be dispersal limited, either via persistent seed bank or (long-distance) dispersal vector (Poschlod et al., 1998; Römermann et al., 2008). Especially some rare and poor dispersed species might be missing in the restored vegetation (Lindborg and Eriksson, 2004b; Ozinga et al., 2005; Piqueray et al., 2011b) which explain why differences in proportion of Red List and habitat-characteristic species still occur. To overcome these differences caused by dispersal limitations of some rare species, functional traits were shown to be good indicators for detecting general patterns of the restoration success (Drobnik et al., 2011; Engst et al., 2016; Kahmen and Poschlod, 2008; Purschke et al., 2013; Pywell et al., 2003). As a next step, looking at the development of meaningful functional traits should be considered in order to estimate restoration success more precisely. Additionally, the historic connectivity was shown to be an important variable for explaining current species richness (Lindborg and Eriksson, 2004a; Purschke et al., 2012), especially with regard to a suspected time lag in extinction debt. Many authors have already proven that an extinction debt, especially on habitat specialists, might occur with a time lag (Diekmann et al., 2019; Piqueray et al., 2011a). Hence, the integration of historic landscape connectivity might give further important implications for estimating long-term success of the restoration measure conducted in this study (Kimberley et al., 2021). Related to this topic, we found small indications for changes in the vegetation composition in the reference grasslands which might occur due to insufficient grazing pressure or due to effects of habitat fragmentation.

A continued monitoring of the study sites will provide more certainty about this issue and is therefore highly recommended.

#### ***IMPLICATIONS FOR NATURE CONSERVATION***

Summed up, we can recommend restoration through clear-cutting followed by grazing which is an especially important outcome for restoration projects that will be initiated under the UN decade 2020 to 2030 on Ecosystem Restoration. Even though species composition did not reach those of the target community completely, we detected an ongoing development towards the target plant community. Additionally, newly created habitats may serve as important stepping stone habitats in the fragmented landscape playing a potential role in long-distance dispersal events, in countering extinction debt and in providing additionally important ecosystem services. Re-introduction and maintaining of grazing is thereby an essential aspect. Based on our findings, we additionally suggest that recently abandoned sites are generally better suited for restoration since they may still contain a large proportion of the typical calcareous grassland species (Poschlod et al., 1998).

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## CHAPTER 4

### CALCAREOUS GRASSLANDS RESTORED BY CLEARANCE AND SUBSEQUENT SHEEP GRAZING SHOW FAST RECOVERY OF PLANT FUNCTIONAL TRAITS – RESULTS FROM A LONG-TERM EXPERIMENT

#### ABSTRACT

The benefits of implementing functional traits in restoration ecology have been shown in numerous studies. Functional traits can be used to detect compositional changes that occur in response to altered environmental conditions. In this context, the classification of species into plant functional groups has proved to be particularly useful.

Using a functional trait approach, the restoration success of calcareous grassland vegetation was investigated at a study site located in the Swabian Alb. The grassland was restored in the 1990s by clearance of a spruce (*Picea abies*) afforestation and subsequent reintroduction of sheep grazing as mobile dispersal vector. Vegetation development was monitored over a period of 25 years, both on the restored and on an adjacent reference grassland. A total of fifteen functional traits, considered to be ecologically meaningful, were included in the analyses.

Changes in the functional composition were investigated by comparing the respective attribute expressions of the restored with the reference (continuously managed and ancient) grassland for each year of observation. In addition, iterative RLQ analyses were carried out to identify those traits that best explained species composition per year. A final RLQ-analysis was conducted based on identified traits and plant functional groups which were extracted using k-mean clustering.

The comparison of attributes expressions showed that differences between the two grassland categories decreased rapidly over time. Lateral spread, life span and growth form were the only traits that differed consistently over a period of at least 20 years. The largest differences were found in persistence-related traits while dispersal- and establishment-related traits recovered faster. The clustering of plant functional groups showed similarities with Grime's CSR-strategy, as cluster analysis grouped species with either ruderal, competitive or stress-tolerant trait attributes. After 20 years since restoration, stress-tolerators dominated, while the ruderal cluster was not detected anymore and only a small cluster of competitive, mostly woody species remained. Furthermore, RLQ analysis revealed no differences in the functional composition between restored and reference grassland in the last observation year.

Based on our results, we conclude that the ecosystem functions of the restored grassland were completely restored to the level of the reference grassland after approximately 20 years. The quick functional recovery is most likely attributed to the traditional low-intensive grazing management but also to the close vicinity of the reference grassland as no spatial isolation prevented dispersal of plant species. Hence, the functional trait approach showed that restoration by clearance and subsequent sheep grazing was a suitable management.

#### KEYWORDS

Iterative RLQ analysis, long-term monitoring, Swabian Alb

## INTRODUCTION

The concept of plant functional traits is an important tool for answering many crucial ecological questions (McGill et al., 2006; Pérez-Harguindeguy et al., 2013) especially in the context of restoration ecology (Clark et al., 2012; Hedberg et al., 2013; Laughlin, 2014; Sandel et al., 2011). Thereby, functional traits are generally defined as environment-responsive plant-specific characteristics (Lavorel et al., 1997) following the idea that species having a similar trait composition respond similarly to changes in the environment, including climate change (Bjorkman et al., 2018; De Bello et al., 2005; Soudzilovskaia et al., 2013), land-use change (Díaz et al., 2007; Kahmen and Poschlod, 2008, 2004; Purschke et al., 2014; Vandewalle et al., 2014) or invasive species (Loiola et al., 2018). Additionally, underlying processes (e.g. the impact of different filtering mechanisms (Zobel, 1997)) can be identified more accurately when the functional compositions of plant communities are compared rather than species compositions (McGill et al., 2006), especially for gaining knowledge on the ecosystem services a certain habitat may provide (Rusch et al., 2003; Zirbel et al., 2017). This is particularly true if some habitat-specific plant species are absent from the vegetation due to dispersal limitations (Öster et al., 2009a; Ozinga et al., 2005) but can be substituted by other species which are characterised by a similar trait composition. Therefore, it can be suspected that estimating restoration success based on species composition can lead to misleading or incomplete conclusions (Derhé et al., 2016; Engst et al., 2016).

One concept, which is particularly useful for understanding vegetation changes, is that of plant functional groups, which has been applied for estimating responses to changes in land-use managements in a variety of habitat types (Drobnik et al., 2011; Gondard et al., 2003; Kahmen and Poschlod, 2008; Römermann et al., 2009; Tozer et al., 2012; Zirbel et al., 2017).

Thereby, species are grouped according to their functional trait composition and thus species, which are allocated to the same functional group, are expected to similarly respond to changes in the environmental conditions (Lavorel et al., 1999).

In the present study, we used such a functional trait approach to gain knowledge on the vegetation development following the implementation of restoration management on calcareous grasslands, which are nowadays protected by European Law (European Commission 1992). Typical calcareous grassland species have adapted to the constant pressure caused by grazing and nutrient-poor, dry habitat conditions over the duration of several centuries or even millennia (Poschlod and Baumann, 2010; Poschlod and WallisDeVries, 2002). Due to major changes in the traditional land-use practices over the past 150 years, these species-rich habitats have rapidly declined in area and number (Poschlod, 2017; Poschlod and WallisDeVries, 2002). Main drivers for the substantial area loss were afforestation, amelioration and abandonment. Consequently, calcareous grasslands became a main issue in restoration and conservation ecology (Poschlod and WallisDeVries, 2002). The current study investigated a grassland patch that was afforested with spruce (*Picea abies*) in the 1960s and restored by clearing in the early 1990s. Subsequently, rotational sheep grazing was reintroduced, connecting the restored patch with an adjacent species-rich calcareous grassland.

It has previously been stressed that there is a gap in evaluating restoration measures in regard to long-term data on permanent observation plots, as on the one hand restoration of grazed grassland is assumed to proceed slowly and on the other hand chronosequences studies are vulnerable to site-specific influences (Bullock et al., 2001; Helsen et al., 2013). To fill this gap, we used vegetation data derived from continuously conducted vegetation surveys on permanent plots on both, restored and reference grasslands, covering in total a time span of 25 years. Given the long-term data set, we then

analysed the recovery of the functional composition in a spruce clear-cut managed by grazing using two approaches:

- 1) A direct comparison of mean functional trait attributes between restored and reference grasslands.
- 2) By finding and analysing plant functional groups.

For the first approach, we looked at differences in the trait attribute expression of ecological meaningful traits between restored and reference grassland. In the second approach, we applied RLQ analyses (Dolédec et al., 1996) to identify plant functional groups. This method allows to correlate vegetation, functional traits and environment conditions, which all may have an important influence on the individual restoration success. A variation of the RLQ analysis, which was shown to be suitable for investigating changes in the functional trait composition as a function of changes in abiotic conditions are iterative RLQ analyses (Bernhardt-Römermann et al., 2008; Drobniak et al., 2011; Römermann et al., 2009). Hereby, the best trait set explaining changes along an environmental gradient is detected by comparing all possible trait combinations. With this trait set we then can detect functional changes during the observation period by conducting a final RLQ for each year and extracting the plant functional groups.

## MATERIAL AND METHODS

### DATA

#### *Vegetation data (L-table)*

Vegetation data originated from a long-term monitoring carried out in the nature reserve 'Haarberg-Wasserberg', federal state of Baden-Wuerttemberg, in the Southwest of Germany (48°37'35.8"N, 9°44'07.8"E). The area, located in the Swabian Alb, is characterised by a mean annual temperature of 7 °C, a mean annual precipitation of 1000 mm and is situated at an

altitude of 1000 m. The nature reserve is known for the comparable high proportion of calcareous grasslands (*Festuca-Brometea*), which have been managed by extensive sheep grazing since at least the early 19<sup>th</sup> century (Kiefer, 1998). In the 1950s and 60s, parts of the 109.6 ha large area were afforested with spruce (*Picea abies*) or pine (*Pinus sylvestris*). In the course of a project investigating the restoration potential of former species-rich calcareous grasslands, which were afforested or had become overgrown due to secondary succession following management abandonment, an area of 400 m<sup>2</sup> of a 35 year old spruce afforestation was logged in winter 1992/93 (Amler et al., 1999; Kiefer, 1998). Vegetation surveys of the afforestation in the year before the clearing did not reveal any herbaceous species in the understorey (Kiefer, 1998). The restored grassland was directly adjacent to a continuously grazed, species-rich calcareous grassland (= reference grassland). Rotational sheep grazing (in late summer) was reintroduced directly after the clearance and served as mobile connecting vector between the reference and restored grassland. Ten permanently marked observation plots (size 2x2m) were installed in each grassland category, restoration and reference, and vegetation was monitored since 1993 using the percentage scale according to Schmidt (1974). More detailed information on the experimental design can be found in Kiefer (1998). Vegetation relevés for the restored and reference grasslands are available for 1993, 1994, 1995, 2001, 2008, 2013 and 2018, thus covering a period of more than 25 years. The long-term vegetation data were used to create the L-table with sites as rows and species abundances in percentages as columns. Species that only occurred with one or two individuals during no more than one observation year were excluded. In total, 172 of the 191 detected species were used for the analyses.



