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Research article

How to successfully improve the biodiversity of city grasslands?

Hassanali Mollashahi [a], Tomasz H. Szymura [b], Magdalena Szymura [a,*]

[a] Wrocław University of Environmental and Life Sciences, Institute of Agroecology and Plant Production, Wrocław, Poland
[b] University of Wrocław. Botanical Garden, Wrocław, Poland

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ABSTRACT

Urban grasslands (UG) are among the most common types of urban green areas. They are usually species poor, and spontaneous plant migration, which can increase biodiversity, is limited. To improve the range of ecosystem services provided by UG, various seed mixtures are applied during the establishment and restoration of UG. These mixtures vary in content, quality, and cost. High-quality seed mixtures are expensive and are usually only available in small amounts. Meanwhile, alternative methods of seed introduction (e.g., fresh hay application, seeds harvested by brush) have not been well studied in UG restoration, and inexpensive commercial mixtures could have low quality and lead to poor restoration outcomes. Here, we tested the effectiveness of different seed sources to create high-quality UG at two study sites. Based on the results, all seed addition methods increased the species richness of restored grasslands. The outcome of seed addition was satisfactory regardless of differences in residual vegetation species composition and soil properties between the sites. The species richness on plots that received a commercial mixture of flower meadow plants dedicated to pollinators decreased after overwintering. The alternative seed sources (fresh hay and seed incidentally collected during mowing) yielded grassland quality that was comparable to that on plots that received high-quality mixtures with known seed origin (a seminatural meadow mixture and a mixture with the addition of grasses).

1. Introduction

Urban grasslands (UG) are among the most common types of urban green areas [1]. They deliver numerous ecosystem services [2–4], including the maintenance of biodiversity [5], often in connection with serving as habitat for pollinators [6,7]. Another group of ecosystem services delivered by city grasslands are cultural aspects, such as recreational, psychological, and aesthetic benefits to city residents [8]. The concept of multifunctionality combines ecological, social, economic (or abiotic), biotic, and cultural functions of green spaces [9]. The relationship between ecosystem multifunctionality and species richness is not straightforward [10], but in the case of grasslands, increased species richness generally increases multifunctionality [11]. The species composition of UG also influences their multifunctionality under climate change; for example, communities with equal proportions of herbs and grasses perform better than communities dominated by one group [12]. To deliver ecosystem services, UG need to have a high diversity of plant species and to be properly managed [13]. However, UG are usually species poor, and the spontaneous species migration that can increase biodiversity is very limited in cities ([14–16], however, see [17]). Therefore, the modern UG should be established and/or managed with the goal of having high biodiversity and including cover with native, local-origin species typical of grasslands with a high fraction of flowering plants that can provide food for pollinators [18–21].

* Corresponding author.
E-mail address: magdalena.szymura@upwr.edu.pl (M. Szymura).

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Unfortunately, most UG are based on seed mixtures with a low species content and a low ecological value [22,23]. Decreased quantity and diversity of flowers, which results in food inadequacy for pollinators, is one of the reasons that pollinators decline in the landscape, including urban areas [6]. As a remedy, "pollinator-friendly" seed mixes are sown in cities, but the floral resources that the constituent plant species provide to pollinators and the seasonal changes in pollen and nectar production are poorly known [19]. Moreover, invasive species and anthropogenic disturbances, such as trampling of vegetation, soil compaction, pollution, and high mowing frequency, affect the habitat quality of grasslands [24,25].

In practice, UG are restored or established using different seed mixtures of varying cost and quality, leading to a range of possible outcomes. High-quality species-rich seed mixtures of local ecotypes are very expensive, and in some regions, such as Poland, they are only available in small amounts [26,27]. Alternative methods of seed introduction (e.g., fresh hay application, seeds harvested by brush) are still in the initial stages of assessment for UG restoration [27–29]. Compared with the two above-mentioned methods of seed acquisition, mixtures of seeds collected from seed plantations carry the risk of unwanted introduction of undesired species, such as invasive species or weeds [28]. These commercial seed mixtures are inexpensive and available in large amounts, but they can contain cultivars that are often alien in origin, as well as species that are not adapted to grasslands, such as arable weeds and/or ruderal species (e.g., 18, 28, see also the composition of seed mixture used in current study). If seed materials are not adequate with regard to the local environment, the restoration may fail because of disrupted plant–pollinator relationships, loss of resiliency and adaptive capacity in coping with environmental stressors, poor regeneration potential, and genetic degradation, for a review, see Ref. [30]. Although the commercial seed mixtures may initially result in high emergence and vigorous growth of grasses, the newly established vegetation can disappear in a few years, likely due to low tolerance to environmental stressors [31]. Moreover, the typical vegetation of seminatural grasslands is dominated by plants that easily regenerate under an extensive mowing regime. The vigorous growth of these species inhibits annual species such as therophytes [32], and it is highly probably that in low-maintenance, species-rich UG, such annuals will be competitively excluded.

