Grassland restoration by local seed mixtures: New evidence from a practical 15-year restoration study

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Abstract
Aim: Local seed mixtures are frequently used to restore species-rich grasslands. However, it has hardly been tested whether local seed mixtures can actually be applied successfully in grassland restoration practice at larger scales and long-term. To close this gap, we report the results of a large-scale restoration study in which grasslands were restored about 15 years ago using different local seed mixtures.

Location: Bavaria, SE Germany.

Methods: To evaluate the efficacy of the local seed mixtures, we compared the species composition of seed mixtures and current vegetation. We then tested whether restoration success depends on site characteristics such as the size and shape (rectangle or stripe) of the grassland, restoration procedures such as topsoil removal, seed density and land use, or species habitat preferences for light, water and nutrients, and species life span (annual, perennial).

Results: On average, the current vegetation contained 62.4% of all species that were present in the local seed mixtures. Species from the local seed mixtures made up on average 69.1% of the total cover in the established vegetation, whereby the species composition of the local seed mixture and vegetation differed significantly from each other. The probability that a sown species would establish increased with seed density up to 300 seeds/m². Furthermore, habitat preferences significantly affected species establishment chances, with species requiring full illumination, dry and nutrient-poor soil being more successful during restoration, reflecting the high proportion of sites with topsoil removal prior to seeding in our study. Annual species had significantly lower establishment chances compared with their perennial counterparts.

Conclusions: Our study provides another piece of evidence that local seed mixtures can be applied successfully in large-scale grassland restoration projects. We provide several practical recommendations of how such practices can be further improved by using specific seed densities and creating new local seed mixtures using species that are ecologically more suitable to the restored sites.

KEYWORDS
local seed mixture, restoration, species diversity, species trait, species-rich grassland, vegetation composition
1 | INTRODUCTION

Grasslands have tremendous ecological significance because they represent a large part of all terrestrial habitats. For example, in Europe, grasslands cover about 1.8 million ha (Carlier et al., 2005), which corresponds to approximately 40% of the land surface. These grasslands provide key ecosystem services including carbon sequestration, protection from erosion and harbour many plant and animal taxa (Dengler et al., 2014).

Currently, grasslands are threatened by modern land use practices (Sala et al., 2000). Increased fertilization and mowing frequencies have caused ongoing loss of species (Klaus et al., 2013; Socher et al., 2012) and this process is enhanced by the increased deposition of atmospheric nitrogen since the middle of the 20th century (Diekmann et al., 2014; Wesche et al., 2012). The restoration of species-rich grasslands is therefore on the nature conservation agenda worldwide.

Modern grassland restoration practices are largely based on the application of local seed mixtures (Jongepierová et al., 2007; Kiehl et al., 2010; Töörö et al., 2010; Walker et al., 2015). The main idea of this approach is that seed mixtures used in restoration should be produced from natural seed material originating in the regions where the restoration practices take place. A main advantage of using local seed material for restoration is that autochthonous plant populations are considerably better adapted to local environmental conditions compared with non-local populations (McKay et al., 2005; van der Mijnsbrugge et al., 2010). Furthermore, the local seed mixtures are recommended for use to avoid potential outbreeding effects (Hufford & Mazer, 2003) and increase restoration success (Sackville Hamilton, 2001). In Germany, for example, the regions for local seed mixture production have been mainly defined based upon region-specific geomorphologic and climatic parameters (Prasse et al., 2010) also taking into account the genetic variability of plant species (Durka et al., 2017; Listl et al., 2017, 2018). These principles have also been implemented in seed production; currently, producers in different parts of the country offer a range of ‘restoration-ready’ local seed mixtures.

Despite their strong relevance for grassland restoration, the success of local seed mixtures in practice has hardly been verified. Previous research has demonstrated that topsoil removal before sowing (Rasran et al., 2007) or disturbance (Freitag et al., 2021), and the use of high-diversity seed mixtures (Kirmer et al., 2012) can increase restoration success. Moreover, sowing density and post-restoration land use can also have an impact on grassland restoration (Kiehl et al., 2010). Furthermore, restoration success may depend on site characteristics such as the size and shape (elongated or regular) of the restored grassland, which in turn affects rates of fertilizer and herbicide deposition from nearby arable fields (Duncan et al., 2008). Finally, species characteristics such as life history, or habitat preferences, e.g. in terms of light or water requirements for successful establishment, may also have an impact on restoration success.