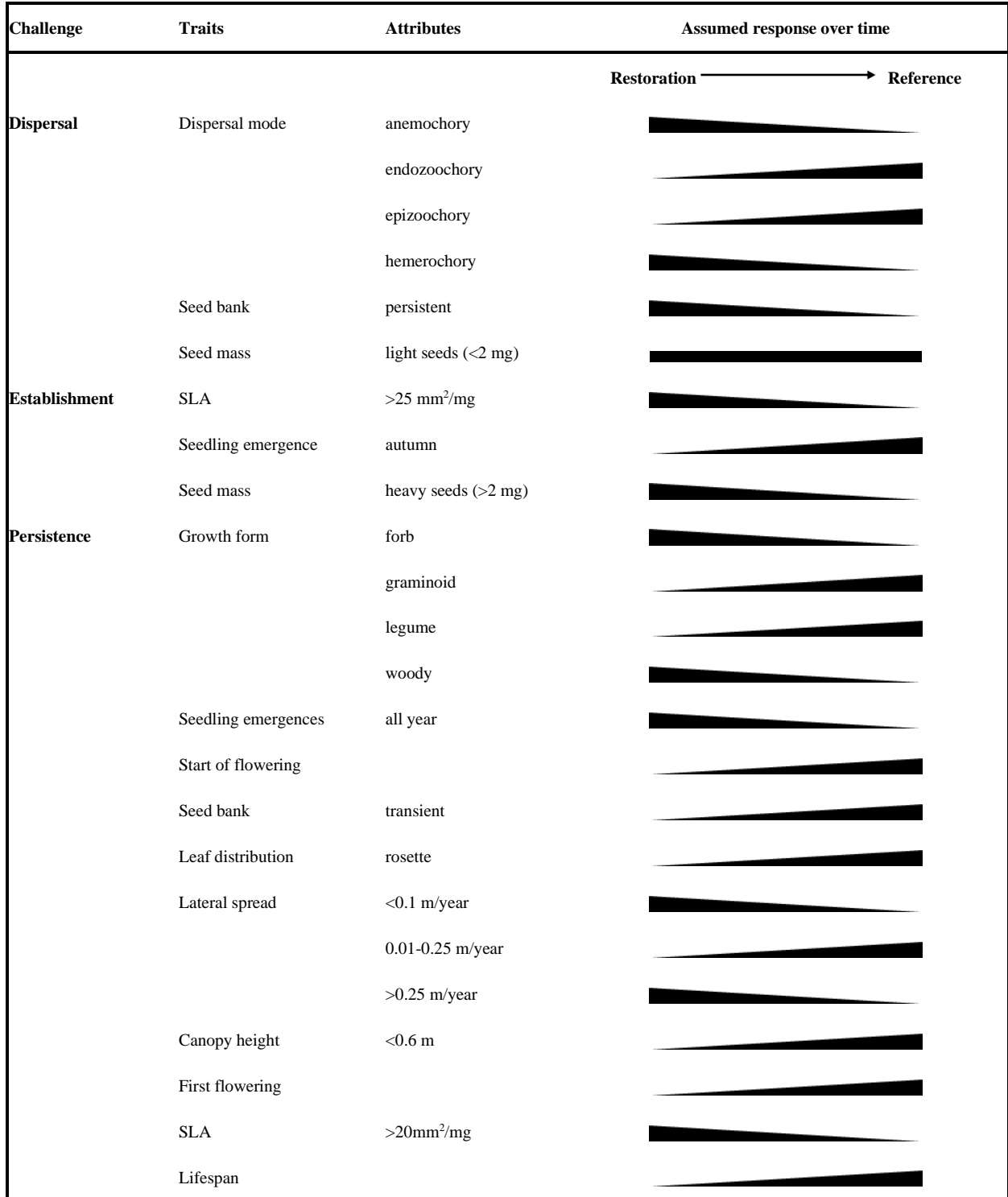


Figure 10: Assumed development of plant functional traits in restored grasslands compared to reference grasslands over time (in accordance to Weiher et al. (1999)).

*Environmental data (R-table)*

Ellenberg indicator values have been shown to accurately represent the local abiotic habitat conditions (Bartelheimer and Poschlod, 2016; Diekmann, 2003). Therefore, we used mean weighted Ellenberg indicator values for light, moisture, nutrients and soil reaction as environmental parameters (Ellenberg et al., 2001). In addition, the management type (restored or reference grassland) was included. The R-table was created with sites as rows and mean indicator values and respective management category in columns.

*Functional traits (Q-table)*

In total, 15 functional plant traits and their respective attributes (Table 11) were extracted from BIOPOP (Poschlod et al., 2003), the LEDA traitbase (Kleyer et al., 2008) and CLO-PLA (Klimešová et al., 2017) while missing values for seed mass and seed number per ramet were supplemented by self-collected data. When extracting plant functional traits, the mean of all available values was taken for metric traits if several data entries were available. For categorical traits, the most frequently mentioned or the highest attribute was taken (e.g. if for seedling emergence ‘spring’ and ‘all year’ were given then ‘all year’ was used). For the traits canopy height, leaf distribution, seed mass and specific leaf area (SLA) classifications were applied as proposed by Kahmen & Poschlod (2004, 2008) and species were assigned to the appropriate category (see Table 11) while the growth form was classified as proposed by Díaz et al. (2007) into forb, graminoid, legume and woody species.

In accordance to Weiher et al. (1999), we differentiated traits according to their relevance for plant species to cope with the challenges of dispersal, establishment and persistence (Figure 10, Table 11). It should be noted that individual trait attributes are in some cases important for different challenges and can therefore be involved in different processes.

The four dispersal modes (anemochory, endozoochory, epizoochory and hemerochory), seed bank and seed mass represent traits that are related to the *dispersal* of plant species. Thereby, the ‘**Dispersal mode**’ not only gives indications on possible spatial seed dispersal distances but also implies whether species may be introduced directional (e.g. by man, by animals) or depend on random dispersal events (e.g. by wind, Cornelissen et al. 2003; Poschlod et al. 2003). In terms of the **seed mass**, it was shown that light seeds tend to be dispersed over greater distances (Cornelissen et al., 2003) and is therefore, important for the dispersal in space (Bakker et al., 1996). The production of persistent seeds (‘**Seed bank**’) is relevant for the reintroduction of plant species in heavily disturbed habitats (dispersal in time, Bakker et al. 1996).

Traits that are correlated with the *establishment* of species were specific leaf area (SLA), season of seedling emergence and again seed mass. High “**SLA**” values represent species with a high relative growth rate which can positively influence the establishment success of seedlings (Weiher et al., 1999). The season of ‘**Seedling emergence**’ is relevant as the disturbance of the vegetation in summer can increase the proportion of species that germinate in autumn (Poschlod et al., 2003) while species that produce heavier seeds (“**Seed mass**”) are more likely to become established due to higher nutrient supply stored in the seed (Cornelissen et al., 2003).

*Persistence* is represented by traits that rather reflect a general trend regarding persistence (growth form and seedling emergence), are connected to the intermediate disturbance by extensive grazing (start of flowering, seed bank and leaf distribution), indicate the ability of the plant species to deal with the stress caused by nutrient poverty (SLA and life span) or are responsive to both, disturbance and nutrient stress (canopy height, age of first flowering, lateral spread).

The differentiation in forbs, graminoids, legumes and woody species (**‘Growth form’**) may provide information on the competitiveness as it is assumed that graminoid and woody species increase with higher nutrient contents while forbs and legumes are favoured by nutrient-poor conditions (Drobnik et al., 2011; Pywell et al., 2003). Species that germinate throughout the year (**‘Seedling emergences’**) are generally more competitive (Poschlod et al., 2003).

The trait **‘Start of flowering’** gives implication whether the plant species are able to produce seeds prior to the destruction of the above ground biomass (e.g. due to grazing) and hence, is important for species persistence. In stable or moderately disturbed habitats, species with short-lived seeds (**‘Seed bank’**) may occur more frequently (Cornelissen et al., 2003; Poschlod et al., 2003) and the proportion of species with rosettes (**‘Leaf distribution’**) is assumed to be positively correlated with an intermediate grazing pressure (Briemle et al., 2002; Cornelissen et al., 2003).

The trait **‘Canopy height’** is positively correlated with the competitive ability of the plant species. In contrast, mean canopy height is negatively affected by disturbance frequencies (e.g. by

grazing intensity) and abiotic stress factors like nutrient poverty (Cornelissen et al., 2003; Weiher et al., 1999). High disturbance frequencies and low nutrient availability are assumed to increase the age of **‘First flowering’** and thus promote species persistence. Contrary, a very early first flowering date can be an alternative way to avoid disturbance (Poschlod et al., 2003; Weiher et al., 1999). The ability to reproduce via clonal organs has a positive effect on plant species persistence, especially in habitats that are subject to disturbance (Cornelissen et al., 2003). Here, we used **‘Lateral spread’** to investigate the relevance of clonality, with a medium ability for lateral spread being indicative for persistence while a high lateral expansion is rather indicative for competitiveness.

The specific leaf area (**‘SLA’**) is positively correlated with growth rate and therefore the competitive ability of plant species while low SLA values indicate resource stress and an investment in long-lived leaves (Cornelissen et al., 2003; Weiher et al., 1999). **‘Life span’** is an important trait for determining the ability to persist in habitat which are exposed to stress like nutrient poverty or grazing pressure (Poschlod et al., 2003; Weiher et al., 1999).

Table 11: Data included into the iterative RLQ analyses. The R-table included environmental parameters, here represented by Ellenberg Indicator Values (EIV) and the respective management, the L-table included the vegetation relevées and the Q-table the functional traits with the respective attributes structured according to the challenges of dispersal, establishment and persistence. Data for the Ellenberg Indicator Values were extracted from Ellenberg et al. (2001) while trait data was extracted from BIOPOP<sup>a</sup> (Poschlod et al. 2003), LEDA<sup>b</sup> (Kleyer et al. 2008) and CLO-PLA<sup>c</sup> (Klimešová et al., 2017). Missing values for seed mass and seed number per ramet were supplemented with self-collected data<sup>d</sup> and plant type was classified based on own observations<sup>e</sup>. Trait attributes of traits that were relevant for several challenges were only described once<sup>f</sup>.

Table	Parameter/ Trait	Scale/ Attributes
<b>R</b>	Weighted EIV Light	metric
	Weighted EIV Nutrients	metric
	Weighted EIV Moisture	metric
	Weighted EIV Soil Reaction	metric
	Category of grassland	reference, restoration
<b>L</b>	Ten vegetation relevées per management; plot size 2x2m; recorded in 1993, 1994, 1995, 2001, 2008, 2013 or 2018	according to Schmidt (1974)
<b>Q</b>	<i>Dispersal-related traits</i>	
	Dispersal mode – anemochory <sup>b</sup>	no, yes
	Dispersal mode – endozoochory <sup>b</sup>	no, yes
	Dispersal mode – epizoochory <sup>b</sup>	no, yes
	Dispersal mode – hemerochory <sup>b</sup>	no, yes
	Seed bank <sup>b</sup>	transient, persistent
	Seed mass <sup>b, d</sup>	<0.5 mg, 0.5-2 mg, >2 mg
	<i>Establishment-related traits</i>	
	SLA <sup>b</sup>	<20 mm <sup>2</sup> /mg, 20-25 mm <sup>2</sup> /mg, >25 mm <sup>2</sup> /mg
	Seedling emergence <sup>a</sup>	spring, autumn, all year
	Seed mass <sup>f</sup>	
	<i>Persistence related traits</i>	
	Growth form <sup>e</sup>	forb, graminoid, legume, woody
	Seedling emergence <sup>a</sup>	
	Start of flowering <sup>b</sup>	May and earlier, after May
	Seed bank <sup>f</sup>	
	Leaf distribution <sup>b</sup>	basal rosette, no rosette
	Lateral spread <sup>c</sup>	<0.01 m/year, 0.01-0.25 m/year, >0.25 m/year
	Canopy height <sup>b</sup>	<0.3 m, 0.3-0.6 m, >0.6 m
	First flowering <sup>a</sup>	<1 year, 1-5 years, >5 years
SLA <sup>f</sup>		
Lifespan <sup>b</sup>	short (annuals & biennials), long (perennials)	

Table 12: Overview of the dimension of the R-, L- and Q-tables used for the calculation of the iterative RLQ analyses for the respective years.

	1993	1994	1995	2001	2008	2013	2018
<b>R-table (plots x environmental factors)</b>	20 x 5	20 x 5	20 x 5	20 x 5	20 x 5	20 x 5	20 x 5
<b>L-table (species x plots)</b>	20 x 83	20 x 119	20 x 119	20 x 121	20 x 102	20 x 103	20 x 103
<b>Q-table (species x traits)</b>	83 x 15	119 x 15	119 x 15	121 x 15	102 x 15	103 x 15	103 x 15

*STATISTICAL ANALYSES*

The proportion of species, which expressed a certain trait attribute, were calculated for restored and reference grasslands for the different observation years. Using Wilcoxon signed-rank tests significant differences between grassland categories were detected and significant changes in trait expression were then plotted.

In this first approach the different traits and their respective attributes hold an unidentified significance for explaining differences in species composition while, additionally environmental conditions were not taken into account. A method which combines all three information (functional traits, environmental conditions, species abundances) is the RLQ analysis as proposed by Dolédec et al. (1996). Hereby, the environmental data (R-table) is linked to the functional trait data (Q-table) via the vegetation data (L-table). The overall aim of this method is not only to investigate the relationship between traits and species abundances or environmental conditions and species abundances but to find a connection between functional traits and environmental conditions by using species abundances as underlying information. A RLQ analysis can be additionally modified into selecting those traits, which are suggested to be the most relevant for explaining species composition. The method for trait selection was proposed and described in detail by Bernhardt-Römermann et al. (2008).

For trait selection, iterative RLQ analyses were carried out separately for each year. The L-table was thereby created by a different set of plant species for the different years, based on the species found during the vegetation surveys. Prior to the analysis, the Ellenberg indicator values included into the R-table were standardised by adjusting the values to zero mean and unit deviance. An overview on the dimensions for the R-, L- and Q-table for the respective years is provided in Table 12.

