Grassland Restoration: Relative effectiveness of different restoration methods on plant and invertebrate diversity

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

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von Augsburg (Deutschland)

Leiterin / Leiter / LeiterInnen der Arbeit:
PD Dr. J.-Y. Humbert
Prof. R. Arlettaz
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Prof. Dr. Z. Balogh

Contents

Summary

Semi-natural grasslands in Central Europe can harbor a big variety of plant and invertebrate species. These grasslands have suffered from a strong decline mainly due to agricultural intensification. Agri-environment schemes have been introduced in Europe to promote farmland biodiversity, but they were only little effective, especially so in grasslands. To mitigate this dramatic decline of farmland biodiversity, active grassland restoration is nowadays widely applied and has gained in importance in research. While there is evidence that active grassland restoration is generally effective in promoting plant diversity, little is known about the effectiveness of the factors involved in such restoration operations, such as soil disturbance intensity, seeding methods and seed source. Furthermore, there are no studies showing whether the soil disturbances that are linked to grassland restoration could be harmful to the resident ground-dwelling invertebrates of a grassland that is going to be restored.

In this PhD thesis I investigated the effects of different factors involved in active grassland restoration on the restoration outcome. I first conducted a systematic review and meta-analysis to evaluate the relative influence of these factors on restoration efficacy, focusing on plant species richness, and to identify research gaps. In parallel, a field-scale restoration experiment replicated across the Swiss Plateau was launched, with this PhD being part of the restoration experiment. This experiment served to study whether hay transfer can be used to effectively transfer invertebrates from one meadow to another and to which degree soil disturbances linked to grassland restoration are harmful to ground-dwelling invertebrates. Furthermore, I studied the short-term outcome of different restoration methods on the plant community. The restoration methods were: (i) control with no seed addition and no soil disturbance, (ii) hay transfer from a species-rich donor meadow on a harrowed receiver meadow, (iii) hay transfer from a species-rich donor meadow on a ploughed receiver meadow, (iv) sowing of a commercial seed mixture on a ploughed receiver meadow and, (v) sowing of a brush- or vacuum harvested seed mixture on a ploughed receiver meadow. Baseline data of plants and ground-dwelling invertebrates (represented by ground beetles and spiders) was collected in 2018, i.e., one year before restoration took place. In early summer 2019 we restored 48 meadows and additionally sampled invertebrates from the hay transfer treatments. One year after restoration (in 2020) we resampled invertebrates and two years after restoration (in 2021) we reconducted vegetation surveys on the restored and control meadows.

The systematic review and meta-analysis revealed the importance of the seed source in grassland restoration, while different soil disturbance intensities or seeding methods (green hay or harvested seeds) do not show differences in the restoration performance on plant species richness. We also identified research gaps, such as little focus on invertebrates in grassland restoration studies and few field scale experiments. With the restoration experiments we could show that invertebrates can be transferred successfully with the hay and that there was no mid-term negative impact on the ground-dwelling invertebrate community due to soil disturbances linked to restoration, no matter of the disturbance intensity. Finally, all four restoration methods that we tested in our experiment significantly increased the plant species richness after two years. All together the present thesis is a contribution to the relatively young research field of restoration ecology with evidence-based recommendations and it comes in a timely moment within the UN decade on restoration.

General introduction

We have entered the Anthropocene (Lewis & Maslin, 2015) and are experiencing already the 6th mass extinction (Ceballos et al., 2015; Pimm et al., 2014). Agriculture was identified among the main drivers for this biodiversity loss. The negative impact of agriculture on biodiversity has different drivers, such as habitat loss through crop farming or pollution through excessive usage of fertilizers and pesticides (Maxwell, Fuller, Brooks, & Watson, 2016). With a growing human population it will therefore remain a huge challenge to maintain food supply while reducing the negative impacts from food production on our environment, plants and animals (Tilman et al., 2011).

In Europe, over 40% of its terrestrial surface is directly under agricultural management (Eurostat, 2018). The European agricultural policy plays thus a decisive role in the protection and maintenance of habitats which are directly or indirectly affected by agricultural practices. Indeed, the important role of agriculture in biodiversity conservation was recognized, which explains why the highest conservation expenditures of the European Union are going into agri-environment schemes (AES, Batáry, Dicks, Kleijn, & Sutherland, 2015). The aim of these AES is to give incentives to farmers to adapt their faming practices in a more ecologically sound way. Most of these AES are under a management-based payment scheme. For example, farmers can reduce the fertilizer input and receive monetary compensation due to lower production of agricultural goods (Herzon et al., 2018). While these schemes showed some improvements on a local scale (such as a decrease in nitrogen leaching), the overall positive effects on biodiversity in Europe was limited (Kleijn et al., 2006). On the contrary, result-based schemes have shown to be more efficient in promoting biodiversity. One example of a result-based payment scheme is the increase in plant species richness on a given surfaces. However, such result-based schemes are more difficult to implement since they require higher control efforts. Thus, they can be found only punctually in Europe (Herzon et al., 2018).

Species-rich, semi-natural grasslands under threat

Grasslands are an important component of the European cultural heritage and are with 17% landcover an essential element of Europe's landscape (Eurostat, 2018; Hejcman et al., 2013). A major part of these grasslands are semi-natural, which implies regular interventions such as mowing or grazing to maintain habitat openness and prevent encroachment (Kuneš et al., 2015). European grasslands have expanded across the continent since the Neolithic agricultural revolution as they were key to the development of livestock farming (Gibson, 2009). Throughout the millennia, plant species which were limited to naturally open grasslands (such as steppes or dry grasslands), have found in these permanently open, semi-natural grasslands ideal conditions through low competition from other plant species. That is why semi-natural grasslands are among the most species rich habitats in Europe (Dengler et al., 2014). This high plant species richness and unique habitat structure of grasslands offer also shelter to a variety of invertebrate species (Batáry et al., 2007; Woodcock et al., 2012). Besides their intrinsic value, semi-natural grasslands have also a high agronomic value in terms of fodder production in form of hay or as pastures (Squires, Dengler, Hua, & Feng, 2018) and provide important ecosystem services, such as water flow regulation, erosion control, nitrogen retention and carbon sequestration (Byrne & delBarco-Trillo, 2019; Yan et al., 2019).

However, these valuable, species-rich grasslands in Europe are exposed to several types of threat since the beginning of agricultural intensification. They can be abandoned because their management is no longer profitable. After abandonment, grasslands get encroached by woody plant species. Or they can be turned into crops. Or finally, grasslands can be intensified through higher fertilizer input and more frequent mowing to achieve higher hay yield. This intensification has led to dwindling populations of grassland habitat specialists which were outcompeted by nitrophilous generalists (Poschlod & WallisDeVries, 2002) and to homogenization across many plant and animal taxa (Gossner et al., 2016). Indeed, the impoverished plant communities have a cascading effect on other taxa. The grassland invertebrates suffer through fewer host and flowering plants (Börschig et al., 2013; Marini et al., 2009). Furthermore, higher mowing frequency and usage of heavy machinery cause high mortality of these invertebrates (Humbert et al., 2010). Consequently, the once widespread mesic hay meadows from low and medium altitudes (*Arrhenatheretalia elatioris*) have become vulnerable (VU) according to the European Red List (Boch et al., 2019). With the UN Decade on Ecosystem Restoration 2021–2030 (UNEP, 2022), policy makers are getting aware that a proactive approach is necessary to tackle the biodiversity crisis. However, grassland restoration has generally received less attention in compared to other habitats such as forest or freshwater (Török et al., 2021).

Need for active restoration

Ecological restoration is the 'process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed' (Gann et al., 2019) and can be classified into three different restoration types (Atkinson & Bonser, 2020). 'Passive' or 'natural' restoration implies the ending of degradation. For grasslands, natural restoration could be the cessation of fertilizer application, which is easy to implement and does not require high financial resources. However, this restoration type is not efficient on an established, species-poor grass sward (van Klink et al., 2017). Another restoration type is 'assisted' restoration, which enters the category of 'active' restoration and consists of two types, either biotic or abiotic. In other words, this implies reintroduction of typical grassland plant species by seeding (biotic) or the application of artificial soil disturbance to promote seed germination (abiotic). It has been shown that seed addition only with no prior soil disturbance is not successful, since the existing grass sward inhibits the new plant seeds from germination (Freitag et al., 2021; Schmiede et al, 2012). On the contrary, soil disturbance without seed addition, or alternatively, the cessation of crop farming, may be a viable and low-cost restoration method if several conditions are met. When the seed bank contains a viable amount of grassland species or ancient, species-rich grasslands are common in the landscape, this abiotic restoration type, i.e., soil disturbance only, may be successful (Prach et al., 2014). Still, it may take several centuries for recovery (Isbell et al., 2019; Nerlekar & Veldman, 2020). In addition, most agricultural areas in Central Europe are dominated by intensive agriculture since a couple of decades. Consequently, the seed bank is depleted and seed sources in surrounding landscape are limited (Münzbergová & Herben, 2005; Turnbull et al., 2000). In such circumstances, abiotic grassland restoration has little chances of success (Freitag et al., 2021; Öster et al., 2009). Finally, 'reconstructive' restoration, the second active restoration type, consists of combining the biotic and abiotic components of restoration. Given that the term 'active' restoration is more widely adopted in the literature, I shall use 'active' restoration throughout this manuscript, keeping in mind that more specifically I refer to 'reconstructive' restoration.

Active grassland restoration overcomes dispersion limitation and is often a success to restore grasslands (Kiehl et al., 2010) or, at least, to accelerate restoration (Kövendi-Jakó et al., 2019). Different factors in active grassland restoration play an important role and have been studied. There is the abiotic component of grassland restoration, i.e., soil disturbance to promote seed germination. Soil disturbance can by achieved superficially, e.g., with a harrow (Durbecq et al., 2021), or more profoundly by tilling, e.g., with a plough

(Freitag et al., 2021). Then there is the biotic component, i.e., seed addition. Seeds for grassland restoration can either be purchased from a commercial seed producer (Freitag et al., 2021) or harvested from a local species-rich donor meadow. If seeds are harvested from a donor meadow, they can be applied on a receiver meadow in form of green hay (hay transfer, Fig. 1) or directly as seeds (Albert et al., 2019). The seeding of local and native seeds is recommended to assure adaptation to local environmental conditions and to maintain genetic structure (Durka et al., 2017). However, native species of local origin are not always widely available (Ladouceur et al., 2018), and the seed mixtures used for revegetation in Europe may be dominated by species of non-local origin. Consequently, transferring local seeds from nearby non-degraded communities is recommended in grassland restoration (Kiehl et al., 2010; Scotton et al., 2012).

Fig. 1 Hay transfer from a species-rich donor meadow on a ploughed receiver meadow.

The Swiss context

Switzerland is situated in Central Europe and is characterized by a heterogenous topography, ranging from lowland flat areas in the Swiss plateau to alpine regions with steep slopes. The focal area of my thesis is the Swiss plateau, stretching between the lakes Geneva and Constance and delimited by the Jura Mountains and the Alps and has an altitudinal range between 300 – 800 m ASL. High-intensity agriculture accounts for ~ 65% of the landscape and urban structures are common (Zingg et al, 2019). The Swiss agricultural landscape is dominated by grasslands (72% of its total agricultural area). However, up to 98% of the species-rich, mesic hay meadows from low altitudes (*Arrhenatheretalia elatioris)* have disappeared since 1900 (Lachat et al., 2010). Consequently, Switzerland has a responsibility in grassland biodiversity protection and restoration.

Similarly as in the European Union, awareness of the environmental damage caused by high-intensity farming has increased in the 1990s, leading to a revision of the Swiss agricultural policy. This resulted among others in the introduction of biodiversity promotion areas (BPA). Examples of BPAs are hedgerows, high-stem fruit trees, wildflower strips, or extensively managed meadows. Farmers can receive direct payments if minimum 7% of their farmland is managed as BPAs (Swiss Federal Council, 1998). Extensively managed meadows are with 52% of the entire BPA surfaces the most common BPAs (Swiss Federal Office of Statistics, 2022). Two different payment schemes are applied within extensively managed meadows, i.e., a management-based scheme (Quality I meadows) and a result-based scheme (Quality II meadows). Quality I meadows receive no fertilization and no pesticides and must be mown after June 15th. Quality II meadows are managed the same way as the Quality I meadows but need additionally "botanical quality" (this term is used and defined in the Swiss decree on direct payments). Botanical quality is achieved when a meadow contains at least six indicator plant species. Indicator species are target species commonly found in species-rich hay meadows. In addition, farmers receive three times more payments when a meadow reaches Quality II, compared to Quality I. A similar result-based approach is also applied in some regions in Germany (Herzon et al., 2018).

Scope of the thesis

The main objective of this thesis was to evaluate the short-term effects of four active grasslands restoration methods of extensively managed meadows on plants and ground-dwelling invertebrates. In 2018, we have launched a field scale grassland restoration experiment in the Swiss plateau. We applied four different restoration treatments which are common in Switzerland and recommended by the Swiss Association for the Development of Agriculture and Rural Areas (AGRIDEA Staub *et al.*, 2015). These treatments differ in seed bed preparation intensity (low vs. high intensity), seeding method (green hay vs. seeds only) and seed origin (local species-rich donor meadow vs. commercial seed producer). The restoration treatments were randomly assigned and applied to the restoration meadows in early summer 2019. Treatments were carried out on field scale (i.e., one treatment per meadow) and replicated across twelve regions. The treatments were:

(i) Control: no seed addition and no soil disturbance (C for control)

(ii) Hay transfer from a species-rich donor meadow on a harrowed receiver meadow (HH for hay harrow)

(iii) Hay transfer from a species-rich donor meadow on a ploughed receiver meadow (HP for hay plough)

(iv) Sowing of a brush- or vacuum harvested seed mixture on a ploughed receiver meadow (SN for seeds natural, Fig. 2)

(v) Sowing of a commercial seed mixture on a ploughed receiver meadow (SC for seeds commercial)

Fig.2 Seed collection on species rich donor meadows with a brush harvester (on the left) and with a vacuum harvester (on the right).

Before restoration, all meadows were managed accordingly to the Swiss BPA scheme "extensively managed meadows" since at least five years, i.e., these meadows received no fertilizer and had their first cut after June 15. In addition, these meadows were registered as Quality I, i.e., these meadows had less than six target species and were relatively species poor. Restoration was implemented in 2019 by the farmers who already managed these meadows and was supervised by us or by practitioners with experience in grassland restoration. After restoration the farmers continued to manage their restored meadows as before. This approach had the advantages that it allowed for feasibility in the implementation of the restoration operations in an agricultural setting and at the same time the farmers would be compensated with higher direct payments if their restored meadows reach Quality II.

To ensure robustness of our study outcomes, we applied the before-after-control-intervention (BACI) framework. Baseline data for plants and invertebrates was collected before restoration in 2018. One year after restoration (in 2020), we resampled invertebrates and two years after restoration (2021), we redid the plant surveys.

The necessity for a study on active grassland restoration arose during accompanying group committee meetings of a grassland management project about the effects of different mowing regimes (Buri et al., 2016). Active restoration through seed addition was identified as the main method to restore species rich grasslands in lower altitudes in Switzerland. The accompanying group was composed of stakeholders such as representatives of local and national environment and agriculture offices. To bridge the gap between science and policy (Arlettaz et al., 2010) we reported about the progression of the restoration experiments to the accompanying group committee on a yearly basis.

Thesis outline

Chapter 1 focuses on synthesizing available knowledge about the effectiveness of different methods for restoring or re-establishing species-rich semi-natural grasslands in temperate Europe. This was done with a systematic review and a subsequent meta-analysis. To begin with, I wrote a systematic review protocol (*sensu* Pullin & Stewart 2006) where I defined the main objective, the search strategy, the screening process, the data extraction and analysis (Chapter 1.1). With this protocol as a guide, I conducted a systematic review on the topic, and then a meta-analysis which compares the effectiveness of different restoration methods for promoting plant diversity (Chapter 1.2). As a result, research gaps were identified and a state-of-the-art on the topic of grassland restoration was provided.

Chapter 2 describes the effects of grassland restoration on invertebrates in two aspects. Firstly, we quantified whether hay transfer from a species-rich donor meadow to a receiver site is effective in transferring invertebrates (Chapter 2.1). Here we show that the type of mowing machine used on the donor meadow and the transport type of the hay are crucial elements in invertebrate survival. Secondly, we studied the effect of different soil disturbance intensities linked to grassland restoration on the resident ground-dwelling invertebrate community in the mid-term (Chapter 2.2). Soil disturbances such as ploughing or harrowing are known to cause direct mortality on soil arthropods in arable land (Thorbek & Bilde, 2004). Given that soil disturbance is necessary to successfully restore the plant community in a grassland (Freitag et al., 2021), there is a risk that this disturbance might be detrimental for the invertebrates already occurring on a grassland. Ground beetles and spiders were chosen as bioindicators for this chapter.

In chapter 3 I tested the relative effectiveness of the different restoration treatments described above on the plant community on the short-term, i.e., two years after restoration. Based on vegetation surveys that I conducted before and after restoration I analyzed how the restoration treatments affected the plant species richness, diversity, and functional traits of the plant community. The outcomes of this chapter are particularly interesting for landowner and farmers of species-poor, extensively managed meadows in Switzerland, where higher plant diversity is compensated through a result-based payment scheme.

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Chapter 1.1

The relative effectiveness of seed addition methods for restoring or re-creating species rich grasslands: a systematic review protocol

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Abstract

Background: Extensively managed grasslands in temperate biomes are capable of harboring a big variety of plant and invertebrate species. Yet, they have suffered from a strong decline in the past decades mainly due to agricultural intensification. Agri-environment schemes have been introduced in Europe in order to promote farmland biodiversity, but they were only little effective, especially so in grasslands. Not surprisingly, grassland restoration and recreation through active seed addition has thus gained in importance in the recent years. The most common methods used rely either on the addition of commercial seed mixes, on the addition of seeds collected from a speciose donor grassland or on transferring hay from a speciose donor grassland after the soil of the receiver site has been prepared (ploughing, harrowing or topsoil removal). While there is evidence that these restoration methods may contribute to improve the biodiversity of grasslands, especially plant diversity, their relative effectiveness remains poorly known. The aim of this systematic review is to scrutinize the peer-reviewed literature for scientific evidence about comparative effectiveness.

Methods: We will search for peer-reviewed journal articles in bibliographic databases and grey literature in search engines and organizational websites dealing with at least one of the above mentioned seed addition methods. We will only include studies which were carried out in temperate Europe. Through a scoping exercise a search string was developed which was based on a previously prepared test-list. The search string was then tested for validity with two independent reference lists. Screening will be done on the title, abstract and full-text level and consistency checking will be done on a random subsample by a second reviewer. After critically appraising internal validity of the retained studies, data on the responses of plants and invertebrates as well as all relevant meta-data will be extracted and coded. A meta-analysis will be conducted on studies with high internal validity whereas a narrative analysis

will be done with descriptive statistics on studies with medium internal validity. Potential effect modifiers like study duration, former land use or local climate will be included in the analysis as moderators.

Keywords: Restoration ecology, Agriculture, Biodiversity, Europe, Conservation

Background

Semi-natural grasslands are open habitats that are dependent on human disturbance, mostly managed for livestock production. Extensively managed grasslands in temperate zones are among the biodiversity richest habitats in the world with up to 89 plant species per m^2 (Wilson et al., 2012). With the massive industrialization that followed World War II, the increase of human population and intensification of agricultural practices, most of the extensively managed grasslands have been converted to croplands or made place to fertilized, nutrient-rich grasslands with an impoverished plant species community (Poschlod & WallisDeVries, 2002). In fact agriculture is now recognized to be among the main drivers for biodiversity loss on this planet (Chaudhary et al., 2016). In Switzerland, for example, up to 98% of the historical *Arrhenatherion* grasslands have disappeared since 1900 (Lachat et al., 2010).

In order to promote biodiversity on farmland, agri-environment schemes (AES) were introduced in Europe in 1992 in which farmers receive payments if they modify their farming practices to promote biodiversity (Kleijn & Sutherland, 2003). Nowadays, more than 20 years after the introduction of AES, their effects on biodiversity are rather sobering, with only little positive to no effects on biodiversity which could be evidenced so far (Aviron et al., 2009; Herzog et al., 2005; Knop et al., 2006). This trend has been observed for the AES in general in Europe (Kleijn et al., 2006; Kleijn & Sutherland, 2003; Pe’er et al., 2014; Pe’er et al., 2017).

One common habitat targeted by AES are extensively managed grasslands, which are widely spread across temperate Europe. This habitat is promoted by fertilizer and/or pesticide reduction, a lower number of cuts per year and/or a later first cut (Caillet-Bois et al., 2015). In highly productive regions intensive agriculture has been in place over several decades. This is one of the main reasons for the depletion of the soil seed bank while recolonization from remnant stands is slow, which impedes passive restoration (Bossuyt & Honnay, 2008; Kiehl et al., 2010; Schmiede et al., 2012). For this reason, grassland restoration/re-creation through active seed addition in order to boost grassland biodiversity has gained in importance in recent years (Albert et al., 2019; Dobson, 1997; Jongepierová et al., 2007; Kiehl & Pfadenhauer, 2007; Losvik & Austad, 2002; Woodcock et al., 2008).

The most common seed addition methods of grassland restoration or re-creation are addition of commercial seeds and addition of collected seeds or hay transfer from a speciose donor grassland (Albert et al., 2019; Bischoff et al., 2018; Engst et al., 2016; Jongepierová et al., 2007; Kiehl & Pfadenhauer, 2007; Rasran et al., 2007; Staub et al., 2015; Török et al., 2011; Woodcock et al., 2008). It plays a role if the grassland which is going to be restored is ploughed or harrowed before the seed addition because an absence of tilling will inhibit the new plants from establishing and jeopardizes chances of success (Schmiede et al., 2012). Many studies tested different seed addition methods for the re-creation of grasslands on former arable lands or restoration of already existing, impoverished grasslands. Literature reviews that were carried out on this topic date back to almost ten years ago or more (Hedberg & Kotowski, 2010; Walker et al., 2004) or focus on re-creation of grasslands rather than on restoration (Kiehl et al., 2010; Török et al., 2011). In these reviews the search strategy is not or only little described, same as the screening process and the eligibility criteria, which impedes the repeatability of these reviews. Hence, our review would be the first to be carried out systematically on the topic of grassland re-creation and restoration while including also more recent studies.

The necessity for an up to date systematic review in the field of active grassland restoration arose as well during accompanying group committee meetings of a grassland management project (Buri et al., 2016; van Klink et al., 2017). Active restoration through seed addition was identified as the main method to restore or re-create species rich grasslands in lower altitudes in Switzerland, representing temperate Europe. The group was composed of experts from multiple disciplines, which included, among others, representatives of local and national environment and agriculture offices. Members of the group provided or will help to access grey literature and gave us some input on technical questions on this topic based on their experience in the field. The systematic review proposed here, with a possible subsequent meta-analysis, will yield a useful overview for various stakeholders. At the same time, this review will help to identify research gaps in the field of grassland restoration and re-creation.

Objective of the Review

The main objective of this review is to compare the effectiveness of three different seed addition methods for the restoration or re-creation of species-rich grasslands which are: 1) seed addition of commercial seeds, 2) seed addition of collected seeds from a species rich donor grassland or hay transfer from a species rich donor grassland and 3) either method combined with different levels of soil disturbance such as ploughing or harrowing. To evaluate effectiveness, we will focus on common biodiversity measures such as species richness and evenness (like the Shannon’s index). We are interested in both plants and invertebrates as response variates. Furthermore, we also want to investigate the influence of different factors such as climate or former land use before the intervention on the effectiveness of the different seed addition methods.

Primary question

Do different seed addition methods for the restoration or re-creation of species rich grasslands differ in their effectiveness to enhance diversity of plants or invertebrates?

Question components

The question components were structured according to the PICO-model (population, intervention, comparator, outcome):

Population:	Grasslands in temperate Europe below the subalpine zone
Intervention:	Restoration or re-creation of species rich grasslands through seed addition by at least one of the following methods: hay transfer, sowing of seed mixture (natural or commercial) and with tillage/ploughing
Comparator:	Control plots that have not been restored and/or reference sites
Outcome:	Changes in biodiversity measures such as species richness, percentage cover (for plants), abundance (for invertebrates) and/or evenness.

Methods

Searching for articles

The final search string will be:

(grassland* OR meadow* OR pasture*) AND (restor* OR seed addition OR seed transfer OR hay transfer OR sow* OR strew*) AND (*diversity OR enhance* OR success OR richness OR establish*)

This search string was developed using the recommendations of the CEE Guidelines (Pullin et al., 2018). The scoping was done on the Web of Science database. Scoping included a first version of the search string which was developed by extracting important terms that were found in our test list, which includes important studies done in this field (see Additional file 1). The hits of the first search string were compared to reference lists of two independent reviews about this topic (Hedberg & Kotowski, 2010; Kiehl et al., 2010). The search string was then adapted accordingly and yielded the final version with population, intervention and outcome terms from the question components. The population terms (grassland* OR meadow* OR pasture*) include the desired final and/or the initial studied population. The intervention terms (restor* OR seed addition OR seed transfer OR hay transfer OR sow* OR strew*) were recognized to be used repeatedly in grassland restoration and re-creation studies and assure the inclusion of the desired intervention methods for our review. The outcome terms (*diversity OR enhance* OR success OR richness OR establish*) cover the variety of different results related to changes in biodiversity. No comparator terms where included in the search string since our desired comparator (control site with no intervention) where not always mentioned in the title or abstract. If the search engine allows it, the search will be restricted to the research area of Ecology, Restoration and Conservation Biology and related areas. Depending on the database being used this will be done by adding further terms or through further refinement in the advanced search modus, e.g. in Web of Science by adding the terms AND SU=(Agriculture OR Biodiversity & Conservation OR Environmental Sciences & Ecology OR Evolutionary Biology OR Plant Sciences OR Zoology).

Relevant literature will be searched in the following bibliographic online databases:

- Web of Science Core Collection

- Cab Abstracts
- JSTOR
- Scopus
- Directory of open access journals (DOAJ)
- eThOs

Using the 'Publish or perish' software, which retrieves references from google scholar (https://scholar.google.ch/), 1000 references will be checked as well.

On 26 April 2019 a pilot run was conducted with Web of Science Core Collection with the above search string and the restrictions in research area (SU=…), which yielded 5'751 hits.

Grey literature

Grey literature, will be searched in the search engines BASE (https://www.base-search.net/) and google (https://www.google.ch/), where the first 500 hits will be retrieved and scanned for relevance (Taylor et al., 2019). Furthermore, we will look for grey literature by asking our stakeholder group and other national and international experts in the field. Finally, the following organizational websites will be searched:

- SALVERE (http://www.salvereproject.eu)
- Regio Flora (https://www.regioflora.ch)
- The Society for Ecological Restoration (https://www.ser.org)
- Pro Natura Switzerland (https://www.pronatura.ch)
- WWF Global, Switzerland, Germany, Austria, France and Poland

Languages

Searches in bibliographic databases will be conducted in English using the above mentioned search string. Using a simplified translated search string in English, German, French and Polish we will conduct additional searches for grey literature in google scholar, google and BASE and go through the above listed organizational websites in their respective languages.

Assembling a library of search results

All results from the above mentioned search will be added to a Mendeley library and duplicates will be removed.

Article screening and study eligibility criteria

Screening process

At the beginning a random sample of 20% of the articles will be screened at the title and afterwards at the abstract level by the main reviewer. Studies that were conducted outside of Europe, that were not restoration studies or generally do not match our research question will be excluded directly at the title or abstract level. For the remaining articles a full-text screening will be performed. A second reviewer will perform the same screening process at each screening stage on the same subset of articles and Cohen's kappa will be used to check for inclusion consistency (Pullin & Stewart, 2006). If the kappa score will reach < 0.6, the inconsistencies among the reviewers will be discussed and the inclusion criteria possibly redefined. Afterwards the screening will be repeated by both reviewers and inclusion consistency checking will be done again. If inclusion consistency is met, the main reviewer will finish the screening with the remaining articles.

Eligibility criteria

The following criteria have to be fulfilled for an article to be included:

Eligible populations: Grasslands in temperate Europe, which we define as being within the Cfb-Zone according to the Köppen-Geiger climate classification system (Kottek et al., 2006).
Eligible interventions: The only seed addition methods to be included are hay transfer from a species rich donor grassland, sowing of seeds originating from a species rich donor grassland from the respective region or sowing of a commercial seed mixture especially designed for restoration or re-creation purposes of grasslands (Kiehl & Pfadenhauer, 2007; Staub et al., 2015). Before seed addition the soil has to be disturbed through either ploughing, harrowing or top soil removal.
Eligible comparators: Control sites/plots with no intervention, i.e. no seed/hay added and managed in the same way as the intervention plots.
Eligible outcomes: Species richness, percentage cover (for plants) or abundance (for invertebrates), or any biodiversity index of at least one taxonomic group.
Eligible types of study design: Only experimental studies will be included. These can be published as journal articles, PhD or MSc theses, book chapters, technical reports or other documents that fulfill our criteria.

A list with all excluded studies at abstract and full text level together with the reasons for exclusion will be provided.

Study validity assessment

Eligible studies will go through critical appraisal of internal validity and will be categorized as having high, medium or low risk of bias, concerning our review question. A similar categorization was done in Jakobsson et al., 2018, but it was adapted to fit the purpose of this review. If a study shows high risk of bias and therefore low internal validity, it will be excluded from the synthesis. This will be the case if a study shows at least one of the following limitations:

- Intervention and comparator sites are not well matched, e.g. soil conditions differ profoundly.
- Severely confounding factors present.

Confounding factors can be the exposure of the intervention and comparator sites to different conditions after restoration/re-creation such as different types of management (mowing vs. grazing or a mix of both). If not excluded so far, a study will be categorized as being of medium internal validity if it matches one of the following conditions:

- Study duration <3 years, i.e. time since restoration/re-creation until last data collection
- No replicates
- Non-random plot allocation

Because many restoration/re-creation studies are site limited, a completely random plot/site-allocation is not always feasible, which increases the risk of selection bias. For this reason, we will also include studies with non-random plot allocation or with no replicates. In addition, if the description of the methods will not be sufficient enough, the data in the results section will be difficult to interpret or if important measurements (these could be any of the ones listed in the Data coding and extraction strategy section below) which were mentioned in the methods are not or only partially reported, we will attempt to contact the corresponding authors in order to obtain the necessary data or explanations. In case of no answer the respective study will be considered as of medium internal validity. Studies with medium internal validity will be analyzed separately in a narrative analysis (see Data synthesis and presentation).

A subset of 20% of the studies will be appraised by two reviewers independently and disagreements and process of resolution will be reported. The remaining studies will be appraised by the main reviewer. A list of the excluded articles with the reason of exclusion will be provided. Studies where none of the above listed conditions apply will be regarded as having low risk of bias and therefore of high internal validity and suitable for data extraction.