Yet, restoration recommendations are mainly based on experimental studies (Freitag et al., 2021; Kiehl et al., 2010) under more or less controlled conditions that can be very different from real-world restoration projects. Here, the restoration process is usually less standardized because of the involvement of different actors with different requirements for the restoration goals (e.g. conservation agencies, farmers, local stakeholders). Furthermore, local abiotic and biotic conditions in specific restoration projects can deviate strongly from published studies because of their local nature (Prach et al., 2013). Therefore, data from real-world restorations are much needed to evaluate the success of grassland restoration under real-life conditions. In our study, we attempted to close this gap by analysing the success of local seed mixtures in a large-scale grassland restoration study conducted at 35 sites in southeastern Germany where about 15 years ago grassland was restored using different local seed mixtures. First, we estimated whether the species composition of the restored grasslands was similar to that of the sown local seed mixtures. We then evaluated the effects of site characteristics, restoration procedures and species characteristics on the establishment success of individual species present in the local seed mixtures. More specifically, we asked the following questions: (a) Are local seed mixtures a tool that can be used successfully to restore species-rich grassland in practice? (b) Is the restoration success of local seed mixtures at the community and species level depending on site characteristics, restoration procedures, species habitat preferences and plant life span? Based on the results of our analyses, we make practical recommendations for the further application of local seed mixtures in grassland restoration.

2 | METHODS

2.1 | Study design

For our investigation we selected 35 study sites at 11 different geographic locations in southeastern Germany (Figure 1; Table 1). Between 2003 and 2006, grasslands were restored on ex-arable fields by application of local seed mixtures within the framework of land consolidation projects. Based upon information from the land consolidation agency (Amt für Ländliche Entwicklung Oberpfalz) and the seed producers, we identified the local seed mixtures used for restoration at each site, their species composition and the total weight of the seed mixture (kg) applied for restoration (Appendix S1). Using the total weight of the applied seed mixture, the relative proportion of each species in a mixture (%) and average seed weight (http://data.kew.org/sid/sidsearch.html), we calculated the seed density for each species and mixture as number of sown seeds/m².

For each study site, the year of the restoration, size and shape (elongated or regular) of the site as well as the potential application of pre-restoration soil preparation (topsoil removal or none) and/or post-restoration land use (mowing, mulching or none) was recorded (Table 1).

The species composition of the restored grasslands was surveyed at each study site (Appendix S2). For this purpose, vegetation surveys were conducted in five randomly distributed study plots with
a size of 2 m × 2 m. All species occurring in the plots were identified and their cover was estimated using a decimal scale (Londo, 1972).

2.2 | Data analysis

Based on data on seed mixture composition and the vegetation surveys we calculated for each plot: (a) the relative proportion of sown species that were in the seed mixture ($SR_{rel}$); and (b) their cumulative abundance ($AB_{rel}$) in the established vegetation, as two simple indices (Table 1).

To estimate the degree of floristic similarity between seed mixture composition and current vegetation of the restored grasslands, we performed Non-metric Multidimensional (Distance) Scaling (NMDS) with species composition of the established vegetation and the corresponding seed mixtures (species presence/absence matrices) based on Bray–Curtis similarity index using PC-ORD (version 7, MJM Software Design, Corvallis, OR, USA).

The NMDS was calculated with 50 runs of real data and 50 randomized (by row) runs with a stability criterion of 0.00001 and a maximum of 200 iterations. We used standard stepdown procedures to find the appropriate number of axes sufficient to reduce
stress. Correlations between seed mixture composition and ordination scores for the established species and sites were quantified using Spearman’s rank correlation as suggested previously (McCune et al., 2002). Visual inspection of the ordination diagrams revealed differences between the composition of the applied seed mixtures and established vegetation. Therefore, a multi-response permutation procedure (MRPP) implemented in the PC-ORD software was used to test whether this difference was statistically significant.