The aim of the current study was to assess the effectiveness of different seed sources to create high-quality UG. We used different

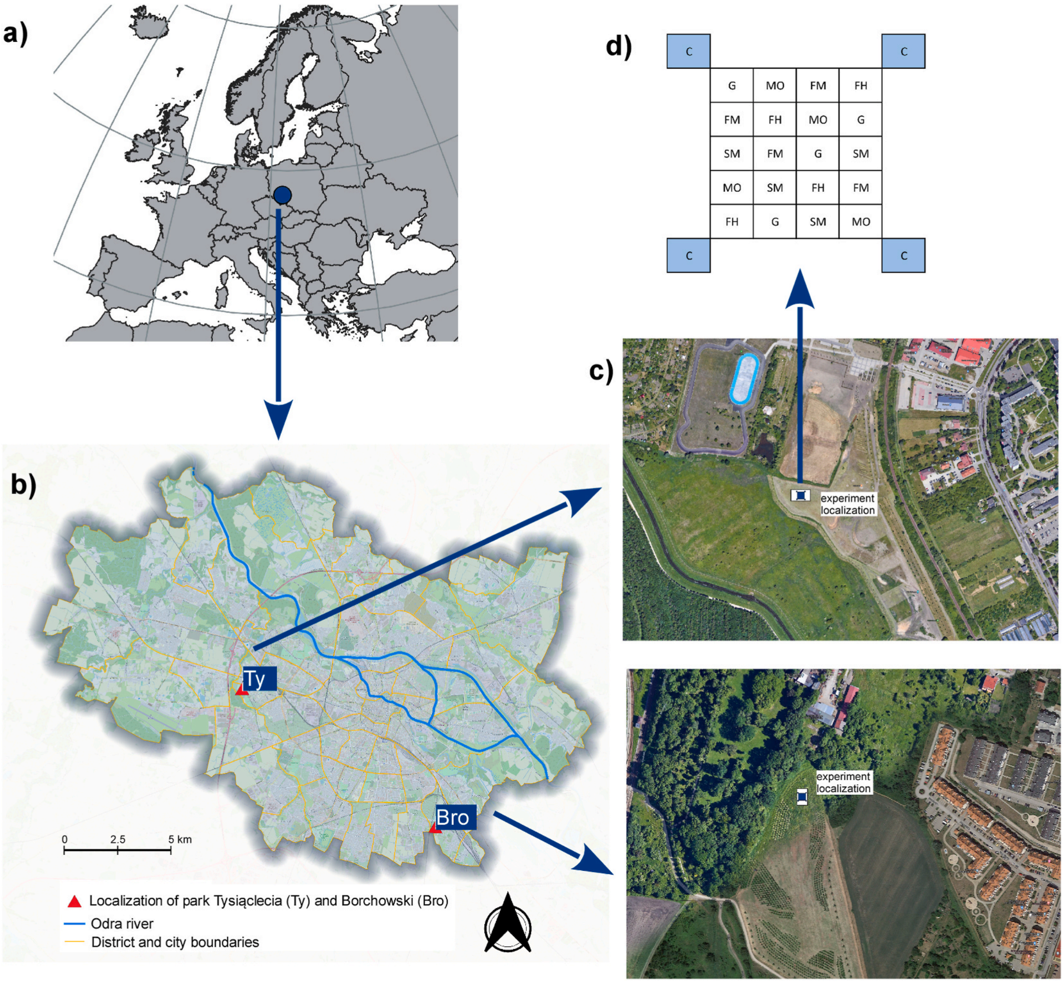

Fig. 1. Locations of the study sites (a), parks in Wrocław city (b), experiments in parks (c), and scheme of the experimental plots (d).

seed mixtures, including a commercial mixture of flowering meadow plants beneficial for pollinators (FM), a seminatural meadow mixture with known seed origin (SM), seeds incidentally collected from mowing (MO), and a mixture with grass species with known seed origin (G), and the spreading of fresh hay (FH). We hypothesized that (1) species richness and cover would decrease after overwintering in plots where FM was applied; (2) the overall value and species richness in SM and G plots would not decrease after overwintering; (3) the overall value of FH and MO would not differ from that of SM and G; and (4) the seeds collected incidentally from mowing and those from fresh hay could contain unwanted species (i.e., invasive species, weeds). We then sought to answer the following question: Which method of seed introduction ensures the highest biodiversity and the highest overall quality after two years?

2. Materials and methods

2.1. Experimental design

To test our hypothesis, we established experimental plots at sites in two parks in Wrocław city, Poland, Central Europe: Tysiąclecia park (Ty) (51°06'59.3″N, 16°56'24.3″E) and Brochowski park (Bro) (51°03'37.0″N, 17°04'24.6″E) (Fig. 1a–d).