Data coding and extraction strategy

As response variables the mean species richness and, if available, the mean evenness (e.g. Shannon's index) will be extracted together with their respective standard deviation. If evenness is not provided, we will calculate it from the reported percentage cover (for plants), abundance (for invertebrates) and species richness, if feasible. Data will be obtained either from tables in the manuscripts or from the text. If other types of variation are provided, such as standard error, they will be converted into standard deviation. If the values are not provided in the manuscript, we will contact the corresponding author asking for these values or for the raw data in order to calculate them.

Meta-data which could potentially be relevant for comparison among studies will be coded and will include:

- Country
- Longitude/latitude
- Altitude
- Mean annual precipitation
- Mean annual temperature
- Establishment year of the study

- Study duration
- Former land use
- Soil conditions before intervention, i.e. pH, N-content and P-content
- Plant community of donor site or targeted community
- Grassland habitat type, such as: dry, wet or mesotrophic grassland
- Number of replicates
- Field/plot size
- Seed addition method, such as: hay transfer, sowing of collected seeds from donor site or sowing of commercial seed mixture
- Soil disturbance, such as: ploughing, harrowing or top soil removal
- Management after initial restoration, such as: grazing, mowing, or mulching

Meta-data will be coded from tables or from the text in the manuscript. If the altitude or the climatological data are not provided in the original study, they will be obtained from the WorldClim database (Fick & Hijmans, 2017). If any of the other data will not be found in the text, the authors will be contacted. The extracted data will be made available as an additional file.

In order to ensure consistency, data of a random set of five articles will be coded and extracted by two reviewers. In case of disagreement in the coding, the results will be discussed among the reviewers. Once agreement is met, data of the remaining articles will be coded and extracted by the main reviewer.

Potential effect modifiers/reasons for heterogeneity

Publications about grassland restoration or re-creation use data from experiments ranging in their study duration from one year (Losvik & Austad, 2002) to over ten years (Woodcock et al., 2012). Especially in the first few years the plant composition can fluctuate from one year to another. For this reason, the study duration has a high potential to be an effect modifier. Also the soil condition such as nutrient content can play an important role in the success of the restoration. Soil measurements are not always performed before the restoration, but the former land use before the restoration can be a good proxy for that, e.g. a highly intensive crop field with regular nutrient input via manure addition versus an extensively managed meadow. Finally, the climatic conditions can also influence the outcome. The list of potential effect modifiers is based on a previous literature research that we conducted and expert knowledge, but is not exhaustive and will be adapted during the review process if necessary.

Data synthesis and presentation

Due to logistic constraints, seed addition experiments for grassland restoration and re-creation are often limited to few or no replicates. Studies with non-random plot allocation, no replicates or where no standard deviation can be retrieved will be used for a narrative analysis (medium internal validity, see Study validity assessment), i.e. including descriptive statistics and brief descriptions from a selection of individual studies and their findings. If enough studies with replicates and their respective means and variances will be found a quantitative meta-analysis will be conducted. Such meta-analysis will be done in R (R Core Team, 2019) with the *metafor* package (Viechtbauer, 2010). Although we will use the species richness as a common measure with the same unit, i.e. number of species, the methods with which the species richness was assessed might differ from study to study, e.g. different plot size for taking the measure. For this reason, we will calculate the standardized mean difference (Hedge’s d) or/and the response ratio for the species richness together with the variances for each study. The same will be done for other measures such as coverage (for plants), abundance (for invertebrates) and species evenness, if enough studies will provide these values. Assuming heterogeneity between the studies we will use for the further inferential analysis the random-effects model with unweighted estimation with the restricted maximum likelihood estimator if we have many studies, i.e. >10, otherwise we will use the fixed-effects model with weighted estimation (Hedges & Vevea, 1998). Moderators will be added (see Potential effect modifiers section above) and their relative importance in explaining the variance will be assessed with the τ^2, I^2 and Q-Values. Furthermore, to check the robustness of the result the risk of publication bias will be determined with funnel-plots and the p-uniform function from the *puniform*-package (van Aert, 2018; van Assen et al., 2015) and sensitivity analysis will be carried out. Finally, knowledge gaps and clusters will be identified in the field of grassland restoration and re-creation. Focus will be given to different species groups included in the studies. While in the reviews that were done on this topic so far mostly plants were included as diversity measures (Hedberg & Kotowski, 2010; Kiehl et al., 2010; Török et al., 2011; Walker et al., 2004), an under representation of other species groups, such as invertebrates, is expected. Moreover, we will check if certain seed addition methods are used more frequently than others. In order to do so, studies with high and medium internal validity will be counted according to the above mentioned categories (i.e. studies on plants or invertebrates, hay transfer vs. direct seeding etc.). The entire protocol complies with the ROSES reporting standards (see Additional file 2).

Additional files

Additional file 1 – Test list

Additional file 2 - ROSES form

Authors' contributions

The protocol was written by DS and edited by JYH and RA. All authors read and approved the final manuscript.

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Additional file 1 – Test list

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Additional file 2 – ROSES form

Section / sub-section	Topic	Description	Further explanation	Checklist/Meta-data	Author response
Title	Title	The title must indicate that it is a systematic review protocol, and must indicate if it is an update/amendment: e.g. "A systematic review update protocol...".	The title should normally be the same or very similar to the review question.	Meta-data	The relative effectiveness of seed addition methods for restoring or re-creating species rich grasslands: a systematic review protocol
Type of review	Type of review	Select one of the following types of review: systematic review, systematic review update, systematic review amendment, systematic review from a systematic map	See CEE Guidance on amendments and updates [1]	Meta-data	systematic review
Authors contacts	Authors contacts	The full names, institutional addresses, and email addresses for all authors must be provided.		Checklist	Yes
Abstract	Structured summary	Abstract must not exceed 350 words and must include two sections 1) Background, the context and purpose of the review, including the review question; 2) Methods, how the review will be conducted and the outputs that are expected (specifically mention search strategy, inclusion criteria, critical appraisal, data extraction and synthesis).		Checklist	Yes

Background	Background	Describe the rationale for the review in the context of what is already known. Protocol must indicate why this study was necessary and what it aims to contribute to the field.	A theory of change and/or conceptual model can be presented that links the intervention or exposure to the outcome.	Checklist	Yes
Stakeholder engagement	Stakeholder engagement	The planned/actual role of stakeholders throughout the review process (e.g. in the formulation of the question) must be described and explained (using a broad definition of 'stakeholder', including e.g. researchers, funders and other decision-makers; see [2])		Checklist	Yes
Objective of the review	Objective	Describe the primary question and secondary questions (when applicable).	The primary question is the main question of the review. Secondary questions are usually linked to sources of heterogeneity (effect modifiers).	Checklist	Yes
	Definitions of the question components	Break down and summarise question key elements e.g. population, intervention(s)/exposure(s),	For other question types see [3,4]	Meta-data	Population: Grasslands ; Intervention: Restoration of

		comparator(s), and outcome(s).			species rich grasslands; Comparator: Control plots; Outcome: Changes in biodiversity
Methods					
Searches	Search strategy	Detail the planned search strategy to be used, including: database names accessed, institutional subscriptions (or date ranges subscribed for each database), search options (e.g. 'topic words' or 'full text' search facility), efforts to source grey literature, other sources of evidence (e.g. hand searching, calls for evidence/submission of evidence by stakeholders).	Details regarding search strategy testing should be provided.	Checklist	Yes
	Search string	Provide Boolean-style full search string and state the platform for which the string is formatted (e.g. Web of Science format)		Meta-data	(grassland* OR meadow* OR pasture*) AND (restor* OR seed addition OR seed transfer OR hay transfer OR sow* OR strew*) AND (*diversity OR enhance* OR

					success OR richness OR establish*); Format: Web of Science Core Collection
	Languages – bibliographic databases	List languages to be used in bibliographic database searches.		Meta-data	English
	Languages – grey literature	List languages to be used in organizational websites searches and web-based search engines.		Meta-data	English, German, French, Polish
	Bibliographic databases	Provide the number of bibliographic databases to be searched.		Meta-data	6
	Web – based search engines	Provide the number of web – based search engines to be searched.		Meta-data	3
	Organisational websites	Provide the number of organisational websites to be searched.		Meta-data	10
	Estimating the comprehensivenes s of the search	Describe the process by which the comprehensiveness of the search strategy was assessed (i.e. list of benchmark articles).		Checklist	Yes
	Search update	Describe any plans to update the searches during the conduct of the review.	Optional. A search update is good practice if original searches were performed more than two years prior to	Checklist	No

		review completion.		
Article screening and study inclusion criteria	Screening strategy	Describe the methodology for screening articles/studies for relevance/eligibility.	Checklist	Yes
	Consistency checking	Describe clearly the process for checking consistency of decisions including the levels at which consistency checking will be undertaken and estimated proportion of articles/studies that will be screened and checked for consistency by two or more reviewers (e.g. Titles (10%), abstracts (10%), full text (10%)).	Checklist	Yes
	Inclusion criteria	Describe the inclusion criteria used to assess relevance of identified articles/studies. These must be broken down into the question key elements (e.g. relevant subject(s), intervention(s)/exposure(s), comparator(s), outcomes, study design(s)) and any other restrictions (e.g. date ranges or languages).	Checklist	Yes
	Reasons for exclusion	State that you will provide a list of articles excluded at full text with reasons for exclusion.	Checklist	Yes
Critical appraisal	Critical appraisal strategy	Describe here the method you propose for critical appraisal of study validity (including assessment of individual studies and the evidence base as a whole).	Checklist	Yes
	Critical appraisal used in synthesis	Describe how the information from critical appraisal will be used in synthesis.	Checklist	Yes
	Consistency checking	Describe how repeatability of critical appraisal of study validity will be tested.	Checklist	Yes

Data extraction	Meta-data extraction and coding strategy	Describe the method for meta-data extraction and coding for studies (potentially providing forms/data sheets (ideally piloted), list if variables to be extracted as meta-data and those that will be coded).		Checklist	Yes
	Data extraction strategy	Describe the method for extraction of qualitative and/or quantitative study findings (potentially providing forms/data sheets (ideally piloted))		Checklist	Yes
	Approaches to missing data	Describe any processes for obtaining and confirming missing or unclear information or data from authors.		Checklist	Yes
	Consistency checking	Describe how repeatability of the meta-data/data extraction process will be tested.		Checklist	Yes
Potential effect modifiers/reasons for heterogeneity	Potential effect modifiers/reasons for heterogeneity	Provide a list of and justification for the effect modifiers /reasons for heterogeneity that will be considered in the review. Also provide details of how the list was compiled (including consultation of external experts).	The list should not be exhaustive but a short list of those variables thought to be most important and amenable to analysis.	Checklist	Yes
Data synthesis and presentation	Type of synthesis	State the type of synthesis conducted as part of the systematic review (narrative only, narrative and quantitative, narrative and qualitative, narrative, qualitative and quantitative, narrative and mixed-methods)		Meta-data	narrative and quantitative
	Narrative synthesis strategy	Describe methods to be used for narratively synthesising the	Vote-counting (tallying of	Checklist	Yes

		evidence base in the form of descriptive statistics, tables (including any map databases) and figures.	studies based on the direction or significance of their findings) must be avoided. Must include a summary of the outputs of critical appraisal of the evidence base as a whole.		
	Quantitative synthesis strategy	If data are appropriate for quantitative synthesis, describe planned methods for calculating effect sizes, methods for handling complex data, statistical methods for combining data from individual studies, and any planned exploration of heterogeneity (e.g. sensitivity analysis, subgroup analysis and meta-regression). If all studies may not be selected for synthesis explain criteria for selection (e.g. incomplete or missing information).	Compulsory if appropriate for data	Checklist	Yes

	Qualitative synthesis strategy	Describe methods to be used for synthesising qualitative data and justify your methodological choice. Describe if and how you plan to analyse subgroups/subsets of data. If all studies may not be selected for synthesis explain criteria for selection (e.g. incomplete or missing information).	Compulsory if appropriate for data	Checklist	Yes
	Other synthesis strategies	Describe any other approaches to be used for synthesising data or combining qualitative and quantitative synthesis (e.g. mixed-methods) and justify your methodological choice.	Compulsory if appropriate for data	Checklist	No
	Assessment of risk of publication bias	Describe planned methods for examining the possible influence of publication bias on the synthesis.	For quantitative syntheses this may be done using diagnostic plots or statistical tests	Checklist	Yes
	Knowledge gap identification strategy	Describe the methods to be used to identify and/or prioritise key knowledge gaps (unrepresented or underrepresented subtopics	Optional	Checklist	Yes

		that warrant further primary research).			
	Demonstrating procedural independence	Describe the role of systematic reviewers (who have also authored articles to be considered within the review) in decisions regarding inclusion or critical appraisal of their own work.	Reviewers who have authored articles to be considered within the review should be prevented from unduly influencing inclusion decisions, for example by delegating tasks appropriately.	Checklist	Yes
Declarations	Competing interests	Describe of any financial or non-financial competing interests that the review authors may have.		Checklist	Yes

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Chapter 1.2

The relative effectiveness of different grassland restoration methods: a systematic review

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Abstract

1. Active grassland restoration or re-creation has gained in importance to mitigate the dramatic decline of farmland biodiversity. While there is evidence that such operations are generally effective in promoting plant diversity, little is known about the effectiveness of the different methods applied, notably in relation to three main factors: seed bed preparation, seed source and technique of seed application.

2. In this systematic review and meta-analysis we screened the scientific literature about the restoration of mesic grasslands in temperate Europe. We focused on active restoration experiments that included a treatment and lasted more than three years. We evaluated the relative influence of these factors on restoration efficacy, focusing on plant species richness.

3. We found 187 articles that investigated the outcome of operations aimed at actively restoring mesic temperate grasslands. Most articles focused on plants, with only 9.6% dealing with other organisms (e.g., beetles, pollinating insects). Many papers had to be excluded due to incomplete data, too short study duration and/or lack of an adequate control. This resulted in only 13 articles fulfilling our criteria for inclusion, yielding a total of 56 data points for the meta-analysis.

4. Restoration actions increased plant species richness by, on average, 17.4%, compared to controls. The seed source explained a significant amount of variation in plant species richness: seeds originating from a speciose donor grassland had a positive effect. This effect was even enhanced when combined with a commercial seed mix, whereas commercial seed mixes alone had no significant effect. We did not observe any effect of other factors, such as the type of seed bed preparation or the seed application method.

5. Synthesis and applications. A seed-source obtained from species-rich grasslands seems to be key to efficient grassland restoration. Even though seeds from a speciose donor grassland should be preferred over commercial seeds, associating natural and commercial seed mixes increases plant species richness. This systematic review further revealed two major research gaps in grassland restoration ecology: a deficit in long-term investigations as well as a deficit in studies focusing on non-plant organisms.

Keywords: Active restoration, temperate Europe, mesic grasslands, seed addition, meta-analysis

Introduction

Grasslands cover more than 25% of Earth's continental biomes (Blair et al., 2014; Wilsey, 2018). They make up 17% of the area of terrestrial ecosystems in Europe (Eurostat, 2018) where they are mostly semi-natural, in the sense that they depend on regular farming interventions such as grazing or mowing to maintain the habitat open and to prevent encroachment by woody vegetation (Hejcman et al., 2013; Kuneš et al., 2015). Semi-natural grasslands progressively expanded since the Neolithic agricultural revolution as they were key to the development of livestock farming (Gibson, 2009). They offer shelter to specialised species that are rare in other habitat types, often originating from grassy steppe formations that occupy drier zones (Dengler et al., 2014). In addition to forage production, semi-natural grasslands provide numerous ecosystem services such as carbon capture and storage, nutrient cycling, reduction of water run-off and soil erosion (Byrne & delBarco-Trillo, 2019; Yan et al., 2019). These ecosystem services and the biodiversity of grasslands are heavily impacted by land-use intensification and land abandonment. From 1975 to 1998, the grassland cover in the EU has declined by 12% (Stoate et al., 2009; Török et al., 2018). Following the declaration of the UN Decade on Ecosystem Restoration 2021–2030 (UNEP, 2022), policy makers are getting aware that a proactive approach is necessary to tackle the biodiversity crisis, although grassland restoration has generally received less attention in contrast to forest or freshwater habitat restoration (Török et al., 2021).

Ecological restoration is the 'process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed' (Gann et al., 2019). 'Passive' or 'natural' restoration (*sensu* Atkinson & Bonser, 2020) of grasslands relies merely on the removal of the main factor responsible for the ongoing degradation, e.g. cessation of fertilizer application. However, passive restoration may be hampered by the poor density of grassland species in the soil seed bank (Buisson et al., 2018; Turnbull et al., 2000; van Klink et al., 2017), by low dispersal capacity of the plants and by the limiting seed sources in the surrounding landscape (Bischoff et al., 2009; Münzbergová & Herben, 2005). This makes passive restoration an extremely slow process that may take several centuries for recovery (Isbell et al., 2019; Nerlekar & Veldman, 2020). 'Active' or 'assisted' grassland restoration overcomes dispersal limitation through seed addition and therefore accelerates the restoration process. In Europe, a multitude of techniques of active grassland restoration are currently being applied and studied. Methods differ in seed source (e.g. from a speciose donor grassland or a commercial seed producer), seed application (seed mix or green hay spread out over the receiving grassland) and seed bed preparation prior to seeding (harrowing or ploughing) (Albert et al., 2019; Auestad et al., 2015; Freitag et al., 2021; Hovd, 2008; Smith et al., 2017).

While several guidelines that describe best practices for successful grassland restoration are available (Kiehl et al., 2014; Scotton et al., 2012), little is known about the relative effectiveness of the different grassland restoration methods, which hampers best practice among practitioners. Literature reviews that have been carried out on this subject date back more than ten years and used a qualitative or narrative synthesis approach (Hedberg & Kotowski, 2010; Kiehl et al., 2010; Török et al., 2011). The need for an actualised systematic review on this topic emerged during stakeholder meetings accompanying a major ongoing grassland restoration experiment performed across Western Switzerland (Stöckli et al., 2021). We thus decided to conduct a quantitative synthesis on this topic, which would not only provide better evidence-based recommendations for management but also allow addressing more specific questions that cannot be answered via non-quantitative syntheses.

In this review we synthesise all the available knowledge about the effectiveness of different methods for restoring or re-establishing species-rich semi-natural grasslands in temperate Europe. We first conduct a systematic review (*sensu* Pullin & Stewart, 2006) on the topic, and second perform a meta-analysis which compares the effectiveness of different restoration methods for promoting plant diversity. More specifically, we were interested in the relative effectiveness of using different sources of seeds, different methods of seeding and different ways of preparing the soil prior to seeding. This systematic review and meta-analysis thus provide a state-of-the-art on the topic of grassland restoration, orienting practitioners towards best practice, while it identifies research gaps to orient future investigations.

Materials and methods

We followed the guidelines of the Collaboration for Environmental Evidence (Pullin & Stewart, 2006) and the ROSES standard. By doing so, we ensure repeatability of our search and screening process (Romanelli, Meli, et al., 2021; Romanelli, Silva, et al., 2021). We prepared a protocol which was peer-reviewed and published (Slodowicz et al., 2019). As some points of the original protocol had to be amended due to some unexpected specific issues, the following section contains the updated protocol.

Literature search

We formulated our research question according to the PICO-structure (population, intervention, control, outcome): Do different seed addition methods for the restoration or re-creation of species rich grasslands differ in their effectiveness to enhance the diversity of plants? (See Table S2 for details on the question components). Based on the question components we developed an initial search string which went through a scoping process. This initial string was used in a search in the Web of Science database. We compared the search result with the reference lists of two reviews on the same topic (Hedberg & Kotowski, 2010; Kiehl et al., 2010). To achieve adequate sensitivity, we adapted the search string, until no references of the reviews were missed. We used the final search string as a template for our database searches and adapted it accordingly to the requirements of the respective databases: (grassland* OR meadow* OR pasture*) AND (restor* OR seed addition OR seed transfer OR hay transfer OR sow* OR strew*) AND (*diversity OR enhance* OR success OR richness OR establish*). We conducted the database searches between 26 November 2019 and 16 March 2020 in Web of Science Core Collection, Scopus, Directory of open access journals (DOAJ) and eThOs. In addition, we used the 'Publish or perish' software (Harzing, 2007) to search articles in Google Scholar and retained the first 1000 hits.

To complement the database search, we looked for other publications and grey literature in Google, organisational websites and through direct requests to authors. The searches and requests were done in English, French, German and Polish. We removed duplicates automatically using the JabRef Reference Manager (JabRef Development Team, 2021).

Article screening

Screening was done on title, abstract and full-text level by two reviewers (DS, AD). A third reviewer (JYH, co-author of this paper) checked for inclusion consistency using Cohen's Kappa (Pullin & Stewart, 2006) on a subsample of 500 articles from each reviewer, respectively. A Kappa score of > 0.6 indicated high consistency between reviewers. At title and abstract level, we included all restoration studies that were conducted within temperate Europe. At full text level the articles had to fulfil our eligibility criteria for inclusion (Table 1). We distinguished three grassland habitat types: dry, wet and mesic. We considered grassland habitats to be "dry" if the substrate was coarse or sandy with low water retention capacity and low amount of nutrients in the soil (e.g., Wolff et al., 2017). We considered grasslands to be “wet” if they were peat or fen meadows (e.g., Klimkowska et al., 2010), or alluvial meadows (e.g., Schmiede et al., 2009). All other grasslands were considered mesic and therefore eligible populations. In our meta-analysis we have intentionally excluded short-term studies of less than three years duration. In effect, the first two years after restoration are typically characterized by a rise in species richness (Freitag et al., 2021). This is often due to the presence of ruderal species, which have become dominant in the seed bank after perturbation. Once the grassland species become more dominant, the number of ruderal species diminishes (Albert et al., 2019). This is reflected in a slight decline in species richness after the second year of restoration (Freitag et al., 2021). For this reason, we focused on the mid-term, thus ensuring that the plant community had become more stable. Yet, to identify research gaps in a later phase, we compiled a separate list of all excluded studies at full-text screening.

Data extraction

The geographic location of each restoration site was recorded and, if necessary, changed into decimal degrees. If the site coordinates were not provided, we looked for a locality (such as a city, village, or a protected area) in the site description of the respective article and determined the coordinates from Google Maps. As potential moderator variables (effect modifiers) we included the control type, type of study design, seed source, seed material, seed bed preparation (seedbed preparation is requisite for efficient seed addition), former land-use before restoration, restoration duration, number of replicates, as well as vegetation survey plot size (see Table 2 for a detailed definition of each moderator variable). These moderator variables are either linked to applied aspects of grassland restoration (e.g., seed source), which are relevant for practitioners, or to experimental aspects (e.g., control type).

As response variable we extracted the mean plant species richness and a measure of variance (which was converted to standard deviation if necessary) from the restored and control plots. We extracted these from summary tables, calculated it from raw data or extracted it from the figures using the WebPlotDigitizer (Rohatgi, 2021). In some cases, an article could include the results of several restoration studies (e.g., Woodcock et al., 2010). In these cases, we attributed to each study its own unique study ID while being nested within the same article ID. We contacted the authors by e-mail to request missing data if relevant data was missing or not extractable from the study.

Data synthesis

To account for scale dependency and to choose the most adequate effect size measure and model type for the meta-analysis, we used the approach recommended by Spake et al., (2021). We tested two effect size measures: log response ratio (lnRR) and Hedge's g. Different modelling approaches were then tested: random effects unweighted, random effects weighted and fixed effects weighted model. Furthermore, we checked to which degree, scale (i.e., the size of vegetation survey plots) influenced any of these effect size measures and models, and finally selected the weighted random effects model using lnRR as effect size (see Figs. S1-S3 for details). Article ID was included as a random factor to account for variation between studies. We fitted the models with restricted maximum likelihood method (REML) and applied the Knapp and Hartung adjustment. With the Knapp and Hartung adjustment the test statistics of individual coefficients are based on the *t* distribution instead on the default *Z* distribution, which in turn may reduce Type I error (Assink & Wibbelink, 2016). To evaluate the effect of moderators on the model, the residual heterogeneity (QE), degrees of freedom and p-value were extracted. To check whether the effect sizes are influenced by a given moderator we extracted the F-value with its degrees of freedom and p-value from the test of moderators (Viechtbauer, 2010). We plotted the effect sizes and 95% confidence intervals of all moderators and their respective categories when they significantly influenced the effect sizes (i.e., $P < 0.05$ at test of moderators). Furthermore, the p-uniform test and the Fail-Safe N Analysis were conducted to check for publication bias. All analyses were performed with R version 4.1.1 (R Core Team, 2021) using the *metafor* (Viechtbauer, 2010) and *puinform* packages (van Aert, 2018).

Results

All searches combined yielded 12’153 literature records. After title and abstract screening, and the removal of duplicates, 532 articles remained (Fig. S4). The Kappa Scores were 0.85 and 0.69 (for DS/JYH and AD/JYH, respectively), indicating high inclusion consistency between reviewers. At full-text screening we identified 187 articles which studied active grassland restoration in temperate Europe. Among these articles, 18 focused on other organisms than plants, in most cases either beetles or pollinating invertebrates. After full-text screening 13 articles remained, which yielded 56 data points from 44 sites for our meta-analysis. Most articles had to be excluded due to, in decreasing order of importance, missing data, a type of grassland habitat different from our target, study duration shorter than three years and inadequate or no experimental control (Fig. 1). Overall, 88% of the study sites were in the United Kingdom, Germany, or the Czech Republic. Further sites were situated in Norway, Ireland, France, and Italy (Fig. 2).

The overall effect of grassland restoration measures on plant species richness was positive (lnRR = 0.34, 95% CI 0.13 - 0.55, $P = 0.002$, Figs. 3 and 4), with a mean increase in plant species richness of 17,4% compared to control. The variance within certain articles was quite large, which was mainly explained by different restoration methods experimentally tested within a single article (Fig. 3). The moderator ‘seed source’ showed a significant moderating effect ($F = 17.48$, $P < 0.001$, AIC = 212.08, Table 3). We observed a positive effect when commercial seeds and seeds from a speciose donor grassland were combined (lnRR = 0.52, 95% CI 0.22 – 0.82, $P < 0.001$, Fig. 4). The effect was less pronounced when the seeds applied originated from a speciose donor grassland only (lnRR = 0.28, 95% CI -0.01 – 0.58, $P = 0.06$). In contrast, the use of commercial seeds alone showed no significant effect on the species richness of restored grasslands (lnRR = 0.31, 95% CI -0.07 – 0.69, $P = 0.11$). The number of seeded plant species in the commercial mixes ranged from 15 to 66, but we could not detect any influence of that seed diversity on the effect size (Fig. S5).

'Restoration duration', ranging from three to 16 years, had a significant moderating effect as well ($F = 13.50$, $P = 0.001$, AIC = 233.09, Table 3) and was slightly negative (lnRR = -0.02, 95% CI -0.03 - -0.01, $P < 0.001$). However, 77% of all data points stemmed from studies whose duration was 3-6 years. Not surprisingly, the moderating effect of 'restoration duration' disappeared when only studies with a 3-6 year duration were included in the model ($F = 2.80$, $P = 0.1$). Furthermore, all data points with a study duration of more than six years originated from three articles; the three experimental studies had been performed on arable land (prior to restoration) and had 'natural regeneration' as control type.

'Land use before restoration' and 'control type' showed significant moderating effects as well ($F = 5.07$, $P = 0.03$, AIC = 239.17 and $F = 4.78$, $P = 0.03$, AIC = 239.30, respectively; Table 3, Fig. 4). In addition, these two moderators were highly correlated ($r = 0.85$, $P < 0.001$) and yielded similar effect sizes. Data points having grasslands as land use before restoration had frequently a species-poor reference as a control (81%) and showed a positive effect of restoration for both land use and control type (lnRR = 0.44, 95% CI 0.23 – 0.64, $P < 0.001$ and lnRR = 0.47, 95% CI 0.26 – 0.69, $P < 0.001$, respectively). Data points with arable land before restoration and natural regeneration as control had a smaller effect (lnRR = 0.21, 95% CI -0.01 – 0.42, $P < 0.07$ and lnRR = 0.24, 95% CI 0.04 – 0.44, $P < 0.02$, respectively). All other potential moderators did not exhibit any effects (Table 3). The vegetation plot size ranged from 0.25 - 25 m^2, but we did not detect scale dependency in our data (Figs. S2, S3). The p-uniform test (L.pb = -3.44, p = 0.99) and the Fail-Safe N Analysis (4603) revealed that there is little evidence for publication bias, indicating that our results are robust.

Discussion

Our systematic review and meta-analysis provides quantitative evidence that operations for active grassland restoration or re-creation that rely on seed addition can definitely enhance plant species richness, corroborating previous narrative reviews on this topic (Hedberg & Kotowski, 2010; Kiehl et al., 2010). Several moderator variables, such as the type of seed source, the past land-use of the site selected for experimental restoration as well as the sort of control employed all emerged as key factors influencing restoration success. However, our analysis could not detect any further difference in effectiveness between the other restoration options under scrutiny: for instance, neither the type of seeding material (direct addition of seeds vs green hay collected from a donor meadow and spread all over the receiving area) nor the intensity of the seed bed preparation (ploughing vs harrowing) were significant factors. Note that we did not consider here over-sowing directly over an extant vegetation cover as this technique is deemed particularly inefficient (Freitag et al., 2021). To our knowledge, the present study is the first quantitative analysis ever carried out on mesic temperate European grasslands. It conveys clear recommendations for conservation and restoration management (see Shackelford et al., 2021 for a meta-analysis on dry grassland restoration).

Mid-term effectiveness depends on seed source

The choice of the seed source – i.e., from a species-rich donor grassland, a commercial seed mixture or a combination of both – had a strong moderating effect on the restoration outcome. The highest positive effect size was obtained when mixing seeds from a speciose donor grassland together with commercial seeds, as already demonstrated by Baasch et al. (2016). These authors added seeds of target plant species, obtained from a local commercial seed producer, that did not originally occur in their donor species-rich grassland. This boosted the final plant species richness that established in the restored meadow. Restoration relying only on seeds from a donor grassland was less effective than the combined approach, but this method might be easier to implement in practice, especially if appropriate donor grasslands are available in the near surrounding landscape. Although the moderator 'commercial seeds' was statistically non-significant (confidence interval just overlapping with 0), its effect size was very close to that of seeds collected from donor grasslands. Despite a wide range in the number of seeded species (15 to 66 (Veen et al., 2018; Freitag et al., 2021), we could not detect any effect of that moderator. The latter study compared the effects of low and high-diversity seed mixes, showing that a more speciose seed mix resulted in higher species diversity in the restored meadow after a few years (Kirmer et al., 2012). In practice, the mixed approach associating natural and commercial seed mixes thus represents the best option for restoration operations, this despite the extra logistic and costs it entails.