<table>
<thead>
<tr>
<th>ST</th>
<th>YE</th>
<th>SI</th>
<th>SH</th>
<th>TR</th>
<th>LU</th>
<th>SD</th>
<th>SRmix</th>
<th>SRrel</th>
<th>SRpro</th>
<th>ABmix</th>
<th>ABrel</th>
<th>ABrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA01</td>
<td>2005</td>
<td>0.27</td>
<td>R</td>
<td>+</td>
<td>NO</td>
<td>970</td>
<td>14.2</td>
<td>21.8</td>
<td>65.5</td>
<td>625.2</td>
<td>808.2</td>
<td>77.0</td>
</tr>
<tr>
<td>HA05</td>
<td>2005</td>
<td>0.17</td>
<td>R</td>
<td>+</td>
<td>NO</td>
<td>147</td>
<td>9.6</td>
<td>16.8</td>
<td>57.0</td>
<td>531.4</td>
<td>649.0</td>
<td>81.8</td>
</tr>
</tbody>
</table>

**Note:** For each site, the density of the sown seeds (SD) is given as number of seeds/m². For each site, the number of species in the applied seed mixture $SR_{mix}$, the total number of species in the established vegetation $SR_{rel}$, and the proportion (%) of sown species in the established vegetation $SR_{pro}$ are given. We also determined the cumulative abundance of sown species in the established vegetation ($AB_{mix}$), the cumulative abundance of all species in the established vegetation ($AB_{rel}$), and the relative abundance of sown species in the established vegetation ($AB_{rel}$). ST: study site; YE: year of grassland restoration; SI: size (ha); SH: shape; TR: soil preparation; +: topsoil removal; -: no topsoil removal; LU: land use; NO: none; MW: mowing; MU: mulching.
To estimate the efficacy of seed mixtures in grassland restoration, we calculated a Generalized Linear Mixed-effects Model (GLMM, family 'Binomial') to analyse whether the establishment of species from the seed mixtures is affected by site characteristics, restoration procedures, density of sown seeds and species characteristics (Table 2). The response variable in the model was relative abundance (from 0 to 1, where 1 corresponds to an abundance of 100%) of a sown species in the established vegetation (Appendix S1). Pre-restoration soil preparation (topsoil removal or not), post-restoration land use (no land use, mowing, mulching), shape of restored grasslands (elongated or regular) and species characteristics were used as fixed factors. Species characteristics included species-specific density of sown seeds (both linear and nonlinear terms), habitat preferences expressed as Ellenberg indicator values (EIV) for light (L), soil moisture (F) and nutrients (N), and four functional traits (life span, specific leaf area, plant height and seed size) (Appendix S1). The EIV are proxies for the habitat requirements of adult plants and, except for the F value (the highest value of which is 12), range from 1 to 9, with the highest numbers indicating high requirements for the corresponding environmental factor (e.g. an N value of 9 indicates a species occurring on soils with high nutrient contents). The EIV for light was included in the model as an interaction term with post-restoration land use to infer the possible positive effects of land use (particularly mowing) on the establishment of sown seeds with different requirements for light. In the same vein, we considered topsoil removal to influence considerably the soil properties in the restored sites. To account for such effects on species establishment from the sown seeds, the EIV values for soil moisture and nutrients were included in the model as interaction terms with the pre-restoration soil preparation. Data on life span were extracted from the LEDA database (Kleyer et al., 2008). The model included sites and plant family as random effects to account for site- and family-specific variation in restoration success, respectively. The seed density values in the models were log-transformed to improve the normality of the residuals. Collinearity was not a problem in all models because the explanatory variables were only weakly correlated with each other. Model assumptions were met in all cases. All statistical analyses were conducted in the R statistical environment (R Core Team, R Foundation for Statistical Computing, Vienna, AT). The GLMM was fitted with the help of the lme4 package (Bates et al., 2015).

### RESULTS

In terms of species richness, seed mixture success varied between 23.2% (site HE_03) and 87.9% (site RA_01) with an average of 62.4% (Figure 2a). The average relative abundance of sown species in the established vegetation was 69.1% (Figure 2b), ranging from 22.7% (site HE_03) to 95% (site NE_02).

The NMDS (two-dimensional, final stress = 16.5) ordination accounted for 75.5% of total variance in seed mixture and established vegetation composition (when correlating the original distance matrix with distances in ordination space) of which 45.4% and 30.1% could be attributed to axis 1 and axis 2, respectively.

