

Grassland Conservation

Global evidence for the effects of selected interventions



**Philip A. Martin, Nancy Ockendon, Anna Berthinussen,
Rebecca K. Smith & William J. Sutherland**

CONSERVATION EVIDENCE SERIES SYNOPSES

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Conservation Evidence Series Synopses



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<https://www.conservationevidence.com/>

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1. About this book

1.1 *The Conservation Evidence project*

The Conservation Evidence project has four main parts:

1. The **synopses** of the evidence captured for the conservation of particular species groups or habitats, such as this synopsis. Synopses bring together the evidence for each possible intervention. They are freely available online and, in some cases, available to purchase in printed book form.
2. An ever-expanding **database of summaries** of previously published scientific papers, reports, reviews or systematic reviews that document the effects of interventions. This resource comprises over 7,650 pieces of evidence, all available in a searchable database on the website www.conservationevidence.com.
3. ***What Works in Conservation***, which is an assessment of the effectiveness of interventions by expert panels, based on the collated evidence for each intervention for each species group or habitat covered by our synopses. This is available as part of the searchable database and is published as an updated book edition each year (www.conservationevidence.com/content/page/79).
4. An online, **open access journal** *Conservation Evidence* publishes new pieces of research on the effects of conservation management interventions. All our papers are written by, or in conjunction with, those who carried out the conservation work and include some monitoring of its effects (www.conservationevidence.com/collection/view).

1.2 The purpose of Conservation Evidence synopses

Conservation Evidence synopses do	Conservation Evidence synopses do not
<ul style="list-style-type: none">• Bring together scientific evidence captured by the Conservation Evidence project (over 7,650 studies so far) on the effects of interventions to conserve biodiversity• List all realistic interventions for the species group or habitat in question, regardless of how much evidence for their effects is available• Describe each piece of evidence, including methods, as clearly as possible, allowing readers to assess the quality of evidence• Work in partnership with conservation practitioners, policymakers and scientists to develop the list of interventions and ensure we have covered the most important literature	<ul style="list-style-type: none">• Include evidence on the basic ecology of species or habitats, or threats to them• Make any attempt to weight or prioritize interventions according to their importance or the size of their effects• Weight or numerically evaluate the evidence according to its quality• Provide recommendations for conservation problems, but instead provide scientific information to help with decision-making

1.3 Who this synopsis is for

If you are reading this, we hope you are someone who has to make decisions about how best to support or conserve biodiversity. You might be a land manager, a conservationist in the public or private sector, a farmer, a campaigner, an advisor or consultant, a policymaker, a researcher or someone taking action to protect your own local wildlife. Our synopses summarize scientific evidence relevant to your conservation objectives and the actions you could take to achieve them.

We do not aim to make your decisions for you, but to support your decision-making by telling you what evidence there is (or isn't) about the effects that your planned actions could have.

When decisions have to be made with particularly important consequences, we recommend carrying out a systematic review, as the latter is likely to be more comprehensive than the summary of evidence presented here. Guidance on how to carry out systematic reviews can be found from the Centre for Evidence-Based Conservation at the University of Bangor (www.cebc.bangor.ac.uk).

1.4 Background

Grasslands are areas in which vegetation is dominated by grasses, with little woody vegetation (Gibson 2009). A key feature of grasslands is their need for regular disturbance to limit colonisation by woody plants that would lead to their conversion to shrubland or forest. Natural grasslands rely on disturbance from recurrent fires or large herds of grazing animals in order to reduce colonisation by woody shrub and tree species while semi-natural grasslands lack these natural disturbances and so rely on anthropogenic disturbances, such as cutting or grazing with livestock, to arrest succession (Gibson 2009).

Grasslands occur on every continent on earth, apart from Antarctica, and cover 31-43% of the earth's surface, making them one of the most widespread biomes in the world (Rosen, 2000). Over the past century, humans have subjected grasslands to particularly severe pressure, leading to losses of 46% of temperate grassland, 24% of tropical and subtropical grassland, and 13% of montane grasslands (Hoekstra *et al.* 2005). Some regions have seen particularly large declines, for example, semi-natural grasslands in the UK declined by an estimated 97% between the 1930s and 1990s (Bullock *et al.* 2011). These changes have largely been the result of conversion of grasslands to croplands or sowing of agriculturally preferred species ('grassland improvement'), though grasslands are also threatened by invasive non-native species, loss of disturbance, increases in livestock, and pollution (Gibson 2009, Bullock *et al.* 2011).

To improve the conservation status of the world's grasslands, it is vital that the most effective interventions for conserving and restoring them are identified. Doing this requires the synthesis of existing studies of interventions. To fulfil this need, this synopsis summarises the available global evidence on the effectiveness of grassland restoration interventions involving seeding and planting. Traditionally this would be done using a targeted review, but these are labour-intensive and expensive. Furthermore, they are ill-suited for areas where the data are scarce and patchy. Here, we use a subject-wide evidence synthesis approach (Sutherland *et al.* 2019) to simultaneously collate the evidence for the wide range of interventions dedicated to the restoration of grasslands and summarize that focussed on seeding and planting. By simultaneously targeting the entire body of interventions, we are able to review the evidence for each intervention cost-effectively, and the resulting synopsis can be updated periodically and efficiently. The synopsis is freely available at www.conservationevidence.com and, alongside the Conservation Evidence online database, is a valuable asset to the toolkit of practitioners and policy makers seeking sound information to support grassland conservation. We plan to summarise the evidence for the interventions not covered in this synopsis and then periodically update the synopsis in the future, to incorporate new research. The methods we used to produce the Grassland Conservation Synopsis are outlined below.

1.5 Scope of the Grassland Conservation synopsis

1.5.1 Review subject

This synopsis focuses on the evidence for the effectiveness of global interventions for the conservation of grasslands and the effects of these interventions on vegetation and plant species in grasslands. For details on other taxonomic groups you may be interested in, see the Conservation Evidence website (www.conservationevidence.com).

The Grassland Conservation synopsis was produced using a subject-wide evidence synthesis approach. This is defined as a systematic method of evidence synthesis that covers entire subjects at once, including all closed review topics within that subject at a fine scale and analysing results through study summary and expert assessment, or through meta-analysis; the term can also refer to any product arising from this process (Sutherland *et al.* 2019).

This synopsis in its current form focuses on grassland restoration interventions that involve seeding or planting. We chose to focus on these interventions because they represent commonly used actions in grassland restoration. The output of the project is an authoritative, freely accessible evidence-base that will support grassland conservation objectives with the latest evidence and help to achieve conservation outcomes.

1.5.2 Advisory board

To assist with the production of the synopsis we brought together international conservationists and academics with expertise in grassland conservation to form an advisory board. These experts provided input for the synopsis at three key stages: a) producing the comprehensive list of conservation interventions for review, b) identifying key journals to search for evidence, and c) reviewing the draft evidence synthesis. The advisory board is listed above and online (<https://www.conservationevidence.com/content/page/119>).

1.5.3 Creating the list of interventions

At the start of the project, a comprehensive list of interventions was developed by searching the literature and in partnership with the advisory board. The list was also checked by Conservation Evidence to ensure that it followed the standard structure. The aim was to include all interventions that have been carried out or advised to conserve or restore grasslands, whether evidence for the effectiveness of an intervention is available or not. During the synthesis process further interventions were discovered and integrated into the synopsis structure.

The list of interventions was organized into categories based on the IUCN classifications of direct threats: (www.iucnredlist.org/technical-documents/classification-schemes/threats-

[classification-scheme](#)) and conservation actions: (www.iucnredlist.org/technical-documents/classification-schemes/conservation-actions-classification-scheme-ver2).

In total, we found 180 conservation and/or management interventions that could be carried out to conserve grasslands (see Appendix 1 for the full list). However, as previously mentioned, in this synopsis we focused on synthesising evidence for the 36 of these interventions that relate to grassland restoration involving sowing seeds or planting. The evidence was reported as 198 summaries from 137 relevant publications found during our searches (see Methods below).

1.6 Methods

1.6.1 Literature searches

Literature was obtained from the Conservation Evidence discipline-wide literature database, and from searches of additional subject-specific literature sources (see Appendices 3 & 4). The Conservation Evidence discipline-wide literature database is compiled using systematic searches of journals (by screening all titles and abstracts) and report series ('grey literature'); relevant publications describing studies of conservation interventions for all species groups and habitats were saved from each and were added to the database. The final list of evidence sources searched for this synopsis is published in this synopsis document (see Appendix 3) and online (<https://www.conservationevidence.com/journalsearcher/synopsis>).

a) Global evidence

Evidence from all around the world was included.

b) Languages included

All journals searched were published in English. A recent study on the topic of language barriers in global science indicates that approximately 35% of conservation studies may be in non-English languages (Amano *et al.* 2016). While only including English-language literature may introduce some biases, resource and time constraints meant that we could not feasibly search non-English language journals.

c) Journals searched

i) From Conservation Evidence discipline-wide literature database

All of the journals (and years) listed in Appendix 3 were searched prior to or during the completion of this project by authors of other synopses, and relevant papers added to the Conservation Evidence discipline-wide literature database. An asterisk indicates the journals most relevant to this synopsis. Others are less likely to include papers relevant to this synopsis, but if they did, those papers were summarised.

ii) Update searches

The authors of this synopsis updated the search of the following journals:

- Acta Oecologia (2013-2017)
- Ecological Indicators (2001-2018)
- Global change biology (2014-2017)

iii) New searches

Additional, focussed searches of journals most relevant to the conservation of grasslands listed in Appendix 3 were undertaken. These journals were identified through expert judgement by the project researchers and the advisory board.

- Grass and Forage Science (1930-2017)
- Preslia (1973-2017)

d) Reports from specialist websites searched

i) From Conservation Evidence discipline-wide literature database

All report series (and years) below have already been systematically searched for the Conservation Evidence project, during the production of previous synopses for example.

- | | | |
|--|-----------|----------------|
| • Amphibian Survival Alliance | 1994–2012 | Vol 9 –Vol 104 |
| • British Trust for Ornithology | 1981–2016 | Report 1–687 |
| • IUCN Invasive Species Specialist Group | 1995–2013 | Vol 1–Vol 33 |
| • Scottish Natural Heritage | 2004–2015 | Reports 1–945 |

ii) Update searches

Updates searches of report series already searched as part of the wider Conservation Evidence project were not undertaken for this synopsis.

No new report searches were undertaken for this synopsis due to time constraints.

e) Other literature searches

The online database (www.conservationevidence.com) was searched for relevant publications that have already been summarised. If such summaries existed, they were extracted and added to this synopsis update.

Where a systematic review was found for an intervention, if the intervention had a small literature (<20 papers), all available English language publications including the systematic review were summarised. If the intervention had a large literature (≥20 papers), then only the systematic review was summarised. Where a non-systematic review (or editorial, synthesis,

preface, introduction etc.) was found for an intervention, all relevant and accessible English language publications referenced within it were included, but the review itself was not summarised. However, if the review also provided new/collective data, then the review itself was also included/summarised. Relevant publications cited in other publications summarised for the synopsis were not included (due to time restrictions).

f) Supplementary literature identified by advisory board or relevant stakeholders

Relevant papers or reports suggested by the advisory board or relevant stakeholders were also included, if relevant.

g) Search record database

A database was created of all relevant publications found during searches. Reasons for exclusion were recorded for all studies included during screening but not summarised for the synopsis.

1.6.2 Publication screening and inclusion criteria

A summary of the total number of evidence sources and papers/reports screened is presented in the diagram in Appendix 4.

a) Screening

We acknowledge that the literature search and screening method used by Conservation Evidence, as with any method, results in gaps in the evidence. The Conservation Evidence literature database currently includes relevant papers from over 330 English language journals as well as over 327 non-English journals (www.conservationevidence.com/content/page/108). Additional journals are frequently added to those searched, and years searched are often updated. It is possible that searchers will have missed relevant papers from those journals searched. Publication bias will not be taken into account, and it is likely that additional biases will result from the evidence that is available. For example, there are often geographic biases in study locations (Christie *et al.* 2020).

b) Inclusion criteria

The following Conservation Evidence inclusion criteria were used.

- There has to be an intervention that conservationists would be likely to do
- Its effects on biodiversity or ecosystem services must have been monitored quantitatively

If the intervention was used for conservation purposes but was done for a different purpose in the study in question, it was included, provided the details of the intervention were the same and the effects on biodiversity or ecosystem services were monitored.

For example, methods to rear bumblebees in captivity for commercial pollination have been used to support conservation of rare bumblebees. All studies testing these methods were included in our bee synopsis. Another example is the construction of artificial wetlands for amphibian conservation. Studies that monitor amphibian numbers in wetlands constructed largely for recreational purposes were included.

Modelling studies that do not actually test the intervention vs a control on the ground are not included.

c) Relevant subject

Studies relevant to the synopsis subject were those focused on the conservation of grasslands.

d) Relevant types of intervention

An intervention has to be one that could be put in place by a manager, conservationist, policy maker, advisor or consultant to protect, manage, restore or reduce the impacts of threats to grasslands. Alternatively, interventions may aim to change human behaviour (actual or intentional), which is likely to protect, manage, restore or reduce threats to grasslands.

e) Relevant types of comparator

To determine the effectiveness of interventions, studies must include a comparison, i.e. monitoring change over time (typically before and after the intervention was implemented), or for example at treatment and control sites. Alternatively, a study could compare one specific intervention (or implementation method) against another. For example, this could be comparing the species richness of a grassland before and after it is restored, or the species richness resulting from different methods of grassland restoration.

Exceptions, which may not have a control but were still included, are, for example, the survival of planted or sown species.

f) Relevant outcomes

Fifteen **core outcomes** (Table 1) have been consistently reported throughout the synopsis. These outcomes were prioritized in order to keep summaries short. They involve direct measures of vegetation community composition, abundance or structure. We aimed to summarize all results related to the key outcomes within individual summary paragraphs. The core outcomes are always included in the Key Messages for an intervention if we found any studies that quantified them.

Four **intermediate outcomes** (Table 2) have been consistently reported for some interventions in the synopsis: interventions where these outcomes provide a useful indication of success, or for which more precise monitoring of vegetation responses is difficult, impossible, or rarely reported. For example, grassland habitat area is an informative outcome for interventions that might be assessed at the landscape scale, such as a comparison of protected area effectiveness in two countries. Another example is the use of 'sown/planted species richness/diversity' or 'sown/planted species abundance' which are commonly measured in studies of grassland restoration. The first sentence of the Key Messages indicates when these outcomes have been tracked for a particular intervention.

Additional outcomes have been summarized for individual studies when they are an important result in a particular study, and/or the summary paragraph is not too long once core outcomes have been summarized. These additional outcomes may be included in Key Messages if they reflect an important result across a particular intervention. If a specific outcome is included in Key Messages for an intervention, results relating to that outcome have been consistently extracted from all studies in that intervention. Additional outcomes that have been included in a least one set of Key Messages include: relative abundance, relative richness/diversity, native/non-target abundance, native/non-target richness/diversity, grass richness/diversity, forb richness/diversity, canopy cover, and individual plant size (e.g. biomass/plant or stems/plant).

Table 1. Summary of **Core Outcomes** and **Intermediate Outcomes** reported throughout the Grassland Conservation synopsis. Terms in *italics* are defined further in the Glossary.

Theme: VEGETATION COMMUNITY	
Community composition	Overall taxonomic composition; how characteristic overall community is of grassland conditions. This could be measured using community similarity indices (e.g. Sorenson similarity index).
Overall richness /diversity	Absolute richness/diversity of plant species/genera. Some measure of overall vegetation: all or vascular plants.
Characteristic plant richness/diversity	Absolute richness/diversity of <i>grassland-characteristic</i> plant species, or species described in a study as characteristic of a particular grassland habitat (e.g. “old meadow characteristic species”, “target steppe species” or “indicator species”).
Sown/planted species richness/diversity	Absolute richness/diversity of <i>sown or planted</i> species. (<i>Intermediate Outcome: reported for selected interventions only</i>).
Theme: VEGETATION ABUNDANCE	
Overall abundance	Absolute vegetation <i>abundance</i> . Some measure of overall vegetation: all or vascular plants.
Characteristic plant abundance	Absolute <i>abundance</i> of <i>grassland-characteristic</i> plant species, or species otherwise defined as characteristic of a habitat type within a study.
Sown/planted species abundance	Overall abundance of <i>sown or planted</i> species. (<i>Intermediate Outcome: reported for selected interventions only</i>).
Grass abundance	Abundance of grasses or grass species overall, or for subgroups e.g. C3/‘cool season’ grasses, C4/‘warm season’ grasses.
Forb abundance	<i>Abundance</i> of herb species that are not grasses or grass-like plants, either overall, or for subgroups.
Tree/shrub abundance	Overall, or for subgroups: trees, shrubs, or dwarf shrubs.
Individual species abundance	<i>Abundance</i> of named plant species. Typically dominant species or species showing largest responses to intervention.
Theme: VEGETATION STRUCTURE	
Height	Maximum or average, across the whole community.
Diameter/perimeter/area	Metrics related to the size of individual trunks or woody stems, or area occupied by individual plants.
Basal area	Cross sectional area multiplied by density, typically measured for trunks or woody stems.
Theme: OTHER	
Germination/Emergence	Proportion of seeds/propagules that produced seedlings or bulbs/rhizomes/tubers that produced above ground parts.
Survival	Survival rate of individual plants, colonies or sods. Includes absence of planted species (i.e. 0% survival).
Growth	Growth rate of individual plants or colonies. Alternatively, change in average size of plants if there is no mortality, only individuals that survived whole experiment are analysed, or it is clear that size was 0 when planted (e.g. when sowing seeds or propagules).
Grassland habitat area	Overall area of grassland habitat. Must quantify outcome of intervention (e.g. “After creating a protected area, the area of grassland increased by 50 ha”) not the intervention itself (e.g. “Three hectares of grassland were created”). (<i>Intermediate Outcome: reported for selected interventions only</i>).
Human behaviour	Difference/change in positive behaviour (e.g. consumer purchasing behaviour; creation of protected areas in response to lobbying) or negative behaviour (e.g. burning, unsustainable harvesting). (<i>Intermediate Outcome: reported for selected interventions only</i>).

Generally, we have not calculated new outcomes from data provided in papers. For example, if a study reports *native plant species richness*, this was not converted to *overall plant species richness* by adding non-native plant species. However, terminology has been harmonized so that all results relating to the same outcome (e.g. measures of tree trunk “diameter” and “width”) are grouped together. In particular, “growth” in this synopsis only includes results that clearly reflect growth of individual plants, such as changes in their biomass, height, diameter, or basal area. Changes in average size have typically been summarized under Vegetation Structure because they do not necessarily reflect growth. The average height of 100 seedlings might increase if the shortest 50 seedlings die, even if there is no change in height of the 50 surviving seedlings.

Outcomes explicitly **not reported** in this synopsis (unless they help interpretation of summarized results for a particular study) include:

- Plant physiology (e.g. gas exchange, nutrient uptake, tissue chemistry), productivity (if not measured as standing biomass), seed/flower production (number or timing, unless used as an estimate of vegetation abundance), nutritional value, genetic richness/diversity.
- Any outcomes related to seeds in the soil (e.g. abundance, richness, diversity).
- Outcomes relating specifically to rare plant species (that are not abundant where they occur).
- Habitat suitability indices, e.g. overall indices of the quality of marsh or swamp habitat for birds.
- Outcomes relating to organisms other than plants, such as birds or amphibians. These are covered in other Conservation Evidence synopses and on www.conservationevidence.com.
- Ecosystem functions (e.g. nutrient cycling) and services (e.g. carbon storage). However, note that these often benefit if vegetation is conserved.
- Outcomes relating to knowledge or awareness, rather than behaviour.
- Vague outcomes such as “successfully restored” or “project objectives were met”, unless clear quantitative objectives were set (Zedler 2007).

We have also excluded studies that aimed to control invasive or other problematic species, and which do not report effects on vegetation other than those species. Such studies are, or will be, summarized in other Conservation Evidence synopses (e.g. Aldridge *et al.* 2017). Thus, when outcomes related to invasive or problematic species have been reported, be aware that these may not give the full picture of effects on these species.

g) Relevant types of study design

Table 2 lists the study designs included. The strongest evidence comes from replicated, randomized, controlled trials with paired-sites and before-and-after monitoring.

Table 2. Relevant study designs

Term	Meaning
Replicated	The intervention was repeated on more than one individual or site. In conservation and ecology, the number of replicates is much smaller than it would be for medical trials (when thousands of individuals are often tested). If the replicates are sites, pragmatism dictates that between five and ten replicates is a reasonable amount of replication, although more would be preferable. We provide the number of replicates wherever possible. Replicates should reflect the number of times an intervention has been independently carried out, from the perspective of the study subject. For example, 10 plots within a mown field might be independent replicates from the perspective of plants with limited dispersal, but not independent replicates for larger motile animals such as birds. In the case of translocations/release of captive bred animals, replicates should be sites, not individuals.
Randomized	The intervention was allocated randomly to individuals or sites. This means that the initial condition of those given the intervention is less likely to bias the outcome.
Paired sites	Sites are considered in pairs, within which one was treated with the intervention and the other was not. Pairs, or blocks, of sites are selected with similar environmental conditions, such as soil type or surrounding landscape. This approach aims to reduce environmental variation and make it easier to detect a true effect of the intervention.
Controlled*	Individuals or sites treated with the intervention are compared with control individuals or sites not treated with the intervention. (The treatment is usually allocated by the investigators (randomly or not), such that the treatment or control groups/sites could have received the treatment).
Before-and-after	Monitoring of effects was carried out before and after the intervention was imposed.
Site comparison*	A study that considers the effects of interventions by comparing sites that historically had different interventions (e.g. intervention vs no intervention) or levels of intervention. Unlike controlled studies, it is not clear how the interventions were allocated to sites (i.e. the investigators did not allocate the treatment to some of the sites).
Review	A conventional review of literature. Generally, these have not used an agreed search protocol or quantitative assessments of the evidence.
Systematic review	A systematic review follows structured, predefined methods to comprehensively collate and synthesise existing evidence. It must weight or evaluate studies, in some way, according to the strength of evidence they offer (e.g. sample size and rigour of design). Environmental systematic reviews are available at: www.environmentalevidence.org/index.htm .
Study	If none of the above apply, for example a study measuring change over time in only one site and only after an intervention. Or a study measuring use of nest boxes at one site.

* Note that “controlled” is mutually exclusive from “site comparison”. A comparison cannot be both controlled and a site comparison. However, one study might contain both controlled and site comparison aspects e.g. study of fertilized grassland, compared to unfertilized plots (controlled) and natural, target grassland (site comparison).

1.6.3 Study quality assessment & critical appraisal

We did not quantitatively assess the evidence from each publication or weight it according to quality. However, to allow interpretation of the evidence, we made the size and design of each study we reported clear.

We critically appraised each potentially relevant study and excluded those that did not provide data for a comparison to the treatment or had obvious errors in their design or analysis. A record of the reason for excluding any of the publications included during screening was kept within the synopsis database.

1.6.4 Data extraction

Data on the effectiveness of the relevant intervention (e.g. mean species richness in sown and unsown areas; vegetation abundance before and after addition of fertilizer alongside seeding/planting) were extracted from, and summarised for, publications that included the relevant subject, types of intervention, comparator and outcomes outlined above. A summary of the total number of evidence sources and papers/reports searched and the total number of publications included following data extraction is presented in Appendix 4.

At the start of each month, authors exchanged three summaries with another author to ensure that the correct type of data had been extracted and that the summary followed the Conservation Evidence standard format.

1.6.5 Evidence synthesis

a) Summary protocol

Each publication usually had just one paragraph for each intervention it tested describing the study. Summaries were written in plain English and, where possible, were no more than 150 words long, though more complex studies required longer summaries. Each summary used the following format:

A [TYPE OF STUDY] in [YEARS X-Y] in [HOW MANY SITES] in/of [HABITAT] in [REGION and COUNTRY] [REFERENCE] found that [INTERVENTION] [SUMMARY OF ALL KEY RESULTS] for [SPECIES/HABITAT TYPE]. [DETAILS OF KEY RESULTS, INCLUDING DATA]. In addition, [EXTRA RESULTS, IMPLEMENTATION OPTIONS, CONFLICTING RESULTS]. The [DETAILS OF EXPERIMENTAL DESIGN, INTERVENTION METHODS and KEY DETAILS OF SITE CONTEXT]. Data was collected in [DETAILS OF SAMPLING METHODS].

Type of study - see terms and order in Table 2.

Site context - for the sake of brevity, only nuances essential to the interpretation of the results are included. The reader is always encouraged to read the original source to get a full understanding of the study site (e.g. history of management, physical conditions, landscape context).

For example:

A replicated study in 1999–2004 in a wetland on an island in Catalonia, Spain (1) found that all 69 bat boxes of two different designs were used by soprano pipistrelles *Pipistrellus pygmaeus* with an average occupancy rate of 71%. During at least one of the four breeding seasons recorded, 96% of boxes were occupied and occupation rates by females with pups increased from 15% in 2000 to 53% in 2003. Bat box preferences were detected in the breeding season only, with higher abundance in east-facing bat boxes (average 22 bats/box) compared to west-facing boxes (12 bats/box), boxes with double compartments (average 25 bats/box) compared to single compartments (12 bats/box) and boxes placed on posts (average 18 bats/box) and houses (average 12 bats/box). Abundance was low in bat boxes on trees (average 2 bats/box). A total of 69 wooden bat boxes (10 cm deep × 19 cm wide × 20 cm high) of two types (44 single and 25 double compartment) were placed on three supports (10 trees, 29 buildings and 30 electricity posts) facing east and west. From July 2000 to February 2004, the boxes were checked on 16 occasions. Bats were counted in boxes or upon emergence when numbers were too numerous to count within the box.

(1) Flaquer C., Torre I. & Ruiz-Jarillo R. (2006) The value of bat-boxes in the conservation of *Pipistrellus pygmaeus* in wetland rice paddies. *Biological Conservation*, 128, 223–230.

A replicated, randomized, controlled, before-and-after study in 1993–1999 of five harvested hardwood forests in Virginia, USA (2) found that harvesting trees in groups did not result in higher salamander abundances than clearcutting. Abundance was similar between treatments (group cut: 3; clearcut: 1/30 m²). Abundance was significantly lower compared to unharvested plots (6/30 m²). Species composition differed before and three years after harvest. There were five sites with 2 ha plots with each treatment: group harvesting (2–3 small area group harvests with selective harvesting between), clearcutting and an unharvested control. Salamanders were monitored on 9–15 transects (2 × 15 m)/plot at night in April–October. One or two years of pre-harvest and 1–4 years of post-harvest data were collected.

(2) Knapp S.M., Haas C.A., Harpole D.N. & Kirkpatrick R.L. (2003) Initial effects of clearcutting and alternative silvicultural practices on terrestrial salamander abundance. *Conservation Biology*, 17, 752–762.

b) Terminology used to describe the evidence

Unless specifically stated otherwise, results reflect statistical tests performed on the data, i.e. we only state that there was a difference if it was a significant difference or state that there was no difference if it was not significant. Table 2 above defines the terms used to describe the study designs.

c) Dealing with multiple interventions within a publication

When separate results were provided for the effects of each of the different interventions tested, separate summaries have been written under each intervention heading.

d) Dealing with multiple publications reporting the same results

If two publications described results from the same intervention but over different periods of time (e.g. after year one vs after one–three years), we only included the publication covering the longest time span. If two publications described at least partially different results, we included both but made it clear they were from the same project in the paragraph, e.g. ‘A controlled study... (Gallagher *et al.* 1999; same experimental set-up as Oasis *et al.* 2001)...’.

e) Taxonomy

Taxonomy was not updated but followed that used in the original publication. Where possible, common names and Latin names were both given the first time each species was mentioned within each summary.

f) Key messages

Each intervention for which evidence is found has a set of concise, bulleted Key Messages at the top, which was written once all the literature had been summarised. These include information such as the number, design and location of studies included.

The first bullet point describes the total number of studies that tested the intervention and the locations of the studies, followed by key information on the relevant metrics presented under the headings and sub-headings shown below (with number of relevant studies in parentheses for each).

- **X studies** examined the effects of [INTERVENTION] on [TARGET HABITAT]. Y studies were in [LOCATION 1]^{1,2} and Z studies were in [LOCATION 2]^{3,4}. *Locations will usually be countries, ordered based on order of studies rather than alphabetically, i.e. USA¹, Australia² not Australia², USA¹. However, when more than 4-5 separate countries, you may group into regions to make it clearer e.g. Europe, North America. You may add distribution of studies amongst habitat types here if relevant.*

VEGETATION COMMUNITY (x STUDIES)

- **Community composition (x studies):**
- **Overall richness/diversity (x studies):**
- **Characteristic plant richness/diversity (x studies):**
- **Sown/planted species richness/diversity (x studies):**

VEGETATION ABUNDANCE (x STUDIES)

- **Overall (x studies):**
- **Characteristic plant abundance (x studies):**
- **Sown/planted species abundance (x studies):**
- **Grass abundance (x studies):**

- Forb abundance (x studies):
- Tree/shrub abundance (x studies):
- Individual species abundance (x studies):

VEGETATION STRUCTURE (x STUDIES)

- Height (x studies):
- Diameter (x studies):
- Basal area (x studies):

OTHER (x STUDIES) *(Included only for interventions/chapters where relevant)*

- Germination/Emergence (x studies):
- Survival (x studies):
- Growth (x studies):
- Grassland habitat area (x studies): *(e.g. "grassland" in a protected area)*
- Human behaviour (x studies): *(for education/awareness chapter)*

If no evidence was found for an intervention, the following text was added in place of the Key Messages above:

- We found no studies that evaluated the effects of [INTERVENTION] on [TARGET POPULATION].

'We found no studies' means that we have not yet found any studies that have directly evaluated this intervention during our systematic journal and report searches. Therefore we have no evidence to indicate whether or not the intervention has any desirable or harmful effects.

g) Background information

Background information for an intervention is provided to describe the intervention and where we feel recent knowledge is required to interpret the evidence. This is presented before the Key Messages and relevant references included in the reference list at the end of the intervention section. In some cases, where a body of literature has strong implications for grassland conservation, but does not directly test interventions for their effects, we may also refer the reader to this literature in the background sections.

1.6.6 Dissemination/communication of evidence synthesis

The information from this synopsis update will be available in three ways:

- This synopsis pdf, downloadable from www.conservationevidence.com, which contains the study summaries, Key Messages and background information on each intervention.
- The searchable database at www.conservationevidence.com, which contains all the summarized information from the synopsis, along with updated expert assessment scores.

- A chapter in *What Works in Conservation*, available as a pdf to download and a book from www.conservationevidence.com/content/page/79, which contains the Key Messages from the synopsis as well as updated expert assessment scores on the effectiveness and certainty of the synopsis, with links to the online database.

1.7 How you can help to change conservation practice

If you know of evidence relating to grassland conservation that is not included in this synopsis, we invite you to contact us via our website www.conservationevidence.com. If you have new, unpublished evidence, you can submit a paper to the *Conservation Evidence Journal* (www.conservationevidencejournal.com/collection/journaldetails). We particularly welcome papers submitted by conservation practitioners.

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2. Habitat restoration and creation: seeding and planting

2.1. Sow grass seeds

- **Thirteen studies** examined the effects of sowing grass seeds on grassland vegetation. Six studies were in North America^{1–3,9,10,12}, five studies were in Europe^{4,5,8,11,13} and one study was in each of Canada⁶ and South Africa⁷.

VEGETATION COMMUNITY (6 STUDIES)

- **Community composition (2 studies):** One of two paired studies (one of which was replicated, randomized and controlled) in Hungary¹³ and the UK⁵ found that, over time, plant communities in areas where grass seeds were sown and in areas where no seeds were sown became more similar to those of intact grasslands¹³. The other study⁵ found that plant communities in sown areas were different from those of ancient grasslands or sites where natural regeneration was allowed.
- **Overall richness/diversity (4 studies):** Three of four studies (three of which were replicated and controlled, and one of which was a site comparison) in the UK⁴, Hungary¹³, Iceland⁸ and the USA³ found that sowing grass seeds did not change^{4,8} or reduced¹³ overall plant species richness. The other study³ found that species richness was lower than that found in nearby intact prairies.
- **Native/non-target species richness/diversity (1 study):** One replicated, controlled study in the USA¹² found that sowing grass seeds did not alter the species richness of native plants.

VEGETATION ABUNDANCE (10 STUDIES)

- **Overall abundance (3 studies):** Two of three studies (including two replicated, controlled studies and one site comparison) in the USA², Iceland⁸ and Norway¹¹ found that sowing grass seeds increased vegetation cover^{8,11}. The other study² found that in two of three years there was no difference in vegetation cover between areas where grass seeds were sown and those where no seeds were sown.
- **Characteristic plant abundance (1 study):** One replicated, randomized, paired, controlled study in Hungary¹³ found that sowing grass seeds reduced the abundance of grassland species.
- **Sown/planted species abundance (1 study):** One replicated, controlled study in South Africa⁷ found that sowing grass seeds increased the abundance of sown species.
- **Grass abundance (4 studies):** Three of four replicated studies (three of which were randomized, controlled studies and the other a site comparison) in the USA^{1,9,10} and Canada⁶ found that sowing grass seeds either reduced native grass cover⁶ or did not alter native grass density^{1,9}. The other study¹⁰ found that after two years grass cover was higher in areas where grass seeds were sown than in areas where no seeds were sown, but after seven years there was no longer a difference between areas.
- **Forb abundance (2 studies):** One of two replicated studies (one randomized, controlled study and one site comparison) in the USA¹ and Canada⁶ found that sowing grass seeds reduced the abundance of native forb species⁶. The other study¹ found no change in the density of native forb species.
- **Tree/shrub abundance (2 studies):** Two replicated studies (one randomized, controlled study and one site comparison) in the USA¹ and Canada⁶ found that sowing grass seeds reduced the abundance of shrub species.
- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA¹² found that sowing grass seeds did not alter the cover of native plant species.

VEGETATION STRUCTURE (0 STUDIES)

Background

Grass seeds are often sown following infrastructure development or in highly degraded land to reduce soil erosion or colonisation by weeds (Deák *et al.* 2011). In some cases, grasses sown include non-native species, as the aim of sowing is often to improve vegetation cover rather than native biodiversity. However, sowing grass seeds may increase the likelihood that other grassland species will colonise a site.

Deák, B., O. Valkó, A. Kelemen, P. Török, T. Miglécz Ph.D, T. Ölvedi, S. Lengyel, and B. Tóthmérész. (2011) Litter and graminoid biomass accumulation suppresses weedy forbs in grassland restoration. *Plant Biosystems*, 145, 730–737.

A replicated, randomized, controlled study in 1972–1976 in a sagebrush grassland affected by wildfire in Nevada, USA (1) found that sowing grass seeds did not alter the density of native grass or forbs but led to a decrease in the number of shrubs. After four years, the density of crested wheatgrass *Agropyron desertorum* and native forbs did not differ significantly between plots sown with grass seeds (wheatgrass: 0.35–0.70 plants/m²; forbs: 0.46–0.48 plants/m²) and unsown plots (wheatgrass: 0.35–1.04 plants/m²; forbs: 0.85–0.88 plants/m²). Total shrub density was lower in plots sown with grass seeds (74–84 shrubs/1,000 m²) than in unsown plots (116–119 shrubs/1,000 m²). In October 1972, eight 12 x 12 m plots were established. Four of the plots were sown with seeds of crested wheatgrass and intermediate wheatgrass *Agropyron intermedium*, while four plots were left unsown. The number of grass, forb and shrub plants was counted within each plot in 1973, 1974 and 1976.

A replicated, controlled study in 1973–1977 in a grassland where powerlines had been constructed in Arizona, USA (2) found that sowing a mixture of native and non-native grass seeds did not alter vegetation cover in most cases. In two of three years, there was no significant difference in vegetation cover between seeded (8–10%) and unseeded areas (10–12%). In one year, seeded areas had significantly higher vegetation cover than unseeded areas in the spring (53% vs 34%) but not in the summer (18% vs 16%). After construction was completed in summer 1973, two areas were sown with a seed mixture of eight grass species at a rate of 15.3 kg/ha, while two areas were not sown with seeds. Vegetation cover was surveyed using eight 6.1-m long line transects in each area in summer 1975, spring and summer 1976, and spring 1977.

A site comparison study in 1992 in a former arable field site in Kansas, USA (3) found that sowing grass seeds resulted in species richness that was lower than that found in intact prairie sites. Plant species richness in the two sites where local seeds were sown was lower (9.2–12.0 species/quadrat) than in a nearby intact prairie (15.0 species/quadrat). In 1957, soil in the arable field was disturbed by disking and sown with *Andropogon gerardii*, *Andropogon scoparius*, *Sorghastrum nutans*, and *Panicum virgatum* seeds. In June 1992, vegetation cover was surveyed in ninety 1-m² quadrats in the site sown with seed, and 30 quadrats in an adjacent intact prairie.

A replicated, randomized, paired, controlled study in 1993–1996 in ex-arable land in the UK (4) found that sowing commercial grass seeds did not alter plant species richness. Plant species richness was the same in sown and unsown areas (28 vs 28 species). No statistical analyses were carried out in this study. A commercial seed mix containing four grass species was sown in 10 plots (size of plots unclear), while no seed

was sown in 10 plots. The survey methods used to assess species richness in this study were not clear.

A paired, site comparison study in 2004 in 40 restored and 40 ancient grasslands in southern England, UK (5) found that sowing grass seeds resulted in plant communities that were different from those of ancient grasslands or sites where natural regeneration was allowed. The plant community of restoration sites where grass seeds were sown was different from the plant communities of paired ancient grasslands, as well as the plant communities of restoration sites where both grass and forb seeds were sown, or where natural regeneration was allowed (results presented as graphical analysis). Between one and >20 years prior to the study, 40 ex-arable sites were seeded with grass seeds only (12 sites), grass and forb seeds (12 sites) or no seeds (natural regeneration; 16 sites). Each site was paired with the closest ancient grassland (0–9 km away; aged >200 years). All sites were grazed, and occasionally mown. In June–August 2004, the cover of plant species was estimated within 50 x 50 cm quadrats placed at 10 m intervals along a 100-m transect at each site.

A replicated, site comparison study in 2004 in 14 sites disturbed by pipeline construction in Alberta, Canada (6) found that areas where grass seeds had been sown had lower cover of native plants, shrubs, grasses and forbs than nearby natural grasslands, but had higher cover of non-native plants. Areas where seeds had been sown after disturbance by pipeline construction had lower cover of native plants (39%) than nearby natural grasslands (71%). There was a similar pattern for native shrubs (seeded: 2%, natural: 7%), native grasses (seeded: 27%, natural: 35%) and native forbs (seeded: 11%, natural: 29%). However, non-native species cover was higher in seeded areas (32%) than in natural grasslands (21%). Fourteen sites where pipelines had been constructed 7–40 years prior were selected for the study. All sites were sown with native grass seeds, including rough fescue *Festuca campestris*, after construction. In June–August 2004, plant cover was surveyed using 10 quadrats along one 30 x 1 m transect in the reseeded area of each site and one transect in adjacent intact grasslands.

A replicated, controlled study in 2004–2006 in a former arable field in Gauteng province, South Africa (7) found that sowing grass seeds increased the abundance of sown species and reduced the abundance of unsown species. After two years, in two comparisons the density of sown species was higher in areas where soil was disturbed and grass seeds were sown (35–80 plants/m²) than in areas where soil was disturbed but no grass seeds were sown (2 plants/m²). The opposite was true for plants whose seed was not sown (disturbed and sown: 51–122 plants/m²; disturbed and unsown 250 plants/m²). In 2004, eight 20 x 10 m plots were ploughed and sown with seeds of five grass species. Eight plots were disturbed with a single toothed ripper and sown with seeds of five grass species, and four plots were ploughed or disturbed with a ripper and not sown with seeds. In March 2005 and 2006, eight 1-m² quadrats were placed in each plot and the number of plants counted.

A replicated, controlled study in 1999–2005 in a formerly overgrazed area in Iceland (8) found that sowing grass seeds increased vegetation cover, but not plant species richness. Vegetation cover was higher in areas where seeds were sown (72–91%) than in areas where they were not (6%). Plant species richness did not differ significantly between areas that were seeded and areas that were not seeded (no data reported). In 1999, some areas of the site were sown with red fescue *Festuca rubra* and Kentucky bluegrass *Poa pratensis* seeds, while other areas were not sown with seeds (levels of

replication were unclear from the study). In August–September 2005, five 10 × 10 m plots were established in seeded areas and five plots were established in unseeded areas. Vegetation cover in ten 0.5 × 0.5 m quadrats within each plot was surveyed.

A replicated, randomized, controlled study in 2006–2011 in arid rangelands in Arizona, USA (9) found that sowing grass seeds did not alter the density of the sown grasses Indian ricegrass *Achnatherum hymenoides* and needle-and-thread grass *Hesperostipa comata*. After five years, there was no significant difference in the average density of Indian ricegrass and needle-and-thread grass between plots where their seeds were sown (0.03 plants/m²) and plots where no seeds were sown (0.01 plants/m²). In November 2006, twenty 3 × 3 m plots were sown with native C3 grass seeds and ten plots were not. Counts of grass species were made in all plots in May 2007, 2010 and 2011.

A replicated, randomized, paired, controlled study in 2005–2012 in two cleared sites in Oregon, USA (10) found that sowing grass seeds initially increased grass cover, but after seven years there was no difference in grass cover between sown and unsown areas. After two years, grass cover was higher in plots where grass seeds were sown (9.7%) than in plots where they were not sown (0.2%). However, after seven years there was no significant difference in the grass cover in sown (1.9%) and unsown plots (0.4%). In 2001–2002, woody vegetation was removed using a masticator, and in 2005, prescribed burning was carried out. Two weeks after burning, native grass seeds of four species were sown in 15 randomly located 1-m² plots at each site, while no seeds were sown in another 15 plots. Grass cover was estimated in each plot in 2006, 2007 and 2012.

