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Weed Composition in Hungarian Phacelia (*Phacelia tanacetifolia* Benth.) Seed Production: Could Tine Harrow Take over Chemical Management?

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Abstract: *Phacelia tanacetifolia*, an excellent cover, green manure and honey crop is now widely cultivated throughout the world. One of its principal European seed production regions is north-western Hungary, where the recent withdrawal of a potent herbicide, linuron, created a new challenge for many growers. The goal of this study is to identify the main factors determining weed species composition in the phacelia fields of the region and to assess the efficiency of tine harrow and clopyralid herbicide in reducing weed abundance and biomass. We carried out a series of weed surveys across the study region following a two-level design: (i) we estimated the cover of all weed species in 205 fields (broad-scale survey, BS); and (ii) in 22 of these fields, we provided more precise biomass measurements (counting the individuals and measuring the dry weights of all weed species) in microplots samples (fine-scale survey; FS). To characterize the fields, 34 background variables were also collected for all of the studied fields. In both investigations, *Chenopodium album* was by far the most abundant weed. Within the BS, using a minimal adequate model containing 11 terms with significant net effects, 20.93% of the total variation in weed species data could be explained. The variation in species composition was determined by environmental factors (soil pH, clay and K; precipitation and temperature), non-chemical management variables (crop cover, preceding crop, irrigation and tillage system) and herbicides (linuron and clopyralid). Variation partitioning demonstrated the dominance of environmental and cultural components in shaping the weed species composition. Although the effect of mechanical treatments was most likely masked in the BS by the soil properties, our FS suggests that tine harrow could efficiently decrease the total number and biomass of weeds and can be a useful tool in the phacelia management of the future.

Keywords: agroecology; arable fields; alternative crops; weed flora; weed management

1. Introduction

Lacy or tansy phacelia (*Phacelia tanacetifolia* Benth.), a versatile plant of North and Central American origin, is now widely cultivated in many regions around the world [1]. It is among the world's top twenty melliferous plants, providing monofloral honey with excellent quality for human consumption [2–4]. It also supports wild pollinators and some biological control agents with pollen and nectar [5–9]. As a cover crop and green manure, it is beneficial for soil structure [10] and soil microorganisms [11]; nevertheless, its effect for weed infestation has been assessed with different outcomes. In Switzerland,

the experiments of Büchi et al. [12] indicated that previous use of phacelia as a cover crop reduced weed abundances in the subsequent planting of maize; however, in Poland, the trials of Gaweda et al. [13] and Kolodziejczyk [14] suggested that phacelia cover crop caused higher weed infestations in the subsequently grown cereals, and that it also resulted in yield loss in potato, when it was sown for living mulch between rows.

In Hungary, phacelia is cropped mainly for its seeds, more than 90% of which are exported for the Western European markets. Its annual growing area has fluctuated between 1500 and 11,000 ha in the last 20 years, with average seed yields ranging between ~400 and 600 kg ha⁻¹ depending on the weather conditions [15]. The germination and initial development of phacelia can be very slow (up to 25–30 days, [16]), and the crop can be easily overwhelmed by faster-emerging and -developing weeds during this period [17]. Although, with proper expertise, phacelia can be successfully grown with low-intensity farming methods [16], in seed production, many farmers assert chemical control to reduce weed pressure. For them, weed control has become a great challenge since June 2018, when their preferred herbicide, linuron, was withdrawn from sale in the European Union (as already prophesized by Hillocks in 2012 [18]).