The ordination diagram revealed clear differences in species composition between applied seed mixtures and established vegetation (Figure 3); the results from the MRPP (A = 0.05, T = -9, p < 0.0001) indicated that these differences were statistically significant. The grass *Dactylus glomerata* was one of the most frequent species in established vegetation, whereas the frequency of many herbs, such as *Campanula rotundifolia*, *Galium verum*, *Hypericum perforatum* and

### TABLE 2

Results of Generalized Linear Mixed Model with the relative abundance of sown species in the established vegetation (AB<br>rel) as the response variable

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Estimate</th>
<th>± Std Err</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.63</td>
<td>0.35</td>
<td>0.405</td>
</tr>
<tr>
<td>Shape (elongated)</td>
<td>0.93</td>
<td>0.35</td>
<td>0.845</td>
</tr>
<tr>
<td>log(Seed density)</td>
<td>1.51</td>
<td>0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log(Seed density)^2</td>
<td>0.95</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Life span (perennial)</td>
<td>7.33</td>
<td>1.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-processing (intact soil): EIV soil moisture</td>
<td>0.86</td>
<td>0.05</td>
<td>0.010</td>
</tr>
<tr>
<td>Pre-processing (topsoil removal): EIV soil moisture</td>
<td>0.92</td>
<td>0.03</td>
<td>0.003</td>
</tr>
<tr>
<td>Pre-processing (intact soil): EIV soil nutrients</td>
<td>1.04</td>
<td>0.04</td>
<td>0.391</td>
</tr>
<tr>
<td>Pre-processing (topsoil removal): EIV soil nutrients</td>
<td>0.88</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Management (no management): EIV light</td>
<td>0.71</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Management (mowing): EIV light</td>
<td>0.76</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Management (mulching): EIV light</td>
<td>0.82</td>
<td>0.07</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Note: Pre-restoration soil preparation (topsoil removal or not), post-restoration land use (no land use, mowing, mulching) and species characteristics were used as fixed factors. Species characteristics included species-specific density of sown seeds (both linear and nonlinear terms), habitat preferences expressed as Ellenberg indicator values (EIV) for light (L), soil moisture (F) and nutrients (N). Entries in bold are significant (p < 0.05).
Origanum vulgare, was much higher in the seed mixtures than in the established vegetation.

The GLMM revealed several significant effects of the restoration procedure and species traits on the abundance of species from the local seed mixtures in the established vegetation (Table 2; Figure 4). To begin with, the number of sown seeds positively affected the success of a species. However, this effect was detected only for the interval from 1 to approximately 300 seeds/m²; seed densities above 300 seeds/m² had a small, yet significant negative effect on the restoration success (Figure 4a). Second, the species occurring in open, fully illuminated habitats (i.e. with a lower EIV for light) had significantly higher success rates; none of the management types had a significant impact on this relationship (Figure 4b). Third, species with low requirements for water (i.e. with lower EIV for soil moisture) also displayed significantly higher success rates (Figure 4c); this relationship was significantly weaker at the sites with pre-restoration soil preparation by topsoil removal. Fourth, species occurring on nutrient-poor soils (i.e. with lower EIV N values) had a significantly higher probability of establishing in the restored grasslands at sites with topsoil removal; this effect was not detected at sites without pre-restoration soil preparation (Figure 4d). Finally, life span was found to have a significant impact on success rates with perennial species being present in higher proportions in the established vegetation compared with annuals (Figure 5).

4 | DISCUSSION

In our study, about two-thirds of all species present in the applied seed mixtures were found at the study sites in relatively high
The observed high proportion of sown species in the established vegetation was largely in line with former studies; the most comprehensive review on grassland restoration using seed mixtures reported establishment rates of 32% to 96% (Kiehl et al., 2010). Similarly, Kirmer et al. (2012) observed an establishment rate of 67% for high-diversity seed mixtures in the ecological restoration of surface mined land to grassland. Moreover, the relative abundance of the sown species in the plots was ca. 70%, which means that on average more than two-thirds of the plots were covered by sown species. Only at 7 of 35 study sites was the relative abundance of spontaneous species larger than the relative abundance of sown species. Our study supported, therefore, the observation that the application of local seed mixtures is, in general, a well-suited approach to restore species-rich grassland in practice.

