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► To cite this version:

Myriam Garrouj, Didier Alard, Jennifer Dudit, Marie-Lise Benot. Transfer seeds, hay, or soil blocks? The importance of the completeness of biological inputs to address dispersal and establishment limitations during the restoration of plant assemblages in floodplain grasslands. *Restoration Ecology*, 2024, 32, 10.1111/rec.14092 . hal-04642204

HAL Id: hal-04642204

<https://hal.inrae.fr/hal-04642204v1>

Submitted on 9 Jul 2024

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RESEARCH ARTICLE

Transfer seeds, hay, or soil blocks? The importance of the completeness of biological inputs to address dispersal and establishment limitations during the restoration of plant assemblages in floodplain grasslands

Myriam Garrouj¹ , Didier Alard¹ , Jennifer Dudit¹, Marie-Lise Benot^{1,2} 

This study investigated different techniques of grassland restoration to overcome dispersal or establishment limitation, which are key processes influencing early-successional plant community assembly. A fully randomized in situ experiment was set up in a former arable land in a floodplain along the Garonne river (south-western France) to test for the effect of (1) the type and completeness of the biological input (any biological material—seed, hay, and soil—transferred to the site under restoration), (2) soil disturbance by deep tillage, and (3) their interaction on plant community dynamics for 5 years. All inputs influenced the plant community structure and composition. The effects depended on the type of the input but not necessarily in link with its completeness. High density and diversity seed mixture led to high levels of richness and relative abundance of target species like soil blocks, the input considered as the most complete. During the first year, hay transfer mostly influenced community assembly through negative litter effects. Delayed germination of several species contributed to buffer these early effects. This study supports the importance of dispersal limitation during early succession in degraded grassland ecosystems whereas soil disturbance had only subtle effects on the seed bank and standing plant community, indicating that competition and establishment limitation were of secondary importance in our study system. Our results suggest that even low-frequency immigration events (e.g. when a few seeds are transferred within hay) can be of great importance providing that a sufficiently long time period is considered for grassland restoration.

Key words: deep tillage, hay transfer, seed mixtures, soil blocks, species composition, species richness

Implications for Practice

- Several types of biological inputs varying in composition and completeness are efficient to increase the richness and relative abundance of target species during the first years of floodplain grassland restoration.
- Sowing high diversity and density seed mixture enables to reach high levels of target species richness and abundance, while limiting heterogeneity among plots.
- Litter can negatively affect plant species establishment with consequences on species composition even 5 years after hay transfer. Though, this input allows the immigration of various species and delayed germination after litter degradation, which both limit negative first-year effects.
- Deep soil tillage only slightly affects the seed bank and mainly controls woody species, with minor consequences on vegetation restoration.

Introduction

Disentangling the processes that underlie vegetation dynamics has been a central subject of ecology for long. Though, in the current context of global change and ecosystem degradation worldwide, understanding the drivers of plant community

restoration trajectories still remains more crucial than ever, in order to answer the growing demand for biodiversity conservation and restoration (UNEP 2021). In particular, ecological restoration studies rooting within key concepts of community ecology should help to identify the drivers of restoration trajectories, thereby providing practical and applicable tools for managers (Perring et al. 2015; Wainwright et al. 2018). Evidence for plant community assembly in early succession has highlighted the central role of dispersal limitation (Myers & Harms 2009;

Author contributions: MG, DA, MLB conceived and designed the research; MG, DA, JD, MLB performed the experiment; MG, MLB analyzed the data; MG, DA, MLB wrote the first version of the manuscript; all authors contributed to the edition of the manuscript.

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doi: 10.1111/rec.14092

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Marteinsdóttir 2014) that influences colonization, and of establishment limitation, which reflects species' ability to establish under given environmental conditions (Nathan & Muller-Landau 2000; Turley et al. 2017). After damage, degraded ecosystems can be highly resilient (Standish et al. 2014; Perring et al. 2015), and the reestablishment of species through spontaneous succession (often referred to as "passive restoration") may take a long time because of the loss of these species from current vegetation and from soil seed bank (Donath et al. 2007). Dispersal limitation is one of the major obstacles to restoration (Turley et al. 2017; Halassy et al. 2019), especially in fragmented landscapes (Butaye et al. 2002). Additionally, species order of arrival can be an important driver of community assembly (Fukami 2015), especially under high nutrient level (Kardol et al. 2013). Such priority effects can profoundly affect restoration trajectories as competitive exclusion by already established vegetation may hamper seed germination and seedling emergence.

Several restoration methods applied in post-cultural lands seek to compensate for seed limitation and decrease competition from early-established, often undesired species (Kiehl 2010; Török et al. 2011). To counteract the lack of target species, biological inputs are usually needed, and seed and plant material transfer is one possible method to re-introduce target communities (Bischoff 2002; Török et al. 2011; Slodowicz et al. 2023). Composition drives the community assembly (Fukami et al. 2005) but restoration success is highly dependent on the sowing rates applied on the restored site (Applestein et al. 2018). Low-diversity seed mixtures are constituted of a few species, which represent dominant matrix species of the target community (sensu Boutin & Keddy 1993), whereas high diversity seed mixtures are generally composed of more than 10 species, containing rarer or interstitial species (Török et al. 2011). Hay transfer from source ecosystems is known as one of the most efficient methods tested in floodplain restoration (e.g. Török et al. 2011; Sengl et al. 2017) as it enables the transfer of seeds and fruits for a large set of species constituting the community (Albert et al. 2019). Finally, topsoil transfer enables to translocate not only seeds from the seed bank, standing vegetation and vegetative buds, but also potentially soil macro-fauna and microorganisms to the restored site, that is, species of importance in plant–soil interactions (Török et al. 2011; Bulot et al. 2014; Gerrits et al. 2023).

In addition to biological inputs, controlling the established vegetation of the site to be restored by, e.g. mowing, plowing and/or harrowing, to open competition-free windows, has also largely been suggested to improve the efficiency of ecological restoration operations on plant community trajectory (Czerwiński et al. 2018). This step is expected to favor the installation of the input brought in the restored site (Czerwiński et al. 2018). Topsoil removal is often proposed for a rapid and efficient reduction of nutrient availability (Jaunatre et al. 2014), but it is also expected to eliminate standing vegetation and topsoil seed bank, thereby reducing competition and preventing priority effects (Durbecq et al. 2021). As this method can be difficult to apply in all restoration schemes, tillage can be an alternative (Glen et al. 2017). At a larger scale, deep tillage of the soil can remove the cultural soil profile without extracting it. It can also be preferred to topsoil

removal as the removal of soil layers, by lowering land altitude, could induce modifications of hydrological functioning especially in floodplain grasslands. Like topsoil removal, deep tillage also supposes a destruction of the vegetation of nontarget species and their seed bank (Jaunatre et al. 2014). However, tillage and its effects on soil conditions for plant community establishment during a floodplain grassland restoration still remains quite unexplored (Czerwiński et al. 2018).