Previous land-use, control type, seedbed preparation and seeding technique

The magnitude of the effect sizes also depends on the type of land-use before restoration, which determined to a large extent the type of control used for calibrating the experiment. The positive effects of restoration operations tended to be less marked when the control was simply undergoing a spontaneous natural regeneration of the vegetation which had merely been harrowed or ploughed. In such a case, treatment and control sites had the same baseline exposure level, i.e., prepared soil done by a harrow or plough, with the difference, that the treatment site was seeded and the control not. This low effect size should be interpreted carefully. Natural regeneration of grasslands (or passive restoration) is a dynamic process in the first years and if the landscape or seed bank provides enough propagules, it might even become as efficient as active restoration, i.e., through seed addition (Prach et al., 2014). This makes it difficult to conclude whether re-creating species-rich grasslands on ex-arable land is less efficient than restoring existing species-poor grasslands. Particularly in our meta-analysis, since in most of our included restoration sites, which had natural regeneration as control, where also previously arable land (and vice versa sites with species-poor grassland as control were previously grasslands).

Our results further suggest that the intensity of the seed bed preparation does not influence the plant species richness that is achieved in the mid-term by restoration. There is a consensus that the seedbed must absolutely be prepared through harrowing or ploughing in order to allow seed germination (Durbecq et al., 2021; Freitag et al., 2021). In effect, over-sowing over an extant vegetation layer is never an option: the seeded plants would mostly be outcompeted by the already established plant community. This is why over-sowing was even not considered in our review. Few studies of mesic grasslands have compared the effects of the intensity of seedbed preparation on restoration outcomes (for wet meadows, see Bischoff et al., 2018). In practice, the method used for seedbed preparation mostly depends on the original soil conditions (e.g., deep vs stony soils), which eventually determine the selection of the agricultural machinery for field operations.

The seeding materials themselves – direct seed sowing vs spread of fresh hay all over the receiving meadow – did not seem to influence species richness in the mid-term. We had predicted that the latter method would boost seed germination, and therefore subsequent plant establishment, by creating a more favourable microhabitat reducing soil evapotranspiration, especially during the dry summer months when restoration experiments take place (Havrilla et al., 2020). Seeding with seeds only might cover a wider range of species, e.g., through collection of different sources or different seasons (Albert et al., 2019). But these differences in the initial phase of establishment seem to be balanced out after several years (Baasch et al., 2016).

Missing data and research gaps

From 187 articles which studied active grassland restoration in temperate Europe we could only include 13 in our meta-analysis, which somehow limits generalisation of the main results. This high exclusion rate was mainly due to deficits in the data provided by authors. We used species richness as a metric, as frequently done for biodiversity syntheses due to its simplicity and wide availability (Marchand et al., 2021; Nerlekar & Veldman, 2020). However, we noticed during the screening process that investigations of grassland restoration often have a different focus. Some look at vegetation differences between the restored area and a nearby reference site (Prach et al., 2014), others focus on ecosystem services (De Cauwer et al., 2006), select target species (Johanidesová et al., 2015) or refer to the cover of different functional groups (Conrad & Tischew, 2011). As a result, species richness is sometimes mentioned in the articles, but without the sufficient quantitative reporting necessary for a proper integration into a meta-analysis. Researchers and practitioners active in restoration ecology should render their data publicly available for future syntheses (FAIR prescriptions; Wilkinson et al., 2016). The Global Restore Project has for objective to standardize such datasets, checking notably for taxonomic consistency, while its platform is publicly available (Ladouceur & Shackelford, 2021). Based on more solid foundations, future syntheses may thus incorporate more information and save the strenuous efforts entailed in data search and acquisition.

We also identified several research gaps. Most studies focused on the effect of restoration measures on the plant community whereas only few focused on other organisms such as beetles (Woodcock et al., 2010, 2012), pollinating insects (Ouvrard et al., 2018; Redpath-Downing et al., 2013) and soil microfauna (Norton et al., 2019; Resch et al., 2019). Invertebrate studies show that the restoration of phytophagous beetles was most successful where grassland restoration achieved the highest diversity of, notably, flowering plants species (Woodcock et al., 2010), which offered also more foraging opportunities for pollinating insects (Ouvrard et al., 2018; Redpath-Downing et al., 2013).

It was rapidly clear to us that there is a lack of long-term studies, which represents a serious impediment to properly assess the success of restoration operations. The majority of studies span 3-6 years, which remains insufficient for a sound evaluation. In effect, it may take decades, if not centuries in extreme conditions, for a re-established grassland to reach its natural state (Isbell et al., 2019; Nerlekar & Veldman, 2020). Astonishingly, the few long-term studies included in our meta-analysis showed no significant effect. However, these studies compared their treatments to natural regeneration, which should does not imply no restoration success. In effect, if the treatment plots are too close to an unseeded, disturbed control or if propagules might arrive from the seed bank or the surrounding landscape, the effect of the treatment might not be detected after several years, as this control increased its species richness as well over time (Prach et al., 2014). All in all, 22 articles, despite being thematically alright, had to be excluded from the analysis for having no proper control or an inadequate control (Torrez et al., 2017). Finally, there was a geographical bias in our dataset. Most studies originated from the United Kingdom, Germany and the Czech Republic (Dolnik et al., 2020; Smith et al., 2017). Other countries which harbour vast areas of grasslands, such as France, Italy, Poland, Switzerland or Ireland harboured only few, if any, restoration studies of mesic grasslands (Chevalier et al., 2018; Fritch et al., 2011; Gentili et al., 2017). This bias cannot be explained by a bias towards papers published in English as our literature search was done in four European languages, covering a wide palette of temperate European countries.

Restoration implications and recommendations

Our results highlight the importance of the seed source when restoring or re-creating grasslands. Restoration success in terms of plant species richness is most likely achieved when combining seeds from a species-rich grassland with commercial seeds. Using seeds from a species-rich grassland only is effective as well, while using commercial seeds only had a slightly lesser success. In practice, grassland restoration can be limited by the availability of seeds, which reduces in some cases the possibility of choosing the appropriate seed source. When no local seed source is available, a commercial seed mix might be the sole option. In Europe, seed transfer zones were recently created to account for local ecotypes and intraspecific variation (Cevallos et al., 2020; Durka et al., 2017) and seed certificates were introduced to make locally produced seeds more widely available for practitioners (Mainz & Wieden, 2019). However, supply remains insufficient to cover the high current demand for restoration operations. This concerns in particular rare or endangered plant taxa, for which seed production is complicated by issues revolving around obtaining permit for plant/seed collection (Ladouceur et al., 2018). We therefore recommend the use of commercial seeds only in areas with a limited provision of a local natural seed source, but a regional origin must then be ensured. Similarly, relying on commercial seeds with an unknown provenance is not an option since it might contribute to introduce genetically different and locally maladapted populations (Höfner et al., 2021). Last but not least, commercial seed mixes can be quite expensive (Török et al., 2011). The reliance on species-rich grasslands as donors appears thus to be the best solution for restoring species-rich mesic grasslands. Beyond its positive effects on the restored plant community, hay transfer also benefits the invertebrate community that might be transported with the freshly mown grass (Elias & Thiede, 2008; Stöckli et al., 2021; Wagner, 2004).

Authors' contributions

DS, JYH and RA conceived the study and designed its methodology; DS, AD, EL and RE collected the data and did the literature screening; DS, EL and JYH analysed the data; DS led the writing of the manuscript with major contributions by AD. All authors contributed critically to the drafts, with thorough editing by JYH and RA, and gave final approval for publication.

Statement on inclusion

Our study is review based on a meta-analysis of secondary rather than primary data. As such, there was no local data collection. However, the geographical distribution of the authorship team broadly represents the major regions of interest in the meta-analysis, supporting the inclusion of data from peer-reviewed studies published in local languages and ensuring the appropriate interpretation of data and results from each region. In addition, the necessity for an up-to-date systematic review in the field of active grassland restoration arose during accompanying group committee meetings of a grassland management project in Switzerland. The group was composed of experts from multiple disciplines, which included, among others, representatives of local and national environment and agriculture offices. Members of the group gave us input on practical issues based on their own field experience.

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Table 1 Eligibility criteria at full-text screening.

Eligible populations	Mesic grasslands in temperate Europe, which we define as being within the Cfb-Zone according to the Köppen–Geiger climate classification system (Kottek et al., 2006).
Eligible interventions	Grassland restoration (e.g., from a species-poor grassland) or re-creation (e.g., on formerly arable land) with one or more of the following seeding methods: • hay transfer from a species-rich donor grassland • seeds originating from a species-rich donor grassland from the respective region • commercial seed mixture especially designed for restoration or re-creation purposes of grasslands AND Seed bed preparation prior to seeding through either ploughing or harrowing.
Eligible comparators	Control sites/plots with no intervention, i.e., no seed/hay added and no seed bed preparation (species-poor reference) or sites/plots with seed bed preparation or ex-arable land, but without seed addition (natural regeneration). The control sites are managed in the same way as the intervention plots. In case of before-after studies, the before-data was used as control.
Eligible outcomes	Mean plant species richness with measure of variance per treatment and study year.
Eligible types of study design	Experimental studies with either before-after or control-intervention design with at least three replicates per treatment and a study duration of at least three years. For field scale studies without replication, there must be at least three vegetation survey plots (we acknowledge that this is considered pseudo replication).

Table 2 Overview of all moderator variables that were extracted from the included studies for the meta-analysis and brief explanations of the different categories. The numbers in brackets after each category indicate the amount of data points of the respective category. The total amount of data points is n = 56.

Moderator variable	Categories	Explanation
Seed bed preparation	Harrow (17)	Soil disturbance up to 10 cm depth-
	Tilling (39)	Soil disturbance beyond 10 cm depth.
Seeding material	Hay (14)	Only possible if seed source was a species-rich donor grassland. The donor grassland was mown, and the fresh hay was spread over the area which was to be restored.
	Seed (42)	Possible for both seed source types. The seeds were harvested if the seed source was a species-rich donor grassland, e.g., with a brush harvester.
Seed source	Species rich donor grassland (21)	A species-rich grassland in the vicinity of the restored area.
	Commercially purchased seed mix (9)	Seeds provided by a seed producer. The seed mix contains typical grassland species.
	Both (26)	Both above mentioned seed sources were applied together.
Study design	Block study (28)	Restoration treatments and control were replicated on one field.
	Field scale study (28)	A whole field/grassland was restored.

Control type	Species-poor reference (17)	A control site on the same or neighbouring grassland which did not undergo any treatment (no seed bed preparation, no seeding) and which was managed the same way afterwards as the restored sites. We considered experiments with before-after design as well as “species-poor reference” if the restored area was formerly already a grassland.
	Natural regeneration (39)	Either a site with seed bed preparation but without seeding on a former grassland or no seeding only if the site was formerly arable land.
Former land-use before restoration	Arable (35)	The area was used as crop before restoration, i.e., regular soil interventions. It can be assumed that the seed bank should be rich in ruderal species. In some of these cases, seeding occurred directly on the open soil with no additional seed bed preparation.
	Grassland (21)	The area was either a hay meadow or pasture before restoration but had a low amount of typical grassland species. In most cases the low species number was due to overexploitation (e.g., high fertilizer input, high mowing frequency).
Restoration duration		Time span between the year of establishment and the year of data collection. When there was a series of time points of data collection, we included only the most recent one.
Replicates		For block studies: amount of treatment replicates For field scale studies: amount of vegetation survey plots.
Vegetation survey plot size		Size of the plot used for the vegetation sampling in m^2

Table 3 Output summary for the moderator analysis. The first column gives the name of a given moderator variable (see Table 2 for details). The following three columns provide the residual heterogeneity (QE) together with its degrees of freedom (df) and p-value (p). The three columns afterwards provide the F-value (F) from the test of moderators with its degrees of freedom (df) and p-value (p). The moderator variables are ranked by their respective AIC value (last column).

	Test for residual heterogeneity			Test of moderators			
Moderator	**df**	**QE**	**p**	**df**	**F**	**p**	**AIC**
Seed source	53	1055.96	<.001	2	17.48	<.001	212.08
Restoration duration	54	897.52	<.001	1	13.50	<.001	233.09
Control type	54	795.59	<.001	1	5.07	0.03	239.17
Former land-use	54	654.25	<.001	1	4.78	0.03	239.30
Plot size	54	950.21	<.001	1	1.24	0.27	240.38
Seed bed preparation	54	847.45	<.001	1	2.91	0.09	240.90
Study design	54	900.76	<.001	1	0.76	0.39	241.32
Full model	55	1102.26	<.001	NA	NA	NA	241.57
Replicates	54	1034.36	<.001	1	0.29	0.59	242.34
Seeding material	54	1005.76	<.001	1	0.29	0.59	245.96

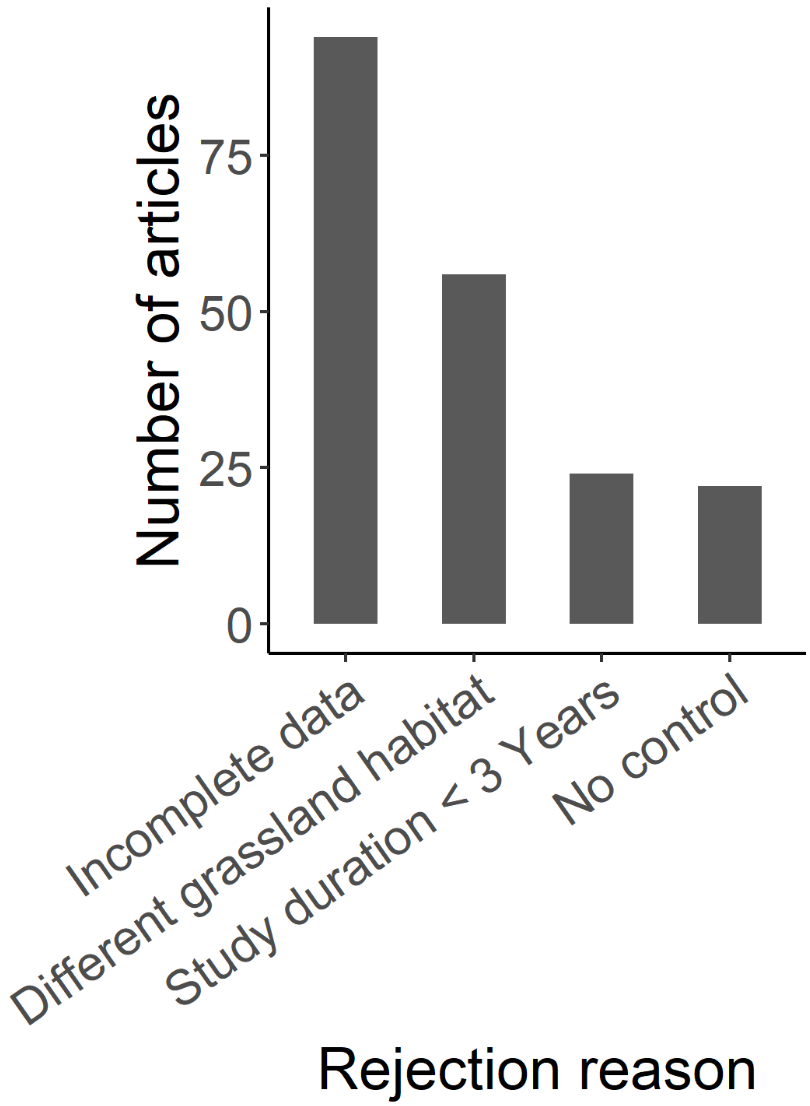

Figure 1 Articles with grassland restoration studies in temperate Europe which were excluded from the meta-analysis and their rejection reason (n = 187). More than one reason can apply to a single article. Articles with missing data did not report all the data that was necessary for our meta-analysis, or the data was not extractable from the figures. A different grassland habitat was a not mesic habitat, i.e., either dry or wet habitat.

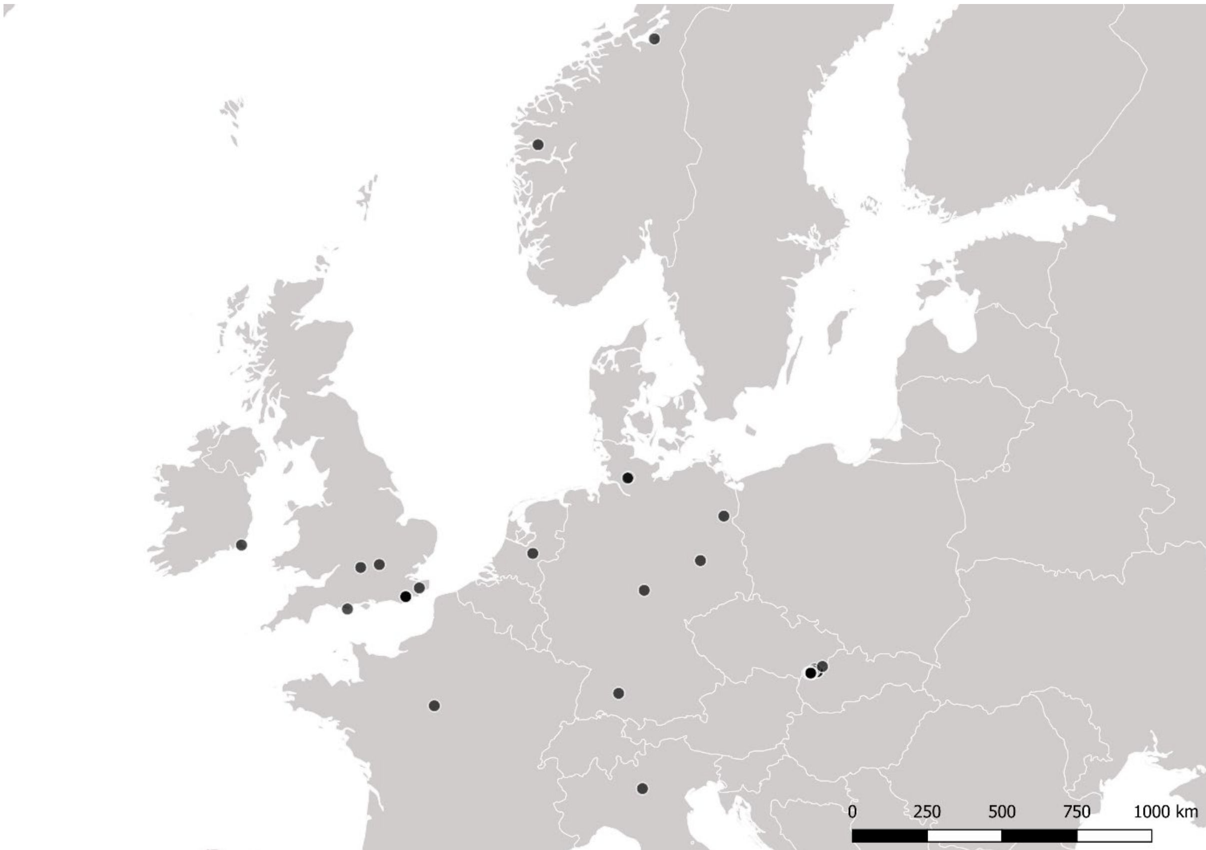

Figure 2 Overview map of study sites included in the meta-analysis.

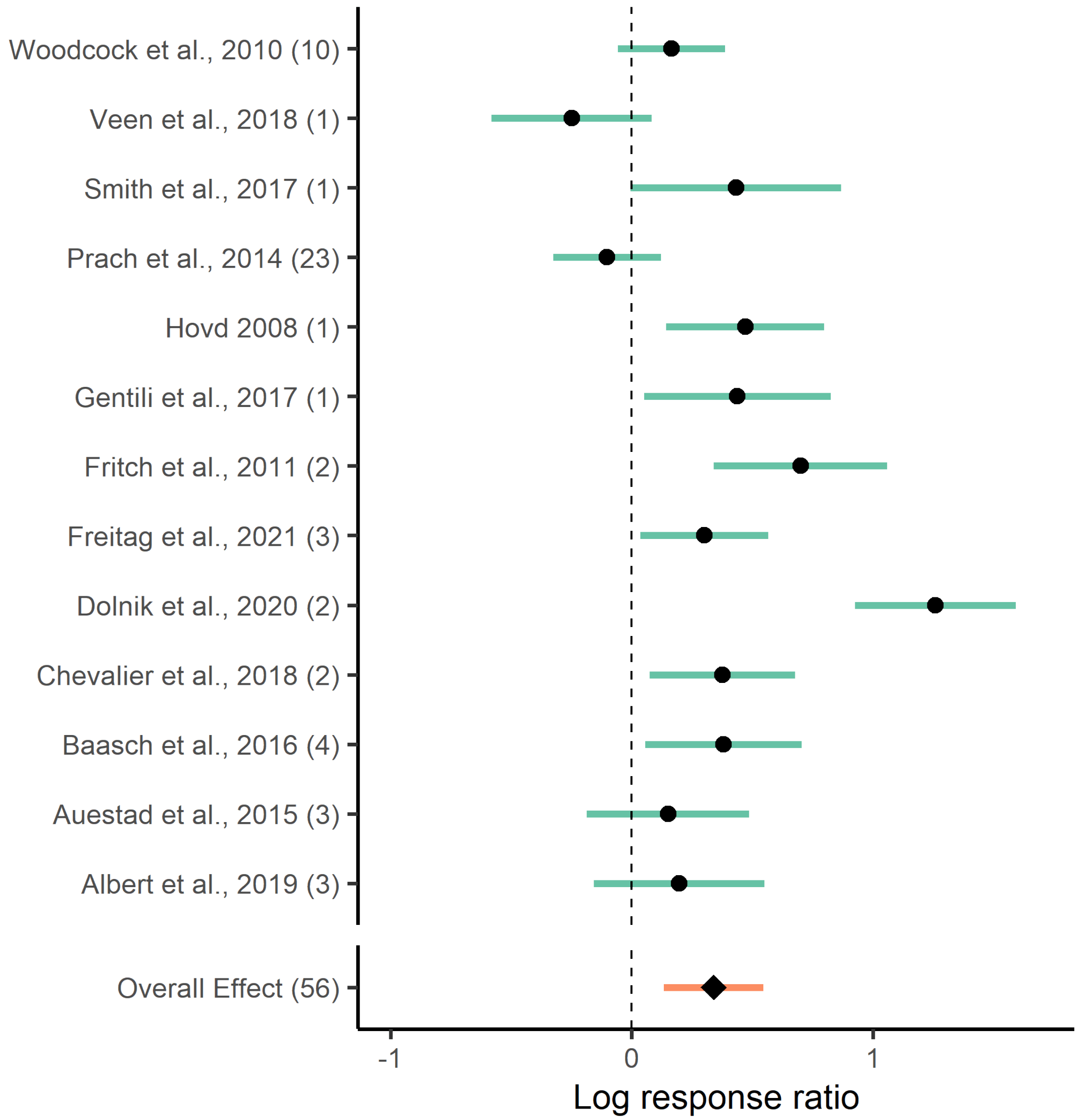

Figure 3 Forest plot with the mean effect sizes and 95% confidence interval per study (in green) and the overall effect of active grassland restoration on plant species richness (in orange). The number in brackets after each article represents the number of data points.

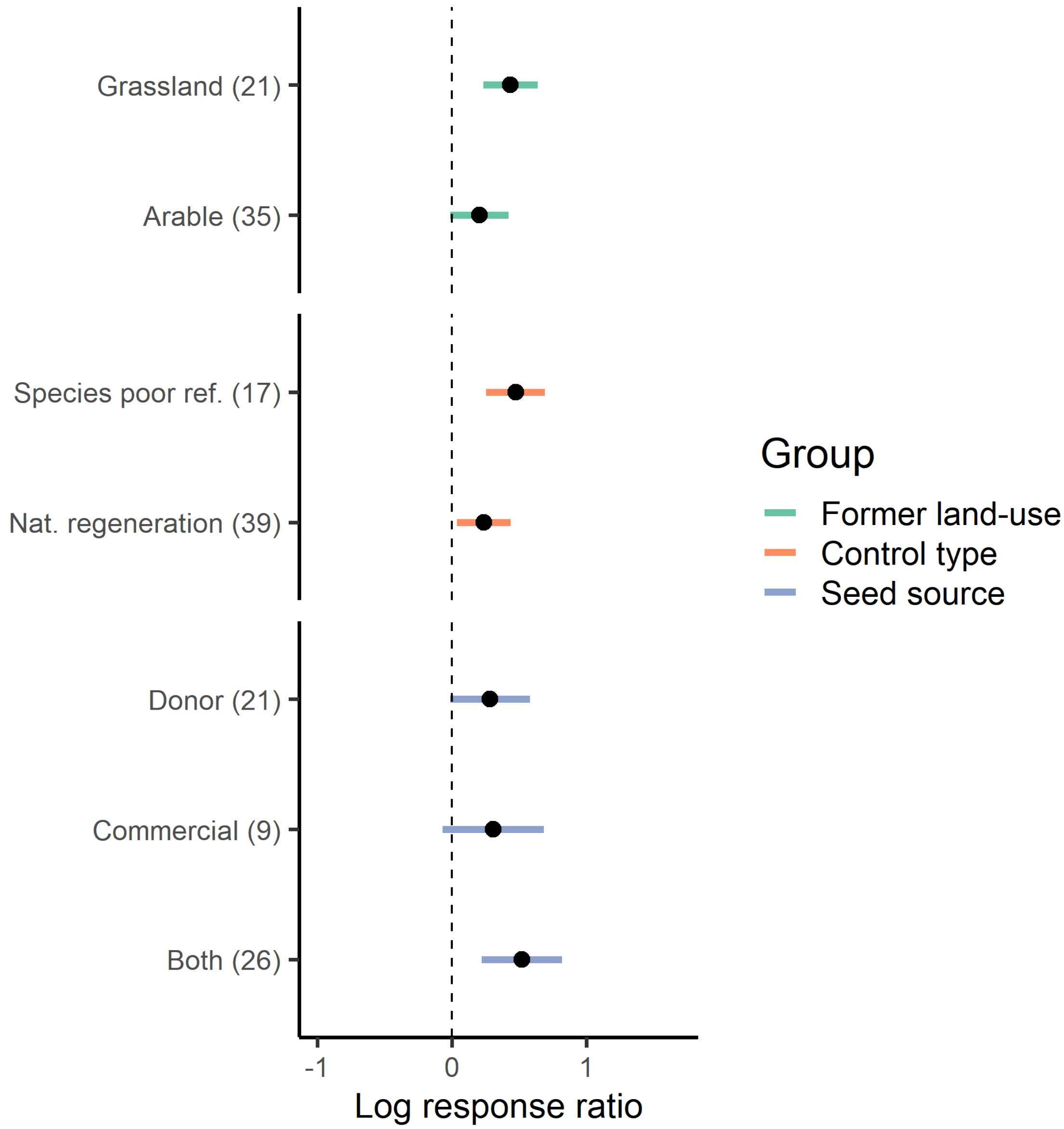

Figure 4 Forest plot with the mean effect sizes and 95% confidence interval of relevant moderators and the overall effect of active grassland restoration on plant species richness. The number in brackets after each category represents the number of data points. See Table 2 for a definition of the different moderators.

Supporting Information

Table S1 PICO structure of the primary question "Do different seed addition methods for the restoration or re-creation of species rich grasslands differ in their effectiveness to enhance diversity of plants?". PICO stands for: Population, Intervention, Comparator, Outcome

Question component	**Content**
Population	Grasslands in temperate Europe below the subalpine zone
Intervention	Restoration or re-creation of species rich grasslands through seed addition by at least one of the following methods: hay transfer, sowing of seed mixture (local or commercial) and with soil preparation before seeding
Comparator	Control plots/fields with no seed addition
Outcome	Changes in plant species richness

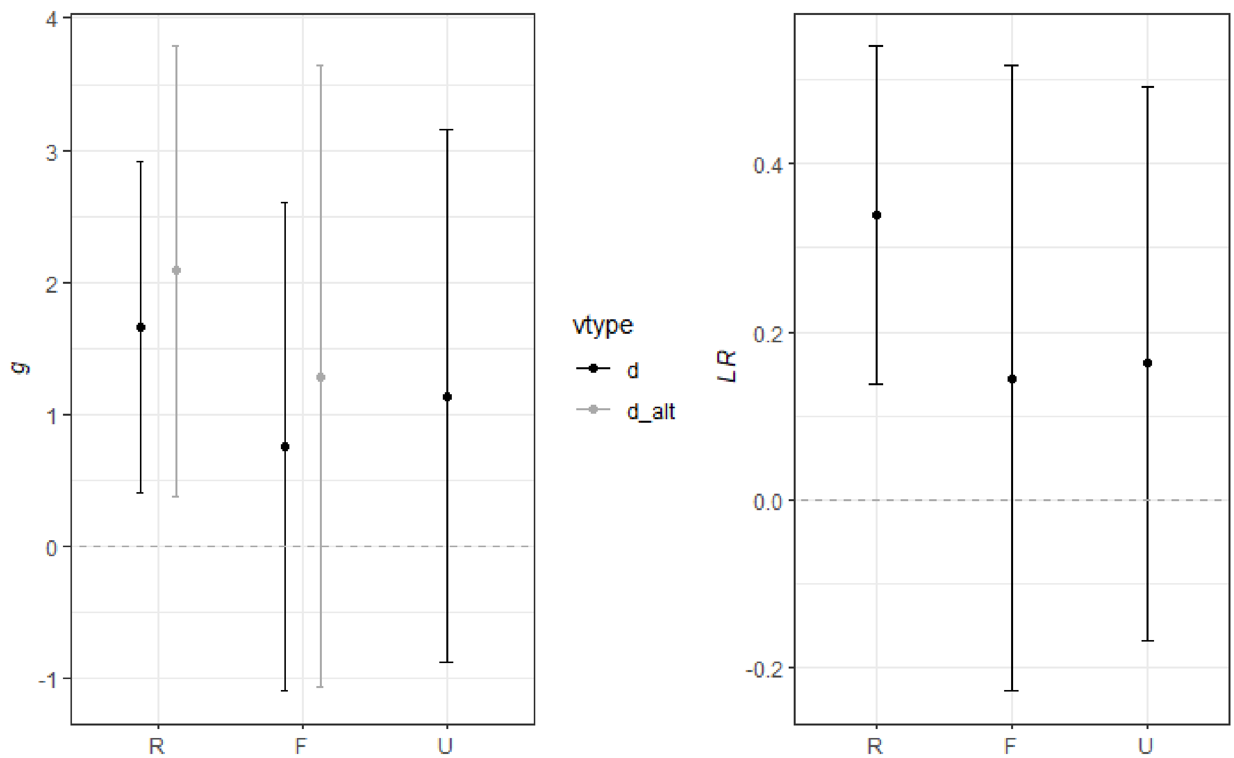

Figure S1 Meta-estimates as calculated with hedges' g (left) and log response ratio LR (right). These are global meta-effect sizes (±95% CI) from models with no moderators. For each effect size measure, we used random effects weighted (R), fixed effects weighted (F) and unweighted (U) models. Weighted meta-analyses of hedges' g (R, F) used variance estimators equal to the conventional variance (black) and also an alternative variance calculation (Hedges, 1982) (grey, d_alt). The difference is that the formula for the conventional variance contains the standardized mean difference, whereas the formula for d_alt is independent of it. There is no effect when the 95% CI band overlaps with zero.