Linuron has a great efficiency against a wide range of weeds [19,20], including the most important weeds of the phacelia crop [21]. Now, in this culture, clopyralid is exclusively licensed for dicotyledonous weeds with a rather narrow spectrum of efficacy, and quizalofop-P-ethyl is used merely against monocots [17]. Hence, this herbicide ban has created a hectic transition period in Hungarian phacelia seed production. Many growers voiced their disappointment over losing their old and trusted technology. Some researchers are exploring substitute chemicals, without a clear winner so far [21]. Some conventional farms, even on highly fertile soils, have turned to extensive management techniques and organic farming, where the application of phacelia has a long history. Some farmers are exploring novel options in mechanical weed control, including cultivating tillage or tine harrow. Tine harrow, in particular, has recently gained some recognition as a promising weed control technique, which is expected to be able to substitute herbicides in the years to come. Spring-tine harrows are generally used in post- and pre-emergence weed control in narrow row crops, such as winter or spring cereals. This method is becoming increasingly popular for mechanical weeding throughout the world [22–26], and its weeding performance is still currently tested in many crops [27–30].

The unique diversity of accompanying circumstances in Hungarian phacelia cultivation offers an excellent opportunity for studying the connections between background variables and weed species composition, as it was previously performed in other minor crops, such as poppy [31], soybean [32] and oil pumpkin [33]. The current situation also provides an excellent opportunity to study the weeding impact of tine harrow on phacelia crops within real, multidimensional farming circumstances. Accordingly, the main goal of this research was twofold: (1) to assess the effect of environmental variables and herbicides on the weed flora of phacelia; and (2) to explore the effects of alternative weed management technologies, in particular tine harrow, on the abundance and biomass of the most burdensome weed species. A better understanding of the impacts of chemical and non-chemical weed management could inspire the development of more environmentally benign weed control strategies, which could also lead to healthier phacelia honeys.

2. Materials and Methods

2.1. Data Collection

The research was carried out in north-western Hungary, in the Little Hungarian Plain. Large-scale Hungarian phacelia seed production started here in the middle of the 1970s, and by the 1990s, this region became a hotspot of phacelia seed production in the EU. The study region still provides more than half of the annual yield of the country. In the blooming period, the phacelia fields of the region attract beekeepers from the whole country [15,34].

To prepare for the work, we first searched for phacelia seed-growing farmers, who gave us access to their fields and were willing to be interviewed about management factors.

In this way, we identified 92 farmers and 205 arable fields throughout the region (Figure 1). In these fields, we conducted two different types of weed surveys: (i) a broad-scale survey (BS) in all the 205 fields and (ii) a more detailed fine-scale survey (FS) in a smaller subset of these fields.