Here, we explore how the completeness of the biological input (i.e. seeds, hay, or soil blocks [SBs]) interacts with soil disturbance to drive a post-cultural early succession. The completeness of the biological input refers, for the biological material transferred to the restored site, to the quality of being whole (complete) and without any missing essential component as concerns, for our case study, the plant community of the reference ecosystem. In our experiment, the kinds of transferred plant material range from seeds mixtures sown in low or higher density and diversity, to hay composed of both plant vegetative parts (stems and leaves) and reproductive parts (fruits and seeds), and finally to SBs containing both aboveground and belowground organs of living plants, and potentially associated microorganisms. We assume that these kinds of biological inputs represent a gradient from a small fraction of the target plant community (seed mixtures) to the entire target plant community (soils blocks) at least at fine scale. The objective of this study was to investigate the effects of (1) the type and completeness of input brought at the beginning of the restoration process, (2) soil disturbance through deep tillage, and (3) their combination, on plant community dynamics during the first stages of a floodplain grassland restoration after several decades of cropping. A 5-year in situ experimental design was set up to test the following hypotheses: (H1) by overcoming dispersal limitation, increasing the completeness of the input constrains and fastens the vegetation dynamics toward the target plant community, (H2) deep soil tillage controls nontarget species already present in standing vegetation and soil seed bank, thereby weakening their potential competitive effect and establishment limitation.

Methods

Study Site

The study site (WGS84 44.901644°N, 0.556966°W) is located in the Ecological Reserve of the Barails, at the North of Bordeaux City (France), between the Garonne River and the National Nature Reserve of Bruges wetlands (NNRB). The site is usually submitted to winter flooding originating from rising groundwater. The experimental area (approximately 0.5 ha) presents a topographic elevation gradient of 40 cm from the South to the North (Fig. S1A).

In 1989, the floodplain grassland that formerly occupied the site was converted into maize and sunflower crops until 2014, when Bordeaux City services reclaimed the area in a compensatory measure program after the construction of new urban infrastructures. The city aims at the recovery of a floodplain function to host specific biodiversity (such as European mink [*Mustela lutreola*]) and the restoration of the floodplain grassland. Since

the cessation of cropping, the plant community has mainly been dominated by *Erigeron* spp., *Ranunculus sardous*, and *Helminthotheca echioides* (Bordeaux Métropole unpublished data).

Reference Site

Due to the absence of historical data on the site, we selected two wet donor grasslands in the NNRB (approximately 3.5 km at the West of the experiment) as a reference for restoration. This reserve, which was created in 1983, is constituted by a habitat mosaics (hedgerows, floodplains, ponds) covering 280 ha. Most of the reserve surface is composed of well-preserved floodplain grasslands that are principally managed by grazing and mowing, and submitted to similar hydrological conditions as the study site.

Plant communities were characterized in donor grasslands by the means of vegetation records carried out in 2013, 2014, and 2015 (NNRB unpublished data), and the vegetation classification and mapping produced by the National Botanical Conservatory of south-western France (CBNSA) in 2015 (SEPANSO 2019). Both grasslands were meso-hygrophilous, one belonging to the EUNIS class E2.211—Atlantic *Arrhenatherum* grasslands (WGS84 44.895993°N, 0.599038°W) and the other to E3.41—Atlantic and sub-Atlantic humid meadows (44.899721°N, 0.593842°W). Grasslands belonging to two habitat types were selected to take the variability of the floodplain grassland reference states into account.

Experimental Design

Vegetation was cut and exported in late August 2017. A fully randomized experiment was set up in September 2017, located at the northwest of the study site (Fig. S1). We tested two soil disturbance treatments, consisting either in soil tillage at 40 cm depth (T) or a control without tillage (NT), and five types of plant material transfer (thereafter “inputs”), including a control (C) without any input. Each combination of the soil treatments and the inputs was replicated on ten 4 m × 4 m plots ($n = 100$). Plots were separated from each other from at least 2 m. Paths of 7 m-wide were also included in the experimental design to enable machine passage (Fig. S1B).

Deep soil tillage was carried out with a backhoe in September 2017 (Fig. S2). The machines did not drive on the experimental plots to avoid soil compaction. Both low and high density and diversity inputs (LDD and HDD, respectively) corresponded to seed mixtures (respectively 5 vs. 13 sown species at a density of 3.4 vs. 4.8 g/m) composed on the basis on the presence and frequencies of the species in the donor grasslands (P. Grisser, NNRB unpublished data, personal communication) (Table 1). LDD input corresponded to a seed mixture of grassland plant species commonly used by local farmers for the sowing of temporary grasslands or grassland rehabilitation. It contained two legumes (20% of the number of seeds in the mixture), and three grasses (80% of the mixture; Table 1). This input also constituted the basis for the three other ones. HDD input consisted of the LDD seed mixture completed with seeds of other plant species encountered at varying frequencies in the donor grasslands. HDD input was thus composed of eight grasses, two legumes,

and three forbs (respectively 81.5, 10, and 8.5% of the mixture; Table 1). Due to the large amount of seeds required, seeds of all species but *Centaurea decipiens* and *Oenanthe pimpinelloides*, which were collected in situ in grasslands located about 16.5 km upstream along the Garonne river (Cadaujac, France, WGS84 44.760035°N, 0.522852°W), originated from organic-labelled crops produced by the Agro Bio Pinault society (Brittany, France). Both LDD and HDD inputs were manually sown in October 2017. The hay input consisted in the sowing of the LDD mixture completed by hay transfer. Hay was harvested in July 2017 in the reference grasslands, air-dried in situ for a few days, and transferred as bales to the experimental site, where it was stored until being manually spread in October 2017. A height of circa 30 cm of hay was spread over the 16 m² plots, representing a volume of circa 4.8 m³ on each plot. The SB input consisted of the sowing of the LDD mixture completed by the transfer of SBs (20 cm × 20 cm) with their standing vegetation extracted from the donor grasslands. Four SBs were transferred into each of the 20 SB experimental plots ($n_{\text{soil blocks}} = 80$). They were positioned in each corner of the plots, 1 m apart the plot side in November 2017 (Fig. S3).