Both parks were recently established on waste lands and abandoned fields and had existing ruderal vegetation. The characteristics of the experimental site soils are shown in Table S1. The experimental design was the same at both parks. The experimental sites were 15 × 12 m in size and enclosed by fencing, and the experimental plots were in full sunlight. Five different seed mixtures were applied: commercial flower meadow with plants beneficial for pollinators (FM), semi-natural meadow mixture with known seed origin (SM), seeds incidentally collected from mowing (MO), mixture with grass species with known seed origin (G), and spreading of fresh hay (FH). The FM was a commercial seed mixture (SAATBAU Company) of 14 floral species of unknown geographical origin normally used as a food source for bees. The SM and G were provided by Rieger-Hofmann GmbH, and these mixtures consisted of native species with a known origin (Lower Saxony). The FH and MO seeds were harvested at the Experimental Station of Wrocław University of Environmental and Life Sciences in Radomierz (50°54'15.1″N, 15°53'58.8″E), Silesia, Poland, Central Europe. A detailed description of FH and MO collection is presented in Ref. [27]. Table S2 presents the species composition of seed mixtures and the site from which the fresh hay was taken. The individual experimental plots were 3 × 3 m in size and arranged randomly, with four replicates for each treatment. In addition, four additional plots were established in each corner of the experimental site as controls. The entire experimental area was mowed and the hay was removed in July 2020, and the soil was then prepared with a rototiller machine. The fresh hay was collected on July 31, 2020, and immediately transported to experimental sites to be spread on the plots receiving the FH treatment. The donor-to-acceptor site area ratio was 1:1, and the layer of fresh hay in the FH plots was ca. 10 cm deep. The seed mixtures were seeded in September 2020 at the seed rates recommended by the producer: 2 $g*m^{-2}$ for SM and 4 $g*m^{-2}$ for G and FM. The rate 4 g/m^2 was also used for the MO plots. Afterward, the seeded soil was rolled.

The vegetation was assessed in June 2021 and 2022. Plants were identified at the species level according to Ref. [33], with the exception of *Taraxacum* microspecies that were aggregated at the genus level (*Taraxacum* sp.). The species were classified with regard to their status in Polish flora, usefulness to pollinators, longevity, and affinity to seminatural grasslands. A list of invasive and cultivar species was created based on [34], and the usefulness of species for pollinators was based on [35,36]. Species with a high conservation value were identified following [37], the affinity to seminatural grassland species was assessed according to Ref. [38], and information on lifespan was drawn from Ref. [39]. Detailed information on the status of particular species is shown in Table S3.

2.2. Statistical methods

The differences between treatments and years, including interactions were checked with the linear mixed effect model using the lme4 package [40] in the R statistical environment [41]. The fixed effects were the seed source, the year, and their interactions, whereas the random effect was the parks. The significance of the main effects and interactions was assessed using type III analysis of variance in the lmerTest package [42]. Post hoc pairwise comparisons were performed using Tukey's multiple comparison test with Benjamini–Hochberg correction [43] in the emmeans package [44]. To briefly summarize the study findings, linear discriminant analyses were performed in the MAAS package [45] on the normalized data, and the results are presented as a biplot. The species composition was analyzed using the nonmetric multidimensional scaling (NMDS) approach in the vegan package [46]. The NMDS the species cover was square root transformed a priori, and the data set was treated by Wisconsin double standardization. The NMDS analyses were performed for different numbers of dimensions, from one to eight, and the observed stress values were plotted against the number of dimensions to compare changes in the associated stress values and to determine the appropriate number of dimensions [47].

3. Results

In total, we found 106 species, with 88 in the first year and 86 in the second year. The vegetation was dominated by native species (96 species), and it had a high fraction (ca. 40%) of short-lived species (44 species). Thirty-nine species were considered typical for seminatural grasslands, and 28 provided food for pollinators. Four species were considered to be invasive (*Erigeron annuus*, *Solidago canadensis*, *Solidago gigantea*, *Bunias orientalis*), and four species were identified as having a high conservation value in Poland, with three (*Bupleurum rotundifolium*, *Camelina sativa*, and *Matricaria chamomilla*) being archeophytes, typical weeds of traditional agriculture systems (Kaźmierczakowa et al., 2016). The fourth species was *Dianthus superbus*, a species that is typical of seminatural grasslands. Detailed information on cover for particular species in the different years of the experiment is shown in Table S4.

We found significant differences among almost all analyzed traits, with the exception of perennial species cover (Table 1). In particular, we observed a significant effect of the year × method interaction. For species cover, grassland species number, and cover, there was a significant effect of year only. Method had a significant effect only for invasive species cover (Table 1).

Each of the seed introduction methods increased the species richness in comparison with the control (Fig. 2a). A similar outcome occurred in terms of the Shannon diversity index, except that FM and MO did not differ from the control in the second year of the experiment (Fig. 2c). FH was distinguished by relatively high species richness and Shannon diversity index. In FM, decreases in both species richness and the Shannon diversity index were observed in the second year of the experiment relative to the first year. In the case of species cover, increases were found in the second year of the experiment for all methods compared with the first year (Fig. 2b).

