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The effect of tillage management and its interaction with site conditions and plant functional traits on plant species establishment during meadow restoration

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Abstract

The restoration of grasslands is a key management practice that supports biodiversity across Europe. On species poor grasslands and ex-arable fields, the establishment of plant species is often limited by the availability of habitat niches, in particular space to germinate. We investigated the impacts of full inversion tillage and its interaction with site conditions and functional traits on the abundance of 51 plant species sown into a 2 ha ex-arable site in Poland. Soils of the donor site were characterized by high levels of heterogeneity in terms of water content and plant availability of N, P and K. One year after sowing the cover of species typical of semi-natural grasslands was significantly higher on the tilled plots than on the non-tilled plots. However, in the case of widespread generalist species the tillage of soil resulted in no significant effect on their establishing percentage cover. The establishment of plants on the tilled area was more successful where soils were relatively rich in mineral N. It was also more successful for species with low Ellenberg's N values. Species indicative of moist soil established poorly where the soil was tilled. This study has clear implications for the applied restoration of grasslands, demonstrating a vital role of soil tillage to promote the establishment of species typical of semi-natural grasslands. This is particularly important where seed mixtures may contain both desirable and undesirable competitive species that would disproportionately benefit from the absence of tillage management.

Keywords: community assembly; ecological filtering; species-rich grassland; plowing; gaps; seed size

Abbreviations: ENIV - Ellenberg's nitrogen (nutrients) indicator value; EMIV - Ellenberg's moisture indicator value

1. Introduction

During the restoration of species-rich grasslands, sowing seeds of the target species is often preceded by plowing, rotovating, harrowing or other methods of mechanical soil disturbance that aim to break up the old vegetation cover and help deplete the soil seed bank (Edwards et al., 2007; Long et al., 2014; Pywell et al., 2007; Schnoor et al., 2015; Wagner et al., 2011). The main theoretical basis for applying mechanical soil disturbance before sowing is that gaps in vegetation are necessary for the regeneration of plant populations. A gap is a competition-free space for seedlings where the requirements for dormancy-breaking, germination and establishment are fulfilled, while the effects of predators, competitors and pathogens are reduced (Bullock, 2000; Grubb, 1977; Harper, 1977). However, the openings created with available farming equipment (e.g. a plow) are different in size, duration, and character from the natural gaps in grasslands that temporarily appear as a result of plants death, livestock trampling and dung deposition. One of the major differences is that with the use of agricultural machinery plant-free spaces at the scale of whole fields can be created almost instantaneously, whereas naturally occurring gaps in grasslands are typically just a few centimeters or decimeters across (Bullock, 2000; Grubb, 1977). Therefore in ecological terms, seedbed preparation for grassland restoration can be considered to be large scale vegetation disturbance that substantially modifies conditions for the establishment of plants by exposing them to direct insolation, wind, air temperature fluctuations, and drying of soil surface.

On emergence, many seedlings of grassland plants require protection from these extreme environmental conditions (Gibson, 2009). It remains unclear the extent to which the presence of few shoots of non-target species co-emerging in a tilled restoration area may provide such protection. Moreover, herbaceous litter, removed through mechanical disturbance of old vegetation, has been shown to promote seedlings emergence by keeping the soil surface moist (Thompson, 1987). However, for some species this surface vegetation litter can act to inhibit species emergence (Donath et al., 2006; Goldberg and Werner; 1983). How soil tillage promotes the establishment of plant species particularly in response to underlying soil conditions remains an important issue in restoration ecology. In the context of the restoration of temperate grasslands, plowing, rotary cultivation and harrowing prior to sowing, have all been shown to increase the rate of target species establishment from sown seed mixes, and in most cases this response was promoted by higher disturbance levels (Donath et al., 2007; Edwards et al., 2007; Hofmann and Isselstein, 2004; Hopkins et al., 1999; Poschlod and Biewer, 2005; Schmiede et al., 2012). However, these studies have typically either focused on a very limited number of species or are related to specific habitat types (e.g. Donath et al., 2006; Edwards et al., 2007; Hofmann and Isselstein, 2004; Hutchings and Booth, 1996). Therefore, the effects of tillage and their interactions with soil conditions on plant species establishment during grassland restoration has remained largely unexplored.

The intrinsic reasons for the differences in establishment success among grassland plant species following sowing into tilled soil when compared to undisturbed sward also need further elucidation. The evidence for such differences has been collated since the 1950s (Black, 1958), but an overwhelming majority of the experiments focus on the importance of small gaps, not larger openings typical of large scale mechanical disturbance. Differences in survival have been observed at either the germination or the seedling emergence stage, but when both these stages of the establishment process were considered together, the results were often complex and inconsistent (Bullock, 2000). In general, the published literature indicates that seed size may be of particular importance in this process. Large seeds are assumed to provide individuals with a competitive advantage in dense turf, as seed reserves allow the seedlings to tolerate prolonged periods of intense competition from the established vegetation (Burke and Grime, 1996; Donath et al., 2006; Goldberg, 1987; Gross, 1984). Where large areas of bare ground are created, differential species establishment on disturbed soil vs. intact vegetation is often better explained by species association with fertile or infertile soils (Pywell et al., 2003), specific ecological guilds (Hopkins et al., 1999; Pywell et al., 2003), tolerance to water stress (Bullock, 2000), as well as again in seed size (Donath et al., 2006). It is also possible that specific leaf area (SLA) may play a role, as low SLA allows young plants to persist during summer drought, while high SLA, by contrast, helps species establishing into existing swards with shady conditions (Lambers et al., 2008).

This paper describes a study investigating the initial establishment of 51 grassland plant species during grassland restoration in response to tillage and mowing management as it interacts with soil moisture and the availability of mineral nutrients. The sown species are characteristic of a wide range of semi-natural vegetation types typical of the surrounding dry calcareous grasslands, mesic lowland meadows, and *Molinia* semi-wet meadows. The study was split into two parts. The first part assessed species level responses and asked how much tillage (temporal vegetation removal) promotes the establishment of plant species introduced by sowing, and how many and which species establish successfully within the sward. In the second part, we tested whether the success of species establishment on tilled soil vs. intact vegetation is associated with their functional traits, realized habitat niche or other soil conditions. Assuming that the main effect of tillage lies in the alleviation of the competitive effect from established vegetation on species establishment, we hypothesize that (H1) this measure favors the establishment of competitively weak species which are typical of low-productive, semi-natural grasslands; (H2) that under dry conditions tillage poses the risk of drought, especially to those species, which are typically associated with wet habitats, whereas this measure should be beneficial to all species in moist sites. With respect to the effect of functional traits of species, we hypothesize that (H3) tillage is more beneficiary for the establishment of small-seeded, small-stature, and low-SLA species, which are less capable of growth under dense canopies dominated by grasses.