The experimental design (experimental plots and paths) was mowed ever year in July with hay removal.

Seed Bank Survey

In early October 2017, that is, after soil tillage but before any input, eight soil cores (6 cm diameter, 15 cm depth) were randomly collected within one quarter of the experimental plots ($n_{\text{tot}} = 25$: $n_{\text{NT}} = 13$, $n_{\text{T}} = 12$) to check the effect of soil tillage on seed bank composition and abundance. For each soil treatment, experimental plots were selected at random. Soil cores were immediately placed into a plastic bag hermetically sealed and transported to the lab. Twenty-five culture trays (30 cm × 44 cm × 6 cm) were then filled with approximately 2 L of autoclave-sterilized river sand and the content of the soil cores collected into an experimental plot was manually mixed and spread into each tray. Experimental trays were installed in climatic chambers (SNIJDERS LABS MICRO CLIMA-SER-IESTM), regularly moistened and moved within and between climatic chambers (Supplement S1).

Seedling growth was surveyed for 13 months (from October 2017 to November 2018), until no further germination was observed. Seedlings were regularly determined to the genus or species level, counted, and removed. Taxon (genus or species) abundance corresponded to the total number of seedlings belonging to that taxon, counted over the whole course of the survey. Total abundance was calculated as the sum of each taxon abundance.

Vegetation Monitoring

In June 2018, 2019, and 2022, vegetation was monitored within each experimental plot, by the means of a pin-point method (Stampfli 1991; Garrouj et al. 2019). Vegetation records consisted of a 4 m² square quadrat located at the center of each experimental plot, 1 m apart from the plot side, to prevent side effects (Fig. S3). The pin-points were spaced from each other by 25 cm, resulting in

Table 1. Composition of the low and high density and diversity seed mixtures (respectively, LDD and HDD).

		Proportion of seeds in the seed mixture (%)	Number of seeds/m ²	Mass (g) of 1000 seeds	Mass (g) of seeds/m ²	Mass (g) of seeds per plot (16 m ²)
Composition of LDD seed mixture						
<i>Schedonorus arundinaceus</i>	Grass	30%	600	2.77	1.6620	26.5920
<i>Dactylis glomerata</i>	Grass	25%	500	0.86	0.4300	6.8800
<i>Lotus corniculatus</i>	Legume	15%	300	1.35	0.4050	6.4800
<i>Lolium perenne</i>	Grass	25%	500	1.68	0.8400	13.4400
<i>Trifolium repens</i>	Legume	5%	100	0.74	0.0740	1.1840
Total		100%	2000	—	3.4110	54.5760
Species added to LDD for HDD seed mixture						
<i>Holcus lanatus</i>	Grass	30%	300	0.27	0.0810	1.2960
<i>Arrhenatherum elatius</i>	Grass	30%	300	1.67	0.5010	8.0160
<i>Anthoxantum odoratum</i>	Grass	10%	100	0.59	0.0590	0.9440
<i>Elytrigia repens</i>	Grass	10%	100	5.25	0.5250	8.4000
<i>Centaurea decipiens</i>	Forb	8%	80	1.40	0.1120	1.7920
<i>Oenanthe pimpinelloides</i>	Forb	8%	80	1.44	0.1152	1.8432
<i>Leucanthemum vulgare</i>	Forb	1%	10	0.53	0.0053	0.0848
<i>Alopecurus pratensis</i>	Grass	3%	30	1.03	0.0309	0.4944
Total		100%	1000	—	1.4294	22.8704
Final composition of HDD seed mixture						
<i>S. arundinaceus</i>	Grass	15%	600	2.77	1.6620	26.5920
<i>D. glomerata</i>	Grass	12.5%	500	0.86	0.4300	6.8800
<i>L. corniculatus</i>	Legume	7.5%	300	1.35	0.4050	6.4800
<i>L. perenne</i>	Grass	12.5%	500	1.68	0.8400	13.4400
<i>T. repens</i>	Legume	2.5%	100	0.74	0.0740	1.1840
<i>H. lanatus</i>	Grass	15%	300	0.27	0.0810	1.2960
<i>A. elatius</i>	Grass	15%	300	1.67	0.5010	8.0160
<i>A. odoratum</i>	Grass	5%	100	0.59	0.0590	0.9440
<i>E. repens</i>	Grass	5%	100	5.25	0.5250	8.4000
<i>C. decipiens</i>	Forb	4%	80	1.40	0.1120	1.7920
<i>O. pimpinelloides</i>	Forb	4%	80	1.44	0.1152	1.8432
<i>L. vulgare</i>	Forb	0.5%	10	0.53	0.0053	0.0848
<i>A. pratensis</i>	Grass	1.5%	30	1.03	0.0309	0.4944
Total		100%	3000	—	4.8394	77.4464

64 points within the record. At each point, a coefficient of one was attributed to every species contacted by a metal rod inserted vertically at that point, and a coefficient of 0.5 was attributed to any species present inside the record but not contacted at any point. Raw species abundance per record thus ranged between 0 and 64. Contacts with bare soil were also recorded.

Data Analyses

Both for seed bank and standing vegetation data, taxonomic nomenclature was homogenized; when several observations were determined only at the genus level and may be confused with species of the same genus, all observations belonging to that genus have been grouped at that level (e.g. *Alopecurus* spp., *Veronica* spp.). However, when determinations made at the genus level could not be confounded with other species of that genus, we kept them distinguished (e.g. *Carex hirta*, *Carex* sp., the latter being with no doubt different from *C. hirta*). All woody species except *Rubus* sp. were grouped under a single denomination (tree species). Undetermined and questionable observations that cannot be grouped with other observations were removed from the dataset for further analyses.

Standing vegetation data were split into three datasets corresponding to each surveyed year. For each vegetation record, the proportion of bare soil was calculated as the number of pin-points where bare soil was observed divided by the total number of contacts (plants species and bare soil) for this record. Bare soil was then removed from the dataset for further analyses on vegetation only. Three groups of species were identified. Target species corresponding to all the species encountered in the donor grasslands and species constituting the LDD and HDD seed mixtures constituted the “target species group” (*Target* species). The species encountered in the non-tilled control (NT.C) plots in 2018, excluding species already defined as *Target* species, constituted the “degraded species group” (*Deg* species). Remaining species that were included in neither of these groups constituted the “other species group” (*Other* species). Due to the localized aspect of vegetation records used to select donor grasslands, some species potentially present might have been missed. Thus, a few species that could be considered as typical of reference floodplain grasslands were classified as *Other* and not *Target* species (*Althaea officinalis*, *Hypericum perforatum*, *Medicago* spp., *Plantago lanceolata*, and *Trifolium pratense*), but they were only found at low frequency

(respectively one, three, seven, three, and seven occurrences over 300 vegetation records). The relative abundance of each species group within each vegetation record was calculated as the sum of raw species abundances of every species belonging to that group divided by the sum of raw species abundances of all species encountered in that record, with bare soil excluded from the calculation. Species richness of each species group and total species richness were also calculated per plot (alpha species richness). To assess potential heterogeneity among plots of a given treatment, overall species richness of each species group (gamma species richness) was calculated for each treatment (interaction of the input and soil disturbance treatments, $n = 10$ replicates) and for each input ($n = 20$ replicates).