We observed that all seed introduction methods increased the number of short-lived species compared with the control (Fig. 3a and b). The number of short-lived species was especially high in the case of FM in 2021, but it was significantly decreased in 2022. The cover of short-lived species was low in the control plots in both years of the experiment, but G, MO, and SM did not differ significantly from the control in 2021. The perennial species number was low in the control and FM in both years of the experiment and also in G in the first year of the experiment (Fig. 3c and d).

Among all plots, the control had the lowest number of native species in both years of the experiment, with FM also having low numbers in the second year of experiment (Fig. 4a). The FM treatment was distinguished by having the lowest cover of native plant species and the highest cultivar species number and cover in both years of experiment (Fig. 4b). However, we also observed a significant decrease in cultivar numbers and an increase of their cover in the second year of the experiment in the FM plots (Fig. 4c and d). With regard to invasive species, cover was the highest in the control plots in both years of the experiment, but the difference was significant only in 2021. For invasive species number, we observed a higher number in FH compared with FM and SM, but only in the first year of the experiment (Fig. 4e and f).

The number of species providing forage for pollinators was the lowest in the control, where species from *Trifolium*, *Taraxacum*, and *Solidago* occurred, but the value in the second year in control plots did not differ from that of FH in the first year. However, other plant species were present in FH and control plots, such as *Knautia arvensis*, *Medicago lupulina*, *Lotus corniculatus*, and *Vicia cracca* (Fig. 5a, Table S4). In the second year, the number of species useful for pollinators was the highest in the FH plots, but the number was not significantly different from that of FM in either year and those of G, MO, and SM in the second year. The cover of species useful for pollinators was the lowest in FH and SM in the first year, but it did not differ from G and MO in first year and from FH in 2022. The highest cover of species for pollinators was in FM in the second year, but it did not differ from control in the first year (Fig. 5b). In FM plots, *Trifolium incarnatum* and *M. lupulina* dominated, and in control, *Trifolium repens*, *Taraxacum* sp., and *Solidago gigantea* were dominant (Table S4).

Control plots also had the lowest number of species typical for seminatural grasslands, but the value did not differ from FM in either year or from G and SM in the first year of the experiment. The highest number of grassland species was in FH plots in the first year, but it did not differ from MO in either year or from SM and G in the second year (Fig. 5c). Grassland species had the lowest cover in FM, but it did not differ from G in either year of the experiment. The lowest cover of grassland species was in C in 2022, but the value did not differ from FH and MO in either year (Fig. 5d). The dominant grassland species present in FH were grasses, including *Arrenatherum elatius*, *Lolium perenne*, *Trisetum flavescens*, and the hemiparasite species *Rhinanthus minor* (Table S4).

The results of the discriminant analysis underlined the contrast between FM and the other treatments and the control (Fig. 6a). The FM plots were characterized by a high fraction of cultivars and short-lived species and the presence of species with high conservation value. A high fraction of invasive species, and species useful for pollinators were observed in control plots (Fig. 6b).

The four-dimension NMDS ordination plots for sites and species are shown in Fig. S1. The first ordination axis can be interpreted as reflecting differences between particular parks; the second, differences between years; and the third and fourth, the interactions among

Table 1
Results of generalized linear models (*F, p*) for year, method, and interaction. Significant values with $p < 0.05$ are in bold type.

trait	year		method		year x method	
	F	p	F	p	F	p
Species richness [N]	1.522	0.221	**5.542**	**<0.001**	**5.502**	**<0.001**
Species cover [%]	**57.418**	**<0.001**	1.698	0.144	1.768	0.129
Shannon index	**8.530**	**0.004**	**7.455**	**<0.001**	**7.357**	**<0.001**
Short lived species number	**50.126**	**<0.001**	**11.912**	**<0.001**	**11.177**	**<0.001**
Short lived species cover	**12.797**	**<0.001**	**2.412**	**0.043**	**2.516**	**0.036**
Perennial species number	**18.208**	**<0.001**	2.163	0.066	**2.342**	**0.048**
Perennial species cover	0.770	0.383	1.607	0.167	1.569	0.178
Native species number	1.38	0.243	**3.506**	**0.006**	**3.554**	**0.006**
Native species cover	**16.579**	**<0.001**	2.024	0.083	**2.439**	**0.041**
Cultivar species number	**10.323**	**0.002**	**43.743**	**<0.001**	**40.798**	**<0.001**
Cultivar species cover	**28.795**	**<0.001**	**20.319**	**<0.001**	**23.867**	**<0.001**
Invasive species number	**9.388**	**0.003**	**3.333**	**0.008**	1.358	0.248
Invasive species cover	0.491	0.486	**9.578**	**<0.001**	0.238	0.945
Species for pollinators number	**24.462**	**<0.001**	**5.374**	**<0.001**	**5.506**	**<0.001**
Species for pollinators cover	**27.331**	**<0.001**	**8.020**	**<0.001**	**7.947**	**<0.001**
Grassland species number	**20.929**	**<0.001**	1.832	0.115	1.986	0.089
Grassland species cover	**9.588**	**0.003**	1.762	0.130	1.837	0.115