A site comparison study in 2010 on 10 road verges in the Dovre Mountains, Norway (11) found that sowing grass seeds increased vegetation cover overall but reduced native vegetation cover. Total vegetation cover was on average higher in areas where a commercial grass seed mixture was sown (85–96%) than in areas where no seeds were sown (65–90%). The opposite was true for native vegetation cover (seeded: 48–72%; unseeded: 65–74%). In 1989, ten road verges were sown with a commercial seed mixture containing four non-native grass species at a rate of 7 kg/1,000 m². Commercial fertilizer was also added at a rate of 50 kg/1,000 m². Ten other areas on each of the 10 road verges were not seeded. All sites were grazed occasionally. In July and August 2010, the abundance of plant species was recorded in five 0.5 × 0.5 m plots in each of the seeded and unseeded areas.

A replicated, controlled study in 2012–2013 in a serpentine grassland in California, USA (12) found that sowing grass seeds did not alter native plant species richness or the cover of native or non-native invasive plant species. Average numbers of native plant species did not differ significantly between plots sown with grass seeds and plots not sown with seeds (both 9–10 species/plot). The same was true for the cover of native plants (sown: 63–77%; unsown: 54–71%) and the cover of non-native invasive plants (sown: 20–29%; unsown: 18–26%). In November 2012, twenty 1 × 1 m plots were sown with 20 seeds of three native grass species (collected onsite 2–3 months prior), while 20 plots were left unsown. All plots were irrigated for 21 days in August 2012. Half of the plots for each treatment were grazed by cattle. Vegetation cover was estimated in March and April 2013 using a 0.25 × 0.25 m quadrat placed in each plot.

A replicated, randomized, paired, controlled study in 2008–2014 in a pine plantation burnt in a wildfire in Hungary (13) found that sowing with grass seeds reduced plant species richness and cover of specialist grassland species, but the plant communities of both seeded and unseeded areas became more similar to that of intact

grasslands over time. After six years, plant species richness of areas where grass seeds were sown was lower (3.4 species/m²) than that in areas where no seeds were sown (7.0 species/m²). Cover of grassland species showed a similar pattern (seeded: 5%, unseeded: 16%). Over six years, the plant community both in areas where seeds were sown and areas where seeds were not sown became more similar to that of intact grasslands (data presented as graphical analysis). These results are not based on tests of statistical significance. In autumn 2008, seeds of two grass species *Festuca vaginata* and *Stipa borysthénica* were sown, at a rate of 1500 seeds/m² and 100 seeds/m² respectively, in twenty 1 x 1 m plots, while in 20 plots no seeds were sown. Cover of all plants was estimated yearly in June between 2008 and 2014. In June 2014, ten intact grassland areas that had also been burnt were surveyed.

- (1) Evans, R.A. & Young, J.A. (1978) Effectiveness of rehabilitation practices following wildfire in a degraded big sagebrush downy brome community. *Journal of Range Management*, 31, 185–188.
- (2) Hessing, M.B. & Johnson, C.D. (1982) Early secondary succession following restoration and reseeded treatments in northern Arizona. *Journal of Range Management*, 35, 667–669.
- (3) Kindscher, K. & Tieszen, L.L. (1998) Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. *Restoration Ecology*, 6, 181–196.
- (4) Manchester, S.J., McNally, S., Treweek, J.R., Sparks, T.H. & Mountford, J.O. (1999) The cost and practicality of techniques for the reversion of arable land to lowland wet grassland - an experimental study and review. *Journal of Environmental Management*, 55, 91–109.
- (5) Fagan, K.C., Pywell, R.F., Bullock, J.M. & Marrs, R.H. (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *Journal of Applied Ecology*, 45, 1293–1303.
- (6) Desserud, P., Gates, C.C., Adams, B. & Revel, R.D. (2010) Restoration of foothills rough fescue grassland following pipeline disturbance in southwestern Alberta. *Journal of Environmental Management*, 91, 2763–2770.
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- (8) Petursdottir, T., Aradottir, A.L. & Benediktsson, K. (2013) An evaluation of the short-term progress of restoration combining ecological assessment and public perception. *Restoration Ecology*, 21, 75–85.
- (9) Bernstein, E.J., Albano, C.M., Sisk, T.D., Crews, T.E. & Rosenstock, S. (2014) Establishing cool-season grasses on a degraded arid rangeland of the Colorado Plateau. *Restoration Ecology*, 22, 57–64.
- (10) Busby, L.M. & Southworth, D. (2014) Minimal persistence of native bunchgrasses seven years after seeding following mastication and prescribed fire in southwestern Oregon, USA. *Fire Ecology*, 10, 63–71.
- (11) Hagen, D., Hansen, T.I., Graae, B.J. & Rydgren, K. (2014) To seed or not to seed in alpine restoration: introduced grass species outcompete rather than facilitate native species. *Ecological Engineering*, 64, 255–261.
- (12) Funk, J.L., Hoffacker, M.K. & Matzek, V. (2015) Summer irrigation, grazing and seed addition differentially influence community composition in an invaded serpentine grassland. *Restoration Ecology*, 23, 122–130.
- (13) Sztár, K., Ónodi, G., Somay, L., Pándi, I., Kucs, P. & Kröel-Dulay, G. (2016) Contrasting effects of land use legacies on grassland restoration in burnt pine plantations. *Biological Conservation*, 201, 356–362.

2.2. Sow grassland forb species

- **One study** examined the effects of sowing grassland forb species on grassland vegetation. The study was in Belgium¹.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (0 STUDIES)

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, controlled study in Belgium¹ found that sowing seeds of grassland forb species did not increase the number of seedlings for three forb species.

Background

Many former pastures are dominated by relatively few grass species. Sowing grassland forbs may help other grassland species colonise these sites as well as helping to increase plant diversity.

A replicated, controlled study in 2006–2007 in five grassland restoration sites in Belgium (1) found that sowing seeds of grassland forbs did not increase the number of seedlings for three forb species. The average number of pasqueflower *Pulsatilla vulgaris* seedlings did not differ significantly between plots where seeds were sown (0.6 seedlings/plot) and plots where seeds were not sown (0 seedlings/plot). Two other species, mountain clover *Trifolium montanum* and prostrate speedwell *Veronica prostrata*, did not germinate in either sown or unsown plots. In May–August 2007, at each of five sites, 25 seeds of pasqueflower, mountain clover or prostrate speedwell were sown in four 1 x 1 m plots, and in four plots no seeds were sown. All sites were former forest stands that were clearcut and restored to grassland 3–14 years before the study. In May 2008, the number of seedlings in each plot was counted.

(1) Piqueray, J., Saad, L., Bizoux, J.-P. & Mahy, G. (2013) Why some species cannot colonise restored habitats? The effects of seed and microsite availability. *Journal for Nature Conservation*, 21, 189–197.

2.3. Sow native grass and forbs

- **Fifty studies** examined the effects of sowing native grass and forb seeds on grassland vegetation. Thirty-six studies were in Europe^{1,2,4,6-8,10-17,22-24,26,28-37,39,40,42,43,46-49}, twelve studies were in North America^{3,5,9,19-21,25,38,41,44,45,50} and one study was in New Zealand¹⁸. One review²⁷ included studies from Europe, North America and Africa.

VEGETATION COMMUNITY (42 STUDIES)

- **Community composition (11 studies):** Five of 11 studies (10 of which were replicated and/or controlled, and three of which were site comparisons) in the UK^{2,11,14,15,24,26}, the Czech Republic^{32,40}, Norway^{29,43} and Germany³¹ found that sowing native grass and forb seeds increased the similarity of plant community composition to that of target communities^{2,11,15,24,26}. Three studies found no increase in community similarity to target communities^{14,29,31}. Two studies^{32,40} found that over time communities became more similar to those of intact grasslands. One study⁴³ found that over time areas sown with native grass and forb seeds became more similar to areas that were not sown with seeds.
- **Overall richness/diversity (28 studies):** Sixteen of 28 studies (24 of which were controlled and four of which were site comparisons) in Europe^{1,3,6,8,10-12,15,23,26,29,30,33-35,37,39,42,43,46,49}, North America^{5,20,21,25,38,45} and New Zealand¹⁸ found that sowing native grass and forb seeds increased overall plant species richness^{5,6,8,10,11,15,18,20,21,25,26,29,34,37,38,49}. Seven studies found that there was no change in plant species richness^{12,35,39,42,45} or mixed effects on plant species richness²³ and plant diversity³⁸. Three studies^{31,33,43} found that sowing native grass and forb seeds increased plant species richness during the first year, but after 3–13 years, species richness did not differ

between sown and unsown areas^{31,43} or was lower in sown areas³³. One study⁴⁶ found that after one year, sowing did not alter plant species richness but after eight years, species richness was higher than in unsown areas. Three studies^{1,3,12} found that species richness was lower in sown areas than in nearby intact grasslands.

- **Characteristic plant richness/diversity (9 studies):** Six of nine studies (eight of which were replicated and/or controlled, and two of which were site comparisons) in Europe^{2,10,16,32,33,37,42,47,48} found that sowing native grass and forb seeds increased the species richness of characteristic grassland plants^{2,10,16,33,37,47}. Two studies^{32,42} found no change in the species richness of characteristic grassland plants. One study⁴⁸ found that sowing native grass and forb seeds increased the species richness of target forbs but not target grasses.
- **Sown/planted species richness/diversity (3 studies):** Three replicated, paired, controlled studies in the UK^{13,17} and the Czech Republic³⁶ found that sowing native grass and forb seeds increased sown species richness.
- **Grass richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK⁴ found that sowing native grass and forb seeds increased grass species richness in 54% of cases.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK⁴ found that sowing native grass and forb seeds increased forb species richness in 71% of cases.
- **Native/non-target richness/diversity (1 study):** One replicated, controlled study in the USA⁴⁴ found that sowing native grass and forb seeds increased the species richness of native plants.

VEGETATION ABUNDANCE (24 STUDIES)

- **Overall abundance (8 studies):** Three of eight replicated, controlled studies (four of which were randomized and paired) in Europe^{7,23,29,33,34,39} and North America^{9,38} found that sowing native grass and forb seeds increased overall vegetation cover⁷, biomass²³ or density³⁴. One study³³ found that sowing native grass and forb seeds increased plant species richness during the first 2–7 years, but after eight years, species richness did not differ between sown and unsown areas. Four studies found that there was no change in overall vegetation abundance in all^{9,29,39} or most cases³⁸.
- **Characteristic plant abundance (5 studies):** Three of five replicated studies (four of which were controlled, and one of which was a site comparison) in Europe^{2,33,42,47,48} found that sowing native grass and forb seeds did not alter the cover of characteristic grassland species^{42,47,48}. The other two studies^{2,33} found an increase in the cover of characteristic or target grassland species.
- **Sown/planted species abundance (6 studies):** Five of six studies (four of which were replicated and controlled, and two of which were reviews) in Europe^{13,17,27,28}, North America^{19,27}, Africa²⁷ and New Zealand¹⁸ found that sowing native grass and forb seeds increased the abundance of sown species in all^{13,17,19} or most cases^{18,28}. The other study²⁷ found mixed effects on sown species abundance.
- **Grass abundance (3 studies):** Two of three replicated, randomized, controlled studies (two of which were paired) in the Czech Republic^{22,36} and the USA⁵⁰ found that sowing native grass and forb seeds increased the cover of grass species^{22,36}. The other study⁵⁰ found no change in the cover of grass species.
- **Forb abundance (4 studies):** Three of four replicated, randomized, controlled studies (three of which were paired) in the Czech Republic^{22,36} and the USA^{41,50} found that sowing native grass and forb seeds increased the cover^{22,50} or density⁴¹ of forb species. The other study³⁶ found that one year after sowing, the cover of forb species increased, but after 10 years it did not differ between sown and unsown areas.

- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA⁴⁴ found that sowing native grass and forb seeds increased the cover of native plant species.
- **Individual plant species abundance (1 study):** One replicated, randomized, paired, controlled study in the UK⁸ found that sowing native grass and forb seeds did not alter yellow rattle abundance.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (2 STUDIES)

- **Germination/Emergence (2 studies):** One of two replicated, controlled studies (one of which was paired) in the USA²⁰ and Germany⁴⁹ found that sowing native grass and forb seeds increased the number of seedlings that emerged²⁰. The other study⁴⁹ found no change in seedling number.

Background

Following land use change, grassland seeds often die. As a result, when grassland restoration is undertaken grass and forb seeds are often added in an attempt to aid the colonisation of grassland species.

This section considers sowing of grass and forb species. This includes studies where soil has been disturbed by ploughing alongside sowing, as this is often an inherent part of sowing seeds. For details on studies that test the impact of soil disturbance on the success of sowing, see the intervention '*Disturb soil before seeding/planting*'. For details of studies that only sowed seeds of grass or forb species, see the actions '*Sow grass seeds*' and '*Sow grassland forb species*'.

A site comparison study in 1984–1994 in a former agricultural field in Essex, UK (1) found that after sowing grass and forb species, plant species richness and diversity were lower than in a nearby old meadow. Four years after sowing, 19 plant species were recorded in the field sown with seeds, while 26 species were present in a nearby old meadow. The same was true 10 years after sowing (sown field: 42 species; old meadow: 57 species). Species diversity was also lower in the field sown with seeds than in the old meadow four and 10 years after sowing (data reported as Shannon-Weiner index). No statistical analysis was done in this study. In 1984, rye grass *Lolium perenne* and white clover *Trifolium repens* seeds were sown in a 3-ha field following agricultural abandonment. In June 1988–1994, vegetation cover was assessed using 40 randomly placed 0.5 x 0.5 m quadrats in the sown field and 30 quadrats in a nearby 1-ha meadow (>40 years old) under similar management (cut in July and grazed in August–October).

A replicated, randomized, paired, controlled study in 1993–1994 in a former arable field in Hampshire, UK (2) found that sowing grass and forb seeds increased cover and species richness of chalk grassland species, and similarity of the plant community to that of the target community, but reduced cover of weed species. After one year, in three of four comparisons cover of chalk grassland species was higher in areas where seeds had been sown (37–98%) than in areas where seeds had not been sown (8%), while in one comparison there was no significant difference (seeded: 19%, unseeded: 8%). Species richness of chalk grassland plants was higher where seeds had been sown (20–33 species/plot) than where no seeds were sown (7 species/plot). Cover of weed species was lower in areas where seeds were sown (2–12%) than in areas where no seeds were sown (23%). Seeded plots resembled the target community more than plots where no seeds were sown (data presented as graphical analysis). Seeds were collected from two

nearby chalk grassland sites using vacuum seed harvesters. In each of four blocks established at the site, four plots were sown with seeds of 47 species at a rate of 0.1 g/m²–4.0 g/m². Cover of each species was estimated in August 1993 and 1994 using two 1-m² quadrats/plot.

A site comparison study in 1992 in a former arable field in Kansas, USA (3) found that sowing grass and forb seeds resulted in lower plant species richness than that found in intact prairie sites. Plant species richness in the two sites where local seeds were sown was lower (8.7–10.3 species/quadrat) than in nearby intact prairies (14.0 species/quadrat). In April 1989, seeds of 33 prairie plant species were sown. Grasses were sown at a rate of 5.8 kg/ha and forbs at a rate of 0.06 kg/ha. In September 1992, vegetation cover was surveyed in sixty 1-m² quadrats in the site sown with seed, and 30 quadrats in an adjacent intact prairie.

A replicated, randomized, paired, controlled study in 1994–1996 at six improved grassland sites in the UK (4) found that sowing grass and forb seeds had mixed effects on grass and forb species richness. No statistical analyses were carried out in this study. In 13 of 24 comparisons, plots where seeds were sown had more grass species (5–12 species/plot) than plots where no seeds were sown (4–10 species/plot), while in 11 comparisons, grass species richness was lower or equal (seeded: 4–13 species/plot, unseeded: 5–13 species/plot). In 17 of 24 comparisons, forb species richness was higher in plots where seeds were sown (7–25 species/plot) than plots where no seeds were sown (5–15 species/plot) while it was lower or equal in seven of 24 comparisons (seeded: 3–8 species/plot; unseeded: 4–8 species/plot). In 1994, at each site, soil was disturbed and seeds sown in eight 6 x 4 m plots and four plots were left unseeded. Seed mixes contained seeds of five grass species and 18 forb species. In May/June of 1995 and 1996, three 40 x 40 cm quadrats were placed in each plot and the frequency of each species recorded.

A replicated, randomized, controlled study in 1995–1997 in a pasture in Iowa, USA (5) found that sowing grass and forb seeds increased plant species richness. The results of this study are not based on statistical analysis. Plant species richness was higher in areas where seeds were sown (8.6–9.4 species/plot) than in areas where no seeds were sown (3.5–5.0 species/plot). In May 1995, in order to remove any vegetation present, glyphosate herbicide was applied to four plots, each of which had an area of at least 500 m². In June 1995, seeds of three native grass species and one native legume were sown by hand or using a seed drill in these four plots, while four other plots were not seeded. Cover of all plant species was monitored in five to eight 0.5-m² quadrats in each plot from 1995–1997.

A replicated, randomized, paired, controlled study in 1993–1996 in ex-arable land in the UK (6) found that sowing grass and forb seeds increased plant species richness. No statistical analyses were carried out in this study. Plant species richness was higher in areas where seeds were sown (36–45 species) than in areas where no seeds were sown (28 species). Seeds of grass and forb species were collected from hay harvested in a nearby intact wet meadow and sown in 10 plots (size of plots unclear), commercial seed mixes containing 11–23 grass and forb species were sown in 20 plots, while no seeds were sown in 10 plots. The survey methods used to assess species richness in this study were not clear.

A replicated, controlled study in 1996–1998 in an area that was previously burned in Galicia, Spain (7) found that sowing grass and forb seeds increased vegetation cover.

No statistical analysis was done in this study. After six to 16 months, vegetation cover in areas sown with seeds was higher (54–95%) than in areas where no seeds were sown (19–76%). In July 1996, a 2,000-m² area was burned using prescribed burning methods. Following this, in September/October 1996, four plots were sown with seeds of *Agrostis truncatula*, *A. capillaris*, bird's-foot trefoil *Lotus corniculatus* and *Lolium multiflorum*, and two plots were not sown with seeds. Vegetation cover was estimated after six, 10, 16, and 19 months using a 50 x 50 cm quadrat placed in each plot.

A replicated, randomized, paired, controlled study in 1990–1998 in improved grassland in North Yorkshire, UK (8 – same experimental set up as 26) found that plots sowed with grass and forb species had more plant species than unseeded plots, but that there was no difference in yellow rattle *Rhinanthus minor* abundance. Eight years after sowing, there were more species in sown plots (17.4 species) than unsown plots (15.6 species). However, the density of yellow rattle plants did not differ significantly between sown (0–52 plants/m²) and unsown plots (0–51 plants/m²), and there was no effect of sowing on annual hay yield (data not given). In 1990, one hundred and eight 6 x 6 m plots were established in three blocks of 36 plots. Fifty-four plots (18 random plots/block) were sown with 7 kg/ha of locally collected seeds and 0.05–1.5 kg/ha of commercial seeds (containing 19 species) each autumn from 1990–1992. Fifty-four plots were left unsown. All plots were cut annually between June and September, and parts of each plot were also grazed, fertilized and/or mowed. Plant species and cover were recorded in two 2 x 2 m quadrats in each plot in summer 1994, 1996 and 1998.

A replicated, randomized, paired, controlled study in 1995–1998 in three degraded riparian meadow sites in Nevada, USA (9) found that sowing native grass and forb seeds did not lead to an increase in overall plant biomass and the establishment of sown species was low. After three years, average plant biomass did not differ significantly between plots sown with native grass and forb seeds (555–825 g/m²) and unseeded plots (580–610 g/m²). In addition, only one of the six sown species (Nebraska sedge *Carex nebrascensis*) was reported to be present in any of the sown plots after three years. In 1995, at each of three sites, one plot measuring 16–25 m² was sprayed with herbicide (Round-up®), tilled to disturb the soil, and sown at a rate of 875 seeds/m² with six riparian meadow species. One plot in each site was not sprayed with herbicide, disturbed or sown with seed. In July 1996–1998, five 0.1-m² quadrats were randomly placed in each plot and vegetation clipped to determine biomass.

A replicated, randomized, paired, controlled study in 1996–1997 in an experimental meadow in Norway (10) found that sowing grass and forb seeds increased species richness as well as richness of traditional meadow plants. Plant species richness was higher in areas where seeds were sown (23 species/plot) than in areas where no seeds were sown (19 species/plot). Species richness of plants that are indicators of traditional meadow management showed a similar pattern (seeded: 7 species/plot; not seeded: 3 species/plot). In April 1996, the soil of all plots was disturbed using a power harrow and 20 kg of calcium oxide was added. Seeds from a nearby hay barn were sown in six 16-m² plots and no seeds were sown in six other plots. All plots were mown in August 1996. In July 1997, three 1 x 1 m quadrats were placed in each plot and the presence of all plant species recorded.

A replicated, randomized, paired, controlled study in 1994–1998 in five arable fields in the UK (11) found that disturbing soil and sowing grass and forb seeds increased plant species richness in most cases and increased the plant community similarity to that

of target habitats. In five of eight comparisons, plant species richness was higher in areas where seeds were sown (16.2–26.4 species/plot) than in areas where seeds were not sown (10.4–18.7 species/plot). However, in three of eight comparisons, plant species richness was lower (seeded: 10.8–14.0 species/plot; unseeded: 10.4–18.7 species/plot). Similarity to target communities was higher in areas that were seeded than in areas that were not seeded (data presented as similarity index). In September 1994, in five sites, four blocks containing five 6 × 4 m plots were established. In each block, the soil of four plots was disturbed using harrows or ploughs and sown with seeds of between six and forty-one plant species. One plot was not disturbed or sown with seeds. Vegetation was cut and removed each year in June or July, and sheep grazed between October and December at a density of 25–40 sheep/ha for six to eight weeks. Vegetation was surveyed in June of each year using three randomly placed 40 × 40 cm quadrats within each plot.

A before-and-after, site comparison study in 1998–2001 in a former arable field and a semi-natural grassland in northern France (12) found that sowing grass and forb seeds did not increase plant species richness, and species richness was lower than in areas in a nearby natural grassland. After three years, the plant species richness of areas where seeds were sown did not differ significantly from that of the same areas before sowing (after: 29 species/plot, before: 26 species/plot). Plant species richness after three years was also lower in areas where seeds were sown (29 species/plot) than in nearby semi-natural grasslands (34 species/plot). Before restoration, the field had been abandoned for five years and had been recolonised by some grassland species. In 1998, the field was mowed and ploughed and in autumn was sown with a commercial seed mix containing *Phleum pratense*, *Lolium perenne*, *Festuca pratensis* and *Trifolium repens* at a rate of 30–35 kg/ha. Before sowing, nine 4-m² plots were established and vegetation was surveyed. In 1999–2001, twenty-one plots in the field were surveyed along with 30 plots in nearby semi-natural grasslands.

A replicated, paired, controlled study in 1993–1999 in an ex-arable field near Aberdeen, Scotland, UK (13) found that sowing native grass and forb seeds led to an increase in the number and cover of sown species. After six years, sown plots on average contained more sown species (4.9 species/m²) and had a greater cover of sown species (97%) than unsown plots (1.8 species/m²; 43%). In April 1993, twelve pairs of plots (each 20 × 40 m) were ploughed and fenced. In May 1993, one plot in each pair was sown with a native seed mix (four grass and 10 forb species sown at a rate of 20 kg/ha), while the other was left unsown. Both plots in each pair received the same grazing and/or cutting treatment each year (six treatments were applied overall; see original paper for details). In June 1994–1999, vegetation was monitored annually within 20 × 1 m² quadrats (number of sown species) and 10 × 0.25 m² quadrats (cover of sown species) randomly placed in each of the 24 plots.

A replicated, randomized, controlled study in 1997–1999 in a grazed wet grassland in London, UK (14) found that sowing native grass and forb seeds did not increase similarity of the vegetation community to that of the target community. The similarity of the vegetation community to that of the target community did not differ significantly between areas sown with seeds and areas that were not sown with seeds (data presented as similarity coefficients). In spring 1997, seeds of eight grass and forb species collected from an existing grassland were sown onto bare soil in twenty 1-m² plots at a rate of 40 kg/ha, and no seeds were sown in 10 other plots. Cover of all species in the plots was estimated in June 1997–1999. Similarity of the plant communities to the

target community, a mesotrophic grassland, was assessed using the UK National Vegetation Classification.

A replicated, randomized, paired, controlled study in 1990–2000 in a grazed meadow in Yorkshire, UK (15) found that sowing grass and forb seeds increased plant species richness and similarity of the plant community to the target habitat type. These results are not based on statistical analyses. In 25 of 30 comparisons, plant species richness was higher in areas where seeds were sown (17.8–26.7 species/4 m²) than in areas where no seeds were sown (15.4–20.5 species/4 m²). However, in five comparisons, species richness in sown areas (16.1–18.6 species/4 m²) was lower or equal to that found in unsown areas (17.2–19.6 species/4 m²). In 26 of 30 comparisons, similarity of the plant community was higher in areas where seeds were sown than in unsown areas, but in four comparisons similarity was equal or lower than that in unsown areas (data reported as model coefficient). The experiment consisted of three 36 × 12 m blocks, each containing twelve 6 × 6 m plots. In autumn 1990–1992, in each block, seeds of four species were sown in six plots and no seeds were sown in another six plots. Seeds were also sown in August 1998 and July 2000. From 1998, all plots were mowed annually in July. Starting in 1994, vegetation was surveyed every two years using four 2 × 2 m quadrats in each plot.

A controlled study from 2001–2002 in a former arable field in northeast France (16) found that sowing grass and forb seeds increased the number of meadow plant species. One year after sowing, there were more plant species on average in the area sown with seeds (8 species/quadrat, including 5 meadow species) than in the area that was not sown with seeds (6 species/quadrat, including 3 meadow species). In part of the field, a commercial seed mixture containing cocksfoot *Dactylis glomerata*, tall fescue *Festuca arundinacea* and common birdsfoot trefoil *Lotus corniculatus* was sown in June 2001 at a rate of 30 kg/ha, while in another part of the field no seeds were sown and natural regeneration was allowed. Vegetation was monitored in 50 × 50 cm quadrats at 1 m intervals along 20-m transects in June 2002. Two transects were surveyed in each of the natural regeneration and commercially seeded areas.

A replicated, randomized, paired, controlled study in 1994–1996 in two ex-arable sites in Scotland, UK (17) found that sowing grass and forb seeds increased the number and cover of sown species and reduced the number and cover of non-sown species. More sown species were present in areas where seeds were sown than areas where no seeds were sown, and the same was true for the cover of sown plant species (no data presented). Similarly, there were fewer non-sown species in areas where seeds were sown when compared to unsown areas, and their cover was also lower (no data presented). Before sowing, sites were ploughed and harrowed. Seeds of 18 species were sown in eight 3 × 9 m plots at a rate of 4 g/m² and no seeds were sown in four plots at each site. Plant cover and species richness were estimated in June/July 1995 and 1996 using a 1 × 1 m quadrat placed in each plot.

A replicated, randomized, controlled study in 1998–1999 in grazed grasslands in Manawatu, New Zealand (18) found that sowing grass and forb seeds increased plant species richness and the cover of five of eight sown plant species. No statistical analyses were carried out in this study. After one year, plant species richness in plots where seeds were sown was higher (8–9 species/plot) than in plots where no seeds were sown (5–6 species/plot). After 21 months, the average cover of five of eight sown plant species was higher in plots where seeds were sown than plots where no seeds were sown: spear

thistle *Cirsium vulgare* (sown: 3–9 %, unsown: 0–1%); ribwort plantain *Plantago lanceolata* (sown: 1–7% , unsown: 0.3–0.5%); bitter dock *Rumex obtusifolius* (sown: 1–7%, unsown: 0–0.3%); white clover *Trifolium repens* (sown: 14–21%, unsown: 11–18%); perennial ryegrass *Lolium perenne* (sown: 74–84%, unsown: 71–80%). The three other species (greater bird's foot trefoil *Lotus uliginosus*, Dallis grass *Paspalum dilatatum* and creeping thistle *Cirsium arvense*) had few or no seedlings in both sown and unsown plots. In March 1998, seeds of eight plant species were sown (1,000 seeds/species/m²) in 120 randomly located plots (each 2 x 2 m), while in another 120 plots no seeds were sown. Plant cover and species richness was estimated in all plots in December 1999.

A replicated, randomized, paired, controlled study in 1999–2001 on former logging roads and agricultural areas in British Columbia, Canada (19) found that sowing native grass and forb seeds increased the total cover of sown plant species. These results are not based on statistical analyses. During two years after sowing, total cover of sown plant species was higher in areas where seeds were sown (4–62%) than in areas where no seeds were sown (0–10%). In autumn 1999, six blocks were established each containing twelve 2.5 × 2.5 m plots. All plots were cleared of rocks and vegetation and tilled to a depth of 12 cm. Ten plots in each block were sown with seeds of six native species at a rate of 375–6,000 seeds/m², and two plots were not sown with seeds. In September 2000 and August 2001, vegetation cover was estimated in three randomly placed 0.5 × 0.5 m quadrats/plot.

A replicated, paired, controlled study in 2003–2004 in a former arable field prairie restoration site in Iowa, USA (20) found that sowing with grass and forb seeds increased the number of seedlings and species richness of seedlings. There were more seedlings in areas where seeds were sown (104–156 seedlings/m²) than in areas where no seeds were sown (85–139 seedlings/m²). The same pattern was seen for seedling species richness (seeded: 3.0–5.8 species/0.1 m²; unseeded: 2.5–2.8 species/0.1 m²). In June 2003 and April 2004, forty-eight 1-m² plots were sown with seeds at a rate of 19,700 seeds/m² and 24 plots were not sown with seed. Seedling numbers and species richness were estimated once a month during the growing season using a 50 x 20 cm quadrat. Herbivore density in the restoration site was approximately 0.1 bison/ha and 0.05 elk/ha. Prescribed burning was carried out at the site every two years and the site was mowed to control weeds. The plots used in the study were not mowed or burned in 2003 or 2004.

A replicated, randomized, paired, controlled study in 2000–2005 in an abandoned pasture in Kansas, USA (21) found that sowing grass and forb seeds increased plant species richness and diversity. After five years, total plant species richness was higher in plots where seeds had been sown (13–15 species/plot) than in plots where no seeds were sown (8–9 species/plot). The same was true for plant diversity (data reported as Shannon diversity index). In January 2000, seeds of 24 native and eight non-native grassland species were sown at a rate of 12,800 seeds/plot in thirty-four 1 x 1 m plots, and no seeds were sown in another 34 plots. Species richness and diversity were assessed from plant biomass harvested from each plot in June and September 2005.

A replicated, randomized, paired, controlled study in 1999–2004 in semi-dry grassland in the Czech Republic (22) found that sowing grass and forb seeds increased the cover of local forb and grass species. Cover of local grass species was higher in areas where seeds were sown (29–53%) than in areas where no seeds were sown (0–2%). The same pattern was seen for local forb species (seeded: 16–32%, unseeded: 0–2%). In 1999,

four blocks were established. In each block, one 55 x 20 m plot was sown with a locally sourced seed mixture containing seven grass species and 20 forb species at a rate of 2 g/m², while one plot was not sown with seeds. In June in 2000–2004, ten 1.5 x 1.5 m quadrats were placed in each plot and all species present and their cover recorded.

A replicated, randomized, paired, controlled study in 1996–2003 in five ex-arable fields in the Czech Republic, the Netherlands, Spain, Sweden, and the UK (23) found that sowing grass and forb seeds had mixed effects on plant species richness but increased biomass. Initially, in one of two comparisons, sowing seeds increased plant species richness (38 species/plot) compared to areas where no seeds were sown (27 species/plot), but in one of two comparisons there was no significant difference (30 species/plot). However, after eight years, species richness of areas sown with seeds (26–27 species/plot) was not significantly different to that found in areas where no seeds were sown (32 species/plot). After two years, plant biomass was higher in areas where seeds were sown (0.59–0.71 kg/m²) than in unsown areas (0.3 kg/m²), and the same was true after eight years (sown: 0.50–0.64 kg/m²; unsown: 0.46 kg/m²). In each site, ten 10 x 10 m plots were sown with a seed mix of either four or 15 grass, legume and other forb species, while five plots were not sown with seeds. Grasses were sown at a rate of 2,500 seeds/m², and legumes and other forbs at a rate of 500 seeds/m². All plots were mown at least once a year. In each plot, the vegetation in twelve 1 x 1 m quadrats was surveyed in 1996–1998 and 2002–2003. Biomass was estimated by cutting and drying all vegetation in twelve 0.25 x 0.25 m quadrats placed within the plots.

A paired, site comparison study in 2004 in 40 restored and 40 ancient calcareous grasslands in southern England, UK (24) found that sowing grass and forb seeds resulted in plant communities similar to ancient grasslands and sites where natural regeneration was allowed. The plant community of restoration sites where grass and forb seeds were sown was similar to the plant communities of paired ancient grasslands and sites where natural regeneration was allowed, but was different from sites where grass seeds alone were sown (results presented as graphical analysis). Between one and >20 years prior to the study, 40 ex-arable sites were seeded with grass and forb seeds (12 sites), grass seeds only (12 sites) or no seeds (natural regeneration; 16 sites). Each site was paired with the closest ancient grassland (0–9 km away; aged >200 years). All sites were grazed, and occasionally mown. In June–August 2004, the cover of plant species was estimated within 50 x 50 cm quadrats placed at 10 m intervals along a 100-m transect at each site.

A replicated, randomized, paired, controlled study in 2003–2004 in a former hay field in Ohio, USA (25) found that sowing native grass and forb seeds increased plant species richness. Average plant species richness was higher in plots where seeds were sown (7–9 species/plot) than in plots where seeds were not sown (4–5 species/plot). In March 2003, in each of six blocks, seeds of 20 native, wet meadow grass and forb species were sown at a rate of 150 seeds/species in each of two 1 x 1 m plots, while two other plots were left unseeded. Seeds were collected from nearby fields or from cultivated plants. In August 2004, vegetation in the central 0.25 m² of each plot was harvested and used to identify plant species.

A replicated, randomized, paired, controlled study in 1990–2004 in three agriculturally-improved grasslands in North Yorkshire, UK (26 – same experimental set up as 8) found that sowing grass and forb seeds increased the number of plant species as well as the similarity of the plant community to the target community. In the four years following sowing, sown plots had more species (22 species) and higher similarity to the

target plant community of upland meadow species (61% similarity) than plots that were not sown with seeds (19 species, 55% similarity). In 1990, thirty-six 6 x 6 m plots were established, with 12 plots in each of three fields. Eighteen plots (six random plots/field) were sown with 15 kg/ha of commercial seed containing bird's foot trefoil *Lotus corniculatus*, quaking grass *Briza media* and bulbous buttercup *Ranunculus bulbosus* in August 1998, and 0.5 kg/ha wood cranesbill *Geranium sylvaticum* in September 1999; eighteen plots were left unsown. Seeded plots had previously been sown with local and commercial seeds in 1990–1992. All plots were grazed in spring and autumn, and cut in July. Vegetation cover was assessed in four 4-m² quadrats in each plot every other summer in 1994–2004. The target plant community was defined as that associated with well-drained permanent upland meadows, characterised by sweet vernal grass.

A review in 1996–2009 of 17 studies of semi-natural grassland restoration in Europe, North America, and Africa (27) found that sowing grass and forb seeds had mixed effects on the introduction of sown species. Eight of 17 studies that carried out seeding to restore semi-natural grasslands reported successful introductions of sown species, while four studies reported limited success, one reported a failed reintroduction, and four studies did not report enough information to allow the success of introductions to be determined. The review used keyword searches to identify studies where semi-natural grassland restoration was carried out. All studies collected seeds from wild or cultivated plants, and often sowed them as multi-species mixes.

A review in 2010 of 13 studies of grassland restoration in Europe (28) found that after sowing seeds most studies reported that more than half of sown species became established and cover of sown species tended to be high. In 14 of 16 sites, more than half of sown plant species became established (53–96%), while in two of 16 sites, establishment was relatively low (28–32%). In eight sites, cover of sown plant species was 33–96%. The studies in the review lasted for 4–21 years and were carried out in a mixture of ex-arable fields, species-poor grasslands and mining sites. The soil was disturbed by ploughing or turf removal before sowing in some sites, and some sites were mowed or grazed by livestock after sowing. The seeds of 11–41 grass and forb species were sown at the restoration sites.

A replicated, randomized, paired, controlled, before-and-after study on a road verge in Norway (29) found that sowing grass and forb seeds did not alter vegetation cover or similarity of the plant community to intact grasslands, but did increase plant species richness. For three of three years, vegetation cover in areas where seeds were sown (32–71%) did not differ significantly from areas where no seeds were sown (27–57%). The same pattern was seen for plant community similarity to a nearby intact grassland (data reported as Bray-Curtis dissimilarity index). However, plant species richness was higher in areas where seeds were sown (10–11 species/plot) than areas where no seeds were sown (8–9 species/plot). In September 2004, five blocks containing six 0.5 × 0.5 m plots were established. In each block, seeds of 17 species from nearby intact grasslands was sown in three plots and no seeds were sown in three plots. In each plot, vegetation cover of each species was estimated in sixteen 12.5 × 12.5 cm quadrats in July/August 2005–2007.

A replicated, randomized, paired, controlled trial in 1988–2000 in arable field margins in Oxfordshire, UK (30) found that plots sown with grass and forb seeds had more perennial but not annual plant species than unsown plots, but the number of species in all plots declined with time. Five months after sowing, there were more species where

seeds were sown (24 species: 8 annual and 16 perennial) than in unsown plots (15 species: 10 annual and 5 perennial). After 13 years, the number of species was not significantly different between sown plots (9 perennial species and 0.5 annual species) and unsown plots (7 perennial species and 0.5 annual species). Forty-eight 50 x 1.5 m plots were established in field margins, with eight plots in each of six fields. Twenty-four plots (four random plots/field) were tilled and sown with a mix of six grass and 17 forb species at a rate of 30 kg/ha in March 1988. The other twenty-four plots were left unseeded. Most plots were cut annually. Plant species were recorded three times/year from 1988 to 1990, and once in July 2000 in three 0.5 x 1 m quadrats/plot.

A replicated, controlled study in 1996–2005 in former arable fields in Saxony-Anhalt, Germany (31) found that sowing grass and forb seeds did not increase plant community similarity to a nearby intact grassland compared to not sowing seeds. After nine years, the similarity of the plant community to a target intact grassland did not differ significantly between seeded and unseeded areas (data reported as Sørensen's similarity index). In 1996, 1997 and 1999, some sites were sown with seed mixes containing cultivars and non-local grass and forb species, while others were not sown with seeds (replication unclear from study). Before sowing, all sites were ploughed and harrowed. Vegetation was surveyed in 5–19 x 25-m² plots/site in May/June 1998–2001, and again in June 2005. Twenty-one plots were sampled in species-rich mesophile grassland within the same region to allow for comparison with the target habitat.

A replicated, site comparison study in 2008 in ex-arable fields in the Czech Republic (32) found that sowing grass and forb seeds did not alter the number of meadow species, but older fields had vegetation communities more similar to those of undisturbed grasslands. The number of meadow or ruderal species in fields that were sown with seeds were not significantly different from those found in fields where no seeds were sown (no data reported). However, in both sown and unsown fields the similarity of vegetation communities to nearby undisturbed grasslands was higher in older fields (data reported as Czekanovski index). Twenty-six fields that were sown with commercial seed mixes containing grass and forb seeds and nine fields that were not sown with seeds were selected for study. Sowing rates in sowed fields were 30–35 kg/ha. All fields were grazed and/or mowed. In June/July 2008, data on plant species was collected in all fields and nearby intact vegetation (replication in surveys unclear).

A replicated, randomized, paired, controlled study in 2000–2009 in a former mine in Saxony-Anhalt, Germany (33) found that sowing native grass and forb seeds increased cover of target grassland species, reduced cover of non-grassland species and initially increased total plant cover and species richness. After nine years, the cover of target grassland plant species was higher in areas where seeds were sown (72%) than areas where no seeds were sown (29%). The opposite was true for non-grassland species (seed: 14%; no seed: 47%). Total plant cover was higher in areas where seeds were sown after two to seven years (43–97%) than where no seeds were sown (6–66%). However, after eight years there was no longer any significant difference in plant cover (seed: 72%; no seed: 66%). After one year, plant species richness was higher in areas where seeds were sown (seed: 65 species/plot; no seed: 43 species/plot), but the number of species declined in seeded plots so that after five years there were fewer species in seeded areas (seed: 18 species/plot; no seed: 48 species/plot). After nine years, there was no longer any significant difference in species richness between seeded (41 species/plot) and unseeded areas (48 species/plot). In September 2000, three blocks were established, each with two 70 x 18 m plots. Seeds of 21 grass and forb species were sown at a density

of 860 seeds/m² in one plot in each block, and no seeds were sown in the other plot. A mulch layer (5 cm thick) was also added to sown plots. In June 2001–2009 (except for 2003), the vegetation cover of all species was estimated using three 25-m² quadrats in each plot.

A replicated, randomized, controlled study in 2009–2010 in an agricultural field in southern Spain (34) found that sowing native grass and forb seeds increased plant density and species richness. In plots where seeds were sown, plant density was higher (1–11 plants/m²) than in plots where no seeds were sown (0 plants/m²). A similar pattern was seen for plant species richness (seeded: 0.2–1.8 species/0.25 m², unseeded: 0 species/0.25 m²). In November 2009, locally collected seeds of seven native grass and forb species were sown in 20 randomly placed 5 x 5 m plots, and 20 plots were left unsown. Four different bedding materials were applied to plots prior to seeding (see original paper for details). Plant density and species richness were estimated in July and October 2010 using fifteen randomly placed 0.5 x 0.5 m quadrats/plot.

A replicated, randomized, controlled, paired, before-and-after study in 2001–2010 in a calcareous grassland previously affected by shrub encroachment in Tuscany, Italy (35) found that sowing grass and forb seeds did not alter species richness. Nine years after sowing, there was no significant difference in species richness between areas where seeds were sown (31 species) and areas where no seeds were sown (31 species). In 1999, shrubs were removed from the entire grassland. In spring 2001, blackthorn *Prunus spinosa* plants across the site were cut. In October 2001, four 3 x 5 m plots were sown with locally collected grass and forb seeds at a rate of 4 g/m² while four plots were not sown with seed. In June/July 2001–2010, sixteen 2 x 1 m quadrats were placed in each plot and a point quadrat used to estimate cover of each plant species.