We tested the effect of soil treatment on total abundance and taxonomic richness of the seed bank using generalized linear models (GLM) with Poisson error distribution and log link. Nonmetric multidimensional scaling (NMDS) ordination was applied on taxon abundances, and differences in seed bank taxonomic composition between non-tilled and tilled plots were tested using permutational multivariate analysis of variance (PERMANOVA) with 199 permutations. Indicator taxa of each soil treatment were searched for using the IndVal method (Duf rene & Legendre 1997).

To test for potential treatment effects on the heterogeneity of species composition of standing vegetation among plots, we calculated the distance of each replicate plot to its group centroid (one group being one experimental treatment, i.e. the combination of input and soil disturbance treatment) for each year, by the means of principal coordinate analysis (Anderson et al. 2006). The greater the average distance to the treatment centroid, the higher the heterogeneity of species composition among plots of that treatment (beta-diversity). Even if some variance heterogeneity was detected, one NMDS was carried out for each surveyed year, and the effect of the input, the soil treatment and their interaction was tested using PERMANOVA ($n = 199$ permutations). We visualized plot dispersion by plotting confidence ellipses (Supplement S2) for a better interpretation of differences between experimental treatments, especially when heterogeneity of variances was previously detected. Multivariate analyses were carried out on Bray–Curtis dissimilarity matrices calculated on raw abundance data. Two reduced dimensions were kept in all NMDS.

For each surveyed year, the effect of the input, the soil treatment and their interaction, was tested using GLM with Poisson error distribution and log link for total and per group species richness, and linear models (LM) with normal error distribution and identity link for bare soil proportion, the proportion of each species group within the vegetation and the distance of the plots to their group centroid. Residuals were visually inspected and, when necessary, data were transformed prior to analyses to meet residual normality requirements. When outliers were detected, models without outliers were also tested in order to identify their effect on the results. Pairwise contrasts among least-square means were used to test for difference between multiple factor levels.

All statistical analyses were performed in R software version 4.1.5 (R Core Team 2022). R packages *car* (Fox & Weisberg 2019) and *emmeans* (Lenth 2022) were used for LM and pairwise contrasts analyses, *vegan* (Oksanen et al. 2020) was used for

multivariate analyses, and *labdsv* (Roberts 2019) was used for the identification of indicator species. Graphical representations were drawn using *ggplot2* (Wickham 2016).

Results

Soil tillage significantly decreased seed bank total abundance (NT 130.1 ± 50.5 , T 104.6 ± 45.0 , $\chi^2 = 508$, p value < 0.001), but had no effect on seed bank taxonomic richness, which was on average 8.9 ± 2.3 per plot. A significant effect of soil treatments on seed bank taxonomic composition was detected despite a small amount of explained variance (Fig. S4), and no indicator taxon (IndVal) detected.

In 2018, the proportion of bare soil varied between 28.1 ± 7.1 and $91.3 \pm 8.3\%$: it was the highest for hay transferred plots, especially when combined with deep tillage (Hay.T), but not different among other inputs (Tables S1 & S2). The proportion of bare soil decreased during the experiment, to a total average of $3.5 \pm 3.3\%$ in 2022 (Tables S1 & S2).

In 2018, with the exception of the hay input, the more complete the input, the higher total species richness (Fig. 1), mostly due to the input effect on the species richness of the *Target* species group (Table S3; Fig. 1). *Deg* species richness was lower in plots submitted to the combination of hay transfer and soil tillage (Hay.T) than for all other treatments (Table S3; Fig. 1). The number of *Other* species was low, but was significantly increased by soil tillage. It was also significantly different between hay and SB inputs, and intermediate for all other inputs (Table S3; Fig. 1). These species represented such a small proportion of the vegetation that it was not possible to test for any treatment effect. In 2018, the vegetation remained mostly dominated by *Deg* species (between 52.4 ± 31.9 and $97.3 \pm 2.1\%$ of the vegetation cover). This proportion was rather variable for the Hay.T plots (important standard error; see also Table S1), but was globally much higher for C than for any other input (Fig. 2A).

In 2019 and 2022, species richness only depended on the input, increasing with its completeness (Table S3; Figs 3 & S5). By the end of the experiment, however, only SB input led to a significantly higher species richness than in C (Table S3; Fig. 3). These patterns were only linked with the effects of the inputs on the *Target* species group (Fig. 3). The relative abundance of *Target* species was significantly higher in LDD, HDD, and SB than in hay and C inputs, both in 2019 and 2022 (Table S1; Figs 2B & S6). In 2022, the relative abundance of *Other* species only depended on the input, being the highest in hay and the lowest in HDD plots (Table S1; Fig. 2B).

From 2018 onward, SB was the input reaching the highest gamma species richness of both *Target* and *Other* species (Table S6). Inter-plot heterogeneity (reflected by the mean distance to centroids) of the hay input was significantly higher than all other inputs in 2018 (Fig. S7). In 2019, deep soil tillage significantly decreased inter-plot heterogeneity for all inputs (Fig. S7). This effect was no longer detected in 2022. At that date, inter-plot heterogeneity was significantly lower for the HDD than for the hay input, and tended to be lower than all other inputs (C, LDD, and SB, but p value < 0.1 only; Fig. S7).