2. Material and methods

2.1. Design of the experiment

A 2-ha experimental site was located in abandoned fields in Bagno Serebryskie Nature Reserve, East Poland (51°10′16′′N, 23°32′01′′E). The terrain is almost flat with height differences of ca. 1 m and mean elevation of 178 m above sea level. The climate of this area is warm, humid continental (Köppen's classification, www.physicalgeography.net), with 574 mm mean annual rainfall and mean annual air temperature of 7.5 °C. For 1.5 ha of the site the underlying soils were Rendzic Cambic Leptosol, with the remaining 0.5 ha - Mollic Gleysol (IUSS Working Group WRB, 2007). Before 1990 the area had been used as an extensive grassland, but was converted to arable agricultural in 1991 and then abandoned in 2005. Soon after the abandonment the former fields were colonized by ruderal and common grassland plants (Appendix A). In the autumn of 2008 the whole area was mown and divided into ca. 8-m-wide strips. Every second strip of land was moldboard plowed, so that the Ap horizon of the soil (average depth of 24 cm) was completely inverted. In this way, 11 parallel strips of plowed land, separated with 11 strips of uncultivated land, were created (Fig. 1a). The introduction of desired plant species was delayed for a year with the aim of reducing the weed burden to a manageable level (UK Rural Development Service staff, 2010). This was achieved by leaving the plowed area in furrows throughout the winter so that the perennating organs of unwanted plant species were exposed to frost. In the following growing season shallow disking or harrowing was carried out every 5–6 weeks from June to October to progressively exhaust the weeds’ food reserves by stimulating regrowth from the rootstock after each cultivation, and homogenize the seedbed.

In December 2009, the experimental area was hand-sown with seed mixture collected by means of vacuum harvesting from nearby meadows that represented *Molinietalia* and *Arrhenatheretalia* orders and *Festuco-Brometea* class (Kącki et al., 2013). The sowing was conducted in bands perpendicular to the plowed lines, again in ca. 8 m wide strips separated by 8 m. These created a lattice work of intermittent tilled and untilled strips going in one directions, overlain with intermittent sown and unsown bands going in the other direction (Fig. 1a). This lattice of sowing and tillage management allowed us to establish four experimental treatments (Fig. 1b) in a replicated 2 (±tillage) × 2 (± sowing) experimental design. These treatment levels were: 1) control with neither soil tillage or the addition of vacuum harvested seed, 2) tillage only, 3) vacuum harvested seed addition only, 4) tillage and vacuum harvested seed addition. Each of these four treatment levels was positioned in adjoining 8 × 8 m plots to form a replicate block. Nineteen replicate blocks (representing 76 experimental plots) were randomly located within this lattice of tillage and sowing management.

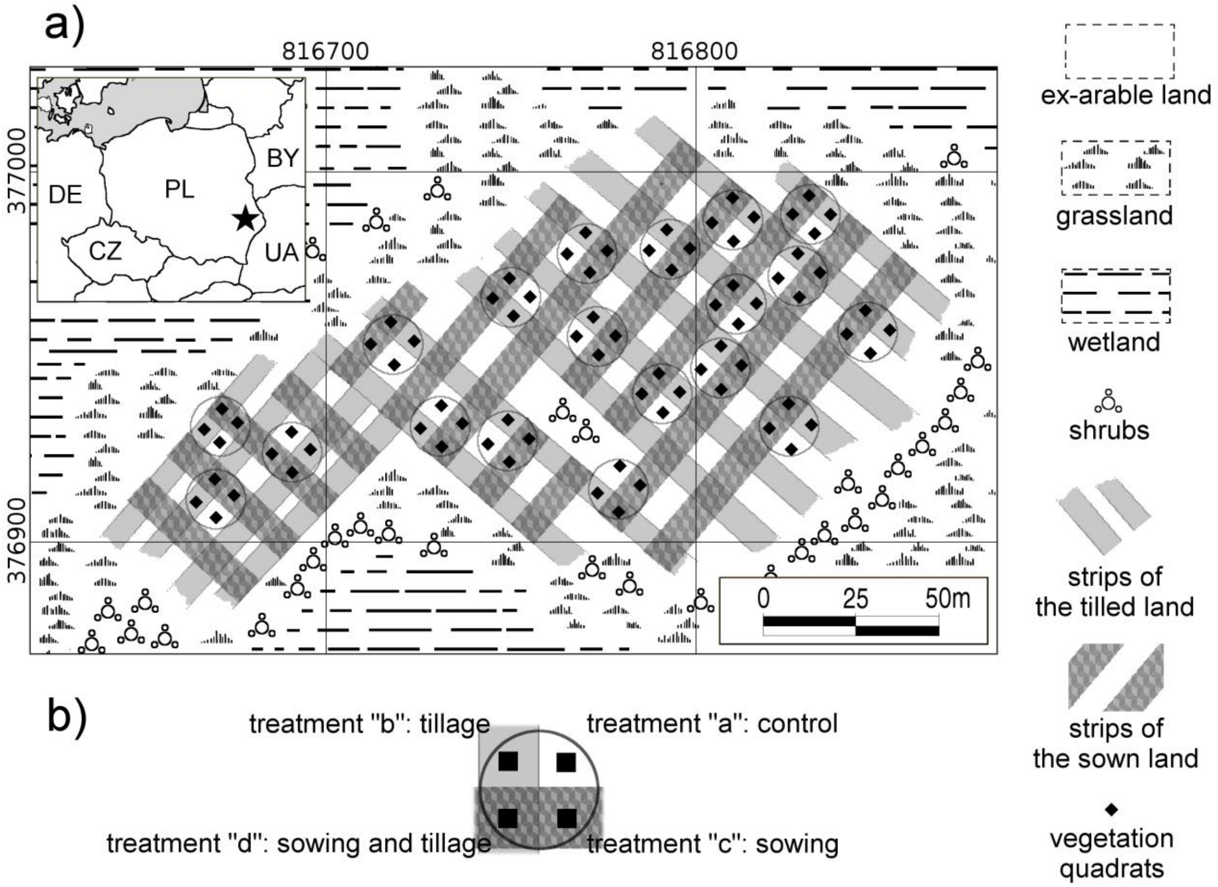

Figure 1 a) The study area and its location; the encircled vegetation quadrats compose a randomized block design. b) Design of each experimental block; there were 19 replicate blocks randomly located within the lattice of sowing and tillage management; each block contained four experimental treatments: “a”: control without soil tillage or the addition of vacuum harvested seed; “b” tillage only; “c” vacuum harvested seed only; “d” tillage and vacuum harvested seed addition.