A replicated, randomized, paired, controlled study in 1999–2009 in semi-dry grassland in the Czech Republic (36) found that sowing with grass and forb seeds increased sown forb and grass species richness and initially the cover of local forb and grass species, but over time forb species cover declined. After one year, species richness of sown forbs (7.9 vs 0 species/plot) and of sown grasses (3.9 vs 0 species/plot) was higher in areas where seeds were sown than in areas where no seeds were sown, and this remained true for both forbs (8.0 vs 3.5 species/plot) and grasses (4.1 vs 1.9 species/plot) after 10 years. The plant community composition of areas sown with seeds was also more similar to ancient hay meadows than that of areas where no seeds were sown. After one year, cover of sown forb species (17% vs 0%) and sown grass species (18% vs 0%) was higher in areas where local seeds were sown than where no seeds were sown. After 10 years, this pattern was still seen for sown grass species (52% vs 9%) but the cover of sown forb species in areas where seeds were sown was no longer significantly different from areas where no seeds were sown (8.7% vs 6.1%). Four 55 x 20 m plots were sown with a locally sourced seed mixture containing seven grass species and 20 forb species at a rate of 2 g/m², while four plots were not sown with seeds. In June 2000–2004 and 2009, ten 1.5 x 1.5 m quadrats were placed in each plot and all species present and their cover recorded.

A replicated, randomized, controlled study in 2008–2010 in 11 urban wasteland sites in Germany (37) found that sowing grass and forb seeds increased plant species richness and the number of local conservation priority species. Average plant species richness in plots where seeds had been sown (43–50 species/plot) was higher than in plots where no seeds were sown (28–31 species). The percentage of vegetation

consisting of local priority grassland species was also higher in plots where seeds were sown (29–46%) than in plots where no seeds were sown (7–9%). In autumn 2008, at each of 11 sites, seeds were sown in one 4 x 4 m plot, while no seeds were sown in another paired plot. All plots were mown and tilled prior to sowing. Seed mixes contained 27 species from the study region. In spring, early and late summer 2009–2010, a 3 x 3 quadrat was placed in the centre of each plot and vegetation cover mapped.

A replicated, randomized, controlled study in 2006–2008 in a former agricultural field in Illinois, USA (38) found that sowing grass and forb seeds increased plant species richness, but had mixed effects on plant species diversity and vegetation cover. In 12 of 12 comparisons, plant species richness was higher in plots where grass and wildflower seeds were sown (5–10 species/plot) than in plots where no seeds were sown (2 species/plot). In five of 12 comparisons, plant species diversity was higher in plots where grass and wildflower seeds were sown than where seeds were not sown, but in seven comparisons there was no significant difference (measured as Shannon-Weiner index). In eight of 12 comparisons, there was no significant difference in plant cover between areas where seeds were sown (90–143%) and not sown (62–136%), but in four comparisons plant cover was higher in sown areas (sown: 105–120%, unsown: 62–76%). In December 2006, fifteen 9 x 9 m plots were sown with a mixture of grass and forb seeds of 128 species (applied at four different rates: 11.2, 33.6, 56.0 and 78.5 kg/ha), and three plots were not sown with seed. In June, July and August 2008, 1 x 1 m quadrats were placed in each plot and plant cover estimated.

A replicated, randomized, controlled study in 2006–2008 in formerly forested grasslands in Slovakia (39) found that sowing grass and forb seeds did not alter plant species richness or cover compared to not sowing with seeds. In each of three years, plant species richness did not differ significantly between plots that were sown with grass and forb seeds (18–26 species) and plots that were not sown (16–25 species). The same pattern was true for vegetation cover (sown: 18–90%; unsown: 47–82%). In spring 2006, all shrubs and trees were cut and wood removed from the site. In three 2.2 x 2.2 m plots, bare ground was raked and seeded with a mixture of four grass and two legume species at a rate of 12.6 kg/ha, while three plots were left unseeded. All plots were grazed by cattle from May to October each year. In May 2006–2008, the cover and species richness of plants was visually estimated within a 1 x 1 m quadrat placed in the centre of each plot.

A replicated, site comparison study in 2009 in 34 restored grassland sites and 20 intact grassland sites in the Czech Republic (40) found that sowing seeds of grass and forb species produced a different plant community to that of intact grasslands, but older sites were more similar to intact grasslands. Sites where seeds were sown had different plant communities to intact grasslands (data reported as ordination analysis). However, sites that were older had a community composition more similar to that of intact grasslands (data reported as ordination analysis). Thirty-four ex-arable sites where a mixture of grasses, legumes and other forbs had been sown 1–12 years previously were selected for study. These sites were compared to nearby species-rich grasslands. In June 2009, at each restored site, three 5 x 5 m plots were established and cover of all plant species estimated visually. Information on intact grasslands was taken from a national database. All sites were mowed once annually in June–August.

A replicated, randomized, paired, controlled study in 1998–2010 in a former arable field in Minnesota, USA (41) found that sowing native grass and forb seeds increased native forb density where seeds were sown, as well as in surrounding areas.

After 10–12 years, the average density of sown forb species was higher in plots where seeds were sown (29–40 plants/m²) than in plots where seeds were not sown (1 plant/m²). Sown forb density was also higher in areas 1–3 m away from plots where seeds were sown (5–19 plants/m²) than in areas a similar distance from plots that were not sown with seeds (<0.1–14 plants/m²). In autumn 1998, in each of five blocks, five 4 x 3 m plots were tilled and sown with a seed mixture containing four native grasses and 12 native forbs at a rate of 400 seeds/m², while one plot was tilled but no seeds were sown. Some of the sown plots also received additional treatments (fertilizer, heat and/or carbon addition). In July–August 2005–2010, density of sown forb species was estimated in each plot, in a 1-m area surrounding each plot, and in three 0.5 x 0.5 m quadrats within each of four 3 x 3 m areas located 3 m away from each plot to the north, east, south and west.

A replicated, site comparison study in 2009–2011 in 47 restored grassland sites and 25 intact grassland sites in the Czech Republic (42) found that sowing grass and forb seeds did not alter plant species richness, or the number or cover of grassland species compared to not sowing seed. After seven years, plant species richness in areas where seeds were sown (31.2 species/plot) did not differ significantly from that found in areas where no seeds were sown (47.8 species/plot), but both had fewer species than nearby intact dry grasslands (62.4 species/plot). The number of grassland species followed a similar trend (seeded: 19.7 species/plot, unseeded: 24.6 species/plot, intact grassland: 55.6 species/plot) as did the cover of grassland species (seeded: 74.3%, unseeded: 68.7%, intact grassland: 102.7%). Between one and 11 years before the start of the study, 35 restored sites were sown with a seed mix containing 44 local plant species at a rate of 17–20 kg/ha and 16 sites were not sown with seed. Twenty-five intact dry grassland sites were also used as a comparison. In 2009–2011, three 5 x 5 m plots were placed in each site and cover of all plant species visually estimated.

A replicated, randomized, controlled, paired study in 2003–2006 in an unvegetated, former grassland in Norway (43) found that sowing native grass and forb seeds initially increased plant species richness compared to areas where seeds were not sown, but this difference declined over time, and species composition of areas where seeds were sown became more similar to that of areas where seeds were not sown. After one year, the number of species in areas where seeds were sown (19 species/plot) was higher than in areas where seeds were not sown (15 species/plot), but after three years there was no longer a significant difference (seeded: 14 species/plot, unseeded: 12 species/plot). The species composition of sown and unsown areas became more similar over time (result based on ordination analysis). In 2003, all vegetation was removed from the site. Seeds were collected from road verges <10 km from the study area. In sixteen 0.5 x 0.5 m plots, seeds of two grass and 13 forb species were sown at a density of 2,300 seeds/m², and in 16 plots no seeds were sown. Plant abundance and species richness were recorded in June–August 2004–2006 in each plot.

A replicated, controlled study in 2008–2013 in a former arable field in Massachusetts, USA (44) found that sowing native grass and forb seeds increased the cover and species richness of native plants compared to unsown areas. During 1–5 years after sowing, plots sown with native grass and forb seeds had on average greater cover (24–59%) and species richness (10–11 species/plot) of native plants than plots not sown with seeds (8–16%, 2–3 species/plot). The same was true for total plant species richness (sown plots: 17–23 species/plot; unsown plots: 10–11 species/plot). Statistical analyses carried out in this study did not test for significant differences between sown and unsown plots. Five 5 x 5 m plots were tilled to a depth of 16cm in June and August 2008 to remove

non-native vegetation. In November 2008, the five plots were sown with seeds of 26 locally collected native species at a rate of 38 kg/ha. Fifteen other plots were not tilled or sown with seeds. Vegetation was surveyed annually in a 3 x 3 m quadrat placed in the centre of each plot in July and August 2007–2013.

A replicated, randomized, paired, controlled study in 2003–2006 in eight prairie restoration sites in Iowa, USA (45) found that sowing grass and forb species did not alter plant species richness. Plant species richness did not differ significantly between areas where seeds were sown (10.7 species/plot) and areas where seeds were not sown (9.8 species/plot). In each site, four 1 x 1 m plots were sown with seeds and two plots were not seeded. All plots were burned in April 2005. In 2003–2006, a 1 x 0.4 m quadrat was placed in each plot and species richness estimated by eye.

A replicated, randomized, controlled, paired study in 2004–2012 on a road verge in Borgund, Norway (46) found that sowing native grass and forb seeds initially did not alter plant species richness, but after eight years there was an increase in species richness. After one year, plant species richness in plots where seeds had been sown did not differ significantly from that found in plots where no seeds were sown (seeded: 15 species/plot, unseeded: 13 species/plot). However, after eight years, species richness was higher in seeded plots (21 species/plot) than in unseeded plots (16 species/plot). In September 2004, in each of five blocks, seeds of 11 grass and forb species were sown at a density of 1,900 seeds/m² in three 0.5 x 0.5 plots, while in one plot/block seeds were not sown. All plots were paired. Seeds were harvested from donor sites (3–16 km away) in late summer/early autumn 2004. The site was mown in July/August 2007–2010. Vegetation cover was recorded in each plot in July–August 2005–2007 and 2012.

A replicated, controlled study in 2009–2015 in a species-poor grassland near Wittenberg, Germany (47) found that sowing grass and forb seeds increased the species richness of target grasses and forbs but did not alter their cover. After six years, plots sown with seeds had more target grass and forb species than unsown plots (data reported as statistical model results). However, the cover of target grass and forb species did not differ significantly between sown and unsown plots (data reported as statistical model results). In 2010–2015, plots sown with seeds had on average 9–19 target grass and forb species/year (6–20% cover), whereas unsown plots had 3–7 target species/year (4–13% cover). In 2009, three 30 x 6 m plots in each of six blocks were rotovated and rolled. In each block, two plots were sown with seeds (obtained by threshing with or without a regional seed mixture added), and one plot was left unsown. On-site threshing was carried out at a meadow 3 km away. All plots were mulched twice and mown once in 2009, and mown twice/year in 2010–2015. Vegetation was recorded annually within a 4 x 4 m quadrat in each of the 18 plots in 2010–2015.

A replicated, randomized, paired, controlled study in 2009–2014 in a species-poor grassland near Wittenberg, Germany (48) found that sowing grass and forb seeds increased the species richness of target forbs, but did not alter target forb species cover or the species richness of target grasses. After five years, plots sown with seeds had on average more target forb species (4.6–6.5 species/plot) than plots not sown with seeds (2.9 species/plot). However, there was no significant difference in average target forb species cover (seeds: <1–3%; no seeds: <1%), or the average number of target grass species (seeds: 1.2–1.4 species/plot; no seeds: 0.8 species/plot). In 2009, six blocks each with three plots measuring 30 x 6 m were established. In each block, two plots were tilled, rolled and sown with seeds (one plot with seeds obtained by threshing only, and the other

with seeds obtained by threshing and combined with a regional seed mixture), while no seeds were added to the third plot. Between 2010 and 2014, the study site was repeatedly flooded and mown twice a year. On-site threshing was carried out at two nearby sites, which were also regularly flooded and mown. Vegetation in each plot was recorded annually from 2010 to 2014 using 4 x 4 m quadrats.

A replicated, controlled study in 2014–2015 in 73 agricultural grasslands in Brandenburg, Thuringia and Baden-Württemberg, Germany (49) found that sowing native grass and forb seeds increased seedling species richness but did not alter the number of seedlings. After 7–19 months, plots that were sown with grass seeds had on average more plant seedling species overall (5 species/quadrat) and more grassland seedling species (data not reported) than plots that were not sown with seeds (all plant species: 4 species/quadrat; grassland species: data not reported). The average number of plant seedlings/quadrat did not differ significantly between sown (94 seedlings) and unsown plots (67 seedlings). Two 7 x 7 m plots were established in each of 73 grasslands. One plot was sown with a mix of native grass, legume and herb seeds (47–66 region-specific species), the other was left unsown. Seeds were mixed with sand and crushed soybean, which was also added to the unsown plots. Sowing was carried out in November 2014 and March 2015. Vegetation was monitored within a 2 x 2 m quadrat in each of the 146 plots on three occasions in May–June 2015.

A replicated, randomized, controlled study in 2008–2015 in an agricultural field in California, USA (50) found that sowing grass and forb seeds increased cover of native forbs, decreased cover of non-native plants, and had no effect on grass cover. After eight years, cover of native forb species was higher in areas where seeds were sown (33%) than in areas where no seeds were sown (17%). However, grass cover did not differ significantly between areas where seeds were sown (9%) and areas where no seeds were sown (2%). Cover of non-native plants was lower in areas where seeds were sown (56%) than areas where no seeds were sown (81%). In 2008, a mix of native grass and forb seeds common to Californian grasslands was sown in twenty-five 1.5 x 1.5 m plots at a rate of 800 seeds/m² and 25 plots were not sown with seeds. Two weeks before sowing all plots were tilled. In November–June of 2008–2010, all plots were weeded. In May 2015, quadrats measuring 1 x 1 m were placed in the centre of each plot and vegetation cover was visually estimated.

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2.4. Plant grassland plants

- **Four studies** examined the effects of planting grassland plants on grassland vegetation. One study was in each of the UK¹, Germany² and the USA⁴. One review³ included studies from the UK and Australia.

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (1 study):** One replicated, controlled study in the USA⁴ found that planting seedlings in addition to sowing seeds increased the number of plant species compared to sowing seeds alone.
- **Grass richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that planting plants increased species richness of grasses in 50% of cases.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that planting plants increased species richness of forbs in 83% of cases.
- **Native/non-target richness/diversity (1 study):** One replicated, controlled study in the USA⁴ found that planting plants in addition to sowing seeds increased the number of native plant species compared to sowing seeds alone.

VEGETATION ABUNDANCE (2 STUDIES)

- **Individual plant species abundance (1 study):** One study in Germany² found that transplanted pepper saxifrage plants increased in number and spread to adjacent grassland.
- **Sown/planted species abundance (1 study):** One review in the UK and Australia³ found that planting grassland plants had mixed effects on planted species abundance.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Survival (1 study):** One study in Germany² found that 44% of new seedlings from transplanted pepper saxifrage plants survived over four months.

Background

This action considers the introduction of grassland plants by planting directly into soil. Plants may be collected from natural grasslands or grown in greenhouses before planting. If successful, this method of restoration may allow for relatively rapid recovery of degraded grasslands.

A replicated, randomized, paired, controlled study in 1994–1996 at six improved grassland sites in the UK (1) found that planting individual plants increased grass and forb species richness in more than half of cases. In six of 12 comparisons, the number of grass species was higher in plots where individual plants had been planted (5–12 species/plot) than in areas where no plants were planted (4–10 species/plot), while in six comparisons the number of grass species was equal to or lower than that found in areas where no plants were planted (5–13 species/plot vs 5–13 species/plot). In 10 of 12 comparisons, forb species richness was higher in areas where plants were planted (7–22 species/plot) than where no plants were planted (4–15 species/plot), while in two comparisons there were equal or fewer numbers of forb species (4–5 species/plot vs 5 species/plot). In 1994, at each site, plants of nine forb species were planted in four 6 × 4 m plots, and no plants were planted in another four plots. In May/June of 1995 and 1996, the frequency of each grass and forb species was recorded in three 40 × 40 cm quadrats in each plot.

A study in 1998–1999 in a grassland restoration site near Holleben, Germany (2) found that transplanted pepper saxifrage *Silene silaus* plants spread to adjacent grassland, and 44% of new seedlings survived over four months. During the year following transplantation, new seedlings emerged up to 4.5 m from transplanted pepper saxifrage plants, although the majority (74%) were recorded within 1.5 m. Forty new seedlings were monitored with 44% surviving for at least four months after germination.

In 1998, fifteen pepper saxifrage plants with almost ripe seeds were transplanted to the centre of each of four 10-m² plots within a floodplain. The site was managed with grazing and cutting. In May and July 1999, new seedlings were recorded within 50 x 0.25 m² quadrats positioned in circles at distances of 1–5 m around each transplant. Ten seedlings in each of the four plots were marked and survival was recorded every 3–4 weeks in May–September 1999.

A review in 1996–2009 of four studies of semi-natural grassland restoration in the UK and Australia (3) found that planting grassland plants had mixed effects on planted species abundance. One of four studies of planting to restore semi-natural grasslands reported successful introductions of planted species, while one study reported limited success, one reported a failed reintroduction, and one did not report enough information to allow the success of introductions to be determined. The review used keyword searches to identify studies where semi-natural grassland restoration was carried out. All studies on planting used plug plants grown in a nursery.

A replicated, controlled study in 1998–2004 in agricultural fields in Indiana, USA (4) found that areas planted with seedlings in addition to seeding with grassland species contained more plant species, and more native plant species, than areas that were only seeded. After one to four years, areas that were planted and seeded contained more species (16–19) than those that were only seeded (6–12). The number of native species was also higher in the planted and seeded plots compared to the seeded only plots (data not given). Both treatments were carried out on former arable land using locally collected seed. The planting and seeding treatment was applied to a different 14 x 24 m plot each year from 2001–2004. Seedlings of 11 species were planted. The seeding treatment was applied to a different parcel of land each autumn from 1998–2004. No information was provided about the size of seeded areas, seeding rates or timing. Seeded plots were mown in the first year after planting, and invasive plants were treated with herbicide. Plant species were recorded within twenty-four 2 x 2 m quadrats in each seeded area, and 18 quadrats in each seeded and planted area.

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- (2) Bischoff, A. (2000) Dispersal and re-establishment of *Silaum silaus* (L.) in floodplain grassland. *Basic and Applied Ecology*, 1, 125–131.
- (3) Hedberg, P. & Kotowski, W. (2010) New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. *Journal for Nature Conservation*, 18, 304–308.
- (4) Middleton, E.L., Bever, J.D. & Schultz, P.A. (2010) The effect of restoration methods on the quality of the restoration and resistance to invasion by exotics. *Restoration Ecology*, 18, 181–187.

2.5. Sow grassland seeds from a local source

- **Five studies** examined the effects of sowing grassland seeds from a local source on grassland vegetation. Two studies were in Germany^{1,4}, two were in the USA^{2,3} and one was in Italy⁵.

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (1 study):** One replicated, controlled study in Italy⁵ found that sowing grassland seeds from a local source increased plant species richness compared to sowing a commercial seed mix.

- **Characteristic plant richness/diversity (1 study):** One replicated, controlled study in Germany¹ found that sowing grassland seeds from a local source, along with increasing the number of species in a seed mix, led to an increase in the species richness of target plants.

VEGETATION ABUNDANCE (3 STUDIES)

- **Overall abundance (2 studies):** One of two replicated studies (including one site comparison and one controlled study) in the USA² and Germany⁴ found that after sowing grassland seeds from a local source vegetation cover increased over time compared to areas sown with non-native seeds, but the density of individual plants declined². The other study⁴ found that vegetation cover did not differ to that in areas sown with commercial grass seed.
- **Characteristic plant abundance (2 studies):** One replicated, controlled study in Germany¹ found that sowing grassland seeds from a local source, along with increasing the number of species in a seed mix, led to an increase in the cover of target plant species. One replicated, randomized, paired, controlled study in Germany⁴ found that sowing grassland seeds from a local source increased the abundance of one of four characteristic plant species that were sown.

VEGETATION STRUCTURE (2 STUDIES)

- **Height (2 studies):** Two replicated studies (including one controlled and one site comparison study) in the USA² and Italy⁵ found that sowing grassland seeds from a local source did not alter vegetation height compared to sowing non-native grass seeds² or a commercial seed mix⁵.

OTHER (1 STUDY)

- **Survival (1 study):** One replicated, randomized, controlled experiment in the USA³ found that sowing Sandberg bluegrass seeds from a local source did not change the survival of sown plants compared to sowing non-local varieties.

Background

Many restoration projects sow seeds harvested from local intact grassland sites rather than using seeds bought from seed suppliers. In some cases, this is done to obtain a mix of species that is more similar to natural grasslands. In other cases, it is done because seeds obtained locally may be better adapted to local environmental conditions, such as local soils and climate.

A replicated, controlled study in 2004–2010 at a former mining site in Saxony-Anhalt, Germany (1) found that sowing grassland seeds from a local source, along with increasing the number of species in a seed mix, led to an increase in the species richness and cover of target plants. After six years, plots sown with a high diversity local seed mix had on average a greater number and cover of target plant species (28 species, 83% cover) than plots sown with a low diversity non-local seed mix (12 species, 36% cover). In December 2004, three blocks were established on an unvegetated area (240 x 50 m) of boulder clay mixed with sand. In each block, one plot was sown with a high diversity mix of seeds from a local source (11 grass and 40 herb species, sown at 36 kg/ha), and one plot was sown with a low diversity mix of non-local seeds (three grass cultivars, sown at 100 kg/ha). Vegetation was recorded annually within a 5-m² quadrat in each plot in 2005–2010.

A replicated, site comparison study in 2005–2007 in 15 former arable fields in Colorado, USA (2) found that sowing grass and forb seeds from a local source had no effect on vegetation height and mixed effects on vegetation cover and plant density compared to sowing non-native grass seeds. In areas where local grass and forb seeds were sown

18–20 years previously, plant cover was higher (20–24%) than in areas sown with non-native grass seeds (12–19%) but there was no significant difference for areas where local seeds had been sown 2–9 years ago (8–12%). There was no significant difference in plant height between areas where local seeds were sown (14–17 cm) and areas where non-native seeds were sown (15–31 cm). Plant density was higher in areas where local seeds were sown 2–9 years ago than in areas where non-native seeds were sown (18–61 plants/m² vs 17–43 plants/m²), but plant density in areas where local seeds were sown 18–20 years ago (18–34 plants/m²) did not significantly differ from areas where non-native seeds were sown. Each field was sown with either a mix of perennial grass and forb seeds of species native to northern Colorado (nine fields), or with seeds of the non-native grasses, wheatgrass *Agropyron intermedium* (three fields) or smooth brome *Bromus inermis* (three fields). In August–September 2005–2007, forty 0.25-m² circular plots were placed in each field and plant height, density and cover were estimated.

A replicated, randomized, controlled experiment in 2012 in a greenhouse in Wyoming, USA (3) found that sowing Sandberg bluegrass *Poa secunda* seeds from a local source resulted in similar survival to that found when non-local varieties were sown. After four months, the survival of Sandberg bluegrass plants from locally sourced seeds (97–98%) was not significantly different from that of plants from non-local seeds (93–100%). In December 2012, wild Sandberg bluegrass seeds sourced from two local sites were sown in 80 pots, while cultivated Sandberg bluegrass seeds from three sources were sown in 120 pots. All pots were filled with sand and peat moss, and watered twice/day. Two weeks after planting, emerged seedlings were thinned to one plant/pot. Plant survival was monitored for four months from December to March 2012.

A replicated, randomized, paired, controlled study in 2011–2013 in a former plant nursery in southern Germany (4) found that sowing locally sourced seeds reduced the abundance of the invasive plant fleabane *Erigeron annuus*, had a mixed effect on the abundance of individual plant species but did not alter plant cover. There were more Carthusian pink *Dianthus carthusianorum* plants in areas where a local grass seeds were sown (56–96 plants/m²) than in areas where commercial grass seeds were sown (31–58 plants/m²). However, there was no significant difference in the abundance of perennial flax *Linum perenne* (local: 84–109 plants/m²; commercial: 72–122 plants/m²) and ox-eye *Bupthalmum salicifolium* (local: 22–38 plants/m²; commercial: 24–40 plants/m²). Vegetation cover did not differ significantly between areas where seeds were sown (43–50%) and where no seeds were sown (34%). There were fewer invasive annual fleabane plants in areas where seeds were sown (108–142 plants/m²) than in areas where no seeds were sown (141 plants/m²). Before seeds were sown, the site was sprayed with glyphosate herbicide and was harrowed and raked. Twelve 3.5 x 1 m plots were sown with locally sourced seeds of the species red fescue *Festuca rubra* at a rate of 0.4 g/m², twelve plots were sown with commercially sourced red fescue seed, and six plots were not sown with grass seed. All plots were sown with seeds of the species perennial flax, Carthusian pink, and ox-eye. In July 2012, seedlings were counted in all plots, and in May 2013, plants of all species were counted.

A replicated, controlled study in 2011–2012 in an abandoned quarry site in northern Italy (5) found that sowing grassland seeds from a local source increased plant species richness, but did not alter the height of herbaceous vegetation, compared to sowing a commercial seed mix. These results are not based on statistical analyses. After one year, areas sown with local seeds had higher average plant species richness (16 species/plot) than areas sown with a commercial seed mix (10 species/plot). The average

height of herbaceous plants was similar between areas sown with local seeds (100 cm) and a commercial seed mix (93 cm). In June 2011, the site was remodelled to create two 200-m² terraced areas that were almost flat and topsoil was added. One area was sown with hay seeds harvested from a nearby grassland at a rate of 36 g/m², while the other was sown with a commercial seed mix of grassland species. Shrubs and trees were planted in both areas. Plants were watered in 2012 during dry periods. In June 2012, vegetation was surveyed in three 3 x 3 m randomly located plots in each area.

- (1) Kirmer, A., Baasch, A. & Tischew, S. (2012) Sowing of low and high diversity seed mixtures in ecological restoration of surface mined-land. *Applied Vegetation Science*, 15, 198–207.
- (2) Munson, S.M. & Lauenroth, W.K. (2014) Controls of vegetation structure and net primary production in restored grasslands. *Journal of Applied Ecology*, 51, 988–996.
- (3) Herget, M.E., Hufford, K.M., Mummey, D.L., Meador, B.A. & Shread, L.N. (2015) Effects of competition with *Bromus tectorum* on early establishment of *Poa secunda* accessions: can seed source impact restoration success? *Restoration Ecology*, 23, 277–283.
- (4) Walker, E.A., Hermann, J.M. & Kollmann, J. (2015) Grassland restoration by seeding: seed source and growth form matter more than density. *Applied Vegetation Science*, 18, 368–378.
- (5) Gilardelli, F., Sgorbati, S., Citterio, S. & Gentili, R. (2016) Restoring limestone quarries: hayseed, Commercial seed mixture or spontaneous succession? *Land Degradation and Development*, 27, 316–324.

2.6. Sow seeds of nurse plants

- **One study** examined the effects of sowing seeds of nurse plants on grassland vegetation. The study was in France¹.

VEGETATION COMMUNITY (1 STUDY)

- **Community composition (1 study):** One replicated, controlled study in France¹ found that sowing seeds of nurse plants reduced the similarity of the plant community to that of nearby intact steppe compared to areas where no seeds were sown.
- **Overall richness/diversity (1 study):** One replicated, controlled study in France¹ found that sowing seeds of nurse plants did not change plant species richness and richness was lower than in nearby intact steppe.

VEGETATION ABUNDANCE (1 STUDY)

- **Overall abundance (1 study):** One replicated, controlled study in France¹ found that sowing seeds of nurse plants did not change vegetation cover compared to areas where no seeds were sown.

VEGETATION STRUCTURE (0 STUDIES)

Background

Nurse plants are plants which are thought to aid the growth of other species by providing relatively benign conditions below their canopy. Examples of these altered conditions may include increases in moisture and nutrients, and decreases in temperature and damage from herbivores (de Toledo Castanho & Prado 2014). Sowing the seeds of nurse plants may facilitate the colonisation of grassland species.

This action involves sowing the seeds of nurse plants only. For studies that sow or plant both nurse plants and grassland plant species, see ‘Sow or plant nurse plants (alongside seeding/planting of grassland species)’.

de Toledo Castanho, C. & Prado, P.I. (2014) Benefit of shading by nurse plant does not change along a stress gradient in a coastal dune. *PLOS ONE*, 9, e105082.

A replicated, controlled study in 2009–2012 in a former orchard in the south of France (1) found that sowing seeds of nurse plants did not alter plant species richness or vegetation cover and reduced the similarity of the plant community to that of intact steppe. Plant species richness did not differ significantly between areas where seeds of nurse plants were sown (10 species/plot) and areas where no seeds were sown (16 species/plot), but both were lower than species richness in an intact steppe site (33 species/plot). The similarity of the plant community to that of the intact steppe site was lower in areas where seeds of nurse plants were sown than in areas where they were not sown (presented as Bray-Curtis similarity). Vegetation cover was not significantly different in areas where the seeds of nurse plants were sown (66%) and areas where they were not (67%). In 2009, all trees were removed from the former orchard and soils were levelled. Sheep were introduced in 2010. Seeds of nurse plants were sown in two 30-ha areas, while no seeds were sown in a 270-ha area. In May 2012, cover of each plant species was recorded in each area in eighteen 2 x 2 m quadrats.

(1) Jaunatre, R., Buisson, E. & Dutoit, T. (2014) Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, southeast France). *Applied Vegetation Science*, 17, 236–245.

2.7. Sow or plant nurse plants (alongside seeding/planting of grassland species)

- **Four studies** examined the effects of sowing or planting nurse plants alongside seeding/planting grassland species on grassland vegetation. Two studies were in Europe^{3,4}, one study was in the USA¹ and one was in Kenya².

VEGETATION COMMUNITY (1 STUDY)

- **Community composition (1 study):** One replicated, controlled study in Slovenia⁴ found that sowing a seed mix containing nurse species resulted in a community composition that was less similar to the target community when compared to sowing a seed mix that did not contain nurse species.
- **Overall richness/diversity (1 study):** One replicated, controlled study in Slovenia⁴ found that sowing a seed mix containing nurse species did not increase species richness compared to sowing a seed mix that did not contain nurse species.

VEGETATION ABUNDANCE (1 STUDY)

- **Grass abundance (1 study):** One replicated, paired, controlled study in the USA¹ found that sowing seeds of nurse plants alongside that of grassland species did not change grass abundance.
- **Forb abundance (1 study):** One replicated, paired, controlled study in the USA¹ found that sowing seeds of nurse plants alongside that of grassland species did not change forb abundance.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (2 STUDIES)

- **Germination/Emergence (1 study):** One replicated, controlled study in Spain³ found that sowing seeds under nurse plants increased seed germination.

- **Survival (2 studies):** One of two replicated, controlled studies (one of which was randomized and paired) in Kenya² and Spain³ found that sowing seeds or planting under nurse plants increased survival of planted plants³. The other study² found that sowing seeds under nurse plants initially increased seedling survival, but there was no difference in survival after two to three years.

Background

Nurse plants are plants which are thought to aid the growth of other species by providing relatively benign conditions below their canopy. Examples of these altered conditions may include increases in moisture and nutrients, and decreases in temperature and damage from herbivores (de Toledo Castanho & Prado 2014). Combining the planting or sowing of seeds of nurse plants with that of focal plants may help to increase focal plant survival and growth. For studies that sow seeds of nurse plants only, see 'Sow seeds of nurse plants'.

de Toledo Castanho, C. & Prado, P.I. (2014) Benefit of shading by nurse plant does not change along a stress gradient in a coastal dune. *PLOS ONE*, 9, e105082.

A replicated, paired, controlled study in 1992–1995 in five former prairie sites destroyed by road widening in Montana, USA (1) found that sowing seeds of nurse plants alongside that of grassland species did not alter forb or grass cover. Cover of native forbs did not differ significantly between areas where nurse plant seeds were sown alongside seeds of grassland species (2.8–10.6%) and areas where seeds of grassland species were sown without nurse plant seeds (3.5–8.1%). The same pattern was true for non-native forbs (nurse: 3.8–21.4%; no nurse: 2.9–22.7%), native grasses (nurse: 7.8–10.0%; no nurse: 5.3–12.1%), and non-native grasses (nurse: 19.4–24.8%; no nurse: 14.1–29.1%). In summer 1992, after construction of a road, original topsoil was replaced at the five sites. In each site, two blocks containing two 8 × 4 m plots were established. In autumn 1992 or 1993, seeds of the nurse plant *Triticum × Agropyron* were sown with native seeds in one plot, and native seeds, but no nurse plant seed, was sown in one plot. In October 1992, all plots were sprayed with a mixture of wood mulch, tackifier, and nitrogen fertilizer. Vegetation cover of all species was estimated in July 1995 using five 50 × 20 cm quadrats in each plot.

A replicated, randomized, paired, controlled study in 2002–2003 in degraded rangelands in north central Kenya (2) found that planting buffel grass *Cenchrus ciliaris* beside aloe *Aloe secundiflora* nurse plants initially increased seedling survival, but after two to three years there was no increase. Two hundred and twenty days after planting, more grass seedlings planted next to aloe bushes were alive (16%) than those without nurse plants (6%). Survival of buffel grass seedlings in the second and third growing seasons after planting did not differ between seedlings next to nurse plants (84–100%) and those without nurse plants (95–100%). In April 2002, nine rows of 14 holes (14 cm diameter and 25 cm deep) were dug in bare ground. Mature aloe bushes were planted in seven holes in each row, and seven holes were refilled without planting aloe bushes. Buffel grass seeds (0.2 g) were sown in furrows 5 cm deep, 20 cm long and 10 cm away on either side of the rows of holes, and covered with soil. Seedling survival was monitored from April 2002–November 2003, which included three complete growing seasons.

A replicated, controlled study in 2006–2007 in arid steppe grassland in North-Eastern Spain (3) found that sowing seeds or planting under nurse plants increased survival of planted plants and increased seed germination. Survival of *Salosa vermiculata* and *Lygeum spartum* was higher when plants were planted under nurse plants (12–73%)

than when they were not planted under nurse plants (0–93%). Seed germination of the two species was also higher (nurse plant: 0–21%; no nurse plant: 0–2%). In the planting experiment, in October 2006, twenty-five *Salosa vermiculata* and *Lygeum spartum* seedlings were planted four metres apart under mature *Suaeda vera* plants, while another twenty-five plants were planted without nurse plants. Seeding was carried out nearby to the seedling experiment (replication unclear). Survival of planted seedlings was recorded in February and September 2007. Seed germination was recorded in March, June and September 2007.

A replicated, controlled study in 1998–2003 on a newly-created motorway verge in southwestern Slovenia (4) found that plots sown with a seed mix containing nurse species contained a similar number of species, but the species composition was less similar to the target community compared to plots sown without nurse species. Three to five years after sowing, plots sown with seed mix with nurse species contained 18–22 species, compared to 24–30 species in plots without nurse species. The composition of plant species in plots sown with nurse species was less similar to the target dry grassland community (19–21%) than plots without nurse species (35–45%) (differences not subject to statistical tests). Vegetation cover was also lower in plots with nurse species than those without nurse species in one out of three years (69% vs 88%). Fifteen 3 x 3 m plots were established on new verges with a slope of approximately 30° constructed of topsoil. In June 1998, ten plots were sown with a mix of six competitive, stress-tolerant species. Five plots were sown with a mix of 12 species which contained ‘nurse’ grasses (*Lolium* species) and legumes (*Trifolium* species) in addition to the stress-tolerant species. Seeds were mixed with organic fertilizers, hay mulch, humic acids and water before seeding (hydroseeded). Plant species and cover were recorded in every plot each June. The target community was dry grassland adjacent to the new motorway.

- (1) Tyser, R.W., Asebrook, J.M., Potter, R.W. & Kurth, L.L. (1998) Roadside revegetation in Glacier National Park, USA: Effects of herbicide and seeding treatments. *Restoration Ecology*, 6, 197–206.
- (2) King, E.G. & Stanton, M.L. (2008) Facilitative effects of *Aloe* shrubs on grass establishment, growth, and reproduction in degraded Kenyan rangelands: Implications for restoration. *Restoration Ecology*, 16, 464–474.
- (3) Pueyo, Y., Alados, C.L., Garcia-Avila, B., Kefi, S., Maestro, M. & Rietkerk, M. (2009) Comparing direct abiotic amelioration and facilitation as tools for restoration of semiarid grasslands. *Restoration Ecology*, 17, 908–916.
- (4) Zelnik, I., Silc, U., Carni, A. & Kosir, P. (2010) Revegetation of motorway slopes using different seed mixtures. *Restoration Ecology*, 18, 449–456.

2.8. Sow seeds of parasitic species (e.g. yellow rattle)

- **Six studies** examined the effects of sowing seeds of parasitic species on grassland vegetation. Four studies were in the UK^{2–5}, one study was in Switzerland¹ and one was in Belgium⁶.

VEGETATION COMMUNITY (6 STUDIES)

- **Overall richness/diversity (6 studies):** Five of six studies (including five controlled studies and one review) in the UK^{2–5}, Switzerland¹ and Belgium⁶ found that sowing seeds of the parasitic plants yellow rattle^{2,4}, European yellow rattle¹ or marsh lousewort⁶ increased plant species richness^{2,4,6} and/or diversity^{1,4}. The other study⁵ found that sowing yellow rattle seeds did not alter plant species richness.

VEGETATION ABUNDANCE (3 STUDIES)

- **Overall abundance (1 study):** One review in the UK³ found that sowing seeds of the parasitic plant yellow rattle led to a decrease in total plant biomass in three of four studies.
- **Characteristic plant abundance (1 study):** One controlled study in Belgium⁶ found that sowing seeds of the parasitic plant marsh lousewort increased the abundance of six target plant species.
- **Grass abundance (1 study):** One replicated, controlled study in Switzerland¹ found that sowing seeds of the parasitic plant European yellow rattle led to a decrease in grass cover.
- **Forb abundance (1 study):** One replicated, controlled study in Switzerland¹ found that sowing seeds of the parasitic plant European yellow rattle did not alter the cover of forbs.

VEGETATION STRUCTURE (0 STUDIES)

Background

Many degraded grasslands are dominated by relatively few grass species. Sowing seeds of parasitic grassland plants, such as yellow rattle *Rhinanthus minor*, may help to restrict the growth of these grasses, potentially allowing other species to increase in abundance.

A replicated, controlled study in 1995–1997 in a former arable field in Lupsingen, Switzerland (1) found that sowing seeds of the parasitic plant European yellow rattle *Rhinanthus alectorolophus* led to an increase in plant species diversity and a decrease in grass cover but did not alter the cover of forbs. After one year, quadrats sown with yellow rattle seeds had on average a greater diversity of plant species than quadrats not sown with yellow rattle seeds (data reported as evenness index). Average grass cover was lower in quadrats sown with yellow rattle (40%) compared to unsown quadrats (51%), whereas there was no significant difference in the cover of forbs (herbs: 34% vs 26%; legumes: 23% vs 26%). In May 1995, two replicate blocks each consisting of 32 plots (8 x 2 m) were sown with different assemblages of local grassland seeds. In October 1996, yellow rattle seeds were sown at a rate of 800 seeds/m² within a 50 x 50 cm quadrat within each plot, while a second quadrat was left unsown. All plots were mown twice/year. In September 1997, vegetation was assessed in each of the two quadrats/plot.

A replicated, randomized, paired, controlled study in 1998–2002 in improved grassland in Oxfordshire, UK (2) found that sowing seeds of the parasitic plant yellow rattle *Rhinanthus minor* along with seeds of grassland forbs increased plant species richness. Species richness was higher in areas where yellow rattle had been sown with seeds of other plants (4.2–5.8 species/plot) than in areas where no seeds of yellow rattle were sown (1.4–1.8 species/plot). In December 1998, five blocks each with four 10 x 10 m plots were established, and livestock allowed to graze the site after which they were removed. In three plots in each block, yellow rattle seeds were sown at a rate of 0.1–2.5 kg/ha, while in one plot no yellow rattle seeds were sown. In October 2000, seeds of 10 forb species were sown in all plots at a rate of 5 kg/ha. Hay was cut every year in July and the field was grazed in autumn by cattle or sheep at a rate of 35–50 animals/ha. In June 2001 and 2002, vegetation was surveyed using seven to ten 1 x 1 m quadrats/plot.

A review in 2005 of four grassland restoration studies in England, UK (3) found that sowing seeds of the parasitic plant yellow rattle *Rhinanthus minor* led to an increase in plant species richness in all of three studies and a decrease in total plant biomass in three of four studies. Three studies found that after 1–4 years, species richness increased in sites sown with yellow rattle for target plants (by 60%; one study), forb species (by

45%; one study) or plants overall (one additional species; one study) compared to sites not sown with yellow rattle. Three of four studies found that after 1–2 years, total plant biomass decreased by 21–44% in sites sown with yellow rattle compared to unsown sites, whereas one study found no significant difference. Four restoration studies were carried out in former arable fields or species-poor grasslands. In each study, yellow rattle seeds were sown in part of the site at a rate of 12–1,000 seeds/m², while another part was left unsown. Vegetation was assessed (species richness in three studies, biomass in all four studies) during 1–4 years after sowing. One study has been summarised individually (2).

A replicated, controlled study in 1998–2000 at a grassland site in West Yorkshire, UK (4) found that sowing seeds of the parasitic plant yellow rattle *Rhinanthus minor* increased plant species richness and diversity. After one year, plots sown with yellow rattle prior to a grassland seed mix had on average a greater number of sown species (4–8.4), unsown species (8.7–11.4) and greater overall plant diversity (data reported as Shannon diversity index) than plots not sown with yellow rattle prior to a grassland seed mix (sown species: 2.7–5.4; unsown species: 7–9.7). In autumn 1998, six plots (each 4 x 4 m) were created on a newly established meadow. Three plots were sown with yellow rattle seeds (1,000 seeds/m²), and three plots were left unsown. In April 1999, all six plots were sown with a commercial seed mix of six grass and six forb species at a rate of 30 kg/ha. All plots were cut in July each year. In May 1999 and 2000, vegetation was recorded within three randomly placed 50 x 50 cm quadrats/plot.