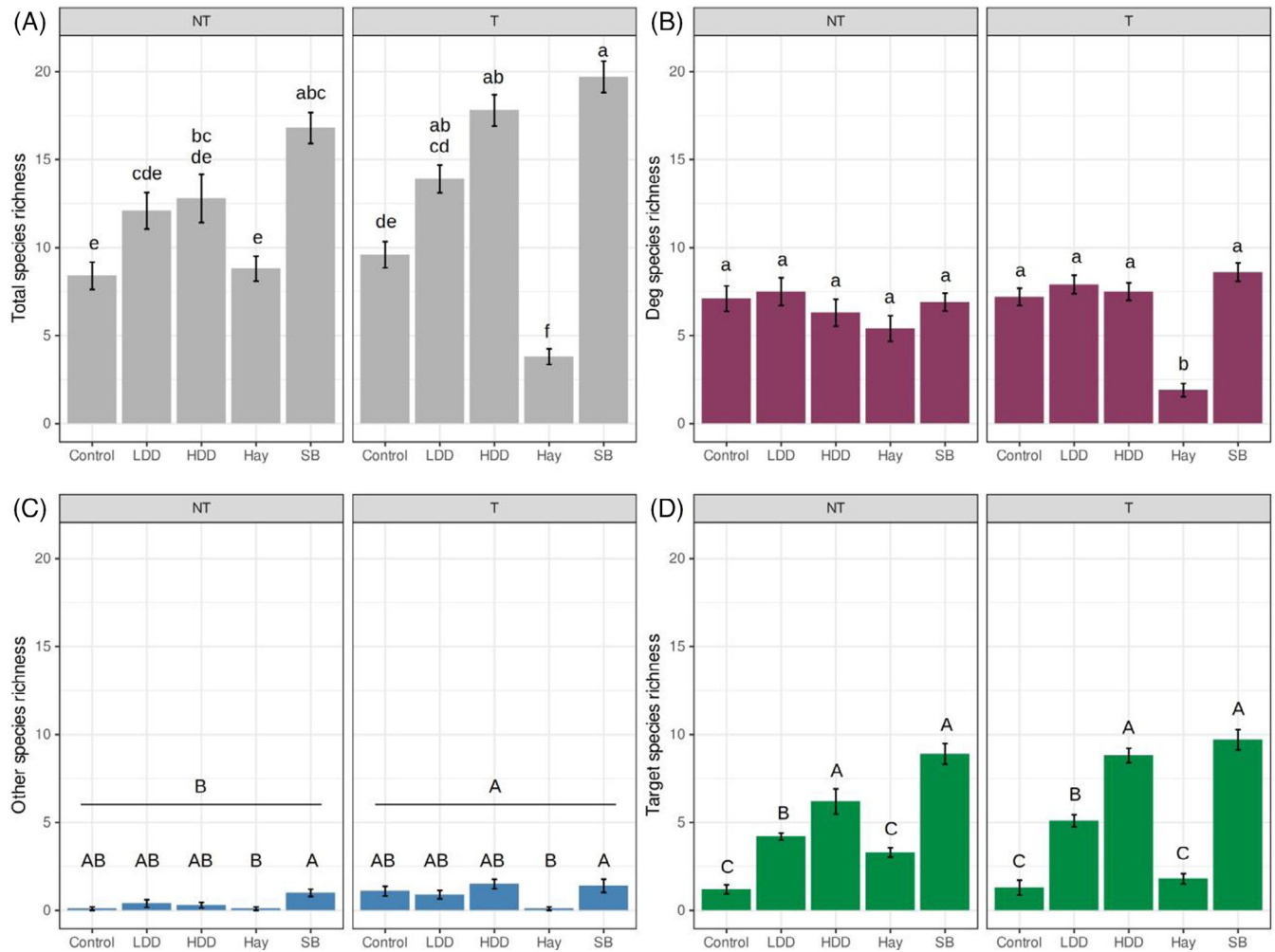


Figure 1. Mean \pm SE total species richness (A) and species richness of the Deg (B), Other (C), and Target (D) species groups in 2018. Upper-case letters indicate significant differences between soil disturbance treatments or inputs; lower-case letters indicate significant differences between the interaction the soil disturbance treatments and inputs.

Species composition was significantly affected both by the type of input and the soil treatment, but not by their interaction (Figs. 4 & S8). In 2018, C and hay inputs were discriminated mostly along the first NMDS axis, while LDD, HDD, and SB plots projected mostly on the negative side of the second NMDS axis (Fig. 4). For that year, one species belonging to the *Deg* species group was significantly indicator of the C input and one species belonging to the *Target* species group was indicator of the hay input, but this species was already present in the seed bank before the onset of the experiment (Tables S4 & S5). One *Deg*, one *Other*, and four *Target* species (all belonging to the LDD seed mixture) were indicators of the LDD input. Three *Target* species belonging to the HDD seed mixture were indicators of the HDD input. Mostly *Target* species were indicators of the SB input. One *Other* species, *Rubus* sp. and other woody species were indicators of NT plots (Tables S4 & S5). Mostly *Target* and *Other* species, half of which were detected in the seed bank (Tables S4 & S5), were indicators of the tilled plots.

In 2022, the first NMDS axis represented a gradient of inputs with, from its negative to its positive sides: C, hay, LDD–HDD,

and SB plots (Fig. 4), a pattern which was already visible in 2019, but with a clearer discrimination between C and hay plots on one side, and LDD, HDD, and SB plots on the other (Fig. S8). One *Target* species, (*Lathyrus nissolia*), which was totally absent from the experimental plots in 2018, was found in three plots in 2019 (one Hay.NT and two Hay.T plots) and occurred in 37 (7 C, 5 LDD, 5 HDD, 11 hay, and 9 SB plots) over the 100 experimental plots in 2022. Three *Target*, one *Other*, and one *Deg* species were indicator of NT plots in 2022 (Table S5). At that date, C plots were characterized by one *Deg* and two *Other* species, while several *Target* and *Other* species were indicators of the other inputs (Table S5).

Discussion

Dispersal Limitation and Effects of the Input Completeness

Biological inputs increased either *Target* species relative abundance, *Target* species richness or both, and resulted in a different species composition from the control plots. However, our first