The vacuum harvested material was thoroughly homogenized and sampled for the analysis of species composition. Seedling emergence tests, which were conducted in a greenhouse, showed that the material contained 70 plant species (see Appendix B). Within the vacuum harvested seed mix 33 species were already identified as being present in the experimental area before sowing. Plant species transfer was planned to maximize the probability that all the species present in the vacuumed seed mix were sown on every experimental plot, and that seed number of each species was similar across the plots. To achieve this, large amount of seeds were sown with a 5:1 ratio of donor to receptor site area used. Seeds were harvested twice in the growing season and harvesting was continued until the majority of seeds were collected from plants. The harvested seed mix was carefully and thoroughly homogenized during sowing.

2.2. Plant community assessments

Plant species composition was assessed within 4 m^2 quadrats situated in the middle of each of the 76 experimental plots. In September 2010 percentage cover was estimated by vertical projection using eight-class scale (0–0.1%, 0.1-1%, 1–5%, 5-12.5%, 12.5–25%, 25-50%, 50-75%, 50–100%) (Appendix C). This was converted into ratio scale by replacing the classes with their middle values. We focus the analysis on only that sub-set of species that were identified as being present within the sown seed mixture, regardless of whether they were or were not present in the experimental area before sowing. We further restricted the analysis to those 52 species that occurred in at least three experimental plots. Note, of the 52 species considered in the study, *Plantago major ssp. intermedia* was excluded from the analysis as the response of this species to tillage was disproportionally high on the tilled and non-sown plots. This was likely caused by massive recruitment of this species from the soil seed bank, however, this made it difficult to detect changes in the abundance as a result of sowing. The exclusion of *P. major ssp. intermedia* did not qualitatively change the overall trends presented in the results. The ‘ initial species establishment' term used in this paper means successful seed germination and seedling emergence as well as the survival of juveniles during the summer drought.

2.3. The effect of tillage on the abundance of the subsequently sown plant species (E_i index)

The cover of individual species was scaled into the range [0, 100] to allow the comparisons of the change of cover among the species as a result of sward destruction and sowing. The following equation was used for scaling:

$$X_i = (x_i - x_{i,min}) / (x_{i,max} - x_{i,min}) \times 100, \quad (1)$$

where: X_i is the scaled percentage cover of species *i*, hereafter referred to as the abundance of plant species *i*; x_i is the recorded percentage cover of species *i*; $x_{i,min}$ and $x_{i,max}$ are the minimum and maximum cover of species *i* recorded within the four variants of plots.

The measure of the effect of tillage that preceded sowing on the abundance of the sown species, E_i was determined by the following equation:

$$E_i = (X_{i,d} - X_{i,b}) - (X_{i,c} - X_{i,a}), \quad (2)$$

where: $X_{i,a}$ is the abundance of sown species *i* in the non-tilled and unsown plots; $X_{i,b}$ is the abundance of sown species *i* in the tilled and unsown plots; $X_{i,c}$ is the abundance of sown species *i* in the non-tilled and sown plots; $X_{i,d}$ is the abundance of sown species *i* in the tilled and sown plots. Therefore ($X_{i,d} - X_{i,b}$) represents the increase in the abundance of sown species *i* on the tilled plots solely as a result of sowing, not as a result of soil diaspore bank activation following tillage. Similarly ($X_{i,c} – X_{i,a}$) represents the increase of the abundance of species *i* on the non-tilled plots solely as a result of sowing, and plants which were present in the sward before sowing are not taken into account. Positive value of E_i indicates a positive effect of tillage on the establishment of a sown species, whereas negative values denotes a negative effect.

The E_i parameter could be used for all sown plant species, regardless of whether they were or were not present in the experimental area before sowing.

2.4. Traits selection

Plant functional traits influence plant's survival, fitness, as well as their establishment success during grassland restorations (Pywell et al., 2003; Woodcock et al., 2011). Trait data was derived from Biolflor traitbase (Klotz et al., 2002), LEDA traitbase (Kleyer et al., 2008) and the database of ecological indicator values of vascular plants of Central Europe and Alps (Ellenberg and Leuschner, 2010). From these data sets we derived for each plant species: (1) guild - grass, sedge, forb or herbaceous legume (Klotz et al., 2002); (2) realised ecological optima of plant species in terms of soil moisture, and soil mineral nitrogen/nutrients content given by Ellenberg's indicator value for N (ENIV) and Ellenberg's indicator value for moisture (EMIV) (Ellenberg and Leuschner, 2010); (3) seed mass (Kleyer et al., 2008); 4) canopy height, defined as the distance between the highest photosynthetic tissue and the base of the plant (Kleyer et al., 2008); 5) SLA (Kleyer et al., 2008), i.e. the one sided area of a fresh leaf divided by its oven-dry mass (Pérez-Harguindeguy et al., 2013). For this paper, we have chosen for the simplicity and consistency to use term "trait" in its broad sense (Pywell et al., 2003) to apply to species Ellenberg's indicator values. It should be noted, though, that Ellenberg's numbers are not basic traits, but attributes that integrate various ecophysiological and morphological characteristics of plants (Bartelheimer and Poschlod, 2016).

2.5. Soil analysis

Seven randomly positioned soil samples were taken in January 2011 from each of the 76 experimental plots after the first season of growth. This soil was sampled from the layer of 0–8 cm, i.e. from the root zone. The depth of rooting was determined in the field by observing the soil profile in a few different places. The seven subsamples collected from each experimental plot were then combined into a single averaged sample. The content of plant-available forms of the main nutrients: nitrogen (N), phosphorus (P) and potassium (K), as well as pH and texture were determined. The measured content of the nutrients was referenced to the thresholds for agricultural plant nutrition levels for the assessment of fertilizer needs (Appendix E).