A replicated, randomized, paired controlled study in 1999–2003 in two species-poor grassland sites in the UK (5) found that disturbing soil and sowing seeds of the parasitic plant yellow rattle *Rhinanthus minor* did not alter the number of plant species compared to disturbing soil alone. In each of four years, plant species richness did not significantly differ between areas where soil was disturbed and yellow rattle seeds were sown (9.1–16.0 species/plot) and areas where soil was disturbed but seeds were not sown (11.0–16.2 species/plot). In September 1999, in eight 15 x 15 m plots at each site, soil was disturbed using a power-harrow to a depth of 5 cm and seeds of the plant yellow rattle were sown at a rate of 2.4 kg/ha. In eight other plots, soil was disturbed but no seeds were sown. All plots were paired. Vegetation composition was recorded in June 1999–2003 using five randomly placed 1 x 1 m quadrats/plot.

A controlled study in 1994–2000 in a degraded fen meadow in Oostkamp, Belgium (6) found that sowing seeds of the parasitic plant marsh lousewort *Pedicularis palustris* increased overall plant species richness and the abundance of six target plant species. After six years, the average number of plant species was higher in the site where lousewort seeds were sown (21 species/quadrat) than the site where no lousewort seeds were sown (14 species/quadrat). The site sown with lousewort also had a greater abundance of six target plant species (45–191 plants/10 m²) than the unsown site (2–74 plants/10 m²; see original paper for details). In July 1994, two adjacent sites measuring 20 x 20 m were established in a fen meadow dominated by acute sedge *Carex acuta*. Marsh lousewort was sown in one site (500 seeds), whereas no seeds were sown in the other. Both sites were mowed 1–2 times/year. Marsh lousewort detected in the unsown site was manually removed. In July 2000, vegetation was surveyed within 10 randomly selected 1-m² quadrats/site.

(1) Joshi, J., Matthies, D. & Schmid, B. (2000) Root hemiparasites and plant diversity in experimental grassland communities. *Journal of Ecology*, 88, 634–644.

- (2) Pywell, R.F., Bullock, J.M., Walker, K.J., Coulson, S.J., Gregory, S.J. & Stevenson, M.J. (2004) Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *Journal of Applied Ecology*, 41, 880–887.
- (3) Bullock, J.M. & Pywell, R.F. (2005) *Rhinanthus*: a tool for restoring diverse grassland? *Folia Geobotanica*, 40, 273–288.
- (4) Westbury, D.B., Davies, A., Woodcock, B.A. & Dunnett, N.P. (2006) Seeds of change: The value of using *Rhinanthus minor* in grassland restoration. *Journal of Vegetation Science*, 17, 435–446.
- (5) Pywell, R.F., Bullock, J.M., Tallowin, J.B., Walker, K.J., Warman, E.A. & Masters, G. (2007) Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology*, 44, 81–94.
- (6) Decler, K., Bonte, D. & Van Diggelen, R. (2013) The hemiparasite *Pedicularis palustris*: ‘Ecosystem engineer’ for fen-meadow restoration. *Journal for Nature Conservation*, 21, 65–71.

2.9. Sow seeds of tree species in savanna

- **One study** examined the effects of sowing seeds of tree species in savanna on grassland vegetation. The study was in Brazil¹.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (0 STUDIES)

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated study in Brazil¹ found that sowing tree seeds in savanna resulted in germination of 52% of the tree seeds.
- **Survival (1 study):** One replicated study in Brazil¹ found that after sowing tree seeds in savanna, 35% of the seeds produced seedlings that survived for more than two years.

Background

Savanna habitats consist of a mixture of grassland and woodland habitats in which trees do not form a closed canopy. Trees are cleared in many savanna areas often to increase the quality of pasture for livestock. Sowing seeds of tree species may help to increase tree abundance.

A replicated study in 2010–2012 in an arable field in Brazil (1) found that over half of tree seeds sown germinated but fewer than half of seeds resulted in trees which survived for more than two years. After 42 days, 3,200 of the 6,180 tree seeds sown had germinated. After 780 days, 2,144 seedlings of the 3,200 that had germinated were alive. In November 2010, tree seeds of six species were sown in rows one metre apart resulting in 6,180 sown seeds. Seedling germination and survival was recorded for each plant after 42, 84, 126, 217, 398 and 780 days. This experiment was also part of a study testing the effect of fertilizer and plant cover on seedling germination and growth.

- (1) Silva, R.R.P., Oliveira, D.R., da Rocha, G.P.E. & Vieira, D.L.M. (2015) Direct seeding of Brazilian savanna trees: effects of plant cover and fertilization on seedling establishment and growth. *Restoration Ecology*, 23, 393–401.

2.10. Sow seeds at a higher density

- **Six studies** examined the effects of sowing seeds at a higher density on grassland vegetation. Four studies were in the USA^{2-4,6}, and one study was in each of the UK¹ and Canada⁵.

VEGETATION COMMUNITY (2 STUDIES)

- **Community composition (1 study):** One replicated, randomized, controlled study in the UK¹ found that sowing grass seeds at a higher density did not increase the similarity of the vegetation community to that of the target community.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the USA² found that sowing seeds at a higher density increased forb species richness.

VEGETATION ABUNDANCE (4 STUDIES)

- **Sown/planted species abundance (1 study):** One replicated, controlled study in the USA⁴ found that sowing seeds at a higher density did not alter the cover of sown plant species.
- **Grass abundance (3 studies):** One replicated, randomized, paired, controlled study in the USA² found that sowing seeds at a higher density increased grass cover.
- **Forb abundance (1 study):** One replicated, randomized, paired, controlled study in the USA² found that sowing seeds at a higher density increased forb cover.
- **Tree/shrub abundance (1 study):** One site comparison study in the USA⁶ found that sowing grass seeds at a higher density reduced the cover of native grassland shrubs.
- **Individual plant species abundance (1 study):** One replicated, controlled study in Canada⁵ found that sowing seeds at a higher density increased the cover of thickspike wheatgrass.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, randomized, paired, controlled study in the USA³ found that sowing seeds at a higher density increased the number of purple needlegrass seedlings.

Background

Grassland restoration often aims to establish grassland vegetation at a degraded site. One method of doing this is sowing the seeds of grassland plants. Increasing the density at which the seeds of grassland plants are sown may help to increase their chances of establishment.

A replicated, randomized, controlled study in 1997–1999 in a grazed wet grassland in London, UK (1) found that sowing grass seeds at a higher density did not increase similarity of the vegetation community to that of the target community. The similarity of the vegetation community to that of the target community did not differ significantly between areas sown with seeds at rates of 10, 25 or 40 kg/ha (data presented as similarity coefficients). In 1997, seeds of eight grass species were sown in varying proportions onto bare soil in each of three 15 x 15 m plots at rates of 10, 25 or 40 kg/ha. Cover of all species in each of the nine plots was estimated within 10 randomly placed 1-m² quadrats in June 1997–1999. Similarity of the plant communities to the target community, a mesotrophic grassland, was assessed using the UK National Vegetation Classification.

A replicated, randomized, paired, controlled study in 2001–2004 in a former agricultural field in Kansas, USA (2) found that sowing seeds at higher densities increased grass and forb cover, and forb species richness. The results of this study did not allow for statistical significance to be assessed. After 1–3 years, average grass cover was higher in plots where grass seeds were sown at high density (35–78%) than in plots where grass seeds were sown at a low density (23–64%). There was a similar pattern for average forb cover (high density: 38–57%; low density: 24–34%) and forb species richness (high density: 7–8 species/plot; low density: 5–6 species/plot) when forb seeds were sown at high and low densities. The field surrounding the experimental area was sown with native species in May 2001. A few days later, the experimental area was sown with forb and grass seeds at three combinations of densities: low grass/low forbs, low grass/high forbs, high grass/low forbs. Each seeding density was sown in six 2 x 2 m plots. Seeds were hand sown and raked into the bare soil. The entire field was mown in June–July 2001 and 2002, and burned in March 2003. Plant species cover was estimated using a 1-m² quadrat in the centre of each plot in May/June and August/September 2001–2004.

A replicated, randomized, paired, controlled study in 2005 in a grassland site in California, USA (3) found that plots sown with a high density of purple needlegrass *Nasella pulchra* seeds had more seedlings and plants than plots sown at a low seeding density. Eight weeks after seeding, there were more seedlings in plots sown at high density (344–517/500 cm²) than plots sown at low density (168–327/500 cm²). Twenty-one weeks after seeding, the number of plants remained higher in plots sown at high density (134–418 plants/500 cm²) than plots sown at low density (79–218 plants/500 cm²). In January 2005, sixty-four 1 x 1 m plots were set up in grassland, in eight blocks of eight plots. In February 2005, locally-sourced purple needlegrass seeds were sown onto the surface of tilled soil. Twenty plots (four random plots in each of five blocks) were seeded at high density (1,000 seeds/m²) and 32 plots (four random plots in each of the eight blocks) were seeded at low densities (500 seeds/m²). Parts of the plots were also subject to different grazing treatments. The number of plants in four 500-cm² areas within each plot was counted in March and June 2005.

A replicated, controlled study in 2006–2009 in a former arable field in Nebraska, USA (4) found that sowing seeds at a higher density did not alter the cover of seeded species, unseeded species or invasive plant species compared to areas that were sown at low density. Cover of seeded species did not differ significantly between areas sown at high density (3–34 cm) and areas sown at low density (3–28 cm). Cover of unseeded plant species (3–35 vs 3–32 cm) and invasive plant species (0–1.4 cm vs 0–1.1 cm) also showed no significant difference between areas seeded at high and low densities. In March–April 2006, twelve 55 x 55 m plots were sown with grass and forb seeds at a high density (328 seeds/m²) and twelve plots were sown at a low density (164 seeds/m²). All plots were burned in March 2008. In July 2008, invasive plants were sprayed with glyphosate herbicide. Vegetation cover was recorded using five 55-m transects in each plot in June 2007–2009. Cover was measured at six points along each transect.

A replicated, controlled study in 2005–2009 in an abandoned field in Saskatchewan, Canada (5) found that sowing thickspike wheatgrass *Elymus lanceolatus* seeds at higher densities resulted in higher thickspike wheatgrass cover and lower cover of non-native species. In two of three comparisons sowing seeds at a higher density increased cover of thickspike wheatgrass (highest seeding rate: 86–98%, lowest seeding rate: 2–25%), however in one comparison there was no change in cover as seeding rate increased (highest seeding rate: 5%, lowest seeding rate: 1%). In two of three

comparisons sowing seeds at a higher density reduced cover of non-native cover (highest seeding rate: 6–13%, lowest seeding rate: 60–94%), but in one comparison there was no change in non-native cover (highest seeding rate: 62%, lowest seeding rate: 59%). In June 2005–2007, thickspike wheatgrass seeds were sown at a rate of 30, 300, 600, 1800, and 3000 seeds/m².

A site comparison study in 1985–2012 at nine former coal mine sites in Montana and Wyoming, USA (6) found that sowing grass seeds at a higher density reduced the cover of native grassland shrubs but did not alter the cover of unseeded non-native grasses and forbs. After 8–20 years, the cover of native grassland shrubs was on average lower in fields that were sown with higher densities of grass seeds (data reported as statistical model results). The average cover of unseeded non-native grasses and forbs did not differ significantly between fields that were sown with low, moderate or high grass seed densities (data reported as statistical model results). In 1985–2005, seed mixes of grasses, forbs and shrubs (average 15 species) were sown in 327 fields at nine former coal mine sites. Grass seeds were sown at low (0–4 kg/ha), moderate (>4–8 kg/ha), or high (>8 kg/ha) densities (number of sites for each not reported). Crushed rock and topsoil were added prior to sowing. In 2011 and 2012, vegetation was sampled within 20 frames (20 x 50 cm, seven sites) or 1–3 areas (4.6 x 45.7 m, two sites) evenly spaced along a transect in each field.

- (1) Gilbert, J.C., Gowing, D.J.G. & Bullock, R.J. (2003) Influence of seed mixture and hydrological regime on the establishment of a diverse grassland sward at a site with high phosphorus availability. *Restoration Ecology*, 11, 424–435.
- (2) Dickson, T.L. & Busby, W.H. (2009) Forb species establishment increases with decreased grass seeding density and with increased forb seeding density in a northeast Kansas, USA, experimental prairie restoration. *Restoration Ecology*, 17, 597–605.
- (3) Orrock, J.L., Witter, M.S. & Reichman, O.J. (2009) Native consumers and seed limitation constrain the restoration of a native perennial grass in exotic habitats. *Restoration Ecology*, 17, 148–157.
- (4) Nemec, K.T., Allen, C.R., Helzer, C.J. & Wedin, D.A. (2013) Influence of richness and seeding density on invasion resistance in experimental tallgrass prairie restorations. *Ecological Restoration*, 31, 168–185.
- (5) Wilson, S.D. (2015) Managing contingency in semiarid grassland restoration through repeated planting. *Restoration Ecology*, 23, 385–392.
- (6) Rinella, M.J., Espeland, E.K. & Moffatt, B.J. (2016) Studying long-term, large-scale grassland restoration outcomes to improve seeding methods and reveal knowledge gaps. *Journal of Applied Ecology*, 53, 1565–1574.

2.11. Increase number of species in seed mix

- **Five studies** examined the effects of increasing the number of species in a seed mix on grassland vegetation. Four studies were in the USA^{1,3–5} and one was in Germany².

VEGETATION COMMUNITY (4 STUDIES)

- **Overall richness/diversity (1 study):** One replicated, site comparison study in the USA³ found that increasing the number of species in a seed mix did not change plant species richness.
- **Characteristic plant richness/diversity (1 study):** One replicated, controlled study in Germany² found that increasing the number of species in a seed mix, along with sowing seeds from a local source, increased the species richness of target plants.
- **Sown/planted species richness/diversity (2 studies):** Two replicated, controlled studies in the USA^{1,5} (one of which was randomized) found that increasing the number species in a seed mix increased the species richness of sown plants.

VEGETATION ABUNDANCE (3 STUDIES)

- **Characteristic plant abundance (1 study):** One replicated, controlled study in Germany² found that increasing the number of species in a seed mix, along with sowing seeds from a local source, increased the cover of target plant species.
- **Sown/planted species abundance (2 studies):** One of two replicated, controlled studies (one of which was randomized) in the USA^{4,5} found that increasing the number of species in a seed mix increased the cover of sown plant species⁵. The other study⁴ found that there was no change in the cover of sown species.

VEGETATION STRUCTURE (0 STUDIES)

Background

Grassland restoration often aims to restore species rich habitats. One method for potentially increasing species richness of restoration sites is to increase the number of species whose seeds are sown.

A replicated, controlled study in 2000–2004 in a former arable field in Kansas, USA (1) found that increasing the number of species in a seed mix increased the species richness of sown species. Species richness of sown plant species was higher in areas sown with the seeds of eight to 16 species (4–9 species/plot) than in areas sown with one to four species (1–3 species/plot). In February 2000, soil at the site was disturbed by harrowing. Five 6 × 6 m plots were each sown with the seeds of one, two, three, four, eight, 12 or 16 plant species. Seeds were a mixture of grasses, nitrogen-fixing species and Asteraceae obtained from local or regional commercial suppliers. Plots were mowed in June 2000, April and June 2001, and November or December 2002, 2003 and 2004. In June each year, species richness and plant cover were estimated using four 0.75 × 0.75 m quadrats placed in each plot.

A replicated, controlled study in 2004–2010 at a former mining site in Saxony-Anhalt, Germany (2) found that increasing the number of species in a seed mix, along with sowing seeds from a local source, led to an increase in the species richness and cover of target plants. After six years, plots sown with a high diversity local seed mix had on average a greater number and cover of target plant species (28 species, 83% cover) than plots sown with a low diversity non-local seed mix (12 species, 36% cover). In December 2004, three blocks were established on an unvegetated area (240 × 50 m) of boulder clay mixed with sand. In each block, one plot was sown with a high diversity mix of seeds from a local source (11 grass and 40 herb species, sown at 36 kg/ha), and one plot was sown with a low diversity mix of non-local seeds (three grass cultivars, sown at 100 kg/ha). Vegetation was monitored annually within a 5-m² quadrat in each plot in 2005–2010.

A replicated, site comparison study in 2004–2011 in 27 restored prairie sites in Michigan, USA (3) found that increasing the number of species sown led to an increase in sown species richness but did not alter overall plant species richness. Four to seven years after seeding, sites sown with seed mixes containing a greater number of species had a higher species richness of sown plants (data reported as statistical model results). However, higher numbers of species in seed mixes did not lead to higher overall plant species richness (data reported as model results). Seeds were sown at all sites in 2004–2008 following removal of all vegetation with herbicide. Information on the seed mixes used were collected from the practitioner who oversaw the restoration projects. In July–September 2011, a 50-m transect was established at each site and a 1 × 1 m quadrat

placed every 5 m on the transect. Vegetation cover of all plants in each 1 x 1 m quadrat was estimated.

A replicated, controlled study in 2006–2009 in a former arable field in Nebraska, USA (4) found that increasing the number of species sown did not alter the cover of seeded species or cover of invasive species, but did reduce the cover of unseeded species compared to areas that were sown with a low diversity seed mix. Cover of seeded species did not differ significantly between areas sown with a high diversity seed mix (3–25 cm) and areas sown with a low diversity seed mix (3–33 cm). Cover of invasive plant species also showed no significant difference (0–1.4 cm vs 0–0.5 cm). Cover of unseeded species was lower in areas where a high diversity seed mix was sown (4–34 cm) than in areas where a low diversity seed mix was sown (12–33 cm). In March–April 2006, twelve 55 x 55 m plots were seeded with a high diversity seed mix containing 97 plant species, and twelve plots were seeded with a low diversity mix containing 15 plant species. All plots were burned in March 2008. In July 2008, invasive plants were sprayed with glyphosate herbicide. Vegetation cover was recorded using five 55-m transects in each plot in June 2007–2009. Cover was measured at six points along each transect.

A replicated, randomized, controlled study in 2007–2012 in a former corn field in Kansas, USA (5) found that increasing the number of species sown increased the species richness and cover of sown plant species, but reduced the number and cover of unsown species. After six years, cover of sown species was higher in areas where high diversity seed mixes were sown (98–127%) than in areas where a low diversity mix was sown (45%). The same was true for the species richness of sown plant species (7–14 species vs 3 species). After six years, cover of unsown species was lower in plots where a high diversity seed mix was sown (6–20%) than in areas where a low diversity mix was sown (55%). In three of four cases, unsown species richness was lower in plots where high diversity seed mixes were sown (6–11 species) than in plots where a low diversity seed mix was sown (5 species). In 2006, the corn field was mowed. In February 2007, twenty-five 900-m² plots were established. In 20 plots, 8–20 species were sown, while in five plots four species were sown. All plots were mown to a height of 10 cm in June 2007 and January 2008. In July 2007–2013, twelve 75 x 75 cm quadrats/plot were used to assess vegetation cover and plant species richness.

- (1) Piper, J.K., Schmidt, E.S. & Janzen, A.J. (2007) Effects of species richness on resident and target species components in a prairie restoration. *Restoration Ecology*, 15, 189–198.
- (2) Kirmer, A., Baasch, A. & Tischew, S. (2012) Sowing of low and high diversity seed mixtures in ecological restoration of surface mined-land. *Applied Vegetation Science*, 15, 198–207.
- (3) Grman, E., Bassett, T. & Brudvig, L.A. (2013) Confronting contingency in restoration: management and site history determine outcomes of assembling prairies, but site characteristics and landscape context have little effect. *Journal of Applied Ecology*, 50, 1234–1243.
- (4) Nemec, K.T., Allen, C.R., Helzer, C.J. & Wedin, D.A. (2013) Influence of richness and seeding density on invasion resistance in experimental tallgrass prairie restorations. *Ecological Restoration*, 31, 168–185.
- (5) Piper, J.K. (2014) Incrementally rich seeding treatments in tallgrass prairie restoration. *Ecological Restoration*, 32, 396–406.

2.12. Sow seeds at start of growing season

- **Three studies** examined the effects of sowing seeds at the start of the growing season on grassland vegetation. Two studies were in the USA^{2,3} and one was in the UK¹.

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (1 study):** One replicated, randomized, controlled study in the USA³ found that sowing seeds in spring increased plant diversity compared to sowing in autumn.
- **Sown/planted richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that sowing seeds in spring increased the number of sown species compared to sowing in autumn.

VEGETATION ABUNDANCE (1 STUDY)

- **Sown/planted species abundance (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that sowing seeds in spring increased the cover of sown grass and forb species compared to sowing in autumn.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, paired, controlled study in the USA² found that sowing seeds in spring led to similar emergence of forb seedlings compared to sowing in winter.

Background

Grassland seeds are commonly sown in autumn or spring in restoration projects in temperate regions. Sowing in autumn may result in early germination of plants allowing them to become established before other non-grassland species. However, sowing in autumn may also result in more seeds dying as a result of waterlogging in winter and so, in some cases, spring sowing may produce better results.

A replicated, randomized, paired, controlled study in 1994–1996 in two ex-arable sites in Scotland, UK (1) found that sowing seeds in the spring increased the cover of sown forb and grass species, as well as the number of sown species, and reduced the cover and species richness of non-sown species when compared to sowing in the autumn. More species that were sown were present in areas where seeds were sown in spring than areas where seeds were sown in the autumn, and the same pattern was true for cover of sown forb and grass species (no data reported). Similarly, there were fewer non-sown species in areas where seeds were sown in spring compared to areas where seeds were sown in autumn, and their cover was also lower (no data reported). Before seeding, sites were ploughed and harrowed. In May 1994, seeds of 18 species were sown at a rate of 4 g/m² in four 3 x 9 m plots, and seeds were sown in another four plots in October 1994 at each site. Plant cover and species richness were estimated in June/July 1995 and 1996 using a 1 x 1 m quadrat placed in each plot.

A replicated, paired, controlled study in 1998–1999 in tallgrass prairie in Iowa, USA (2) found that sowing a local seed mix in spring did not change the number of forb seedlings that emerged when compared to sowing in winter. Plots sown in spring contained 18–32 seedlings/m², while those sown in winter contained 14–24 seedlings/m². Six 15 x 20 m plots were sown with 23 native forb species at a rate of 350 seeds/m² in November 1998 (winter seeding), and six plots were sown in April 1999 (spring). All plots were burned before seeding and mowed weekly from May to September. All plants in seven randomly placed 0.25-m² quadrats/plot were identified every month from June to September.

A replicated, randomized, controlled study in 2005–2010 in two grassland sites in Iowa, USA (3) found that sowing seeds in spring increased plant diversity compared to

sowing seeds in autumn. In areas where seeds were sown in spring, plant species diversity was higher than in areas where seeds were sown in autumn (data reported as Simpson's diversity index). In 2005, at each site, fifty-eight 5 x 5 m plots were sown with grass and/or prairie seeds in spring and 58 plots were sown with grass and/or prairie seeds in autumn. In 2006–2010, point intercept sampling was used to estimate species diversity in each plot.

- (1) Lawson, C.S., Ford, M.A. & Mitchley, J. (2004) The influence of seed addition and cutting regime on the success of grassland restoration on former arable land. *Applied Vegetation Science*, 7, 259–266.
- (2) Williams, D.W., Jackson, L.L. & Smith, D.D. (2007) Effects of frequent mowing on survival and persistence of forbs seeded into a species-poor grassland. *Restoration Ecology*, 15, 24–33.
- (3) Martin, L.M. & Wilsey, B.J. (2012) Assembly history alters alpha and beta diversity, exotic–native proportions and functioning of restored prairie plant communities. *Journal of Applied Ecology*, 49, 1436–1445.

2.13. Sow seeds in part of site

- **Three studies** examined the effects of sowing seeds in part of a site on grassland vegetation. Two studies were in the USA^{1,3} and one was in the Czech Republic².

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (1 study):** One replicated study in the USA³ found that sowing seeds in part of a site resulted in an increase in plant species richness over time.
- **Sown/planted species richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the Czech Republic² found that sowing seeds in part of a site did not alter species richness for sown grass and herb species.

VEGETATION ABUNDANCE (2 STUDY)

- **Sown/planted species abundance (2 studies):** One study in the USA¹ found that after sowing seeds in part of a site, new patches of two of three sown plant species were recorded in unsown areas. One replicated, randomized, paired, controlled study in the Czech Republic² found that sowing seeds in part of a site did not alter the cover of sown grass and herb species.

VEGETATION STRUCTURE (0 STUDIES)

Background

In some grassland restoration projects, it may not be economically viable to sow entire sites with the seeds of grassland species. In these cases, it may make sense to sow part of the site with seeds in the hope that sown species will colonise areas they are not sown.

A study in 1998–2003 at two reclaimed mine sites in southeast Montana, USA (1) found that two of three native plant species sown within plots spread to unsown areas at each site. After 3–4 years, 113–146 narrow-leaved purple cornflower *Echinacea angustifolia* patches and 6–14 white sagebrush *Artemisia ludoviciana* patches were recorded in unsown areas of the sites at average distances of 50–67 m and 32–46 m from the nearest sown plot, respectively. After four years, large Indian breadroot *Pediomelum esculentum* plants were not recorded in unsown areas at either site. In autumn 1998, eighteen 9-m² plots (64 m apart) were tilled at each of the two sites. In February 1999, purple cornflower (2,133 seeds/m²), white sagebrush (161 seeds/m²) or Indian breadroot (161 seeds/m²) was sown in the plots (number of plots for each not reported).

Both sites were strip-mined in 1980–1983 and reseeded with 12 native grass species and five native forbs in 1990–1993. One of the two sites was grazed on rotation during the study. In 2002 and 2003, new patches (individual plants or plant clusters) of each of the three plant species were mapped within unsown areas of each site.

A replicated, randomized, paired, controlled study in 1999–2009 in semi-dry grassland in the Czech Republic (2) found that sowing seeds in strips within each site did not alter sown grass and herb cover or species richness compared to unsown areas. Cover of sown grass species (0–20% vs 0–9%) and herb species (0–8% vs 0–6%) did not differ significantly between areas where seeds were sown in strips and areas where no seeds were sown. The same pattern was true for grass species richness (seeded strips: 0–2.5 species/plot; unseeded: 0–1.9 species/plot) and herb species richness (seeded strips: 0–3.5 species/plot; unseeded: 0–3.5 species/plot). The plant community composition of areas sown with seeds in strips was also more similar to areas where no seeds were sown than that of ancient meadows. In eight 55 × 20 m plots, a 2.5-m central strip was sown with a seed mixture containing seven grass species and 20 herb species at a rate of 2 g/m², while four plots were not sown with seeds. In June in 2000–2004 and 2009, ten 1.5 × 1.5 m quadrats were placed in each plot and all species present and their cover recorded.

A replicated study in 2006–2011 in nine arable fields in Iowa, USA (3) found that after sowing seeds in strips within the fields, the total number of plant species and the number and cover of native plant species increased with time. From 1–4 years after strips were seeded, there were increases in the total number of plant species (from 38 to 55 species), and the number (from 25 to 39 species) and cover (from 38 to 69%) of native plant species. The cultivated part of fields with planted strips had similar numbers (11–15 species) and cover (5–7%) of weeds to fields without strips (13 species, 4% cover). Twelve arable fields of 0.5–3.2 ha were studied. In nine fields, one to three strips, comprising 10–20% of the field area, were tilled and seeded with 31 species of locally-collected tallgrass prairie seeds in July 2007, and mowed annually. Three fields were left as entire crop fields cultivated with a corn-soybean rotation, and treated with synthetic fertiliser and glyphosate. Plant cover and species were recorded in twelve 0.5-m² quadrats within the planted strips and 12 quadrats within the crop area of each field each summer from 2008–2011.

- (1) Reeve Morghan, K., Sheley, R., Denny, M. & Pokorny, M. (2005) Seed islands may promote establishment and expansion of native species in reclaimed mine sites (Montana). *Ecological Restoration*, 23, 214–215.
- (2) Mitchley, J., Jongepierová, I. & Fajmon, K. (2012) Regional seed mixtures for the re-creation of species-rich meadows in the White Carpathian Mountains: Results of a 10-yr experiment. *Applied Vegetation Science*, 15, 253–263.
- (3) Hirsh, S.M., Mabry, C.M., Schulte, L.A. & Liebman, M. (2013) Diversifying agricultural catchments by incorporating tallgrass prairie buffer strips. *Ecological Restoration*, 31, 201–211.

2.14. Sow seeds in prepared gaps within vegetation

- **One study** examined the effects of sowing seeds in prepared gaps within vegetation on grasslands. The study was in Hungary¹.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (1 STUDY)

- **Sown/planted species abundance (1 study):** One replicated study in Hungary¹ found that sowing seeds in large gaps within vegetation led to a greater cover of sown target plant species than sowing in smaller gaps.

VEGETATION STRUCTURE (0 STUDIES)

Background

Seeds may be sown within gaps (sometimes referred to as ‘establishment gaps’) created within existing grassland. Gaps are prepared prior to sowing by breaking up the grass sward and disturbing the soil, e.g. by digging. The aim is to reduce competition by resident grasses and provide optimal conditions for the establishment of sown target grassland species. This may be particularly beneficial within dense species-poor grasslands.

A replicated study in 2013–2015 in eight species-poor grassland sites in east Hungary (1) found that sowing seeds in large gaps created in grassland led to a greater cover of sown target plant species than sowing in smaller gaps but the cover of weeds was similar. During the first two years after sowing, the average cover of target plant species was higher when seeds were sown in large gaps (4 x 4 m: 52–59%) than in smaller gaps (1 x 1 m: 27–31%; 2 x 2 m: 16–39%). The cover of weed species did not differ significantly between the three gap sizes (1 x 1 m: 19–26%; 2 x 2 m: 16–29%; 4 x 4 m: 19%). In October 2013, gaps of three sizes (1 x 1 m, 2 x 2 m, 4 x 4 m) were created >50 m apart within existing grassland at each of eight sites. All sites were former arable fields sown with a low diversity grass seed mix in October 2005. Gaps were prepared by digging, rotary hoeing and raking the soil. All gaps were sown with a seed mixture of 35 native grassland species at a rate of 10 g/m² and grazed by cattle in April–October each year. Vegetation cover was recorded in each of the 24 gaps in June 2014 and 2015.

(1) Valkó, O., Deák, B., Török, P., Kirner, A., Tischew, S., Kelemen, A., Tóth, K., Miglécz, T., Radócz, S. & Sonkoly, J. (2016) High-diversity sowing in establishment gaps: a promising new tool for enhancing grassland biodiversity. *Tuexenia*, 36, 359–378.

2.15. Drill seed rather than seeding by hand

- **Five studies** examined the effects of drill seeding rather than sowing by hand on grassland vegetation. The studies were in the USA^{1–5}.

VEGETATION COMMUNITY (3 STUDIES)

- **Overall richness/diversity (3 studies):** Two of three replicated, controlled studies (two of which were paired) in the USA^{2,3,4} found that sowing seeds with a seed drill did not alter plant species richness^{2,3}. The other study found mixed effects⁴.

VEGETATION ABUNDANCE (4 STUDIES)

- **Sown/planted species abundance (3 studies):** One of three replicated, controlled studies (two of which were randomized and one paired) in the USA^{1,4,5} found that sowing seeds with a seed drill increased the density of two sown grass species compared to sowing by hand⁵. The two other studies found that in most cases sowing seeds with a seed drill led to no change or a reduction in the abundance of sown plants compared to hydroseeding¹ or sowing by hand⁴.

- **Grass abundance (1 study):** One replicated, paired, controlled study in the USA² found that sowing grassland seeds with a seed drill increased the abundance of warm-season grass species compared to sowing by hand.

VEGETATION STRUCTURE (0 STUDIES)

Background

Seed-drilling is the sowing of seeds in uniform rows to a standard soil depth (Bufton 1978). This is usually done with the aid of specialized machinery called a seed drill. Sowing at depth rather than sowing seeds by hand on the soil's surface may reduce seed removal by seed predators, such as birds. Rolling after sowing with seed drills may allow light to reach seeds to allow germination.

Bufton, L. (1978) *The influence of seed-drill design on the spatial arrangement of seedlings and on seedling emergence*. Symposium on the Timing of Field vegetable Production, 72.

A replicated, randomized, paired, controlled study in 1998–1999 in a former arable field in California, USA (1) found that drill seeding did not increase the abundance of four of six plant species compared to applying a slurry of mulch and seeds ('hydroseeding'). The abundance of three of six plant species was lower in areas where drill seeding was used (0.2–10.0 plants/m²) than in areas where seeds were applied in a slurry (0.3–17.0 plants/m²). However, the abundance of two species was higher where drill seeding was used (drill: 2–18 plants/m²; seeds in slurry: 1–12 plants/m²), and in one case there was no significant difference (drill: 313–370 plants/m²; seeds in slurry: 228–368 plants/m²). In February 1998, eight blocks each with three 27 × 4.5 m plots were established. In each block, one plot was seeded with a seed drill to a depth of 6–12 mm, and one plot had a slurry of seed, water, and wood fibre applied at a rate of 560 kg/ha. A straw mulch was applied to all plots at a rate of 1,680 kg/ha and a hydromulch slurry of water, wood fibre, and soil stabilizer was sprayed over the straw. In July 1998 and January and May 1999, plant abundance in each plot was estimated using six 4 × 4 m quadrats.

A replicated, paired, controlled study in 2005–2007 in three former arable fields in Iowa, USA (2) found that sowing grassland seed by drill seeding resulted in similar plant species richness to sowing by hand, but higher abundance of warm-season grasses. Two years after seeding, there was no significant difference in species richness between drill-seeded plots (6.3–7.2) and plots seeded by hand (5.1–6.3). However, native warm-season grasses were more abundant in drilled plots (relative abundance: 0.23–0.34) than plots seeded by hand (relative abundance: 0.11–0.28). In spring 2005, seventy-two 12 × 12 m plots across three sites were seeded with a commercial mix of 13 forb and seven grass species at a rate of 430 seeds/m². Half of the paired plots were drill seeded, and half were seeded by hand. Abundance of plant species was recorded in July 2007 in a randomly placed 1-m² quadrat in each plot.

A replicated, paired, controlled study in 1999–2007 in two former arable fields in Iowa, USA (3) found that drill seeding resulted in similar plant species richness to broadcast seeding at both sites, but drill seeded areas had fewer native warm-season grass and more non-native species at one of two sites. At both sites, species richness was similar in drilled (10–15 species) and broadcast-seeded areas (10–18 species). However, at one site, drill-seeded areas had a lower relative cover of native warm-season grasses (16%) and a higher relative cover of non-native species (72%) than broadcast-seeded

areas (native warm-season grasses: 54%, non-native species: 35%), while at the other site there was no significant difference (native warm-season grasses: 87% vs 89%; non-native species: 9% vs 7%). At two sites, one area was drill-seeded and another was broadcast-seeded, using the same seed mix in both areas. At Site 1, one 1.9-ha area was drilled, and one 3.5-ha area was broadcast, with a local seed mix of 20 native species (sown at 16–17 kg/ha) in autumn 1999. The site was burned in spring 2004–2006. At Site 2, one 1-ha area was drilled, and one 1-ha area was broadcast, with a seed mix containing 37 forbs and nine grasses (sown at 12 kg/ha) in spring 2003. The site was mowed twice yearly. Plant species and cover were recorded in 10 random 1-m² quadrats in each area in June 2007.

A replicated, controlled study in 2005–2010 in nine ex-arable fields in Minnesota and Iowa, USA (4) found that using drill seeding had mixed effects on the cover of sown and non-native plants and plant species richness compared with sowing by hand. In four of 10 comparisons, cover of sown plant species was higher where a seed drill was used (35–71%) than where seeds were sown by hand (33–50%). In six of 10 comparisons, cover was lower or not significantly different where a seed drill was used (drill: 0–36%; hand: 0–52%). In three of 10 comparisons, the cover of non-native plant species was lower in areas where a seed drill was used (11–34%) than where seeds were sown by hand (25–41%), but in seven of ten comparisons it was not significantly different (drill: 11–34%, hand: 11–45%). In two of four comparisons, species richness was lower where a seed drill was used (10 species) than where seeds were sown by hand (12–14 species), and in two comparisons, there was no significant difference (both 10 species). In each of nine fields, thirty-six 6 x 2 m plots were established. Seeds were sown in 12 plots using a seed drill, while in 24 plots seeds were sown by hand. Seed mixes contained 10–36 species representing a mixture of grasses, legumes and non-legume forbs. In mid-June to August 2005–2007 and 2010, vegetation cover in each plot was estimated using a 4 x 0.25 m quadrat, while species richness was estimated using 6 x 2 m plots.

A replicated, randomized, controlled study in 2006–2011 in arid rangelands in Arizona, USA (5) found that drill seeding increased the density of the sown grasses Indian ricegrass *Achnatherum hymenoides* and needle-and-thread grass *Hesperostipa comata* compared to broadcast seeding by hand. After five years, the average density of Indian ricegrass and needle-and-thread grass was higher in drill-seeded plots (0.09–0.11 plants/m²) than in plots where broadcast seeding was done by hand (0.01–0.02 plants/m²). In November 2006, twenty 3 x 3 m plots were sown with native C3 grass seeds. Drill seeding was simulated by using a hoe to create furrows (40 cm apart, 0.6–10.1 cm deep) in half of the plots, and broadcast seeding was done by hand in the other half. Counts of grass species were made in all plots in May 2007, 2010 and 2011.

- (1) Montalvo, A.M., McMillan, P.A. & Allen, E.B. (2002) The relative importance of seeding method, soil ripping, and soil variables on seeding success. *Restoration Ecology*, 10, 52–67.
- (2) Yurkonis, K.A., Wilsey, B.J., Moloney, K.A., Drobney, P. & Larson, D.L. (2010) Seeding method influences warm-season grass abundance and distribution but not local diversity in grassland restoration. *Restoration Ecology*, 18, 344–353.
- (3) Yurkonis, K.A., Wilsey, B.J., Moloney, K.A. & van der Valk, A.G. (2010) The impact of seeding method on diversity and plant distribution in two restored grasslands. *Restoration Ecology*, 18, 311–321.
- (4) Larson, D.L., Bright, J.B., Drobney, P., Larson, J.L., Palaia, N., Rabie, P.A., Vacek, S. & Wells, D. (2011) Effects of planting method and seed mix richness on the early stages of tallgrass prairie restoration. *Biological Conservation*, 144, 3127–3139.
- (5) Bernstein, E.J., Albano, C.M., Sisk, T.D., Crews, T.E. & Rosenstock, S. (2014) Establishing cool-season grasses on a degraded arid rangeland of the Colorado Plateau. *Restoration Ecology*, 22, 57–64.

2.16. Use slot/strip seeding

- **Two studies** examined the effects of using slot/strip seeding on grassland vegetation. Both studies were in the UK^{1,2}.

VEGETATION COMMUNITY (1 STUDY)

- **Grass richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that strip seeding increased grass species richness.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that strip seeding increased forb species richness.

VEGETATION ABUNDANCE (1 STUDY)

- **Sown/planted species abundance (1 study):** One review in the UK² found that in the majority of cases strip seeding resulted in failed introductions of sown species.

VEGETATION STRUCTURE (0 STUDIES)

Background

Slot or strip seeding involves drilling of seeds into shallow slots using specialized machinery. This potentially aids germination of grassland seeds by reducing seed removal by seed predators as well as allowing for lower application rates of seeds.

A replicated, randomized, paired, controlled study in 1994–1996 at six improved grassland sites in the UK (1) found that strip seeding increased grass and forb species richness in most cases compared to not sowing seeds. No statistical analyses were carried out in this study. In 11 of 12 comparisons, strip seeded plots had more grass species (5–14 species/plot) than unsown plots (4–13 species/plot), while in one comparison, there were fewer grass species in strip seeded plots (7 species/plot) than unsown plots (8 species/plot). In eight of 12 comparisons, forb species richness was higher in strip seeded plots (7–25 species/plot) than unsown plots (4–15 species/plot), while in four comparisons, forb species richness in strip seeded plots was equal to or lower than that in unsown plots (both 5–8 species/plot). In August 1994, at each site, strip seeding (at a spacing of 23 cm and depth of <2 cm) was carried out in four 6 x 4 m plots, while four plots were left unseeded. Seed mixes contained seeds of five grass species and 18 forb species. In May/June of 1995 and 1996, three 40 x 40 cm quadrats were placed in each plot and the frequency of each grass and forb species recorded.

A review in 1996–2009 of three studies of semi-natural grassland restoration in the UK (2) found that strip seeding resulted in failed introductions of grassland species in the majority of cases. Two of three studies of strip seeding to restore semi-natural grasslands reported failed reintroductions, while one study did not report enough information to allow the success of the introduction to be determined. The review used keyword searches to identify studies where semi-natural grassland restoration was carried out. All studies of strip seeding used machinery to drill seeds into the soil.

(1) Hopkins, A., Pywell, R.F., Peel, S., Johnson, R.H. & Bowling, P.J. (1999) Enhancement of botanical diversity of permanent grassland and impact on hay production in Environmentally Sensitive Areas in the UK. *Grass and Forage Science*, 54, 163–173.

(2) Hedberg, P. & Kotowski, W. (2010) New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. *Journal for Nature Conservation*, 18, 304–308.

2.17. Spray slurry of seed, mulch and water ('hydroseeding')

- **Four studies** examined the effects of spraying a slurry of seed, mulch and water ('hydroseeding') on grassland vegetation. Two studies were in Spain^{2,3}, one study was in the USA¹ and one was in Italy⁴.

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (2 studies):** One of two replicated, controlled studies (one of which was randomized and paired) in Spain³ and Italy⁴ found that hydroseeding with non-native seeds did not alter plant diversity in most cases³. The other study⁴ found that hydroseeding increased plant species richness in one of two cases.