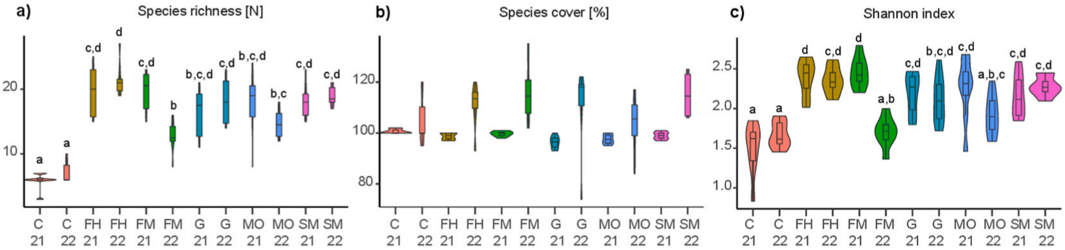

Fig. 2. Species richness (a), cover (b), and Shannon diversity index (c) for different types of seed introduction methods and years of observation with results of post hoc tests. Different lowercase letters indicate significant differences among the factors. Notice that for species cover, only the year effect was significant. The median value (horizontal line), upper and lower quartiles (box), minimum and maximum (whiskers), and kernel density estimation (filed area) are shown.

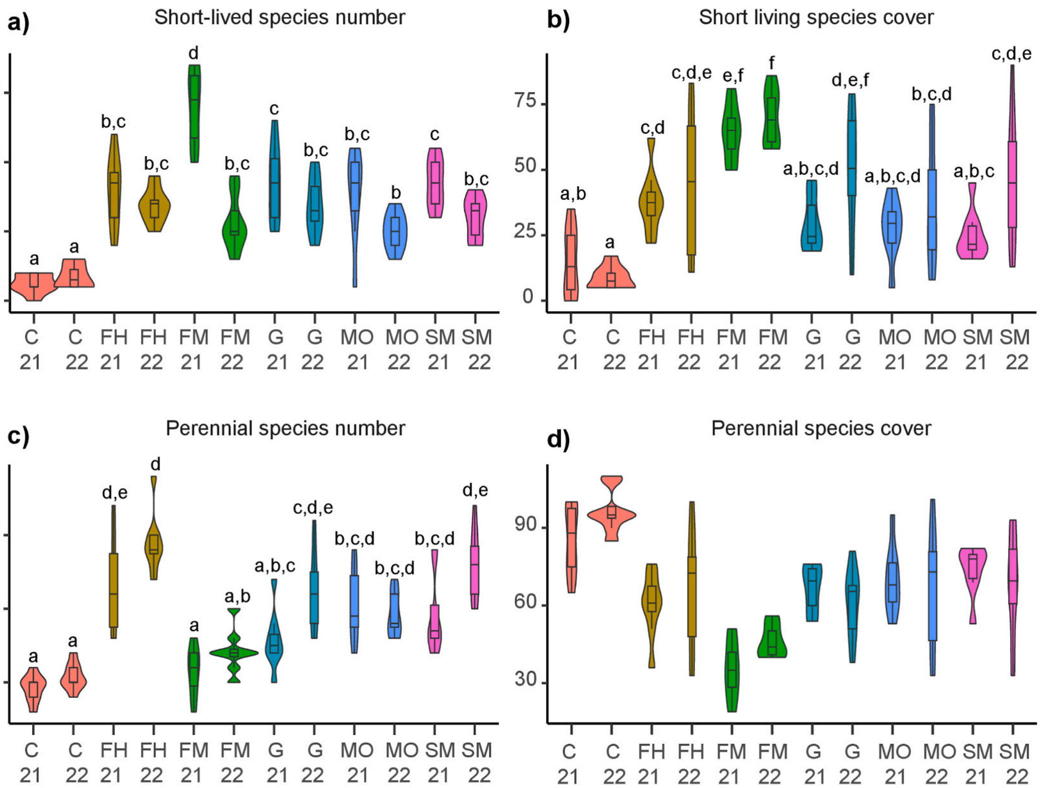

Fig. 3. Number and cover of short-lived (a, b) and perennial (c, d) species for different types of seed introduction methods and years of observation with results of post hoc tests. Different lowercase letters indicate significant differences among the factors. The median value (horizontal line), upper and lower quartiles (box), minimum and maximum (whiskers), and kernel density estimation (filed area) are shown.

seed introduction methods and years (Fig. S1). The interpretation of changes in stress values associated with the number of dimensions suggests that one-dimension ordination gives almost random results and two-dimension ordination is poor, while results for three and more dimensions are good (Fig. S2).

4. Discussion

Our results show that different seed addition methods resulted in differentiation of vegetation characteristics. We also observed significant differentiation between years of the experiment, and the vegetation was shaped by interaction between treatments and years in practice.