Soil moisture was determined in the field with a ‘FOM/mts’ meter (the Institute of Agrophysics of the Polish Academy of Sciences, Lublin). The meter measures volumetric soil moisture content by responding to changes in the apparent dielectric constant of moist soil. The moisture was measured in the layer 0-11 cm, six times during the growing season in 2010 and twice in 2011. Although the recorded relative differences in soil moisture across experimental plots were broadly similar for both these years, we used only data collected in 2011, because it met the minimum sample size criteria (Appendix F). In 2011 the measurements were performed in May and July, in four points that were distributed regularly along the diagonal of each plot, and the results were averaged for each plot.

The availability of soil N for plants was expressed by means of the content of ammonium (NH_4-N) and nitrate (NO_3-N) forms, assayed with the method of segmented-flow colorimetry. In subsequent regression analysis, the sum of both these forms (soil mineral N) was used as a predictor. Determination of plant-available forms of P and K was made with the use of the Egner-Riehm DL method. The methodology of soil analysis is described in detail in our previous paper (Czerwiński et al., 2015). As the response metric E_i is derived from the four plots within each block an average value of each of the soil parameters was determined for each of the 19 blocks.

2.6. Data analysis

2.6.1. The response of individual species to the conditions caused by tillage

The size of the effect of tillage on the abundance of each of the sown species *i* was expressed by E_i value. The significance of this effect was estimated by calculating the significance of the difference in the abundance of a species *i* between the tilled plots ($X_{i,d} - X_{i,b}$) and the non-tilled plots ($X_{i,c} - X_{i,a}$). A paired two-sided *t*-test at 95% confidence level was used to test the significance of the difference. There were 51 species so the *t*-test was performed 51 times.

2.6.2. The response of the target vs. non-target species to the site conditions changed by tillage

We also analyzed how the conditions caused by tillage affected the establishment of particularly desired, semi-natural grassland species (“target species”) and non-priority species (“non-target species”). These were considered as two separate groups. Target species were those that represent species-rich, semi-natural grasslands, particularly of *Molinion caerulae* alliance and *Festuco-Brometea class* (Kącki, 2013). All the other species were considered non-target, because they were ubiquitous in the region and did not need to be transferred to the restoration area. To compare the response of the two species groups, average E_i value across each of these groups was used. In addition, for each group the significance of the difference in multivariate species abundance between the tilled plots ($X_{i,d} – X_{i,b}$) and the non-tilled plots ($X_{i,c} – X_{i,a}$) was calculated, using a multivariate Hotteling's T^2 test (Zar, 2007).

2.6.3. The effects of species attributes and site conditions on the establishment success of the sown species on tilled vs. non-tilled soil

We tested which trait characteristics of plant species in combination with underlying soil conditions would predict the effect of tillage on the abundance of plant species (E_i index). This was undertaken using a multi-model inference approach with MuMIn (Bartoń, 2013) in R version 3.0 (R Core Team, 2015) with linear mixed effects models defined by the lme4 package (Bates et al., 2015). The E_i score of each species within each block was treated as a single data point so that the sample size for the analysis was 19 (number of blocks) × 51 (number of sown species). Fixed effects included in the model were seed mass, canopy height, SLA, guild, as well as habitat requirements in terms of soil moisture and plant-available forms of N, P, and K, whereas random effect were blocks. Soil pH was excluded from the model because it proved to be almost the same across all experimental plots. The approach runs all possible combinations of these models excluding interactions (1024 models) and uses Akaike's Information Criterion (*AIC*) to compare model fit (Burnham and Anderson, 2002). Models were ranked on the basis of their *AIC* value. For each of these models an *AIC* difference (Δ_i) was calculated as $\Delta_i = AIC_i - AIC_{min}$, where AIC_{min} is the lowest recorded value for any model, and AIC_i is the model specific *AIC* value. Δ_i indicates the relative support for each model and is used to derive Akaike weights (w_i) (Burnham and Anderson, 2002), which describe the probability that model *i* would be selected as the best fitting model if the data were collected again under identical conditions. The w_i of all N models sums to 1, so that the higher the value of this parameter the greater is the weight of evidence that it has an effect on the response variable of interest. Following Burnham and Anderson (2002) any model with a Δ_i of less than 2 has equivalent power in explaining variation in the data relative to the identified best fit model. This is referred to as the ΔAIC<2 model sub-set. Within this sub-set individual fixed effects will be represented to different extents, from inclusion within all models present in the ΔAIC<2 model sub-set to none. To assess the relative weight of evidence in support of each fixed effect a variable importance score was calculated as the sum of the w_i scores of models containing a given explanatory factor over the sum of w_i scores from all models within that $\Delta AIC<2$ subset. In addition, averaged parameter estimates weighted by individual model w_i scores were calculated (Burnham and Anderson, 2002). Finally, as *AIC* provides a relative measure of model fit we also followed the recommendations of Symonds and Moussalli (2011) and derived a marginal R^2 value for the global model. This provides an indication of goodness of fit of the models to the data, and allows an objective assessment of the importance of the considered variables in explaining responses in E_i.

3. Results

3.1. Effects of tillage management on the establishment of individual species

The difference in species abundance between the tilled and non-tilled plots (E_i) show a high level of variation across the sown species, which means that full inversion tillage had a variable effect on the success of species establishment. Over a quarter of plant species were significantly more abundant on the tilled area. For over two-thirds of species no significant difference between the control and treatment area was detected (the 95% confidence interval includes zero). Considerable variation of species response within the 19 blocks was observed, as indicated by wide confidence intervals (Fig. 2). Most of the plant species that were significantly more abundant on the tilled area were target species, characteristic of semi-natural grasslands. Also, an overwhelming majority of the species, for which no significant difference between the control and treatment area was detected, are common, generalist, non-target plants that do not need to be introduced during grassland restoration (Fig. 2).