VEGETATION ABUNDANCE (3 STUDIES)

- **Overall abundance (2 studies):** One of two controlled studies (one of which was replicated, randomized and paired) in Spain^{2,3} found that hydroseeding with non-native seeds increased overall plant cover in most cases³. The other study² found that hydroseeding did not alter vegetation cover.
- **Sown/planted species abundance (1 study):** One replicated, randomized, paired, controlled study in the USA¹ found that hydroseeding increased the abundance of half of the sown plant species compared to drill seeding.

VEGETATION STRUCTURE (1 STUDY)

- **Height (1 study):** One replicated, controlled study in Italy⁴ found that hydroseeding led to an increase in the height of herb species.

Background

Hydroseeding is a method of sowing seeds that involves spraying a slurry of seed, mulch and water over an area. Often an adhesive is added to fix the mulch and seeds onto slopes to prevent erosion. Fertilizer is also sometimes added to this mix. It is often used following construction of roads or dikes to increase vegetation cover and prevent soil erosion. The method is potentially useful in arid areas as added mulch retains moisture, potentially facilitating germination and colonization by grassland plants.

A replicated, randomized, paired, controlled study in 1998–1999 in a former arable field in California, USA (1) found that hydroseeding increased the abundance of half of plant species compared to drill seeding. The abundance of three of six plant species was higher in areas where hydroseeding was used (0.3–17.0 plants/m²) than where drill seeding was used (0.2–10.0 plants/m²). However, abundance of two species was lower (hydroseeded: 1–12 plants/m²; drill: 2–18 plants/m²), and in one case there was no significant difference (hydroseeded: 228–368 plants/m²; drill: 313–370 plants/m²). In February 1998, eight blocks each with three 27 × 4.5 m plots were established. In each block, one plot was hydroseeded using a slurry of seed, water, and wood fibre at a rate of 560 kg/ha, while in one plot seeds were drilled to a depth of 6–12 mm. A straw mulch was applied to all plots at a rate of 1,680 kg/ha. A hydromulch slurry of water, wood fibre, and soil stabilizer was sprayed over the straw. In July 1998 and January and May 1999, plant abundance in each plot was estimated using six 4 × 4 m quadrats.

A controlled study in 1992–1995 in a disused mine in Salamanca, Spain (2) found that hydroseeding did not alter total vegetation cover. In five of six comparisons, there was no significant difference in vegetation cover between areas where hydroseeding was used (126–161%) and areas where hydroseeding was not used (122–142%), but in one

comparison vegetation cover was higher (hydroseeded: 163%; not hydroseeded: 90%). Vegetation cover in nearby dahesas, the target habitat, was similar to that found in both areas that were hydroseeded and areas that were not (125–140%). In autumn 1992, one spoil dump was hydroseeded with a slurry containing mulch, fertilizer, tackifier, rhizobacteria, and a commercial grass seed mix containing 13 species. Another spoil heap was not hydroseeded and no seeds were sown in this area. In June 1993–1995, cover of all plant species in eight permanent 0.25-m² quadrats located on each spoil heap was estimated.

A replicated, randomized, paired, controlled study in 2006–2008 on five motorway verges in central Spain (3) found that hydroseeding with non-native grass species led to an increase in plant cover but not plant diversity in most cases. No statistical tests were carried out in this study. In seven of ten comparisons, overall plant cover was on average higher in plots where non-native grass species were hydroseeded (49–61%) than in plots not sown with seeds (42–61%), while in three comparisons vegetation cover was lower in hydroseeded plots (40–58% vs 43–60%). In four of ten comparisons, plant diversity was higher in plots where non-native grass species were hydroseeded than in plots where no seeds were sown, while in six comparisons plant diversity was lower (data reported as Shannon diversity index). In December 2006, six random blocks containing two 1 × 1 m plots were established at each of five sites. In each block, one plot was hydroseeded with a mix of non-native seeds (30 g/m²), soil stabilizer (10 g/m²), wood fiber mulch (100 g/m²) and water (3 l/m²), and one plot was not sown with seed. In May 2007 and 2008, the cover of all plants was visually assessed in each plot.

A replicated, controlled study in 2011–2012 in an abandoned quarry site in northern Italy (4) found that hydroseeding had mixed effects on plant species richness but increased the height of herbaceous vegetation. These results are not based on statistical analyses. After one year, areas where hydroseeding was carried out using local seeds had higher average plant species richness (16 species/plot) than areas where no seeds were sown (13 species/plot). However, plant species richness was lower in areas where hydroseeding was carried out using a commercial seed mix (10 species/plot). The average height of herbaceous plants was greater in areas that were hydroseeded with local seeds (100 cm) and commercial seeds (93 cm) than in areas where no seeds were sown (16 cm). In June 2011, the site was remodelled to create three 200-m² terraced areas that were almost flat and topsoil was added. Two areas were hydroseeded at a rate of 36–40 g/m² with either a commercial grassland seed mix or locally-collected hay seeds, while one area was not seeded. Shrubs and trees were planted in all three areas. Plants were watered in 2012 during dry periods. In June 2012, vegetation was surveyed in three 3 × 3 m randomly located plots in each area.

- (1) Montalvo, A.M., McMillan, P.A. & Allen, E.B. (2002) The relative importance of seeding method, soil ripping, and soil variables on seeding success. *Restoration Ecology*, 10, 52–67.
- (2) Martinez-Ruiz, C., Fernandez-Santos, B., Putwain, P.D. & Fernandez-Gomez, M.J. (2007) Natural and man-induced revegetation on mining wastes: Changes in the floristic composition during early succession. *Ecological Engineering*, 30, 286–294.
- (3) Garcia-Palacios, P., Soliveres, S., Maestre, F.T., Escudero, A., Castillo-Monroy, A.P. & Valladares, F. (2010) Dominant plant species modulate responses to hydroseeding, irrigation and fertilization during the restoration of semiarid motorway slopes. *Ecological Engineering*, 36, 1290–1298.
- (4) Gilardelli, F., Sgorbati, S., Citterio, S. & Gentili, R. (2016) Restoring limestone quarries: hayseed, Commercial seed mixture or spontaneous succession? *Land Degradation and Development*, 27, 316–324.

2.18. Disturb soil before seeding/planting

- **Seven studies** examined the effects of disturbing soil before seeding/planting on grassland vegetation. Five studies were in Europe^{1,3,4,6,7} and one study was in each of the USA² and China⁵.

VEGETATION COMMUNITY (3 STUDIES)

- **Overall richness/diversity (2 studies):** One of two replicated, controlled studies in the UK⁴ and Germany⁷ found that disturbing soil before sowing seeds increased plant and seedling species richness⁷. The other study⁴ found no change in plant species richness or diversity.
- **Grass richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that rotovating soil before sowing seeds increased grass species richness in most cases compared to harrowing before sowing.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that rotovating soil before sowing seeds increased forb species richness in most cases compared to harrowing before sowing.

VEGETATION ABUNDANCE (2 STUDIES)

- **Overall abundance (1 study):** One replicated, controlled study in the UK⁴ found that disturbing soil before sowing seeds did not alter total plant biomass.
- **Forb abundance (1 study):** One replicated, randomized, paired, controlled study in the USA² found that disturbing soil before sowing forb seeds increased the cover of forb species.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (5 STUDIES)

- **Germination/Emergence (5 studies):** Four of five replicated, controlled studies (three of which were randomized and paired) in the USA², Germany^{3,7}, China⁵ and Spain⁶ found that disturbing soil before sowing seeds increased plant emergence in most cases compared to sowing alone^{2,3,5,7}. The other study⁶ found no change in seed germination.
- **Survival (2 studies):** One of two replicated, controlled studies (one of which was randomized and paired) in China⁵ and Spain⁶ found that disturbing soil before sowing seeds increased the survival of seedlings⁵. The other study⁶ found that ploughing to disturb soil followed by planting did not alter the survival of planted species.
- **Growth (1 study):** One replicated, randomized, paired, controlled study in the USA² found that disturbing soil before planting forb seedlings had no effect on seedling growth.

Background

Before sowing seeds of grassland plants, soil is regularly disturbed by ploughing or tilling. This disturbance may help plants to become established by reducing competition with other plants, such as grasses. The studies detailed in this intervention are direct tests of the effectiveness of disturbing soil before seeding or planting (e.g. by comparison with an undisturbed but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions '*Sow grass seeds*', '*Sow grassland forb species*' or '*Sow native grass and forbs*'.

A replicated, randomized, paired, controlled study in 1994–1996 in six improved grassland sites in England and Wales, UK (1) found that disturbing soil by rotovating before sowing seeds increased grass and forb species richness in most cases compared to harrowing before sowing. No statistical analyses were carried out in this study. In

seven of 12 comparisons, there were more grass species in plots where soil was rotovated and seeds sown (5–12 species/plot) than in plots where soil was harrowed and seeds sown (4–9 species/plot). In the five other comparisons, the number of grass species did not differ between plots that were rotovated or harrowed before sowing (both 8–13 species/plot). In all of 12 comparisons, there were more forb species in plots where soil was rotovated and seeds sown (7–25 species/plot) than in plots where soil was harrowed and seeds sown (3–21 species/plot). In 1994, at each site, eight 6 x 4 m plots were mown. In four plots/site, the soil was disturbed by rotovating (to give 50% bare ground), while the other four plots were harrowed. Seed mixes (five grass species and 18 forb species) were sown at a rate of 12–14 kg/ha in all plots in early August 1994. In May/June of 1995 and 1996, three 40 x 40 cm quadrats were placed in each plot and the frequency of each grass and forb species recorded.

A replicated, randomized, paired, controlled study in 1993 on road verges in California, USA (2) found that disturbing soil before sowing forb seeds increased the emergence and cover of sown forbs compared to sowing seeds alone, but disturbing the soil before planting forb seedlings had no effect on seedling growth. Plots where soil was disturbed before sowing had greater emergence (11%) and cover (97%) of sown forbs than plots where soil was not disturbed before sowing (7% and 14% respectively). However, the proportion of planted forb seedlings showing aboveground growth did not differ significantly between disturbed and undisturbed plots (data not reported). In January 1993, five blocks, each with three 7.62 x 7.62 m plots, were established on grass verges. In each block, one plot was tilled to a depth of 10–15 cm and sown with seeds of seven native forb species, while one plot was not tilled but was sown with seeds. In January–April 2003, thirty seedlings of each of two forb species, narrow-leaf milkweed *Asclepias fascicularis* and blue-eyed-grass *Sisyrinchium bellum*, were planted 30 cm apart in each plot. In April 1993, cover and emergence of forbs was estimated in two 0.5 x 0.25 m quadrats/plot. Aboveground growth of seedlings was assessed in each plot in March–May 1993.

A replicated, randomized, paired controlled study in 1998–1999 in a species-poor grassland near Göttingen, Germany (3) found that disturbing soil before sowing seeds increased the emergence of seedlings for seven of eight wildflower species compared to sowing alone. After one year, the average percentage of seedlings that emerged for seven of eight wildflower species was higher in plots where soil was disturbed before sowing (19–32%) than in plots where soil was not disturbed before sowing (11–22%). For one wildflower species, seedling emergence did not differ significantly between plots where soil was disturbed (14%) or not disturbed (13%) before sowing. In 1998, blocks were established at the site (replication unclear from study). A rake was used to disturb the soil and vegetation in 0.5 x 0.5 m plots and wildflower seeds were sown, while in other plots seeds were sown but soil and vegetation were not disturbed (number for each not reported). Emergence of seedlings was recorded in each plot in July 1999.

A replicated, controlled study in 1998–2000 at a grassland site in West Yorkshire, UK (4) found that disturbing soil before sowing seeds did not alter plant species richness, diversity, or total biomass compared to sowing alone. After 1–2 years, plots that were disturbed before sowing had on average a similar number of total plant species (12.7–14.4), sown species (2.6–6.2), unsown species (8.2–10.1), plant diversity (data reported as Shannon diversity index) and above-ground biomass (140–450 g/m²) to plots that were not disturbed before sowing (total plant species: 12.3–12.4; sown species: 2.7–5.4; unsown species: 7–9.7; biomass: 210–490 g/m²). In autumn 1998, six plots (each 4 x 4

m) were created on a newly established meadow. Three plots were disturbed using a lawn scarifier to a depth of 2 cm, while three plots were left undisturbed. In April 1999, all six plots were sown with a commercial seed mix of six grass and six forb species at a rate of 30 kg/ha. All plots were cut annually in July. In May 1999 and 2000, vegetation was assessed within three randomly placed 50 x 50 cm quadrats/plot.

A replicated, randomized, paired, controlled study in 2005–2006 in a degraded steppe grassland in Hebei province, northern China (5) found that disturbing soil before sowing seeds increased plant emergence, survival, and seedling density compared to sowing alone. The percentage of seeds from which plants emerged was higher where soil had been disturbed prior to seeding (57%) than in areas where soil had not been disturbed before seeding (40%). After one year, a similar pattern was also seen for seedling survival (disturbed: 5.4%; undisturbed: 0.4%) and seedling density (disturbed: 14 seedlings/m²; undisturbed: 3 seedlings/m²). Before seeding, soil was disturbed in half of all 2 × 2 m plots using a rake and half of the plots remained undisturbed (replication of experiment unclear). Seeds were collected in Saibei administrative region in autumn 2004. In June 2005, seeds were sown in all plots at a density of 400–1,200 seeds/m² and soil compressed using a roller. Plots were fenced to prevent damage from livestock and sprayed with pesticides. Seedling density and survival was monitored between June 2005 and August 2006.

A replicated, controlled study in 2006–2007 in arid steppe grassland in northeastern Spain (6) found that disturbing soil by ploughing and sowing seeds or planting did not alter survival of planted plants or seed germination compared to sowing alone. Survival of Mediterranean saltwort *Salsola vermiculata* and esparto grass *Lygeum spartum* plants did not differ significantly between areas that were ploughed (22–70%) and areas that were not ploughed (0–73%). Seed germination of the two species also did not differ significantly (ploughed and seeded: 0%; seeded, not ploughed: 0–2%). In the planting experiment, in October 2006, the soil was ploughed to a depth of 30–40 cm and twenty-five Mediterranean saltwort and esparto grass seedlings were planted four metres apart, while another twenty-five plants were planted in an area that was not ploughed. Seeding was carried out nearby to the planting experiment. Survival of planted seedlings was recorded in February and September 2007. Seed germination was recorded in March, June and September 2007.

A replicated, controlled study in 2014–2015 in 73 agricultural grasslands in Brandenburg, Thuringia and Baden-Württemberg, Germany (7) found that disturbing soil before sowing seeds led to an increase in plant and seedling species richness and number of seedlings compared to sowing alone. After 7–19 months, plots that were disturbed before seeds were sown had on average a greater species richness of plants (36 species/quadrat) and seedlings (14 species/quadrat) than plots that were not disturbed before seeds were sown (27 plant species/quadrat; 5 seedling species/quadrat). Disturbed and sown plots also had more seedlings/quadrat (average 251 seedlings) than undisturbed and sown plots (average 94 seedlings). Two 7 x 7 m plots were established in each of 73 grasslands. The soil was disturbed in one plot (using rotovation tilling or a rotary harrow in October 2014) before seeds were sown, the other was left undisturbed. A mix of native grass, legume and herb seeds (47–66 region-specific species) combined with sand and crushed soybean was sown in each plot in November 2014 and March 2015. Vegetation was monitored within a 2 x 2 m quadrat in each of the 146 plots on three occasions in May–June 2015.

- (1) Hopkins, A., Pywell, R.F., Peel, S., Johnson, R.H. & Bowling, P.J. (1999) Enhancement of botanical diversity of permanent grassland and impact on hay production in Environmentally Sensitive Areas in the UK. *Grass and Forage Science*, 54, 163–173.
- (2) Brown, C.S. & Bugg, R.L. (2001) Effects of established perennial grasses on introduction of native forbs in California. *Restoration Ecology*, 9, 38–48.
- (3) Hofmann, M. & Isselstein, J. (2004) Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science*, 7, 193–200.
- (4) Westbury, D.B., Davies, A., Woodcock, B.A. & Dunnett, N.P. (2006) Seeds of change: The value of using *Rhinanthus minor* in grassland restoration. *Journal of Vegetation Science*, 17, 435–446.
- (5) Liu, G.X., Mao, P.S., Huang, S.Q., Sun, Y.C. & Han, J.G. (2008) Effects of soil disturbance, seed rate, nitrogen fertilizer and subsequent cutting treatment on establishment of *Bromus inermis* seedlings on degraded steppe grassland in China. *Grass and Forage Science*, 63, 331–338.
- (6) Pueyo, Y., Alados, C.L., Garcia-Avila, B., Kefi, S., Maestro, M. & Rietkerk, M. (2009) Comparing direct abiotic amelioration and facilitation as tools for restoration of semiarid grasslands. *Restoration Ecology*, 17, 908–916.
- (7) Klaus, V.H., Schäfer, D., Kleinebecker, T., Fischer, M., Prati, D. & Hölzel, N. (2016) Enriching plant diversity in grasslands by large-scale experimental sward disturbance and seed addition along gradients of land-use intensity. *Journal of Plant Ecology*, 10, 581–591.

2.19. Remove leaf litter before seeding/planting

- **Three studies** examined the effects of removing leaf litter before seeding/planting on grassland vegetation. One study was in each of Germany¹, Belgium² and Hungary³.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (2 STUDIES)

- **Sown/planted species abundance (1 study):** One replicated, randomized, paired, controlled study in Hungary³ found that removing leaf litter before sowing seeds did not increase the cover of either of two sown grass species.
- **Individual plant species abundance (1 study):** One replicated, controlled study in Germany¹ found that removing leaf litter before planting did not alter the biomass of ragged robin and marsh birdsfoot trefoil transplants in most cases.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (3 STUDIES)

- **Germination/Emergence (3 studies):** Two of three replicated, controlled studies (one of which was also randomized and paired) in Germany¹, Belgium² and Hungary³ found that removing leaf litter, and in one study also removing vegetation², before sowing seeds had mixed effects on the number of seedlings of sown plant species^{1,2}. The other study³ found no change in the number of seedlings of either of two grass species.

Background

Traditional management of grasslands, such as mowing and grazing, can help to prevent a build-up of plant litter. However, many grasslands have been abandoned leading to accumulation of litter which may reduce the growth and germination of some grassland plants. Removing this litter may, consequently, aid growth and germination of grassland plants.

The studies detailed in this intervention are direct tests of the effectiveness of removing leaf litter before seeding or planting (e.g. by comparison with a seeded or planted plot with leaf litter left in place). Studies that represent comparisons of seeding to unseeded

plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

A replicated, controlled study in 2002–2004 in seven grassland sites in northern Germany (1) found that removing leaf litter before sowing and planting had mixed effects on the number of sown ragged robin *Silene flos-cuculi* and marsh birdsfoot trefoil *Lotus pedunculatus* seedlings and the biomass of transplants. During the first year after sowing, the average number of ragged robin seedlings was higher in plots with litter removed (2–3 seedlings/plot) than in plots without litter removed (0 seedlings/plot) at two of seven sites. The same was true for marsh birdsfoot trefoil seedlings at four of seven sites (litter removed: 1–11 seedlings/plot; litter not removed: 0.1–3 seedlings/plot). After one year, the average biomass of ragged robin and marsh birdsfoot trefoil transplants was higher in plots with litter removed than without at one of seven sites and none of the sites, respectively (data not reported). There were no significant differences at any of the other sites. Two plots (1 x 2 m) within each of six blocks were established at each of seven fen-grassland sites. Two hundred ragged robin and marsh birdsfoot trefoil seeds were sown within an area (0.25 x 0.25 m) in each plot in autumn 2002. Leaf litter was removed from one plot/block prior to sowing. Four equal-sized juvenile plants of each species were transplanted to each plot in April 2003. Vegetation was monitored in each plot at the end of summer in 2003 and 2004. Biomass was sampled in August 2004.

A replicated, controlled study in 2006–2007 in five grassland restoration sites in Belgium (2) found that removing leaf litter, along with removing vegetation, before sowing forb seeds increased the number of seedlings for one of three sown species. For one sown species, pasqueflower *Pulsatilla vulgaris*, the average number of seedlings was higher in plots where litter and vegetation were removed before sowing (3 seedlings/plot) than in plots where litter and vegetation were not removed before sowing (0.6 seedlings/plot). The two other sown species, mountain clover *Trifolium montanum* and prostrate speedwell *Veronica prostrata*, did not germinate in sown plots with or without litter and vegetation removal. In May–August 2007, at each of five sites, leaf litter and vegetation were removed in four 1 x 1 m plots after which 25 seeds of *Pulsatilla vulgaris*, *Trifolium montanum* or *Veronica prostrata* were sown. In another four plots, seeds were sown but litter and vegetation were not removed. All sites were former forest stands that were clearcut and restored to grassland 3–14 years before the study. In May 2008, the number of seedlings in each plot was counted.

A replicated, randomized, paired, controlled study in 2008–2014 in a pine plantation burnt by wildfire in Hungary (3) found that removing litter before sowing native grass seeds did not increase the number of seedlings or cover of either of two sown grass species compared to sowing alone. After one year, the average number and cover of *Festuca vaginata* seedlings was lower in sown plots where litter was removed (96 seedlings/m², 8%) than in sown plots where litter was not removed (125 seedlings/m², 19%). For *Stipa borysthénica*, after one year, the average number and cover of seedlings did not differ significantly between sown plots with litter removed (45 seedlings/m², 2%) and litter not removed (38 seedlings/m², 2%). In autumn 2008, two 1 x 1 m plots were established in each of twenty 3 x 3 m blocks. In one plot/block, litter was removed with a rake and seeds of two grass species *Festuca vaginata* and *Stipa borysthénica* were sown, while in the other plot, litter was not removed before seeds were sown. Cover of all plants was estimated in each plot yearly in June between 2008 and 2014.

- (1) Rasran, L., Vogt, K. & Jensen, K. (2007) Effects of litter removal and mowing on germination and establishment of two fen-grassland species along a productivity gradient. *Folia Geobotanica*, 42, 271–288.
- (2) Piqueray, J., Saad, L., Bizoux, J.-P. & Mahy, G. (2013) Why some species cannot colonise restored habitats? The effects of seed and microsite availability. *Journal for Nature Conservation*, 21, 189–197.
- (3) Sztár, K., Ónodi, G., Somay, L., Pándi, I., Kucs, P. & Kröel-Dulay, G. (2016) Contrasting effects of land use legacies on grassland restoration in burnt pine plantations. *Biological Conservation*, 201, 356–362.

2.20. Remove topsoil or turf before seeding/planting

- **Six studies** examined the effects of removing topsoil or turf before seeding/planting on grassland vegetation. Three studies were in the UK^{1,2,5}, two studies were in the USA^{3,4} and one was in France⁶.

VEGETATION COMMUNITY (3 STUDIES)

- **Community composition (1 study):** One replicated, randomized, controlled study in France⁶ found that removing topsoil before sowing seeds increased plant community similarity to that of intact steppe.
- **Overall richness/diversity (1 study):** One replicated, randomized, controlled study in France⁶ found that removing topsoil before sowing seeds increased plant species richness.
- **Sown/planted species richness/diversity (1 study):** One replicated, controlled study in the UK⁵ found that removing topsoil before sowing seeds increased the species richness of sown plants.
- **Grass richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that removing turf before sowing seeds increased grass species richness in most cases compared to disturbing the soil before sowing.
- **Forb richness/diversity (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that removing turf before sowing seeds increased forb species richness in most cases compared to disturbing the soil before sowing.

VEGETATION ABUNDANCE (2 STUDIES)

- **Overall abundance (1 study):** One replicated, randomized, controlled study in France⁶ found that removing topsoil before sowing seeds did not alter overall vegetation cover.
- **Sown/planted species abundance (1 study):** One replicated, randomized, controlled study in the UK² found that removing topsoil before planting seedlings led to higher cover of planted species.
- **Individual species abundance (1 study):** One replicated, randomized, controlled study in the UK² found that removing topsoil before planting seedlings led to lower cover of common knapweed.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (2 STUDIES)

- **Survival (2 studies):** Two replicated, controlled studies (one paired and one randomized) in the USA^{3,4} found that removing topsoil before planting California oatgrass³ or sowing and planting purple needlegrass⁴ increased the survival of seedlings and plants.

Background

Conversion of grasslands to arable agriculture or pasture often leads to soils with high nutrient content. These high nutrient content soils promote the growth of grass species which can outcompete forb species. In addition, these soils may contain seeds of undesirable plants (e.g. crop seeds). Removing topsoil may help to reduce soil nutrient

content as well as eliminating the seeds of undesirable species. Sowing seeds after the removal of topsoil may aid colonisation by grassland plants.

The studies detailed in this intervention are direct tests of the effectiveness of removing topsoil/turf before seeding or planting (e.g. by comparison with a seeded or planted plot with topsoil/turf left in place). Studies that represent comparisons of seeding to unseeded plots can be found in the actions '*Sow grass seeds*', '*Sow grassland forb species*' or '*Sow native grass and forbs*'.

A replicated, randomized, paired, controlled study in 1994–1996 in six improved grassland sites in England and Wales, UK (1) found that removing turf before sowing seeds increased grass and forb species richness in most cases compared to disturbing soil before sowing. No statistical analyses were carried out in this study. In eight of 12 comparisons, there were more grass species in plots where turf was removed and seeds sown (10–15 species/plot) than in plots where soil was disturbed and seeds sown (4–13 species/plot). In the four other comparisons, the number of grass species was similar in plots that had turf removed or soil disturbed before sowing (both 7–12 species/plot). In 10 of 12 comparisons, there were more forb species in plots where turf had been removed and seeds sown (11–28 species/plot) than in plots where soil was disturbed and seeds sown (3–25 species/plot). In the two other comparisons, the number of forb species was similar in plots that had turf removed (10–12 species/plot) or soil disturbed (8–13 species/plot) before sowing. In 1994, at each site, twelve 6 x 4 m plots were mown. In four plots/site, turf was removed to a depth of 3 cm. In eight plots/site, soil was disturbed by harrowing (four plots) or rotovating (four plots). Seed mixes (five grass species and 18 forb species) were sown at a rate of 12–14 kg/ha in all plots in early August 1994. In May/June of 1995 and 1996, three 40 x 40 cm quadrats were placed in each plot and the frequency of each grass and forb species recorded.

A replicated, randomized, controlled study in 1994–1999 in a species-poor wet pasture in the UK (2) found that removing topsoil before planting seedlings led to higher cover of planted species and lower cover of common knapweed *Centaurea nigra* compared to planting without topsoil removal. No statistical analyses were carried out in this study. Cover of planted *Cirsio-Molinietum* species was higher in plots where topsoil was removed before planting (41–68%) than in plots where topsoil was not removed before planting (29–40%). Cover of common knapweed was lower in plots where topsoil was removed before planting (5–12%) than in plots where topsoil was not removed (50–68%). In May 1994, ten 2 x 2 m plots were rotovated. Topsoil was removed to a depth of 15–20 cm in five plots, while topsoil was not removed in the five other plots. In May 1995, all plots were sprayed with glyphosate herbicide and seedlings of 14 species planted. Cover of all species was assessed in each plot every year between 1997 and 1999.

A replicated, randomized, controlled study in 2002–2004 in two coastal prairies in California, USA (3) found that removing topsoil before planting California oatgrass *Danthonia californica* plants increased their survival compared to planting without topsoil removal. After 18 months, survival of California oatgrass plants was higher in areas where topsoil was removed and plants planted (39%) than in areas where topsoil was not removed and plants were planted (12%). In each site, in August 2002 topsoil was removed from twenty-four 1.5 x 1.5 m plots to a depth of 10 cm by tilling, while topsoil was not removed from 24 plots. In January 2003, four California oatgrass plants were

planted in each plot. Survival of plants in each plot was recorded in March, June and December 2003 and June 2004.

A replicated, paired, controlled study in 2002–2004 in a coastal grassland in California, USA (4) found that removing topsoil before sowing and planting purple needlegrass *Nassella pulchra* increased seedling and transplant survival. After 17 weeks, more purple needlegrass seedlings had emerged and survived in plots where topsoil was removed before sowing (41–53%) than in plots where topsoil was left intact before sowing (13–27%). After 1.5 years, the survival of purple needlegrass transplants was higher in plots where topsoil was removed before planting (20–28%) than in plots where topsoil was left intact (5–15%). In August 2002, twenty-four 0.75 x 1.5 m plots were established in exotic grass and forb patches. Topsoil was removed to a depth of 10 cm from 12 of the plots by tilling and scraping, while in 12 plots topsoil was left intact. Half of each plot was sown with 25 locally-collected purple needlegrass seeds in October 2002, and half was planted with four purple needlegrass transplants in January 2003. Seedling number and survival were recorded in each plot until March 2003. Transplant survival was recorded until June 2004.

A replicated, controlled study in 2001–2007 in a former landfill site in Somerset, UK (5) found that removing topsoil before sowing with a commercial seed mix increased the number of seeded species compared to seeding alone. The number of seeded plant species was higher in areas where topsoil was removed and seeds sown (6.4–10.2 species/plot) than in areas where topsoil was not removed but seeds were sown (1.7–5.2 species/plot). In 1998, the landfill site was decommissioned and covered with clay to a depth of 1 m to which topsoil and compost were added to a depth of 15–20 cm. In June 2001, topsoil was removed from six 10 x 10 m plots to a depth of 15 cm, while soil was not removed from six other plots. In June 2001, a commercial seed mix of 22 species was sown at a rate of 1.8 g/m² in all plots. The presence of all plant species was recorded annually in June 2003–2007 using two randomly located 1-m² quadrats in each plot.

A replicated, randomized, controlled study in 2009–2012 in a former orchard in the south of France (6) found that removing topsoil before sowing seeds increased plant species richness and the similarity of the plant community to that of intact steppe, but did not alter vegetation cover. After three years, average plant species richness was higher in plots where topsoil was removed before seeds were sown (23 species/quadrat) than in plots where topsoil was not removed before seeds were sown (10 species/quadrat). Plant community similarity to an intact steppe site was also higher in plots where topsoil was removed before sowing than in plots where topsoil was not removed (data reported as Bray-Curtis similarity). Vegetation cover did not differ significantly between plots where topsoil was removed (55%) or not removed (67%) before sowing. In 2009, all trees were removed from the site and soils were levelled. Topsoil was removed (to a depth of 20 cm) and seeds of three nurse plants were sown in three randomly selected 10 x 10 m plots. No topsoil was removed and seeds were sown in two 30-ha plots. Sheep grazing was reintroduced in 2010. In May 2012, vegetation was recorded in nine 2 x 2 m quadrats in plots where topsoil was removed and seeds sown, and in eighteen 2 x 2 m quadrats in plots where no topsoil was removed and seeds sown and in an adjacent intact steppe site.

- (1) Hopkins, A., Pywell, R.F., Peel, S., Johnson, R.H. & Bowling, P.J. (1999) Enhancement of botanical diversity of permanent grassland and impact on hay production in Environmentally Sensitive Areas in the UK. *Grass and Forage Science*, 54, 163–173.
- (2) Tallowin, J. & Smith, R. (2001) Restoration of a *Cirsio-Molinietum* fen meadow on an agriculturally improved pasture. *Restoration Ecology*, 9, 167–178.

- (3) Buisson, E., Holl, K.D., Anderson, S., Corcket, E., Hayes, G.F., Torre, F., Peeters, A. & Dutoit, T. (2006) Effect of seed source, topsoil removal, and plant neighbor removal on restoring California coastal prairies. *Restoration Ecology*, 14, 569–577.
- (4) Buisson, E., Anderson, S., Holl, K.D., Corcket, E., Hayes, G.F., Peeters, A. & Dutoit, T. (2008) Reintroduction of *Nassella pulchra* to California coastal grasslands: effects of topsoil removal, plant neighbour removal and grazing. *Applied Vegetation Science*, 11, 195–204.
- (5) Carrington, L.P. & Diaz, A. (2011) An investigation into the effect of soil and vegetation on the successful creation of a hay meadow on a clay-capped landfill. *Restoration Ecology*, 19, 93–100.
- (6) Jaunatre, R., Buisson, E. & Dutoit, T. (2014) Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, southeast France). *Applied Vegetation Science*, 17, 236–245.

2.21. Remove vegetation before seeding/planting

- **Two studies** examined the effects of removing vegetation before seeding/planting on grassland vegetation. One study was in each of the UK¹ and Belgium².

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (0 STUDIES)

VEGETATION STRUCTURE (0 STUDIES)

OTHER (2 STUDIES)

- **Germination/Emergence (2 studies):** One of two replicated, controlled studies in the UK¹ and Belgium² found that removing vegetation before sowing seeds increased the germination rate of sown species¹. The other study² found that removing vegetation, along with removing leaf litter, before sowing seeds increased the number of seedlings for one of three species.

Background

Following conversion to arable agriculture or pasture, many grasslands are dominated by a relatively small number of plant species. Removal of this vegetation before sowing seeds of grassland species may aid the germination and colonization of grassland plants.

The studies summarised below removed vegetation using mechanical methods. For evidence relating to the removal of vegetation using herbicide, see ‘*Apply herbicide before seeding/planting*’ or for burning, see ‘*Burn vegetation before seeding/planting*’.

A replicated, randomized, controlled study in 1986 in a former arable field in Sussex, UK (1) found that removing vegetation before sowing seeds increased the germination of four grassland plant species compared to sowing without vegetation removal. The average percentage of seeds that germinated for four grassland plant species was higher in plots where vegetation was removed and seeds sown (7.0–45.5%) than in plots where seeds were sown but vegetation was not removed (0.1–1.8%). In April 1986, all vegetation was removed (cut at ground level) from twenty 7 x 5 m plots, and seeds were sown of either *Achillea millefolium*, *Pimpinella saxifraga*, *Plantago media* or *Scabiosa columbaria* (five plots sown/species). In twenty other plots, seeds were sown but vegetation was not removed. Plots were monitored for seed germination every 1–2 weeks until September 1986.

A replicated, controlled study in 2006–2007 in five grassland restoration sites in Belgium (2) found that removing vegetation, along with removing leaf litter, before sowing forb seeds increased the number of seedlings for one of three sown species

compared to sowing alone. For one sown species, pasqueflower *Pulsatilla vulgaris*, the average number of seedlings was higher in plots where vegetation and litter were removed before sowing (3 seedlings/plot) than in plots where vegetation and litter were not removed before sowing (0.6 seedlings/plot). The two other sown species, mountain clover *Trifolium montanum* and prostrate speedwell *Veronica prostrata*, did not germinate in sown plots with or without vegetation and litter removal. In May–August 2007, at each of five sites, vegetation and litter were removed in four 1 x 1 m plots after which 25 seeds of *Pulsatilla vulgaris*, *Trifolium montanum* or *Veronica prostrata* were sown. In another four plots, seeds were sown but vegetation and litter were not removed. All sites were former forest stands that were clear cut and restored to grassland 3–14 years before the study. In May 2008, the number of seedlings in each plot was counted.

- (1) Hutchings, M.J. & Booth, K.D. (1996) Studies of the feasibility of re-creating chalk grassland vegetation on ex-arable land .2. Germination and early survivorship of seedlings under different management regimes. *Journal of Applied Ecology*, 33, 1182–1190.
- (2) Piqueray, J., Saad, L., Bizoux, J.-P. & Mahy, G. (2013) Why some species cannot colonise restored habitats? The effects of seed and microsite availability. *Journal for Nature Conservation*, 21, 189–197.

2.22. Burn vegetation before seeding/planting

- We found no studies that evaluated the effects of burning vegetation before seeding/planting on grassland vegetation.

'We found no studies' means that we have not yet found any studies that have directly evaluated this intervention during our systematic journal and report searches. Therefore we have no evidence to indicate whether or not the intervention has any desirable or harmful effects.

Background

Burning of vegetation in degraded grasslands can reduce dense swards and the encroachment of woody shrubs or invasive non-native species. When carried out prior to seeding or planting, this may aid the germination and colonisation of grassland plants.

For studies that remove vegetation prior to sowing or planting using mechanical methods, see '*Remove vegetation before seeding/planting*', or for studies that apply herbicide, see '*Apply herbicide before seeding/planting*'.

2.23. Apply herbicide before seeding/planting

- **Four studies** examined the effects of applying herbicide before seeding/planting on grassland vegetation. Two studies were in the USA^{1,4} and one study was in each of Germany² and the UK³.

VEGETATION COMMUNITY (1 STUDY)

- **Sown/planted species richness/diversity (1 study):** One replicated, controlled study in the UK³ found that applying herbicide before sowing seeds increased sown species richness.

VEGETATION ABUNDANCE (2 STUDIES)

- **Sown/planted species abundance (1 study):** One replicated, randomized, controlled study in the USA⁴ found that spraying with herbicide before sowing seeds increased the cover of sown grass species.

- **Forb abundance (1 study):** One replicated, randomized, controlled study in the USA¹ found that spraying with herbicide before sowing grass seeds did not alter or reduced the density of native forb species.
- **Tree/shrub abundance (1 study):** One replicated, randomized, controlled study in the USA¹ found that spraying with herbicide before sowing grass seeds led to an increase in the density of shrubs.
- **Individual plant species abundance (1 study):** One replicated, randomized, controlled study in the USA¹ found that spraying with herbicide before sowing grass seeds did not alter the density of crested wheatgrass.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, randomized, paired, controlled study in Germany² found that spraying with herbicide before sowing seeds increased seedling emergence for five of eight wildflower species.

Background

Many degraded grasslands have few species and are dominated by grasses. In order to restore species-rich grasslands, herbicide may be applied to kill any vegetation that is present. Sowing seeds of grassland species after application of herbicide may help grassland species colonise the site more effectively. However, herbicide residues may also affect the germination and establishment of sown species.

For studies that remove vegetation prior to sowing or planting using mechanical methods, see '*Remove vegetation before seeding/planting*' or for burning, see '*Burn vegetation before seeding/planting*'.

A replicated, randomized, controlled study in 1972–1976 in a sagebrush grassland affected by wildfire in Nevada, USA (1) found that spraying with herbicide followed by sowing grass seeds had mixed effects on native grass, forb and shrub density compared to sowing without spraying. After four years, crested wheatgrass *Agropyron desertorum* density did not differ significantly between plots sprayed (0.35–0.40 plants/m²) and not sprayed (0.35–0.70 plants/m²) with herbicide before sowing. In one of two comparisons, native forb density was lower in sprayed plots (0.48 plants/m²) than unsprayed plots (0.31 plants/m²), and in one comparison there was no significant difference (sprayed: 0.34 plants/m², unsprayed: 0.46 plants/m²). Total shrub density was higher in sprayed plots (112–148 shrubs/1,000m²) than unsprayed plots (74–84 shrubs/1,000m²). In October 1972, eight 12 x 12 m plots were sown with seeds of crested wheatgrass and intermediate wheatgrass *Agropyron intermedium*. Four of the plots were sprayed with herbicide (2,4-D), while the other four plots were left unsprayed. The number of grass, forb and shrub plants was counted in each plot in 1973, 1974 and 1976.

A replicated, randomized, paired, controlled study in 1998–1999 in a species-poor grassland near Göttingen, Germany (2) found that spraying with herbicide followed by sowing seeds increased the emergence of seedlings for five of eight wildflower species compared to sowing alone. After one year, the average percentage of seedlings that emerged for five of eight wildflower species was higher in plots sprayed with herbicide before seeds were sown (22–35%) than in plots not sprayed with herbicide before seeds were sown (11–22%). For the other three species, seedling emergence did not differ

significantly between sprayed (14–20%) and unsprayed plots (13–20%). In 1998, blocks were established at the site (replication unclear from study). The herbicide glufosinate was sprayed on vegetation in 0.5 × 0.5 m plots and six weeks later wildflower seeds were sown, while in other plots seeds were sown but herbicide was not sprayed (number for each not reported). Emergence of seedlings was recorded in each plot in July 1999.

A replicated, controlled study in 2001–2007 in a former landfill site in Somerset, UK (3) found that applying herbicide before sowing seeds increased the number of seeded species compared to seeding alone. After two years, the average number of seeded plant species was higher in areas where herbicide was applied and seeds were sown (7.8 species/plot) than in areas where no herbicide was applied but seeds were sown (5.0 species/plot). The same pattern was true after 3–6 years (with herbicide: 2.4–7.2 species/plot; without herbicide: 1.7–5.1 species/plot), although statistical significance was not reported. In 1998, the landfill site was decommissioned and covered with clay to a depth of 1 m to which topsoil and compost were added to a depth of 15–20 cm. In June 2001, herbicide was applied and dead vegetation raked from six 10 × 10 m plots, while herbicide was not applied to six other plots. In June 2001, a commercial seed mix of 22 species was sown at a rate of 1.8 g/m² in all plots. The presence of all plant species was recorded annually in June 2003–2007 using two randomly located 1-m² quadrats in each plot.

A replicated, randomized, controlled study in 2005–2008 in two former agricultural fields in South Dakota, USA (4) found that spraying with herbicide followed by sowing native grass seeds increased the cover of sown grass species and reduced the cover of unsown grass species compared to plots that were seeded but not sprayed with herbicide. After three years, the average cover of sown grass species was higher in plots where herbicide was sprayed and seeds were sown (57–70%) than in unsprayed plots where seeds were sown (1%). The opposite was true for unsown grass species (sprayed plots: 1–3%; unsprayed plots: 59%). In 2005, four 3 × 10 m areas were established in two fields where soybeans *Glycine max* were previously grown. Herbicide ('Roundup' containing imazapic and glyphosate) was randomly applied to three areas, while in one area herbicide was not applied. Two weeks later, a seed mix containing native grass seeds was sown in all areas. Vegetation cover was measured in eight 1-m² plots in each of the four areas at the end of the growing season in 2006–2008.

- (1) Evans, R.A. & Young, J.A. (1978) Effectiveness of rehabilitation practices following wildfire in a degraded big sagebrush downy brome community. *Journal of Range Management*, 31, 185–188.
- (2) Hofmann, M. & Isselstein, J. (2004) Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science*, 7, 193–200.
- (3) Carrington, L.P. & Diaz, A. (2011) An investigation into the effect of soil and vegetation on the successful creation of a hay meadow on a clay-capped landfill. *Restoration Ecology*, 19, 93–100.
- (4) Bahm, M.A., Barnes, T.G. & Jensen, K.C. (2015) Native grass establishment using Journey® herbicide. *Natural Areas Journal*, 35, 69–73.

