Establishment of wildflower strips in a wide range of environments: a lesson from a landscape-scale project

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Wildflower strips established on arable fields or intensively used grassland are an important tool for supporting biodiversity in impoverished landscapes. They provide ecosystem services like resources for pollinators, and, if they are based on perennial native plants, also plant diversity as well as nesting and wintering habitats for insects. To successfully create such wildflower strips, it is important to understand parameters that restrain establishment of the target plant species. Here we studied initial plant establishment at 45 experimental plots located in recently established wildflower strips across a broad variety of environmental conditions, complemented by 32 control plots in adjacent intensively used grasslands. All strips were seeded with a nearly identical seed mixture of native species, and thus, differences in vegetation were largely driven by environmental factors. We recorded vegetation and related it to soil parameters, seeding season, and previous use. Establishment of wildflower strips nearly doubled plant species richness in comparison with adjacent grasslands. Seeded species established better on former arable land than in intensively used grasslands, but also in grasslands, nearly half of the species from the seed mixture established. Sown species were more abundant in wildflower strips than in intensively used grasslands, but also in grasslands, nearly half of the species from the seed mixture established.

During the last decades, the intensification of agriculture has led to a massive loss of biodiversity (Díaz et al. 2019). The biodiversity of arable fields decreased due to fertilization, improved seed cleaning technologies, and use of herbicides. Because of excessive nutrients, field margins are nowadays mostly dominated by productive grasses and nitrophytes (Kleijn et al. 2009; Schmidt et al. 2020). As a consequence, flower-rich plant communities drastically declined in many agricultural landscapes and with this, also resources for pollinators, other insects, and many farmland birds (Tscharntke et al. 2005; Butchart et al. 2010). To counteract this loss and boost biodiversity, Common Agricultural Policy of the European Union introduced agri-environmental schemes, a program that provides payments for farmers in exchange for environmental services (Haaland et al. 2011; Science for Environment Policy 2017).

Key words: arable land, biodiversity, farmland, grassland, plant establishment, seed-based restoration, wildflower strips

Implications for Practice

- Perennial wildflower strips can be established in a wide range of soil conditions and in both former arable land and species poor grasslands. Yet, the success is higher in former arable land.
- Higher establishment rate can be achieved by seeding in fall rather than in spring.
- Further monitoring may be necessary because the soil quality can possibly influence the development of the vegetation over subsequent years.

Introduction

A common agri-environmental scheme in Europe is to set up strips of flowering plants, which increase plant diversity in the landscape and provide resources for pollinators (Buhr et al. 2018). The seed mixtures used for the creation of such wildflower strips vary in their species composition and life span. While annual seed mixtures consist only of short-lived plants, perennial seed mixtures are composed of species with different life span to maintain a flowering aspect over the planned period, which is usually 5 years (Ministeriums für Klimaschutz, Umwelt, Landwirtschaft, Natur- und Verbraucherschutz des Landes NRW 2015). Annual and biennial species dominate the plant community in the first year to provide nectar for the pollinators from the beginning and to suppress unwanted species emerging from

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the seed bank. From the second year onward, flowering perennial species gain cover (Schmidt et al. 2020). The best results can be achieved with using species-rich seed mixtures of regional origin (Bucharova et al. 2019; Schmidt et al. 2020).

Successful establishment of target communities after seeding depends on environmental factors (Pywell et al. 2003). Seeding to sites with already existing vegetation has usually limited success because germinating seedlings face high levels of competition from already established plants (Fry et al. 2017). Community establishment is also affected by soil characteristics, especially the soil nutrient content (e.g. Hözel & Otte 2003; Bakker et al. 2012). High amounts of nutrients (especially N, P, and K) can reduce diversity by promoting growth of fast-growing perennial grasses, which may outcompete target forbs germinating from the added seeds (Grime 1973; Kleijn et al. 2009; van Dobben et al. 2019). Another important factor is water availability. As water is one of the basic needs for plants to germinate and survive (Bradford 1990), a sufficient water availability enhances species establishment and restoration success (Groves & Brudvig 2019).