As we hypothesized, biodiversity measures in FM decreased after overwintering, but contrary to our expectations, the vegetation cover increased. The decrease of biodiversity was related to low survival of short-lived species, often cultivars, such as *Calendula officinalis*, *Coriandrum sativum*, *Helianthus annuus*, and *Phacelia tanacetifolia*. Fortunately, the species lost did not result in a decrease of the vegetation cover because the gaps were filled by the surviving species, especially *Trifolium incarnatum*, for which coverage almost

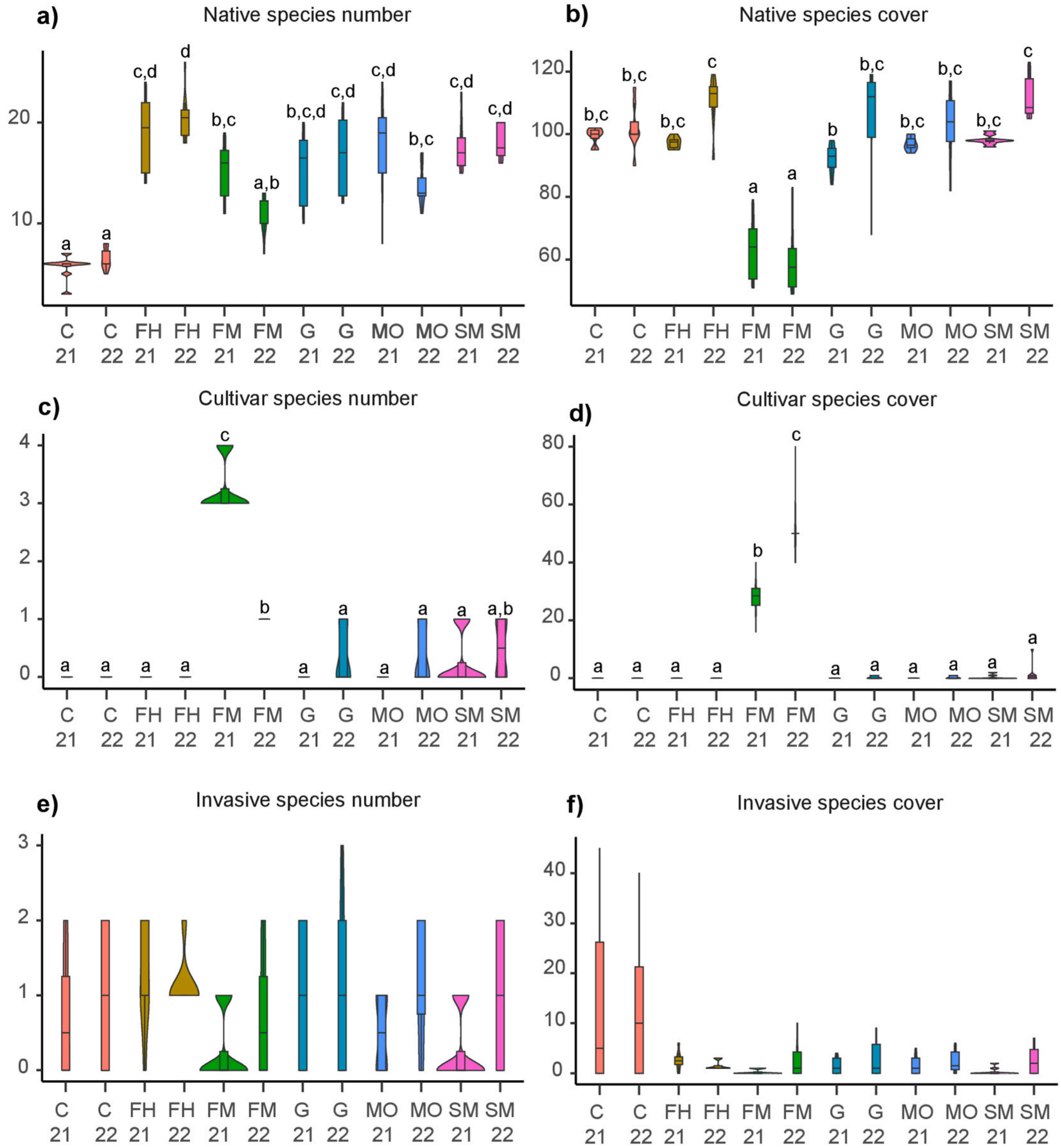

Fig. 4. Number and coverage of native (a, b), cultivar (c, d), and invasive (e, f) species for different types of seed introduction methods and years of observation with results of post hoc tests. Different lowercase letters indicate significant differences among the factors. The median value (horizontal line), upper and lower quartiles (box), minimum and maximum (whiskers), and kernel density estimation (filed area) are shown.