2.24. Mow before or after seeding/planting

- **Ten studies** examined the effects of mowing before or after seeding/planting on grassland vegetation. Nine studies were in Europe^{1–7,9,10} and one was in China⁸.

VEGETATION COMMUNITY (5 STUDIES)

- **Community composition (1 study):** One replicated, site comparison study in Hungary⁹ found that annual mowing after sowing seeds increased plant community similarity to that of natural grassland.
- **Overall richness/diversity (1 study):** One replicated, controlled study in the UK¹ found that cutting vegetation yearly after sowing seeds increased plant species richness compared to grazing with livestock.
- **Characteristic plant richness/diversity (1 study):** One replicated, controlled study in Germany¹⁰ found that cutting vegetation three times/year after sowing seeds increased the richness of characteristic grassland species compared to cutting once/year.
- **Sown/planted species richness/diversity (2 studies):** One replicated, randomized, paired, controlled study in the UK⁵ found that mowing after sowing seeds increased the richness of sown species. One replicated study in the UK³ found that cutting sown plots each year and removing cut vegetation increased sown grass and forb species richness compared to cutting and not removing cut vegetation.

VEGETATION ABUNDANCE (4 STUDIES)

- **Sown/planted species abundance (3 studies):** One replicated, randomized, paired, controlled study in the UK⁵ found that mowing after sowing seeds increased the abundance of sown forb species. One replicated, randomized, paired, controlled study in Germany⁶ found that mowing more frequently after sowing seeds increased the abundance of five of seven sown forb species. One replicated study in the UK³ found that cutting sown plots each year and removing cut vegetation reduced the cover of sown grass and forb species compared to cutting and not removing cut vegetation.
- **Individual plant species abundance (1 study):** One replicated, controlled study in Germany⁷ found that mowing after planting increased the biomass of transplanted ragged robin and birdsfoot trefoil plants at 2–3 of seven sites.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (4 STUDIES)

- **Germination/Emergence (3 studies):** One of three replicated, controlled studies (including two randomized and one paired study) in the UK², Germany⁷ and China⁸ found that mowing after sowing seeds increased the germination of four grassland plant species². One study⁷ found that mowing after sowing seeds increased the number of ragged robin and birdsfoot trefoil seedlings at 1–2 of seven sites. One study⁸ found that cutting grass after sowing seeds did not alter the emergence rate or density of seedlings.
- **Survival (2 studies):** One of two replicated, randomized, paired, controlled studies in Germany⁴ and China⁸ found that mowing more frequently after sowing seeds increased seedling survival for seven sown forb species⁴. The other study⁸ found that cutting grass after sowing seeds did not alter seedling survival.

Background

Mowing involves cutting grass to a uniform height with specialized machinery. This may help to maintain or increase grassland diversity as well as reducing the abundance of woody plant species. Combining mowing with sowing of seeds or planting may further help to promote diversity by introducing plant species that were not previously present.

The studies detailed in this intervention are direct tests of the effectiveness of mowing before or after seeding or planting (e.g. by comparison with an unmown but seeded or

planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M. & Steinauer, E.M. (1998) Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science*, 280, 745–747.

A replicated, controlled study in 1986–1992 in a former opencast mine in Northumberland, UK (1) found that cutting vegetation yearly after sowing seeds increased plant species richness compared to grazing with livestock after sowing. After one year, plant species richness did not differ significantly between areas where seeds were sown and then cut yearly (20 species/m²) and area where seeds were sown and grazed by livestock (21 species/m²). However, after two years, species richness was higher in areas that were cut annually, and this remained the case for the following two years (seeded and cut: 22–24 species/m²; seeded and grazed: 20–22 species/m²). In 1986, topsoil that had been removed during mining was replaced and sown with a temporary cover crop. The cover crop was removed by ploughing in autumn 1987 and soil disturbed using a power harrow in April 1988. Two 1,500 m² plots were fenced and cut every year in mid-July, while two plots were grazed by livestock throughout the summer. All plots were grazed in spring. In July 1989–1992, vegetation cover for each species was estimated using fifteen 1-m² quadrats in each plot.

A replicated, randomized, controlled study in 1986 in a former arable field in Sussex, UK (2) found that cutting vegetation before sowing seeds increased the germination of four grassland plant species compared to sowing without cutting. The average percentage of seeds that germinated for four grassland plant species was higher in plots where vegetation was cut and seeds sown (5.8–21.0%) than in plots where seeds were sown but vegetation was not cut (0.1–1.8%). In April 1986, vegetation was cut to a height of 3 cm in twenty 7 x 5 m plots, and seeds were sown of either *Achillea millefolium*, *Pimpinella saxifraga*, *Plantago media* or *Scabiosa columbaria* (five plots sown/species). In twenty other plots, seeds were sown but vegetation was not cut. Plots were monitored for seed germination every 1–2 weeks until September 1986.

A replicated study in 1993–1999 in an ex-arable field near Aberdeen, Scotland, UK (3) found that cutting sown plots each year and removing cut vegetation led to a greater number, but lower cover, of sown grass and forb species compared to cutting and not removing cut vegetation. After six years, sown plots that were cut each year and had cuttings removed had on average more sown species (3.4 species/m²) than sown plots that were cut and had cuttings left in place (2.3 species/m²). However, plots with cuttings removed had lower cover of sown species (63%) than plots with cuttings left in place (76%). In April–May 1993, four 20 x 40 m fenced plots were ploughed and sown with a native seed mix (four grass and 10 forb species sown at a rate of 20 kg/ha). Each year in 1994–1999, all plots were cut in early August. Half of the plots had cuttings removed, while half had cuttings left in place. In 1994–1999, vegetation was monitored annually within 20 x 1 m² quadrats (number of sown species) and 10 x 0.25 m² quadrats (cover of sown species) randomly placed in each plot.

A replicated, randomized, paired, controlled study in 1998–1999 in a species-poor grassland in Göttingen, Germany (4; same study site as 6) found that mowing more frequently after sowing seeds increased seedling survival for seven sown forb species. After 12 months, the average percentage of seedlings that survived for seven sown forb species was higher in plots mown once every 1–3 weeks (55–84%) than in plots mown

once every nine weeks (10–51%). In July 1998, multiple 2 x 6 m plots (number not reported) were sown with seeds of seven local forb species and mown once every one, three or nine weeks for 12 months. Emergence and survival of seedlings was recorded by marking seedlings in 0.5 x 0.5 m subplots within each plot from April 1998 to July 1999.

A replicated, randomized, paired, controlled study in 1994–1996 in two ex-arable sites in Scotland, UK (5) found that mowing after sowing seeds increased the abundance of sown forb species and the number of sown species when compared to areas that were seeded but not mown. The cover of sown forb species was higher in areas that had been sown with seeds and mown (10%) than areas that were sown with seeds but not mown (6%). The number of sown species was also higher in areas that were sown with seeds and mown (no data reported). Before seeding, both sites were ploughed and harrowed. In May 1994, seeds of 18 species were sown at a rate of 4 g/m² in eight 3 x 9 m plots. Half of the area of these plots was mowed once or twice in 1994, while half remained unmown. Plant cover and species richness were estimated in June/July 1995 and 1996 using a 1 x 1 m quadrat placed in each plot.

A replicated, randomized, paired, controlled study in 1998–1999 in a species-poor grassland near Göttingen, Germany (6; same study site as 4) found that mowing more frequently after sowing seeds increased the abundance of five of seven sown forb species. After 12 months, five of seven sown forb species were more abundant in areas that were mown once/week than areas mown once every nine weeks: autumn hawkbit *Leontodon autumnalis* (16–36 vs 4–6 plants/m² respectively), ribwort plantain *Plantago lanceolata* (4–19 vs 1 plant/m²), goatsbeard *Tragopogon pratensis* (8–19 vs 3–7 plants/m²), common bird's-foot trefoil *Lotus corniculatus* (4–9 vs 1–4 plants/m²), red clover *Trifolium pratense* (3–11 vs 0–4 plants/m²). For the two other species, there was no significant difference in the number of plants in areas mown once/week or once every nine weeks: wild carrot *Daucus carota* (5–8 vs 4–8 plants/m²), brown knapweed *Centaurea jacea* (13–30 vs 8–16 plants/m²). In July 1998, eight 2 x 2 m plots were sown with seeds of seven local grassland forb species. In 1998 and 1999, four of the plots were mown once/week, and four plots were mown once every nine weeks. In July 1999, the number of plants of each species was counted in each plot.

A replicated, controlled study in 2002–2004 in seven grassland sites in northern Germany (7) found that mowing after sowing and planting led to an increased number of ragged robin *Silene flos-cuculi* and marsh birdsfoot trefoil *Lotus pedunculatus* seedlings and a greater biomass of transplants at less than half of the sites. After two years, the average number of sown ragged robin seedlings was higher in mown plots (2–5 seedlings/plot) than unmown plots (0–3 seedlings/plot) at two of seven sites. The same was true for marsh birdsfoot trefoil seedlings at one of seven sites (data not reported). After one year, the average biomass of ragged robin and marsh birdsfoot trefoil transplants was higher in mown than unmown plots at three of seven and two of seven sites, respectively (data not reported). There were no significant differences at the other sites. Two plots (1 x 2 m) within each of six blocks were established at each of seven fen-grassland sites. Two hundred ragged robin and marsh birdsfoot trefoil seeds were sown within an area (0.25 x 0.25 m) in each plot in autumn 2002. Four equal-sized juvenile plants of each species were transplanted to each plot in April 2003. One plot/block was mown in June 2003 and 2004, the other was left unmown. Vegetation in each plot was monitored at the end of summer in 2003 and 2004. Biomass was sampled in August 2004.

A replicated, randomized, paired, controlled study in 2005–2006 in a degraded steppe grassland in Hebei province, northern China (8) found that cutting grass after sowing seeds did not alter the emergence rate of plants, their survival, or seedling density compared to sowing and not cutting. The percentage of seeds from which plants emerged did not significantly differ between areas that were cut after seeding (48%) and areas that were not cut after seeding (48%). Similarly, there was no significant difference for seedling survival after one year (cut: 3.0%; uncut: 3.5%) or seedling density after one year (cut: 5.6 seedlings/m²; uncut: 11.0 seedlings/m²). Seeds were collected in Saibei administrative region in autumn 2004. In June 2005, seeds were sown in all plots at a density of 400–1200 seeds/m² and soil compressed using a roller. In half of all plots, vegetation was cut to a height of 5 – 10 cm (replication of experiment unclear). Plots were fenced to prevent damage from livestock and sprayed with pesticides. Seedling density and survival was monitored between June 2005 and August 2006. This study was also part of an experiment testing the effects of fertilizer addition and soil disturbance on seedling performance.

A replicated, site comparison study in 2006–2008 in 10 former arable fields and six natural grasslands in Hungary (9) found that annual mowing after sowing grassland species increased plant community similarity to that of natural grassland. No statistical analyses were carried out in this study. After three years, the plant communities in areas that were sown with seeds and subsequently mowed were more similar to those of natural grasslands than after one year (data presented as graphical analysis). In 2006, ten fields were ploughed. Six fields were sown with seeds of *Festuca rupicola*, *Poa angustifolia*, and *Bromus inermis* and four fields were sown with seeds of *P. angustifolia* and *Festuca pseudovina* at a rate of 25 kg/ha. The fields were mowed in June 2007 and 2008. In each field, and six nearby intact loess and alkali grasslands four 1-m² plots were used to measure cover of plant species in June 2006–2008.

A replicated, controlled study in 2011–2012 in a species-poor hay meadow in Germany (10) found that cutting vegetation three times/year after sowing seeds resulted in more species characteristic of hay meadows than cutting once/year after sowing seeds. After one year, the number of species characteristic of hay meadows was higher in areas where seeds were sown and vegetation was cut three times/year (4.5 species/plot) than in areas where seeds were sown and vegetation was cut once/year (3.2 species/plot). In September 2011, four blocks consisting of twelve 4 × 4 m plots were established. All plots were ploughed and vegetation removed, following which they were sown with the seeds of 18 plant species. In each block, vegetation was cut three times/year in six plots and once/year in six other plots. Vegetation cover in a 0.5 × 0.5 m quadrat in each plot was surveyed in autumn 2012.

- (1) Chapman, R. & Younger, A. (1995) The establishment and maintenance of a species-rich grassland on a reclaimed opencast coal site. *Restoration Ecology*, 3, 39–50.
- (2) Hutchings, M.J. & Booth, K.D. (1996) Studies of the feasibility of re-creating chalk grassland vegetation on ex-arable land .2. Germination and early survivorship of seedlings under different management regimes. *Journal of Applied Ecology*, 33, 1182–1190.
- (3) Warren J., Christal A. & Wilson F. (2002) Effects of sowing and management on vegetation succession during grassland habitat restoration. *Agriculture, Ecosystems & Environment*, 93, 393–402.
- (4) Hofmann, M. & Isselstein, J. (2004) Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science*, 7, 193–200.
- (5) Lawson, C.S., Ford, M.A. & Mitchley, J. (2004) The influence of seed addition and cutting regime on the success of grassland restoration on former arable land. *Applied Vegetation Science*, 7, 259–266.

- (6) Hofmann, M. & Isselstein, J. (2005) Species enrichment in an agriculturally improved grassland and its effects on botanical composition, yield and forage quality. *Grass and Forage Science*, 60, 136–145.
- (7) Rasran, L., Vogt, K. & Jensen, K. (2007) Effects of litter removal and mowing on germination and establishment of two fen-grassland species along a productivity gradient. *Folia Geobotanica*, 42, 271–288.
- (8) Liu, G.X., Mao, P.S., Huang, S.Q., Sun, Y.C. & Han, J.G. (2008) Effects of soil disturbance, seed rate, nitrogen fertilizer and subsequent cutting treatment on establishment of *Bromus inermis* seedlings on degraded steppe grassland in China. *Grass and Forage Science*, 63, 331–338.
- (9) Török, P., Deák, B., Vida, E., Valkó, O., Lengyel, S. & Tóthmérész, B. (2010) Restoring grassland biodiversity: Sowing low-diversity seed mixtures can lead to rapid favourable changes. *Biological Conservation*, 143, 806–812.
- (10) John, H., Dullau, S., Baasch, A. & Tischew, S. (2016) Re-introduction of target species into degraded lowland hay meadows: how to manage the crucial first year? *Ecological Engineering*, 86, 223–230.

2.25. Graze with livestock after seeding/planting

- **Seven studies** examined the effects of grazing with livestock after seeding/planting on grassland vegetation. Five studies were in Europe^{1–3,5,7}, one study was in New Zealand⁴ and one was in the USA⁶.

VEGETATION COMMUNITY (5 STUDIES)

- **Overall richness/diversity (2 studies):** One replicated, randomized, paired, controlled study in Italy⁵ found that grazing with livestock after sowing seeds increased plant species richness compared to sowing without grazing. One replicated, controlled study in the UK² found that grazing with livestock after sowing seeds reduced plant species richness compared to cutting vegetation after sowing.
- **Sown/planted species richness/diversity (2 studies):** One replicated study in the UK³ found that grazing with cattle after sowing seeds increased sown species richness compared to grazing with sheep. One replicated, randomized, controlled study in New Zealand⁴ found that grazing with sheep continuously after sowing seeds did not alter sown species richness compared to grazing on rotation.
- **Native/non-target species richness/diversity (1 study):** One replicated, controlled study in the USA⁶ found that grazing with cattle after sowing seeds increased native plant species richness compared to sowing without grazing.

VEGETATION ABUNDANCE (4 STUDIES)

- **Characteristic plant abundance (1 study):** One replicated, controlled study in Hungary⁷ found that grazing with livestock after sowing seeds did not alter the cover of target plant species compared to sowing without grazing.
- **Sown/planted species abundance (2 studies):** One replicated study in the UK³ found that grazing with cattle after sowing seeds reduced the cover of sown species compared to grazing with sheep. One replicated, randomized, controlled study in New Zealand⁴ found that grazing with sheep continuously after sowing seeds increased the cover of four of eight sown species compared to grazing on rotation.
- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA⁶ found that grazing with cattle after sowing seeds reduced the cover of native plant species compared to sowing without grazing.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Survival (1 study):** One replicated, randomized, controlled study in the UK¹ found that grazing in the winter after sowing seeds resulted in higher survival of cut-leaved cranesbill seedlings.

Background

Grazing with livestock may reduce vegetation height, and disturb soil and vegetation, due to trampling. This may result in small-scale differences in the suitability of grassland habitat to different plant species, and as a result increase species diversity. Sowing seeds may further increase the probability of grassland species establishing successfully.

The studies detailed in this intervention are direct tests of the effectiveness of grazing with livestock after seeding or planting (e.g. by comparison with an ungrazed but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions '*Sow grass seeds*', '*Sow grassland forb species*' or '*Sow native grass and forbs*'.

A replicated, randomized, controlled study in 1987–1988 in a grassland in Oxfordshire, UK (1) found that allowing grazing in the winter after sowing of cut-leaved cranesbill *Geranium dissectum* seeds resulted in higher seedling survival than in areas that were not grazed but where seeds were sown. Fourteen months after sowing, the percentage of surviving seedlings was higher in plots that were grazed in winter and sown with cut-leaved cranesbill seeds (9%) than in plots that were not grazed but where seeds were sown (2%). Four 50 x 50 m paddocks were grazed in winter while four paddocks were not grazed. In June 1987, three 25 x 25 cm quadrats in each paddock were sown with 121 cut-leaved cranesbill seeds. The number of seedlings in the quadrats was counted in October 1987 and March and August 1988.

A replicated, controlled study in 1986–1992 in a former opencast mine in Northumberland, UK (2) found that grazing with livestock after sowing seeds reduced plant species richness compared to cutting vegetation after sowing. After one year, plant species richness did not differ significantly between plots sown with seeds and grazed by livestock in summer (21 species/m²) and plots sown with seeds and cut once/year (20 species/m²). However, after two years, species richness was lower in plots that were grazed by livestock each summer and this remained the case for the following two years (seeded and grazed: 20–21 species/m²; seeded and cut: 22–23 species/m²). In 1986, topsoil that had been removed during mining was spread over the site and sown with a temporary cover crop. The cover crop was removed by ploughing in autumn 1987 and soil was disturbed using a power harrow in April 1988. Two 1,500-m² plots were fenced and grazed by livestock throughout the summer, while two plots were cut every year in mid-July. All plots were grazed in spring. In July 1989–1992, vegetation cover for each species was estimated using fifteen 1-m² quadrats in each plot.

A replicated study in 1993–1999 in an ex-arable field near Aberdeen, Scotland, UK (3) found that grazing with cattle after sowing grass and forb seeds led to a greater number, but lower cover, of sown species compared to grazing with sheep. After six years, plots that were grazed with cattle after seeds were sown had on average more sown species (4.8 species/m²) than plots grazed by sheep after seeds were sown (2.2 species/m²). However, cattle-grazed plots had lower sown species cover (46%) than sheep-grazed plots (91%). In April–May 1993, four 20 x 40 m fenced plots were ploughed and sown with a native seed mix (four grass and 10 forb species sown at a rate of 20 kg/ha). Each year in 1994–1999, two plots were grazed by cattle in May–October, and

two plots were grazed by sheep in April–October. Vegetation was monitored annually within twenty 1-m² quadrats (number of sown species) and ten 0.25-m² quadrats (cover of sown species) randomly placed in each plot in June 1994–1999.

A replicated, randomized, controlled study in 1998–1999 in grazed grasslands in Manawatu, New Zealand (4) found that grazing with sheep continuously after sowing seeds did not alter the species richness of sown plants but increased the cover of four of eight sown plant species compared to grazing on rotation. After 21 months, sown species richness did not differ significantly in plots that were grazed continuously (8.5 species/4 m²) or grazed on rotation (7.5–8.8 species/4 m²). Average cover of four of eight sown plant species was higher in plots that were grazed continuously than in those grazed on rotation: spear thistle *Cirsium vulgare* (continuous: 9%; rotation: 3–4%); ribwort plantain *Plantago lanceolata* (continuous: 7%; rotation: 1–2%); bitter dock *Rumex obtusifolius* (continuous: 7%; rotation: 1–3%); white clover *Trifolium repens* (continuous: 21%; rotation: 14–16%). Cover of perennial ryegrass *Lolium perenne* did not differ significantly between plots that were grazed continuously (74%) or on rotation (81–84%). Three other species (greater bird's foot trefoil *Lotus uliginosus*, Dallis grass *Paspalum dilatatum*, creeping thistle *Cirsium arvense*) had few or no seedlings in any plots. In March 1998, seeds of eight plant species were sown in ten 36 x 24 m plots. Sheep grazed continuously all year round in two plots, while in the other eight plots grazing was rotated at intervals of 12–63 days. In December 1999, plant cover and species richness were estimated in twenty-four 2 x 2 m quadrats placed in each plot.

A replicated, randomized, paired, controlled study in 2001–2010 in a calcareous grassland previously affected by shrubland encroachment in Tuscany, Italy (5) found that grazing with livestock after sowing of locally sourced seeds increased plant species richness compared to sowing without grazing. Nine years after sowing and the start of grazing, plots that were seeded and grazed had on average higher plant species richness (39 species/plot) than plots that were seeded but not grazed (31 species/plot). In 1999, shrubs were removed from the entire site, and in spring 2001, blackthorn *Prunus spinosa* plants were cut. In October 2001, four 5 x 3 m plots were sown with locally collected seeds at a rate of 4 g/m² and subsequently grazed by livestock. Four plots were sown with seeds and fenced to exclude livestock. In June/July 2001–2010, sixteen 2 x 1 m quadrats were placed in each plot and a point quadrat method used to estimate cover of each plant species.

A replicated, controlled study in 2012–2013 in a serpentine grassland in California, USA (6) found that grazing with cattle after sowing grass seeds led to a greater number, but lower cover, of native plant species compared to not grazing after sowing. Plots grazed with cattle after sowing seeds had on average more native plant species (10 species/plot) than plots not grazed after sowing (9 species/plot). However, grazed plots had lower native plant species cover (63%) than ungrazed plots (77%). The cover of non-native invasive species did not differ significantly between grazed (20%) and ungrazed plots (29%). In November 2012, twenty 1 x 1 m irrigated plots were sown with seeds of three native grass species. Half of the plots were grazed by cattle (0.25 cow-calf pairs/ha) from November 2012 to May 2013, while the other half were fenced and not grazed. Vegetation cover was estimated in March and April 2013 using a 0.25 x 0.25 m quadrat placed in each plot.

A replicated, controlled study in 2013–2015 at eight species-poor grassland sites in east Hungary (7) found that grazing with livestock after sowing seeds did not alter the

cover of target plants or weeds compared to not grazing after sowing. During the first two years after sowing, there was no significant difference in the average cover of sown target plant species or weed species between plots that were grazed by cattle (target species: 52–59%; weed species: 23–31%) and plots left ungrazed (target species: 51–66%; weed species: 19%). In October 2013, two 4 x 4 m plots located >50 m apart were established in each of eight sites. All plots were prepared (by digging, rotary hoeing and raking the soil) and sown with a seed mixture of 35 native grassland species at a rate of 10 g/m². One plot/site was grazed by cattle (0.5 livestock units/ha) in April–October each year, the other was fenced and left ungrazed. Vegetation cover was recorded in each of the 16 plots in June 2014 and 2015.

- (1) Silvertown, J., Watt, T.A., Smith, B. & Treweek, J.R. (1992) Complex effects of grazing treatment on an annual in a species-poor grassland community. *Journal of Vegetation Science*, 3, 35–40.
- (2) Chapman, R. & Younger, A. (1995) The establishment and maintenance of a species-rich grassland on a reclaimed opencast coal site. *Restoration Ecology*, 3, 39–50.
- (3) Warren, J., Christal, A. & Wilson, F. (2002) Effects of sowing and management on vegetation succession during grassland habitat restoration. *Agriculture, Ecosystems & Environment*, 93, 393–402.
- (4) Edwards, G.R., Hay, M.J.M. & Brock, J.L. (2005) Seedling recruitment dynamics of forage and weed species under continuous and rotational sheep grazing in a temperate New Zealand pasture. *Grass and Forage Science*, 60, 186–199.
- (5) Maccherini, S. & Santi, E. (2012) Long-term experimental restoration in a calcareous grassland: Identifying the most effective restoration strategies. *Biological Conservation*, 146, 123–135.
- (6) Funk, J.L., Hoffacker, M.K. & Matzek, V. (2015) Summer irrigation, grazing and seed addition differentially influence community composition in an invaded serpentine grassland. *Restoration Ecology*, 23, 122–130.
- (7) Valkó, O., Deák, B., Török, P., Kirmer, A., Tischew, S., Kelemen, A., Tóth, K., Migléc, T., Radócz, S. & Sonkoly, J. (2016) High-diversity sowing in establishment gaps: a promising new tool for enhancing grassland biodiversity. *Tuexenia*, 36, 359–378.

2.26. Add topsoil before seeding/planting

- **One study** examined the effects of adding topsoil before seeding/planting on grassland vegetation. The study was in the USA¹.

VEGETATION COMMUNITY (1 STUDY)

- **Overall richness/diversity (1 study):** One replicated, controlled study in the USA¹ found that adding topsoil before sowing seeds increased plant species richness.

VEGETATION ABUNDANCE (1 STUDY)

- **Sown/planted species abundance (1 study):** One replicated, controlled study in the USA¹ found that adding topsoil before sowing seeds increased the biomass of sown species in most cases.

VEGETATION STRUCTURE (0 STUDIES)

Background

When restoring former grasslands converted to agriculture it is often necessary to introduce seeds of some or all species. One way to do this is through the addition of topsoil from intact grasslands. Translocating topsoil may help to increase the number of seeds in the soil, thereby increasing the probability of grassland plants becoming established. The addition of topsoil may also help to increase soil fertility or reduce the effects of processes that dramatically alter soil structure, such as mining or quarrying.

Sowing seeds and planting may further increase the likelihood of colonisation by grassland plants.

The studies detailed in this intervention are direct tests of the effectiveness of adding topsoil before seeding or planting (e.g. by comparison with a seeded or planted plot without topsoil added). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

Hendry, G.A.F., Thompson, K. & Band, S.R. (1995) Seed survival and persistence on a calcareous land surface after a 32-year burial. *Journal of Vegetation Science*, 6, 153–156.

A replicated, controlled study in 1977–1981 in a formerly mined site in Wyoming, USA (1) found that adding topsoil before seeding increased plant species richness and biomass of seeded species in most cases, and reduced biomass of species that were not sown in most cases. Areas where topsoil was added and seeds sown had higher plant species richness (4.7–5.2 species/plot) than areas where no topsoil was added but seeds were sown (4.0 species/plot). In six of nine comparisons, the biomass of seeded species was higher in areas where topsoil was added and seeds were sown (445–984 kg/ha) than in areas where no topsoil was added but seeds were sown (180–410 kg/ha). In five of nine comparisons, the biomass of non-seeded species was lower in areas where topsoil was added and seeds were sown (189–952 kg/ha) than in areas where topsoil was not added but seeds were sown (448–1,115 kg/ha). In 1977, topsoil was added to a depth of 20–60 cm to fifteen 45.7 x 4.9 m plots, and no topsoil was added to five plots. Seeds of 'Critana' thickspike wheatgrass *Agropyron dasystachyum*, green needlegrass *Stipa viridula*, slender wheatgrass *A. trachycaulum* and 'Rosana' western wheatgrass *A. smithii* were sown at a rate of 15.5 kg/ha in all plots. Biomass was sampled by hand in 4–8 x 0.18-m² quadrats/plot in 1979–1981. Plant species richness was estimated using 50 x 20 cm quadrats (replication unclear).

(1) Pinchak, B.A., Schuman, G.E. & Deput, E.J. (1985) Topsoil and mulch effects on plant-species and community responses of revegetated mined land. *Journal of Range Management*, 38, 262–265.

2.27. Add mulch before or after seeding/planting

- **Six studies** examined the effects of adding mulch before or after seeding/planting on grassland vegetation. Two studies were the USA^{1,3}, two were in Canada^{5,6}, and one study was in each of the UK² and Germany⁴.

VEGETATION COMMUNITY (1 STUDY)

- **Characteristic plant richness/diversity (1 study):** One replicated, controlled study in Germany⁴ found that adding mulch before sowing seeds did not alter the species richness of target plants.

VEGETATION ABUNDANCE (4 STUDIES)

- **Overall abundance (1 study):** One replicated, controlled study in Canada⁶ found that adding mulch before sowing seeds did not increase plant cover.
- **Characteristic plant abundance (1 study):** One replicated, controlled study in Germany⁴ found that adding mulch before sowing seeds increased the cover of target plant species.
- **Sown/planted species abundance (2 studies):** One of two replicated, randomized, controlled studies in the USA¹ and UK² found that adding mulch before sowing seeds did not alter the density

of six sown plant species in most cases¹. The other study² found that adding mulch before planting seedlings reduced the cover of planted species.

- **Individual species abundance (1 study):** One replicated, randomized, controlled study in the UK² found that adding mulch before planting seedlings did not alter the cover of common knapweed.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (2 STUDIES)

- **Germination/Emergence (1 study):** One replicated, controlled study in Canada⁵ found that adding mulch before sowing seeds increased the number of seedlings in most cases.
- **Growth (1 study):** One replicated, randomized, controlled study in the USA³ found that adding mulch after planting native prairie plants did not alter the growth of any of seven plant species.

Background

Mulch is organic material, such as leaves or bark, which is spread over soil with the aim of improving germination and survival of target plants. Mulch may increase soil water availability, thereby aiding plant germination (Donath *et al.* 2007) as well as protecting against erosion. However, thick mulch layers may also prevent the germination of seeds that respond to changes in light and temperature, and survival of seedlings that are shaded by mulch (Facelli & Pickett 1991, Suding & Goldberg 1999).

The studies detailed in this intervention are direct tests of the effectiveness of adding mulch before or after seeding or planting (e.g. by comparison with an unmulched but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

Donath, T.W., Bissels, S., Hölzel, N. & Otte, A. (2007) Large scale application of diaspore transfer with plant material in restoration practice – Impact of seed and microsite limitation. *Biological Conservation*, 138, 224–234.

Facelli, J.M. & Pickett, S.T.A. (1991) Plant litter: Its dynamics and effects on plant community structure. *The Botanical Review*, 57, 1–32.

Suding, K.N.H. & Goldberg, D.E. (2001) Variation in the effects of vegetation and litter on recruitment across productivity gradients. *Journal of Ecology*, 87, 436–449.

A replicated, randomized, controlled study in 1981–1982 in two former pits used for disposal of waste from oil extraction in Texas, USA (1) found that adding mulch after sowing seeds did not alter the density of six sown plant species in most cases compared to sowing without mulch. In 11 of 13 comparisons, after 6–7 months, the density of six plant species did not differ significantly between mulched and seeded areas (0–27 plants/m²) and unmulched and seeded areas (0–18 plants/m²). In two of 13 comparisons, there were more plants in mulched and seeded areas than in unmulched and seeded areas for king ranch bluestem *Bothriochloa ischaemum* (18 vs 2 plants/m² respectively) and kleingrass *Panicum coloratum* (28 vs 1 plants/m²). Before seeding, each pit was covered with soil which was then disturbed using a tractor and fenced to exclude herbivores. At each site, in six 6.1 x 6.1 m plots, the seeds of either king ranch bluestem, Lehmann lovegrass *Eragrostis lehmanniana*, kleingrass, alkali sacaton *Sporobolus airoides*, kochia *Kochia scoparia*, or fourwing saltbush *Atriplex canescens* was sown, and mulch was applied to half of the plots. After 6–7 months, ten 0.5 x 0.5 m quadrats were placed in each plot and the number of seedlings counted.

A replicated, randomized, controlled study in 1994–1999 in a species-poor wet pasture in the UK (2) found that adding mulch before planting seedlings led to lower cover of planted species and similar cover of common knapweed *Centaurea nigra* compared to planting without mulch. No statistical analyses were carried out in this study. Cover of planted *Cirsio-Molinietum* species was lower in plots where mulch was added before planting (22–34%) than in plots where mulch was not added before planting (29–40%). Cover of common knapweed was similar in plots where mulch was added (54–67%) or not added (50–68%) before planting. In May 1994, ten 2 x 2 m plots were rotovated. Cereal straw was added as a mulch to five plots, while five other plots were left unmulched. In May 1995, all plots were sprayed with glyphosate herbicide and seedlings of 14 species were planted. Cover of all species was assessed in each plot every year between 1997 and 1999.

A replicated, randomized, controlled study in 1995–1997 at a former landfill site in Seattle, USA (3) found that adding mulch after planting native prairie plants did not alter the growth of any of seven plant species. During two years after planting, there was no significant difference in the growth of Idaho fescue *Festuca idahoensis*, prairie lupine *Lupinus lepidus*, camas *Camassia quamash*, cinquefoil *Potentilla gracilis*, white-top aster *Aster curtus*, Oregon sunshine *Eriophyllum lanatum*, or long stolonated sedge *Carex inops* between fertilized and unfertilized plots (see original paper for data). In May 1995, twelve circular 4-m² plots at the landfill site were each planted with four individuals of seven native prairie species. Six plots were covered with 10 cm of mulch (a locally produced composted yard waste), and six plots were left untreated. The landfill site had been decommissioned in 1966 and sown with grass in 1971. All surviving plants were measured in June and September 1995, and in July 1996 and 1997. Measurements included height, diameter, area, spread, and/or branch and stem length depending on the plant species.

A replicated, controlled study in 2004–2005 at a former mining site in Saxony-Anhalt, Germany (4) found that adding mulch before sowing seeds increased the cover of target plant species but did not alter target plant species richness compared to sowing without mulch. After one year, plots that were mulched and seeded had on average a greater cover of target plant species (67–70%) than plots that were unmulched and seeded (25–33%). However, the average number of target plant species was similar between mulched (7–23 species) and unmulched plots (6–22 species). In December 2004, three blocks were established on an unvegetated area (240 x 50 m) of boulder clay mixed with sand. In each block, two plots had a layer of mulch added (3–5 cm thick), and two plots were left unmulched. Mulch was obtained from a second cut of species-poor grassland. One of each of the two mulched and unmulched plots/block was sown with a high diversity mix of local seeds, and the other with a low diversity mix of non-local seeds. Vegetation was monitored within a 5-m² quadrat in each plot in 2005.

A replicated, controlled study in 2012 in a former arable field in Alberta, Canada (5; same experimental setup as 6) found that adding mulch before sowing seeds increased the number of seedlings in most cases compared to sowing without mulch. In three of four comparisons, there were more seedlings in plots where mulch was added alongside sowing of seeds (39–54 seedlings/m²) than in plots where seeds were sown but no mulch was added (9 seedlings/m²). However, in one of four comparisons, seedling emergence did not differ significantly in plots that were mulched and seeded (25 seedlings/m²) compared to those that were not mulched and seeded (9 seedlings/m²). In May 2012, the site was sprayed with glyphosate herbicide, tilled to a depth of 10 cm, and fenced to

exclude livestock. Mulch (wheat straw or hay) was added to twenty-four 2 × 2 m plots, following which plots were sown with five grass species at a rate of 250 seeds/plot. In six plots, seeds were sown but no mulch was added. Seedling emergence was recorded in each plot every two weeks in July–September 2012.

A replicated, controlled study in 2012–2014 in a former arable field in Alberta, Canada (6; same experimental setup as 5) found that adding mulch before sowing seeds did not increase vegetation cover compared to sowing seeds but not adding mulch. Vegetation cover did not differ significantly between plots where mulch was added and seeds were sown (23–82%) and plots where seeds were sown but mulch was not added (29–70%). In May 2012, the entire site was sprayed with glyphosate herbicide, tilled to a depth of 10 cm, and fenced to exclude livestock. Mulch (wheat straw or hay) was added to twenty-four 2 × 2 m plots, following which plots were sown with five grass species. In six plots, seeds were sown but no mulch was added. In August 2013 and 2014, vegetation cover was estimated using 1 × 1 m quadrats placed in each plot.

- (1) McFarland, M.L., Ueckert, D.N. & Hartmann, S. (1987) Revegetation of oil-well reserve pits in west Texas. *Journal of Range Management*, 40, 122–127.
- (2) Tallowin, J. & Smith, R. (2001) Restoration of a *Cirsio-Molinietum* fen meadow on an agriculturally improved pasture. *Restoration Ecology*, 9, 167–178.
- (3) Ewing, K. (2002) Mounding as a technique for restoration of prairie on a capped landfill in the Puget Sound lowlands. *Restoration Ecology*, 10, 289–296.
- (4) Kirmer, A., Baasch, A. & Tischew, S. (2012) Sowing of low and high diversity seed mixtures in ecological restoration of surface mined-land. *Applied Vegetation Science*, 15, 198–207.
- (5) Mollard, F.P.O., Naeth, M.A. & Cohen-Fernandez, A. (2014) Impacts of mulch on prairie seedling establishment: Facilitative to inhibitory effects. *Ecological Engineering*, 64, 377–384.
- (6) Mollard, F.P.O., Naeth, M.A. & Cohen-Fernandez, A. (2016) Mulch amendment facilitates early revegetation development on an abandoned field in northern mixed grass prairies of North America. *Ecological Engineering*, 97, 284–291.

2.28. Add woody debris to protect seeds/plants

- **One study** examined the effects of adding woody debris to protect seeds/plants on grassland vegetation. The study was in Kenya¹.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (0 STUDIES)

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Survival (1 study):** One replicated, randomized, controlled study in Kenya¹ found that sowing buffel grass seeds beside woody debris did not affect seedling survival.

Background

In natural grasslands, the survival and growth of some plant species may be positively influenced by another plant species as a result of protection from drought or from grazing (Brooker *et al.* 2008). This observation has led to the use of nurse plants – where the seeds of plants are sown underneath other existing plants in the hope that this will improve their chances of establishment. Adding woody debris for protection is a modification of this idea, where woody debris is added to increase the likelihood that plants sown underneath the debris will survive.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, 96, 18–34.

A replicated, randomized, paired, controlled study in 2002–2003 in degraded rangelands in north central Kenya (1) found that sowing buffel grass *Cenchrus ciliaris* beside piles of branches did not affect seedling survival. At the end of the first growing season, there was no significant difference in survival between buffel grass seedlings planted next to piles of thorn branches (9%) and those without protection (6%). Survival in the second and third growing seasons after planting also did not differ significantly between seedlings with (91–98%) and without (95–100%) thorn branches. In April 2002, prior to seasonal rains, nine rows of 14 holes (14 cm diameter and 25 cm deep) were dug in bare ground. Seven holes in each row were piled with thorny *Acacia* branches, and seven holes were refilled as untreated controls. Buffel grass seeds (0.2 g) were sown in furrows 5 cm deep, 20 cm long and 10 cm away on either side of the rows of holes, and covered with soil. The site was grazed at a density of 0.8 sheep and goats/ha. Seedling survival was monitored from April 2002–November 2003, which included three complete growing seasons.

(1) King, E.G. & Stanton, M.L. (2008) Facilitative effects of *Aloe* shrubs on grass establishment, growth, and reproduction in degraded Kenyan rangelands: Implications for restoration. *Restoration Ecology*, 16, 464–474.

2.29. Transfer plant material from intact grassland alongside seeding/planting

- **Four studies** examined the effects of transferring plant material from intact grassland alongside seeding/planting on grassland vegetation. Three studies were in Germany^{2–4} and one was in Hungary¹.

VEGETATION COMMUNITY (3 STUDIES)

- **Characteristic plant richness/diversity (3 studies):** Two of three replicated, controlled studies (including two randomized studies, one of which was paired) in Germany^{3,4} and Hungary¹ found that transferring hay alongside sowing seeds did not alter target grass and forb species richness^{3,4}. The other study¹ found that transferring hay alongside sowing seeds increased the species richness of target plants.

VEGETATION ABUNDANCE (3 STUDIES)

- **Characteristic plant abundance (3 studies):** Two of three replicated, controlled studies (including two randomized studies, one of which was paired) in Germany^{3,4} and Hungary¹ found that transferring hay alongside sowing seeds did not alter the cover of target grass and forb species^{3,4}. The other study¹ found that transferring hay alongside sowing seeds increased the cover of target plant species.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, randomized, paired, controlled study in Germany² found that transferring plant material alongside sowing seeds did not alter seedling

emergence when small amounts of plant material were added, but seedling emergence was reduced when large amounts of plant material were added.

Background

One method of introducing grassland seeds is by spreading plant material from intact grasslands. Combining this with sowing may further increase the chances of colonisation by grassland plants.

A replicated, randomized, controlled study in 2008–2011 in three former arable fields in east Hungary (1) found that transferring hay alongside sowing seeds led to an increase in the number and cover of target plant species and a decrease in weed species compared to sowing seeds without hay. During three years after sowing seeds, plots with hay added had a greater number and cover of target plant species than plots with no hay added (data reported as statistical model results). The opposite was true for weed species. In October 2008, three fields were prepared (by disking and smoothing) and sown with seeds of *Festuca pseudovina* at a rate of 20 kg/ha. Following sowing, two 15 x 15 m plots were randomly selected within each field. Hay (from a species-poor native grassland) was spread over one plot/field to a thickness of 5 cm, while the other plot had no hay added. All plots were mown annually. Vegetation was monitored once/year in eight 1-m² quadrats randomly placed within each plot in 2009–2011.

A replicated, randomized, paired, controlled study in 2006–2008 in a mesic grassland in Germany (2) found that transferring plant material alongside sowing seeds had mixed effects on seedling emergence compared to sowing seeds without plant material. When low amounts of plant material were added (400–800 g/m²), seedling emergence did not differ significantly between plots where plant material was added and seeds were sown (5–8%) and areas where no plant material was added but seeds were sown (7%). However, at high rates of plant material addition (1,600–3,200 g/m²), emergence was lower in plots where plant material was added and seeds were sown (0–1%) than in areas where no plant material was added but seeds were sown (7%). In February 2007, five blocks each containing five 3 × 3 m plots were ploughed and levelled with a harrow. Plant material from an intact grassland was added at a rate of 400, 800, 1,600, or 3,200 g/m² to one plot in each block, while one plot received no plant material. Seeds of eight species were sown at a rate of 1,600 seeds/plot. Seedling emergence was assessed in each plot in July and October 2007 and April, July and October 2008.