In the first step of the RLQ analysis, the L-table was arcsine-square-root transformed and a

correspondence analysis was performed on this table. Then, Hill-Smith ordinations (Hill and Smith, 1976) were carried out on the R-table. Hereby, the row scores of the correspondence analysis on the L-table were used as canonical factor. The same was done for the Q-table. However, here the column scores were used as canonical factor. Next, two separate analyses were performed: one between the constrained ordination on the Q-table and the correspondence analysis on the L-table and one between the constrained ordination on the R-table and the correspondence analysis of the L-table. In the following iterative process, testing all possible combinations of traits, functional traits were extracted that describe the ecosystem response of the plant species along the environmental gradient best (Bernhardt-Römermann et al., 2008). This process was repeated for each year separately and the optimal trait set was identified individually for each of the seven observation years.

A final RLQ analysis was then conducted for each year based on the trait set which was identified in the iterative RLQ analyses. Besides using the altered Q-table, the RLQ analysis was performed as previously described. For detecting functional plant groups, the species scores of the final RLQ analysis per year was subsequently clustered by k-mean clustering. The ideal number of clusters was calculated based on 30 different indices and the number of clusters which was proposed most often was consequently used. Species allocation to clusters and cluster stability were additionally tested by using bootstrapping (Römermann et al., 2009). Once the best clustering for the plant species was calculated, characteristics of the plant functional groups were extracted by summarizing the number of species expressing each attribute. Significant differences between the plant functional groups were tested by using Chi<sup>2</sup>-tests for categorical values. As final step, the results of the RLQ analysis per year and the k-mean clustering were plotted based on species scores and cluster allocation and the correlation of the

restoration plots based on site scores was included (Management Category).

All analyses were conducted using R v.3.5.2 (R Core Team, 2020) and the packages ‘ade4’ v.1.7-13 (Dray and Dufour, 2007), ‘exactRangTest’ v0.8-31 (Hothorn and Hornik, 2015), ‘fpc’ v.2.2-3 (Hennig, 2019), ggplot2 v.3.32 (Valero-Mora, 2010) and ‘NBClust’ v.3.0 (Charrad et al., 2014).

## RESULTS

### *DIFFERENCES IN TRAIT ATTRIBUTES BETWEEN GRASSLAND CATEGORIES OVER THE STUDY PERIOD*

The comparison of functional traits showed clearly that more attributes differed significantly between the reference and restored grassland in the first years of observation than in later years (Table 13). Of the 15 investigated traits, 10 showed significant different trait attribute expressions in 1993. In 1994, 1995, 2001, 2008, 2013 and 2018, the number of significantly different traits amounted to 11, 9, 8, 5, 3 and 3, respectively.

Of the *dispersal*-related traits, hemerochory as well as light seed mass (< 2mg) showed significant differences between reference and restored grasslands during the first two years after restoration, while significant differences for epizoochory were only detected in 2018. Seed bank persistence differed at least until 2008.

All traits, correlated with the *establishment* of plant species, showed significant differences during the first year after restoration. Of these, only heavy seeded species differed until 2008, while autumn germinators showed an inconsistent pattern with additional significant differences in 2001, 2008 and 2018.

*Persistent*-related traits all showed significant differences for at least the first three years (1993, 1994, 1995) after restoration, with the exception of seedling emergence throughout the year (only significant in 1993) and start of flowering and leaf distribution (not significant in 1993). Growth form

was the only trait that still showed significant differences in 2018, while traits reflecting the moderate grazing pressure (start of flowering, seed bank and leaf distribution) differed until 2001 or 2008, respectively. Traits, that indicate plant species persistence like SLA and life span differed until 2008, while canopy height and age of first flowering were only significantly different until 1995 (the latter additionally in 2013). Lateral spread showed significant differences until 2013. A graphical illustration of the development of trait attributes displaying significant differences between reference and restored grassland is given in Figure 11 A-L (except epizoochory as differences were detected in 2018 for the first time).

### *RLQ- AND CLUSTER ANALYSES*

#### *Optimal trait sets identified by iterative RLQ analyses*

The traits that were identified as the optimal set of traits for explaining species composition were identified in the iterative RLQ analyses for each date separately (Table 14). The identified traits were then used to calculate the final RLQ for each year of observation.

Hemerochory and seed bank persistence were the only two dispersal-related traits that were identified for the optimal traits for several dates (1993-2008 and 1993-2013, respectively). Contrarily, anemochory (1993), endozoochory (2018), epizoochory (1993) and seed mass (1994) were traits that entered the final RLQ analysis only during one date.

For establishment-related traits, again seed mass was only identified for one date (1994) while SLA and seedling emergence entered the final RLQ analysis for five and four dates, respectively (Table 14).

Concerning the persistence of plant species, most frequently identified traits were canopy height (not in 2008), age of first flowering (not in 1993) and seed bank persistence (not in 2018) while plant life span (not 2001, 2013) and SLA (not

1993, 2013) were found for five dates and seedling emergence (not in 1994, 1995, 2013) and growth form (not in 1993, 1995, 2018) were found for four dates. Both, lateral spread and leaf distribution entered the final RLQ analyses in 2008 and 2013 while start of flowering was not identified for any date.

#### *Final RLQ analyses*

An overview on the results of the final RLQ analyses, which were calculated separately for each year based on the optimal final trait set (Table 14), is given in Table 15. Over the course of the study, the total inertia explained by the final RLQs declined. In 1993, the total inertia, which represents the variability of the data set, amounted to 1.717 while in 2018, the total variability decline to 0.128.

#### *Cluster identification and characterisation*

Three clusters of plant functional groups were detected by k-mean clustering in the years 1993, 1994, 1995, 2001 and 2008, whereas only two remained in 2013 and 2018 (Appendix Table A.5, Figure 12 A-G).

For the first five years of observation (Figure 12 A-E), Cluster 1 and Cluster 3 were separated from each other along the first axis which explained between 94.1% and 81.6% of the projected inertia (Table 15). Cluster 1 was thereby placed in the opposite direction of the restored grasslands while Cluster 3 showed a strong correlation with the restored grasslands. In addition, Cluster 2 was placed between Cluster 1 and Cluster 3 along the first axis and was separated from the other two clusters by the second axis (explaining between 4.9% and 13.6%).

For 2013 and 2018 (Figure 12 F-G), the two Clusters were split up along the first axis (54.3% and 65.3% of projected inertia, respectively) while the second axis explained differences within the clusters (37.3% and 22.4%, respectively; Table 14). There was no correlation between the restored grassland and the two clusters.

Significant differences between cluster based on the traits that were used for the final RLQ calculations and thus, cluster identification (Table 14), were calculated based on Chi<sup>2</sup>-tests. Thereby, we found no significant differences between identified clusters for hemerochory in 1993, for lateral spread and seed bank in 2013 and for endozoochory and SLA in 2018. All other traits showed significant differences between clusters (Appendix Table A.5).

Species allocations to the respective clusters is given in the Appendix (Appendix Table A.6). Based on significantly different trait attributes and associated plants species, characterisation of the three clusters is provided in the following. Since the functional traits that best describe species composition varied between dates, general patterns were used to define the clusters.

#### *Cluster 1 – ‘Grassland cluster’*

Persistence-related trait attributes that characterised Cluster 1 included the occurrence of small (<0.6 m), mostly perennial species which flowered early in their life cycle (<5 years) and had small SLA values (<25 mm<sup>2</sup>/mg). Moreover, the cluster contained the highest proportion of rosette and graminoid species and was dominated by species with transient seeds and by forbs. Concerning dispersal, hemerochorously dispersed species were underrepresented while with regard to establishment-related traits autumn germinators occurred most frequently in Cluster 1. Species belonging to Cluster 1 amounted to 40, 60, 38, 41, 63, 88 and 78 species in 1993, 1994, 1995, 2001, 2008, 2013 and 2018, respectively.

Species, which were assigned to Cluster 1 in all seven years were *Briza media*, *Campanula rotundifolia*, *Carex flacca*, *Cirsium acaulon*, *Festuca ovina*, *Gentiana verna*, *Helianthemum nummularium*, *Medicago falcata*, *Plantago media*, *Polygala amarella*, *Primula veris*, *Prunella grandiflora* (not recorded in 2008), *Sanguisorba minor* and *Scabiosa columbaria*.

Based on the strong negative correlation of Cluster 1 with the restoration plots in the RLQ analyses (Figure 12 A-G), this Cluster was defined as ‘Grassland cluster’.

*Cluster 2 – ‘Tall species cluster’*

Cluster 2 was characterised by tall (>0.6 m), perennial species, which flowered late in their life cycle (>1 year) and produced transient seeds. Further trait attributes that were related to persistence was the high proportion of woody species and the dominance of species with small SLA values. Concerning establishment, this cluster was dominated by spring germinators while for dispersal-related traits hemerochorously dispersed species only had the low frequencies in Cluster 2 (with the exception of 1995 and 2001). Species belonging to Cluster 2 amounted to 23, 14, 50, 40, 10, 15 and 25 species in 1993, 1994, 1995, 2001, 2008, 2013 and 2018, respectively.

Some species, which occurred exclusively in Cluster 2 were *Acer campestre*, *Carpinus betulus* (since 2001), *Crataegus monogyna*, *Fagus sylvatica*, *Fraxinus excelsior*, *Juniperus communis*, *Lonicera xylosteum* (since 1995), *Prunus spinosa*, *Quercus robur* (since 2001) and *Sorbus aria* (since 1994).

The highest species number for Cluster 2 was found in 1995 and 2001. Cluster analysis put only in these two years some more competitive species in this cluster, which were found in previous years for Cluster 1 (e.g. *Achillea millefolium*, *Dactylis glomerata*, *Knautia arvensis*, *Pimpinella saxifraga*) or Cluster 3 (*Cirsium arvense*, *Daucus*

*carota*),. This Cluster was defined as the ‘Tall species cluster’.

*Cluster 3 – ‘Restoration cluster’*

Regarding persistence-related traits, Cluster 3 was characterized by small (<0.6 m) species, which flowered early in their life cycle and produced persistent seeds. Furthermore, forbs and legumes dominated this cluster while species were indifferent in terms of life span. Trait attributes reflecting the establishment success were the high frequency of species with high SLA values that germinating throughout the year. Regarding dispersal, Cluster 3 generally contained many hemerochorously dispersed species (except 2001). Species belonging to Cluster 2 amounted to 20, 45, 31, 40 and 29 species in 1993, 1994, 1995, 2001 and 2008, respectively.

Species, which only occurred in Cluster 3 included for example *Alopecurus myosuroides* (1994/95), *Capsella bursa-pastoris* (1993-95), *Galeopsis ladanum/tetrahit* (1994/95), *Hieracium murorum* (1994/95), *Lactuca serriola* (1994-2001), *Myosotis arvensis* (1993-2001), *Poa annua* (1993/94), *Sonchus asper* (1993-95), *Stellaria media* (1993-95) and *Veronica arvensis* (2001-08). In addition, some species were assigned to Cluster 3 until 2008 and were then reassigned to Cluster 1 since 2013 (e.g. *Cerastium holosteoides*, *Linum catharticum*, *Medicago lupulina*, *Trifolium repens* or *Veronica chaemedrys*)

Due to the strong correlation of Cluster 3 with the restored grassland (Figure 12 A-G), this cluster was defined as ‘Restoration cluster’.