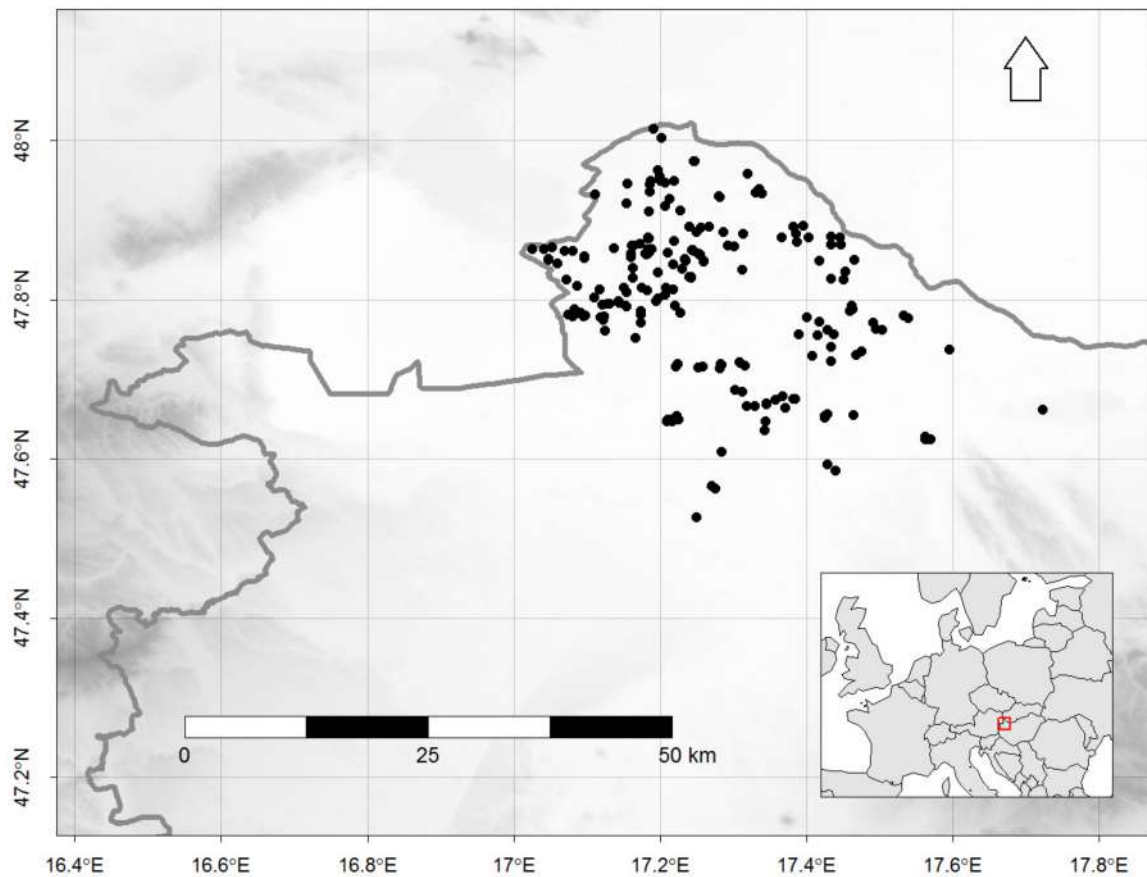


Figure 1. The distribution of the 205 surveyed phacelia fields across the region (a single point may represent multiple fields).

For the BS, weed data were recorded once during the flowering peak of phacelia (between the end of May and the end of June) in the 2017–2021 period. In each field, the weed vegetation was sampled in a single randomly selected rectangular plot of 200 m², located at least 10 m far from the field edges, and the percentage ground cover of all plant species was estimated visually. A soil sample of 1000 cm³ was also collected from the top 10 cm layer in each of the BS field surveys.

For the FS studies, we selected a subset of 22 fields from the two last years of the surveys (2020–2021), in which a more intensive measurement of crop and weed biomass was performed. To examine deeply the effects of mechanical and chemical weed control, half of the selected fields received a treatment with tine harrow, and the other half received either clopyralid herbicide or no weed control at all. We restricted this selection to three major farm holdings in the vicinity of Mosonmagyaróvár, all of which were located within a circle with a radius of about 3 km, thus ensuring relatively similar management and environmental conditions. In these fields, six microplots of 0.25 m² (50 cm × 50 cm) were randomly selected within the 200 m² plots of the BS survey. Then, the above-ground parts of the crops and weeds were cut in each microplot in the first week of June each year. Weeds were separated by species, and for each of the species found, the number of individuals (cuttings) was first counted, and then the samples were oven-dried at 75 °C for 72 h to obtain their dry weights, similarly to other European weed survey studies [35]. A soil sample of 1000 cm³ from the top 10 cm layer was also collected from each microplot.

Soil samples were analyzed in the laboratory of Széchenyi University using an Agrocares scanner, with near-infrared spectroscopy (NIRS) [36,37]. Climatic conditions were represented by annual mean temperature and the precipitation sum of 1970–2000 taken from the WorldClim 2.0 database [38] at a 30" (~1 km) horizontal resolution. Management information was received directly from the farmers.

Altogether, 34 predictor variables (11 *environmental*: 2 climate, 9 soil; 19 *non-chemical management*: 14 cultural, 5 mechanical management; and 4 *chemical weed control factors*) were recorded during the surveys (Table 1). Management variables were grouped following the classification of Blackshaw et al. [39] and Cloutier et al. [40]; accordingly, cultural and mechanical weed management variables together were considered to be the elements of 'non-chemical management', and we considered chemical weed control to be a different group, following the logic of the key questions of this study.