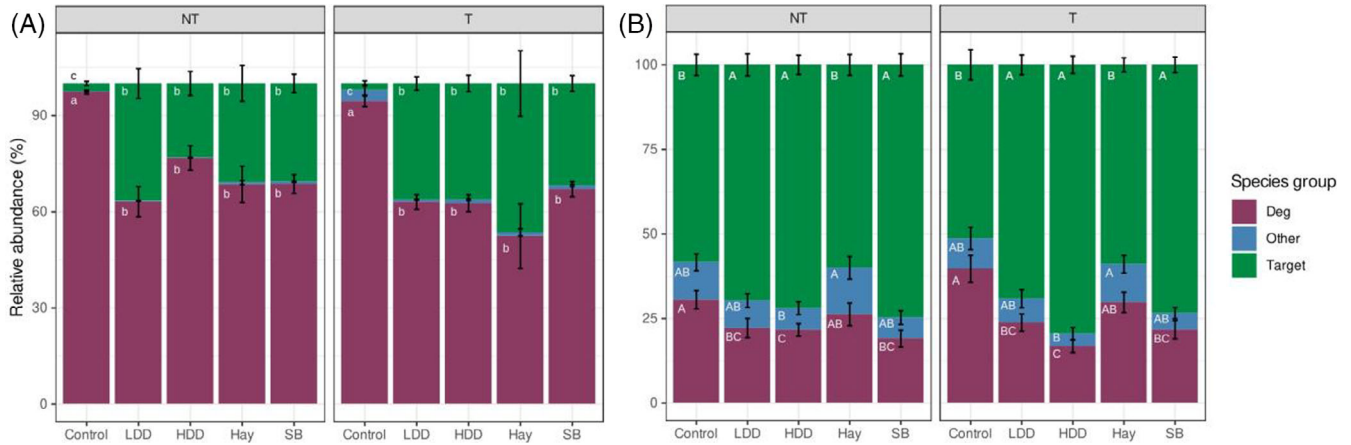


Figure 2. Mean \pm SE relative abundance of each species group within the vegetation in 2018 (A) and 2022 (B). See Figure 1 for the meaning of different letters.

hypothesis was only partially supported, as the effect of the inputs on species composition and on the *Target* species group, was not clearly linked with their expected completeness.

Seed sowing enabled a rapid and significant installation of *Target* species together with a decrease of the relative abundance of initial nontarget (*Deg*) species (Kiehl 2010; Török

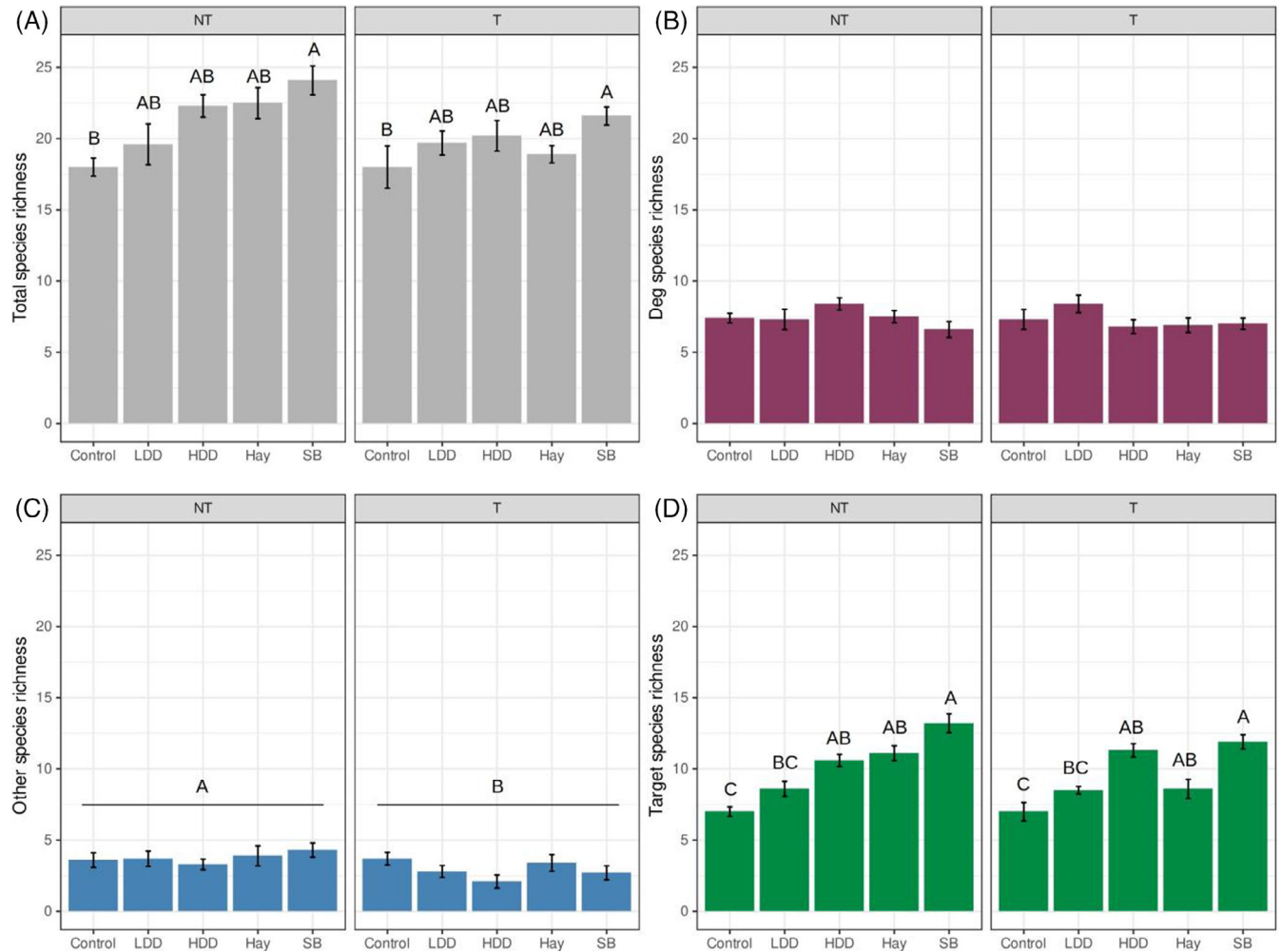


Figure 3. Mean \pm SE total species richness (A) and species richness of the Deg (B), Other (C), and Target (D) species groups in 2022. See Figure 1 for the meaning of different letters.