Table 13: Significant differences in the mean expression of functional traits attributes between the reference and restoration grasslands for the seven different observation years. For each grassland category 10 plots were available. Results are based on Wilcoxon signed-rank tests. Given are the respective p-values. Significant differences are illustrated in bold with  $p < 0.5^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$

Trait	Attribute	1993	1994	1995	2001	2008	2013	2018
<i>Dispersal-related</i>								
Dispersal mode	anemochory	0.447	0.725	0.105	0.956	0.839	0.218	0.383
	endozoochory	0.617	0.929	0.783	0.868	0.541	0.868	0.840
	epizoochory	0.671	0.912	0.225	0.955	0.271	0.669	<b>0.019*</b>
	hemerochory	<b>0.022*</b>	<b>0.003**</b>	0.105	0.435	0.669	0.516	0.739
Seed bank	persistent	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001**</b>	<b>&lt;0.001**</b>	<b>0.004**</b>	0.280	0.566
Seed mass	<0.5mg	<b>0.006**</b>	<b>0.024*</b>	0.671	0.128	0.342	0.184	0.684
	0.5 -2mg	<b>&lt;0.001***</b>	<b>0.004**</b>	0.47	0.118	0.565	0.517	0.239
<i>Establishment-related</i>								
SLA	>25 mm <sup>2</sup> /mg	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>0.018*</b>	0.566	0.725
Seedling Emergence	spring	<b>0.037*</b>	0.740	0.225	0.782	0.578	0.726	0.425
	autumn	<b>0.001***</b>	0.897	0.467	<b>&gt;0.001**</b>	<b>0.045*</b>	0.210	<b>0.040*</b>
Seed mass	>2mg	<b>&lt;0.014**</b>	0.516	1.000	0.839	1.000	0.382	0.184
<i>Persistence-related</i>								
Growth form	forb	<b>&lt;0.001***</b>	<b>0.002**</b>	<b>&lt;0.010**</b>	<b>0.005**</b>	<b>0.403**</b>	0.403	<b>0.016*</b>
	graminoid	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>0.002**</b>	0.755	<b>0.020*</b>
	legume	<b>0.030*</b>	0.148	0.426	0.093	0.171	0.565	0.697
	woody	0.059	0.529	0.078	0.21	1.000	0.448	0.780
Seedling Emergence	all year	<b>0.005**</b>	0.670	0.218	0.182	1.000	0.469	0.837
Start of Flowering		0.896	<b>0.011*</b>	<b>0.020*</b>	<b>0.045*</b>	0.403	0.853	0.343
Seed bank	transient	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001**</b>	<b>&lt;0.001**</b>	<b>0.004**</b>	0.280	0.566
Leaf distribution		0.779	<b>&lt;0.001***</b>	<b>0.002**</b>	<b>0.020*</b>	0.271	0.645	0.470
Lateral spread	<0.01 m/year	0.698	0.325	0.109	<b>0.002**</b>	<b>&lt;0.050*</b>	0.591	0.224
	0.01-0.25 m/year	<b>0.028*</b>	<b>0.037*</b>	0.403	<b>0.024*</b>	0.403	0.424	0.289
	>0.25 m/year	0.810	0.079	<b>&lt;0.001***</b>	<b>0.011*</b>	<b>&lt;0.001***</b>	<b>0.004**</b>	0.343
Canopy Height	<0.3 m	<b>0.001***</b>	<b>0.007**</b>	<b>&gt;0.001**</b>	0.644	0.643	0.796	0.753
	0.3-0.6 m	<b>&lt;0.001***</b>	0.248	0.566	0.628	0.956	0.986	0.631
	>0.6 m	<b>0.009*</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	0.353	0.955	0.725	0.645
First Flowering	<1 year	<b>0.005**</b>	<b>&lt;0.001***</b>	<b>0.004**</b>	0.143	0.867	0.271	0.753
	1-5 years	<b>0.035*</b>	<b>0.002**</b>	<b>0.005**</b>	0.342	0.305	0.063	0.239
	>5 years	<b>0.002**</b>	<b>&gt;0.001**</b>	0.159	0.342	0.240	<b>0.034*</b>	0.210
SLA	<20 mm <sup>2</sup> /mg	<b>0.006**</b>	<b>0.020**</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>0.043*</b>	0.927	0.869
	20-25 mm <sup>2</sup> /mg	<b>&lt;0.001***</b>	<b>0.043*</b>	0.305	0.085	0.136	0.363	0.898
Life span		<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>	0.184	<b>0.045*</b>	0.065

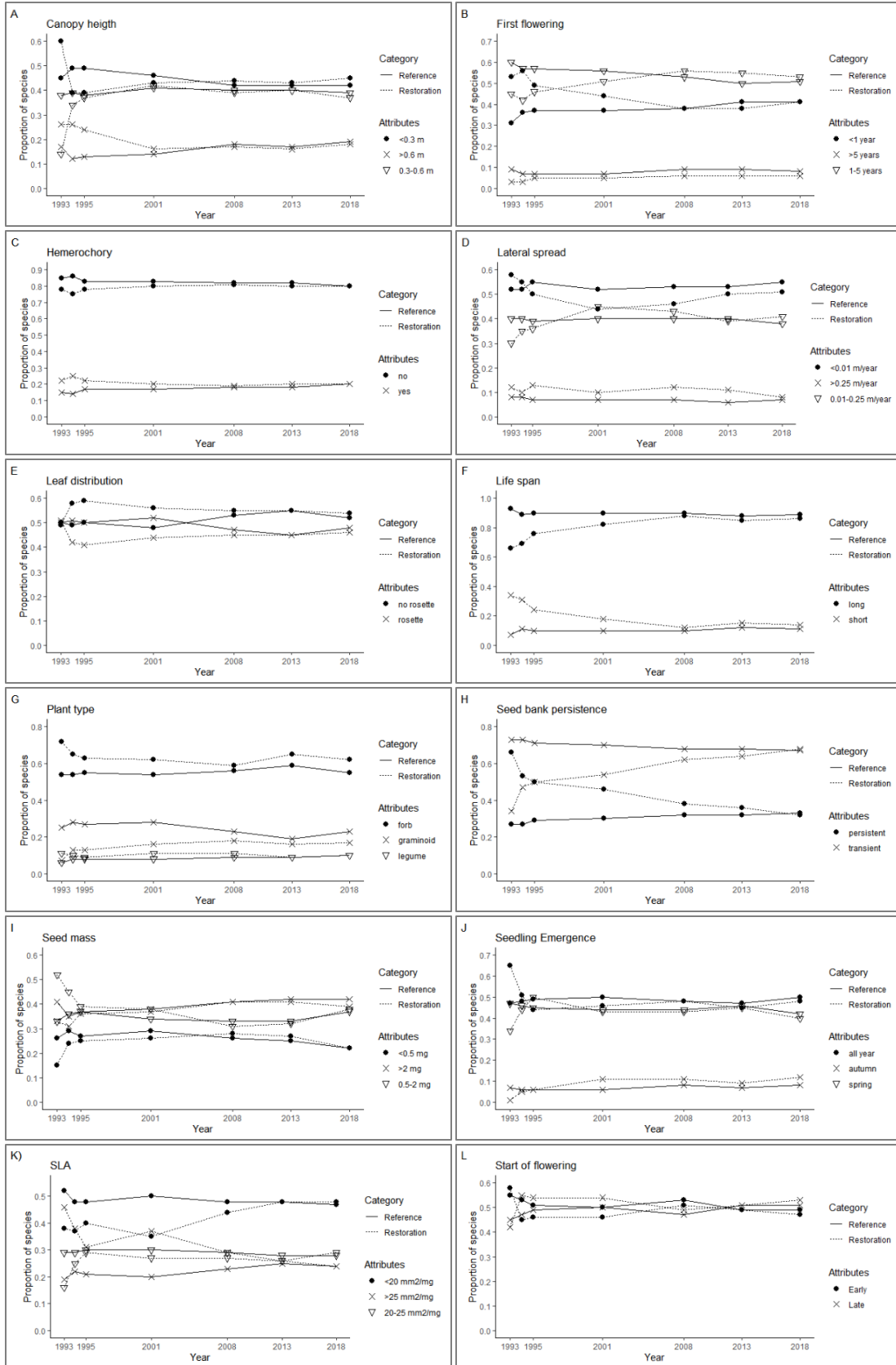


Figure 11 A-L: Development of trait attributes over the observation period. Included are all trait attributes (except epizoochory) that showed significant differences between reference and restoration plots (compare Table 2).

CHAPTER 4

Table 14: Overview on traits that were identified by the iterative RLQ-analyses, which were conducted for each year separately, as optimal trait set. Based on the identified traits the final RLQ analysis was calculated for each year. Grey shaded traits were relevant for two challenges and therefore appear twice in the list.

Trait	1993	1994	1995	2001	2008	2013	2018
<b>Number of traits</b>	7	8	6	7	9	6	6
<i>Dispersal-related traits</i>							
Dispersal mode – anemochory	final RLQ	-	-	-	-	-	-
Dispersal mode – endozoochory	-	-	-	-	-	-	final RLQ
Dispersal mode – epizoochory	final RLQ	-	-	-	-	-	-
Dispersal mode – hemerochory	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	-	-
Seed bank	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	-
Seed mass	-	final RLQ	-	-	-	-	-
<i>Establishment-related traits</i>							
SLA	-	final RLQ	final RLQ	final RLQ	final RLQ	-	final RLQ
Seedling emergence	final RLQ	-	-	final RLQ	final RLQ	-	final RLQ
Seed mass	-	final RLQ	-	-	-	-	-
<i>Persistence related traits</i>							
Growth form	-	final RLQ	-	final RLQ	final RLQ	final RLQ	-
Seedling emergence	final RLQ	-	-	final RLQ	final RLQ	-	final RLQ
Start of flowering	-	-	-	-	-	-	-
Seed bank	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	-
Leaf distribution	-	-	-	-	final RLQ	final RLQ	-
Lateral spread	-	-	-	-	final RLQ	final RLQ	-
Canopy height	final RLQ	final RLQ	final RLQ	final RLQ	-	final RLQ	final RLQ
First flowering	-	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ	final RLQ
SLA	-	final RLQ	final RLQ	final RLQ	final RLQ	-	final RLQ
Lifespan	final RLQ	final RLQ	final RLQ	-	final RLQ	-	final RLQ

Table 15: Overview of the results of the final RLQ analyses for the different observation years. Given are the number of traits as identified by the iterative RLQ, the total inertia of the final RLQ and the projected inertia for the first and second axis.

Results of final RLQ	1993	1994	1995	2001	2008	2013	2018
<b>Total inertia</b>	1.717	1.558	0.771	0.638	0.297	0.120	0.128
<b>Projected inertia (%) – Axis 1</b>	88.5	93.8	94.1	87.6	81.6	54.3	65.3
<b>Projected inertia (%) – Axis 2</b>	8.8	5	4.9	10.1	13.6	37.3	22.4

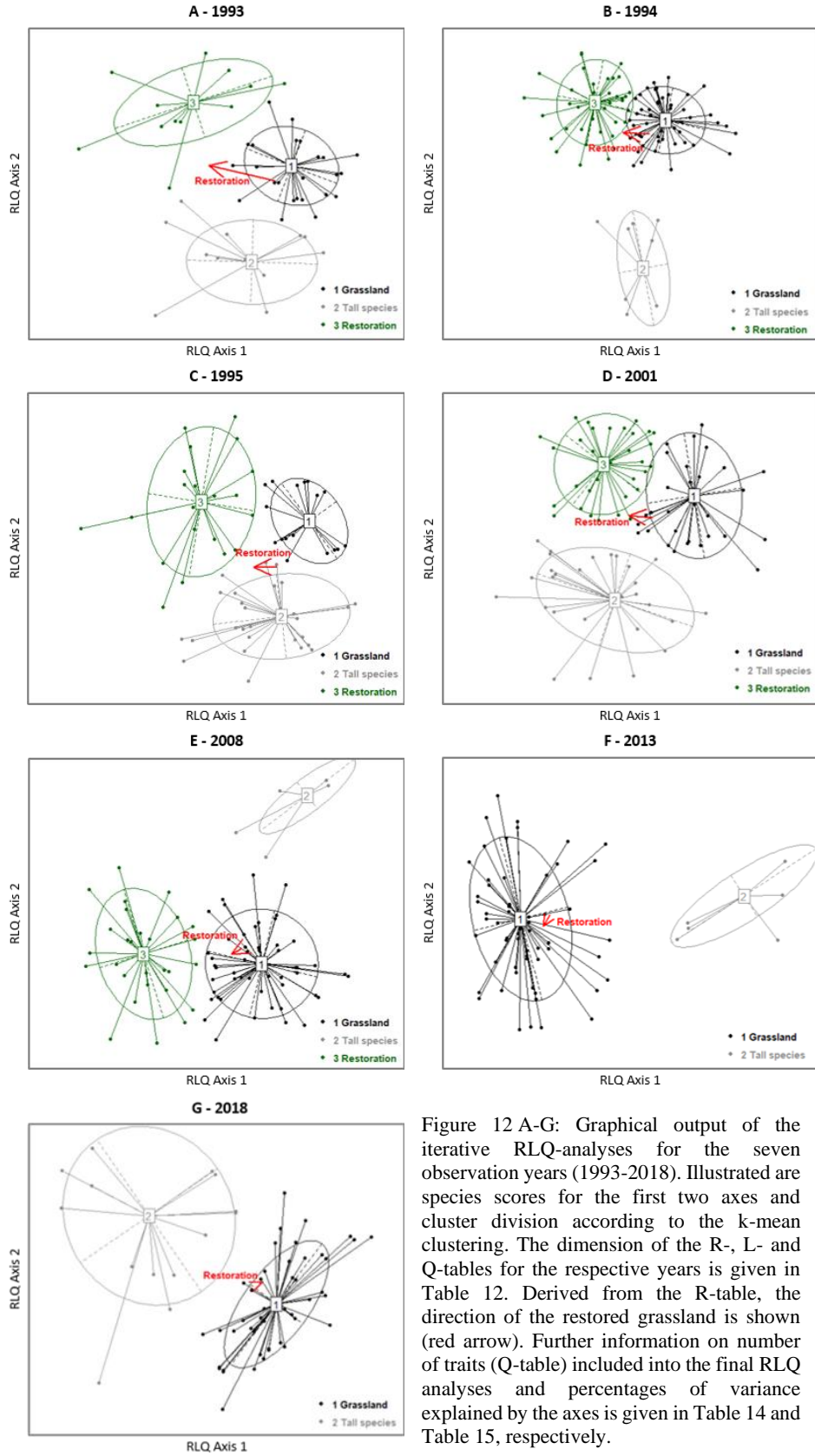


Figure 12 A-G: Graphical output of the iterative RLQ-analyses for the seven observation years (1993-2018). Illustrated are species scores for the first two axes and cluster division according to the k-mean clustering. The dimension of the R-, L- and Q-tables for the respective years is given in Table 12. Derived from the R-table, the direction of the restored grassland is shown (red arrow). Further information on number of traits (Q-table) included into the final RLQ analyses and percentages of variance explained by the axes is given in Table 14 and Table 15, respectively.