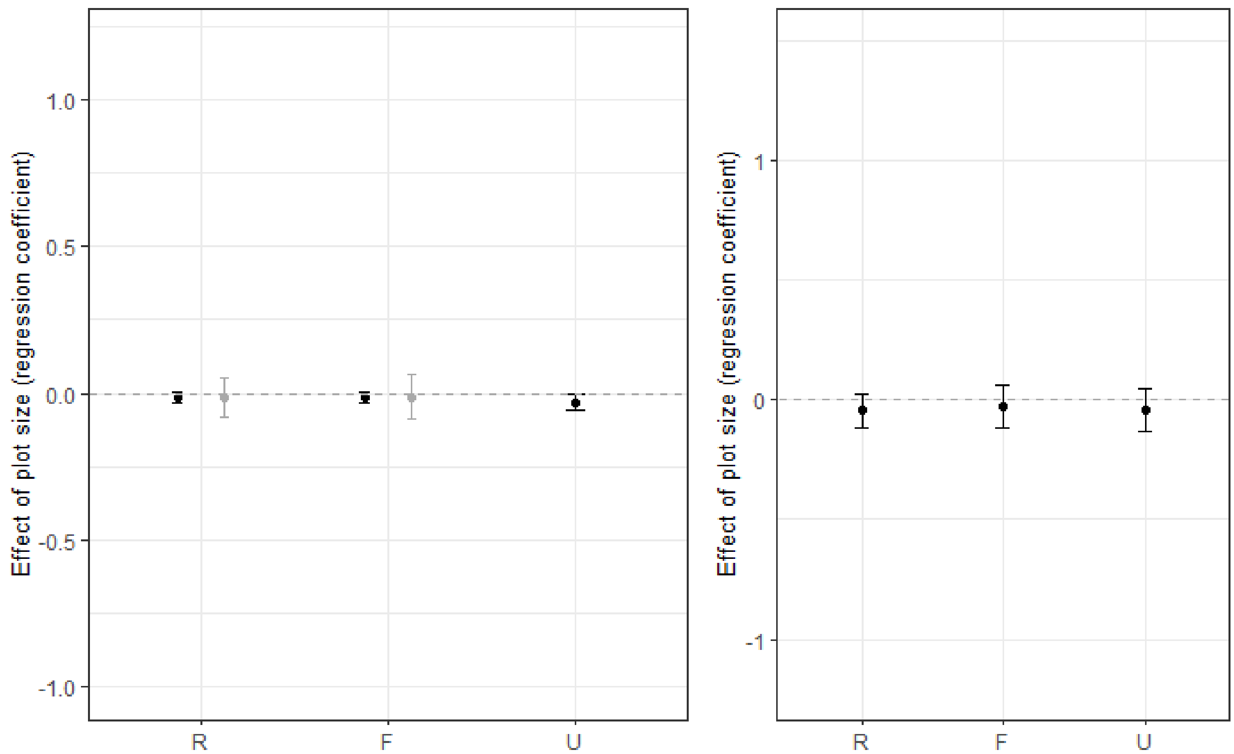

Figure S2 Effects of plot size on effect sizes of active grassland restoration on plant species richness. See figure caption of Fig. S1 for explanations.

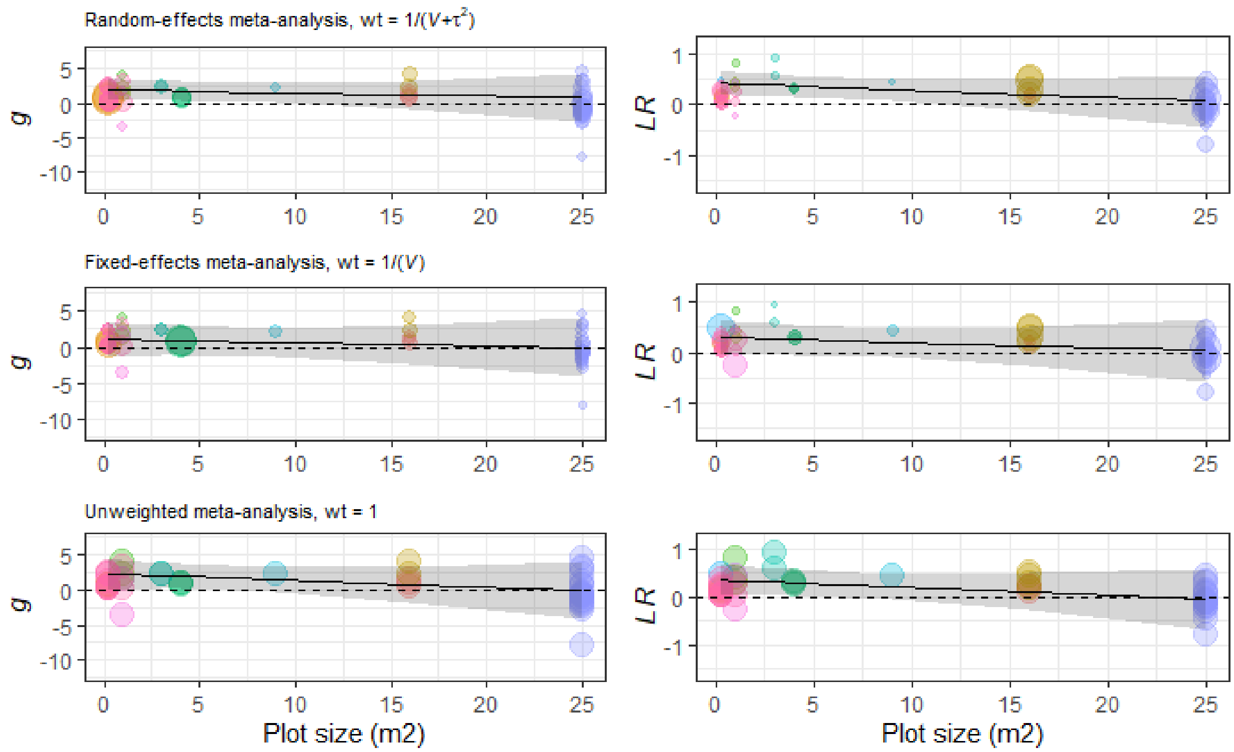

Figure S3 Influence of scale (plot size) on effect-size metric (columns) and model type (rows), for active grassland restoration on plant species richness. Meta-regressions have 95% prediction intervals (grey shading) based on uncertainty only in the plot-size effect. Point size is proportional to relative study weight for each meta-regression, with colours distinguishing different publications. Variances for hedges' g were estimated by the conventional variance measure (see figure caption of Fig. 1).

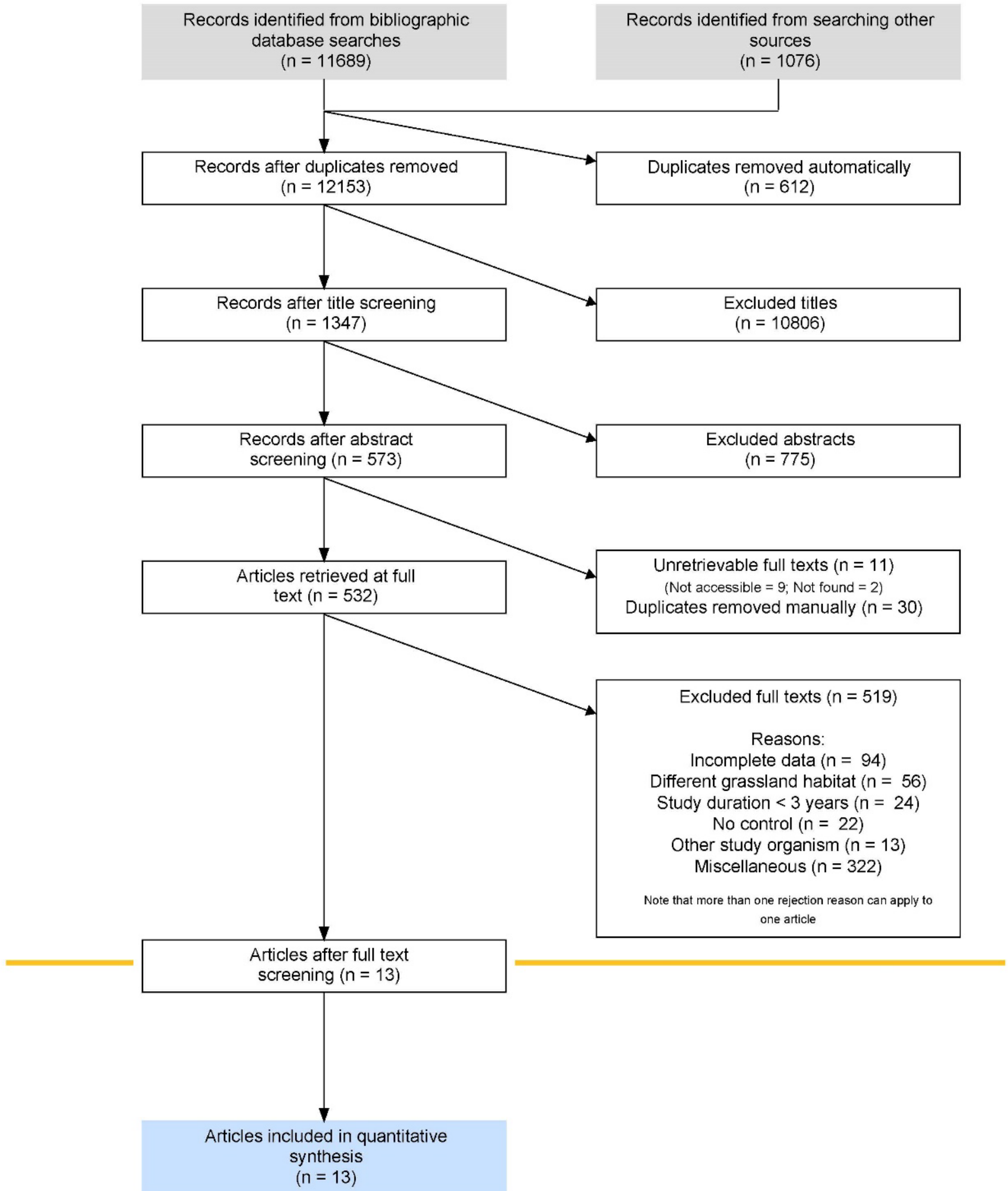

Figure S4 Flow chart showing the different screening steps. At full-text screening we compiled a separate list with excluded grassland restoration studies from temperate Europe to identify research gaps (n = 187, not shown here).

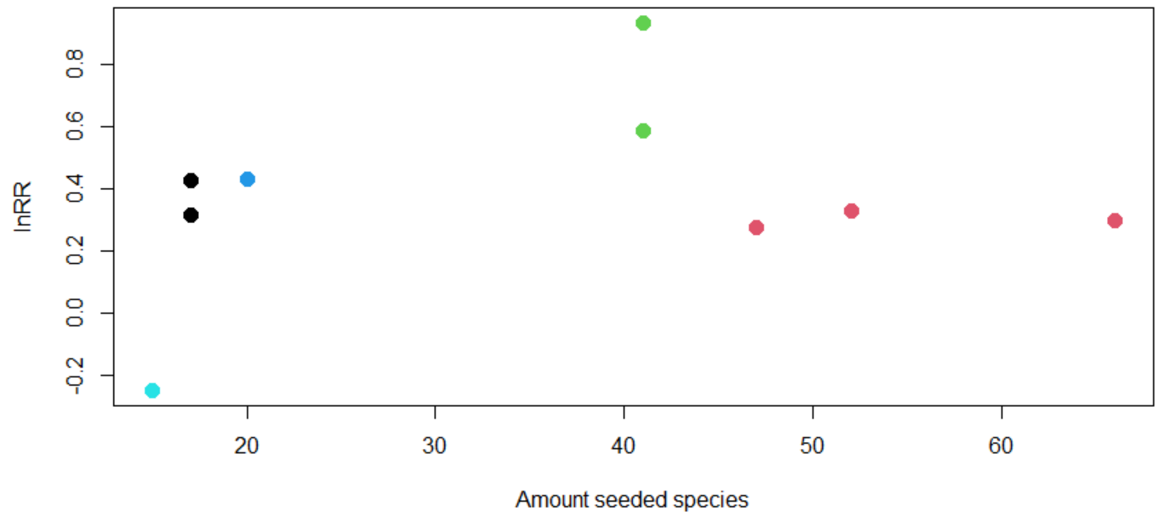

Figure S5 Scatterplot for the number of seeded species (x-axis) and the effect size in log response ration (lnRR, y-axis). Each point represents a single data point from the meta-analysis and the colours represent articles, meaning that points with the same colour belong to the same article. The data points in this plot are only having commercial seed mixes as seed source. There was no effect of the amount seeded species on the effect size (F(7) = 0.52, P = 0.5).

Chapter 2.1

Transfer of invertebrates with hay during restoration operations of extensively managed grasslands in Switzerland

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Abstract

Introduction Hay transfer from a speciose donor meadow to a species-poor receiver grassland is an established method to restore species-rich grassland plant communities. However, it has rarely been investigated to which extent invertebrates can be transferred with hay during such operations, which was the aim of this study.

Methods Sampling was conducted in eight sites of the Swiss lowlands with one donor meadow and two receiver sites each. On the receiver sites, three to four white bed sheets of one square meter each were deployed on the ground to receive a standard quantity of fresh hay just transferred from the donor meadow. All living invertebrates were collected from these sheets with an aspirator and subsequently identified to order level.

Results On average (± SD), 9.2 ± 11.3 living invertebrates per square meter were transferred with the hay. Beetles were the most abundant species group, representing 46.9% of all transferred invertebrates, followed by true bugs (8.9%) and spiders (7.0%). More individuals were transferred when the donor meadow was mown with a hand motor bar mower than with a rotary disc mower. Similarly, more invertebrates were transferred when the hay was transported loosely with a forage wagon than compacted as bales.

Discussion While this study demonstrates that living invertebrates can be transferred with the hay, their subsequent survival and establishment remains to be explored.

Implications for insect conservation We recommend using a hand motor bar mower and a forage wagon for increasing the survival probability of invertebrates in hay transfer.

Introduction

Several methods exist to actively restore or re-create grasslands. One commonly used method is the transfer of green, i.e. freshly mown hay from a species-rich donor grassland to a former arable land or species-poor receiver grassland, which was harrowed or ploughed beforehand (see Kiehl et al., 2010 for detailed description of the hay transfer method).The efficiency of the hay transfer method to increase plant or invertebrate diversity has been demonstrated in several studies (reviewed in Török et al., 2011 for plants, see Woodcock et al., 2010 for invertebrates). For example, Kiehl and Wagner (2006) found that 69–89% of the plant species from the donor grassland are transferred this way with the hay, with ca 66% being permanently established on the restored grassland after five years.

Invertebrates can also be trapped and transferred with the fresh hay in the same way. Indeed, Wagner (2004) demonstrated that *Metrioptera bicolor*, a grasshopper, can be directly transferred with this method. With a capture-mark-recapture approach, he established that 4.6% of the individuals capable to reproduce were transferred to a restored meadow. To the best of our knowledge, Wagner (2004) is the only study that investigated the potential of translocating invertebrates with hay. Furthermore, it remains unknown if other invertebrates than grasshoppers can be transferred this way.

The aim of this study was to identify and quantify, in terms of relative abundance, which invertebrates are effectively transferred with the hay from a donor to a receiver site. In effect, invertebrates have to survive several operations, including mowing, transportation and spreading of the hay (Humbert et al., 2010). Therefore, we hypothesized that the chances for a successful transfer of invertebrates are greater (1) when the donor meadow is mown with a lighter mowing machine (e.g. a bar mower instead of a rotary disc mower) and (2) when the hay is transported loosely and not compacted in bales.

Materials and methods

Experimental setup

The hay transfer and data collection were performed in June 2019 under warm and dry weather conditions. They took place in eight study sites located on the Swiss Plateau, an intensively-farmed lowland belt situated between the Alps and the Jura mountain ranges (elevation of study sites 423–712 m a.s.l.,Fig. 1). Each site included one plants speciose donor meadow (with 52–68 vascular plant species per meadow over the whole meadow and 26–47 vascular plant species within 2 × 4 m plots per meadow, meadow size 0.9–3.3 ha) and two receiver grasslands with a lower plant species richness (with 14–30 vascular plant species within 2 × 4 m plots per meadow, meadow size 0.2–0.9 ha). This resulted in a total of eight donor and 16 receiver meadows. Donor meadows were mesic hay meadows belonging to the *Arrhenatherion elatioris* community with a slight influence of the *Mesobromion* community. These meadows were managed extensively since at least 20 years, i.e. without fertilizer input and a first cut after June 15th. Receiver grasslands were also extensively managed since at least seven years.

Prior to restoration, receiver meadows were either ploughed in March–April or harrowed just a few days before the transfer of the hay. To make the hay transfer possible within one day (i.e. mowing the donor meadow, transport the hay and spread it on the receiver site) and to avoid loss of seeds, the distance between the donor and receiver sites within a study site was not more than 10 km. In two sites, the donor meadows were mown with a hand motor bar mower, whereas at the other six sites a rotary disc mower was used. The transport of the hay was done for 13 meadows with a forage wagon and for three meadows as hay bales (Fig. 2). On each receiver site the hay was spread in a proportion of 1:1, i.e. 1 m^2 of hay of the donor meadow was scattered on 1 m2 of the receiver site.

Invertebrate sampling

Invertebrate sampling was carried out during the hay spreading operation. The hay was spread over three or four white 1 m^2 linen bed sheets that were placed on the ground of any receiver meadow before the transfer. Each sheet received the freshly mown grass collected from 1 m^2 of the donor meadow (Fig. 3a). Just after spreading the hay we closed the sheets to avoid invertebrates to escape (Fig. 3b). Next, we carefully opened the sheets and collected with an aspirator every living invertebrate that we could detect (i.e. > 1–2 mm). Ants were not collected because no survival was expected without their colony. Afterwards, the samples were stored in a freezer. In the lab we sorted and counted all sampled invertebrates to order level (in total 16 taxa).

Data analyses

We analysed the quantity of transferred invertebrates with generalised linear mixed-effects models. Models were always run with the rounded average number of transferred invertebrates per meadow (two meadows per region) as response variable, whereas study site (spatial replicates) was set as a random effect. We first analysed the influence of the transfer technique by comparing the total number of invertebrates that were found after being transferred with a forage wagon ($n = 13$) or as hay bales ($n = 3$). Since the residuals were overdispersed, we corrected for it by adding an observer ID as a random effect. Secondly, the model was applied to assess the effect of the mowing machine, i.e. bar mower ($n = 4$) vs disc mower ($n = 9$). Due to the low sample size for hay bales (3 out of 16 receiver meadows) and the significant effect of the transport technique, only the data of forage wagon were used as an explanatory variable for the mowing machine analysis. All statistical analyses were performed with R version 3.5.1 (R Core Team, 2018).

Results

In total we sampled 429 invertebrates belonging to 16 taxa (Table 1, Appendix Fig. 5). The average number of transferred invertebrates per square meter ± SD (standard deviation) ranged between 9.2 ± 11.3 (n = 13) when the hay was transported from the donor to the receiver site with a forage wagon and 0.8 ± 1.2 (n = 3) with hay bales (estimate = 2.304, SE = 0.911, z = 2.529, P = 0.011; Fig. 4a). Beetles were the most abundant species group, representing 46.9% of all transferred invertebrates, followed by true bugs (8.9%) and spiders (7.0%). Although snails were the second most abundant group (9.3%), their fraction was lower than 1% when one site with super abundant snails was discarded. Larvae included all juvenile specimens, irrespective of whether they were attributable to a taxon or not (except for five sampled orthopterans that were all nymphs). Likewise, the type of mower had an influence on the number of transferred invertebrates: more invertebrates were transferred when the donor meadow was cut with a bar mower (n = 4) than with a disc mower (n = 9; estimate = 1.153, SE = 0.374, z = 3.08, P = 0.002; Fig. 4b).

Discussion

This study shows that a variety of living invertebrate taxa can be successfully transferred from a donor to a receiver meadow with the hay transfer method. It further suggests that when a forage wagon is used for transporting the freshly cut hay, 9.2 invertebrates per m2, on average, were transferred. Extrapolated to one hectare this figure sums up to 92,000 transferred individuals. Given that the detectability of smaller invertebrates is generally low, this figure should be considered as conservative.

We do not know the original invertebrate densities in the donor meadows for 2019, but true bugs and spiders were sampled in these same eight donor meadows in 2018 using suction sampling (as in Buri et al., 2016). Looking only at the donor meadows for which a forage wagon had been used, we sampled, in 2018, on average, 21 adult true bugs and 49 adult spiders per m^2 (unpublished data). Therefore, assuming similar population densities in 2018 and 2019, we can estimate an average transfer rate of 2.5% (median 1%, range 0–10%) for true bugs and 2.3% (median 0.7%, range 0–14%) for spiders. Regarding beetles, we have no previous quantitative estimates of densities as they were sampled with pitfall traps, which cannot be related to a reference sampling area.

Ten times more living invertebrates were transferred when a forage wagon was used compared to bales, although sample size for the latter method was small. This was expected as baler machines compact the hay, including animals trapped in it, much harder than forage wagons. Although we could not find studies on the effect of baling on the survival of invertebrates, we expect it to be much lower due to the impact of compaction. Similarly, fewer invertebrates were transferred when the donor meadows were cut with a rotary disc mower than with a hand motor bar mower. This is probably due to the higher mortality induced by rotary mowers, which are powered by tractors, than by hand motor bar mowers that have light engines (Humbert et al., 2010). Although the type of mowing machine had a significant influence on the number of transferred invertebrates, it has to be taken into account that the overall sample size was also disproportionally smaller for the bar mower.

Although this study demonstrates that many living invertebrates are transported via the hay transfer method typically applied in active grassland restoration operations, it measured neither the survival nor the establishment success of the translocated invertebrates. To constitute a new viable population, a minimum number of individuals should be transferred (Shaffer, 1981). Gardiner (2010) showed that translocating 40 adult individuals (sex ratio of 1:1) of the orthopteran *Myrmeleotetrix maculatus* led to reproduction the following year. Berggren (2001) obtained a minimum population size of

32 individuals for efficiently translocating the orthopteran *Metrioptera roeseli* to previously uninhabited meadows. However, at the time of hay transfer, the vegetation is very scarce or not present, which might represent a serious impediment to invertebrate installation, notably of herbivorous species. Especially for less mobile species it is more difficult to move to more densely vegetated field margins or adjacent meadows (Thorbek & Bilde, 2004). To circumvent the issue of a non-vegetated receiver site, a second hay transfer after the restoration of the plant community may be foreseen as an option to further increase invertebrate diversity and abundance (Kiehl & Wagner, 2006). Another option would be to set aside an unploughed vegetated meadow patch or strip on the receiver site, which can serve as refuge during the vegetation free period (Humbert et al., 2012). In addition, Woodcock et al. (2010) found that invertebrates can recolonize restored meadows after hay transfer, once that a more diverse plant community is established. The recolonization rate of invertebrates, however, depends on the landscape and connectivity to other source populations.

In light of our results, we recommend to mow the donor meadow with a hand motor bar mower and transport the fresh hay with a forage wagon. This will maximize the total number of transferred living invertebrates and thus increase the probability of establishment. Given that hand motor bar mowers are smaller and therefore more time consuming in mowing grass, this approach is only feasible on small meadows.

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Table 1 The proportion of transferred invertebrates on the receiver sites per taxa. In total 16 taxa were identified from 429 individuals. Juveniles of each taxa were pooled in "larvae", expect for orthopterans where only nymphs were found and are represented as an own group.

Taxa	Proportion (%)
Beetles	46.9
Snails	9.5
Larvae	9.3
True bugs	8.9
Spiders	7.0
Sternorrhyncha	5.1
Flies	4.4
Earwigs	3.3
Auchenorrhyncha	1.9
Orthopterans	1.2
Hymenopterans	1.2
Isopods	0.5
Caddisflies	0.2
Net-winged insects	0.2
Lepidopterans	0.2
Springtails	0.2

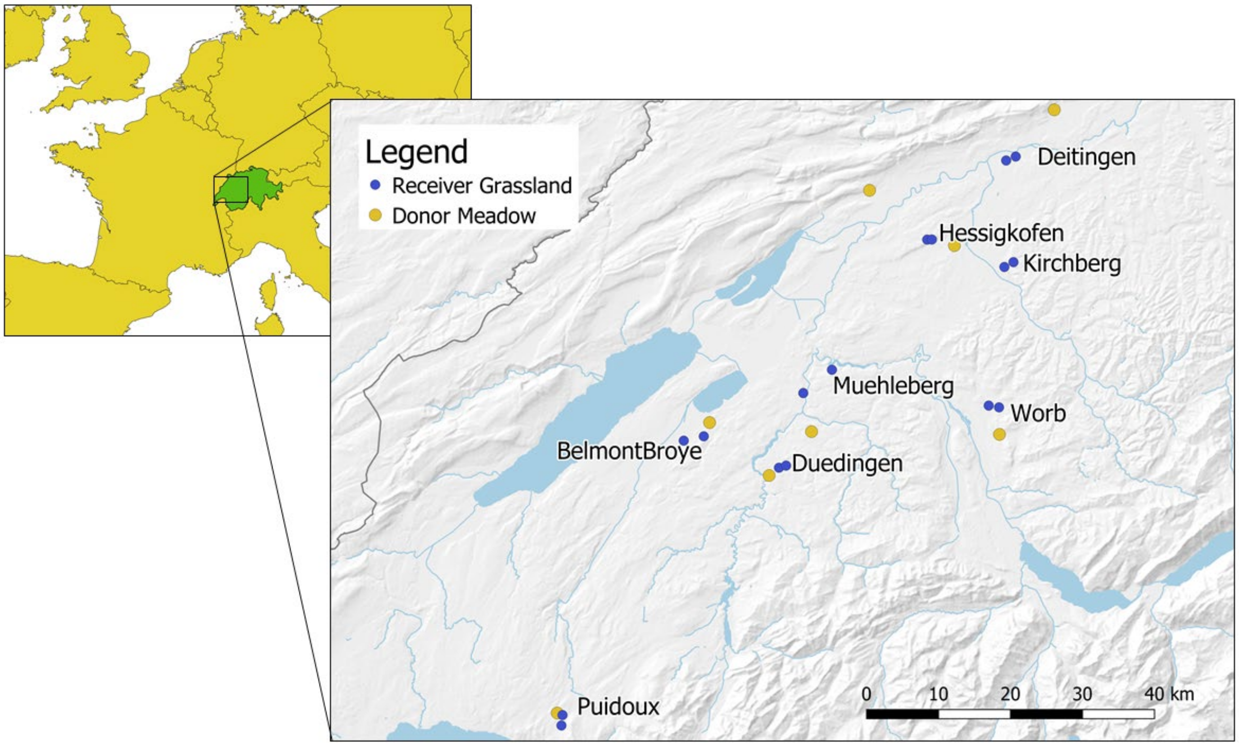

Figure 1 Study area in Switzerland. The donor meadows are represented with yellow dots, the receiver sites are represented with blue dots.

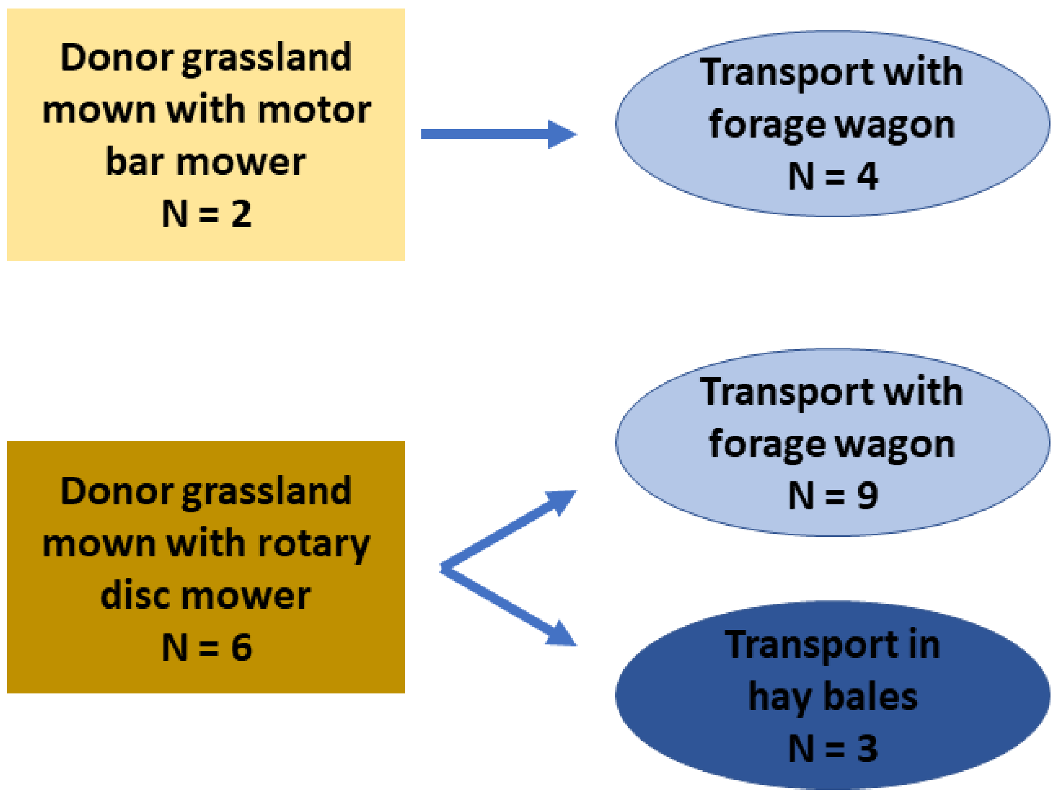

Figure 2 Overview of the mowing and hay transport techniques used in our experiment. Yellow squares represent donor meadows, blue circles represent receiver site.

Figure 3 Field material: (a) a sampling linen bed sheet with the equivalent of 1 m 2 of spread hay. In the background a forage wagon unloading the transferred hay onto the meadow; (b) sampling sheet closed to avoid living invertebrates to escape.