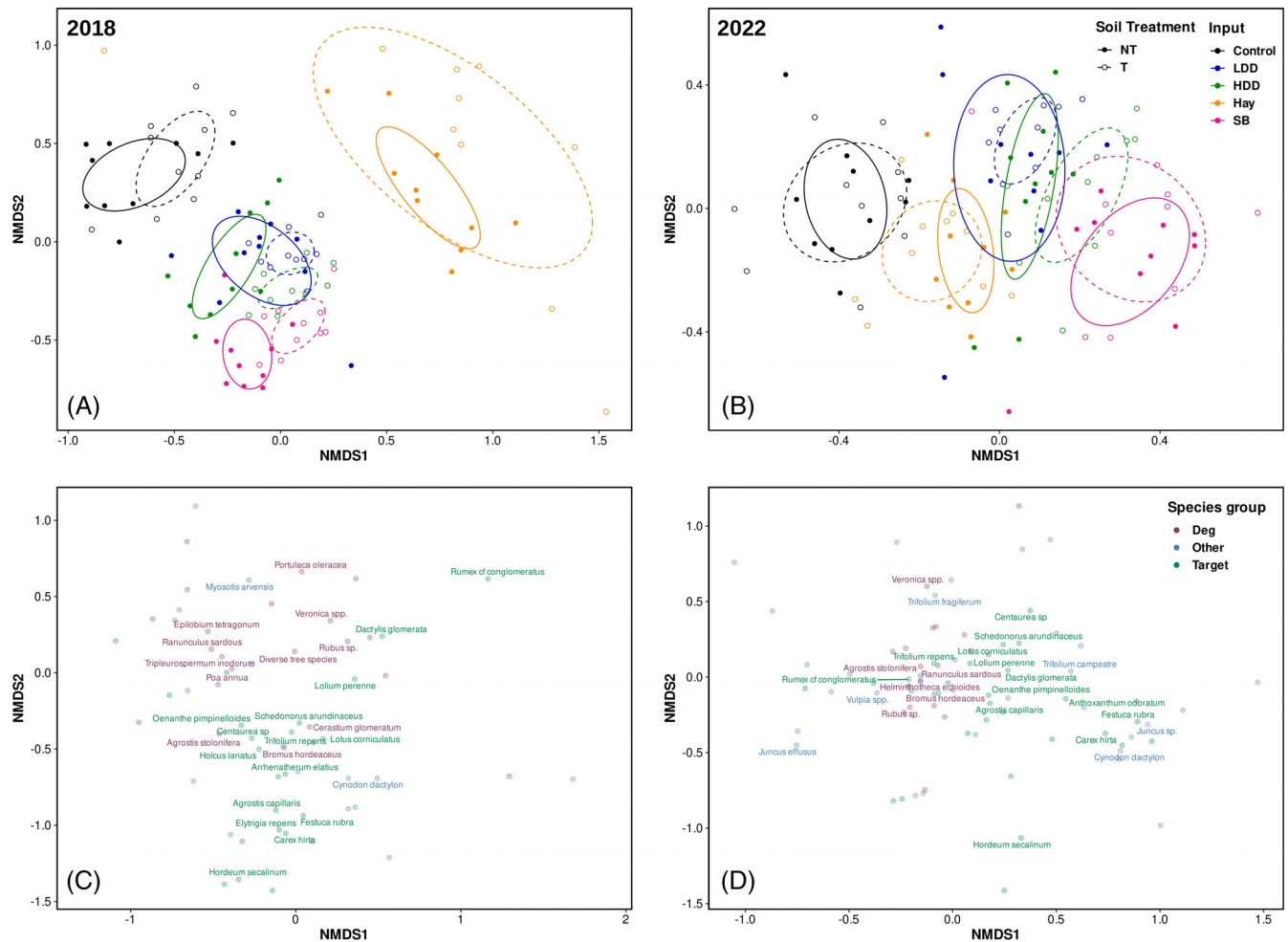


Figure 4. Nonmetric multidimensional scaling ordinations of standing vegetation taxonomic composition in 2018 (A, C) and 2022 (B, D). Plot representation highlighting the soil disturbance treatments and inputs (A, B) and species representation highlighting the three species groups (C, D). Only the names of species being significantly indicator of at least one treatment (Table S5) are shown.

et al. 2012; Sengl et al. 2017). Species composition of the vegetation matrix and proportions of species groups were similar between LDD and HDD inputs. However, only HDD input reached levels of total and *Target* species richness similar to the highest ones, which were recorded for the SB input. It also resulted in the lowest level of inter-plot heterogeneity, presenting the advantage to control species composition of the sown mixture, which constrains grassland restoration trajectory toward the reference. However, this also increases the risk to rapidly saturate the plant community and reach stable states where chance events of colonization are less likely. Moreover, although phenotypic plasticity may support plant ability to establish under contrasting environmental conditions (e.g. soil pH; Rupprecht et al. 2021), studies claim for the need to clearly delineate the region of plant material collection, based on knowledge about abiotic, mostly climatic and geological, variables but also on habitat connectivity and consistency in the genetic pool (Baasch et al. 2016; Kaulfuß & Reisch 2021; Rupprecht et al. 2021). Using commercial seed mixtures of

unknown origin for grassland restoration is problematic as their establishment may be hampered by local conditions to which those seeds are not adapted. Moreover, introducing plant material of unknown origin may be detrimental to local populations through outbreeding depression linked with genetic introgression (Frankham et al. 2011). Thus, seed sowing as a tool for grassland restoration would greatly be improved by using locally collected or cultivated plant material (Bucharova et al. 2019).

Soil blocks were expected to contain viable seeds, vegetative buds (Bulot et al. 2014; Jaunatre et al. 2014; Fowler et al. 2015), and associated microorganisms (Jaunatre et al. 2014; Emsens et al. 2022; Gerrits et al. 2023). As such, they were considered as the most complete input, which was expected to improve both plant immigration and establishment and to constraint most rapidly the vegetation dynamics toward the reference. Although among the most efficient for the restoration of the plant community, this input did not significantly differ from the HDD seed mixture at least regarding the effect on species richness and proportion of *Target* species. These results suggest either that plant

species establishment was not or little affected by the soil biotic and abiotic properties of our degraded site, or that SBs were not sufficient to restore these properties and improve plant establishment. Emsens et al. (2022) demonstrated that soil inoculation differentially affected soil microbes, and these effects were limited and dependent on the environmental properties, such as soil nutrient content. In our study, even if microorganisms were transferred within SBs, their establishment and dispersal may have further been limited by differences in soil abiotic properties between the donor grassland and the experimental plots. Moreover, SBs were restrained in space as they only represented 1% of the experimental plot area. Thus, even if the transfer of SBs can be a source of *Target* species and microorganisms, large dispersion in the restored site may be a rather long process.

However, SB had particular effect on species composition as it was the only input that allowed the immigration of some grassland species such as *Carex hirta*, *Festuca rubra*, or *Hordeum secalinum*. Soil block transfer enables the immigration of species relying mostly on vegetative growth and producing a small amount of seeds. Contrary to the sowing of seed mixtures, for which species richness and abundances are strongly controlled, SBs offer the opportunity of immigration of a larger, although less controlled, pool of species including species reproducing mainly through clonal growth. However, as it is highly destructive for the donor site, this technique is only applicable to small areas. Our results rather advocate for a moderate and thoughtful use of the transfer of SBs as its benefits appeared to be too limited to outweigh its costs, at least at short term.