## DISCUSSION

### **DEVELOPMENT OF PLANT FUNCTIONAL TRAITS IN THE RESTORED GRASSLAND IN COMPARISON TO THE REFERENCE GRASSLAND**

To estimate restoration success based on the functional composition, traits related to plant species dispersal, establishment and persistence (Weiher et al., 1999) were compared between the restored and the reference grassland (Laughlin, 2014). To do so we estimated, based on their ecological significance, the temporal development of the individual traits on the restored grassland (Figure 10 & 2.1.3 *Functional traits (Q-table)*), which is discussed in the following.

#### *Dispersal related traits*

To test the importance of dispersal for the restoration success of a typical calcareous grassland vegetation, we compared traits that are important for dispersal in space and time. We proposed that the dispersal modes anemochory, endozoochory and epizoochory favoured the likelihood of seed dispersal in space while a persistent seed bank, the dispersal mode hemerochory and a low seed mass were attributes correlated with the dispersal of species in time.

We thereby assumed that immediately after restoration, anemochorously dispersed species would dominate due to their high spatial dispersal potential, which allows them to arrive at the restored grassland very fast (Bakker et al., 1996; Von Blanckenhagen and Poschlod, 2005). In contrast, for epi- and endozoochorously dispersed species we assumed a slower arrival rate (Pärtel et al., 1998; Poschlod et al., 1998). However, we found no significant differences between restored and reference grasslands for all three dispersal modes because there were already similar proportions of species during the first year after restoration. We speculate this happened due to the close vicinity of the donor grassland, which in combination with the small size of the restored

grassland, enabled the fast dispersal of plant species with wind and grazing animals (Helsen et al., 2013). The rapid arrival of these species (at least zoochorously dispersed ones) is nevertheless unexpected based on previous observations (Piqueray et al., 2015; Von Blanckenhagen and Poschlod, 2005), thus the dispersal in space should perhaps be examined using further characteristics. However, further traits for dispersal in space (e.g. realised dispersal distances) are very difficult to generate and often not available for many species (Cornelissen et al., 2003).

For three other trait attributes (hemerochorously dispersed species, small seed mass, persistent seeds), however, we found higher incidences in the restored grassland during the first years of observation. A correlation of low seed weight with seed persistence has been reported from previous studies (Bekker et al., 1998a; Thompson et al., 1977) while an initial high proportion of species with persistent seeds was also found for other clear-cut restoration studies (Poschlod et al., 1998). However, the proportion of typical calcareous grassland species in the long-term persistent seed bank was reported to be comparable low (Bakker et al., 1996; Von Blanckenhagen and Poschlod, 2005) and accordingly, we rather detected many arable weed species (e.g. *Capsella bursa-pastoris*, *Myosotis arvensis*). The occurrence of these species, characterised with a long-term persistent seed bank, were not unexpected when considering that in earlier times the transition from grazed calcareous grassland and arable field was more common (Poschlod and WallisDeVries, 2002). Thus, we speculate that these species were most likely relicts of former arable farming and persisted as seeds in the soil until they were disturbed by the restoration measure, leading to the reactivation of these seeds (Aksoy et al., 1998; Davies and Waite, 1998; Karlík and Poschlod, 2014; Oberdorfer, 2001). Therefore, this finding reflected the land use history of grasslands, which

can be revealed based on species that survived in the soil seed bank (Karlík and Poschlod, 2014). Summed up, we detected no differences in traits related to dispersal ‘in space’ (wind and animal-mediated dispersal, Bakker et al. 1996), but found strong evidence, that, at least during the first two years after the clearance, species composition of the restored grassland was influenced by the occurrence of mostly non-target species that were dispersed ‘in time’ (persistent, light seeds that were transported by men, Bakker et al. 1996).

#### *Establishment related traits*

We compared traits that have been shown as predictive of plant species establishment (heavy seed mass, seedling emergence season in autumn, high SLA values) for estimating the importance of establishment for the restoration success of calcareous grassland vegetation.

Thereby, high seed mass is supposed to be correlated with nutrient supply for the seedling and thus heavy seeds are assumed to establish more easily (Cornelissen et al., 2003). In our study, however, heavy seeds were underrepresented compared to the reference during the first year after restoration while in later years no differences between grassland categories were found. This is supported by the earlier finding that species with low seed mass usually predominate in grazed calcareous grasslands (Kahmen et al., 2002) and thus, in this habitat type species with heavy seeds are generally of lesser importance.

Additionally, previous studies indicated that the occurrence of grazing pressure in late summer promotes the availability of gaps in the vegetation for species establishment increasing the frequency of autumn germinators (Kahmen et al., 2002; Piqueray et al., 2015). Recurring differences in the proportion of species germinating in autumn were detected throughout the study period. Thereby, a higher proportion of species germinating in autumn were found for the restored grassland since the third year after restoration rather

indicating that germination conditions for typical calcareous grassland species were more favourable in the restored grassland than in the reference grassland.

High SLA values are assumed to be positively correlated with the growth rate of the seedling (Cornelissen et al., 2003) and accordingly we found a much higher proportion of species with high SLA values in the restored grassland for at least sixteen years after the initial restoration. However, high SLA values are generally correlated with the overall growth rate of a plant species and hence, are not only important for the seedling establishment itself but also for the overall competitiveness of a plant species (Cornelissen et al., 2003) which makes it difficult to identify the importance of this trait attribute for the establishment. As heavy seeds showed nearly no significant differences and the pattern for autumn germination was inconsistent, we propose that high SLA values in our study are rather an indicator for the general species competitiveness than establishment alone.

Summed up, no final conclusion on the changes in establishment related traits can be given. However, as seed mass recovered quickly and autumn germinators showed higher incidences in the restored grassland since the second year after restoration, species establishment was probably not a limiting factor for species recovery on the restored grassland.

#### *Persistence related traits*

Most of the traits, which were investigated for estimating the restoration success, were relevant for explaining plant species persistence in calcareous grasslands in response to moderate grazing disturbance, nutrient poverty and concurrence by more competitive species (canopy height, first flowering, lateral spread, leaf distribution, life span, plant types, seed bank, SLA, start of flowering and seedling emergence). Numerous studies have already shown that the occurrence of small plant species with basal

rosettes, the ability to spread laterally, the high proportion of species producing transient seeds and the occurrence of autumn germinators are typical adaptations to small scale disturbances caused by moderate grazing pressure (Díaz et al., 2007; Kahmen et al., 2002; Piqueray et al., 2015; Poschlod et al., 1998). Accordingly, differences within restored and reference grasslands were detected for three of the four trait attributes (small, rosette species and the ability for lateral spread) until 2001, while the proportion of autumn germinating species was still higher in the restored grassland in 2018.

Trait attributes associated with the nutrient poverty of calcareous grassland habitats were a belated first flowering age, a prolonged life span, low SLA values, a high proportion of legumes and in general, a higher proportion of forbs than of graminoids (Díaz et al., 2007; Dupré and Diekmann, 2001; Kahmen et al., 2002; Piqueray et al., 2015; Wesuls et al., 2012). In accordance, the proportion of early flowering species decreased while the proportion of perennial species and of species with low SLA values increased, both reaching reference grassland proportions within the third, ninth and sixteenth year, respectively. Non-compliant was the proportions of legumes, which was significantly higher in the restored grassland during the first observation year while the proportion of forbs and graminoids both still showed significant differences between restored and reference grasslands after 25 years. However, the first finding was probably attributed to the occurrence of persistent seeds in the seed bank of the restored grassland (e.g. *Genista sagittalis*) while for the latter finding a possible explanation was provided by Diacon-Bolli et al. (2013) who showed that graminoids were dispersed better than forbs (but see also Chapter 2). That graminoids were still overrepresented in the last year is also in line with the floristic analysis of Chapter 3, where it was shown that the species composition of restored clear-cut grasslands, including Haarberg-

Wasserberg, was not completely restored, even after a period of 25 years.

Trait attributes that were correlated with the competitiveness of plant species were lateral spread, high canopy height, early first flowering, seedling emergences throughout the year, high SLA values and early start of flowering (Cornelissen et al., 2003). In 2013, there was only one plant trait attribute, associated with competitive behaviour, that had a higher incidence in the restored grassland, namely high lateral spread ( $>0.25$  m/year), while all other attributes converged more quickly.

For persistence related traits (or contrary traits correlated with the competitiveness of plant species), we general detected longer recovery rates in the restored grassland than for traits related to dispersal while establishment and persistence related traits could not be clearly separated from each other (high SLA, autumn germinators). However, we detected no trait attributes that indicated severe functional differences between restored and reference grassland after a period of 25 years since restoration.

#### **PLANT FUNCTIONAL GROUPS**

For analysing the extracted functional groups, we re-encountered the concept of the CSR-strategy (Grime, 1974). The three identified plant functional groups ('Grassland', 'Tall species, 'Restoration') largely resembled the classification of plant species according to this strategy in which competitive (C), stress tolerant (S) and ruderal (R) species are distinguished (Grime, 1977, 1974; Hodgson et al., 1999).

Thereby, Grime (1988) stated that short-lived, fast growing and rapidly reproducing species (= ruderals) are best suited to colonise highly disturbed habitats. The description matched the characteristics of the 'Restoration cluster', as most informative trait attributes were high SLA values as proxy for a fast relative growth rate, an early onset of first flowering, the production of persistent seeds and a high proportion of short-

lived species. The high proportion of hemerochorously dispersed species in this plant functional group was linked to the frequent occurrence of arable weeds, which is also in line with the concept of ruderal strategists (Grime, 1988).

According to the CSR-classification, competitive species are characterised by tall and long-lived plant species that reproduce comparatively late in their life cycle and put considerably more effort into enlarging their surface than into reproduction, which allows species to persist in nutrient-rich and relatively undisturbed habitats (Grime, 1988). Accordingly, we found that tall species with transient seeds and a late reproduction were dominant in the plant functional group of 'Tall species'. The high occurrence of tree and shrub species is thereby also consistent with the concept of the competitive strategy (Grime 1988).

Stress-tolerators are small and slowly growing plant species characterised by attributes promoting species resilience towards environmental stress factors in moderately undisturbed habitats (Grime, 1988). Trait attributes of the 'Grassland Cluster' complied with these characteristics. The plant functional group contained small species with low SLA values as proxy for low growth rates while additionally, a high proportion of rosette species occurred as adaptation to the disturbance by grazing.

Hence, in compliance with the CSR-strategy, stress-tolerant species made up the 'Grassland cluster', while the 'Tall species cluster' consisted of competitive and the 'Restoration cluster' of ruderal species. Calcareous grasslands, which were in the focus of this study, are characterised by the occurrence of high stress due to the nutrient-poor and dry habitat conditions, while the extensive grazing management caused moderate disturbances of the aboveground biomass (Poschlod and WallisDeVries, 2002). Therefore, it can be expected that stress-tolerant species will cope best with these environmental conditions due to their functional adaptations and will therefore

be the dominant in calcareous grassland habitats (Grime, 1988; Moog et al., 2005). Indeed, the development of the plant functional groups in this study was in accordance with this assumption.