A replicated, paired, controlled study in 2009–2015 in a species-poor grassland near Wittenberg, Germany (3) found that transferring hay alongside sowing seeds did not alter the species richness or cover of target grasses and forbs compared to sowing seeds without hay. During six years after sowing seeds, plots with hay added had on average a similar number and cover of target grass and forb species (13–19 species, 12–26%) to plots that had no hay added (14–19 species, 12–20%). In 2009, two 30 x 6 m plots in each of six blocks were rotovated (10 cm depth) and rolled. In each block, green hay and a regional seed mixture were added to one plot. Seeds (obtained from threshing and a regional seed mixture) were sown in the other plot but no hay was added. Hay was obtained from a meadow 3 km away. All plots were mulched twice and mown once in 2009, and mown twice/year in 2010–2015. Vegetation was recorded annually within a 4 x 4 m quadrat in each of the 12 plots in 2010–2015.

A replicated, randomized, paired, controlled study in 2009–2014 in a species-poor grassland near Wittenberg, Germany (4) found that transferring hay alongside sowing seeds did not alter the richness or cover of target grass and forb species compared to sowing seeds without hay. After five years, there was no significant difference in the average number and cover of target forb and grass species between plots with hay added and seeds sown (forbs: 8 species, 6.7%; grasses: 1.3 species, 2.4%) and plots without hay added and seeds sown (forbs: 6.5 species, 3.2%; grasses: 1.2 species, 2.7%). In 2009, six blocks, each with two plots measuring 30 × 6 m, were established. In each block, hay and a regional seed mixture was added to one tilled and rolled plot. In the other plot, seeds (obtained from threshing and a regional seed mixture) were sown but no hay was added. In 2010–2014, the study site was repeatedly flooded and mown twice a year. Hay was obtained from two nearby sites, which were also regularly flooded and mown. Vegetation in each plot was recorded annually from 2010 to 2014 using 4 x 4 m quadrats.

- (1) Török, P., Migléc, T., Valkó, O., Kelemen, A., Tóth, K., Lengyel, S. & Tóthmérész, B. (2012) Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer. *Ecological Engineering*, 44, 133–138.
- (2) Schmiede, R., Ruprecht, E., Eckstein, R.L., Otte, A. & Donath, T.W. (2013) Establishment of rare flood meadow species by plant material transfer: Experimental tests of threshold amounts and the effect of sowing position. *Biological Conservation*, 159, 222–229.
- (3) Baasch, A., Engst, K., Schmiede, R., May, K. & Tischew, S. (2016) Enhancing success in grassland restoration by adding regionally propagated target species. *Ecological Engineering*, 94, 583–591.
- (4) Engst, K., Baasch, A., Erfmeier, A., Jandt, U., May, K., Schmiede, R. & Bruehlheide, H. (2016) Functional community ecology meets restoration ecology: Assessing the restoration success of alluvial floodplain meadows with functional traits. *Journal of Applied Ecology*, 53, 751–764.

2.30. Add charcoal to soil before seeding/planting

- **One study** examined the effects of adding charcoal to soil before seeding/planting on grassland vegetation. The study was in the Netherlands¹.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (1 STUDY)

- **Overall abundance (1 study):** One replicated, randomized, paired, controlled study in the Netherlands¹ found that adding charcoal to soil before sowing seeds did not alter overall plant biomass.
- **Grass abundance (1 study):** One replicated, randomized, paired, controlled study in the Netherlands¹ found that adding charcoal to soil before sowing seeds did not alter grass cover.
- **Forb abundance (1 study):** One replicated, randomized, paired, controlled study in the Netherlands¹ found that adding charcoal to soil before sowing seeds increased the cover of legumes but did not alter the cover of other forbs.

VEGETATION STRUCTURE (0 STUDIES)

Background

Charcoal can be added to soil to increase its fertility, carbon storage, and water retention. When charcoal is used for soil amendment it is commonly known as biochar. Charcoal has a long history of use as a fertilizer, and was used by pre-Colombian Amazonians to enhance soil productivity. Adding charcoal to soil can increase crop yield in agricultural

systems (Jeffery *et al.* 2011), and so addition of charcoal to soil alongside seeding in grasslands may help to increase survival and growth of grassland plants.

The studies detailed in this intervention are direct tests of the effectiveness of adding charcoal to the soil before seeding or planting (e.g. by comparison with an untreated but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

Jeffery, S., Verheijen, F.G., van der Velde, M. & Bastos, A.C. (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, ecosystems & environment*, 144, 175–187.

A replicated, randomized, paired, controlled study in 2011 in a restored grassland in the Netherlands (1) found that addition of charcoal to soil before sowing seeds increased the cover of legumes, but did not alter the cover of grasses or other forbs or total plant biomass. Plots where charcoal was added to soil before sowing had higher cover of legumes (39%–41%) than those where no charcoal was added before sowing (14%). However, there was no significant effect of charcoal addition on cover of other forbs (charcoal: 86–90%; untreated: 93%), cover of grasses (charcoal: 6%; untreated: 8%), or total plant biomass (charcoal: 486–495 g/m²; untreated: 465 g/m²). In April 2011, six 4 x 4 m plots were treated with charcoal, produced from grass cuttings heated to either 400°C or 600°C, at a rate of 10 Mg/ha. Plots were paired with untreated plots. All plots were then rotovated and sown with a grassland seed mixture. In August 2011, vegetation cover was assessed in four 1 x 1 m quadrats/plot. In October 2011, vegetation was clipped in two 0.5 x 1 m quadrats/plot to determine plant biomass.

(1) Van de Voorde, T.F.J., Bezemer, T.M., Van Groenigen, J.W., Jeffery, S. & Mommer, L. (2014) Soil biochar amendment in a nature restoration area: effects on plant productivity and community composition. *Ecological Applications*, 24, 1167–1177.

2.31. Add carbon to soil before or after seeding/planting

- **Two studies** examined the effects of adding carbon to soil before or after seeding/planting on grassland vegetation. Both studies were in the USA^{1,2}.

VEGETATION COMMUNITY (0 STUDIES)

VEGETATION ABUNDANCE (2 STUDIES)

- **Sown/planted species abundance (2 studies):** Two replicated, randomized, controlled studies in the USA^{1,2} found that adding carbon to soil after sowing seeds either reduced¹ or did not alter² the density of sown forb species.

VEGETATION STRUCTURE (0 STUDIES)

Background

Carbon amendments, such as sugar and/or sawdust, can be added to soil to reduce the nitrogen available to plants. This may reduce competition from fast-growing non-native and invasive plants, which may increase the survival and growth of native species after seeding or planting.

The studies detailed in this intervention are direct tests of the effectiveness of adding carbon to soil before or after seeding or planting (e.g. by comparison with an untreated but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

A replicated, randomized, controlled study in 2000–2006 in a former arable field in Minnesota, USA (1; same study site as 2) found that adding carbon to soil after sowing seeds led to a decrease in the density of seeded and unseeded forb species compared to sowing without carbon. After 1–5 years, forb density was lower in plots where carbon was added and seeds were sown (seeded forbs: 9–35 plants/m²; unseeded forbs: 13–70 plants/m²) than in plots where no carbon was added and seeds were sown (seeded forbs: 30–128 plants/m²; unseeded forbs: 32–186 plants/m²). In autumn 2000, two 2.8 × 2.8 m plots in each of ten blocks were tilled and seeded with a combination of native grasses and forbs at a rate of 25 kg/ha. In spring 2001, carbon was added (granular sugar at a rate of 0.5 kg/m²) to one plot/block, while no carbon was added to the other plot. Vegetation was sampled in August 2002–2006 using four randomly placed 0.25-m² quadrats/plot.

A replicated, randomized, paired, controlled study in 1998–2010 in a former arable field in Minnesota, USA (2; same study site as 1) found that adding carbon to soil after sowing seeds did not alter the density of sown native forb species. After 10–12 years, the average density of sown forb species did not differ significantly between plots where carbon was applied after seeds were sown (29 plants/m²) and plots where carbon was not applied after seeds were sown (40 plant/m²). In autumn 1998, two 4 x 3 m plots in each of five blocks were tilled and sown with a seed mixture containing four native grasses and 12 native forbs. In spring 1999, one plot/block had carbon applied (granular sugar at a rate of 0.5 kg/m²), while the other plot had no carbon applied. In July–August 2005–2010, the density of sown forb species was estimated in each of the 10 plots.

- (1) Grygiel, C.E., Norland, J.E. & Biondini, M.E. (2012) Can carbon and phosphorous amendments increase native forbs in a restoration process? A case study in the Northern Tall-grass Prairie (U.S.A.). *Restoration Ecology*, 20, 122–130.
- (2) Grygiel, C.E., Norland, J.E. & Biondini, M.E. (2014) Using Precision Prairie Reconstruction to drive the native seeded species colonization process. *Restoration Ecology*, 22, 465–471.

2.32. Add fertilizer to soil before or after seeding/planting

- **Seventeen studies** examined the effects of adding fertilizer to soil before or after seeding/planting on grassland vegetation. Nine studies were in North America^{1,3-8,14,15}, six studies were in Europe^{2,10-13,17}, one study was in China⁹, and one was in Brazil¹⁶.

VEGETATION COMMUNITY (3 STUDIES)

- **Overall richness/diversity (3 studies):** One replicated, randomized, paired, controlled study in Spain¹⁰ found that adding fertilizer alongside sowing of non-native plant seeds increased plant diversity in 40% of cases. Two replicated, controlled studies in Spain¹³ and Italy¹⁷ found that plant species richness¹³ and diversity¹⁷ were not altered by organic matter or fertilizer addition alongside seeding.

VEGETATION ABUNDANCE (13 STUDIES)

- **Overall abundance (8 studies):** Six of nine replicated, controlled studies (five of which were also randomized and paired) in North America^{3–5,7} and Europe^{10–13,17} found that adding fertilizer alongside sowing or planting increased vegetation cover in all^{3,11,12} or some cases^{4,5,10}. Three studies^{7,13,17} found no change in vegetation cover or plant density.
- **Characteristic plant abundance (1 study):** One replicated, randomized, paired, controlled study in the UK¹² found that adding fertilizer and sowing seeds increased the abundance of specialist grassland species.
- **Sown/planted species abundance (3 studies):** Two replicated, randomized, controlled studies in the USA^{14,15} found that adding fertilizer after sowing seeds did not alter the density of sown forbs^{14,15}. One replicated, randomized, paired, controlled study in the USA¹ found that adding fertilizer after sowing seeds increased the cover but not the density of four sown plant species.
- **Grass abundance (1 study):** One replicated, controlled study in the USA⁸ found that adding fertilizer and sowing seeds increased the biomass of three native grass species.

VEGETATION STRUCTURE (0 STUDIES)

OTHER (4 STUDIES)

- **Germination/Emergence (1 study):** One replicated, randomized, paired, controlled study in China⁹ found that adding fertilizer and sowing seeds did not increase seedling emergence or density.
- **Survival (3 studies):** Three replicated, controlled, paired studies (one of which was randomized) in the UK², China⁹ and Brazil¹⁶ found that adding fertilizer alongside sowing seeds did not alter the survival of seedlings.
- **Growth (1 study):** One replicated, randomized, controlled study in the USA⁶ found that adding fertilizer after planting native prairie plants reduced the diameter of prairie lupine plants and did not alter the growth of six other plant species.

Background

The addition of fertilizer includes addition of any substance to the soil that may increase its fertility. This includes addition of chemical fertilizers, as well as compost and other organic matter. Addition of fertilizers to soils can help to increase nutrient concentration. Addition of organic matter can also increase soil microbe abundance in degraded soils (Ros *et al.* 2003). These changes in soil conditions may increase the likelihood of colonisation of a degraded habitat by grassland species, particularly when combined with sowing seeds.

The studies detailed in this intervention are direct tests of the effectiveness of adding fertilizer to soil before or after seeding or planting (e.g. by comparison with an unfertilized but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions ‘*Sow grass seeds*’, ‘*Sow grassland forb species*’ or ‘*Sow native grass and forbs*’.

Ros, M., Hernandez, M.T. & Garcia, C. (2003) Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry*, 35, 463–469.

A replicated, randomized, paired, controlled study in 1975–1980 in a former coal mine in Montana, USA (1) found that adding fertilizer after sowing seeds increased the cover, but not the density, of four sown plant species compared to sowing without fertilizer. After five years, average cover of four sown plant species (thickspike wheatgrass *Elymus lanceolatus*, crested wheatgrass *Agropyron cristatum*, alfalfa

Medicago sativa, and fourwing saltbush *Atriplex canescens*) was higher in plots where fertilizer was added and seeds were sown (10–43%) than in plots where seeds were sown without fertilizer (2–28%). However, the average density of each of the four sown species did not differ significantly between fertilized (12–88 plants/m²) and unfertilized plots (8–82 plants/m²). In 1975, eight 9 x 10 m plots were each sown with thickspike wheatgrass, crested wheatgrass, alfalfa, or fourwing saltbush seeds. Nitrogen and phosphorus fertilizer was applied to 12 of the plots, while 12 plots had no fertilizer applied. In July 1980, plant density and cover were estimated in at least 14–16 randomly placed 20 x 20–50 cm quadrats/plot.

A replicated, paired, controlled study in 1986–1987 in a meadow in Hampshire, UK (2) found that adding compost and sowing seeds of 12 grassland species did not increase survival of seedlings compared to sowing without fertilizer. During the first two growing seasons, the survival of seedlings did not differ between plots where compost was added to the soil alongside sowing of seeds and plots where seeds were sown without compost (no data presented). In March 1986, the meadow was sprayed with herbicide and plots dug to remove all dead vegetation. In three 2.6 m x 1.2 m plots, a 2.5 cm layer of compost was added to the soil surface and seeds of 12 grassland species were sown. In three other plots, no compost was added but seeds were sown. Survival of plants in each plot was recorded during the growing seasons in 1986 and 1987.

A replicated, paired, controlled study in 1988–1991 in a largely unvegetated area on the island of Kaho'olawe, USA (3) found that adding fertilizer and sowing seeds increased vegetation cover compared to sowing without fertilizer. After 25 months, average vegetation cover was higher in plots where fertilizer was added and seeds sown (9–48%) than in plots where seeds were sown without fertilizer (3%). Plots where rates of fertilizer addition were higher also had greater vegetation cover (see original paper for details). In November 1988, the site was ploughed to a depth of 10 cm. In four 60 x 1.8 m plots in each of five blocks, fertilizer was added at three different rates and seeds (a mix of six grasses and one legume) were sown using a seed drill in. Seeds were sown without fertilizer in one plot/block. In January 1991, thirty 0.25-m² quadrats were placed in each plot and vegetation cover estimated by eye.

A replicated, randomized, paired, controlled study in 1992–1994 in a former mine in Minnesota, USA (4) found that adding fertilizer before sowing seeds had a mixed effect on plant cover depending on fertilizer type and time since treatment. After two to three years, plant cover was higher in plots where compost was spread and seeds were sown (40–73%) than in plots where seeds were sown without compost (12–14%). However, after two years, plant cover did not differ significantly between plots where ammonium phosphate fertilizer was added (34–35%) and plots where it was not added (27%). After three years, plant cover was higher in plots where ammonium phosphate fertilizer was added (50–52%) than in plots where fertilizer was not added (43%). In May 1992, four blocks were established. In each block, compost was spread in two plots at a rate of 22.4–44.8 tonnes/ha, ammonium phosphate fertilizer was applied in two plots at a rate of 224–448 kg/ha, and one plot was left unfertilized. All plots were sown with a mixture of native plant species at a rate of 30.4 kg/ha. Plant cover was measured in all plots in August 1993 and 1994 along three 1-m wide transects.

A replicated, randomized, paired, controlled study in 1993–1997 on three road verges in Colorado, USA (5) found that adding fertilizer alongside seeding and planting increased vegetation cover in half of cases compared to seeding and planting alone. In

three of six comparisons, vegetation cover was higher in plots where fertilizer was added, seeds were sown, and plants were planted (11.1–24.4%) than in plots where no fertilizer was added but seeding and planting was undertaken (2.7–8.6%). In two of six comparisons there was no significant difference (fertilized: 3.3–21.1%; unfertilized: 1.5–10.5%), and in one of six comparisons vegetation cover was lower in fertilized plots (fertilized: 0.6%; unfertilized: 8.9%). In October 1993 blocks, each with two plots, were established on three road verges. BioSol® organic fertilizer was added at a rate of 2242 kg/ha to one plot that was then sown with seeds of 13 grass, forb, and shrub species and planted with the same plant species. One plot was seeded and planted but no fertilizer was added. Vegetation cover in each plot was recorded in June 1994–1997.

A replicated, randomized, controlled study in 1995–1997 at a former landfill site in Seattle, USA (6) found that adding fertilizer to soil after planting native prairie plants either reduced or did not alter the growth of seven plant species. After one year, prairie lupine *Lupinus lepidus* plants in fertilized plots had smaller diameters (average 35 cm) than those in unfertilized plots (average 40 cm). After 1–2 years, there was no significant difference in the growth of six other planted prairie species between fertilized and unfertilized plots (see original paper for data). In May 1995, twelve circular 4-m² plots at the landfill site were each planted with four individuals of seven native prairie species. A granular NPK fertilizer was added to six plots, and six plots were left untreated. The landfill site had been decommissioned in 1966 and sown with grass in 1971. All surviving plants were measured in June and September 1995, and in July 1996 and 1997. Measurements included height, diameter, area, spread, and/or branch and stem length depending on the plant species.

A replicated, randomized, paired, controlled study in 2000–2001 in a mining area in the Northwest Territories, Canada (7) found that adding fertilizer and sowing of seeds did not alter vegetation cover compared to seeding alone. Vegetation cover did not differ significantly between areas where fertilizer was added and seeds were sown (2–6%) and areas where no fertilizer was added but seeds were sown (4%). In August 2000, blocks were established containing four 5 × 2 m plots (number of blocks unclear from study) on areas of mining waste. Gypsum (2,173 kg/ha), rock phosphate (2,080 kg/ha) and calcium carbonate (1,667 kg/ha) were each added to one plot in each block, while one plot received no fertilizer. All plots were sown with the seeds of seven native grass species. In August 2000 and 2001, vegetation cover was assessed using three 50 × 20 cm quadrats/plot.

A replicated, controlled study in 2004 at a severely disturbed serpentine site in northern California, USA (8) found that adding fertilizer before sowing seeds increased the biomass of three native grass species compared to sowing seeds without fertilizer. After five months, above-ground biomass of sown perennial grasses Chinook brome *Bromus laevipes* and squirreltail *Elymus elymoides*, and the annual grass small fescue *Vulpia microstachys*, was higher in plots with compost added (Chinook brome: 0.28 kg/m²; squirreltail: 0.20 kg/m²; small fescue: 1.19 kg/m²) compared to plots without compost (Chinook brome: 0.07 kg/m²; squirreltail: 0.03 kg/m²; small fescue: 0.01 kg/m²). In winter 2004, garden waste compost was added to nine 0.7-m² plots. No compost was added to nine other plots. All plots were then tilled to a depth of 30 cm. Six plots were broadcast-seeded with each of Chinook brome (500 seeds/m²), squirreltail (300 seeds/m²) or small fescue (1,500 seeds/m²). All seeds were collected locally. Plots were harvested to calculate biomass 165 days after seeding.

A replicated, randomized, paired, controlled study in 2005–2006 in a degraded steppe grassland in Hebei province, northern China (9) found that adding fertilizer and sowing seeds did not increase seedling emergence, survival or density compared to seeding alone. The percentage of seeds from which plants emerged did not significantly differ between areas where fertilizer was applied alongside seeding (51%) and areas that were seeded but no fertilizer was applied (45%). Similarly, after one year, there was no significant difference in seedling survival (fertilized: 3.1%; unfertilized: 2.6%) or seedling density (fertilized: 7.2 seedlings/m²; unfertilized: 9.5 seedlings/m²). In June 2005, seeds were sown in one hundred and twenty 2 × 2 m plots at a density of 400–1,200 seeds/m² and soil compressed using a roller. Seeds were collected locally in autumn 2004. In 80 plots, fertilizer was applied, and in 40 plots, no fertilizer was applied. Plots were fenced to prevent damage from livestock and were sprayed with pesticides. Seedling density and survival was monitored in one 50 × 50 cm quadrat in each plot between June 2005 and August 2006.

A replicated, randomized, paired, controlled study in 2006–2008 on five motorway verges in central Spain (10) found that adding fertilizer and sowing seeds of non-native plants increased plant cover and plant diversity in four of 10 comparisons compared to sowing alone. No statistical tests were carried out in this study. In four of 10 comparisons, plots where fertilizer was added and seeds were sown had on average greater overall plant cover (65–72%) and plant diversity (data reported as Shannon diversity index) than plots where seeds were sown but no fertilizer was added (plant cover: 61–71%). In the other six comparisons, sown plots with fertiliser added had lower or equal plant cover (49–55%) and diversity compared to sown plots without fertilizer added (plant cover: 49–65%) In December 2006, at each of five sites, two 1 × 1 m plots in each of six random blocks were sown with a commercial non-native seed mixture. A slow-release inorganic fertilizer was added to one plot/block in December 2006 and January 2008, while the other plot was not fertilized. In May 2007 and 2008, the cover of all plants was visually assessed in each plot.

A replicated, controlled study in 2007–2008 in a landfill site and on the site of a former factory in north-west England, UK (11) found that adding compost before sowing seeds increased vegetation cover compared to sowing without compost. No statistical analyses were carried out in this study. Vegetation cover was higher in areas where compost was added and seeds were sown (22–100%) than in areas where seeds were sown but no compost was added (8–92%). In May 2007, three blocks, containing fifteen 5 × 5 m plots, were established at each site. In each block, compost was added to soil in nine plots and no compost was added to soil in six plots. Wildflower seeds were sown in all plots at a rate of 4 g/m². In April, July, August, September and October 2007, vegetation cover was estimated in two 1 × 1 m quadrats/plot.

A replicated, randomized, paired controlled study in 2006–2007 on the site of a former steelworks in Flintshire, UK (12) found that adding fertilizer and sowing seeds increased vegetation cover and cover of specialist grassland species compared to sowing without fertilizer. Vegetation cover was higher in areas where compost was added to the soil alongside sowing of seeds (38–64% cover) than in areas where no compost was added to the soil but seeds were sown (10–22%). Cover of specialist grassland species was higher in areas where compost was added to the soil alongside sowing (6.4% cover) than in areas with seeding but no compost (0.3%). Compost produced from green waste (biosolids) or paper was added to twenty-four 8 × 3 m plots using a muck spreader along with seeds of 24 grassland species. In six plots, no compost was added but seeds were

sown. Wood chip was also added to all compost mixtures prior to the composting process. In July 2007, a 1 x 1 m quadrat was established in each plot and the percentage cover of each plant species was assessed.

A replicated, randomized, controlled study in 2009–2010 in an agricultural field in southern Spain (13) found that adding organic matter and sowing seeds did not alter plant density or species richness compared to sowing seeds without organic matter. In each of four comparisons, plant density and species richness were similar in plots where organic matter was added and seeds were sown (1–13 plants/m²; 0.3–2.1 species/0.25 m²) and in plots where organic matter was not added and seeds were sown (1–11 plants/m²; 0.2–1.8 species/0.25 m²). In November 2009, forty 5 x 5 m plots were sown with locally collected seeds of seven native grass and forb species. Organic matter (a commercial substrate) was randomly added to half of the plots (160 l/plot), while the other half had no organic matter added. Four different bedding materials were also applied to plots prior to seeding (see original paper for details). Plant density and species richness were estimated in July and October 2010 using fifteen randomly placed 0.5 x 0.5 m quadrats/plot.

A replicated, randomized, controlled study in 2000–2006 in a former arable field in Minnesota, USA (14; same study site as 15) found that adding fertilizer after sowing seeds did not alter the density of seeded or unseeded forb species compared to sowing without fertilizer. After 1–5 years, forb density did not differ significantly between plots where fertilizer was added and seeds were sown (seeded forbs: 30–137 plants/m²; unseeded forbs: 33–233 plants/m²) and plots where no fertilizer was added and seeds were sown (seeded forbs: 30–128 plants/m²; unseeded forbs: 32–186 plants/m²). In autumn 2000, two 2.8 x 2.8 m plots in each of ten blocks were tilled and seeded with a combination of native grasses and forbs at a rate of 25 kg/ha. In spring 2001, slow-release phosphorus fertilizer was added to one plot/block at a rate of 22 g/m², while no fertilizer was added to the other plot. Vegetation was sampled in August 2002–2006 using four randomly placed 0.25-m² quadrats/plot.

A replicated, randomized, paired, controlled study in 1998–2010 in a former arable field in Minnesota, USA (15; same study site as 14) found that adding fertilizer to soil after sowing seeds did not alter the density of sown native forb species. After 10–12 years, the average density of sown forb species did not differ significantly between plots where fertilizer was applied after seeds were sown (33 plants/m²) and plots where fertilizer was not applied after seeds were sown (40 plant/m²). In autumn 1998, two 4 x 3 m plots in each of five blocks were tilled and sown with a seed mixture containing four native grasses and 12 native forbs. In spring 1999, one plot/block had slow-release phosphorous fertilizer applied at a rate of 14 g/m², while the other plot had no fertilizer applied. In July–August 2005–2010, the density of sown forb species was estimated in each of the 10 plots.

A replicated, controlled, paired study in 2010–2012 in an arable field in Brazil (16) found that adding fertilizer and sowing tree seeds did not increase seedling survival compared to sowing without fertilizer. After 780 days, survival did not differ significantly for seedlings where fertilizer had been added (13–35%) and seedlings where it had not been added (27–52%). In November 2010, soil was ploughed in eight 1-m wide rows. In four of these rows, 30 seeds of six tree species were sown and 253 g of fertilizer and 84 g of phosphate/m were added. In the four other rows, seeds were sown but no fertilizer was applied. Holes were then refilled with soil and a straw mulch was spread. Seedling

germination and survival was recorded for each plant after 42, 84, 126, 217, 398, and 780 days.

A replicated, controlled study in 2010–2012 in an experimental field in Tuscany, Italy (17) found that adding fertilizer before sowing seeds did not alter plant density or diversity compared to sowing alone. Plant density did not differ significantly between areas where compost was added to the soil and seeds were sown, and areas where seeds were sown but no compost was added (no data reported). The same pattern was seen for plant diversity (data reported as diversity indices). In October 2010, eight 2 × 1 m boxes were filled with soil collected from a nearby floodplain. Compost derived from household waste was added to four boxes at a rate of 2 kg/m², while no compost was added to the other four boxes. In November 2010, seeds of 26 native herb species were sown and the soil was raked. Any weeds that grew were removed. The number of plants in each plot was counted in July and October 2011 and plant diversity was assessed in July and October 2011 and 2012.

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- (2) Fenner, M. & Spellerberg, I. (1988) Plant species enrichment of ecologically impoverished grassland: a small scale trial. *Field Studies*, 7, 153–158.
- (3) Warren, S.D. & Aschmann, S.G. (1993) Revegetation strategies for Kahoolawe Island, Hawaii. *Journal of Range Management*, 46, 462–466.
- (4) Noyd, R.K., Pflieger, F.L. & Norland, M.R. (1996) Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil*, 179, 89–97.
- (5) Paschke, M.W., DeLeo, C. & Redente, E.F. (2000) Revegetation of roadcut slopes in Mesa Verde National Park, USA. *Restoration Ecology*, 8, 276–282.
- (6) Ewing, K. (2002) Mounding as a technique for restoration of prairie on a capped landfill in the Puget Sound lowlands. *Restoration Ecology*, 10, 289–296.
- (7) Reid, N.B. & Naeth, M.A. (2005) Establishment of a vegetation cover on tundra kimberlite mine tailings: 2. A field study. *Restoration Ecology*, 13, 602–608.
- (8) O'Dell, R.E. & Claassen, V.P. (2006) Relative performance of native and exotic grass species in response to amendment of drastically disturbed serpentine substrates. *Journal of Applied Ecology*, 43, 898–908.
- (9) Liu, G.X., Mao, P.S., Huang, S.Q., Sun, Y.C. & Han, J.G. (2008) Effects of soil disturbance, seed rate, nitrogen fertilizer and subsequent cutting treatment on establishment of *Bromus inermis* seedlings on degraded steppe grassland in China. *Grass and Forage Science*, 63, 331–338.
- (10) Garcia-Palacios, P., Soliveres, S., Maestre, F.T., Escudero, A., Castillo-Monroy, A.P. & Valladares, F. (2010) Dominant plant species modulate responses to hydroseeding, irrigation and fertilization during the restoration of semiarid motorway slopes. *Ecological Engineering*, 36, 1290–1298.
- (11) Sparke, S., Putwain, P. & Jones, J. (2011) The development of soil physical properties and vegetation establishment on brownfield sites using manufactured soils. *Ecological Engineering*, 37, 1700–1708.
- (12) Tandy, S., Wallace, H.L., Jones, D.L., Nason, M.A., Williamson, J.C. & Healey, J.R. (2011) Can a mesotrophic grassland community be restored on a post-industrial sandy site with compost made from waste materials? *Biological Conservation*, 144, 500–510.
- (13) Ballesteros, M., Cañadas, E.M., Foronda, A., Fernández-Ondoño, E., Peñas, J. & Lorite, J. (2012) Vegetation recovery of gypsum quarries: Short-term sowing response to different soil treatments. *Applied Vegetation Science*, 15, 187–197.
- (14) Grygiel, C.E., Norland, J.E. & Biondini, M.E. (2012) Can carbon and phosphorous amendments increase native forbs in a restoration process? A case study in the Northern Tall-grass Prairie (U.S.A.). *Restoration Ecology*, 20, 122–130.
- (15) Grygiel, C.E., Norland, J.E. & Biondini, M.E. (2014) Using Precision Prairie Reconstruction to drive the native seeded species colonization process. *Restoration Ecology*, 22, 465–471.
- (16) Silva, R.R.P., Oliveira, D.R., da Rocha, G.P.E. & Vieira, D.L.M. (2015) Direct seeding of Brazilian savanna trees: effects of plant cover and fertilization on seedling establishment and growth. *Restoration Ecology*, 23, 393–401.
- (17) Vannucchi, F., Malorgio, F., Pezzarossa, B., Pini, R. & Bretzel, F. (2015) Effects of compost and mowing on the productivity and density of a purpose-sown mixture of native herbaceous species to revegetate degraded soil in anthropized areas. *Ecological Engineering*, 74, 60–67.

2.33. Add sulphur to soil before seeding/planting

- **Two studies** examined the effects of adding sulphur to soil before seeding/planting on grassland vegetation. One study was in the UK¹ and one was in the USA².

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (1 study):** One of two replicated, controlled studies (one of which was randomized and paired) in the UK¹ and USA² found that adding sulphur to soil before sowing seeds reduced plant species richness¹. The other study² found no change in overall plant species richness.
- **Native/non-target species richness/diversity (1 study):** One replicated, controlled study in the USA² found that adding sulphur to soil before sowing seeds did not alter the number of native plant species.

VEGETATION ABUNDANCE (2 STUDIES)

- **Overall abundance (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that adding sulphur to soil before sowing seeds reduced overall vegetation cover.
- **Sown/planted species abundance (1 study):** One replicated, randomized, paired, controlled study in the UK¹ found that adding low amounts of sulphur to soil before sowing seeds increased the cover of three of six sown species.
- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA² found that adding sulphur to soil before sowing seeds did not alter the cover of native plant species.

VEGETATION STRUCTURE (0 STUDIES)

Background

Adding sulphur to soils can increase their acidity (Nielsen *et al.* 1993). Alongside seeding or planting of grassland species, adding sulphur may increase the establishment and colonisation of plant species that are adapted to acidic conditions.

The studies detailed in this intervention are direct tests of the effectiveness of adding sulphur to soil before seeding or planting (e.g. by comparison with an untreated but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions 'Sow grass seeds', 'Sow grassland forb species' or 'Sow native grass and forbs'.

Nielsen, D., Hogue, E., Hoyt, P. & Drought, B. (1993) Oxidation of elemental sulphur and acidulation of calcareous orchard soils in southern British Columbia. *Canadian Journal of Soil Science*, 73, 103–114.

A replicated, randomized, paired, controlled study in 1993–1996 in a former arable field in Suffolk, UK (1) found that adding sulphur to soil before sowing seeds reduced overall vegetation cover and species richness but low amounts of sulphur increased the cover of three of six sown species. After two years, plots where sulphur was added to the soil before sowing had on average lower overall vegetation cover (0–93%) and fewer plant species (0–15 species/plot) than plots where no sulphur was added before sowing (118%; 19 species/plot). Low rates of sulphur addition (1–4 tonnes/ha) increased the cover of three of six sown species (common bent *Agrostis capillaris*, sheep sorrel *Rumex acetosella*, buck's-horn plantain *Plantago coronopus*), while the three other species (sheep fescue *Festuca ovina*, velvet grass *Holcus lanatus*, sheep's-bit *Jasione montana*) had the highest cover in untreated plots (see original paper for details).. In August 1993, sulphur was added to six 2.5 x 2.5 m plots within each of three blocks (at

rates of 1, 2, 4, 8, 10 and 12 tonnes/ha) and rotovated into the soil to a depth of 5–10 cm. One plot/block had no sulphur added. In October 1994, all plots were sown with a seed mixture of 16 plant species at a rate of 45 kg/ha. In August and September 1995 and 1996, species richness and vegetation cover were estimated using a 1 x 1 m quadrat randomly placed in the centre of each plot.

A replicated, controlled study in 2008–2013 in a former arable field in Massachusetts, USA (2) found that adding sulphur to the soil before sowing native grass and forb seeds did not alter the cover and species richness of native plants or total plant species richness compared to sowing without sulphur. In the first year after sowing, the average cover and richness of native plant species and total plant species richness did not differ significantly between plots with sulphur added (native plants: 23 % cover, 8–10 species/plot; total: 20–21 species/plot) and plots with no sulphur added (native plants: 36% cover, 11 species/plot; total plants: 23 species/plot). The same was true five years after sowing (native plants: 40–64% vs 59% cover, 11 vs 10 species/plot; total plants: 18–21 vs 17 species/plot). In October–November 2008, fifteen 5 x 5 m plots had sulphur (91–273 g/m²) tilled into the soil before native grass and forb seeds of 26 species were sown, while in five other plots seeds were sown but no sulphur was added. All plots were tilled before treatment/seeding to remove non-native plants. Vegetation was surveyed in a 3 x 3 m quadrat placed in the centre of each plot in July and August 2009 and 2013.

(1) Owen, K.M. & Marrs, R.H. (2000) Acidifying arable soils for the restoration of acid grasslands. *Applied Vegetation Science*, 3, 105–116.

(2) Neill, C., Wheeler, M.M., Loucks, E., Weiler, A., Von Holle, B., Pelikan, M. & Chase, T. (2015) Influence of soil properties on coastal sandplain grassland establishment on former agricultural fields. *Restoration Ecology*, 23, 531–538.

2.34. Inoculate soil with mycorrhiza before seeding/planting

- **Five studies** examined the effects of inoculating soil with mycorrhiza before seeding/planting on grassland vegetation. Four studies were in the USA^{1,2,4,5} and one was in Germany³.

VEGETATION COMMUNITY (1 STUDY)

- **Overall richness/diversity (1 study):** One replicated, randomized, controlled study in Germany³ found that inoculating soil with mycorrhizal fungi and sowing seeds of grassland species did not alter plant species richness.

VEGETATION ABUNDANCE (4 STUDIES)

- **Overall abundance (2 studies):** One replicated, randomized, paired, controlled study in the USA¹ found that inoculating soil with mycorrhizal fungi before sowing seeds initially increased vegetation cover, but after three years, vegetation cover did not differ between areas that were and were not inoculated. One controlled study in the USA⁵ found that adding soil microbes and nutrients when planting grass plugs did not change the overall cover of herbaceous species.
- **Characteristic plant abundance (2 studies):** One replicated, randomized, controlled study in Germany³ found that adding mycorrhiza to the soil and sowing seeds of grassland species increased the abundance of target species that were considered a local conservation priority. One controlled study in the USA⁵ found that adding soil microbes and nutrients when planting grass plugs increased the cover of three of 38 native prairie species.

- **Tree/shrub abundance (1 study):** One controlled study in the USA⁵ found that adding soil microbes and nutrients when planting grass plugs did not change the cover of woody species.
- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA⁴ found that adding mycorrhizal fungi to soil before sowing seeds did not alter the biomass of three native grass and forb species.

VEGETATION STRUCTURE (1 STUDY)

- **Height (1 study):** One replicated, controlled study in the USA² found that adding mycorrhizal fungi to soil before sowing seeds increased the height of giant sacaton plants.
- **Individual plant size (1 study):** One replicated, controlled study in the USA² found that adding mycorrhizal fungi to soil before sowing seeds did not increase the biomass of giant sacaton plants.

OTHER (1 STUDY)

- **Germination/Emergence (1 study):** One replicated, controlled study in the USA² found that adding mycorrhizal fungi to soil before sowing seeds did not increase the emergence of giant sacaton plants.

Background

Modern agricultural practices such as ploughing and application of pesticides and fertilizer may reduce the abundance of mycorrhizal fungi (Helgason *et al.* 1998, Johnson 1993). As a result, addition of mycorrhiza to degraded habitats may help natural plant communities to establish.

Inoculating soil with mycorrhizal fungi often involves the transfer of soil or plant material where mycorrhiza are already present to new areas. These microorganisms can increase nutrient uptake and protect against root pathogens (Smith & Read 2008). In grasslands, mycorrhizal fungi can increase species diversity and productivity (Van der Heijden *et al.* 1998).

The studies detailed in this intervention are direct tests of the effectiveness of inoculating soil with mycorrhiza before seeding or planting (e.g. by comparison with an untreated but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions ‘*Sow grass seeds*’, ‘*Sow grassland forb species*’ or ‘*Sow native grass and forbs*’.

Helgason, T., Daniell, T., Husband, R., Fitter, A. & Young, J. (1998) Ploughing up the wood-wide web? *Nature*, 394, 431.

Johnson, N.C. (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecological applications*, 3, 749–757.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal symbiosis*. Academic Press, USA.

Van der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I.R. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69.

A replicated, randomized, paired, controlled study in 1992–1994 in a former mine in Minnesota, USA (1) found that inoculating soil with mycorrhizal fungi before sowing seeds had mixed effects on plant cover. After two years, plant cover was higher in areas that were inoculated with mycorrhizal fungi and sown with seeds (36%) than in areas where seeds were sown but no mycorrhizal fungi were added (28%). However, after three years, plant cover was not significantly different in areas that were inoculated with mycorrhizal fungi and sown with seeds (50%) and areas where seeds were sown but no

mycorrhizal fungi were added (37%). In May 1992, four blocks consisting of two 4 × 2.5 m plots were established. In each block, one 4 × 2.5 m plot was inoculated with mycorrhizal fungi by applying 2.5 g of *Sorghum sudanense* root material infected with fungi, and one plot was not inoculated. All plots were sown with a mixture of native plant species at a rate of 30.4 kg/ha. Plant cover was measured in all plots in August 1993 and 1994 using three 1-m wide transects.

A replicated, controlled study in 1998–1999 in a greenhouse and a former arable field in Arizona, USA (2) found that adding mycorrhizal fungi to soil before seeding did not alter the biomass or emergence success of giant sacaton *Sporobolus wrightii* plants, although after one year plants with added mycorrhiza were taller. Eight weeks after seeding in a greenhouse, emergence and biomass did not differ significantly between seeds sown in soil with added mycorrhiza (emergence: 71%; biomass: 0.44 g) and those sown in untreated soil (emergence: 62%; biomass: 0.46 g). Twelve months after the plants were transplanted to a field, those with added mycorrhiza were taller (79–85 cm) than those grown in untreated soil (63–79 cm). Survival of plants grown in soil with added mycorrhizal fungi was 90–100% compared to 82–95% for plants grown in untreated soil, although these results were not tested for statistical difference. Plants were grown in pots filled with heat-treated soil. Mycorrhizal fungi from local soil was added to the soil in 105 pots, while 105 pots were left untreated. Pots were sown with either three or ten seeds and watered regularly. Emergence was monitored weekly for eight weeks. Plants were transplanted into a former arable field in July 1998 and measured after 2, 4, 10 and 12 months.

A replicated, randomized, controlled study in 2008–2010 in 11 urban wasteland sites in Hellersdorf, Germany (3) found that inoculating soil with mycorrhizal fungi and sowing seeds of grassland species did not alter overall plant species richness or the proportion of target species. In the three years after sowing, average plant species richness did not differ significantly between plots where seeds were sown in soil inoculated with mycorrhizal fungi (47–52 species) and plots where seeds were sown in untreated soil (43–50 species). The percentage of vegetation consisting of target species of local conservation priority also did not differ significantly (inoculated plots: 35–50%; untreated plots: 29–46%). In autumn 2008, one 4 × 4 m plot at each of 11 sites was sown with seeds and the soil inoculated with mycorrhizal fungi (0.75 l/plot). One plot at each site was sown with seeds and the soil was left untreated. Plots were mown and tilled prior to sowing. Seed mixes contained 27 species from the study region. In spring, early and late summer in 2009–2010, a 3 × 3 m quadrat was placed in the centre of each plot and plant cover mapped.

A replicated, controlled study in 2008–2011 in a greenhouse in Wisconsin, USA (4) found that adding mycorrhizal fungi to soil before seeding did not alter the dry weight of three native grass and forb species. Dry weight of plants did not differ significantly between those grown in soil with added mycorrhizal fungi and those grown in untreated soil for the native grasses Canada wild rye *Elymus canadensis* (mycorrhiza added: 0.06–0.15 g; untreated: 0.09–0.13 g) and little bluestem *Schizachyrium scoparium* (mycorrhiza added: 0.02–0.07 g; untreated: 0.02–0.04 g), or the forb heath aster *Aster ericoides* (mycorrhiza added: 0.01–0.11 g; untreated: 0.05–0.06 g). Seeds of each species were sown into twenty 50-ml tubes containing sieved soil in December 2008. Sixteen tubes were treated with one of four commercial mycorrhizal treatments at a rate of 149–597 g/m³, and four were left untreated. All species were over-seeded and then thinned to one

individual/tube, and watered regularly. Total plant dry weight was calculated 83 days after sowing.