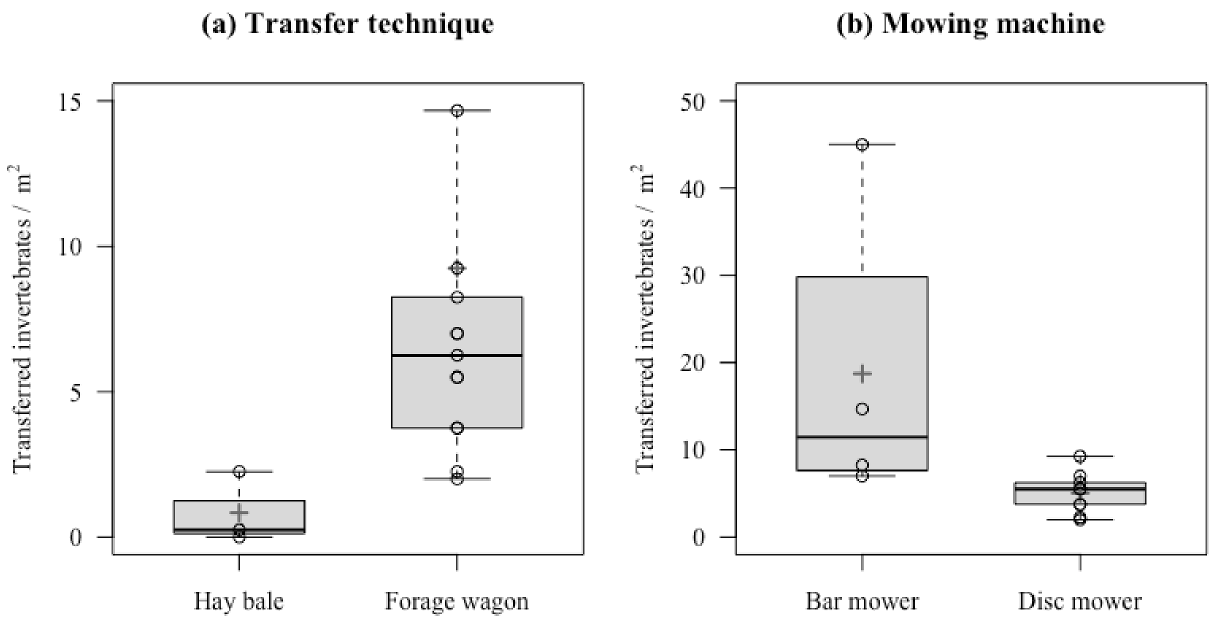

Figure 4 (a) Total number of transferred invertebrates per m 2 with respect to the transportation technique: hay bale: 0.8 ± 1.2, n = 3 and forage wagon, (mean ± SD) 9.2 ± 11.3, n = 13. (b) Number of transferred invertebrates per m 2 depending on the mowing machine: bar mower (mean ± SD) 18.7 ± 17.8, n = 4; and disc mower: 5.0 ± 2.3, n = 9. Means are represented as grey crosses.

Appendix

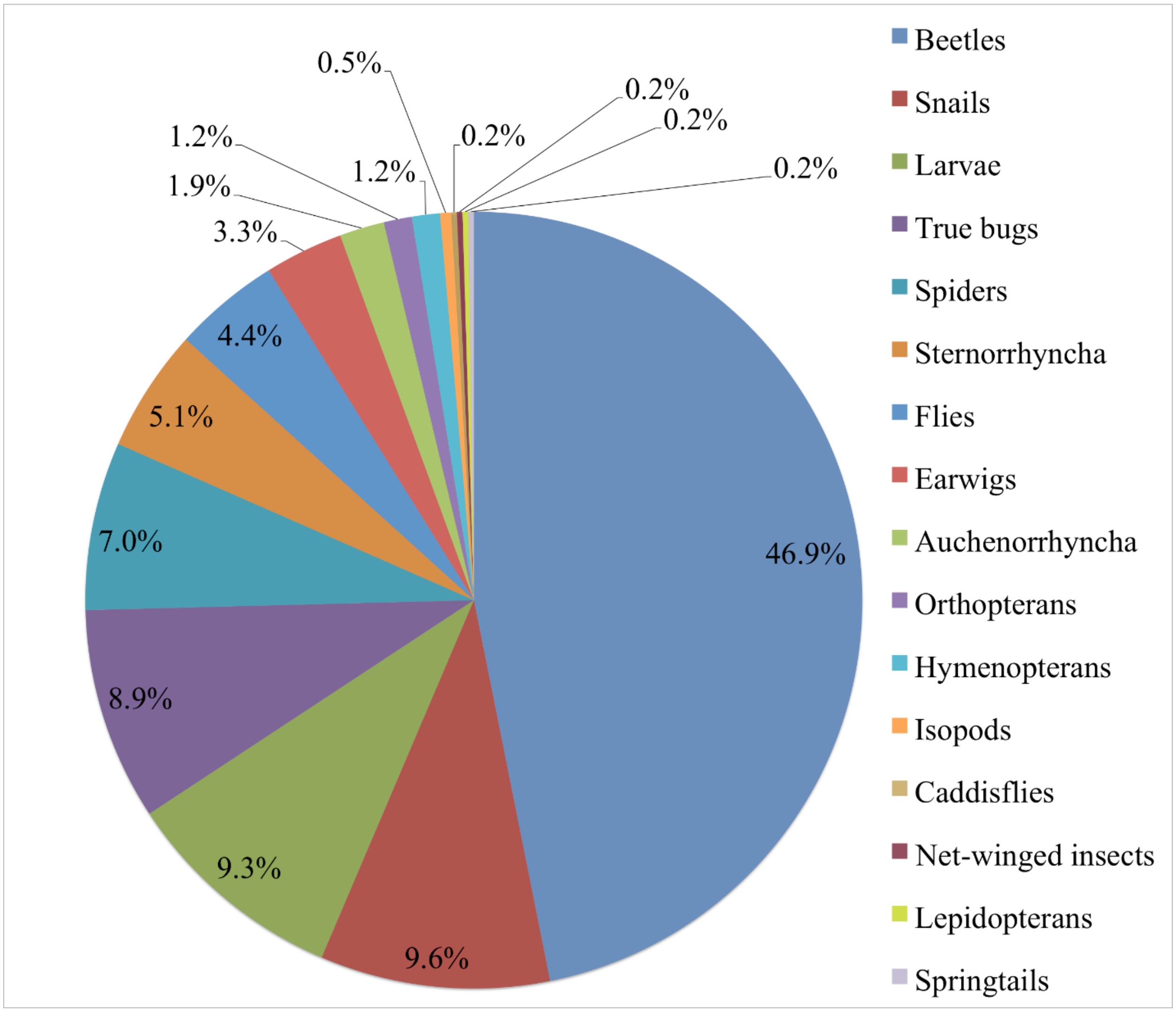

Figure 5 Overview of transferred invertebrates on the receiver meadows. In total 16 taxonomic groups were identified. Juveniles of each group were pooled in the category "larvae", except for orthopterans where only nymphs were found and are thus represented as an own group.

Chapter 2.2

No mid-term detrimental effects of soil disturbance linked to grassland restoration on established ground-dwelling invertebrates

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Abstract

Active grassland restoration requires soil disturbance by harrowing or ploughing prior to seeding to create favourable conditions for plant germination. Yet, it is still unknown if these soil interventions are detrimental to the local ground-dwelling invertebrate fauna. We evaluated how ground beetle (Carabidae) and spider (Araneae) communities responded to three common grassland restoration methods, differing in soil disturbance intensity and seed application method. The study was carried out in extensively managed species-poor mesic meadows using a before-after-control-intervention design. It was applied at the field scale and replicated twelve times across selected Swiss lowland sites. In addition, the potential mitigating effect of leaving an undisturbed area around the restored meadows was investigated. One year after restoration, abundance and species richness of ground beetles and spiders were the same on restored and control meadows. At the community level we observed a slight shift towards a preference for wetter habitat (for both invertebrate groups), and restored meadows harboured a smaller weighted mean body size of spiders than control meadows. The latter was mainly driven by a higher abundance of some pioneer species typically found in frequently disturbed habitats, like in arable fields. No effects of surrounding undisturbed areas were found. Our results suggest that one year after restoration action, the ground-beetle and spider communities recovered almost entirely to their pre-disturbance state, indicating that harrowing or ploughing can be applied when restoring plant species-poor grasslands without being concerned about detrimental effects on the local ground-dwelling invertebrates.

Key words: Ground beetles, spiders, hay transfer, plough, seeding, meadows

Introduction

Temperate grasslands are recognized as important habitats for plants (Wilson et al. 2012), invertebrates (Batáry et al., 2007; Woodcock et al., 2012) and play an important role in feeding livestock. In Europe, most grasslands are semi-natural, which means that mowing and grazing regimes are key factors for the maintenance of high grassland biodiversity (Hejcman et al., 2013). To achieve higher hay yield, fertilizer input and mowing frequency have increased drastically in the past decades. This intensification has led to a biodiversity decline and homogenization across many plant and animal taxa in grasslands (Gossner et al., 2016; Robinson & Sutherland, 2002). To counteract the negative impact of high intensity agriculture on biodiversity, agri-environment schemes were introduced in the 90s in Europe. These schemes led to a partial decrease of high intensity farming practices (e.g., through lower fertilizer input), but showed only limited success on grassland biodiversity (Kleijn et al., 2006; Pe’er et al., 2014). Thus, a more proactive approach is necessary to tackle the biodiversity crisis, which is emphasized with the launch of the UN Decade on Ecosystem Restoration 2021 – 2030 (UNEP 2022)

Active grassland restoration, i.e., the reintroduction of plant species, is often necessary in areas with a long history of intensive agriculture, which is the case in Central Europe. This is due to low availability of propagules in the seed bank and few remnant species-rich grasslands in the surrounding landscape (Bakker et al. 1996). To successfully restore the plant community of a species-poor grassland, mechanical soil disturbance is necessary prior to seeding. This disturbance is required to open the grass sward to reduce competition from the existing plant community and to create optimal germination conditions (Freitag et al., 2021; Schmiede et al., 2012). Although, grassland restoration has gained in importance in practice and research (Török et al. 2021), the majority of grassland restoration studies focus on the effects of restoration treatments on the plant community (Kiehlet et al., 2010), while some studies looked at the recolonization of invertebrates after restoration (DiCarlo & DeBano, 2019; Woodcock et al., 2010). Nowadays it is still unknown if mechanical soil disturbance linked to grassland restoration have a negative effect on the existing ground-dwelling invertebrate community. This is particularly important in current times, where invertebrate declines are becoming more evident (Seibold et al., 2019; van Klink et al., 2020).

Our main objective was to find out whether different intensities of soil disturbances linked to grassland restoration have a detrimental effect on the ground-dwelling invertebrate community. We were also interested in whether an undisturbed part of a meadow, which was exposed to restoration, will have a mitigating effect on the invertebrates. Such an undisturbed area could serve as a refuge or harbour an intact source population, which could recolonize the meadow after restoration, similarly as with uncut refuges during mowing operations (Humbert et al., 2012). To address these questions, we launched a field-scale restoration experiment in twelve selected Swiss lowland sites in 2018 with three different restoration treatments and an unrestored control. The restoration treatments differed in soil disturbance intensity and seeding method. Soil disturbance was achieved either with a rotary harrow, i.e., superficial disturbance up to 10 cm depth, or with a plough, i.e., tilling beyond 20 cm depth. Seeding was done in form of green hay or seeds. All experimental sites were extensively managed, but plant species-poor, mesic hay meadows and were restored in early summer 2019.

We selected for our study ground beetles (*Carabidae*) and spiders (*Araneae*) as bioindicators to represent the ground-dwelling invertebrate community. Both groups have proven to be valuable bioindicators to monitor disturbances and restoration success (DiCarlo & DeBano, 2019; Koivula, 2011). Furthermore, these organisms provide important ecosystem services such as natural pest control agents feeding on a wide variety of prey and food source of higher trophic levels (Lövei & Sunderland, 1996; Luff, 1987; Nyffeler &

Sunderland, 2003). Previous studies have shown that intensive soil interventions can cause both direct and indirect mortality of these soil arthropods (Thorbek & Bilde, 2004). On the community trait level, shifts have been observed after soil disturbances for the traits body size and trophic level (Hanson et al., 2016; Kosewska et al., 2018). A shift in the community body size of spiders will also have an indirect effect on the community mobility. Smaller spider species tend to use aerial dispersion (ballooning) more frequently than bigger spiders (Thomas et al., 2003). For the ground beetle community, soil disturbances have shown mixed effects on the overwintering strategy, i.e., overwintering as larvae or as adults (Purvis & Fadl, 2002).

We predicted no differences in ground beetles and ground-dwelling spider species richness and abundance one year after restoration, irrespective of the soil disturbance intensity. While direct mortality due to harrowing or ploughing has been observed for ground-dwelling arthropods in the short-term (i.e., within few weeks after the disturbance event) on arable land (Shearin et al., 2007; Thorbek & Bilde, 2004), recolonization from the surrounding landscape occurred within days after disturbance, due to the high mobility of these arthropods (Pfingstmann et al., 2020). However, we expected to detect changes at the community level, such as a shift in the community body size. We further expect a shift in the habitat preferences of both ground beetles and spiders, given that grassland habitat conditions are prone to changes directly after restoration due to the arrival of new plant species (Albert et al., 2019; Woodcock et al., 2008). Finally, we expect no changes in the community heterogeneity. However, most of the above-mentioned effects on the ground dwelling arthropod assemblages have been studied in arable sites, where soil disturbances occur frequently. Given that in our restoration study these soil disturbances happen only once, we expect that the arthropod community will mostly recover in the mid-term, i.e., one year after restoration.

Methods

Study Sites and Experimental Design

In this study, we selected twelve study sites in the lowlands of the Swiss Plateau with an altitudinal range between 420 – 760 m (Fig. 1). This region is dominated by an Atlantic climate with a mean annual temperature range of 13.7° to 16.3°C, and an annual precipitation of 845 to 1,148 mm. High-intensity agriculture accounts for ~ 65% of the landscape, which is representative of Central Europe (Zingg et al., 2019). For our experiment we selected four extensively managed, mesic hay meadows within each study site, which makes a total of 48 meadows. These meadows were registered as biodiversity promotion areas (a Swiss equivalent to European agri-environment schemes), which implies no fertilization, no use of pesticides and a first cut not before 15 June. Selected meadows have been managed extensively for at least five years and their surface was on average 0.5 ha, ranging from 0.14 ha to 1.0 ha. The minimum distance between the meadows was 330 m, while staying within a 3 km radius. In addition, one species-rich donor meadow per study site served as a seed source for restoration.

Restoration actions took place in May and June 2019. Within each study site, three different restoration treatments plus an unrestored control were assigned at random to the four meadows (Table 1). Restoration occurred on a field-scale to ensure independence between treatments, i.e., one restoration treatment was assigned per meadow. The harrowed treatment HH had the lowest soil disturbance intensity among all. The respective HH meadows were harrowed with a rotary harrow one week before seeding occurred and then they were re-harrowed on the day of seeding. The two plough treatments HP (Fig. 2) and SN had the strongest soil disturbance intensity. Three months before seeding, these meadows were ploughed beyond a depth of 20 cm and then harrowed every 4–6 weeks until seeding to inhibit the establishment of undesired weeds. All restoration methods complied with the guidelines proposed by the Swiss Association for the Development of Agriculture and

Rural Areas (AGRIDEA, Staub et al. 2015). One restoration with the SN method failed, which left us 47 meadows in total.

Due to logistic constrains or the proximity of hedgerows or forest hedges, some of the receiver meadows were not entirely restored. Thus, the meadow parts that were neither ploughed nor harrowed might have served as refuge areas for invertebrates during the restoration, similarly to what is observed during mowing events (Humbert et al. 2012). To include the effect of this refuge opportunity in our analyses, we calculated a refuge opportunity index. The refuge opportunity index is a ratio of the non-restored area divided by the entire meadow area and ranged from 0 to 1, where the value of 0 represented meadows with its entire surface restored (i.e., no opportunistic refuge available) and where the hypothetical value of 1 represented meadows with none of its surface restored (i.e., entire surface available as opportunistic refuge).

Invertebrate Sampling

Our experiment followed the before-after-control-intervention (BACI) framework. All meadows were sampled in 2018, i.e., one year before restoration, and again in 2020 one year after restoration. We used pitfall traps to assess invertebrate communities during four sampling sessions of one week each. The first two sampling sessions were carried out between mid-May and mid-June before the first cut of the meadows. The second two sessions were carried out shortly after the first cut, i.e., from July on. Four pitfalls were buried in the corners of a 10 x 10 m square and at least 10 m from the meadow edge to avoid edge effects. To prevent micromammals from being trapped, the pitfalls were directly covered with a metal grid with a mesh size of 2.2 cm. To avoid water overflow by rain, the pitfalls were covered with a transparent plastic cover fixed with nails 5 cm above the trap. To kill and preserve the invertebrates the traps were filled with propylene glycol diluted with water with a ratio of 2:1. In addition, a pinch of odour-free detergent (sodium dodecyl sulphate) was added to reduce the surface tension and allow trapped specimen to drown. From a total of 1,520 traps from both sampling years, we had to discard 47 traps because micromammals were caught, or the pitfalls were dug out. Finally, we sorted the remaining pitfalls in the lab, counted all individuals and stored them in 60% ethanol for later identification. We identified adult specimens of ground beetles and spiders to species level from one out of four pitfalls per session following the nomenclature of Müller-Motzfeld 2004, Nentwig et al. 2020 and Trautner & Geigenmüller 1987. In addition, we checked for occurrence of red listed ground beetle species from the red list in Switzerland (Duelli, 1994). There is no red list for spiders in Switzerland.

Statistical Analyses

Abundance and species richness

For all analyses based on the abundance of ground beetles and spiders we used the mean abundance per pitfall trap per meadow and year. Using the mean abundance per pitfall and not the sum allowed us to limit the significance of incidental factors affecting trapping. The species richness for ground beetles and spiders was pooled per meadow and year using the data of all identified individuals (always based on four pitfall traps per meadow and year). This resulted in 47 mean abundance and total species richness values per year for all meadows for ground beetles and spiders.

To account for a potential year effect, we calculated the differences between the years 2020 and 2018 of both abundance and species richness per meadow. We then tested if the difference in abundance or species richness of a restoration treatment was different from the difference of the control. If a difference in the control is detected, a year effect can be assumed. We fitted univariate linear mixed-effects models (LMMs) with restoration treatment as explanatory variable using restricted maximum likelihood (REML) and study area as random effect. As response variables the differences between 2020 and 2018 in abundance or species richness of ground beetles or spiders were used. Furthermore, to test the effect of refuge opportunities during restoration, we used only the data from 2020 (after restoration) for ground beetle and spider abundance and species richness as response variables and the refuge opportunity index as explanatory variable. The control treatment was excluded from the refuge opportunity analysis. Model assumptions were fulfilled, therefore a normal error distribution was kept. All analyses were performed with the statistical software R version 4.1.1 (R Core Team, 2021) and the package *lme4* (Bates et al. 2015).

Traits and community

We extracted traits that could be affected by the restoration interventions from the literature for most ground beetle and spider species (Tables 2, S2 and S3). To include these traits into our analysis, we calculated a trait-based community weighted mean (CWM) for each trait separately for ground beetles and spiders for each meadow. The community indices *CI* were calculated as follows:

$$CI = \sum_{i=1}^{n} \frac{N_i}{N_{tot}} * SI_i$$

Where N_i is the abundance of the species *i*, N_{tot} is the summed abundance of all species and SI_i is the specific index of the species *i*. To calculate the *CI*, we used the *bat* function from the *BAT* package (Cardoso et al., 2021). The use of ranked discrete categories created gradients. To test the effect of the restoration methods on the CWM of a respective trait of ground beetles or spiders the same modelling approach as with abundance and species richness was used, i.e., we fitted univariate LMMs with the difference between 2020 and 2018 of the CWM of a trait as explanatory variable and restoration method as response variable.

Furthermore, multivariate analyses were performed to assess how the ground beetle and spider community were affected by the restoration treatments. We conducted these analyses separately for ground beetles and spiders for the years 2018 (before restoration) and 2020 (one year after restoration). First, permutational tests for homogeneity of multivariate dispersions (PERMDISP) with Tukey honest significant differences (TukeyHSD) were conducted for change of heterogeneity between treatments as a measure of β-diversity (Anderson, Ellingsen, & McArdle, 2006). Second, permutational multivariate analysis of variance (PERMANOVA) was used with 9,999 permutations and the Bray-Curtis dissimilarity measure as a distance metric to test for significance in a community shift. All PERMANOVA outcomes were plotted on the first two principal coordinate analysis (PCoA) axes. We used the *vegan* package (Oksanen et al., 2020) for the multivariate analyses.

Results

In 2018, we collected a total of 12,258 ground beetles and 25,179 spiders. In 2020, i.e., one year after restoration actions, we collected 12,402 ground beetles and 21,513 spiders. We identified 70 species of ground beetles being represented by 6,465 specimens (3,070 ground beetles in 2018 and 3,395 in 2020, respectively). For spiders, we identified 68 species represented by 11,394 specimens (6,243 spiders in 2018 and 5,151 in 2020, respectively). The most abundant ground beetle species was *Amara fulvipes* with 1,516 individuals (23% of the whole community). The most abundant spider species was *Pardosa palustris* with 5,564 individuals (49% of the whole community). Among the identified ground beetles, seven species were red listed. Among these, five species were very low abundant with $\leq$ 10 individuals. The two remaining red listed species, *Amara kulti* and *Anisodactylus nemorivagus*, were present across all treatments before and after restoration (Table S1). A full species list with their respective abundances is provided in the supporting information (Tables S2 and S3)

Abundance and species richness

We did not observe any difference in abundance for ground beetles and spiders for any restoration treatment compared to the control (Tables 3 and 4, Figs. 3A and C). Ground beetle species richness was not affected by any restoration method (Table 3, Fig. 3B). We found that the spider species richness was positively affected by the HH treatment versus the control (P = 0.038, Table 4, Fig. 3D). These outcomes did not change after re-running the same analyses without the most abundant species (*Amara fulvipes* for ground beetles or *Pardosa palustris* for spiders). The refuge opportunities, i.e., the proportion of unrestored meadow area, had no effect on ground beetle and spider abundance or species richness (Table S4).

Traits and community

For ground beetles, no restoration method showed an effect on the community indices body size, hibernation index and trophic level. The habitat preference index of ground beetles became slightly higher in the SN treatment compared to the control, indicating a higher preference towards wetter habitats (P = 0.009, Table 3). For spiders, all restoration methods had a negative effect on the community weighted mean body size (C vs. HH: P = 0.015, C vs. HP: P = 0.009, C vs. SN: P = 0.013, Table 4, Fig. 4A), i.e., the average size of spider species in the community became on average 0.7 mm smaller than before restoration. This effect became more pronounced when we removed the most abundant spider species *Pardosa palustris* from the analysis. We noticed that two small spider species, *Erigone dentipalpis* and *Oedothorax apicatus*, increased in abundance in all restoration treatments after restoration (Figs. 4B and S2). The habitat preference index for spiders was slightly lower in the treatments HP and SN compared to the control meadows, indicating higher preference of the spider community towards a wetter habitat after restoration (C vs. HP: P = 0.040, C vs. SN: P = 0.023, Table 4). The mobility index was higher for the HP treatment only compared to the control, indicating more frequent ballooning (P = 0.008, Table 4).

Our PERMDISP revealed that the heterogeneity of both ground beetle and spider communities was not significantly affected by any restoration treatment (Figs. 5, S3). Similarly, the PERMANOVA showed a consistent overlap and sizing of the polygons between all treatments and years for both ground beetle and spider communities (Fig. 5). Before restoration, there was no difference in community identity between the treatments for both ground beetles (F = 0.707, Pr(>F) = 0.817) and spiders (F = 0.760, Pr(>F) = 0.775). However, we observed a significant shift in community identity for both ground beetles (F = 1.867, Pr(>F) < .001) and spiders (F = 1.366, Pr(>F) = 0.045) after restoration.

Discussion

Active grassland restoration through seed addition combined with soil disturbance has been shown to be an effective tool to enhance the plant community (Kiehl et al., 2010). However, little is known on the mid-term effect of such restoration methods on the established ground-dwelling invertebrate community. In our multi-site real-scale experiment we found no evidence of a negative impact of any restoration method including soil disturbance by harrowing or ploughing on ground beetle and ground-dwelling spider species richness and specimen abundance in mesic meadows. We observed some minor trait shifts in the respective invertebrate communities, which were mainly due to relative changes in species abundances. The novelty of our study resides in its focus of a potential negative effect caused by soil disturbance within grassland restoration operations on resident ground-dwelling arthropods. Our results suggest a recovery of the ground-dwelling arthropod community one year after disturbance by either harrowing or ploughing, which reduces the conflict of interest between enhancing the plant diversity of a grassland and not damaging the resident invertebrate community (Bell et al., 2001).

Except for a small increase in spider species richness after the harrowed treatment, the species richness and abundance of both ground beetles and spiders remained stable before and one year after restoration as well as in comparison to the control meadows. This is well in line with our first expectation. We assume that the soil disturbances induced immediate high mortality for the studied invertebrates. Direct mortality of soil invertebrates can reach up to 60% of the arthropod population due to ploughing (Thorbek & Bilde, 2004). Despite this high mortality, grassland invertebrate communities can recover relatively quickly. A similar mortality rate with subsequent recovery was observed as well after mowing events (Humbert et al., 2010). Given the high mobility of ground beetles and spiders, recolonization from the surrounding landscape can happen as fast as within a few days (Pfingstmann et al., 2020). In this regard, direct mortality was beyond the scope of our study aims since immediate effects of soil disturbance intensities would allow only for limited conclusion of the impact of a treatment on the invertebrate population. Further, our study design does not allow for conclusions about direct mortality since we sampled only one year after restoration. This study design is probably the same reason why we did not detect an effect of an unrestored refuge on the invertebrate abundance and species richness. For instance, leaving an undisturbed area may have accelerated recolonisation of the disturbed area, but this effect is likely not detected anymore after one year. Several studies have highlighted the importance of connectivity between habitats in the landscape for the recolonization of invertebrates (Knop et al., 2011; Woodcock et al., 2010).

Indeed, the Swiss agricultural landscape is dominated by semi-natural grasslands (58% of utilised agricultural area, Swiss federal office of statistics, 2022), which makes the vicinity of such a grassland with an intact invertebrate source population to our restored meadows very likely. Further studies would be necessary to confirm if our findings are consistent across different landscapes.

Minor shifts in the community traits confirmed our prediction, that community changes will be visible on the community trait level but not on the abundance and species richness level. Our results showed that the community weighted mean body size decreased for spiders within all restored meadows. At the same time, we observed an increase in number of two small sized spider species *Erigone dentipalpis* and *Oeodothorax apicatus* from the Linyphiid family which are often found in disturbed habitats such as arable lands (Rushton et al., 1989). The higher occurrence of these small spider species could also be an explaining factor in the increase in spider mobility, i.e., ballooning frequency, in the hay plough treatment, where we observed the highest increase in both species. Small spiders from the *Linyphiid* family are known to be good aerial dispersers (Bell et al., 2005). Given that both species are pioneer species, we expect their number to decrease in the following years, when the plant community will be fully established, which will reduce habitat openness. For both ground beetles and spiders, we noted a slight increase in humidity preference in the ploughed treatments, indicating a wetter habitat after restoration. A similar trait shift of spiders towards wetter habitats was observed as well after soil disturbances in xeric grasslands (Hamřík & Košulič 2021). Given that in our case the soil disturbance will no longer be repeated, we assume that, in the years to come, the habitat preference indices will return towards values found in the undisturbed control meadows. Our multivariate analyses on the community level further consolidate what we have found on the trait level. The community heterogeneity was not affected, while community compositions changed slightly due to abundance changes of some species. However, no distinct trend was visible for any of the treatments.

Finally, we could not find evidence that rare or threatened invertebrates were affected neither positively nor negatively by soil disturbance. However, there is no red list for spiders for Switzerland at the moment and the Swiss red list for ground beetles dates from 1994 (Duelli, 1994). Based on this list, we identified some low abundant rare species before and after restoration. Due to their low number (less than ten individuals over the entire study period) it was not possible to conclude how they reacted on the treatments. One nearly threatened ground-beetle species, *Amara kulti*, increased in abundance.

In conclusion, our results suggest that a single soil disturbance event has no mid-term detrimental effects on the local grassland ground-dwelling invertebrate community. We have included in our experiment three grassland restoration methods, differing in soil disturbance intensity, which are commonly used in Central Europe (Kiehl et al. 2010). Knowing that these restoration methods do not damage the existing invertebrate community in the mid-term is reassuring. Although our study focused on a potential mid-term damaging effect of soil disturbance on the resident invertebrates, we can expect that on a long-term more species will immigrate from the surrounding landscape into the restored meadows (DiCarlo & DeBano 2019; WallisDeVries & Ens 2010; Woodcock et al. 2012). There is also evidence that hay transfer can translocate invertebrates from one meadow to another and therefore accelerate the colonization process (Kiehl & Wagner 2006; Stöckli et al. 2021). In spite of our results, it remains to be experimentally established to what extent other grassland taxa such as snails or plant-dwelling invertebrates are affected by these restoration measures. The first years after grassland restoration are characterized by a changing plant community, which usually stabilizes after the third year of restoration (Albert et al., 2019; Freitag et al., 2021). Yet, vegetation-dwelling arthropods are more affected by changes in the plant community than the ground-dwelling arthropods and might therefore show a different response to the restoration treatments (Ebeling et al., 2018).

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Table 1 Overview of the restoration methods. The hay or seeds for the restoration treatments originated from a nearby, species-rich donor meadow (< 10 km).

	Control (C)	Hay harrow (HH)	Hay plough (HP)	Seeds natural (SN)
Seeding material	None	Hay	Hay	Seeds
Soil disturbance intensity	None	Low (Rotary harrow)	High (Plough)	High (Plough)

Table 2 Overview of traits that we included in our analysis. For all categorical traits we used integer numbers which were necessary for calculating the community weighted means. Trait information was extracted from the literature for ground beetles (Cole et al., 2002; Fazekas, 1997; Luka et al., 2009; Lundgren, 2009; Marggi, 1992; Müller-Motzfeld, 2004) and spiders (Cardoso et al., 2011; Entling et al., 2007; Macías-Hernández et al., 2020; Nentwig et al., 2020), respectively.

Trait	**Ground beetles**	**Spiders**
Body size	Continuous, in mm	Continuous, in mm
Habitat preference	Categorical 1 = xerophilous 2 = mesophilic 3 = hygrophilous	Continuous 0 = moist 1 = dry
Trophic level	Categorical 1 = herbivore 2 = predator	NA
Hibernation index	Categorical 1 = overwintering as larvae only 2 = overwintering either as larvae or as adults 3 = overwintering as adults only	NA
Mobility	NA	Categorical The frequency of a species to use ballooning: 1 = rare 2 = occasional 3 = frequent

Table 3 Pairwise test results for the effect of the three restoration methods compared to an unrestored control on ground beetles. We used the differences between the year 2020 (after restoration) and 2018 (after restoration) of a respective variable as input, to account for the year effect. Estimates plus-or-minus standard error are provided. Significant p-values (P < 0.05) are highlighted in bold. See Table 1 for details on the restoration methods and Table 2 for details on the traits.