Generally, many ruderal species appeared during the first years after restoration, showing suitable adaptations to the massive disturbance caused by the implementation of the clearance measure. However, ruderal species could not establish themselves permanently due to the lack of severe disturbance events providing gaps in the vegetation for establishment and thus gradually disappeared (Hodgson et al., 1999; Moog et al., 2005). After a period of sixteen years, the functional group of ruderals could no longer be detected. Three years after the restoration, competitive species became increasingly dominant, especially in the period between 1995 and 2001. The restored grassland probably had a higher nutrient availability than the reference grassland (Chapter 3), which promoted the growth of competitive species (Pywell et al., 2003). However, due to the extensive sheep grazing, competitive species were also reduced as nutrients were constantly depleted. As of the year 2013, approximately 20 years after clearance was applied as restoration measure, the most dominant plant functional group contained stress-tolerant species while a small group of competitive, mostly woody species, additionally persisted. It can thus be concluded that the functional composition of the restored grassland was in equilibrium with the reference grassland.

However, it must be kept in mind that the characterisation of the plant functional groups was based on the dominant traits found to be significantly different between identified clusters. Although traits correspond to the CSR-strategy classifications, it may be possible that species showed intermediate strategies, e.g. species that were characterised by typical traits of ruderal and stress-tolerant species or of competitive and stress-tolerant species or even all three strategists (Hodgson et al., 1999; Moog et al., 2005). This



was for example found for *Fragaria vesca* or *Phleum pratensis*, which both have an affiliation towards all three strategies (CSR-strategists, Hodgson et al. 1999) and hence, were not consistently put into the same plant functional group in the different observation years. However, we mainly recorded species that were assigned constantly to the same group and had a high affiliation towards the respective strategy (e.g. *Briza media* or *Carex flacca* – S-strategists; *Fraxinus excelsior* – C-strategists; *Capsella bursa-pastoris* and *Stellaria media* – R-strategists, Hodgson et al. 1999).

#### **IMPLICATIONS GIVEN BY THE FUNCTIONAL TRAIT APPROACHES**

After approximately 25 years, we only found few trait attributes that still showed significant differences between reference and restored grasslands while plant functional groups showed no longer a correlation with any grassland category. Therefore, the two functional trait approaches we used in this study showed that the functional composition of a calcareous grassland, which was restored on a spruce clear-cut and managed by subsequent sheep grazing, was successfully re-established within the observation period.

Overall, the functional trait composition of the restored grassland showed a clear development towards the reference grassland, with differences in traits related to persistence and competitiveness recovering less quickly than those related to dispersal and establishment. That the functional composition of restored grasslands developed towards donor grasslands was also observed in a chronosequence study, which was conducted on grasslands restored by clearance and subsequent grazing by sheep (Piqueray et al., 2015). However, in the study of Piqueray et al. (2015), the maximum age of the restored grasslands covered a period of 15 years and the authors concluded that the restoration process was still ongoing in terms of functional trait recovery. Our results were in

line with their conclusion, however, after a period of more than 25 years, significantly differing traits could rather be attributed to floristic differences (Chapter 3, proportion of graminoids and forbs). This finding indicated that the functional composition recovered much faster than the floristic composition, which was shown to be still ongoing even after 25 years (Chapter 3). The recovery of the functional composition was moreover supported by the development of plant functional groups over the study period. Here, the importance of functional differences were shown to decrease over the course of the study as the total differences explained by the axes of the ordination plots constantly declined (Table 14). In addition, the plant functional group characterised by trait attributes that were associated with ruderality could no longer be detected after more than 20 years of observation. The two remaining groups, stress tolerators and competitors, showed no differences in trait attribute composition between restored and reference grassland indicating that the functional composition of both grassland categories was in equilibrium. Hence, the combination of both functional approaches showed a clear convergence of the two grassland categories and thus indicated a successful restoration.

#### **CONCLUSION**

Using two different functional trait approaches, we could demonstrate that in terms of functional composition, a former grassland which was restored by clearance of a spruce afforestation and subsequently managed by extensive rotational sheep grazing, could be successfully restored over a period of 25 years. Overall, the present study supports the assumption that functional traits in general, and plant functional groups in particular, can be very good predictors of recovery success. In our study, restored and reference grassland were directly adjacent to each other, explaining the comparable fast recovery of the trait

composition. It was shown that with increasing spatial isolation the restoration success of both floristic and functional composition is slowed down (Helsen et al., 2013). Therefore, dispersal limitations, as indicated by the low response of dispersal related trait attributes, were probably of minor importance for the vegetation recovery in this study, on the one hand due to the small size of the restored grassland, on the other hand also due to the close vicinity of the donor grassland (Helsen et al., 2013). Over the study period, the nutrient content of the soil might likely had an more severe influence (Bullock et al., 2001), as traits correlated with the competitive character of significantly differed for more than 15 years while during the same time competitive species, which are promoted by high nutrient availability (Grime, 1988), were more abundant. In the course of the study, differences related to competitiveness and hence, higher nutrient content, gradually decreased. The overall functional recovery was thus much faster than it was recorded for other studies that investigated restoration success on former semi-natural grasslands characterised by a higher soil nutrient content which included in particular restored ex-arable fields (>50 and >35 years, respectively; Fagan et al. 2008; Helm et al. 2019).

To the best of our knowledge, this study is an exceptional example for the investigation of the functional recovery of calcareous grassland, restored by clearance and sheep grazing, where vegetation data was collected on permanent plots continuously and therefore, is available for an extended period (more than 25 years). The results of this study thus supported once more the importance of long-term observations, preferably using permanent plots, as changes may develop over long time (Bullock et al., 2001) and restrictions of chronosequence approaches can be overcome (Helsen et al., 2013).

**CHAPTER 5**  
**GENERAL DISCUSSION,**  
**CONCLUSION AND PERSPECTIVES**



Calcareous grasslands, characterised by a high biodiversity, have declined throughout Europe over the past 150 years (Poschlod and WallisDeVries, 2002) and thus, are nowadays in the focus of conservation and restoration ecology. Important information about these habitats, however, is still missing to guide restoration and conservation measures in the best possible way (WallisDeVries et al., 2002).

The aim of the thesis at hand was to fill important knowledge gaps, especially regarding the individual dispersal capability of three typical calcareous grassland grazers, namely cattle, goat and sheep, for typical calcareous grassland species (Chapter 2) as well as the functional and floristic long-term restoration success of calcareous grassland habitats, which were restored by clearance and sheep grazing (Chapter 3 & 4).

#### **SPATIAL CONNECTIVITY**

The results of Chapter 2 clearly supported that sheep with their long fur are better epizoochorous dispersal vectors than cattle and goats with rather short hair. The finding, that sheep are the most important epizoochorous seed dispersers, is not completely new (see also Fischer et al., 1996; Mouissie et al., 2005a; Poschlod et al., 1998; Römermann et al., 2005), however, we showed it here for the first time in a direct comparison of the historically three main calcareous grassland grazers (Poschlod and Baumann, 2010; Poschlod and WallisDeVries, 2002).

Moreover, the comparison of the endozoochorous seed dispersal indicated the high potential for all three animal types. In particular, we could show that cattle transported the highest proportion of species as well as of Red List species (*Alyssum montanum*, *Dianthus carthusianorum* and *Gentiana cruciata*), which were not detected in goat or sheep dung neither in this study nor in previous studies (Kleyer et al., 2008), supporting the high potential of cattle grazing as a tool for spatial seed dispersal.

By investigating the zoochorous dispersal potential in Chapter 2, important conclusions for future restoration planning and conservation measures can be derived. These results clearly support that, besides epizoochorous dispersal by sheep (e.g. Fischer et al., 1996), rotational cattle grazing can serve as important seed dispersal vector, particularly in terms of rare and habitat-characteristic species, and should therefore be considered for future conservation and restoration strategies. Considering that the specific study region was grazed by cattle until the middle of the 20th century (Baumann et al., 2005), it is reasonable to assume that many of the locally occurring species have adapted to extensive cattle grazing. Thus, the re-introduction of cattle grazing is especially beneficial for the conservation of the high biodiversity of calcareous grasslands in regions which have former been grazed by cattle. Such regions are for example in Southern Germany, where cattle grazing is hardly practised anymore. In areas that were previously connected mainly by sheep grazing (e.g. Swabian Alb, Poschlod and WallisDeVries, 2002), it is recommended to validate the potential of cattle grazing for seed dispersal first.

Moreover, due to the high biodiversity in the dung of all three animal types, endozoochorous seed dispersal could be particularly useful for connecting strongly isolated habitats as seed dispersal in the intestinal tract of livestock depends on the timing of the release of seeds from the intestinal tract and, hence, is more reliable than the stochastic epizoochorous dispersal (Bullock et al., 2011; Nathan et al., 2008). Using genetic markers, Rico et al. (2014) could for instance show that the effectiveness of spatial connectivity in terms of gene flow between habitats is depending on the distance between species-rich habitat patches. Therefore, endozoochorous seed dispersal can be particularly beneficial in regions with severely fragmented grassland habitats where species-rich migration routes are no longer present and long distances need to be covered. Thus, the

directed endozoochorous seed dispersal of plant species between grassland fragments, with respect to the individual retention times in the intestinal tract of the animal types, in particular of cattle, has the potential to prevent the local extinction of calcareous grassland species. Further studies with more animals and longer observation periods are however needed to confirm the finding as, in contrast, evidence was found that epizoochorous dispersal by sheep and endozoochorous dispersal by cattle might be complementary, at least for some plant species.

### ***RESTORATION SUCCESS***

The results of Chapter 3 and Chapter 4 raise strong evidence that restoration of former calcareous grasslands by clearance and subsequent sheep grazing is, independent of the cleared initial afforestation or succession, a successful measure. By comparing the vegetation composition of restored grasslands with adjacent donor grasslands covering a period of 25 years, we showed that the floristic composition gradually developed towards the target vegetation. Differences in nutrient indicators, rare species and habitat-characteristic species, however, indicated that the restoration process in terms of species richness was not yet completed. Based on other studies, which analysed the vegetation recovery on former arable fields, the process of species reestablishment can last for several decades (Fagan et al., 2008; Karlík and Poschlod, 2019; Redhead et al., 2014). In these studies, the slow recovery was attributed to an increased nutrient supply due to the former intensified arable land use, which in turn can suppress the establishment of nutrient-avoiding habitat characteristic species. Accordingly, we also found such a negative influence of high nutrient content for the recovery of calcareous grasslands vegetation on former afforestations or successions even after 25 years in our floristic analysis. In order to find out, how long the higher nutrient content influences the vegetation composition on clear-cut restorations, the

continuation of the monitoring is highly recommended.

In contrast, the functional analysis of only one study location ('Haarberg-Wasserberg') showed that the plant functional group, containing competitive species which are favoured by enhanced nutrient availability, was not detected after a period of 20 years since restoration. This result is in accordance with the assumption that the functional composition reflect changes more quickly than species richness (McGill et al., 2006). However, the general trend of a rapid recovery of the calcareous grassland vegetation, which was in the focus of the study, is of clear evidence on both, floristic and functional analysis.

The comparison of the functional trait composition between restored and reference grasslands further supports that nutrient availability had the strongest influence on the vegetation recovery of the restored grasslands. Moreover, it shows that dispersal-related trait attributes and most establishment-related traits are quickly recovering within the first years after restoration while persistence-related traits differed for a period of at least 20 years. These results imply two different things: First, the focus of future restoration measures which aim to restore adjacent grasslands that are connected by sheep grazing should focus on nutrient impoverishment. Secondly, as plant species showed differences in dispersal-related traits only for a short period, zoochorous dispersal by sheep is important and sufficient for species dispersal when restoring nearby grassland habitats (Poschlod et al., 1998). In contrast, Helsen et al. (2013) showed that spatial isolation slowed the recovery of the target species richness down. Therefore, it is likely that dispersal limitations become more relevant for restoration projects that aim to restore grasslands that are further apart from each other (Piqueray et al., 2015), in particular in the context that the spatial connectivity provides by migrating livestock seems to be crucial for the conservation of the biodiversity (Kimberley et al., 2021).

### **OUTLOOK**

The spatial connectivity between calcareous grassland patches is extremely important factor for the maintenance of species-rich habitats which was strongly supported in the thesis at hand. Rotational sheep grazing proved to be important for the recovery of restored calcareous grasslands while cattle, in particular, showed great promise as dispersers for endangered plant species. Given these indications, further research into the significance of rotational cattle grazing for the dispersal of typical calcareous grassland species is recommended.

Spatial connectivity is, however, not only provided by grazing livestock but might also be realized by wild animals (e.g. Pakeman, 2001), birds or beetles. So far, little research has been done in this context, especially in recent times, however, as the connectivity of calcareous grasslands is a key issue, the potential of these mobile vectors for seed dispersal should also be taken into account.

In conclusion, further investigations on the spatial dispersal potential of livestock as well as species groups which might act as seed dispersers should be conducted in the future.