Delayed Effects of Hay Transfer

Eight months after the inputs, hay transfer increased neither total nor *Target* species richness. As evidenced by bare soil cover, hay transfer clearly hampered seed germination and seedling establishment. The vegetation litter introduced by hay transfer is known to potentially have contrasting effects. On the one hand, as a physical barrier to light penetration, it can inhibit seed germination or cause young seedling death (Donath et al. 2007; Albert et al. 2019; Hansen et al. 2022). While this can be favorable to the restoration process by lowering the ruderal species cover (Albert et al. 2019), it can also be detrimental to the installation of target species contained in sown seed mixtures or hay itself (Valkó et al. 2022). On the other hand, this litter layer can improve local abiotic conditions and protect seeds and young seedlings from extreme events such as frosts or droughts (Durbecq et al. 2022), or from predation (Reed et al. 2006). In our experiment, the litter layer created by hay transfer had a much greater effect on seed germination and seedling establishment than pre-restoration standing vegetation and remaining seed bank.

Consistent with studies showing a delayed establishment of target species, in particular specialists (Wagner et al. 2021; Valkó et al. 2022), the negative effect of the hay input was rather transient. Our results provide evidence for the transfer of a *Target* species, *Lathyrus nissolia*, within the hay. This species was

not detected the first year, probably because seed germination was delayed (Donath et al. 2007), but, 3 years later, it had dispersed throughout the whole experimental design. Hay transfer offers the opportunity for the immigration of species from donor sites, which, even if they are introduced at low density, can further disperse widely into the restored site (Török et al. 2011).

Yet, even 5 years after the input, the legacy of hay transfer remained visible as the largest proportion of *Other* species (e.g. *Galega officinalis*, classified as exotic invasive in the study region [Caillon & Lavoué 2016], and *Vulpia* spp.), or a high frequency of occurrence of *Rubus* sp. Hay transfer success depends on the quality of transferred material, especially its content in viable seeds and plant phenology (Bischoff et al. 2018). In our experiment, although originating from species-rich grasslands, the hay did not contain a lot of fruits and seeds (M. Garrouj and M.-L. Benot 2007, University of Bordeaux, personal observations). The efficiency of hay transfer is hardly predictable and can be improved by increasing the number of transfers (Kiehl et al. 2006; Scotton et al. 2011).

Establishment Limitation: More Subtle Effects of the Soil Disturbance Treatment

The effects of the soil treatment were less pronounced than expected, either alone or combined with the input. Contrary to our expectations, deep soil tillage had almost no effect on *Deg* species, but mostly influenced bare soil relative cover and species composition. It disfavored *Rubus* sp. and tree species, probably through the removal of almost all plant parts, including the rooting system, thereby preventing regrowth and resprouting. The weak effects of deep tillage on *Target* species recorded in our experiment are contradictory with previous studies (Schnoor et al. 2015; Bischoff et al. 2018; Freitag et al. 2021). Deep soil tillage was tested as an alternative to topsoil removal, which is usually applied on a relatively small scale due to its high cost (Hedberg et al. 2014; Klimkowska et al. 2015). But our results show that this technique is rather limited in controlling the seed bank. As suggested by the large proportion of bare soil recorded in 2018, space availability was probably not a limiting factor for early seedling installation.

Contrary to dispersal limitation, establishment limitation linked with competition from plant species already present in standing vegetation or occurring in the seed bank did not emerge as a major process influencing grassland community restoration in our study site. As deep tillage can have negative effects on soils (e.g. erosion, biological processes, and soil organisms; Johnston et al. 2018), its relevancy for former arable land restoration seems rather questionable.

Acknowledgments

This study is part of the REZOH project funded by Bordeaux Métropole, Département de la Gironde, Agence de l'Eau Adour Garonne. M.G. received a grant from the French Ministère de l'Enseignement Supérieur et de la Recherche (MESR). The authors are grateful to the staff of Bordeaux Métropole, especially of the Direction de la Nature, the Direction des Espaces

Verts, and the Réserve Ecologique des Barails, who enabled to set up and maintain the experimental design, as well as C. Bodénès, L. Lacombe, S. Le Stradic, P. Massart, M. Walbott, and the interns (L. Barrail, C. Boufflet, A. Castagnos, P. Faure, T. Folituu, I. Gechoud, V. Gomez, M. Langlois, A. San Millan, and M. Vignaud) who took part to field campaigns. We thank the staff of the NNR of Bruges wetlands for access to the reserve, the biologic material, existing data, and advices along the restoration operation. Data are available on INRAE data portal under doi <https://doi.org/10.57745/F7RJQR>.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Map of the experimental site representing variations in elevation (A) and the experimental plots with their assigned treatment (B).

Figure S2. Pictures of the engine used for deep soil tillage.

Figure S3. Schematic representation of an experimental plot showing the sampling method for vegetation monitoring, and the location of the four soil blocks (SB input).

Figure S4. Nonmetric multidimensional scaling ordination of seed bank taxonomic composition highlighting the two soil disturbance treatments (NT non-tilled, T tilled) and showing the result of PERMANOVA testing for soil disturbance effect.

Figure S5. Mean \pm SE total species richness (A) and species richness of the Deg (B), Other (C) and Target (D) species groups in 2019.

Figure S6. Mean \pm SE relative abundance of each species group within the vegetation in 2019.

Figure S7. Mean \pm SE distance of each plot to the centroid of its treatment (interaction between the biological inputs and soil treatments), for the three surveyed years.

Figure S8. Nonmetric multidimensional scaling ordinations of standing vegetation taxonomic composition in 2019.

Table S1. Results of linear model ANOVAs testing for the effect of biological input treatment, soil disturbance treatment and their interaction on the relative abundance of bare soil.

Table S2. Mean \pm SD proportion of bare soil.

Table S3. Results of generalized linear model ANOVAs (Poisson error distribution) testing for the effect of biological input treatment, soil disturbance treatment, and their interaction on total species richness.

Table S4. List of species (taxons) encountered in the seed bank and in the standing vegetation grouped according to their corresponding species group.

Table S5. Indicator species of each biological input and soil disturbance treatment, for each surveyed year.

Table S6. Total number of species (gamma species richness) recorded per year and experimental treatment, including all replicate plots.

Supplement S1. Details about seed bank survey.

Supplement S2. R script used to draw ellipses in NMDS.

Coordinating Editor: Stephen Davis

Received: 25 June, 2023; First decision: 25 August, 2023; Revised: 21 November, 2023; Accepted: 16 December, 2023