	Control vs. Hay harrow			Control vs. Hay plough			Control vs. Seeds natural		
	Estimate	*t*	*P*	Estimate	*t*	*P*	Estimate	*t*	*P*
Abundance	2.25 ± 3.37	0.67	0.509	0.19 ± 3.37	0.06	0.954	-1.45 ± 3.46	-0.42	0.678
Species richness	1.67 ± 1.56	1.07	0.292	0.92 ± 1.56	0.59	0.560	2.46 ± 1.59	1.54	0.133
Body size	0.04 ± 0.50	0.08	0.939	0.99 ± 0.50	1.99	0.055	0.72 ± 0.51	1.42	0.165
Habitat preference	0.00 ± 0.09	-0.03	0.975	0.18 ± 0.09	2.02	0.052	0.25 ± 0.09	2.77	**0.009**
Trophic level	0.10 ± 0.09	1.04	0.306	0.19 ± 0.09	2.03	0.051	0.17 ± 0.10	1.81	0.080
Hibernation index	0.03 ± 0.17	0.20	0.841	-0.06 ± 0.17	-0.36	0.725	-0.02 ± 0.17	-0.12	0.906

Table 4 Pairwise test results for the effect of the three restoration methods compared to an unrestored control on spiders. We used the differences between the year 2020 (after restoration) and 2018 (after restoration) of a respective variable as input, to account for the year effect. Estimates plus-or-minus standard error are provided. Significant p-values (*P* < 0.05) are highlighted in bold. See Table 1 for details on the restoration methods and Table 2 for details on the traits.

	Control vs. Hay harrow			Control vs. Hay plough			Control vs. Seeds natural		
	Estimate	*t*	*P*	Estimate	*t*	*P*	Estimate	*t*	*P*
Abundance	-0.90 ± 4.31	-0.21	0.837	-8.69 ± 4.31	-2.02	0.052	2.38 ± 4.43	0.54	0.594
Species richness	2.58 ± 1.20	2.16	**0.038**	-0.17 ± 1.20	-0.14	0.890	1.28 ± 1.23	1.04	0.306
Body size	-0.70 ± 0.28	-2.56	**0.015**	-0.77 ± 0.28	-2.79	**0.009**	-0.74 ± 0.28	-2.62	**0.013**
Habitat preference	-0.03 ± 0.02	-1.53	0.135	-0.04 ± 0.02	-2.15	**0.040**	-0.05 ± 0.02	-2.38	**0.023**
Mobility	0.11 ± 0.07	1.48	0.148	0.21 ± 0.07	2.83	**0.008**	0.13 ± 0.08	1.66	0.107

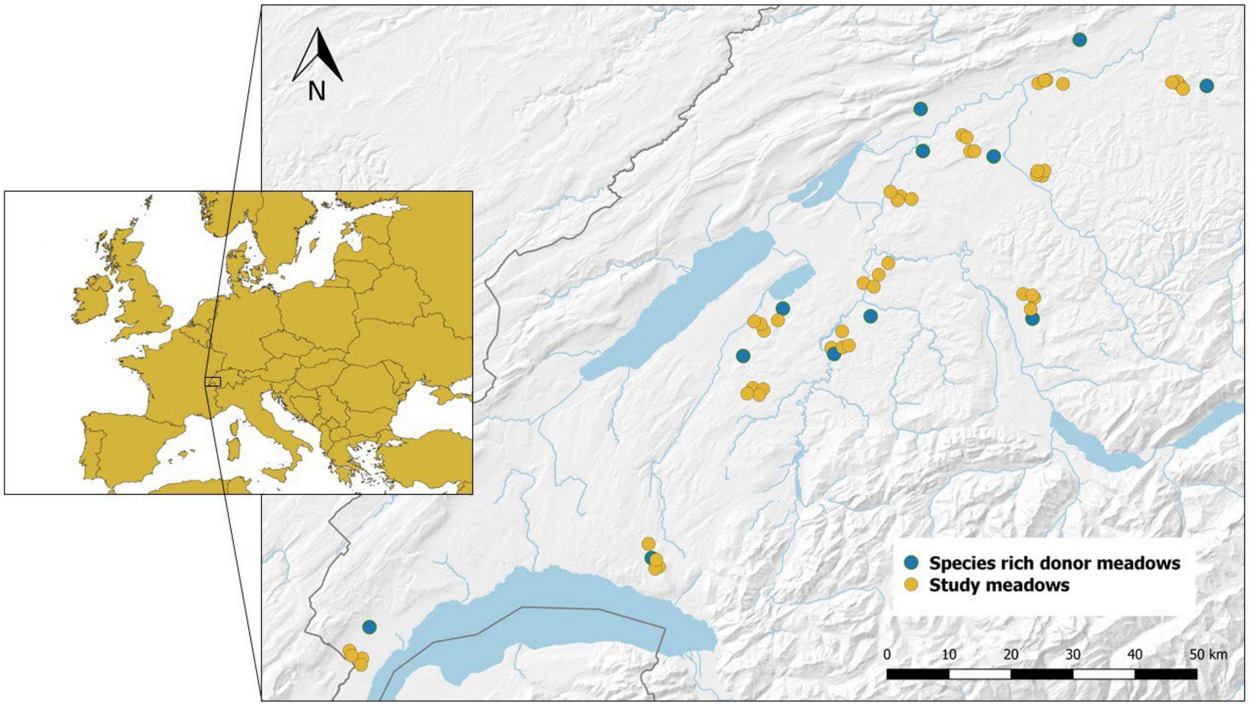


Figure 1 Overview of the 12 study sites in the Swiss lowlands.

Figure 2 Hay transfer on a ploughed receiver meadow (i.e., treatment hay plough HP) in the Swiss lowlands in June 2019. The hay was mown and transferred on the same day from a species rich donor meadow in close vicinity. This is a common restoration method of active grassland restoration in Central Europe.

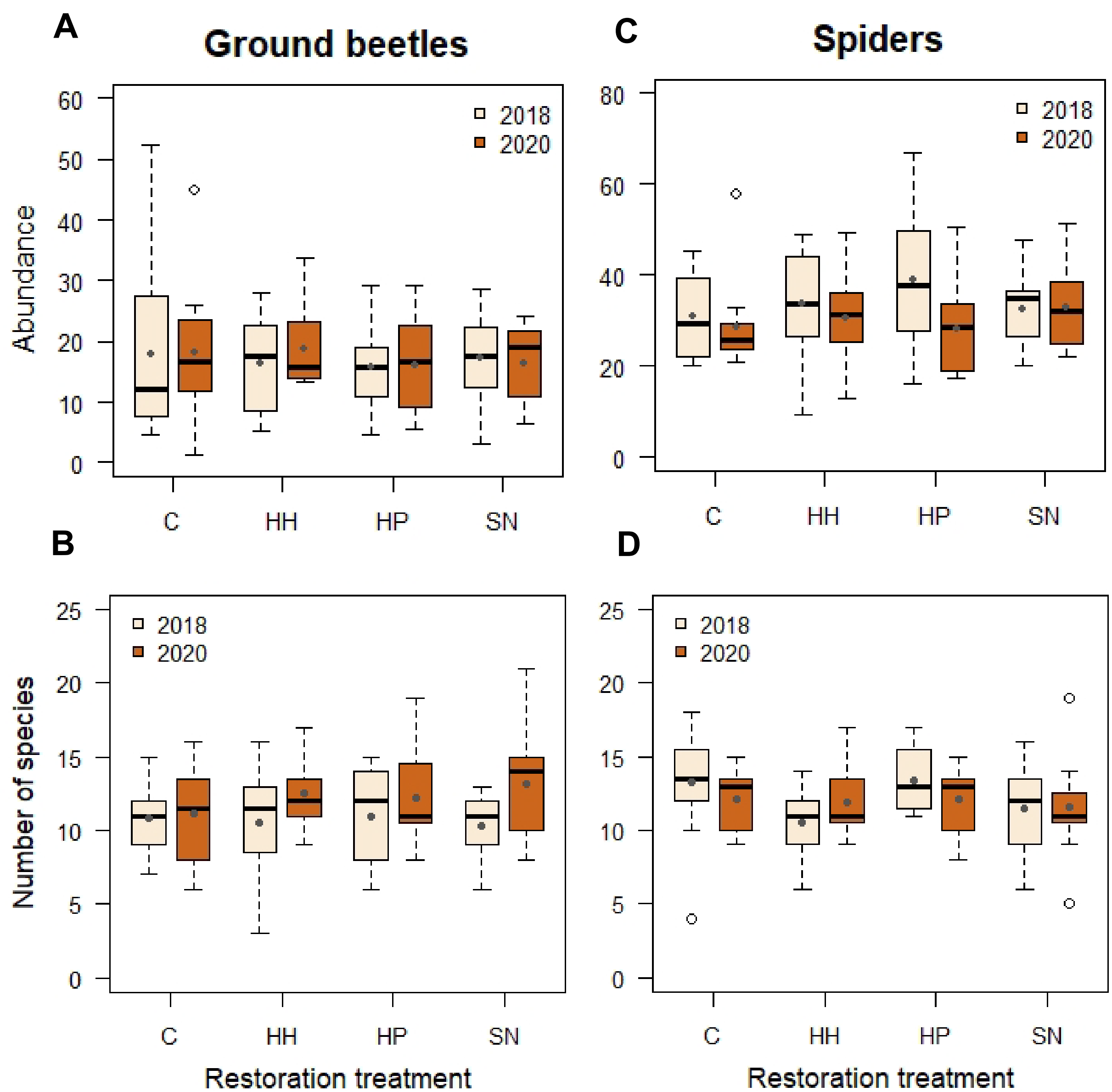


Figure 3 Ground beetle and spider abundance (A and C, respectively) and ground beetle and spider species richness (B and D, respectively) with respect to restoration treatment. The data from 2018 is one year before restoration and 2020 is one year after. Restoration treatment C stands for control, HH for hay harrow, HP for hay plough and SN for seeds natural with plough. Subplots A and C are based on the mean abundance per pitfall trap per meadow and year from all pitfalls (from 16 pitfalls per meadow and year). Subplots B and D are based on pooled data per meadow and year using the data of all identified individuals (from four pitfalls per meadow and year).

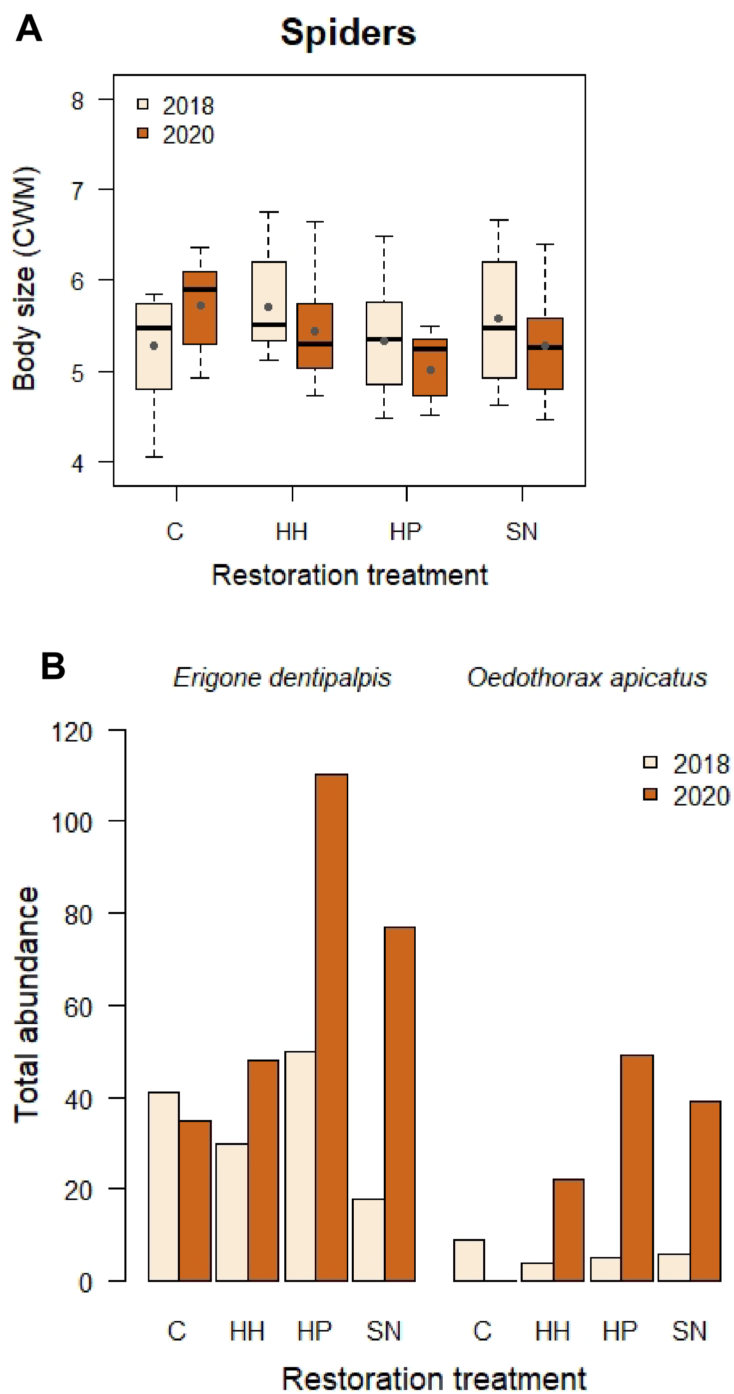


Figure 4 Community weighted mean (CWM) of spider body size with respect to restoration treatment (A). Abundance changes of two small spider species, *Erigone dentipalpis* and *Oedothorax apicatus*, for all treatments and both study years (B). See figure caption of Fig. 3 for a description of the treatments.

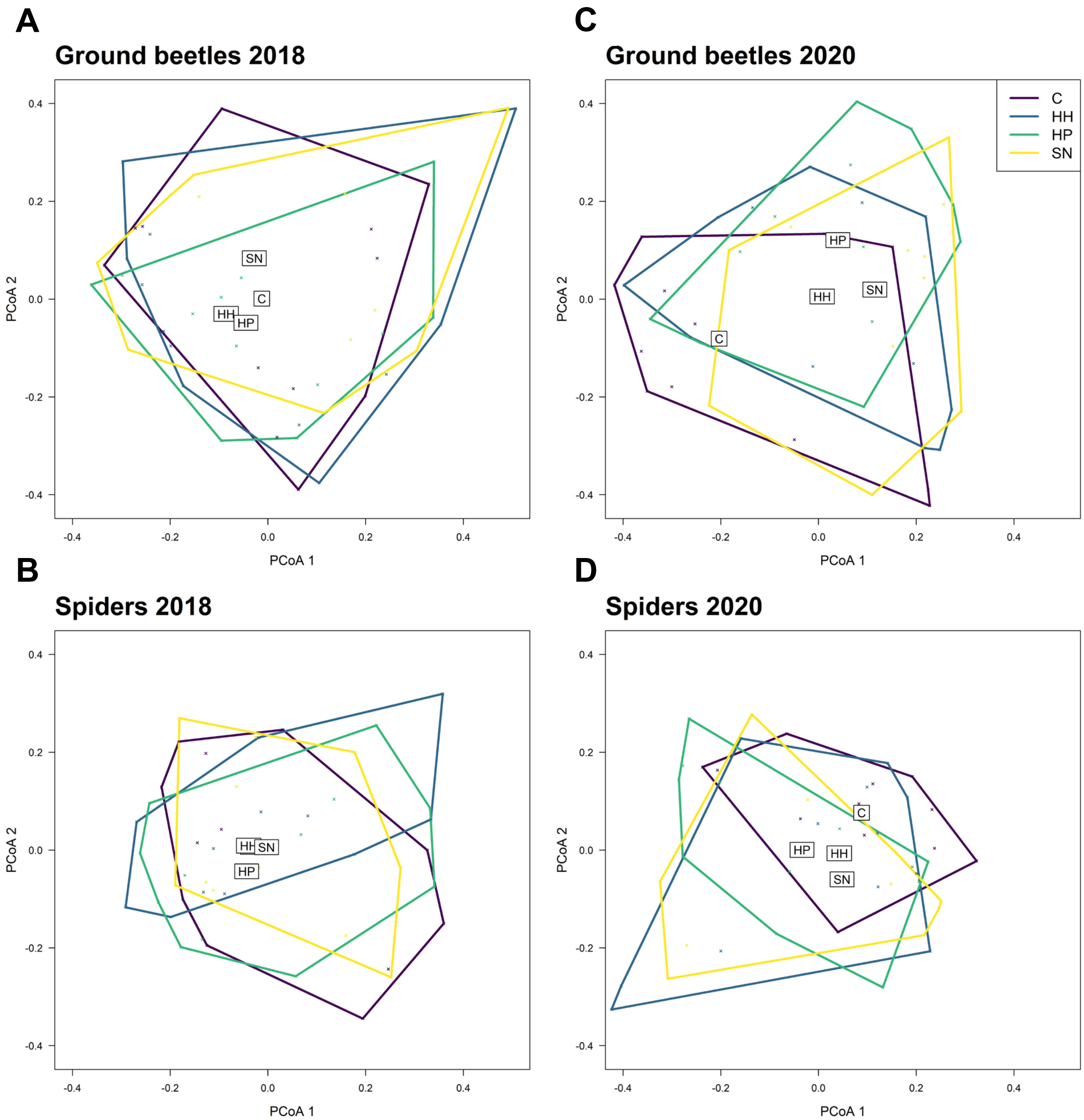


Figure 5 Invertebrate community composition using PERMANOVA of ground beetles and spiders for the years 2018 (A and B, respectively) and 2020 (C and D, respectively). Each x represents one meadow. The positions of the outmost meadows are indicated with polygons. The centroid of a polygon is represented with a label of a respective treatment. C stands for control, HH for hay harrow, HP for hay plough and SN for seeds natural with plough. The year 2018 represents sampling before restoration and 2020 sampling one year after restoration.

Supporting information

Table S1 Red listed *Carabidae* species in Switzerland, which were sampled during our restoration experiment. The column "CH" the provides the Swiss red list categories for each species, and the column "IUCN" provides the equivalent IUCN red list categories. None of these species were listed in the current IUCN red list (https://www.iucnredlist.org/, accessed on 17.02.2022). Abundances are given for the control meadows (n = 12) and receiver meadows (n = 36) in 2018, i.e., before restoration (Ab. contr. 2018 and Ab. restor. 2018, respectively) and in 2020, i.e., after restoration (Ab. contr. 2020 and Ab. restor. 2020, respectively).

Species	**CH**	**IUCN**	**Ab. contr. 2018**	**Ab. restor. 2018**	**Ab. contr. 2020**	**Ab. restor. 2020**
Agonum viridicupreum (Goeze, 1777)	1	CR	0	1	0	0
Amara kulti (Fassati, 1947)	4	NT	21	53	46	206
Anisodactylus nemorivagus (Duftschmid, 1812)	4	NT	56	78	36	29
Brachinus elegans (Chaudoir, 1842)	3	VU	2	3	3	2
Carabus auratus (Linnaeus, 1761)	3	VU	0	3	0	3
Harpalus marginellus (Gyllenhal, 1827)	4	NT	0	1	0	0
Harpalus smaragdinus (Duftschmid, 1812)	3	VU	0	0	0	2

Table S2. List of the ground beetle species with the abundances before (in 2018) and after (in 2020) restoration. The table also provides all species-specific traits. See table 2 for details on traits.

Species	Abundance 2018	Abundance 2020	Body size	Trophic level	Habitat preference	Hibernation index
Abax ovalis (Duftschmid, 1812)	3	0	13	2	3	3
Abax parallelepipedus (Piller & Mitterpacher, 1783)	1	0	20	2	2	NA
Acupalpus meridianus (Linnaeus, 1760)	0	1	3.5	2	2	3
Agonum muelleri (Herbst, 1784)	8	1	8	2	2	3
Agonum sexpunctatum (Linnaeus, 1758)	1	0	8.5	2	2	3
Agonum viridicupreum (Goeze, 1777)	1	0	9	2	3	3
Amara aenea (DeGeer, 1774)	192	326	7.5	1	1	3
Amara communis (Panzer, 1797)	21	7	7	1	2	2
Amara convexior (Stephens, 1828)	65	22	8	1	2	3
Amara familiaris (Duftschmid, 1812)	3	17	6.5	1	2	3
Amara fulvipes (Audinet-Serville, 1821)	745	771	10.5	1	1	3
Amara kulti (Fassati, 1947)	74	252	9.5	1	2	1
Amara lucida (Duftschmid, 1812)	2	1	5.75	1	2	3
Amara lunicollis (Schiødte, 1837)	395	235	7.5	1	2	3
Amara montivaga (Sturm, 1825)	3	17	8.5	1	1	3
Amara ovata (Fabricius, 1792)	0	11	8.75	1	2	3
Amara plebeja (Gyllenhal, 1810)	5	5	7	1	2	3
Amara similata (Gyllenhal, 1810)	0	16	8.75	1	2	3
Anchomenus dorsalis (Pontoppidan, 1763)	9	42	6.8	2	2	3
Anisodactylus binotatus (Fabricius, 1787)	214	222	11	1	2	3
Anisodactylus nemorivagus (Duftschmid, 1812)	134	65	9	1	1	3
Anisodactylus signatus (Panzer, 1796)	0	3	11.75	1	2	1
Badister bullatus (Schrank, 1798)	1	0	5.5	2	2	3
Bembidion guttula (Fabricius, 1792)	0	1	3.3	2	3	3
Bembidion lampros (Herbst, 1784)	3	8	3.4	2	2	3
Bembidion quadrimaculatum (Linnaeus, 1760)	5	0	3.05	2	1	3
Brachinus crepitans (Linnaeus, 1758)	1	0	8.7	2	1	NA
Brachinus elegans (Chaudoir, 1842)	5	5	7.75	NA	2	3
Brachinus explodens (Duftschmid, 1812)	39	35	6	2	1	3
Calathus fuscipes (Goeze, 1777)	1	4	12.25	2	2	2
Calathus melanocephalus (Linnaeus, 1758)	1	0	7.5	2	1	2
Carabus auratus Linnaeus, 1761	3	3	23.5	2	2	NA
Carabus cancellatus carinatus Charp. 1825	0	1	23	2	2	NA
Carabus coriaceus Linnaeus, 1758	0	1	37	2	3	2
Carabus granulatus Linnaeus, 1758	0	2	19.5	2	3	3

Carabus monilis (Fabricius, 1792)	42	24	24.5	2	2	2
Clivina fossor (Linnaeus, 1758)	3	3	6.25	2	2	3
Diachromus germanus (Linnaeus, 1758)	83	66	8.45	1	2	3
Harpalus affinis (Schrank, 1781)	13	58	10.5	1	2	3
Harpalus dimidiatus (P.Rossi, 1790)	20	58	12.5	1	1	3
Harpalus distinguendus (Duftschmid, 1812)	6	50	9.55	1	1	2
Harpalus latus (Linnaeus, 1758)	5	5	9.5	1	2	2
Harpalus luteicornis (Duftschmid, 1812)	62	23	6.75	1	2	3
Harpalus marginellus Gyllenhal, 1827	1	0	10.65	1	2	NA
Harpalus rubripes (Duftschmid, 1812)	19	9	10	1	2	2
Harpalus serripes (Quensel, 1806)	8	30	10.5	1	1	3
Harpalus smaragdinus (Duftschmid, 1812)	0	2	9.25	1	1	2
Harpalus subcylindricus (Dejean, 1829)	56	121	6.75	1	1	NA
Harpalus tardus (Panzer, 1796)	13	3	9.45	2	1	NA
Loricera pilicornis (Fabricius, 1775)	2	1	7.4	2	3	3
Metallina properans (Stephens, 1828)	244	165	3.95	2	2	3
Microlestes minutulus (Goeze, 1777)	3	21	3.1	2	1	3
Nebria brevicollis (Fabricius, 1792)	1	10	12	2	3	2
Nebria salina Fairmaire & Laboulbène, 1854	0	3	11	2	2	2
Notiophilus palustris Sturm, 1826	0	3	4.75	2	3	3
Ophonus azureus (Fabricius, 1775)	2	2	7.5	1	1	3
Panagaeus cruxmajor (Linnaeus, 1758)	1	0	8.25	2	3	3
Parophonus maculicornis (Duftschmid, 1812)	63	52	6.65	NA	2	3
Poecilus cupreus (Linnaeus, 1758)	101	238	11	2	2	3
Poecilus lepidus (Leske, 1785)	1	4	12	2	1	1
Poecilus versicolor (Sturm, 1824)	353	222	9.75	2	2	3
Pseudoophonus griseus (Panzer, 1796)	0	3	10.5	1	1	1
Pseudoophonus rufipes (De Geer, 1774)	16	121	13.5	1	2	2
Pterostichus melanarius (Illiger, 1798)	9	5	15	2	3	2
Pterostichus niger (Schaller, 1783)	1	0	18.5	2	3	NA
Pterostichus vernalis (Panzer, 1796)	15	5	6.85	2	2	3
Semiophonus signaticornis (Duftschmid, 1812)	1	1	6.5	1	1	1
Stenolophus teutonus (Schrank, 1781)	5	10	6.25	2	2	3
Syntomus truncatellus (Linnaeus, 1760)	3	2	3.15	2	2	3
Trechus quadristriatus (Schrank, 1781)	0	1	4	2	2	1

Table S3 List of the spider species with the abundances before (in 2018) and after (in 2020) restoration. The table also provides all species-specific traits. See table 2 for details on traits.

Species	Abundance 2018	Abundance 2020	Body size	Habitat preference	Mobility
Agyneta affinis	7	1	1.5	0.51	3
Agyneta rurestris	12	16	2.1	0.38	3
Agyneta simplicitarsis	0	1	1.65	0.68	3
Alopecosa cuneata	10	2	7.5	0.59	2
Alopecosa pulverulenta	107	12	9	0.42	2
Araeoncus humilis	3	2	1.4	0.31	3
Arctosa leopardus	111	129	3.4	0.28	2
Arctosa lutetiana	7	3	9	0.53	2
Argiope bruennichi	1	0	14.4	0.44	3
Asagena phalerata	17	14	5	0.81	2
Atypus affinis	1	0	11	0.7	NA
Aulonia albimana	50	29	3.8	0.52	2
Centromerita bicolor	0	1	3.25	NA	3
Clubiona neglecta	1	0	6.5	NA	1
Dicymbium nigrum	7	0	1.8	0.31	3
Diplostyla concolor	7	2	2.5	0.32	3
Drassodes cupreus	0	1	NA	NA	NA
Drassyllus lutetianus	7	7	5.7	0.5	1
Drassyllus praeficus	139	137	6.5	0.5	1
Drassyllus pusillus	65	72	4	0.5	1
Enoplognatha thoracica	3	1	5.6	0.65	2
Erigone atra	12	22	1.9	0.29	3
Erigone dentipalpis	140	270	2.2	0.23	3
Euophrys frontalis	9	1	3.2	NA	2
Hahnia nava	1	0	1.8	0.56	NA
Haplodrassus signifer	6	1	8.2	0.6	1
Harpactea lepida	1	1	6	NA	1
Histopona torpida	1	2	9.2	0.39	NA
Mangora acalypha	0	1	4.5	0.62	3
Mermessus trilobatus	21	7	1.6	NA	3
Micaria micans	7	5	NA	NA	1

Micaria pulicaria	2	5	3.2	0.36	1
Oedothorax apicatus	25	110	2.5	0.28	3
Oedothorax fuscus	35	14	2.5	0.22	3
Oedothorax retusus	0	1	2.6	NA	3
Ozyptila simplex	48	66	3.5	0.39	2
Pachygnatha clercki	54	4	5	0.26	3
Pachygnatha degeeri	1330	365	3.5	0.38	3
Panamomops sulcifrons	0	1	NA	NA	NA
Pardosa agrestis	120	219	4.5	0.3	2
Pardosa amentata	17	3	6.5	0.26	2
Pardosa hortensis	8	16	5	NA	2
Pardosa lugubris	0	1	6.1	0.42	2
Pardosa paludicola	0	1	7.8	0.33	2
Pardosa palustris	3032	2532	6	0.32	2
Pardosa prativaga	4	6	6.2	0.26	2
Pardosa pullata	31	23	5	0.36	2
Pardosa saltans	2	11	5.6	NA	2
Pardosa tenuipes	132	428	4.8	NA	2
Pelecopsis parallela	37	48	1	0.31	3
Philodromus aureolus	1	0	NA	NA	NA
Phlegra fasciata	3	7	5.8	0.54	2
Phrurolithus festivus	3	7	2.7	0.43	2

Table S4 Test results for the effect of refuge opportunities on ground beetle and spider abundance and species richness.

	Ground beetles			Spiders		
	Estimate	t	*P*	Estimate	t	*P*
Abundance	-1.15 $\pm$ 5.52	-0.21	0.836	-0.40 $\pm$ 7.65	-0.05	0.959
Species richness	-0.59 $\pm$ 2.23	-0.26	0.793	-0.59 $\pm$ 2.23	-0.26	0.793

Figure S1 Contingency table between ground beetle abundance per species and treatment and year. Only species with a total abundance of >50 individuals overall are included here.

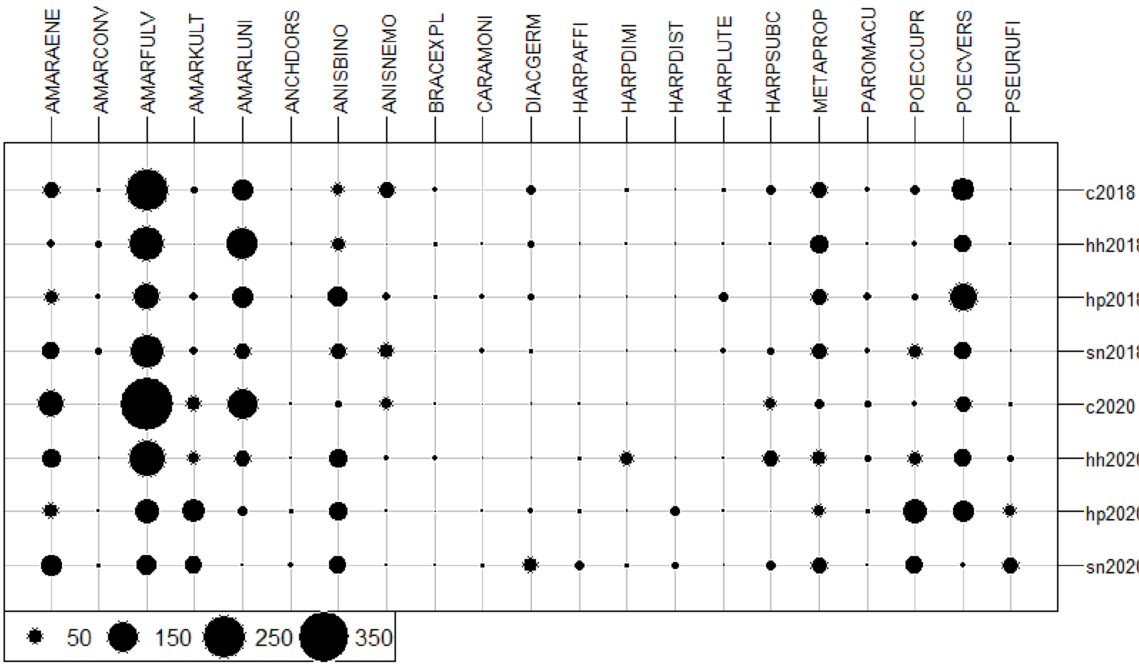


Figure S2 Contingency table between spider abundance per species and treatment and year. Only species with a total abundance of >50 individuals overall are included here.