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## APPENDIX

### Chapter 3:

Table A.1 Complete Table for Indicator species analysis on restored grasslands

Table A.2 Complete Table for Indicator species analyses on reference grasslands

Table A.3 Correlation matrix for environmental parameters in NMDS

### Chapter 4:

Table A.4 Correlation matrix of the environmental parameters with the RLQ axes

Table A.5 Cluster characterisation by trait attributes

Table A.6 Species allocation to the three clusters



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Table A.1: Complete results for the restored sites obtained in the Indicator Species Analyses. Given are the total number of significant indicator species and the respective indicator value (stat) and p-value for each species.

Signif. codes: \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$

	Year 1			Year 2			Year 8			Year 15			Year 20			Year 25		
Total number of Species	26			29			28			15			12			7		
Species	stat	p		stat	p		stat	p		stat	p		stat	p		stat	p	
<i>Agrostis stolonifera</i>							0.454	0.0221	*									
<i>Angelica sylvestris</i>	0.365	0.0257	*															
<i>Arrhenatherum elatius</i>				0.39	0.0279	*												
<i>Bromus sterilis</i>							0.394	0.0128	*									
<i>Bryonia cretica</i>	0.394	0.0131	*															
<i>Carduus nutans</i>				0.444	0.0031	**												
<i>Cerastium holosteoides</i>										0.5	0.003	**						
<i>Cirsium arvense</i>	0.665	0.0001	***	0.816	0.0001	***	0.497	0.0092	**				0.494	0.0006	***	0.394	0.0225	*
<i>Cirsium palustre</i>	0.447	0.003	**							0.412	0.0226	*						
<i>Cirsium vulgare</i>	0.65	0.0001	***	0.745	0.0001	***	0.422	0.0062	**									
<i>Clematis vitalba</i>	0.825	0.0001	***	0.855	0.0001	***	0.789	0.0001	***	0.72	0.0001	***	0.68	0.0001	***	0.619	0.0002	***
<i>Clinopodium vulgare</i>										0.436	0.0449	*				0.368	0.037	*
<i>Cornus sanguinea</i>	0.698	0.0001	***	0.844	0.0001	***	0.748	0.0001	***	0.715	0.0001	***	0.719	0.0001	***			
<i>Crataegus monogyna</i>				0.444	0.019	*	0.516	0.0003	***	0.504	0.0105	*	0.518	0.0101	*			
<i>Deschampsia cespitosa</i>							0.471	0.0011	**	0.387	0.0198	*				0.4	0.0055	**
<i>Dipsacus fullonum</i>	0.471	0.0017	**															
<i>Epilobium tetragonum</i>				0.394	0.0129	*												
<i>Erigeron canadensis</i>	0.447	0.0026	**	0.537	0.0003	***	0.493	0.002	**	0.467	0.0078	**	0.468	0.0049	**			
<i>Euphorbia verrucosa</i>	0.468	0.0239	*															
<i>Fragaria spp.</i>	0.447	0.0024	**	0.516	0.0001	***	0.594	0.0001	***	0.546	0.0006	***	0.529	0.0004	***	0.447	0.0027	**
<i>Galium aparine</i>				0.494	0.0004	***	0.471	0.0015	**									
<i>Galium mollugo</i>	0.455	0.0072	**	0.564	0.0001	***							0.565	0.0045	**	0.607	0.0006	***
<i>Galeopsis tetrahit</i>	0.494	0.0005	***	0.558	0.0001	***												
<i>Geranium sanguineum</i>							0.394	0.011	*									
<i>Geum urbanum</i>	0.632	0.0001	***	0.632	0.0001	***	0.516	0.0004	***	0.483	0.0247	*	0.417	0.0156	*			
<i>Helleborus foetidus</i>				0.38	0.0374	*												
<i>Hypericum hirsutum</i>	0.494	0.0004	***	0.486	0.0026	**												
<i>Hypericum perforatum</i>				0.554	0.0098	**	0.596	0.0156	*	0.621	0.0039	**						
<i>Inula britannica</i>							0.394	0.0136	*									
<i>Lactuca serriola</i>	0.394	0.0114	*	0.577	0.0001	***												
<i>Linaria vulgaris</i>				0.365	0.0233	*												
<i>Mentha longifolia</i>	0.391	0.0372	*				0.394	0.0119	*	0.422	0.0047	**	0.394	0.0125	*	0.447	0.0011	**

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<i>Myosotis arvensis</i>						0.447	0.0028	**							
<i>Phleum pratense</i>			0.365	0.0249	*	0.441	0.01	**	0.383	0.033	*				
<i>Picris hieracioides</i>						0.499	0.0309	*							
<i>Plantago major</i>	0.394	0.0137	*	0.422	0.0061	**									
<i>Poa trivialis</i>						0.464	0.0053	**							
<i>Prunella vulgaris</i>						0.386	0.0316	*							
<i>Pulicaria dysenterica</i>	0.422	0.0063	**												
<i>Ranunculus repens</i>						0.365	0.0261	*							
<i>Ribes uva-crispa</i>			0.365	0.0268	*										
<i>Rubus idaeus</i>	0.394	0.0103	*	0.471	0.0014	**	0.365	0.0284	*						
<i>Rubus ssp.</i>	0.683	0.0001	***	0.712	0.0001	***	0.632	0.0001	***	0.556	0.0002	***	0.515	0.0012	**
<i>Sambucus nigra</i>	0.65	0.0001	***	0.615	0.0001	***									
<i>Senecio jacobaeae</i>						0.394	0.0126	*				0.365	0.0301	*	
<i>Sonchus asper</i>	0.758	0.0001	***	0.816	0.0001	***									
<i>Sonchus oleraceum</i>	0.447	0.0028	**	0.471	0.0012	**									
<i>Sorbus aria</i>									0.419	0.0175	*				
<i>Taraxacum officinale agg.</i>	0.764	0.0001	***	0.832	0.0001	***	0.738	0.0001	***			0.549	0.0099	**	
<i>Torilis japonica</i>	0.422	0.0065	**	0.516	0.0008	***									
<i>Veronica arvensis</i>						0.365	0.0272	*							
<i>Viola hirta</i>						0.794	0.0003	***							

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Table A.2: Complete results for the reference sites obtained in the Indicator Species Analyses. Given are the total number of significant indicator species and the respective indicator value (stat) and p-value for each species.

Signif. codes: \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$

Year	1			2			8			15			20			25		
Total number of Ind. Species	43			36			21			12			14			9		
Species	stat	p		stat	p		stat	p		stat	p		stat	p		stat	p	
<i>Achillea millefolium</i>	0.868	0.0001	***	0.88	0.0001	***	0.831	0.0001	***	0.821	0.0002	***				0.8	0.0001	***
<i>Acinos arvensis</i>							0.331	0.0363	*									
<i>Anthoxanthum odoratum</i>	0.337	0.0246	*	0.472	0.0021	**	0.337	0.0265	*									
<i>Anthyllis vulneraria</i>	0.475	0.0025	**	0.495	0.0072	**	0.447	0.0033	**									
<i>Arrhenatherum elatius</i>													0.426	0.002	**			
<i>Asperula cynanchica</i>	0.678	0.0002	***	0.57	0.0054	**	0.552	0.0018	**	0.513	0.0035	**						
<i>Briza media</i>	0.905	0.0001	***	0.945	0.0001	***	0.872	0.0001	***	0.769	0.0001	***	0.746	0.0001	***			
<i>Bromus erectus</i>	0.957	0.0001	***	0.979	0.0001	***												
<i>Carlina acaulis</i>	0.337	0.0261	*	0.369	0.0115	*	0.369	0.0118	*									
<i>Carex caryophylla</i>	0.708	0.0001	***	0.763	0.0001	***	0.697	0.001	***				0.666	0.0002	***			
<i>Carex flacca</i>										0.694	0.0005	***						
<i>Carex ornithopoda</i>	0.369	0.0131	*															
<i>Carlina vulgaris</i>	0.337	0.0246	*															
<i>Centaurea jacea</i>	0.797	0.0001	***	0.741	0.0002	***												
<i>Centaurea scabiosa</i>							0.369	0.0134	*				0.456	0.0297	*			
<i>Cirsium acaule</i>	0.719	0.0001	***	0.636	0.0011	**	0.652	0.0001	***	0.578	0.0044	**	0.59	0.0002	***	0.528	0.0134	*
<i>Dactylis glomerata</i>	0.585	0.0003	***															
<i>Dianthus carthusianorum</i>	0.5	0.0005	***	0.518	0.0004	***	0.426	0.002	**				0.382	0.0495	*			
<i>Euphrasia officinalis</i>	0.5	0.0002	***	0.522	0.0001	***												
<i>Fagus sylvatica</i>	0.337	0.0294	*															
<i>Festuca ovina</i>	0.97	0.0001	***	0.985	0.0001	***	0.961	0.0001	***	0.812	0.0001	***	0.659	0.003	**			
<i>Festuca pratensis</i>	0.423	0.0216	*	0.45	0.016	*												
<i>Fraxinus excelsior</i>																0.436	0.0486	*
<i>Galium verum</i>	0.886	0.0001	***	0.853	0.0001	***												
<i>Helianthemum nummularium</i>	0.64	0.0001	***	0.631	0.0001	***	0.646	0.0001	***	0.586	0.0008	***	0.586	0.0031	**			
<i>Helictotrichon pratense</i>				0.424	0.0398	*	0.369	0.0126	*									
<i>Hieracium pilosella</i>	0.652	0.0001	***	0.572	0.0016	**												
<i>Hippocrepis comosa</i>	0.417	0.0123	*	0.446	0.005	**												
<i>Hypericum maculatum</i>																0.319	0.0258	*
<i>Knautia arvensis</i>	0.513	0.007	**															
<i>Koeleria pyramidata</i>	0.887	0.0001	***	0.954	0.0001	***	0.814	0.0001	***									
<i>Listera ovata</i>	0.337	0.0277	*	0.369	0.0125	*												

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<i>Lotus corniculatus</i>	0.863	0.0001	***	0.91	0.0001	***													
<i>Luzula campestris</i>	0.369	0.0117	*	0.399	0.0052	**													
<i>Ononis repens</i>	0.399	0.0052	**	0.385	0.0303	*													
<i>Quercus robur</i>																0.533	0.0045	**	
<i>Phleum phleoides</i>	0.452	0.0017	**																
<i>Pimpinella saxifraga</i>	0.874	0.0001	***																
<i>Plantago media</i>	0.855	0.0001	***	0.832	0.0001	***	0.766	0.0001	***										
<i>Poa pratensis</i>																			
<i>Potentilla erecta</i>				0.337	0.0252	*	0.426	0.0026	**	0.44	0.0349	*	0.469	0.013	*	0.493	0.0044	**	
<i>Potentilla tabernaemontani</i>	0.788	0.0001	***	0.7	0.0001	***							0.65	0.0025	**	0.317	0.0404	*	
<i>Prunella grandiflora</i>	0.739	0.0001	***	0.758	0.0001	***	0.729	0.0001	***	0.545	0.0173	*	0.644	0.0004	***	0.65	0.0056	**	
<i>Prunella vulgaris</i>										0.382	0.0452	*	0.386	0.0231	*				
<i>Ranunculus bulbosus</i>	0.802	0.0001	***	0.808	0.0001	***													
<i>Rhinanthus glacialis</i>	0.496	0.0013	**	0.477	0.0208	*													
<i>Sanguisorba minor</i>	0.825	0.0001	***																
<i>Scabiosa columbaria</i>	0.723	0.0002	***	0.723	0.0003	***													
<i>Taraxacum laevigatum</i>				0.337	0.0269	*													
<i>Teucrium chamaedrys</i>	0.588	0.003	**	0.577	0.0078	**	0.608	0.0052	**										
<i>Thymus pulegioides</i>	0.92	0.0001	***	0.895	0.0001	***	0.854	0.0001	***	0.794	0.0001	***	0.695	0.0001	***				
<i>Trifolium campestre</i>	0.369	0.0115	*	0.392	0.0285	*										0.464	0.0139	*	
<i>Trisetum flavescens</i>	0.337	0.0254	*																
<i>Trifolium pratense</i>							0.545	0.0065	**										
<i>Trifolium repens</i>	0.485	0.03	*																
<i>Vicia angustifolia</i>	0.463	0.0067	**	0.536	0.0005	***													
<i>Vincetoxicum hirsutifolium</i>										0.399	0.0057	**	0.424	0.0085	**				

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Table A.3: Correlation matrix obtained for parameter fitting for the NMDS. Given are the correlations with the first axis (NMDS1) and the second axis (NMDS2), the squared correlation coefficient ( $r^2$ ) and respective p-value. Signif. code: \*\*\*  $p \leq 0.001$ . Fes.-Bro.: typical species for the *Festuca-Brometea* class

	NMDS-Axis 1	NMDS-Axis 2	$r^2$	p	
Fes.-Bro.	0.98	0.19	0.61	0.001	***
Red List	0.77	-0.63	0.34	0.001	***
EIV Moisture	-0.89	-0.44	0.76	0.001	***
EIV Light	0.95	-0.31	0.48	0.001	***
EIV Soil Reaction	0.48	0.88	0.44	0.001	***
EIV Nutrients	-1.00	-0.02	0.73	0.001	***
Reference	0.31	-0.02			
BeforeBlack locust	-0.95	0.63			
BeforeBlackthorn	-0.37	0.13	0.45	0.001	***
BeforePine	-0.11	0.18			
BeforeSpruce	-0.20	-0.68			

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Table A.4: Correlation of environmental parameters with the first two axes of the final RLQ analysis for the respective years of observation.