Environmental limits of species establishment from seeds was mostly studied in the context of ecosystem restoration, but the results cannot simply be transferred to wildflower strips (Kiehl et al. 2010; Klaus et al. 2018). The goal of restoration is to recreate natural or semi-natural plant communities and maintain these for long time periods. Wildflower strips, however, are neither natural nor semi-natural because they are designed to provide specific ecosystem services, especially resources for pollination. In their species compositions, they differ from natural communities as they contain mainly insect-pollinated plants and only low proportions of grasses (Haaland et al. 2011). Furthermore, due to the short funding period of 5 years for wildflower strips within the agri-environmental schemes (Ministeriums für Klimaschutz, Umwelt, Landwirtschaft, Natur- und Verbraucherschutz des Landes NRW 2015), it is crucially important that species establish and flower rapidly to provide the desired ecosystem services.

Because wildflower strips are a fairly new element and were introduced only after 1990 (Haaland et al. 2011), few studies examined their ecology, mostly focusing on insects, especially pollinators (e.g. Tschumi et al. 2015; Warzecha et al. 2018). The limited amount of works that studied the vegetation of wildflower strips mainly addressed the importance of the seed mixture (Schmidt et al. 2020) and the management regime (Piqueray et al. 2019), but knowledge about the influence of site conditions on initial species establishment and species composition is missing.

In this study, we investigated several dozens of newly established wildflower strips scattered across a region, with up to 30 km distance between the strips. The wildflower strips were seeded in spring or fall to former agricultural fields and intensively used grasslands, and differed in soil conditions. Because the seed mixture was nearly the same for all strips, differences in species composition of the establishing wildflower strips can mainly be attributed to environmental conditions. We recorded species establishment as the richness and cover of species and tested the importance of the previous use and soil characteristics for the establishment of seeded species. We hypothesized that (1) the establishment success is higher on former arable land than on former grassland and (2) is affected by the soil conditions.

Methods

Study Site

The study was located in northwestern Germany (Fig. 1), in an area dominated by intensive agriculture. The mean annual temperature in the region is 9.9°C and annual precipitation 782 mm (Deutscher Wetterdienst 2020). Dominating soils are podsol gleys and plaggen soils, both on poor Pleistocene sands. Fewer study sites are situated on sandy gley podsols and cambisols on loamy sand (Geologischer Dienst Nordrhein-Westfalen 2021). The study sites were part of a large-scale project aiming to the establishment of perennial wildflower strips on arable land and species-poor grasslands (Biological Station of the county Steinfurt 2019). The wildflower strips were created by disturbing soil by a rotator and seeding a seed mixture consisting of 28–31 regionally adapted species (25–28 herbs, three grasses; 13% annuals and biennial, 87% perennials). The seeding took place in 2019, either in spring or fall, with slightly different seed mixture for the two seeding dates (28 species in both seeding dates, three additional species in fall 2019), but the proportion of grasses and annuals was the same (Table S1). The seeding density was 2 g/m², which corresponds to approximately 4,700 seeds/m².

We studied 33 wildflower strips (11 sown in spring 2019, 22 in fall 2019) that were at maximum of 30 km apart and ranged from 152 to 8,522 m² in size, mostly shaped as a long, 3–5 m wide strip (Fig. 1). Depending on size, we examined either one or two plots per flower strip, resulting in 45 plots. Because of the considerable length of the seeding strips, the
two plots in one strip were usually far apart, at minimum 50 m, but mostly more than 100 m. Thirteen plots were located on former arable soil and 32 in species-poor grasslands. For each grassland plot, we selected a control plot in an adjacent grassland that has not been disturbed and seeded. We did not establish controls for the plots at former arable fields because these would be located in a cultivated field. Thus, our dataset contained in total 77 plots: 45 wildflower strip plots and 32 control plots (Table S2). As many of the wildflower strips were rather narrow, the plots had size of 1 m × 5 m, with the longer dimension in the direction of the strip to reduce edge effects.