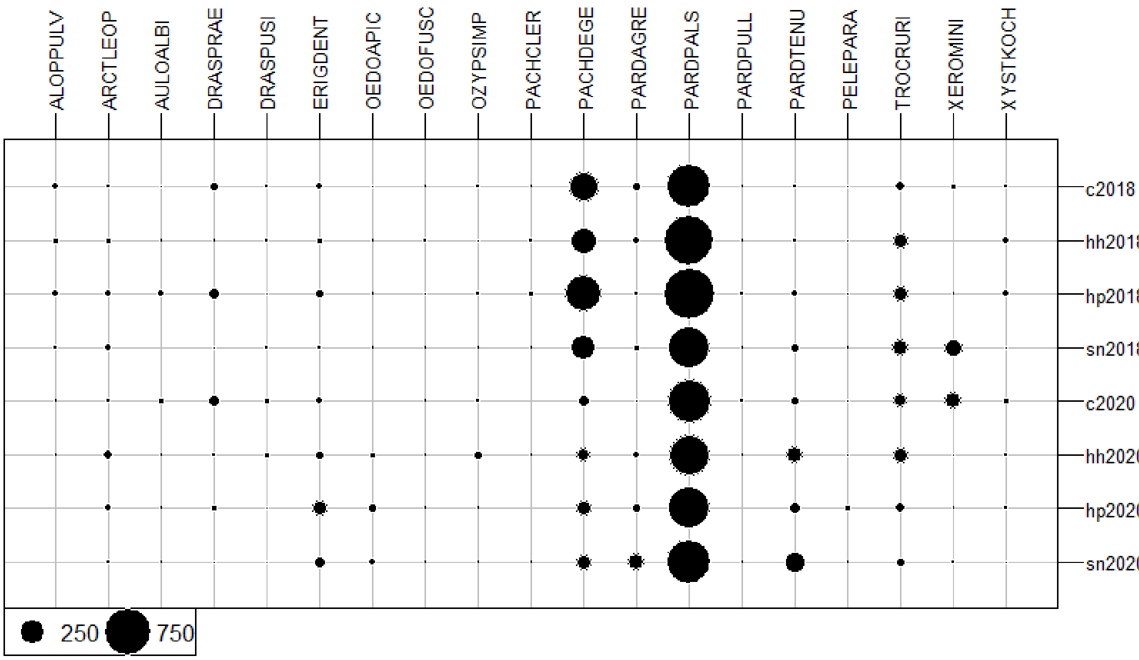
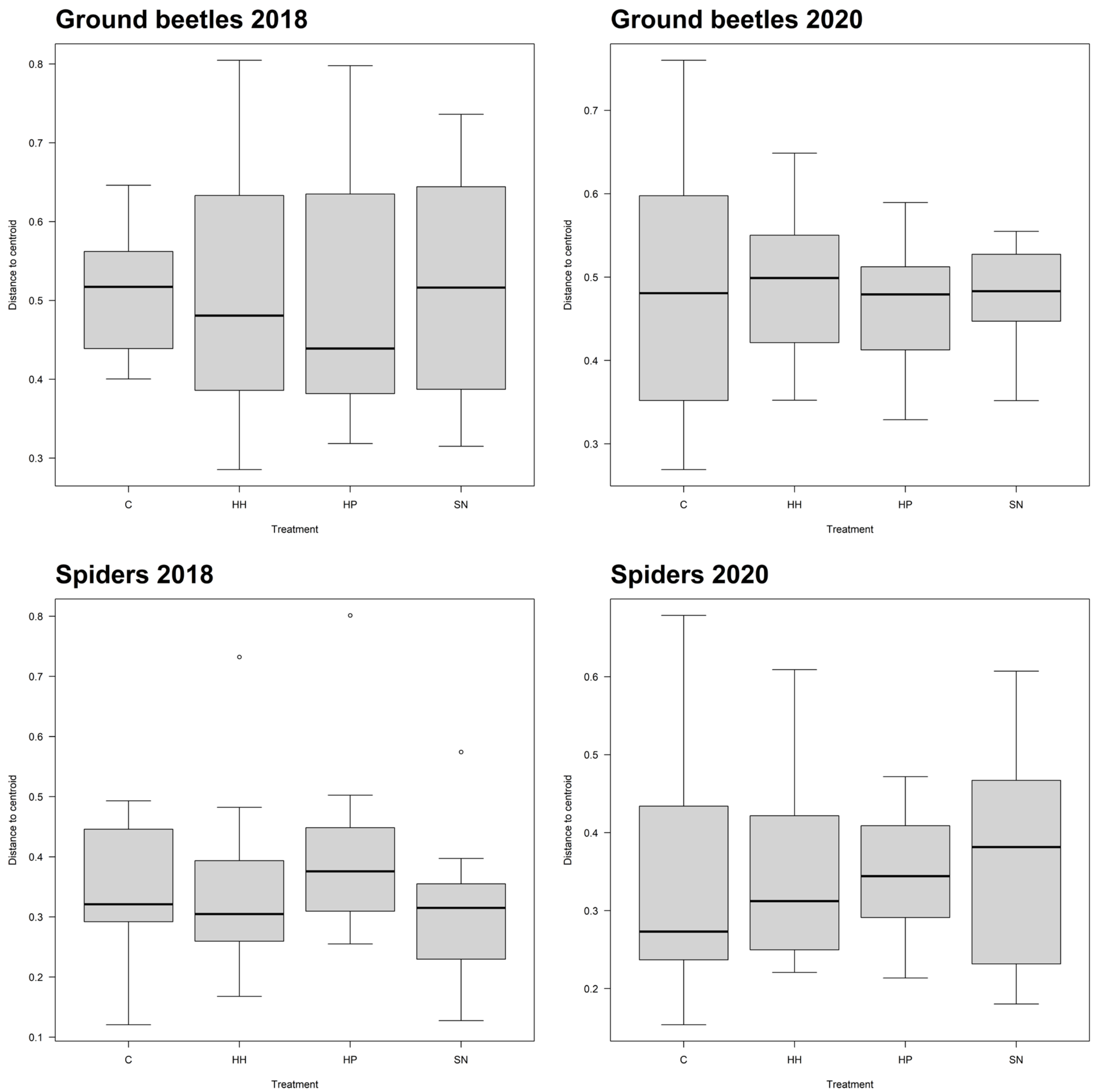


Figure S3 Boxplots with the outcome of the permutational tests for homogeneity of multivariate dispersions for ground beetles and spiders for the year 2018 (before restoration) and 2020 (one year after restoration). Pairwise post hoc tests with tukey honest significant differences (TukeyHSD) revealed no differences between treatments.



Chapter 3

Active restoration promotes plant diversity of semi-natural grasslands in the Swiss Plateau

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Abstract

In Central Europe, active restoration of semi-natural grasslands is often necessary due to propagule limitation in the landscape. While studies highlight the importance of soil disturbance prior to seeding, seeding method and seed source, only few studies directly compare these factors against each other. In a field-scale trial, which was replicated twelve times across the Swiss plateau, we experimentally tested the short-term effect of four active restoration methods of species-poor, mesic grasslands on plant species richness, diversity, and community traits. The restoration methods differed in soil disturbance intensity (deep tillage vs. superficial harrowing), seeding method (green hay vs. seeds) and seed source (locally harvested vs. from a commercial provider). In addition, there was an unrestored control meadow per region. We collected plant data one year before restoration and two years after. Total plant species richness increased on average by 9 ± 1 species after restoration across all methods. The highest plant species richness was obtained for meadows which were tilled and seeded with a commercial seed mix, while the diversity remained low. Meadows that used seeds from a local species-rich meadow did not differ in their species richness after restoration, regardless of soil disturbance intensity or if seeding occurred in form of green hay or as harvested seeds. Furthermore, we found that the community specific leaf area was smaller on meadows sown with a commercial seed mix and harvested seeds compared to the control. A less strong soil disturbance resulted in a higher grass cover after restoration. Our results suggest that all active grassland restoration methods investigated in this study are effective, at least on the short-term.

Key words: active restoration, hay transfer, mesic grasslands, result-based scheme, Switzerland

Introduction

Grasslands are an essential element of the cultural landscape in Europe (Dengler et al., 2014). In total, 17% of the European surface are covered by grasslands (Eurostat, 2018). Besides having a high agronomic value in terms of fodder production (Squires et al., 2018), these grasslands provide also important ecosystem services, such as erosion control, water flow regulation, nitrogen retention and carbon sequestration (Byrne & delBarco-Trillo, 2019; Yan et al., 2019). Until a few decades ago, grasslands belonging to the class of mesic hay meadows from low and medium altitudes (*Arrhenatheretalia elatioris*) used to be widespread across Central Europe (Hejcman et al., 2013). However, land-use intensification and land-use conversion have led to a strong decline of this valuable habitat, making it vulnerable (VU) according to the European Red List (Boch et al., 2019). In Switzerland for example up to 98% of the historical lowland *Arrhenatheretalia elatioris* have disappeared since 1900 (Lachat et al., 2010).

In this context Switzerland introduced biodiversity promotion areas (BPA) in the early 1990s to counteract the biodiversity loss which is caused by intensive agriculture. Farmers can receive direct payments only if minimum 7% of their farmland is managed as BPAs (Swiss Federal Council, 1998). The most common BPAs are extensively managed meadows, constituting 52% of the entire BPA surfaces (Swiss Federal Office of Statistics, 2022). These extensively managed meadows are classified into two distinct payment schemes, that is a management-based scheme (Quality I meadows) and a result-based scheme (Quality II meadows). Quality I meadows receive no fertilization and no pesticides and must be mown after June 15th. Quality II meadows are managed the same way as the Quality I meadows but need additionally “botanical quality” (this term is used and defined in the Swiss decree on direct payments). Botanical quality is achieved when a meadow contains at least six indicator plant species. Indicator species are target species commonly found in species-rich hay meadows. In addition, farmers receive three times more payments when a meadow reaches Quality II, compared to Quality I. It has been shown that mere extensification does not lead to an increase in plant species richness (van Klink et al., 2017). This could be due to propagule limitation such as a depleted seed bank or low occurrence of species-rich grasslands in the landscape (Buisson et al., 2018; Münzbergová & Herben, 2005). To overcome this propagule limitation and to accelerate plant species establishment, active restoration through seed addition is necessary.

Active grassland restoration consists of two main components: seed bed preparation and seed addition. Seed bed preparation is necessary to reduce competition pressure from the resident plant community and to create favorable conditions for seedling germination (Freitag et al., 2021). Common seed bed preparation methods are either harrowing, i.e., superficial soil disturbance (Durbecq et al., 2021), or ploughing, i.e., tilling of the soil (Hovd, 2008). Seed addition can be done either in the form of green hay containing seed(also known as hay transfer) or directly as seeds, whereas the seeds can originate from a species-rich donor meadow or from a seed producer (Kiehl et al., 2010). Given the costs and efforts that are involved in grassland restoration (Török et al., 2011), it is important to know which restoration methods are the most effective ones. Thus, comparative field experiments are necessary. Several studies have compared different seeding methods, e.g., green hay vs. threshing material (Albert et al., 2019; Baasch et al., 2016). To our knowledge, there is no comparative restoration study for mesic grasslands which took also the soil disturbance intensity into account (but see Bischoff *et al.*, 2018 for floodplain meadows). Furthermore, many restoration studies were conducted on a single site with replicated blocks (Albert et al., 2019; Auestad et al., 2015), while only few conducted field scale studies (Prach et al., 2014). On the one hand, replicated field scale restoration studies have that advantage of reducing the influence of confounding factors such as edge effects. On the other hand, field scale studies are difficult to implement due to logistical constraints.

In 2018, we have launched a field-scale grassland restoration experiment in the Swiss plateau. We applied four different restoration treatments which are common in Switzerland and recommended by the Swiss Association for the Development of Agriculture and Rural Areas (AGRIDEA Staub *et al.*, 2015). These treatments differ in seed bed preparation intensity (low vs. high intensity), seeding method (green hay vs. seeds only) and seed origin (local species-rich donor meadow vs. commercial seed producer). Before restoration, all meadows were managed according to the Swiss BPA scheme and belonged to the Quality I class, i.e., these meadows had less than six target species and were relatively species poor. Restoration was implemented in 2019 by the farmers who already managed these meadows and was supervised by us or by practitioners with experience in grassland restoration. After restoration the farmers continued to manage their restored meadows as before.

Our study aim was to test the relative effectiveness of different restoration treatments on the plant community on the short-term (two years after restoration). In particular, we were interested: a) if we could observe a difference in species richness and diversity between restoration treatments, b) if there will be a difference on the trait community level between treatments and c) if the amount of Quality indicator species reflects the total plant species richness.

Methods

Study sites

We selected twelve study regions which were distributed over the western part of the Swiss Plateau with a minimal distance of 10 km between each region (Fig. 1). Each study region contains five restoration meadows and one species-rich donor meadow. All meadows in this study were extensively managed, mesic meadows. The donor meadows were required to host high botanical diversity, which was defined by the presence of at least ten indicator plant species in the entire meadow according to the Swiss decree on direct payments (Swiss Federal Council, 1998). In contrast, restoration meadows had a low botanical diversity, i.e., these meadows were listed as Quality I only, and were managed extensively since at least five years. Note that grazing in autumn was permitted. All meadows are on farmland with productive soils and relatively low inclination for Swiss standards (mean slope = 5° ± 1). The size of the restoration meadows varies from 0.14 ha to 1.1 ha, with a mean of 0.5 ha. Most meadows were formerly used as cropland, intensively managed meadows, or pasture.

Experimental design

Five restoration treatments were randomly assigned and applied to the restoration meadows in early summer 2019. Treatments were carried out on field scale (i.e., one treatment per meadow). The five treatments were:

(i) Control: no seed addition and no soil disturbance (C)

(ii) Hay transfer from a species-rich donor meadow on a harrowed receiver meadow (HH)

(iii) Hay transfer from a species-rich donor meadow on a ploughed receiver meadow (HP)

(iv) Sowing of a commercial seed mixture on a ploughed receiver meadow (SC)

(v) Sowing of a brush- or vacuum harvested seed mixture on a ploughed receiver meadow (SN)

Prior to seeding, the meadows which were assigned to the SC, SN and HP treatments, were ploughed below a depth of 20cm in early spring 2019. These meadows were then harrowed every four to six weeks until seeding to prevent establishment of undesired weeds. For the HH treatment, the meadows were mown and harrowed with a rotary harrow about a week before seeding and then harrowed one more time on the day of seeding. For the hay transfer (HH and HP treatments), green hay was cut early in the day on the donor meadows and directly distributed on the restoration meadows. Seeds for the SN treatments were harvested on the same donor meadows one year before restoration in 2018 and seeded by hand in 2019. The commercial seed mixture (SC treatment) was provided by the Swiss seed producer “UFA Samen”. The mix contained 38 plant species of Swiss origin and was seeded at an amount of 10 g/m2 (see Table S2 for the species composition).

Data collection

We recorded vascular plant species richness and cover per species estimated in percent. Two vegetation survey plots were placed within two subplots of each 2 m × 4 m and with each plot 8m apart (Fig. 2). To avoid potential edge effects, we placed the survey plots randomly within each meadow and 10m away from the edge. We conducted vegetation surveys of all restoration meadows in May and June 2018, i.e., one year before restoration. We resurveyed the restoration meadows in 2021, i.e., two years after restoration. One meadow from the SN treatment had to be excluded due to failure of the restoration treatment. This resulted in 60 surveyed meadows in 2018 and 59 surveyed meadows in 2021. In 2021 we also recorded the presence of quality indicator species within a circle with a radius of 3m to define whether a meadow reached Quality II or not. The official list of quality plant indicators also contains species groups, e.g., “yellow flowering *Asteraceae* with several heads” contains species like *Picris hieracioides* or *Crepis biennis*. The presence of both species would officially be counted as one presence point. For our purpose, we noted all quality indicator plant species, i.e., also the ones within the same group.

Statistical analysis

For all analyses we pooled the data of the two subplots per meadow. All statistical analysis were performed using R version 4.1.1 (R Core Team, 2021). For the linear mixed-effect models (LMM) we used the package *lme4* (Bates et al., 2015) and model assumptions were tested using the package *DHARMa* (Hartig, 2022). We first tested whether there was a difference in species richness in the control meadows only between the years 2018 and 2021, to verify if the vegetation has undergone changes within these years. No difference could be detected, so we continued the analysis with the data from 2021 only, i.e., two years after restoration.

We tested the effect of all restoration treatments on a) total plant species richness, b) beta-diversity (absolute species turnover), and c) community weighted means of plant functional traits. The beta-diversity of a meadow was calculated by taking the difference between the overall species richness within a treatment and the total species richness per meadow. Based on data availability, ecological importance and preliminary results, the following traits were analysed: specific leaf area (SLA; leaf area per leaf dry mass [mm^2/mg]), number of seeds, seed mass [mg], phenology (first month of flowering) and functional groups (forbs and grasses). Functional trait data was extracted from the LEDA trait database (Kleyer et al., 2008) and from a previous research project from our group (van Klink et al., 2017).

The mean species cover of each plant in a meadow was used to calculate community-weighted means (CWM) using the formula:

$$CWM = \sum_{i=1}^{n} \frac{c_i}{c_{tot}} * F_i$$

Where c_i is the cover of the species i, c_{tot} is the summed cover over all species and F_i is the median functional trait value. The categorical value (i.e., grass or forb) was calculated by taking the sum of c_i for each category.

For all analyses we used linear mixed-effect models (LMM), with region as random effect and Gaussian distribution. We checked for differences between control and treatments, as well as among treatments by comparing least-square means (Package *emmeans*; Lenth, 2022). Model assumptions were tested for normal distribution of residuals as well as zero-inflation by dispersion test, a QQ-Plot of the residuals, and the residual plot vs. predicted treatments. We tested for the presence of spatial autocorrelation using Moran's I, but it was never significant.

In addition, we were interested whether the amount of quality indicator species also reflects the plant species richness. We tested this using linear mixed-effect models with the plant species richness as response variable and the amount of quality indicator species as explanatory variable.

Results

Species richness and diversity

In 2018, we recorded a total of 88 plant species and a mean species richness of 24.8 per meadow (min = 9, max = 32, control meadows included, within $16 m^2$). Two years after restoration in 2021, we recorded 129 species and a mean species richness of 32.2 per meadow (min = 15, max = 44). Mean species richness significantly increased after restoration (i.e., control excluded) by 9.15 species (SE = 0.96, $P < 0.001$).

All treatments had a significantly higher plant species richness than the control in 2021. The difference in species richness between the respective treatment and the control ranged from 4.75 species in HH (P = 0.016) to 11.8 species in SC (P < 0.001) with a mean between all treatments of 7.25 species. Thus, SC resulted in the highest species richness and performed significantly better than all other treatments in terms of species richness (Fig. 3a, Table S1). There was no difference between the treatments which used seeds from a species-rich donor meadow (HH, HP and SN). Treatment explained 41% of the variance of species richness (marginal R^2 = 0.41). Beta diversity was significantly higher for the treatments HP and SN compared to the control (HP vs. C: P = 0.001; SN vs. C: P = 0.002, Fig. 3b). The beta diversity of the treatments HH and SC was not significantly different from the control (Table S1).

Plant functional traits and groups

CWM of plant functional traits differed significantly among treatments. Species with low SLA were predominantly present in treatments using seed mixtures (SC and SN, Fig. 4a). The mean number of seeds produced per plant did not differ significantly from the treatments to the control but within the treatments (Table S1). HP had a higher CWM of seed number than both HH and SN. CWM of seed mass was highest in HP (Fig 4c). The mean month of first flowering was highest for SN (P = 0.002), while the other treatments did not differ from the control. The percentage cover of graminoids only differed significantly between HH and HP (P = 0.035, Fig. 4). We observed no significant difference in the summed cover of forbs between all treatments and control and among treatments.

Quality indicator species

The number of quality indicator species per restoration meadow increased from a mean of 2.7 indicators in 2018 (min = 1, max = 6) to a mean of 8.4

indicators in 2021 (min = 3, max = 16), excluding the control. The number of quality indicator species also showed a positive linear relationship with plant species richness ($P < 0.001$, Fig. 5).

Discussion

All restoration methods from our experiment led to a significant increase in vascular plant species two years after restoration, which is in line with previous studies and reviews (Hedberg & Kotowski, 2010; Kiehl et al., 2010). The novelty of our experiment is the replicated field-scale, i.e., real-scale approach, which makes our outcomes robust. However, we observed differences between the treatments in terms of species richness, diversity, and prevalence of plant functional traits after restoration. These positive short-term effects are of high importance for farmers whose meadows are under a result-based payment scheme, where higher plant diversity is compensated, as for example in Switzerland.

Effects of restoration methods on species richness and diversity

The restoration treatment which resulted in the highest plant species richness among all methods was the sowing of a commercial seed mixture on a ploughed surface. However, this same method was also among the ones with the lowest diversity. This is not surprising, given that the same seed mixture was applied for this method across all regions. Many studies underline the importance of introducing regional (autochthonous) seeds to ensure local ecotypes of plant species remain in a region (Albert et al., 2019). However, the low availability of regional seed mixtures in a landscape with relatively few species-rich donor meadows, as in the Swiss Plateau, is a strong limiting factor in grassland restoration. This is less of an issue in other regions such as the Czech Republic with ancient grasslands hosting some of the most species-rich areas in Europe (Biurrun et al., 2021). The low availability of local propagules highlights the importance of seed production for grassland restoration

(Ladouceur et al., 2018). Indeed, the quality in the seed production has increased in the recent years, e.g., by defining seed transfer zones which take into consideration intraspecific variation and local ecotypes (Cevallos et al., 2020; Durka et al., 2017).

The three other restoration treatments which used seeds from a local species-rich donor meadow were all effective in increasing the species richness but did not differ among each other. For instance, the soil disturbance intensity had no effect on the species richness when the same seeding method, i.e., hay transfer, was applied. Similarly, the soil disturbance intensity did not play a big role in the restoration of floodplain meadows (Bischoff et al., 2018; Schmiede et al., 2012). With the same soil disturbance intensity, but with different seed application methods, i.e., hay transfer or seed of locally harvested seeds showed no difference as well, which is in line with previous findings (Baasch et al., 2016). However, the soil disturbance intensity showed a significant effect on the plant diversity. Meadows which received more intense soil disturbance with a plough displayed a higher diversity compared to the meadows that were only harrowed. Indeed, the plant diversity of the harrowed meadows did not differ from the control. While the competition of resident plants is very low after deep soil tillage, superficial harrowing does not destroy entirely the grass sward which may inhibit the establishment of new species (Edwards et al., 2007). The high grass cover of our harrowed treatments confirms that the grasses could possibly re-establish relatively quickly. Thanks to our BACI design we could further show that passive restoration, i.e., the continuation of extensive management with no soil disturbance nor seed addition, did not change the plant community after three years. It has been already previously shown, that passive restoration is not efficient in the Swiss Plateau even after five years (van Klink et al., 2017).

Plant functional traits

The studied functional traits show distinct variability between treatments. This indicates differences in plant composition, which were not detected by analysing species richness or indicators alone. The lower SLA values were mainly observed in treatments with high species richness, which indicates that restoration indeed favoured specialized species rather than fast-growing generalists. This was also observed by similar studies, which found better establishment of species with tougher leaves (low SLA), especially on low-productivity sites (Albert et al., 2019; Freitag et al., 2021). The number of seeds had similar values for restored meadows and the undisturbed control. In contrast to our result, Albert *et al.*, (2021) found much higher seed numbers in ancient grasslands compared to restored sites. Likewise, the study by Albert et al., (2019) found that green hay supported the transfer of species with larger seeds. Meadows that we seeded with locally harvested seeds had a plant community which displayed later first flowering compared to the other treatments. This difference can be explained by the harvesting methods. While the green hay for the hay transfer treatments was harvested on a single day in early summer, we collected the seeds throughout the summer, which makes the presence of later flowering species likely. Other studies point out that plant phenology is rather important for restoration and linked to establishment on the species level (Engst et al., 2017). We recorded all plots before mowing and our plots were characterized by a dominance of grasses (graminoids) compared to forbs (non-graminoids). This is reflected by findings of Albert et al., (2021) who recorded a dominance of grasses before mowing and forbs after mowing.

Conclusions

With our study we could show that the most common active grassland restoration methods can substantially increase the plant species richness in the short term, i.e., two years after restoration. Considering that active restoration actions are costly and require a substantial amount of effort (reviewed in Török *et al.*, 2011), restoration practitioners need the certainty that a given method will work. Quick restoration outcomes are especially important in a result-based payment scheme, where farmers or landowners receive higher payments after achieving a predefined goal, such as a higher number of target plant species in their meadows (Herzon et al., 2018; McDonald et al., 2018). However, we anticipate further changes in plant species richness and plant composition in the following years, as a decline in species richness has been confirmed by other studies two to three years after restoration (Albert et al., 2019; Freitag et al., 2021). We can therefore recommend, that the restoration methods can be chosen accordingly to the possibilities and financial resources to restore mesic grasslands, if the aim is to obtain positive effects on the short term. Yet, at this stage it is not guaranteed that the plant community will be maintained in the coming years.

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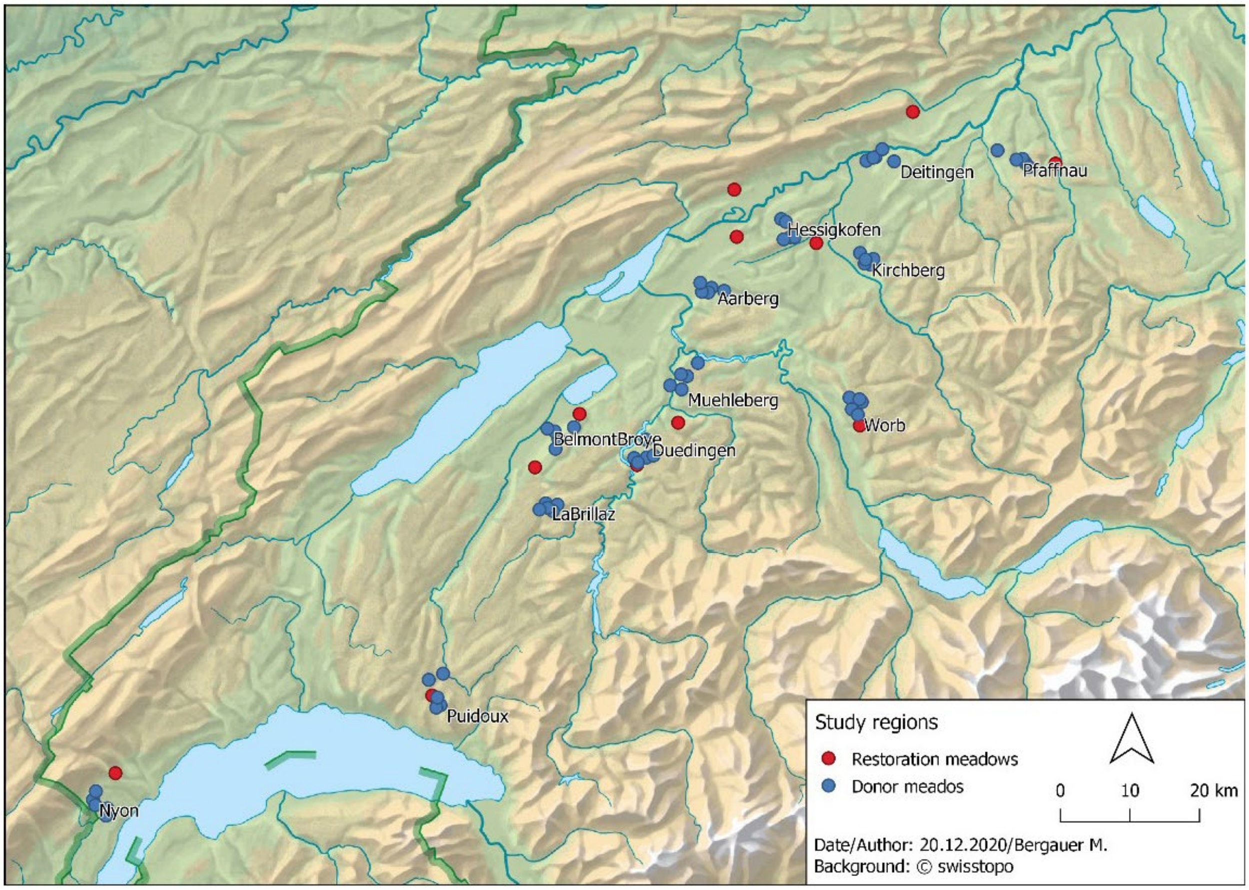

Figure 1 Overview of our study regions (n = 12) and all meadows included in our experiment.

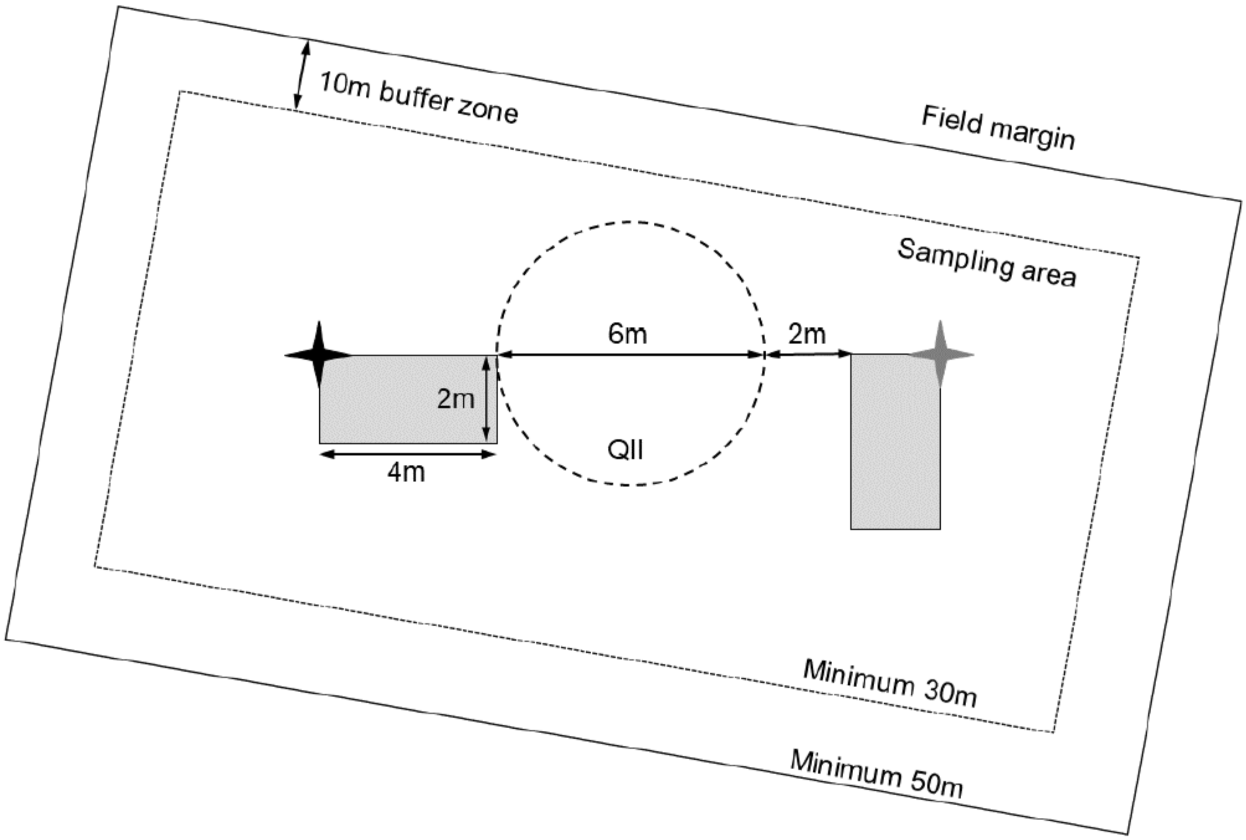

Figure 2 Sampling scheme. The placement of the permanent plots inside a restoration- or donor meadow. Random point 1 (black star) was set 10 m away from the meadow border. Random point two (gray star) was placed 14 m away from random point 1 in a random direction (north, east, south or west). In between the centre the quality assessment plot was placed, 7m apart from random point 1.