Abbr.: L = Ellenberg indicator value for light; F = Ellenberg indicator value for moisture, N = Ellenberg indicator value for nutrients, R = Ellenberg indicator value for soil reaction, Manag.M = reference grasslands, Manag.R = restored grasslands.

<b>Year</b>	<b>Environmental variable</b>	<b>RLQ-Axis 1</b>	<b>RLQ-Axis 2</b>
<b>1993</b>	L	-0.674	0.162
	F	-0.478	-0.227
	N	-0.544	-0.214
	R	-0.168	-0.005
	Manag.M	0.372	-0.085
	Manag.R	-1.377	0.314
<b>1994</b>	L	-0.314	0.209
	F	-0.347	0.058
	N	-0.692	-0.054
	R	-0.457	-0.163
	Manag.M	0.748	-0.035
	Manag.R	-0.740	0.035
<b>1995</b>	L	-0.209	0.134
	F	-0.398	0.069
	N	-0.480	-0.065
	R	-0.222	-0.105
	Manag.M	0.528	0.003
	Manag.R	-0.461	-0.002
<b>2001</b>	L	-0.109	0.173
	F	-0.248	-0.057
	N	-0.352	-0.153
	R	-0.357	0.075
	Manag.M	0.523	-0.049
	Manag.R	-0.448	0.042
<b>2008</b>	L	0.220	-0.039
	F	-0.199	0.104
	N	-0.258	0.063
	R	-0.197	-0.095
	Manag.M	0.234	0.130
	Manag.R	-0.207	-0.115
<b>2013</b>	L	0.000	0.008
	F	0.100	-0.142
	N	0.277	-0.048
	R	-0.034	-0.032
	Manag.M	0.122	0.217
	Manag.R	-0.123	-0.217
<b>2018</b>	L	0.186	0.029
	F	-0.078	0.112
	N	-0.153	-0.089
	R	0.133	-0.084
	Manag.M	-0.046	-0.018
	Manag.R	0.047	0.019

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Table A.5 Characterisation of the differentiated clusters for each year. Given are attributes for significantly differing functional traits between clusters as identified by Chi<sup>2</sup>-tests (\* p< 0.05, \*\* p<0.01 and \*\*\* p<0.001).

Year	Functional trait	attribute	Cluster 1 Grassland	Cluster 2 Tall Species	Cluster 3 Restoration	Chi-test
1993	Canopy Height	<0.3 m	23	3	15	38.0 ***
		0.3-0.6 m	16	6	3	
		>0.6 m	1	14	2	
	Dispersal mode	anemochory - no	33	11	19	14.8 **
		anemochory - yes	7	12	1	
		epizoochory - no	20	23	1	
		epizoochory - yes	20	0	19	
	Life span	short	0	5	10	22.8 ***
		long	40	18	10	
	Seed bank	transient	32	21	1	42.63 ***
persistent		8	2	19		
Seedling Emergence	spring	15	18	2	24.0 ***	
	autumn	5	0	1		
	all year	20	5	17		
1994	Canopy Height	<0.3 m	27	0	19	58.9 ***
		0.3-0.6 m	30	0	16	
		>0.6 m	3	14	10	
	Dispersal mode	hemerochory – no	49	12	24	11.7 *
		hemerochory - yes	11	2	21	
	First flower	<1 year	23	1	36	68.7 ***
		1-5 years	36	6	9	
		>5 years	1	7	0	
	Life span	short	3	0	25	41.4 ***
		long	57	14	20	
	Plant type	forb	41	0	36	121.9 ***
		graminoid	12	0	4	
		legume	7	0	5	
		woody	0	14	0	
	Seed bank	transient	49	12	9	45.1 ***
		persistent	11	2	36	
	Seed mass	<0.5 mg	22	0	17	28.4 ***
		0.5-2 mg	17	0	18	
		>2 mg	21	14	10	
	SLA	<20 mm <sup>2</sup> /mg	28	11	8	29.3 ***
20-25 mm <sup>2</sup> /mg		18	2	8		
>25 mm <sup>2</sup> /mg		14	1	29		
1995	Canopy Height	<0.3 m	30	1	14	57.9 ***
		0.3-0.6 m	8	29	11	
		>0.6 m	0	20	6	
	Dispersal mode	hemerochory – no	38	32	19	18.9 ***
		hemerochory - yes	0	18	12	
	First Flower	<1 year	21	11	27	39.9 ***
		1-5 years	17	29	4	
		>5 years	0	10	0	
	Life span	short	4	0	21	56.6 ***
		long	34	50	10	
Seed bank	transient	27	41	8	27.4 ***	
	persistent	11	9	23		
SLA	<20 mm <sup>2</sup> /mg	19	26	5	32.1 ***	
	20-25 mm <sup>2</sup> /mg	16	11	5		
	>25 mm <sup>2</sup> /mg	3	13	21		
2001	Canopy Height	<0.3 m	21	0	25	63.9 ***
		0.3-0.6 m	18	15	14	
		>0.6 m	2	25	1	
	Dispersal mode	hemerochory – no	40	15	39	55.7 ***
		hemerochory - yes	1	25	1	
	First Flower	<1 year	16	12	26	25.1 ***
		1-5 years	24	18	14	
		>5 years	1	10	0	
	Plant type	forb	24	16	33	62.6 ***
		graminoid	15	4	0	
legume		2	3	7		
woody		0	17	0		

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Year	Functional trait	attribute	Cluster 1 Grassland	Cluster 2 Tall Species	Cluster 3 Restoration	Chi-test
<b>2001</b>	Seed bank	transient	38	27	11	37.4 ***
		persistent	3	13	29	
	Seedling Emergence	spring	9	27	11	24.0 ***
		autumn	3	2	7	
		all year	29	11	22	
	SLA	<20 mm <sup>2</sup> /mg	21	19	8	16.4 **
20-25 mm <sup>2</sup> /mg		14	11	11		
>25 mm <sup>2</sup> /mg		6	10	21		
<b>2008</b>	Dispersal mode	hemerochory – no	54	9	17	9.5 *
		hemerochory - yes	9	1	12	
	First Flower	<1 year	26	1	16	82.4 ***
		1-5 years	36	0	13	
		>5 years	1	9	0	
	Lateral spread	<0.01 m/year	37	9	6	22.4 ***
		0.01-0.25 m/year	24	0	17	
		>0.25 m/year	2	1	6	
	Life span	short	5	0	8	8.5 *
		long	58	10	21	
	Leaf distribution	basal rosette	41	0	4	29.9 ***
		no rosette	22	10	25	
	Plant type	graminoid	17	0	2	74.6 ***
		herb	38	0	19	
		legume	4	0	7	
		woody	4	10	1	
	Seed bank	transient	53	9	6	38.7 ***
		persistent	10	1	23	
	Seedling Emergence	spring	23	10	8	17.5 **
		autumn	8	0	3	
all year		32	0	18		
SLA	<20 mm <sup>2</sup> /mg	31	8	4	37.8 ***	
	20-25 mm <sup>2</sup> /mg	19	2	2		
	>25 mm <sup>2</sup> /mg	13	0	23		
<b>2013</b>	Canopy Height	<0.3 m	41	0		61.1 ***
		0.3-0.6 m	39	0		
		>0.6 m	8	15		
	First Flower	<1 year	42	1		45.9 ***
		1-5 years	44	5		
		>5 years	2	9		
	Leaf distribution	rosette	46	0		14.2 ***
		no rosette	42	15		
	Plant type	forb	61	0		103.0 ***
		graminoid	16	0		
		legume	11	0		
		woody	0	15		
	<b>2018</b>	Canopy Height	<0.3 m	42	0	
0.3-0.6 m			34	2		
>0.6 m			2	23		
First Flower		<1 year	37	2		42.2 ***
		1-5 years	41	12		
		>5 years	0	11		
Life span		short	14	0		5.2 *
		long	64	25		
Seedling Emergence		spring	22	17		12.8 **
		autumn	11	2		
	all year	45	6			



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Table A.6: Species allocations to the three clusters over the course of the study (Explanation for species abbreviations can be found in the electric appendix)

Year	'Grassland cluster'							'Tall species cluster'							'Restoration cluster'				
	93	94	95	01	08	13	18	93	94	95	01	08	13	18	93	94	95	01	08
acecam								2	2	2	2	2	2	2					
achmil	1	1		1	1	1	1			2	2				3				
agrcap																			
agreup	1	1			1	1	1			2									
agrgig														2					
agrpro														2					
agrsto					1									2					
alomyo															3	3			
anapyr		1		1		1				2				2					
antodo		1		1	1	1	1			2									
antvul		1	1																
areser															3				
arrela					1	1					2			2			3		
aspcyn						1	1												
brapin	1	1		1	1	1	1			2									
brimed	1	1	1	1	1	1	1												
broere	1	1		1	1	1	1			2									
bupsal							1	1											
camrot	1	1	1	1	1	1	1												
capbur															3	3	3		
caraca		1	1	1	1	1	1		2										
carbet											2	2	2	2					
carcar		1	1																
carfla	1	1	1	1	1	1	1												
carmon			1	1	1	1	1												
carorn	1	1	1	1	1	1	1												
carvul			1																3
cenjac		1		1	1	1			2		2			2					
cerhol							1	1								3		3	3
chamin																			
chasag							1	1							3	3	3	3	3
cicint																3			
ciraca	1	1	1	1	1	1	1												
cirarv										2	2				3	3			
cireri								1											
cirole																			
cirvul				1	1	1			2							3	3		
clevit	1									2	2			2	2				
clivul				1			1				2								
corave											2	2							
corsan					1				2	2	2	2		2	2				
cramon									2	2	2	2	2	2					
crecap																3	3		
dacglo	1	1			1	1	1				2	2							
daucar							1	1			2	2			3	3			3
epiang			1															3	
epihel	1	1								2									
epimon																			
epipar		1									2								
epitet											2						3		
eupcan									2							3	3		
eupcyp	1						1	1								3	3	3	3
eupoff					1	1	1											3	
fagsyl																			
fesovi	1	1	1	1	1	1	1			2	2	2	2	2					
fesrub					1														
fraexc																			
fraves	1	1	1		1	1	1			2	2	2	2					3	
gallad																3	3		
galmol							1	1								3	3	3	3
galtet																3	3		
galver	1	1		1	1	1	1			2									
gencil	1	1	1		1	1	1											3	
genger		1	1	1															
genver	1	1	1	1	1	1	1			2									



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roscan							2												
rubida								2	2	2									
rumobt																			
salpra		1				1	1	1									3	3	
samnig									2	2								3	
sanmin	1	1	1	1	1	1	1	1											
scacol	1	1	1	1	1	1	1	1											
secvar	1									2								3	3
senjac																		3	3
shearv																			
solcan		1																3	
sonasp																			
sonole																		3	3
sorari									2	2	2	2	2	2					
stager											2								
stemed																		3	3
taroff	1					1	1	1										3	3
thypul		1	1															3	3
torjap																			1
trapra									2										1
trifla																			1
trimed		1	1			1	1	1											2
tripra																			1
trirep																			1
tusfar			1						2										3
verarv																			3
vercha																			3
verper																			3
verteu	1					1	1	1											3
vibopu						1			2	2	2	2							2
vicang									2										2
vicra																			2
vicrat																			2
vicsep		1																	2
vinhir		1				1	1		2	2	2								2
viohir	1	1	1			1	1	1											3

## **ELECTRONIC APPENDIX**

Attached as CD.

Chapter 2:

S1.1 Basic Data (Excel-tables with vegetation data, epizoochorous data, endozoochorous data, trait data)

S1.2 Analysis (in R (with Scripts) and Excel)

Chapter 3:

S2.1 kmz-file - locations

S2.2 Basic data (Vegetation relevées for 1993-2018)

S2.3 Analysis (with R-Scripts)

S2.4 Table with species abbreviations

Chapter 4:

S3.1 Basic data and analysis