**Vegetation and Soil Sampling**

During 2 weeks at the turn of May and June 2020, which was the first growing season after seeding, we recorded all vascular plant species present in each plot and estimated their percentage cover. For soil analysis, we took five 10-cm deep soil samples at each plot using a corer with an inner diameter of 3 cm and pooled them. We stored the soil in a cooler for the day and froze them at −20°C in the evening. Using a “Pürckhauer” drill, we extracted an additional 1-m long soil core at each site. We inspected the color, temperature, and texture of the extracted soil and determined the depth at which the soil started to be moist.

**Soil Analysis**

We analyzed the soil samples for mineral nitrogen, phosphate, potassium, pH, and the weight proportions of coarse and fine soil particles. We crumbled the soil samples and sieved them to a grain size of <2 mm. To extract the nitrate and the ammonium, we added 100 mL of a 0.01 mol/L CaCl₂ solution to 10 g of soil and placed the samples on a horizontal shaker for...
1 hour. We measured the amount of nitrate and ammonium in the solution with the San++ Continuous-Flow Analyzer (Skalar Analytical B.V.). To determine plant available phosphate and potassium, we air-dried the soil samples until their weight was stable, and extracted them following the calcium acetat lactate extraction method (CAL, Schüller 1969). In the extract we quantified the amount of phosphate using a spectral photometer and the amount of potassium using a flame photometer. To determine the soil pH, we added 25 mL of 0.01 mol/L CaCl₂ solution to 10 g of dried soil. After 2 hours we measured the pH in the supernatant. We determined the weight proportions of coarse (>63 μm) and fine (<63 μm) soil particles, by passing the dried soil samples through sieves of different mesh sizes.

Statistical Analysis
All analyses were done in R (R Core Team 2020).
First, we tested whether the seed addition had any effect on the species richness per plot in comparison to grasslands that were already present in the landscape, and whether this effect depended on the former land use and the season of seeding. We related the total number of species per plot to the type of the plot (factor with three levels: control, seeded to former arable land, seeded to species poor grassland) in a linear model. We used multcomp package (Hothorn et al. 2008) for comparison among the three factor levels. In this and all subsequent models, we visually estimated whether model assumptions are met (Zuur et al. 2009). This and all subsequent models had normal error distribution, and when necessary, we transformed the response variable to approximate the normal distribution, as indicated below.

Second, we tested whether establishment and cover of sown (part of seed mixture) and spontaneously establishing species (not part of the seed mixture) depends on previous use and time of seeding. This analysis was carried out only with seeded plots. As the seed mixture slightly differed between the spring and fall seeding, we expressed establishment of seeded species as the proportion of species that established relative to the total number of species that were already present in the landscape.
species in the seed mixture. We created four linear models, one for each of these response variables: proportion of established sown species, cover of sown species, number of spontaneously established species, and cover of spontaneously established species. Cover was log transformed to achieve normality. Explanatory variables were, in all four models, time of seeding (spring vs. fall), previous use (arable soil vs. grassland), and interaction of these two variables.

Third, we tested whether soil properties affect establishment and cover of sown and spontaneous species. This analysis was carried out only with seeded plots. We created four linear models for the same response variables as above, and related them to soil properties, specifically content of K, P, mineral nitrogen, pH, moisture depth, and proportion of fine soil. All explanatory variables were standardized before the analysis, and all response variables were corrected for the effect of time of seeding and previous land use. For visualization, we used package relaimpo (Grömping 2006) to obtain averaged proportion of explained variability per variable, and package arm (Gelman & Su 2020) to obtain posterior distributions of model estimates, which were subsequently plotted using package ggrides (Wilke 2021).