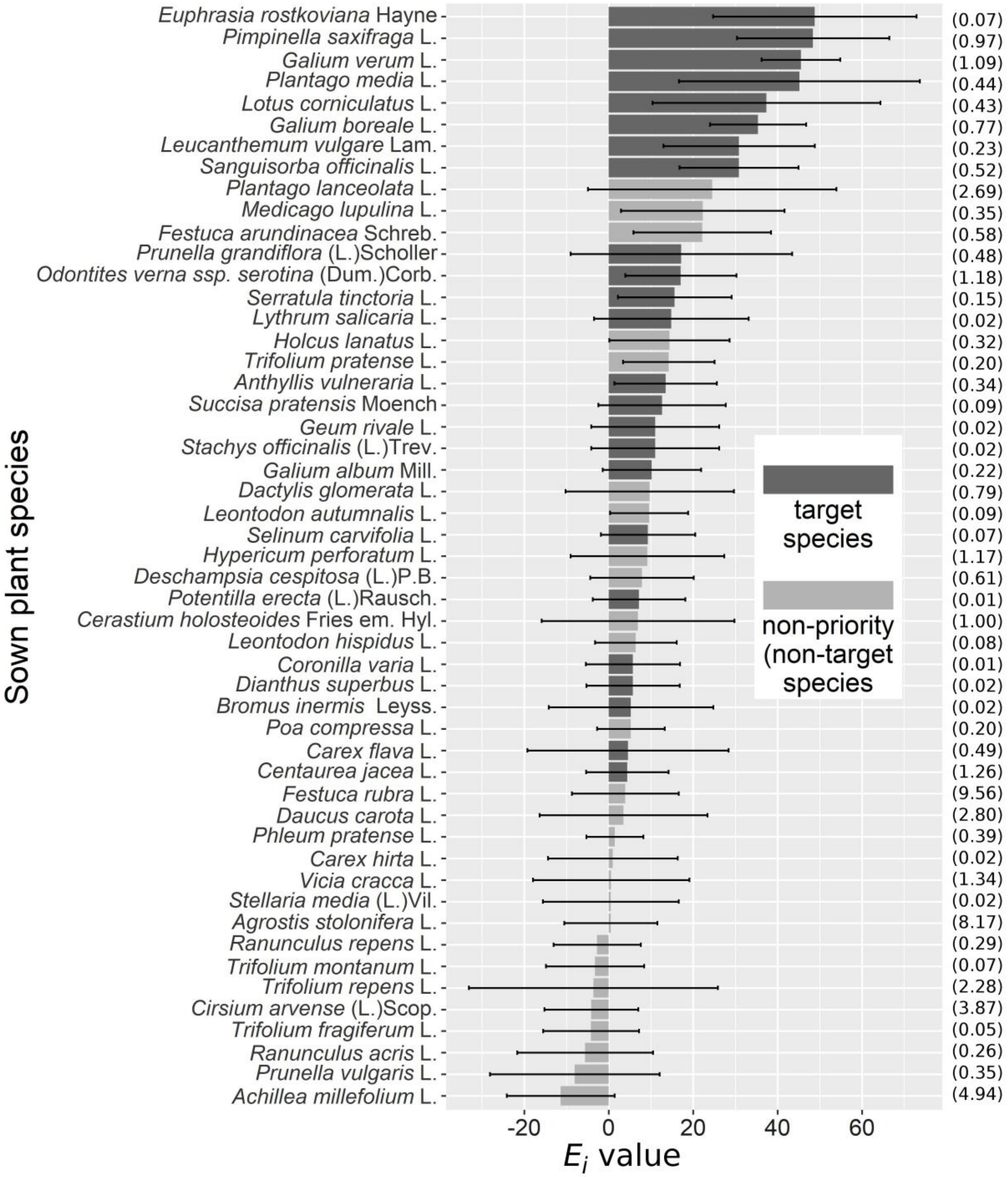

Fig. 2. The difference (E_i) between the abundance of sown species on the tilled plots (without plants which emerged as a result of soil diaspore bank activation during the tillage) and on the non-tilled plots (without

plants which were present in the sward before sowing). Species abundance is normalized percentage cover (see the main text for further details). The size of the difference, averaged across all 19 blocks, is denoted by the gray bars. The error bars are confidence intervals constructed for the difference between the means (paired two-sided t-test at the 95% confidence level). If the confidence interval includes zero, the difference between the means is non-significant. The numbers in brackets on the right denote species percentage cover averaged across all blocks and treatments at the experimental site.

The observed pattern in species response to the conditions caused by tillage was confirmed by the multivariate T^2 test, which showed that the abundance of the target species is significantly higher on the tilled plots than on the non-tilled plots, whereas the abundance of the non-target species between these two experimental treatments was not significantly different (Table 1).

Table 1

Statistical difference in the abundance of the sown plant species between the group of the tilled and non-tilled (control) plots. The significance was assessed separately for the semi-natural grassland species and for the other (non-target) species, using the multivariate two-sided Hotelling's T^2 test.

Group of the sown species	**Mean abundance in the non-tilled plots, ($X_{i,c} - X_{i,a}$), (%)**	**Mean abundance in the tilled plots, ($X_{i,d} - X_{i,b}$), (%)**	**Test statistic, T^2**	**Degrees of freedom**	***p*-value**
Semi-natural grassland species	1.2	21.4	9.89	24, 13	<0.001
The other (non-target) species	0.1	3.3	1.15	28, 9	0.438

3.2. Predicting the effect of tillage on plant establishment based on site conditions and species functional traits

Of 1024 models explaining the size of the effect of tillage on the abundance of the sown plants (E_i), only 17 were represented within the $\Delta AIC<2$ confidence set (Table 2). The global model for this relationship explained 7.6% of the variance in the data. Only two explanatory variables were present in all 17 models within the $\Delta AIC<2$ confidence set. These were ENIV and soil mineral N content (Table 2).

The establishment from seed on tilled soil was more successful for plant species with low ENIV (from 1 to 3), i.e. *Pimpinella saxifraga*, *Galium verum*, *Plantago media*, *Lotus corniculatus*, *Galium boreale*, *Leucanthemum vulgare*, *Prunella grandiflora*, *Serratula tinctoria*, *Anthyllis vulneraria*, *Succisa pratensis* and *Stachys officinalis* (Appendix D). Moreover, species establishment from seed on a tilled soil was more successful for soils that are richer in mineral N (Table 2), or more specifically, NH_4-N (Appendix E).

The dependence of E_i on species ENIV and soil mineral N content was caused by the response of species that were sown on the tilled plots. The response of the species that were sown into the non-tilled area was similar across the whole gradient of soil N availability and over the whole range of species ENIV (Figures 4a and 4b).