In order to avoid rare levels of categorical variables and to make the data appropriate for a multivariate analysis, several modifications were implemented. For the BS, preceding crop species occurring fewer than ten times were considered to be 'miscellaneous'. Due to the low frequency of their use, two herbicides ('quizalofop-P-ethyl' and 'quizalofop-P-tefural') were also unified into a binary variable ('quizalofop herbicides'). In the case of the BS, rare types of mechanical weed control ('cultivating tillage', 'manual weed control' and 'tine harrow') were also merged into a single variable ('mechanical weed control'). However, this last simplification was not applied in the FS analysis, where 'tine harrow' was the only form of mechanical weed control.

2.2. Statistical Analysis

The statistical analysis of the BS followed the same lines as the analysis described in Pinke et al. [41]; thus, here, we only present a brief summary thereof. The collinearity between the environmental, management and herbicide variables (potential model terms) were assessed prior to the analysis by calculating their generalized variance inflation factor (GVIF; [42]). Soil N, Ca, Mg and cation exchange capacity, as well as cultivar and K fertilizer, had to be dropped during this process, while the rest of the variables showed only slight collinearity, which should not bias the analysis (the highest GVIF score adjusted by degree of freedom was 1.75). Cover values were subjected to a Hellinger transformation [43] and were examined in a redundancy analysis (RDA) together with the retained background variables data. Only the species with >10 occurrences were included in the analyses. The number of explanatory variables was decreased via stepwise backward selection using a $p < 0.01$ threshold for type I errors, which led to a minimal adequate model containing 11 terms (out of 33). As a next step of the multivariate analysis, we estimated the gross and net effects of each explanatory variable of the reduced model, as carried out by Lososová et al. [44]. In most of the partial RDAs, there was only one constrained axis, except for the preceding crop, where five constrained axes were tested. Given the results, a common rank of 'importance' was settled among all explanatory variables according to the R^2_{adj} values of the net effects of the pRDA models. To show the responses of the weed species to the significant factors, for each pRDA model, we identified those 10 species that represented the highest explained variation in the constrained axes ('strongly associated' species). Variation partitioning based on partial RDA [43] was applied to establish the relative effects of the different groups of explanatory variables on species composition.

Table 1. Units and ranges of continuous variables and values of categorical variables within the surveys.

Variable (Unit)	Broad-Scale Survey (BS) Range/Values	Fine-Scale Survey (FS) Range/Values
ENVIRONMENTAL		
<i>Climate</i>		
Mean annual precipitation (mm)	535–582	552–562
Mean annual temperature (°C)	10.07–10.47	10.25–10.47
<i>Soil</i>		
Soil pH (KCl)	6.2–8.1	7–8
Soil clay (%)	8–41	11.8–38.1
Soil N (g kg ^{−1}) ^a	0.9–3.9	0.2–2.7
Soil P (mg kg ^{−1}) ^b	18.1–69.1	32.8–47.4
Soil K (mmol kg ^{−1})	2.8–11.2	0.9–9.6
Soil Ca (mmol kg ^{−1}) ^a	78.8–461.6	137.4–330.8
Soil Mg (mmol kg ^{−1}) ^a	5.8–79.5	17.2–54.6
Soil cation exchange capacity (mmol kg ^{−1}) ^a	64–461	98.3–317.8
Soil organic matter (%) ^b	1.8–6.4	0.8–5.1
NON-CHEMICAL MANAGEMENT^c		
<i>Cultural</i>		
Farming type ^b	Conventional, organic	Conventional
Crop cover (%)	10–100	50–100
Crop dry weight (g 0.25 m ^{−2})	<i>Not measured</i>	49.65–251.85
Seeding rate (kg ha ^{−1}) ^b	4.7–12	5–12
Crop row spacing (cm) ^b	12–45	12–30
Field size (ha) ^b	0.2–71	0.22–15.45
Cultivar ^a	Angélia, Balo, Faktotum, Júlia, Lilla, Liza, Mira.	Angélia, Júlia, Lilla
Date of sowing ^b	27 February–15 April	12–19 March
Preceding crop	Cereal, maize, miscellaneous, phacelia, rape, sunflower	Cereal, maize, soybean, rape
Irrigation (mm)	0–35	0–35
Organic manure (t ha ^{−1}) ^b	0–50	0
Amount of fertiliser (kg ha ^{−1})		
N ^b	0–118	0–45
P ₂ O ₅ ^b	0–90	0–45
K ₂ O ^a	0–120	0–45
<i>Mechanical</i>		
Primary tillage depth (cm) ^b	2–65	25–30
Tillage system	No-tillage, ploughing	No-tillage, ploughing
Tine harrow (times) ^b	0–1	0–1
Cultivating tillage (times) ^b	0–2	0
Manual weed control (times) ^b	0–1	0
CHEMICAL WEED CONTROL		
<i>Herbicides</i>		
Linuron (g a.i. ha ^{−1})	0–810	0
Clopyralid (g a.i. ha ^{−1})	0–240	0–150
Quizalofop-P-ethyl (l a.i. ha ^{−1}) ^b	0–0.75	0
Quizalofop-P-tefuryl (g a.i. ha ^{−1}) ^b	0–150	0