A controlled study in 2009–2011 in an urban prairie restoration site in San Antonio, Texas, USA (5) found that adding soil microbes and nutrients when planting increased cover of two of 38 native prairie species but did not alter overall cover of herbaceous or woody species. Two years after planting, two of 38 native prairie species had higher average cover in the area with microbes and nutrients added (Texas cupgrass *Eriochloa sericea*: 3.4%; bluegrama *Boutouloua gracilis*: 2.2%) than in the untreated area (Texas cupgrass: 1.2%; bluegrama: 0.6%). Cover of the other 36 plant species did not differ significantly between areas (see original paper for data). There was also no significant difference in average overall cover of herbaceous plant species (with microbes: 53%; untreated: 56%) and woody species (with microbes: 10%; untreated: 9%). Woody vegetation was cleared from the 9.4-ha site, and in September 2009, grass plugs of seven native species were planted at 4 plants/m². Half the area was planted with plugs from seeds inoculated with nutrients and a slurry containing 17 microbial strains, at a rate of 1 g slurry/1,600 g seed. The other half of the area was planted with plugs from untreated seeds. The treated plants were also sprayed with fertiliser one month after planting. The whole site was sown with a native prairie seed mix (0.001 kg/m²) before and after planting, and mowed in December 2010. Vegetation cover was surveyed in October 2011 in ten 1 x 1 m quadrats placed along five 50-m transects in the inoculated and untreated areas.

- (1) Noyd, R.K., Pfleger, F.L. & Norland, M.R. (1996) Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil*, 179, 89–97.
- (2) Richter, B.S. & Stutz, J.C. (2002) Mycorrhizal inoculation of big sacaton: Implications for grassland restoration of abandoned agricultural fields. *Restoration Ecology*, 10, 607–616.
- (3) Fischer, L.K., Lippe, M.v.d., Rillig, M.C. & Kowarik, I. (2013) Creating novel urban grasslands by reintroducing native species in wasteland vegetation. *Biological Conservation*, 159, 119–126.
- (4) Paluch, E.C., Thomsen, M.A. & Volk, T.J. (2013) Effects of resident soil fungi and land use history outweigh those of commercial mycorrhizal inocula: Testing a restoration strategy in unsterilized soil. *Restoration Ecology*, 21, 380–389.
- (5) Leonard, W.J. & Lyons, K.G. (2015) The use of commercial bacterial soil inoculant regime in an urban prairie restoration. *Natural Areas Journal*, 35, 9–17.

2.35. Irrigate before or after seeding/planting

- **Two studies** examined the effects of irrigating before or after seeding/planting on grasslands. One study was in Spain¹ and one in the USA².

VEGETATION COMMUNITY (2 STUDIES)

- **Overall richness/diversity (2 studies):** One of two replicated, controlled studies (one of which was randomized and paired) in Spain¹ and the USA² found that irrigating after sowing non-native seeds increased plant diversity in four of 10 cases. The other study² found that irrigating after sowing native seeds did not alter plant species richness.
- **Native/non-target species richness/diversity (1 study):** One replicated, controlled study in the USA² found that irrigating after sowing seeds did not alter the species richness of native plants.

VEGETATION ABUNDANCE (2 STUDIES)

- **Overall abundance (1 study):** One replicated, randomized, paired, controlled study in Spain¹ found that irrigating after sowing non-native seeds increased vegetation cover in six of 10 cases.

- **Native/non-target species abundance (1 study):** One replicated, controlled study in the USA² found that irrigating after sowing seeds did not alter the cover of native plant species.

VEGETATION STRUCTURE (0 STUDIES)

Background

In many arid areas low rainfall may limit the success of grassland restoration. Irrigating with water alongside seeding and planting may help to improve the probability of grassland plant establishment and colonization.

The studies detailed in this intervention are direct tests of the effectiveness of irrigating after seeding or planting (e.g. by comparison with an unirrigated but seeded or planted plot). Studies that represent comparisons of seeding to unseeded plots can be found in the actions '*Sow grass seeds*', '*Sow grassland forb species*' or '*Sow native grass and forbs*'.

A replicated, randomized, paired, controlled study in 2006–2008 on five motorway verges in central Spain (1) found that irrigating after sowing non-native seeds increased plant cover but not plant diversity in most cases compared to sowing without irrigating. No statistical tests were carried out in this study. In six of 10 comparisons, overall plant cover was on average higher in plots that were irrigated and sown with seeds (54–89%) than in plots that were sown with seeds and not irrigated (49–71%), while in four comparisons plant cover was lower in irrigated plots (54–58% vs 55–65%). In four of 10 comparisons, plant diversity was higher in plots that were irrigated and sown with seeds than in sown plots that were not irrigated, while in six comparisons plant diversity was lower (data reported as Shannon diversity index). In December 2006, at each of five sites, two 1 × 1 m plots in each of six random blocks were sown with a commercial non-native seed mixture. One plot/block was irrigated in March–June 2007 and 2008 at a rate equivalent to 50% of the average monthly precipitation recorded in 1971–2000, while the other plot was not irrigated. In May 2007 and 2008, the cover of all plants was visually assessed in each plot.

A replicated, controlled study in 2008–2013 in a former arable field in Massachusetts, USA (2) found that adding water after sowing native grass and forb seeds did not alter the cover and species richness of native plant species or total plant species richness compared to sowing without adding water. In the first year after sowing, the average cover and richness of native plant species and total plant species richness did not differ significantly between plots with water added (native plants: 25–32% cover, 8–10 species/plot; total plants: 19–20 species/plot) and plots with no water added (native plants: 36% cover, 11 species/plot; total plants: 23 species/plot). The same was true five years after sowing (native plants: 51–58% vs 59% cover, 10 vs 10 species/plot; total plants: 18 vs 17 species/plot). In November 2008, fifteen 5 x 5 m plots were sown with locally collected native grass and forb seeds of 26 species. All plots were tilled before sowing to remove non-native vegetation. Water was added to 10 plots (190–380 l/plot) between June and August 2009, and five plots had no water added. Vegetation was surveyed in a 3 x 3 m quadrat placed in the centre of each plot in July or August 2009 and 2013.

(1) Garcia-Palacios, P., Soliveres, S., Maestre, F.T., Escudero, A., Castillo-Monroy, A.P. & Valladares, F. (2010) Dominant plant species modulate responses to hydroseeding, irrigation and fertilization during the restoration of semiarid motorway slopes. *Ecological Engineering*, 36, 1290–1298.

- (2) Neill, C., Wheeler, M.M., Loucks, E., Weiler, A., Von Holle, B., Pelikan, M. & Chase, T. (2015) Influence of soil properties on coastal sandplain grassland establishment on former agricultural fields. *Restoration Ecology*, 23, 531–538.

2.36. Use erosion blanket after seeding/planting

- **Three studies** examined the effects of using erosion blankets after seeding/planting on grassland vegetation. Two studies were in the USA^{1,2} and one study was in Spain³.

VEGETATION COMMUNITY (1 STUDY)

- **Overall richness/diversity (1 study):** One replicated, randomized, controlled study in Spain³ found that using an organic blanket after sowing seeds increased plant species richness.

VEGETATION ABUNDANCE (3 STUDIES)

- **Overall abundance (3 studies):** Two of three replicated, randomized, controlled studies (two of which were paired and two randomized) in the USA^{1,2} and Spain³ found that using an erosion blanket after seeding and planting did not alter vegetation cover^{1,2}. The other study³ found that using an organic blanket after sowing seeds increased plant density.

VEGETATION STRUCTURE (0 STUDIES)

Background

Many degraded grasslands suffer from erosion due to lack of vegetation. One method of reducing this is by using erosion blankets – biodegradable materials made of natural or synthetic fibres. Combining this with sowing or planting may aid the establishment of grassland plants.

The studies detailed in this intervention are direct tests of the effectiveness of adding an erosion blanket after seeding or planting (e.g. by comparison with a seeded or planted plot without an erosion blanket). Studies that represent comparisons of seeding to unseeded plots can be found in the actions ‘*Sow grass seeds*’, ‘*Sow grassland forb species*’ or ‘*Sow native grass and forbs*’.

A replicated, paired, controlled study in 1988–1991 in a largely unvegetated area on the island of Kaho‘olawe, USA (1) found that using an erosion blanket after sowing seeds did not alter vegetation cover compared to seeding alone. After 25 months, average vegetation cover did not differ between sown plots with or without an erosion blanket (both 3%). In November 1988, the site was ploughed to a depth of 10 cm. In December 1988, seeds (a mix of six grasses and one legume) were sown using a seed drill in two 60 x 1.8 m plots in each of five blocks. One plot/block was covered with woven jute netting, and the other plot was left uncovered. In January 1991, thirty 0.25-m² quadrats were placed in each plot and vegetation cover estimated by eye.

A replicated, randomized, paired, controlled study in 1993–1997 on three road verges in Colorado, USA (2) found that using an erosion control blanket after seeding and planting did not alter vegetation cover compared to seeding and planting alone. Vegetation cover was not significantly different in areas where an erosion control blanket was used, seeds were sown, and plants were planted (0.6–12.3%) and areas where no erosion control blanket was used but seeds were sown and plants planted (2.7–10.5%). In October 1993, three blocks each containing two plots, were established on road verges.

An erosion control blanket made of aspen *Populus* sp. shavings and plastic netting was added to one plot that was then sown with seeds of 13 grass, forb, and shrub species and planted with the same plant species. One plot was planted with plants and sown with seeds but no erosion control blanket was added. Vegetation cover in each plot was recorded in June 1994–1997.

A replicated, randomized, controlled study in 2009–2010 in an agricultural field in southern Spain (3) found that using an organic blanket after sowing seeds increased plant density and species richness compared to sowing without an organic blanket. In four of four comparisons, plant density was higher in plots where seeds were sown and an organic blanket was used (4–34 plants/m²) than in plots where seeds were sown and no blanket was used (1–11 plants/m²). A similar pattern was seen in four of four comparisons for plant species richness (with organic blanket: 0.4–3.2 species/0.25 m²; without organic blanket: 0.2–1.8 species/0.25 m²). In November 2009, forty 5 x 5 m plots were sown with locally collected seeds of seven native grass and forb species. An organic blanket (biodegradable net made of straw and alpha grass *Stipa tenacissima*) was randomly added to half of the plots, while the other half had no blanket added. Four different bedding materials were applied to plots prior to seeding (see original paper for details). Plant density and species richness were estimated in July and October 2010 using fifteen randomly placed 0.5 x 0.5 m quadrats/plot.

- (1) Warren, S.D. & Aschmann, S.G. (1993) Revegetation strategies for Kahoolawe Island, Hawaii. *Journal of Range Management*, 46, 462–466.
- (2) Paschke, M.W., DeLeo, C. & Redente, E.F. (2000) Revegetation of roadcut slopes in Mesa Verde National Park, USA. *Restoration Ecology*, 8, 276–282.
- (3) Ballesteros, M., Cañadas, E.M., Foronda, A., Fernández-Ondoño, E., Peñas, J. & Lorite, J. (2012) Vegetation recovery of gypsum quarries: Short-term sowing response to different soil treatments. *Applied Vegetation Science*, 15, 187–197.

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Appendix 1: Threats to grassland ecosystems and potential interventions

This section lists all threats and potential interventions identified for grasslands. Threats are organised into broad, numbered categories, based on IUCN threat categories.

- Specific threats are identified using this type of bullet point
 - Interventions are identified using this type of bullet point

1. Threat: Residential & commercial development

- Legally protect habitat from development

2. Threat: Agriculture & Aquaculture

- Livestock farming and ranching
 - Use fences to exclude grazing livestock from grasslands
 - Modify grazing intensity by altering density of livestock
 - Modify grazing intensity/impacts by changing type of livestock
 - Modify grazing intensity/impacts by adjusting the period during which livestock can graze / seasonal grazing
 - Provide financial incentives to prevent or reduce livestock grazing
 - Introduce mowing/cutting to mimic livestock grazing
 - Manage timing of cutting regime
 - Manage cutting height
 - Modify cutting technique (e.g. grass trimmer vs bar mower vs heavy machinery)
 - Stop or reduce addition of fertilizer to grassland
 - Remove or invert topsoil to reduce fertility
 - Grow annual crops without fertilizers to reduce soil fertility
 - Plough soils to reduce fertility
 - Add rubble to soils to reduce their fertility
 - Introduce annual cutting and remove cuttings to reduce fertility
 - Sow hemiparasitic species that decrease competition of grasses induced by higher fertility
 - Add carbon to soil to reduce fertility (sucrose, starch, cellulose, sawdust)
 - Use forage harvesting to remove nutrients
 - Pay farmers to cover the cost of conservation measures (e.g. agri-environment schemes)
 - Use AES to encourage establishment or maintenance of native grassland
 - Use AES or other policy tools to encourage traditional pastoral farming and skills
 - Regulation to reduce or prevent use of slurry on grasslands
 - Use salt or 'cow chips' to discourage livestock from grazing certain areas
 - Stop or reduce supplementary feeding of livestock

- Annual/perennial non-timber crops
 - Increase the proportion of semi-natural grassland habitat in the farmed landscape
 - Pay farmers to cover the cost of conservation measures (e.g. agri-environment schemes)
 - Implement policies to encourage grassland restoration programmes, including landscape-based habitat creation
 - Create protected areas
- Wood/pulp plantations
 - Increase the proportion of semi-natural grassland habitat in the forestry landscape
 - Implement policies to discourage tree-planting on grassland habitat

3. Threat: Energy production and mining

- Maintain grassland habitat corridors in areas of energy production or mining
- Increase the proportion of semi-natural grassland habitat in areas of energy production or mining
- Maintain / manage areas of semi-natural grasslands near to mining areas to promote natural re-colonization
- Retain grassland topsoil before exploitation, and spread it once mining is finished
- Add topsoil to reduce the effects of toxic chemicals on former mining sites
- Add elemental sulphur to reduce pH in acidic grassland
- Add $\text{Fe}(\text{OH})_3$ to reduce available P
- Introduce grazing or other disturbance regime on former mining sites

4. Threat: Transportation and service corridors

- Maintain grassland habitat corridors along roads and other transportation corridors
- Create grassland buffer zones beside roads and other transportation corridors using commercial seed mix
- Create grassland buffer zones beside roads and other transportation corridors using local seed mix
- Create grassland buffer zones beside roads and other transportation corridors using passive restoration

5. Threat: Biological resource use

- Gathering of terrestrial plants or their seeds
 - Plant plugs or sow seed of species affected by gathering
 - Legally protect the species affected by gathering
 - Place signs to deter gathering of terrestrial species

6. Threat: Human intrusions and disturbance

- Re-route paths to reduce grassland habitat disturbance
- Place signs to discourage access to sensitive areas of grassland habitat
- Plant spiny shrubs to act as barriers to people

7. Threat: Natural system modification

- Abstraction of surface or ground water
 - Introduce irrigation
- Increase in frequency / extent of flooding
 - Introduce drainage
- Construction of coast defences
 - Allow natural flooding/inundation
- Increase in fire frequency/intensity
 - Use prescribed burning approaches to reduce potential for large wild fires
 - Reduce use of prescribed fire
- Reductions in traditional managed burning
 - Reinstate or replicate the use of traditional burning practices
 - Alter or control seasonal timing of prescribed fire
- Suppression of fire frequency/intensity
 - Introduce or increase prescribed burning to mimic natural or historic fire cycle

8. Threat: Invasive and other problematic species

- Problematic shrub or tree species
 - Control problem shrub or tree species by modifying grazing intensity or type of livestock
 - Control problem shrub or tree species with herbicide
 - Control problem shrub or tree species using biological control
 - Control problem shrub or tree species with herbicide, followed by sowing seed
 - Control problem shrubs or trees by cutting
 - Control problem shrubs or trees by cutting, followed by sowing seed
 - Control problem shrubs or trees by cutting, followed by an increase in grazing intensity
 - Control problem shrubs or trees by cutting, followed by application of herbicide
 - Control problem shrubs or trees by cutting, followed by prescribed burning
 - Control problem shrubs or trees by cutting, followed by removal of seedlings
 - Control problem shrubs or trees using prescribed burning
 - Control problem shrubs or trees using prescribed burning, followed by herbivory
 - Control problem shrubs or trees by excavating roots after cutting or using herbicide
 - Control problem shrubs or trees by sowing nurse species to reduce shrub and seeding recruitment

- Sow native grassland species in invaded habitat
- Problematic grass or forb species (native or non-native invasive)
 - Control problem grass species by modifying grazing intensity or type of livestock
 - Control problem grass species by mowing
 - Control problem grass species by mowing followed by sowing native seed
 - Control problem grass species by mowing followed by increasing grazing intensity
 - Control problem grasses by mowing, followed by prescribed burning
 - Control problem grasses by mowing, followed by application of herbicide
 - Control problem grass species by stripping turf
 - Control problem grass species by tilling/using rotovator
 - Plant parasitic species to control dominant grass species e.g. *Pedicularis palustris*, *Rhinanthus minor*
 - Control problem grass species with herbicide
 - Control problem grass species with herbicide, followed by sowing native seed
 - Control problem grasses using prescribed burning
 - Control problem grass species by sowing native seed or planting native plugs
 - Control problem grass species by sowing nurse species to reduce invasion by problem herbaceous species
 - Add mulch to control problem grass species
 - Add mulch followed by fertilizer to control problem grass species
 - Add mulch to control invasive grass species, followed by sowing native seed
 - Remove or invert topsoil to control problem grass or forb species
 - 'Flush' exotic grass seed by summer watering in Mediterranean climate
 - Prime native grass seed to germinate earlier to outcompete non-native species
 - Impoverish soils to shift from non-native invasives to native grasses and forbs
- Bracken
 - Control bracken using herbicide
 - Control bracken using herbicide followed by increased grazing
 - Control bracken using biological control
 - Control bracken by cutting / crushing / bruising
 - Control bracken by cutting followed by applying herbicide
 - Control bracken by applying herbicide followed by cutting
 - Control bracken by increasing grazing intensity of livestock
 - Control bracken using rotovator
 - Remove or invert topsoil to control bracken roots and rhizomes
- Problematic native or non-native animals
 - Use fences to exclude large native herbivores
 - Control populations of large native herbivores

- Reintroduce carnivores to control herbivores
- Reduce numbers of problematic native small mammals by trapping/hunting
- Reduce numbers of problematic native herbivorous invertebrates using biological control

9. Threat: Pollution

- Agricultural/forestry/industrial effluents
 - Plant vegetation to act as a buffer to exclude pollution
 - Reduce pesticide use on nearby agricultural/forestry land
 - Reduce herbicide use on nearby agricultural/forestry land
 - Reduce fertilizer use on nearby agricultural/forestry land
 - Mow grassland and remove hay/clippings to reduce concentration of pollutants (e.g. N, P, K)
 - Burn grassland to reduce concentration of pollutants (e.g. nitrogen)
 - Plant parasitic species to control dominant grass species e.g. *Pedicularis palustris*, *Rhinanthus minor*
- Airborne pollution
 - Add lime to grassland to reduce the impacts of sulphur dioxide pollution
 - Introduce policies or regulation to reduce air pollution from vehicles
 - Enforce international and national policies for the reduction of pollutants and carbon dioxide

10. Threat: Climate change

- Habitat shifting and alteration
 - Restore grassland habitat in area predicted to have suitable climate into the future
 - Create connectivity between areas of grassland to allow species movements in response to climate changes
 - Plant a wide diversity of grassland species and ecotypes to provide resilience to future climate change

11. Habitat protection

- Protect grassland habitats
- Retain connectivity between habitat patches
- Retain buffer zones around core habitat

12. Habitat restoration and creation

Seeding and planting

- Sow grass seeds
- Sow grassland forb species

- Sow native grass and forbs
- Plant grassland plants
- Sow native grassland species from a local source
- Sow seeds of nurse plants
- Sow or plant nurse plants (alongside seeding/planting of grassland species)
- Sow seeds of parasitic species (e.g. yellow rattle)
- Sow seeds of tree species in savanna
- Sow seeds at a higher density
- Increase number of species in seed mix
- Sow seeds at start of growing season
- Sow seeds in part of site
- Sow seeds in prepared gaps within vegetation
- Drill seed rather than seeding by hand
- Use slot/strip seeding
- Spray slurry of seed, mulch and water ('hydroseeding')
- Disturb soil before seeding/planting
- Remove leaf litter before seeding/planting
- Remove topsoil or turf before seeding/planting
- Remove vegetation before seeding/planting
- Burn vegetation before seeding/planting
- Apply herbicide before seeding/planting
- Mow before or after seeding/planting
- Graze with livestock after seeding/planting
- Add topsoil before seeding/planting
- Add mulch before or after seeding/planting
- Add woody debris to protect seeds/plants
- Transfer plant material from intact local grassland site (alongside seeding/planting)
- Add charcoal to soil before seeding/planting
- Add carbon to soil before or after seeding/planting
- Add fertilizer to soil before or after seeding/planting
- Add sulphur to soil before seeding/planting
- Inoculate with mycorrhiza before seeding/planting
- Irrigate before or after seeding/planting
- Use erosion blanket after seeding/planting

Physical modification of habitat

- Remove vegetation
- Remove trees/shrubs
- Plant sporadic trees to restore wood pasture or savanna
- Add fertilizer

- Disturb soil
- Disturb topsoil and add topsoil from intact grassland
- Remove topsoil
- Remove topsoil and add topsoil from intact grassland
- Add carbon to soil
- Add fertilizer to soil (including compost/manure)
- Add mulch to soil
- Irrigate
- Inoculate with mycorrhiza
- Transfer turf from intact grassland site
- Translocate topsoil to encourage recolonization
- Transfer small ecosystem patches (soil plates) from undisturbed grasslands
- Transfer plant material from intact local grassland site

Passive restoration

- Allow grassland to recover from disturbance (passive restoration)

Restoration of wider landscape

- Restore or create habitat connectivity of grasslands
- Increase diversity of habitats in wider landscape

13. Education and awareness

- Raise awareness amongst the general public through campaigns and public information and demonstration sites
- Provide education campaigns about grasslands
- Provide training to grassland site managers
- Provide traditional livestock management training to farmers / land-owners / shepherds etc.
- Community action

Appendix 2: Glossary

Abundance: any measurement of the amount of vegetation present, including *cover*, *biomass*, volume, frequency, plant density, stem/shoot density, and area or coverage of vegetation stands. Unless specified, refers to all standing vegetation (live and dead).

Biomass: the total mass of all the organisms of a given type and/or in a given area. It is normally measured in terms of grams of dry mass/m². In this synopsis, only results relating to above ground plant biomass are reported. Results clearly based on, or including, below ground biomass are not included.

Control: (a) noun: plot/site not treated with intervention. (b) verb: any action to manage a population of a problematic species – eradication, suppression, or containment.

Cover: the proportion of ground that is occupied by the aerial parts of plants, or the perpendicular projection of them on to the ground (Allaby 2012). May be measured for each species individually, with the sum of individual species' cover potentially exceeding 100% or may be measured for vegetation overall, so maximum cover is 100% (Wilson 2011). So, note that overall cover results are not necessarily comparable between studies.

Forb: A herbaceous flowering plant that is not a grass or grass-like plant (graminoid).

Genus (*pl. genera*): A category used in the classification of organisms, consisting of a number of similar or closely related species (Daintith & Martin 2010). For example, the genus *Equus* contains horses (e.g. *Equus ferus*), zebras (e.g. *Equus quagga*) and donkeys (e.g. *Equus africanus*).

Grass: A herb plant that has a cylindrical (occasionally flattened), hollow, jointed stem and long narrow leaves.

Grass-like plants: Herbaceous plant species with a grass-like morphology, also known as a graminoids. This grouping includes grasses, sedges, and rushes.

Grassland-characteristic plants: plant species that always or usually grow in grasslands.

Harvest: cut or pull up plant material and remove it from site.

Herb: a seed-bearing plant that has no, or little, permanent woody tissue. Above-ground tissue usually dies back at the end of each growing season (Allaby 2012). For the purposes of this synopsis, herbs are considered in their broadest possible sense: including grass-like plants (graminoids), forbs (non-graminoid herbs), succulents (fleshy plants), subshrubs (non-woody tissue growing from a woody base) and some vines.

Invasive species: established non-native species that have negative impacts on the environment and/or humans, usually at considerable distance from the original site of introduction (IUCN/ISSG 2020). Compare *non-native species*.

Native species: species that have evolved in a given area or that arrived there by natural means from an area where they are native, without the intentional or accidental intervention of humans (Richardson *et al.* 2011). Compare *non-native species*.

Non-native species: species whose presence in a region is attributable to human actions that enabled them to overcome fundamental biogeographical barriers (e.g. mountain ranges or oceans) (Richardson *et al.* 2011). Compare *native species* and *invasive species*.

Propagule: Any cellular structure produced by an organism that is capable of dispersing and surviving in the environment before developing into a new individual (Allaby 2012). For

example, seeds and spores. The propagules of many mangrove trees are small, mature plants (unlike seeds which are embryonic plants enclosed in a protective outer coating).

Restoration: returning a habitat from a disturbed or altered condition towards a previously existing condition. In this sense restoration may, but almost always does not, return the vegetation exactly to that previous condition. This may be impossible due to changes in the physical habitat.

Rhizome: A horizontal underground stem (Daintith & Martin 2010). It enables the plant to survive from one growing season to the next and in some species it also serves to propagate the plant vegetatively. It may be thin and wiry or fleshy and swollen.

Shrub: A perennial woody plant which branches below or near ground level into several main stems (Allaby 2012).

Species: A category used in the classification of organisms. According to the biological species concept, a species is a group of individuals that can usually breed among themselves and produce fertile offspring (Daintith & Martin 2010). For example, all humans belong to the species *Homo sapiens*.

Stolon: A long aerial side stem that gives rise to a new daughter plant when the bud at its apex touches the soil (Daintith & Martin 2010). Plants that multiply in this way include strawberries *Fragaria* spp. and grasses like creeping bent-grass *Agrostis stolonifera*.

Taxon (pl. taxa): A group of organisms at any level in the hierarchical classification of organisms (Daintith & Martin 2010). For example, the species *Homo sapiens* is a taxon, as is the genus *Homo*, and the class Mammalia.

Tree: a perennial plant with an elongated woody trunk, supporting branches and leaves.

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Appendix 3: Journals (and years) searched

Journals (and years) searched and for which relevant papers have been added to the Conservation Evidence discipline-wide literature database. An asterisk indicates the journals most relevant to this synopsis.

Journal	Years Searched	Topic
Acta Chiropterologica	1999–2017	All biodiversity
Acta Herpetologica	2006–2016	All biodiversity
Acta Oecologica-International Journal of Ecology	1990–2017	All biodiversity
Acta Theriologica	1977–2014	All biodiversity
African Bird Club Bulletin	1994–2017	All biodiversity
African Journal of Ecology	1963–2016	All biodiversity
African Journal of Herpetology	1990–2016	All biodiversity
African Journal of Marine Science	1983–2017	All biodiversity
African Primates	1995–2012	All biodiversity
African Zoology	1979–2013	All biodiversity
Agriculture, Ecosystems & Environment	1983–2017	All biodiversity
Ambio	1972–2011	All biodiversity
American Journal of Primatology	1981–2014	All biodiversity
American Naturalist	1867–2017	All biodiversity
Amphibia-Reptilia	1980–2012	All biodiversity
Amphibian and Reptile Conservation	1996–2012	All biodiversity
Animal Biology	2003–2013	All biodiversity
Animal Conservation	1998–2017	All biodiversity
Annales Zoologici Fennici	1964–2013	All biodiversity
Annales Zoologici Societatis Zoologicae Botanicae Fennicae Vanamo	1932–1963	All biodiversity
Annual Review Ecology and Systematics	1970–2017	All biodiversity
Anthrozoos	1987–2013	All biodiversity
Apidologie	1958–2009	All biodiversity
Applied Animal Behaviour Science	1998–2014	All biodiversity
Applied Herpetology	2003–2009	All biodiversity
Applied Vegetation Science*	1998–2017	All biodiversity
Aquaculture Research	1972–2008	All biodiversity
Aquatic Botany	1975–2017	All biodiversity
Aquatic Conservation: Marine and Freshwater Ecosystems	1991–2017	All biodiversity
Aquatic Ecology	1968–2016	All biodiversity
Aquatic Ecosystem Health & Management	1998–2016	All biodiversity
Aquatic Invasions	2006–2016	All biodiversity
Aquatic Living Resources	1988–2016	All biodiversity
Aquatic Mammals	1972–2017	All biodiversity
Arid Land Research and Management	1987–2013	All biodiversity
Asian Primates	2008–2012	All biodiversity
Auk	1980–2016	All biodiversity
Austral Ecology	1977–2017	All biodiversity
Australasian Journal of Herpetology	2009–2012	All biodiversity
Australian Mammalogy	2000–2017	All biodiversity

Avian Conservation and Ecology	2005–2016	All biodiversity
Basic and Applied Ecology	2000–2017	All biodiversity
Behavior	1948–2013	All biodiversity
Behavior Ecology	1990–2013	All biodiversity
Bibliotheca Herpetologica	1999–2017	All biodiversity
Biocontrol	1956–2016	All biodiversity
Biocontrol Science and Technology	1991–1996	All biodiversity
Biodiversity and Conservation	1994–2017	All biodiversity
Biological Conservation	1981–2017	All biodiversity
Biological Control	1991–2017	All biodiversity
Biological Invasions	1999–2017	All biodiversity
Biology and Environment: Proceedings of the Royal Irish Academy	1993–2017	All biodiversity
Biology Letters	2005–2017	All biodiversity
Biotropica	1990–2017	All biodiversity
Bird Conservation International	1991–2016	All biodiversity
Bird Study	1980–2016	All biodiversity
Boreal Environment Research	1996–2014	All biodiversity
Bulletin of the Herpetological Society of Japan	1999–2008	All biodiversity
Canadian Journal of Fisheries and Aquatic Sciences	1901–2017	All biodiversity
Canadian Journal of Forest Research	1971–2013	All biodiversity
Caribbean Journal of Science	1961–2013	All biodiversity
Chelonian Conservation and Biology	2006–2016	All biodiversity
Collinsorum	2012–2014	All biodiversity
Community Ecology	2000–2012	All biodiversity
Conservation Biology*	1987–2017	All biodiversity
Conservation Evidence*	2004–2018	All biodiversity
Conservation Genetics	2000–2013	All biodiversity
Conservation Letters	2008–2017	All biodiversity
Contemporary Herpetology	1998–2009	All biodiversity
Contributions to Primatology	1974–1991	All biodiversity
Copeia	1910–2016	All biodiversity
Cunninghamia	1981–2016	All biodiversity
Current Herpetology	1964–2016	All biodiversity
Dodo	1977–2001	All biodiversity
Ecological and Environmental Anthropology	2005–2008	All biodiversity
Ecological Applications*	1991–2017	All biodiversity
Ecological Indicators	2001–2007	All biodiversity
Ecological Management & Restoration*	2000–2017	All biodiversity
Ecological Restoration*	1981–2016	All biodiversity
Ecology	1936–2017	All biodiversity
Ecology Letters	1998–2013	All biodiversity
Ecoscience	1994–2013	All biodiversity
Ecosystems	1998–2013	All biodiversity
Emu	1980–2016	All biodiversity
Endangered Species Bulletin	1966–2003	All biodiversity
Endangered Species Research	2004–2017	All biodiversity
Environmental Conservation	1974–2017	All biodiversity

Environmental Evidence	2012–2017	All biodiversity
Environmental Management	1977–2017	All biodiversity
Environmentalist	1981–1988	All biodiversity
Ethology Ecology and Evolution	1989–2014	All biodiversity
European Journal of Soil Science	1950–2012	Soil Fertility
European Journal of Wildlife Research	1955–2017	All biodiversity
Evolutionary Anthropology	1992–2014	All biodiversity
Evolutionary Ecology	1987–2014	All biodiversity
Evolutionary Ecology Research	1999–2014	All biodiversity
Fire Ecology	2005–2016	All biodiversity
Fisheries Management and Ecology	1994–2018	All biodiversity
Fisheries Research	1990–2018	All biodiversity
Folia Primatologica	1963–2014	All biodiversity
Folia Zoologica	1959–2013	All biodiversity
Forest Ecology and Management	1976–2013	All biodiversity
Freshwater Biology	1975–2017	All biodiversity
Freshwater Science	1982–2017	All biodiversity
Functional Ecology	1987–2013	All biodiversity
Genetics and Molecular Research	2002–2013	All biodiversity
Geoderma	1967–2012	Soil Fertility
Gibbon Journal	2005–2011	All biodiversity
Global Change Biology	1995–2017	All biodiversity
Global Ecology and Biogeography	1991–2014	All biodiversity
Grass and Forage Science*	1980–2017	All biodiversity
Herpetofauna	2003–2007	All biodiversity
Herpetologica	1936–2012	All biodiversity
Herpetological Bulletin	2000–2013	All biodiversity
Herpetological Conservation and Biology	2006–2012	All biodiversity
Herpetological Journal	2005–2012	All biodiversity
Herpetological Monographs	1982–2012	All biodiversity
Herpetological Review	1967–2014	All biodiversity
Herpetology Notes	2008–2014	All biodiversity
Human Wildlife Interactions	2007–2017	All biodiversity
Hydrobiologia	2000–2017	All biodiversity
Hystrix, the Italian Journal of Mammalogy	1986–2017	All biodiversity
Ibis	1980–2016	All biodiversity
ICES Journal of Marine Science	1990–2018	All biodiversity
iForest	2008–2016	All biodiversity
Integrative Zoology	2006–2013	All biodiversity
International Journal of Pest Management (formerly PANS Pest Articles & News Summaries 1969–1975, PANS 1976–1979 & Tropical Pest Management 1980–1992)	1969–1979	All biodiversity
International Journal of the Commons	2007–2016	All biodiversity
International Journal of Wildland Fire	1991–2016	All biodiversity
International Wader Studies	1970–1972	All biodiversity
International Zoo Yearbook	1960–2015	Management of Captive Animals
Invasive Plant Science and Management	2008–2016	All biodiversity

Israel Journal of Ecology & Evolution	1963–2013	All biodiversity
Italian Journal of Zoology	1978–2013	All biodiversity
Journal for Nature Conservation	2002–2017	All biodiversity
Journal of Animal Ecology	1932–2017	All biodiversity
Journal of Apicultural Research	1962–2009	All biodiversity
Journal of Applied Ecology*	1964–2017	All biodiversity
Journal of Aquatic Plant Management	1962–2016	All biodiversity
Journal of Arid Environments	1993–2017	All biodiversity
Journal of Avian Biology	1980–2016	All biodiversity
Journal of Bat Conservation and Research	2000–2017	All biodiversity
Journal of Cetacean Research and Management	1999–2012	All biodiversity
Journal of Ecology	1933–2017	All biodiversity
Journal of Environmental Management	1973–2017	All biodiversity
Journal of Experimental Marine Biology & Ecology	1980–2016	All biodiversity
Journal of Field Ornithology	1980–2016	All biodiversity
Journal of Forest Research	1996–2017	All biodiversity
Journal of Great Lakes Research	1975–2017	All biodiversity
Journal of Herpetological Medicine and Surgery	2009–2013	All biodiversity
Journal of Herpetology	1968–2015	All biodiversity
Journal of Kansas Herpetology	2002–2011	All biodiversity
Journal of Mammalian Evolution	1993–2014	All biodiversity
Journal of Mammalogy	1919–2017	All biodiversity
Journal of Mountain Science	2004–2016	All biodiversity
Journal of Negative Results: Ecology & Evolutionary Biology	2004–2016	All biodiversity
Journal of Ornithology	2004–2017	All biodiversity
Journal of Primatology	2012–2013	All biodiversity
Journal of Raptor Research	1966–2016	All biodiversity
Journal of Sea Research	1961–2017	All biodiversity
Journal of the Japanese Institute of Landscape Architecture	1934–2017	All biodiversity
Journal of the Marine Biological Association of the United Kingdom	1887–2006	All biodiversity
Journal of Tropical Ecology	1986–2017	All biodiversity
Journal of Vegetation Science	1990–2017	All biodiversity
Journal of Wetlands Ecology	2008–2012	All biodiversity
Journal of Wetlands Environmental Management	2012–2016	All biodiversity
Journal of Wildlife Diseases	1965–2012	All biodiversity
Journal of Zoo and Aquarium Research	2013–2016	All biodiversity
Journal of Zoology	1966–2017	All biodiversity
Jurnal Primatologi Indonesia	2009	All biodiversity
Kansas Herpetological Society Newsletter	1977–2001	All biodiversity
Lake and Reservoir Management	1984–2016	All biodiversity
Land Degradation and Development	1989–2016	All biodiversity
Land Use Policy	1984–2012	Soil Fertility
Latin American Journal of Aquatic Mammals	2002–2016	All biodiversity
Lemur News	1993–2012	All biodiversity
Limnologica - Ecology and Management of Inland Waters	1999–2017	All biodiversity
Mammal Research	2001–2017	All biodiversity
Mammal Review	1970–2017	All biodiversity

Mammal Study	2005–2017	All biodiversity
Mammalia	1937–2017	All biodiversity
Mammalian Biology	2002–2017	All biodiversity
Mammalian Genome	1991–2013	All biodiversity
Management of Biological Invasions	2010–2016	All biodiversity
Mangroves and Salt Marshes	1996–1999	All biodiversity
Marine Ecological Progress Series	2000–2018	All biodiversity
Marine Environmental Research	1978–2017	All biodiversity
Marine Mammal Science	1985–2017	All biodiversity
Marine Pollution Bulletin	2010–2017	All biodiversity
Mires and Peat	2006–2016	All biodiversity
Natural Areas Journal	1992–2017	All biodiversity
Neobiota	2011–2017	All biodiversity
Neotropical Primates	1993–2014	All biodiversity
New Journal of Botany	2011–2013	All biodiversity
New Zealand Journal of Zoology	1974–2017	All biodiversity
New Zealand Plant Protection	2000–2016	All biodiversity
Northwest Science	2007–2016	All biodiversity
Oecologia	1969–2017	All biodiversity
Oikos	1949–2017	All biodiversity
Ornitologia Neotropical	1990–2018	All biodiversity
Oryx	1950–2017	All biodiversity
Ostrich	1980–2016	All biodiversity
Pacific Conservation Biology	1993–2017	All biodiversity
Pakistan Journal of Zoology	2004–2013	All biodiversity
Plant Ecology	1948–2007	All biodiversity
Plant Protection Quarterly	2008–2016	All biodiversity
Polish Journal of Ecology	2002–2013	All biodiversity
Population Ecology	1952–2013	All biodiversity
PLOS	1980–2018	Key word: bat*
Preslia*	1973–2017	All biodiversity
Primate Conservation	1981–2014	All biodiversity
Primates	1957–2013	All biodiversity
Rangeland Ecology & Management (previously Journal of Range Management 1948–2004)*	1948–2016	All biodiversity
Raptors Conservation	2005–2016	All biodiversity
Regional Studies in Marine Science	2015–2017	All biodiversity
Restoration Ecology*	1993–2017	All biodiversity
Revista Chilena de Historia Natural	2000–2016	All biodiversity
Revista de Biología Tropical	1976–2013	All biodiversity
River Research and Applications	1987–2016	All biodiversity
Russian Journal of Herpetology	1994–2000	All biodiversity
Slovak Raptor Journal	2007–2016	All biodiversity
Small Ruminant Research	1988–2017	All biodiversity
Soil Biology & Biochemistry	1969–2012	Soil Fertility
South African Journal of Botany	1982–2016	All biodiversity
South African Journal of Wildlife Research	1971–2014	All biodiversity

South American Journal of Herpetology	2006–2012	All biodiversity
Southern Forests: a journal of Forest Science	2008–2013	All biodiversity
Southwestern Naturalist	1956–2013	All biodiversity
Strix	1982–2017	All biodiversity
Systematic Reviews Centre for Evidence-Based Conservation*	2004–2017	All biodiversity
The Canadian Field-Naturalist	1987–2017	All biodiversity
The Condor	1980–2016	All biodiversity
The Journal of Wildlife Management	1945–2017	All biodiversity
The Open Ornithology Journal	2008–2016	All biodiversity
The Rangeland Journal	1976–2016	All biodiversity
Trends in Ecology and Evolution	1986–2017	All biodiversity
Tropical Conservation Science	2008–2014	All biodiversity
Tropical Ecology	1960–2014	All biodiversity
Tropical Grasslands*	1967–2010	All biodiversity
Tropical Zoology	1988–2013	All biodiversity
Turkish Journal of Zoology	1996–2014	All biodiversity
Vietnamese Journal of Primatology	2007–2009	All biodiversity
Wader Study Group Bulletin	1970–1977	All biodiversity
Waterbirds	1983–2016	All biodiversity
Weed Biology and Management	2001–2016	All biodiversity
Weed Research	1961–2017	All biodiversity
West African Journal of Applied Ecology	2000–2016	All biodiversity
Western North American Naturalist	2000–2016	All biodiversity
Wetlands	1981–2016	All biodiversity
Wetlands Ecology and Management	1989–2016	All biodiversity
Wildfowl	1948–2016	All biodiversity
Wildlife Biology	1995–2013	All biodiversity
Wildlife Monographs	1958–2013	All biodiversity
Wildlife Research	1974–2017	All biodiversity
Wildlife Society Bulletin	1973–2017	All biodiversity
Wilson Journal of Ornithology	1980–2016	All biodiversity
Zhurnal Obshchei Biologii	1972–2013	All biodiversity
Zoo Biology	1982–2016	All biodiversity
ZooKeys	2008–2013	All biodiversity
Zoologica Scripta	1971–2014	All biodiversity
Zoological Journal of the Linnean Society	1856–2013	All biodiversity
Zootaxa	2004–2014	All biodiversity

Appendix 4: Literature reviewed for the Grassland Conservation synopsis

The diagram below shows the total numbers of journals and report series searched for this synopsis, the total number of publications searched (title and abstract) within those, and the number of publications that were summarized from each source of literature.