Soil moisture measured in the field was the third most important co-variable, being a component of 14 out of 17 models explaining species response to tillage. A slightly negative effect of tillage on the establishment of the introduced plant species was detected for the experimental blocks where soil was the moistest (Table 2 and Fig. 3c).

The fourth factor that diversified plant species composition of the experimental area in the first year after sowing was EMIV. This variable occurred in 11 out of 17 best fit models. The moister the realized habitat niche of a species, the poorer was the establishment from sown seed into tilled soil. This result was due largely to the unsuccessful establishment of plants that are indicative of moist habitats, such as *Carex flava*, *Lythrum salicaria*, *Trifolium fragiferum*, *Ranunculus repens*, *Potentilla erecta* and *Selinum carvifolia* (where the EMIV score is 7 or 8). Typically greater establishment success was observed for plants that occur in semi-dry habitats (EMIV is 3 or 4): *Plantago media*, *Pimpinella saxifraga*, *Prunella grandiflora*, *Medicago lupulina*, *Hypericum perforatum*, *Daucus carota*, *Leucanthemum vulgare* (Appendix D). The relationship between E_i and species EMIV was shaped mainly by the response of species that were sown on the tilled plots (Fig. 3d).

Table 2. The 17 linear mixed models (M1-M17) within the $\Delta AIC<2$ confidence set that explain the response of individual species establishment success on tilled soil to the fixed effects of individual species traits and soil conditions. The inclusion of a fixed effect within each of these models is indicated by 1, while *AIC* scores, delta weight (Δ_i) and the model selection probabilities (w_i) are provided. Parameter estimates (β) were generated by averaging across all models within the $\Delta AIC<2$ confidence set and using the selection probabilities to weight this process. VI-scores refer to the variable importance scores derived from summed w_i values. Abbreviations: : ENIV = Ellenberg's indicator value for N / nutrients (-); Soil N = Soil mineral N; EMIV = Ellenberg's moisture (-); SLA = Specific leaf area; Soil K = Plant-available K content in soil; Soil P – Plant-available P content in soil.

	ENIV	**Soil N**	**Soil moisture**	**EMIV**	**SLA**	**Seed mass**	**Soil K**	**Canopy height**	**Soil P**	***AIC***	**Δ_i**	**w_i**
M1	1	1	1	1						9682.2	0	0.10
M2	1	1	1							9682.3	0.12	0.10
M3	1	1	1	1	1					9682.4	0.24	0.09
M4	1	1	1		1					9683.0	0.84	0.07
M5	1	1	1	1		1				9683.2	1.05	0.06
M6	1	1	1			1				9683.3	1.15	0.06
M7	1	1		1						9683.5	1.32	0.05
M8	1	1	1	1			1			9683.6	1.38	0.05
M9	1	1								9683.6	1.45	0.05
M10	1	1	1	1	1	1				9683.6	1.47	0.05
M11	1	1	1				1			9683.7	1.50	0.05
M12	1	1		1	1					9683.7	1.55	0.05
M13	1	1	1	1	1		1			9683.8	1.62	0.05
M14	1	1	1					1		9684.0	1.82	0.04
M15	1	1	1	1				1		9684.0	1.84	0.04
M16	1	1	1	1	1			1		9684.1	1.93	0.04
M17	1	1	1	1					1	9684.1	1.97	0.04
***VI*-score**	1.00	1.00	0.85	0.63	0.35	0.17	0.15	0.12	0.04			
β	-3.013	1.090	-0.342	-1.949	-0.239	-0.779	0.055	-4.984	0.023			