Results
We detected in total 147 species during the study, of which 29 were part of the seed mixture. Only two seeded species (Agri- monia eupatoria and Scrophularia nodosa) did not establish on any of the observed plots, which translates into an establishment success rate of approximately 94% at landscape scale. Seed addition significantly increased the overall species richness per plot from 16.4 ± 1.1 SE species per 5 m² in the control plots to 33.4 ± 2.2 SE species in the study plots sown to agricultural field and 31.0 ± 1.3 SE in the study plots sown to intensive grasslands ($R^2 = 0.53, p < 0.001$; Fig. 2). The overall species richness did not differ between the wildflower strip plots in grasslands and former arable field (Fig. 2).

Establishment of sown species was affected by both previous use and season of seeding. On former arable fields, the proportion of established sown species was 34.2% higher in comparison to former grassland (Fig. 3, Table S3) and on the plots where the seed mixture was added in fall 2019, the proportion of established sown species was 44.7% higher than on the plots that were sown in spring 2019 (Fig. 3, Table S3). Cover of seeded species was 56.1% higher in plots at former arable fields, but there was no difference between the seeding seasons. In contrast to sown species, the establishment of spontaneous species was not affected by seeding season and previous land use (Fig. 3, Table S3). The interaction between seeding season and previous use was not significant in any of the models above.

Soil properties had only limited effect on the establishment of sown species. The proportion of established sown species did not depend on any of the soil parameters, cover of sown species increased with moisture depth, but the overall proportion of explained variability remained low ($R^2 = 0.22$; Fig. 4, Table S4). In contrast, soil properties explained variance in the number and the cover of spontaneously established species by 51.8% and 46.2%, respectively. Both the number and the cover of spontaneously establishing species decreased with increasing supply of plant available phosphorus in the soil and increased with soil pH. Cover of the spontaneous species increased with finer soil texture (Fig. 4, Table S4).

Discussion
We studied early establishment of species seeded as wildflower strips in an agricultural landscape. Establishment success was high, with 94% of all sown species recorded at least once, and wildflower strips hosted on average twice as many species as adjacent grasslands without seed enrichment. Increased species richness was independent of the previous type of land use and the season of seeding, which corroborates the existing evidence that soil disturbance followed by seeding species rich mixtures is an excellent technique to increase plant species richness in agricultural landscapes with impoverished species pools (Kiehl et al. 2010; Klaus et al. 2016; Freitag et al. 2021).

Previous Use
The increase in overall species richness after seeding was the same at former arable fields and former species-poor grasslands. However, species from the seed mixture established better and had a higher cover at former arable land. The higher success of sown species on former arable land is probably a result of different levels of competition. At arable land, the competition was likely minimal because there was no previous vegetation except few agricultural weeds and the seed bank was impoverished (Prach et al. 2007; Carmona et al. 2020). At grasslands, however, many species were already present and even after soil disturbance, the resident species could quickly regenerate from rhizome fragments or the existing seed bank, and limit resources available for seeded species (Klaus et al. 2018; Bucharova & Krahulec 2020). In this context, the belowground competition was likely more important than aboveground, because we did not record higher cover of spontaneously established species in plots at former grasslands, a pattern which would indicate increased aboveground competition.

Sowing Season
Richness and cover of sown species were higher when seeds were sown in fall. The reduced establishment success of spring seeding was probably caused by excessive seedling mortality of vulnerable species during the exceptionally hot and dry summer 2020 (Deutscher Wetterdienst 2019). While the legacy of seeding season can last for many years (Groves et al. 2020), it is also possible that the difference will diminish with time. Some of the sown seeds could have delayed germination and may germinate in subsequent years (Kiehl & Pfadenhauer 2007).

Nutrient Content in the Soil
Nutrient content in the soil did not affect establishment success of the sown species. This result may be counterintuitive, because
Soil pH
Analogous to nutrients in the soil, soil pH affected only spontaneously establishing species. This is probably again legacy of the vegetation prior the creation of the wildflower strips, as in mesic grasslands, soils with higher pH tend to generally host higher plant diversity (Riesch et al. 2018). The underlying effects of acidic soils, e.g. aluminum toxicity, calcium deficiency, and a high ammonium/nitrate ratio in acidic soils (Bartelheimer & Poschlod 2016), can impair plant survival, while intermediate pH levels provide decent conditions for more species (Schuster & Diekmann 2003; Crawley et al. 2005). The seeded species were mostly generalists that can tolerate a wide range of soil reaction, and thus, it is not surprising that their establishment was not affected by soil pH.