doubled in the second year. This species is considered useful for pollinators, thus its spread caused a significant increase in the cover of pollinator-friendly species (Table S4). *Trifolium incarnatum* is used as a catch crop for forage or as a green manure crop (winter cover crops) in field rotation [48,49]. In Central Europe, seeds of *T. incarnatum* are added to seed mixtures used as flower meadows in urban greenery (e.g., by Łąki Kwietne, SAATBAU, and Werbena-ar companies), because of its nice smell and blood-red flowers [50]. This species is native to southeastern Europe and southwestern Asia Minor, while in Poland, it is considered an ephemerophyte [34,49], and in China and United States, it is invasive [51,52]. The future of this species in Poland is not clear. On the one hand, it can be invasive, but on the other hand, its cultivation as a forage plant is limited to regions with a long period of relatively mild, moist weather [50]. Therefore, its long-term survival without seed addition (naturalization) is uncertain. Moreover, the flowering period seems to be very short, about one to two weeks (Szymura M, personal observations), which suggests that its function for pollinators is rather restricted.

In accordance with our hypothesis, the species richness and diversity of SM and G plots did not decrease after overwintering, and the cover increased significantly. We also did not observe a decrease in other measures of utility (pollinator species, grassland species). As briefly mentioned in the introduction, the seeds of grassland species of local origin are well adapted to the local climate, which is beneficial for long-term survival [29,53]. Moreover, the mixture consists of typical grassland species that are adapted to ecological conditions typical for grasslands, such as interspecific competition and resistance to low-intensity mowing [54,55]. Additionally, plant

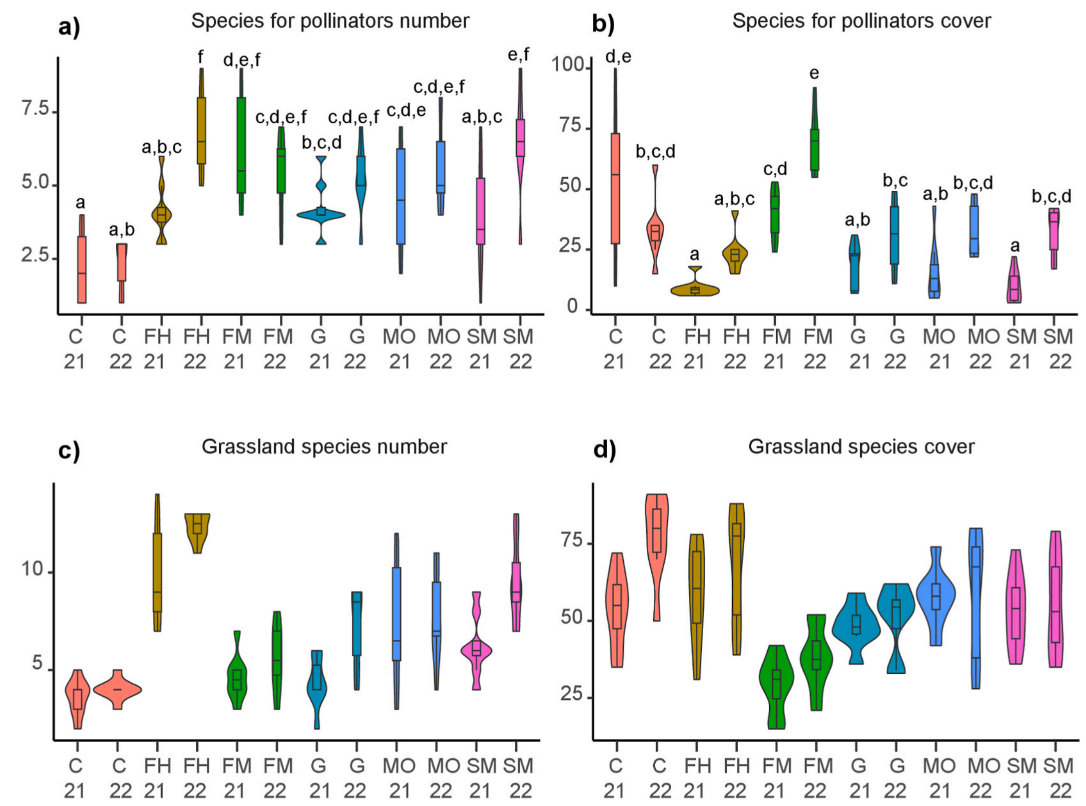

Fig. 5. Number and cover of species useful for pollinators (a, b) and species typical for grasslands (c, d) for different types of seed introduction methods and years of observation with results of post hoc tests. Different lowercase letters indicate significant differences among the factors. The median value (horizontal line), upper and lower quartiles (box), minimum and maximum (whiskers), and kernel density estimation (filed area) are shown.