Despite the comparatively high success rates, multivariate analysis revealed considerable differences in the species composition of seed mixtures and restored vegetation. The most parsimonious explanation is that many herbaceous species, which established in the first few years after application of the local seed mixture, disappeared in later years because of competition with competitive grasses. The GLMM results suggest that uncompetitive annual species were most affected by this process. Consequently, light-demanding herbs included in the seed mixtures, such as *Campanula rotundifolia*, *Galium verum*, *Hypericum perforatum* or *Origanum vulgare*, declined over time, whereas competitive grasses like *Arrhenatherum elatius*, *Festuca pratensis* or *Dactylis glomerata* increased. This line of argument is supported by research showing...
that grasses may out-perform herbaceous species in grassland restoration (Jongepierová et al., 2007; Pywell et al., 2003; Török et al., 2010). Moreover, the immigration of species from nearby located grasslands and the loss of species not compatible with the mowing regime (Prach et al., 2013), may also have contributed to the observed differences between the species composition of seed mixtures and current vegetation.

The GLMM revealed a strong impact of seed density on restoration success at the species level. In our investigation, at 24 of 35 study sites grassland was restored with less than 200 seeds/m², which is a significantly lower number of seeds than reported in previous studies (Kiehl et al., 2010). It is, therefore, obvious that we observed a strong relationship between restoration success and the number of sown seeds. Our results support previous studies showing that increasing seed density boosts the number of established individuals (Sheley & Half, 2006). Consequently, Carter and Blair (2012) and Barr et al. (2017) showed that higher seed density results in more successful grassland restoration.

The GLMM also revealed a strong impact of species habitat requirements on restoration success. Previous studies demonstrated that an appropriate balance of species with different ecological requirements is required to ensure quick restoration of species-rich grassland (Staab et al., 2015). In our study, we also observed that the establishment of species from seed mixtures depends on their habitat requirements. First, we found that annual species decline over time since the beginning of the restoration. This may be ascribed to the decline in short-lived and colourful species like Papaver rhoeas or Centaurea cyanus, which were added to the seed mixtures to create visually appealing grasslands soon after application of the seed mixture. These species, which are typical arable field weeds, disappear from the restored grasslands as the vegetation becomes gets denser, and habitat conditions are no longer suited to annual species such as arable weeds - a process that is often observed in the course of vegetation succession (Boscotti et al., 2017). Second, and even more interesting, we observed that the success of individual species in grassland restoration depends on the light, water and nutrient conditions required for establishment and persistence. Plant species adapted to bright, dry and nutrient-poor habitat conditions had a significantly higher probability of establishing and persisting in the restored grasslands, compared with species favouring other ecological conditions, particularly at study sites with topsoil removal. Therefore, our results corroborate the observation that top soil removal before restoration supports the establishment of grassland plant species adapted to relatively nutrient-poor site conditions (Rasran et al., 2007). Therefore, species composition of the restored grasslands, even after 20 years, still reflects the habitat conditions at the beginning of the restoration process. The open, dry and nutrient-poor environmental conditions shortly after topsoil removal represent a strong filter, selecting those species that were able to cope with these conditions, whereas other species were deleted from the species pool. Therefore, our results illustrate clearly that the interaction of habitat conditions and realized niche requirements has a large impact on the development of the restoration process.

Based on our results, two general recommendations can be made for the successful restoration of species-rich grassland with local seed mixtures in the future. First, seeding density should always be high enough to ensure successful restoration – following our results there is probably no additional benefit from sowing much in excess of 300 seeds per m² per species because establishment success levels off thereafter.

Second, it is advantageous when seed mixtures contain mainly species favouring light, dry and nutrient-poor habitat conditions, particularly when topsoil removal has been applied to prepare the restoration sites, because these species have a significantly higher probability of establishing and persisting in the restored grasslands.

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AUTHOR CONTRIBUTIONS
CR conceived of the research idea. FK collected the data. SR performed statistical analyses. All authors contributed to manuscript writing.

DATA AVAILABILITY STATEMENT
Data are available as supplementary files (Appendix S1 and S2).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

Appendix S1. List of species from seed mixtures, site characteristics and species traits

Appendix S2. Vegetation surveys