Water Availability
Contrary to our expectations, the sowing success was greater at sites with low water availability, as indicated by the increasing cover of sown species at sites with deeper soil moisture. The sown species probably do not truly prefer dry conditions, but this relationship is rather caused by elevated competition of spontaneous species under wet conditions, leading to a suppression of the sown species (Groves et al. 2020). In our study, the cover of spontaneous species indeed slightly increased with decreasing moisture depth, yet this effect was not significant. We further observed higher cover of spontaneous species on soils with finer soil texture, which typically have a higher water holding capacity and improved water availability for plants (Scheffer et al. 2010). However, fine soil particles have an influence on other soil characteristics that are important for plants as well (e.g. cation exchange capacity, Scheffer et al. 2010), and it is unclear whether water availability is the actual factor benefitting the cover of spontaneously established species. We are also aware that our measures of water availability were imperfect, and that further research that includes factors such as precipitation and water holding capacity of the soil is needed to determine the effect of water availability on the establishment of wildflower strips.

In summary, wildflower strips are a common measure to increase biodiversity in agriculture-dominated landscapes. Here we show that establishment of perennial wildflower strips doubled plant biodiversity in comparison with existing intensively used grasslands, and that their initial establishment did not depend on soil properties. These are good news, because wildflower strips could successfully establish across a broad variety of soil conditions. The establishment of seeded species was best at former arable land, that is at sites with severely impoverished resident biodiversity, where these measures bring the largest benefit. In intensively used grasslands, the success of seeded species was lower, but still on average nearly 40% of the sown species established per plot. Seeding to grassland thus can be considered an effective measure as well (Klaus et al. 2016; Freitag et al. 2021). In general, better results are likely to be achieved when seeding in fall rather than spring.

Although our results are promising, it is necessary to keep in mind that we recorded vegetation only in the first season after establishment of the perennial wildflower strips. The vegetation will likely further develop, and the soil properties may increasingly influence species performance. Specifically, the cover of competitive grasses may boost at nutrient-rich plots (Freitag et al. 2021). To be sure that the increased species richness persists for the planned lifetime of the flower strips, it is necessary to continue data collection and evaluation. However, data from other studies are encouraging, e.g. Schmidt et al. (2020) demonstrated that perennial wildflower strips established by seeding regional seeds persist for at least 7 years.

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High nutrient content in the soil is one of the main factors constraining the successful species establishment in restoration (Walker et al. 2004; Bakker et al. 2012). However, restoration projects are mostly evaluated after many years and with proceeding time, few strong competitors can profit from high availability of nutrients, suppress subordinate species, and reduce plant diversity (Grime 1973; McCain et al. 2010). Our data come from the first year after seeding, and the competitive species were probably not fully able to use the higher nutrient contents to their advantage yet (Schäfer et al. 2019). It is possible that the nutrient content in the soil will influence richness and cover of the seeded species in subsequent years (Freitag et al. 2021). As wildflower strips are mostly designed for the relatively short duration of 5 years, the limited time will likely not allow comparable development as in permanently restored ecosystems, but long-term monitoring of the plots is necessary to provide data on this.

Although there was no effect of nutrient supply on sown species, the number of species that spontaneously established in the wildflower strips decreased with plant-available phosphorus in the soil. As high phosphorus supply typically correlates with a reduced species richness (Crawley et al. 2005), the number of spontaneously establishing species obviously reflects the species richness of the original community.
Wildflower stripes in various environments

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

Table S1. Species composition of the seed mixtures used for seeding for the plots in spring 2019 and fall 2019.
Table S2. Experimental plots broken according sowing season and previous use.
Table S3. The effect of previous land use and season of sowing on establishment of sown and spontaneous species.
Table S4. The effect of soil parameters on establishment of sown and spontaneous species.

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