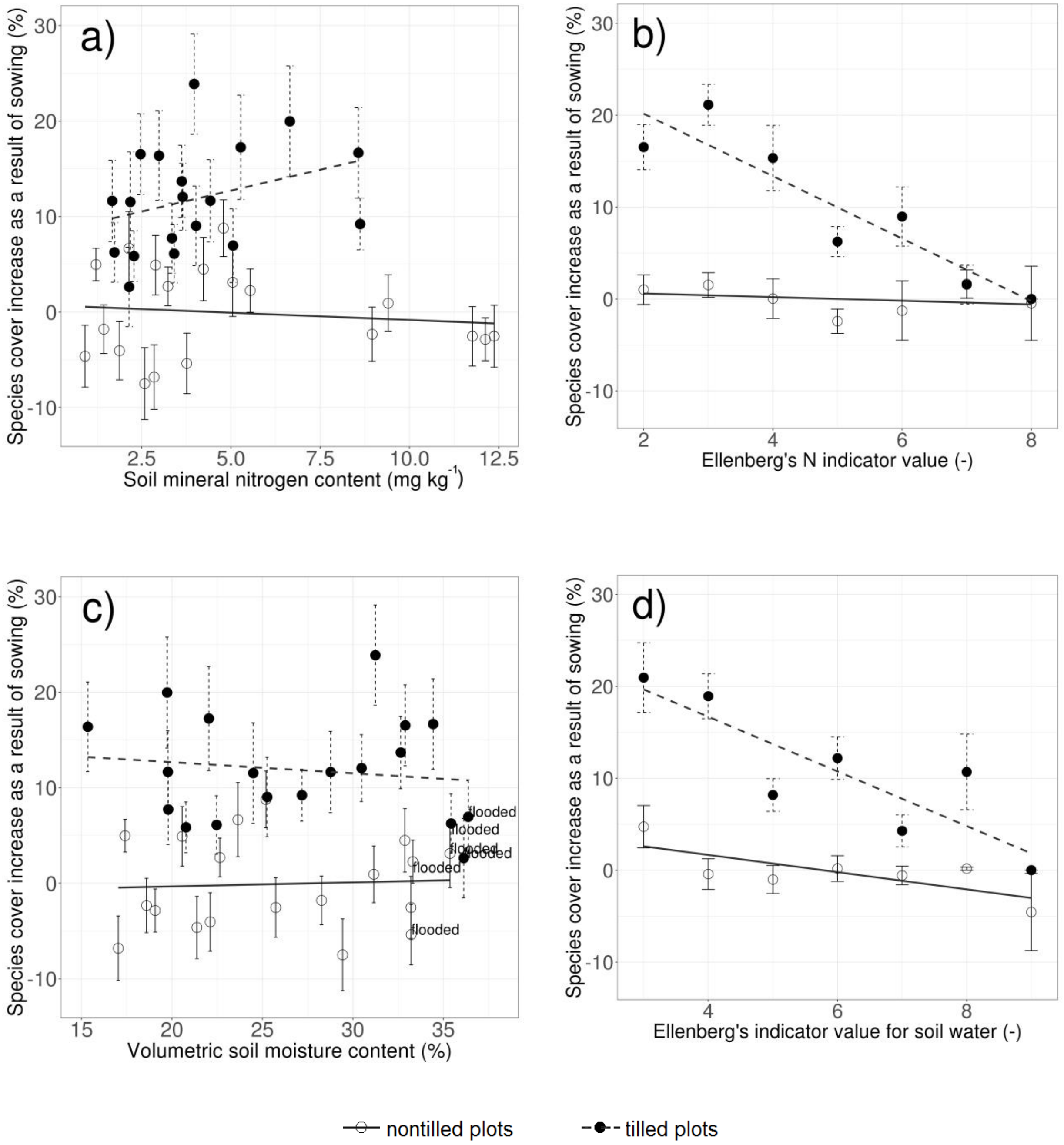

Fig. 3.

The abundance of the sown plant species on the tilled and non-tilled plots in the function of the most important predictors identified in the previous analysis (Table 2). The abundance of the species on the tilled plots refer to ($X_{i,d}$ – $X_{i,b}$) term in the equation 2; the abundance of the species on the non-tilled plots refer to ($X_{i,c}$ – $X_{i,a}$) term in the equation 2. The difference between these two terms is the effect of tillage on the abundance of the subsequently sown plant species (E_i). The following regressors were used: soil mineral nitrogen/nutrients content (a), Ellenberg's

N indicator value (b), volumetric soil moisture content (c), and Ellenberg's indicator value for soil moisture (d). Species abundance, i.e. the terms ($X_{i,d} - X_{i,b}$) and ($X_{i,c} - X_{i,a}$), were averaged across all plant species having the same Ellenberg's indicator value (plots a and c) or across all species recorded in the same pair of plots (plots b and d). The error bars denote the standard error of the mean. Regression lines (dashed line) are for univariate relationships only and are included to provide a visual reference for the relationship.

In addition to these key variables that dominated the best fit models of the $\Delta AIC<2$ confidence set, other factors were also seen to affect establishment. However, these all had low importance score (<0.4) and weak slope estimates (β), indicating that these variables were unlikely to be important predictors of predictors of E_i. This included SLA, seed mass, canopy height, and the availability of K and P in the soil. The tillage favored the establishment of species with relatively low SLA, small seeds and low canopy. Also, the sown species established more successfully on plots where the soil was richer in plant available K and P. None of the 17 models that were represented within the $\Delta AIC<2$ confidence set included guild (Table 2).

4. Discussion

4.1. Effects of tillage management on the establishment of sown plant species

The restored vegetation was observed after the sown plant species had survived the first phases of development: seed germination, seedling emergence and the growth of juveniles. Plant survival rates in this period shape long-term trajectories of community development and can determine the ultimate success of the restoration project as small differences in initial species establishment can result in priority effects with permanent impacts on community composition (Galatowitsch, 2008; Fukami et al., 2005; Young et al., 2005).

In general the comparison of the abundance of individual sown plant species between tilled and non-tilled plots suggests that soil tillage, used as a restoration management practice before sowing seeds promoted the establishment of semi-natural grassland species (Fig. 2). The multivariate test of the response of the target vs. non-target species provided evidence for this hypothesis (Table 1). These findings match results obtained in previous studies (Hofmann and Isselstein, 2004; Hopkins et al., 1999; Pywell et al., 2007; Schmiede et al., 2012) and are also in agreement with the rule formulated by Bullock (2000) whereby most species in most communities will establish better in gaps, although most species can also establish some seedlings in intact vegetation.

4.2. Predicting the effect of tillage on plant establishment: explanatory power of the model

Although percentage cover varied considerably across the sown species and experimental blocks, only 7.6% of the variation in cover was explained by extrinsic environmental conditions (e.g. soils) and intrinsic species traits. This means that the variations in the abundance among the sown plant species in the first year following sowing cannot be attributed merely to the characteristics of those species, so the exclusion of plant species from the sown pool in continuous sward represents a habitat and trait independent process to a certain degree. However, ecological filtering (Keddy, 1992) can still continue during the reproductive phase of plants' life histories. Independent of this, full inversion tillage represents a seedbed preparation measure that increases the chance that sown semi-natural grassland species will be able to outcompete common grassland plants.

It is highly likely that the variation in E_i metric, which describes the effect of tillage on the abundance of the subsequently sown plant species, was influenced by the timing of tillage and seed sowing. On the non-tilled area, the conditions for plant establishment were relatively constant throughout the first growing season after sowing, whereas on the tilled area, the chance of successful plant establishment from seed was highest in the spring in the first year following tillage. This was before the canopy of the developing vegetation had shaded soil surface. By the following summer and autumn, the conditions for seed germination and seedling emergence were not so favorable. The time niche for plant establishment which was created by tillage might have facilitated the establishment of species for which regeneration takes place in spring the year after their production, while inhibiting species that establish typically in autumn, shortly after seed shedding (Grime, 2002). The time window created by tillage might have also supported the establishment of species that quickly respond to the favorable environmental conditions by massive and rapid recruitment from the released seed, and do not rely on persistent soil seed bank (Grime, 2002). Since we had monitored the developing vegetation in spring or early summer after sowing, we were not able to investigate these effects. Nevertheless, they could have decrease the accuracy (R^2) of our model, and, given the possible priority effects, they should be investigated in future studies.