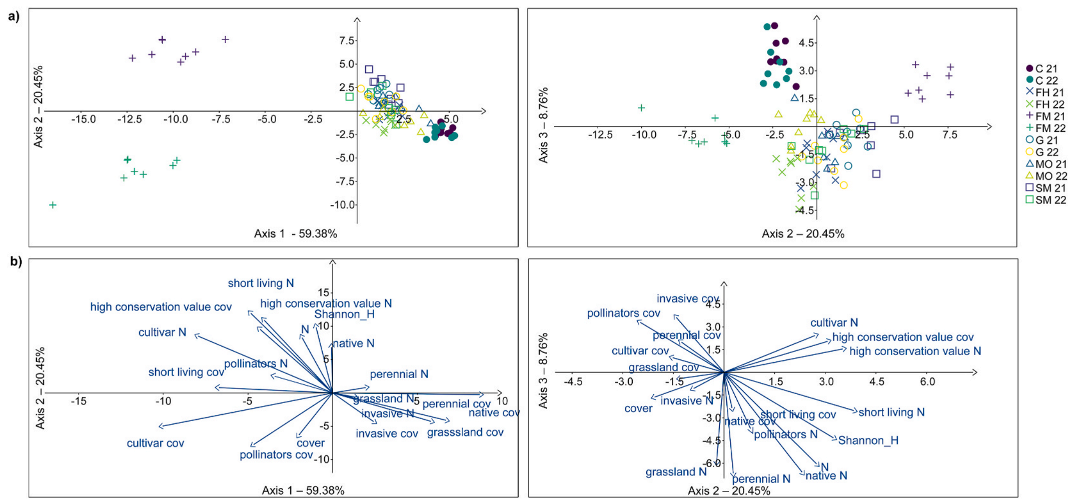

Fig. 6. The biplots of discriminatory analysis results for plots (a) and vegetation characteristics (b) for four discriminant axes.

provenances differ substantially in terms of their interactions with local pollinators. Therefore, the selection of plant provenance should be considered when planning restoration projects for supporting pollinators [56].

As we expected, the alternative methods of UG restoration, namely FH and MO, generally did not differ from SM and G plots in terms of overall quality. Specifically, they did not caused the introduction of unwanted species (hypothesis 4). The majority of invasive species (*Erigeron annuus*, *Solidago canadensis*, and *Solidago gigantea*) that occurred in the experiment emerged from resident vegetation and were present mostly in the control. Only *Bunias orientalis* was introduced accidently with FH on a single plot in first year, and it disappeared in the second year of the experiment. In light of the high prices for species-rich high-quality seed mixtures [26], the FM and MO seem to be reasonable alternatives for UG restoration, but an experienced botanist should choose the site and time of seed or fresh hay collection [27]. To our knowledge, FM and MO are rarely used in UG restoration and establishment, (but see [16,57]) however, they seem to constitute a promising approach.

The results presented here should be considered as preliminary because we expect that further vegetation development under regular, low-intensity mowing will lead to competitive exclusion of some species and overall vegetation structure changes [58]. The cover of grassland species can be expected to increase with time, and species composition between plots may homogenize due to unassisted seed migration.

The results of our two-year experiment suggest that any method of seed introduction would increase the biodiversity indexes and the number of plant species useful for pollinators. The lack of seed introduction resulted in a high increase in invasive species cover, especially during the first year of experiment, and quite surprisingly, in high cover of species such as *Trifolium repens* that are useful for pollinators. We considered the two phenomenon as idiosyncrasies resulting from the composition of residual vegetation in the restored areas. The effect of parks is clearly visible in the differentiation in species composition along the first NMDS axis (Fig. S1), and the processes during UG restoration could be expected to be influenced by the legacy of resident vegetation and differences in soil properties. Nonetheless, the changes of NMDS stress values with an increase in model dimensions (Fig. S2) and the results of the mixed effect model, with parks considered as a random effect, suggest that, in addition to the differentiation between parks, the effects of seed addition and year significantly influenced the results. In particular, the high stress value for one-dimension NMDS and the considerable decrease of this value with more dimensions (Fig. S2) suggest that the observed species composition is shaped simultaneously by effects of the park, the seed source, and the year, and the park effect is not especially prominent.

5. Conclusions and practical implications

1) Any of the applied seed addition methods increased species richness of the restored grasslands.
2) The species richness in plots with commercial flower meadow plants beneficial for pollinators (FM) decreased after overwintering, due to the low survival rate of the species in the mixture. The gaps in vegetation were filed by *T. incarnatum*, an alien species with questionable applicability for UG.
3) The vegetation developed on plots with seeds coming from alternative sources (FH, MO) was comparable to the overall quality of vegetation on plots seeded with high-quality, but expensive seed mixtures.
4) There was an observable effect of site (different parks) on species composition, but the effect of seed source and years was significant. Thus, the effectiveness of different seed mixtures and fresh hay application for establishment of high-biodiversity UG is satisfactory regardless of the differences in residual vegetation species composition and soil properties observed here.

Data availability statement

All data used in the present study are included in the manuscript.

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CRediT authorship contribution statement

Hassanali Mollashahi: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis. **Tomasz H. Szymura:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Magdalena Szymura:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27810.

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