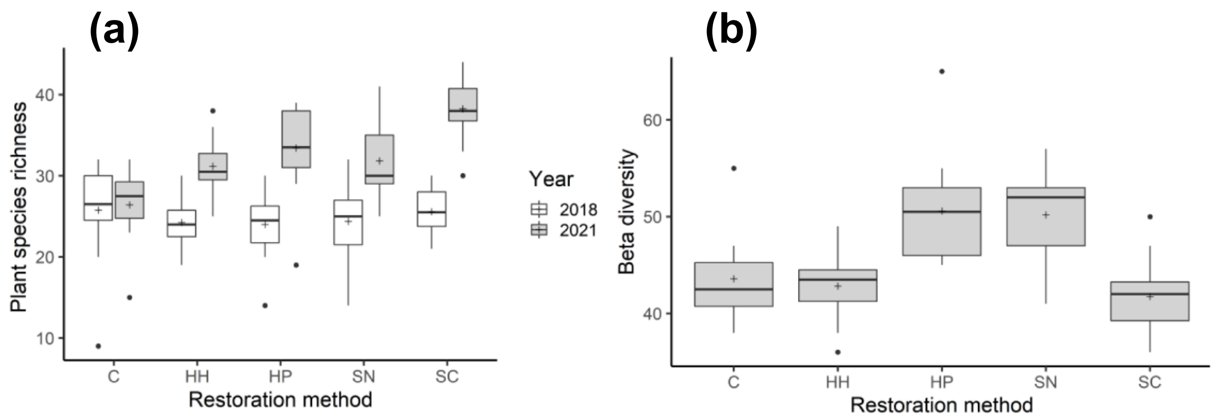

Figure 3 Plant species richness before restoration in 2018 and after restoration in 2021 (a) and plant beta diversity after restoration in 2021 (b) with respect to restoration treatment. Treatment abbreviations: C = control, HH = hay harrow, HP = hay plough, SN = seed natural, and SC = seed commercial.

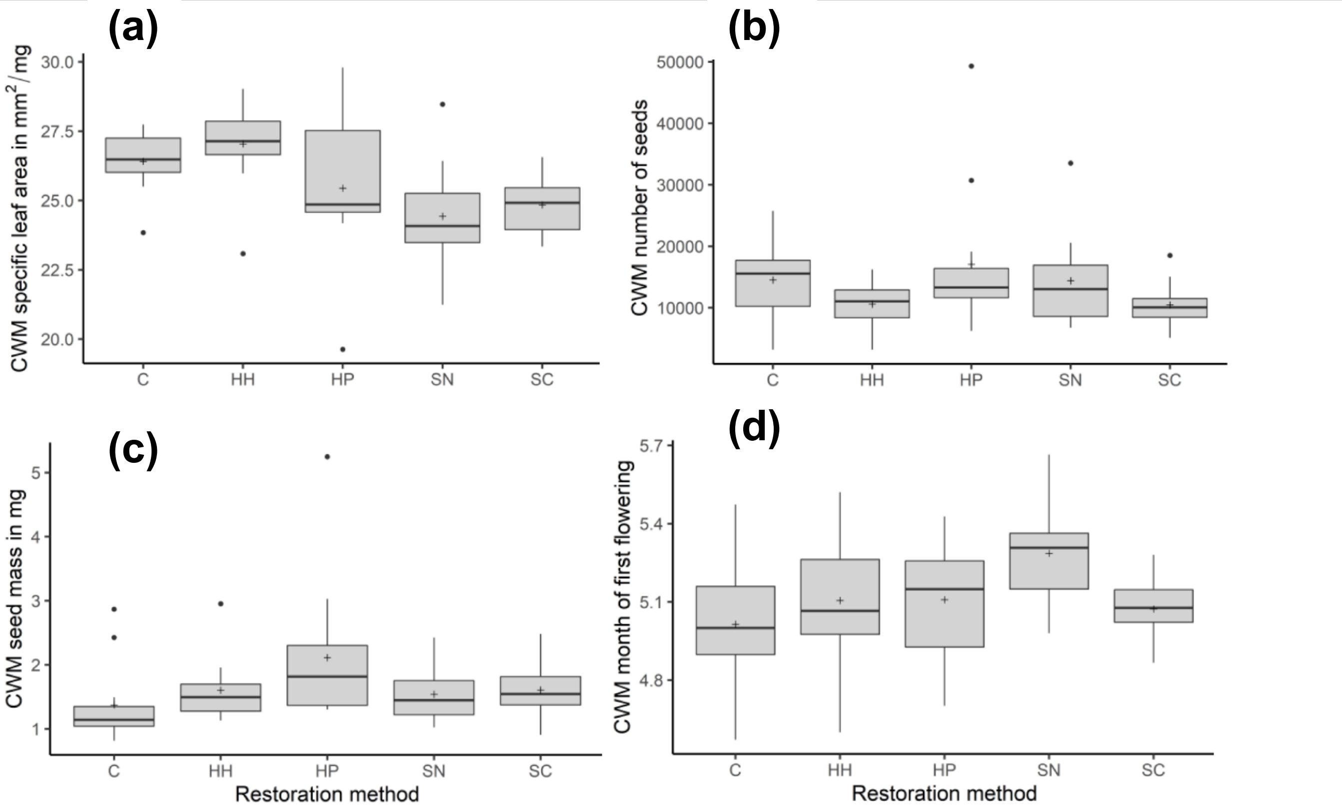

Figure 4 Community-weighted means of (a) specific leaf area, (b) number of seeds, (c) seed mass and (d) month of first flowering with respect to restoration treatment after restoration in 2021. Treatment abbreviations: C = control, HH = hay harrow, HP = hay plough, SN = seed natural, and SC = seed commercial.

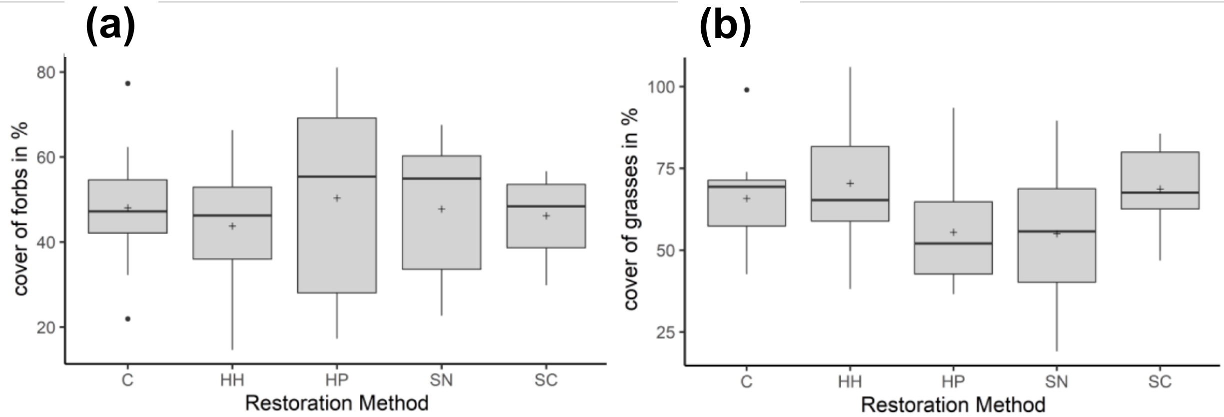

Figure 5 Proportion of (a) forbs and (b) grasses with respect to restoration treatment after restoration in 2021. Treatment abbreviations: C = control, HH = hay harrow, HP = hay plough, SN = seed natural, and SC = seed commercial.

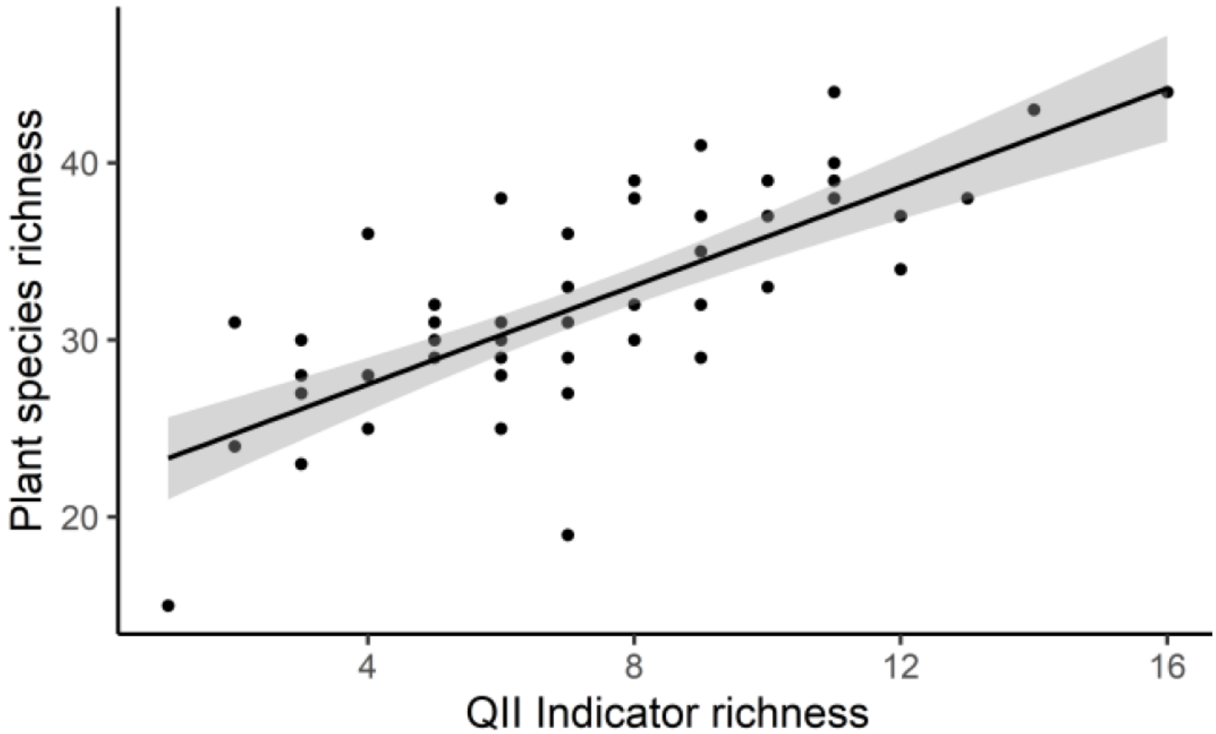

Figure 6 Linear regression between plant species richness and number of quality indicator species (black line) and 95% confidence intervals (grey shading). Quality indicator species are a selection of target species which typically occur extensively managed, species-rich grasslands and which are rarely found in intensified grasslands.

Supplementary material

Table S1 Pairwise test results for the effect of the four restoration methods compared to an unrestored control and against each other for (a) plant species richness, (b) beta diversity, (c) community weighted mean (CWM) of specific leaf area, (d) CWM of seed mass, (e) number of seeds, (f) flowering start, (g) cover of forbs, and (h) cover of graminoids. Estimates with standard error are provided. Significant p-values (P < 0.05) are highlighted in bold. Degrees-of-freedom method: Kenward-roger

(a)

Plant species richness				
Treatment	*mean*	*std. Error*	*df*	*CI*
C (Intercept)	26.4	1.34	54	23.7 – 29.1
HH	31.2	1.34	54	28.5 – 33.9
HP	33.4	1.34	54	30.7 – 36.1
SC	38.2	1.34	54	35.6 – 40.9
SN	31.8	1.41	54	29 – 34.6
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	-4.75	1.9	43	**0.016**
C - HP	-7	1.9	43	**0.001**
C - SC	-11.833	1.9	43	**<.0001**
C - SN	-5.402	1.95	43.8	**0.008**
HH - HP	-2.25	1.9	43	0.243
HH - SC	-7.083	1.9	43	**0.001**
HH - SN	-0.652	1.95	43.8	0.739
HP - SC	-4.833	1.9	43	**0.015**
HP - SN	1.598	1.95	43.8	0.416
SC - SN	6.432	1.95	43.8	**0.002**
Marginal R^2	0.41			

(b)

Treatment	mean	std. Error	df	CI
Beta diversity				
C (Intercept)	43.6	1.34	54	40.9 – 46.3
HH	42.8	1.34	54	40.1 – 45.5
HP	50.6	1.34	54	47.9 – 53.3
SC	41.8	1.34	54	39.1 – 44.4
SN	50.2	1.34	54	47.4 – 53
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	0.75	1.9	43.0	0.695
C - HP	-7	1.9	43.0	**0.001**
C - SC	1.833	1.9	43.0	0.340
C - SN	-6.598	1.95	43.8	**0.002**
HH - HP	-7.75	1.9	43.0	**0.000**
HH - SC	1.083	1.9	43.0	0.572
HH - SN	-7.348	1.95	43.8	**0.001**
HP - SC	8.833	1.9	43.0	**<.0001**
HP - SN	0.402	1.95	43.8	0.838
SC - SN		1.95	43.8	**<.0001**
Marginal R^2	0.403			

(c)

Predictors [Treatment]	mean	std. Error	df	CI
CWM SLA				
C (Intercept)	26.4	0.502	54	25.4 – 27.4
HH	27	0.502	54	26 – 28
HP	25.4	0.502	54	24.4 – 26.5
SC	24.8	0.502	54	23.8 – 25.8
SN	24.4	0.526	54	23.4 – 25.5
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	-0.631	0.711	43	0.380
C - HP	0.965	0.711	43	0.182
C - SC	1.569	0.711	43	**0.033**
C - SN	1.98	0.728	43.8	**0.009**
HH - HP	1.595	0.711	43	**0.030**
HH - SC	2.2	0.711	43	**0.003**
HH - SN	2.611	0.728	43.8	**0.001**
HP - SC	0.604	0.711	43	0.400
HP - SN	1.015	0.728	43.8	0.170
SC - SN	0.411	0.728	43.8	0.575
Marginal R^2	0.238			

(d)

CWM seed mass				
Predictors [Treatment]	*mean [seed mass]*	*std. Error*	*df*	*CI*
C (Intercept)	1.37	0.194	54	0.98 – 1.76
HH	1.6	0.194	54	1.21 – 1.99
HP	2.11	0.194	54	1.72 – 2.5
SC	1.61	0.194	54	1.22 – 2
SN	1.54	0.203	54	1.13 – 1.95
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	-0.2302	0.275	43	0.406
C - HP	-0.7386	0.275	43	**0.010**
C - SC	-0.2327	0.275	43	0.402
C - SN	-0.1659	0.281	43.8	0.558
HH - HP	-0.5084	0.275	43	0.071
HH - SC	-0.0025	0.275	43	0.993
HH - SN	0.0643	0.281	43.8	0.820
HP - SC	0.5060	0.275	43	0.072
HP - SN	0.5727	0.281	43.8	**0.048**
SC - SN	0.0668	0.281	43.8	0.814
Marginal R^2	0.123			

(e)

CWM number of seeds				
Predictors [Treatment]	*mean*	*std. Error*	*df*	*CI*
C (Intercept)	14534	2114	51.6	10292 – 18776
HH	10588	2114	51.6	6346 – 14830
HP	17071	2114	51.6	12829 – 21313
SC	10469	2114	51.6	6227 – 14711
SN	14720	2209	52.2	10287 – 19152
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	3946	2822	43	0.169
C - HP	-2537	2822	43	0.374
C - SC	4065	2822	43	0.157
C - SN	-185	2894	43.6	0.949
HH - HP	-6483	2822	43	**0.027**
HH - SC	119	2822	43	0.966
HH - SN	-4131	2894	43.6	0.161
HP - SC	6602	2822	43	**0.024**
HP - SN	2351	2894	43.6	0.421
SC - SN	-4251	2894	43.6	0.149
Marginal R^2	0.11			
Conditional R^2	0.21			

(f)

CWM flowering start (phenology)

Predictors [Treatment]	*mean [phenology]*	*std. Error*	*df*	*CI*
C (Intercept)	5.01	0.0655	42.8	4.88 – 5.15
HH	5.11	0.0655	42.8	4.97 – 5.24
HP	5.11	0.0655	42.8	4.98 – 5.24
SC	5.07	0.0655	42.8	4.94 – 5.2
SN	5.29	0.0681	44.8	5.15 – 5.42
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	-0.09	0.080	43	0.261
C - HP	-0.09	0.080	43	0.244
C - SC	-0.06	0.080	43	0.466
C - SN	-0.27	0.082	43.4	**0.002**
HH - HP	0.00	0.080	43	0.967
HH - SC	0.03	0.080	43	0.689
HH - SN	-0.18	0.082	43.4	**0.033**
HP - SC	0.04	0.080	43	0.659
HP - SN	-0.18	0.082	43.4	**0.036**
SC - SN	-0.21	0.082	43.4	**0.013**
Marginal R^2	0.135			
Conditional R^2	0.358			

(g)

Cover of forbs

Predictors [Treatment]	*mean [forb cover]*	*std. Error*	*df*	*CI*
C (Intercept)	48.1	4.59	51.6	38.9 – 57.3
HH	43.8	4.59	51.6	34.6 – 53
HP	50.4	4.59	51.6	41.1 – 59.6
SC	46.2	4.59	51.6	37 – 55.4
SN	47.6	4.8	52.2	38 – 57.2
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	4.321	6.13	43	0.485
C - HP	-2.267	6.13	43	0.713
C - SC	1.9	6.13	43	0.758
C - SN	0.469	6.29	43.6	0.941
HH - HP	-6.588	6.13	43	0.288
HH - SC	-2.421	6.13	43	0.695
HH - SN	-3.852	6.29	43.6	0.543
HP - SC	4.167	6.13	43	0.500
HP - SN	2.736	6.29	43.6	0.666
SC - SN	-1.431	6.29	43.6	0.821
Marginal R^2	0.019			
Conditional R^2	0.13			

(h) **Cover of graminoids**

Predictors [Treatment]	*mean*	*std. Error*	*df*	*CI*
C (Intercept)				
	65.8	4.95	53.6	55.9 – 75.7
HH	70.4	4.95	53.6	60.5 – 80.4
HP	55.5	4.95	53.6	45.6 – 65.4
SC	68.7	4.95	53.6	58.8 – 78.6
SN	55.2	5.18	53.7	44.8 – 65.5
Pairwise differences	*Estimates*	*std. Error*	*df*	*p. value*
C - HH	-4.646	6.85	43	0.501
C - HP	10.275	6.85	43	0.141
C - SC	-2.921	6.85	43	0.672
C - SN	10.63	7.02	43.7	0.137
HH - HP	14.921	6.85	43	**0.035**
HH - SC	1.725	6.85	43	0.802
HH - SN	15.275	7.02	43.7	**0.035**
HP - SC	-13.196	6.85	43	0.061
HP - SN	0.355	7.02	43.7	0.960
SC - SN	13.55	7.02	43.7	**0.060**
Marginal R^2	0.128			

Table S2 List of the commercial seed mix for the SC treatment. The mix was provided from the Swiss seed producer "UFA Samen" (Winterthur, Switzerland). All seeds are of Swiss origin.

Anthoxanthum odoratum	*Knautia arvensis*
Arrhenatherum elatius	*Lathyrus pratensis*
Briza media	*Leontodon hispidus*
Bromus erectus	*Leucanthemum vulgare*
Dactylis glomerata	*Lotus corniculatus*
Festuca pratensis	*Medicago lupulina*
Festuca rubra rubra	*Onobrychis viciifolia*
Helictotrichon pubescens	*Picris hieracioides*
Poa pratensis	*Pimpinella major*
Trisetum flavescens	*Plantago lanceolata*
Anthyllis carpatica	*Primula veris*
Campanula patula	*Salvia pratensis*
Campanula rotundifolia	*Sanguisorba minor*
Carum carvi	*Scabiosa columbaria*
Centaurea jacea	*Silene vulgaris*
Centaurea scabiosa	*Stachys officinalis*
Clinopodium vulgare	*Tragopogon orientalis*
Crepis biennis	*Trifolium pratense*
Daucus carota	*Vicia sepium*

General discussion

Although there is consensus that active grassland restoration is efficient in boosting the agricultural biodiversity, which is supported by the availability of practical guidelines (Scotton et al. 2012; Staub et al. 2015), numerous field studies and literature reviews on this topic (Kiehl et al. 2010; Hedberg & Kotowski 2010), the need for further research in the field of grassland restoration has been urged recently (Török et al. 2021). With this PhD thesis I contribute to the relatively young research field of restoration ecology, and more precisely, the restoration of grasslands. I conducted a systematic review and meta-analysis on the topic of restoration of mesic grasslands in Central Europe (Chapter 1). Thanks to this review I identified research gaps, such as the lack of considering invertebrates in restoration studies, few replicated field-scale studies, and a geographical bias, where Switzerland was underrepresented. I helped towards filling these gaps with a field-scale, grassland restoration experiment where I further studied the effects of different restoration methods on invertebrates (Chapter 2) and plants (Chapter 3). The studied methods were: (i) control with no seed addition and no soil disturbance, (ii) hay transfer from a species-rich donor meadow on a harrowed receiver meadow, (iii) hay transfer from a species-rich donor meadow on a ploughed receiver meadow, (iv) sowing of a commercial seed mixture on a ploughed receiver meadow and, (v) sowing of a brush- or vacuum harvested seed mixture on a ploughed receiver meadow. I could show the potential of transferring invertebrates with the hay during hay transfer operations and that soil disturbances linked to grassland restoration have no detrimental effect on the residing ground-dwelling invertebrate community in the mid-term. Furthermore, I showed that all restoration methods that were included in this experiment effectively increased the plant species richness, although there were differences on the trait level.

There are several points that distinguish our approach from previous grassland restoration studies. For one, there is the big scale at which the experiments were carried out. Here we have tested four treatments on field-scale replicated across twelve regions which were all established in the same year. This is unprecedented in grassland restoration studies. Previous studies that included several restoration methods were replicated in blocks on one field (Baasch et al. 2016; Albert et al. 2019) and had sometimes a small restored area of less than $40m^2$ (Fritch et al. 2011; Auestad et al. 2015). These experimental scales may be adequate for studying the short-term effects of plant communities, such as establishment rates. However, these kind of designs are unfit for studying treatment effects on invertebrates due to spill-over effects between treatment plots that could blur the outcomes (Lessard-Therrien et al. 2018). Other restoration studies which were replicated across different regions tested few methods (Freitag et al. 2021) or were exposed to inconsistent conditions, such as different management regimes after restoration (Woodcock et al. 2010). Our study design reduces the influence of these confounding factors by its real scale, the number of replicates, and similar site conditions across the study area. Site independence is essential especially for studying the effect on mobile species, which we accounted for by a minimal distance between sites.

In addition, it was important to us that our tested restoration methods can be implemented realistically by farmers and restoration practitioners. A group of stakeholders and experts consulted us on this subject and helped us in the selection of donor and restoration sites. All meadows in our experiment are managed and were restored by farmers, who voluntarily participate in our project. This way we can be sure that our recommendations are also applicable.

However, one of the major drawbacks of our study design was that all restoration treatments were established in the same year. Given that the meteorological conditions play an important role in seedling establishment, the possibility of a confounding factor caused by temperature and precipitation during and after restoration cannot be entirely excluded. With the before-after-control-intervention approach we can partly account for this bias. Furthermore, our study setup was not fully factorial, which limits the statistical power of our results. This is a common trade-off between field-scale and common garden experiments. There are two reasons why we did not apply a fully factorial design to our study. First, a fully factorial approach would imply treatment combinations which are known to be inefficient, such as overseeding on a undisturbed meadow (Freitag et al. 2021; Schmiede et al. 2012). It would be difficult to convince farmers to put effort into a treatment which is already known that it will not succeed. Second, to achieve all combinations of our treatment factors (i.e., soil disturbance, seeding method and seed source) we would have to increase the number of meadows to an amount which would make the realization of our experiment impossible with our resources.

In the following I will discuss in more detail the different factors concerning grassland restoration that were included in our study, namely soil disturbance intensity, seeding method and seed origin.

Soil disturbance intensity: Ploughing vs. harrowing

We could not observe any differences in terms of plant species richness between the ploughed and harrowed treatments, meaning that both treatments performed equally well. This confirms what previous studies have shown already in wet meadow restoration, that is, there is little difference between a more intense soil disturbance (plough) and superficial soil disturbance (harrow) concerning plant species establishment (Bischoff et al. 2018; Schmiede et al. 2012). This finding is further consolidated by our meta-analysis. To our knowledge, our study is the first one to compare the effect of soil disturbance intensity on the plant community in mesic grassland restoration. Furthermore, the ground-dwelling invertebrate community, represented by ground beetles and spiders, recovered one year after both types of soil intervention. Therefore, on the short term, a superficial or deep soil intervention prior to seeding perform equally well while not disrupting the soil invertebrate community.

In practice, using a harrow instead of a plough for soil disturbance exhibits several advantages. This method involves less interventions and preparation can be done within less than two weeks before seeding, while using a plough involves more interventions over several months. Therefore, this method could be preferred due to less effort and reduced costs. In addition, a shorter time span of open soil also reduces the risk of soil erosion, making it a good option on steeper sites.

Seeding method: Green hay vs. harvested seeds

Both seeding methods were equally effective to increase the plant species richness. In our meta-analysis we did not find a difference in performance between these two methods as well. Other studies found a similar performance (Baasch et al. 2016) or that hay transfer performed better than seeding of harvested seeds (Albert et al. 2019). We could further show that hay transfer can be used as well to transfer invertebrates from a donor to a receiver meadow. In practice, hay transfer might be an easier and cheaper option than sowing of harvested seeds. For the hay transfer we needed only one day, i.e. mowing the donor meadow early in the morning and then transporting and spreading the hay on the receiver meadow. The seed harvest method, however, required overall more effort, i.e., harvesting the donor meadow with a brush- or vacuum-harvester on one day and additional hand collections in the following months, cleaning the harvested material and storage (in our case the harvested seeds were stored for one year until sowing). All these points make this method more expensive than hay transfer. However, there is a higher flexibility in choosing the sowing day when using harvested seeds, which is a major advantage of the harvested seeds in terms of practicability. The right moment for the hay transfer depends on the overall ripeness of the meadow (usually early summer for mesic hay meadows) and the meteorological conditions during the hay transfer. A recent study discovered, that using hay as a seeding method reduces the risk of soil erosion compared to seeds only, which might be advantageous in steeper slopes (Durbecq et al. 2022).

Seed origin: Local vs. commercial seeds

The use of a commercial seed mix outperformed the use of local seeds (as harvested seeds or as green hay) in terms of plant species richness significantly, which contrasts with our findings from our meta-analysis. Therefore, if the main goal of a restoration project is to increase the plant species richness (vs. re-creating the local plant community), the usage of commercial seed mixes might be a viable option if some conditions are met. The seed mix should account for local ecotypes and intraspecific variation. This can be achieved by defining seed transfer zones (Cevallos et al. 2020; Durka et al. 2017). If commercial seeds are of unknown provenance their usage should be avoided since it might introduce genetically different and locally maladapted populations (Höfner et al. 2021).

Management recommendation

Our restoration experiment showed that all our tested restoration methods are efficient in enhancing the plant community after two years, while there was very little difference at the invertebrate community level between the treatments. Despite of this, at this stage it would be too early to give final recommendations for which method should be preferred or not, given that the plant community is exposed to variations in the first couple of years after restoration (Freitag et al. 2021; Albert et al. 2019). Nevertheless, based on our outcomes from the experiment and meta-analysis and taking into consideration other studies, we recommend:

- to use local seed sources to maintain the local community composition and genetic variation
- to prefer hay transfer over seed harvest to increase the chances of invertebrate transfer
- soil disturbance (i.e., seed bed preparation) should be adapted to the local conditions, at which both, ploughing or harrowing can be applied.

These recommendations strongly depend on the availability of a species-rich donor meadow in the vicinity of the grassland that should be restored. If such species-rich grasslands are lacking the use of a commercial seed mix can be used.

Our results and recommendations also apply for the neighboring regions around Switzerland, given that the climatic and agricultural conditions are quite similar. This might be particularly interesting in regions which apply a result-based payment scheme, where farmers or landowner are compensated when their meadows achieve a certain plant diversity (Herzon et al. 2018). Concerns about restoration failure, reluctance towards some methods (e.g., some of the farmers from our project did not like the idea of ploughing their meadows) and the costs and efforts that are involved in restoration grasslands might impede certain landowners to restore their species-poor grasslands. Having the choice between a set of reliable tools for restoration and receiving a compensation for successful restoration could be helpful to further increase the acceptance of grassland restoration.

Further research

As we pointed out already in our meta-analysis, few grassland restoration studies focus on above- and below-ground invertebrates. To better understand how grassland restoration affects the entire community of the grassland habitat, we must widen our scope to other taxa. In a next step, the plant-dwelling invertebrate community as well as pollinators should be studied in our restored and control meadows. Pollinating insect are an essential part of our agro-ecosystem and it would be important to know whether one restoration method favors more the pollinators than another. Furthermore, below-ground invertebrates are only rarely studied within grassland restoration experiments (Resch et al. 2019; Norton et al. 2019). Although we could show that the different soil disturbance intensities were not harmful to the ground-dwelling invertebrates in the mid-term, the organisms living permanently below the soil surface might be affected differently.

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