4.3. Predicting the effect of tillage on plant establishment based on site conditions

4.3.1 Soil nitrogen and Ellenberg's N indicator value

Among the intrinsic and extrinsic (environmental) predictors of species response to tillage, soil mineral N content was one of the most important factors. On the tilled part of the experiment area, lower availability of N in soil was accompanied by lower abundance of the sown species (Table 2, Fig. 3a). The other most important predictor was ENIV. The higher ENIV of a species so the lower was the abundance of this species on a tilled soil (Table 2, Fig. 3b). It is not clear what kind of environmental limitation underlies the latter relationship, as ENIV may reflect species response to the availability of nutrients in general, not only to the availability of N (Bartelheimer and Poschlod, 2016; Ellenberg and Leuschner, 2010; Schaffers and Sykora, 2000). However, the following findings suggest that ENIVs reflect species response to the availability of N: (1) the role of soil availability of P and K on species abundance was negligible; (2) the impact of soil N availability was relatively high, and its direction was the same as that seen for the relationship with ENIVs (Table 2). Moreover, the significant relationship between ENIVs of sown species and their abundance cannot be explained in terms of seedling adaptation to emerge in dense sward. This is because the relationship was determined by the response of species that were sown on the tilled plots, whereas the response of the species that were sown on the non-tilled area was similar over the whole range of ENIVs (Fig. 3b). Altogether, the results indicate that plants establishing on the tilled part of the experiment area grew under N deficiency in the year following tillage. Plowing grasslands in autumn greatly increases mineralization of soil N and this effect lasts for about a year. At this time, from 60 to 350 kg ha^{-1} is leached in the form of nitrate (Besnard et al., 2007; Conijn and Taube, 2004; Hatch et al., 2004; Shepherd et al., 2001). Certainly, the intensity of N mineralization on the studied ex-arable area was not as intense as reported for fertilized grasslands, because the studied soils were already poor in mineral N before tillage (Table 1). Also, the biomass which was plowed down was probably smaller than for fertilized grasslands, because it was produced by ruderal vegetation, which did not develop dense sod. Nevertheless, the literature cited above supports the hypothesis that in the experimental area, the rate of N mineralization was elevated in the year following soil inversion, but its resources liberated to the soil were rapidly leached from the surface layer and were no longer available for plants during the establishment of the sown species. Chemical analysis did not detect any difference in soil mineral N content between the tilled and non-tilled plots (Czerwiński et al., 2015). The failure to detect significant effects may have been due to the limited number of soil samples, with the many values below detection limits in the analyzed data set, necessitating the use of non-parametric tests which tend to be less sensitive to small differences than parametric tests (Czerwiński et al., 2015).

We were not able to unambiguously identify ecophysiological and morphological characteristics which lied behind the ENIVs of the sown species and influenced their abundance on the tilled area. However, among many traits of plant species that correlate with their ENIVs (Bartelheimer and Poschlod, 2016), the following seem to have played a significant role in our experiment: (1) N requirements for germination (species with high ENIVs germinate better at high N availability in soil while species with low ENIVs have optimum germination at lower N availability); (2) N requirements for the formation of leaves (the requirements are higher for species that have higher ENIVs); (3) relative growth rate (which is limited by the availability of N or nutrients in general).

4.3.2 Soil moisture and Ellenberg's M indicator value

The predictors of species response to tillage linked to soil moisture were the other two most important factors. The establishment success on the tilled vs. non-tilled area was lower for species that are indicative of moist habitats and for sites (experiment blocks) where the soil was moister. These two relationships seem to be contradictory, but further analysis shows that they complement each other (Fig. 3c and 3d). The former relationship can be easily explained by the effects of drought that occurred in July in the year when the introduced plants were establishing. The drought seem to have limited the development of the introduced plants, particularly those that are typical of moist habitats and this limitation must have been stronger on the tilled area because of the exposure of plants to direct insolation and wind (Fig. 3d). This explanation is consistent with the second hypothesis of our study. The latter dependence, which does not support this hypothesis, was shaped by low E_i scores obtained for three blocks that were flooded in the first spring after sowing seeds. Within these blocks many introduced plants must have died from soil anoxia and the mortality was higher on bare soil surface, which was situated a few centimeters lower that on the non-tilled area due to the lack of the layer of turf and litter (Fig. 3c).

4.3.3 Other soil conditions

The positive effect of tillage was more pronounced on the experimental plots where soil K was more available (Table 2). This indicates that the plants introduced into a tilled area grew under K deficiency during their establishment. Indeed, in our previous study we observed a decrease in the content of mobile K in the surface layer of the soil under the influence of tillage operations (Czerwiński et al., 2015). This decrease should be attributed chiefly to the acceleration of the chemical weathering of the primary minerals in which nearly all of the soil K is bound, and the accompanying leaching of K into the deeper soil layers (Mengel, 2007).

The influence of soil P content on the cover of the sown plant species on the tilled vs. non-tilled area was found to be marginal. This could be due to plant growth being limited principally by the availability of soil mineral N (Table 2), and so according to the Liebig’s law of the minimum, this element determined the results. Alternatively, soil P content was similar for ca. 80% of the experimental plots (Appendices E and G), which could have hampered the detection of the effect of this element.

4.4. Predicting the effect of tillage on plant establishment based on species functional traits

The difference in cover of the sown plant species between the tilled and non-tilled plots decreased with the increasing SLA of the species. This relationship can be interpreted in view of the severeness of the conditions that prevailed on the tilled land, and in the context of plant species characteristics that are associated with SLA. High SLA is typical for plants that are relatively sensitive to drought, and occur in shady and wind-sheltered places. Also, plants with high SLA fail to dominate on nutrient-poor places (Lambers et al., 2008).

Tillage was advantageous for the establishment of smaller-seeded species, but the observed relation was quite weak (Table 2). It is worth noting that the results of studies investigating the establishment of grassland plants in naturally appearing gaps also failed to sufficiently support the hypothesis that seed size plays important role in this process (Bullock, 2000).

We found that the relationship between canopy height and the effect of tillage on the abundance of the subsequently sown plant species was quite weak. This may seem somewhat surprising, since canopy height, similarly to seed mass, affects the competitive vigor of plants. What is more, for the analyzed species pool these two traits were positively correlated (r=0.39). It should be noted, though, that seed mass influences species competitiveness during seedling establishment (Kotowski et al., 2010), the phase which was crucial for the outcome of our experiment, whereas canopy height is associated with competitive vigor throughout the whole plant life.

4.5. Implications for practice

The results of this study have important implications for grassland restoration, particularly where seed mixtures contain a diverse range of species. This may be the case where seeds are harvested from existing species-rich grasslands, using indiscriminate and extensive suction or brushing methods. Where soil tillage precedes sowing of these seed mixtures, and the time period between these two restoration measures is sufficiently long, plants typical of low-productive but diverse communities will be the principal beneficiaries. However, the absence of tillage is likely to select against such species, creating an establishment bias for common generalist herbs that while present in many grasslands, do not represent a key target for restoration. These results favor the inclusions of tillage into environmental management schemes aimed at promoting grassland restoration.

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