^a Variables not included in the data analysis of BS due to multicollinearity. ^b Variables dropped during the backward selection process during the data analysis of BS. ^c All management excluding herbicides.

In the analysis of the FS data, we focused on the effectiveness of mechanical (by tine harrow) and chemical weed management. Since none of the studied fields applied both tine harrow and clopyralid, and dosage of clopyralid was always 150 g ha^{−1}, a categorical variable called ‘weed control’ with 3 levels (‘chemical’, ‘mechanical’ and ‘neither’) was created and used as a fixed factor in the fitted generalized linear mixed models (GLMM). For controlling their potential effect on weed abundance, the seeding rate and phacelia biomass were also included in the model; however, the significance of their effect was not tested.

Farm ID was included as a random factor, in this way controlling the differences among farm holdings in environmental conditions and management practice beyond weed control and seeding rate. The response variables were total weed biomass, the total abundance (i.e., number of individuals) of weed species and the abundance of seven weed species occurring in more than 15 fields. Biomass values were log-transformed and then analyzed with a linear mixed model assuming a normal (Gaussian) distribution. For abundance data, GLMM with negative binomial distribution and log-link were fitted. The assumptions of the fitted models were checked using diagnostic plots. Then, the mean differences among the three levels of the variable ‘weed management’ were calculated along with their 95% confidence intervals. To change the relative differences and their confidence intervals back to their original scale (i.e., difference in percentage of the reference category), the values were back-transformed using the same transformations (logarithmic transformation or log-link).

Data processing and statistical analyses were conducted in the R statistical software [45] using its packages ‘abind’ [46], ‘car’ [47], ‘DHARMA’ [48], ‘glmmTMB’ [49], ‘multcomp’ [50], ‘raster’ [51], ‘sf’ [52], ‘vegan’ [53] and ‘VennDiagram’ [54].

3. Results

During the BS, altogether, 159 weed species were found. *Chenopodium album* L., *Ambrosia artemisiifolia* L., *Polygonum aviculare* L., *Convolvulus arvensis* L., *Stachys annua* L. and *Sinapis arvensis* L. had the highest mean cover. Moreover, *C. hybridum* L. and *Fallopia convolvulus* (L.) Á. Löve were also among the top six most frequent weeds (Figure 2). The full RDA model (comprising 24 explanatory variables) explained 27.32% of the variance, while the reduced model (comprising 11 explanatory variables) still explained 20.93% of the total variation in species data. According to the pRDA, all of the 11 remaining variables have significant net effects, with two soil parameters (pH and clay content) being the most influential (Table 2). In addition, the effects of three further environmental parameters (precipitation, temperature and soil K), four non-chemical management variables (crop cover, preceding crop, irrigation and tillage system) and two herbicides (linuron and clopyralid) were significant (Table 2).

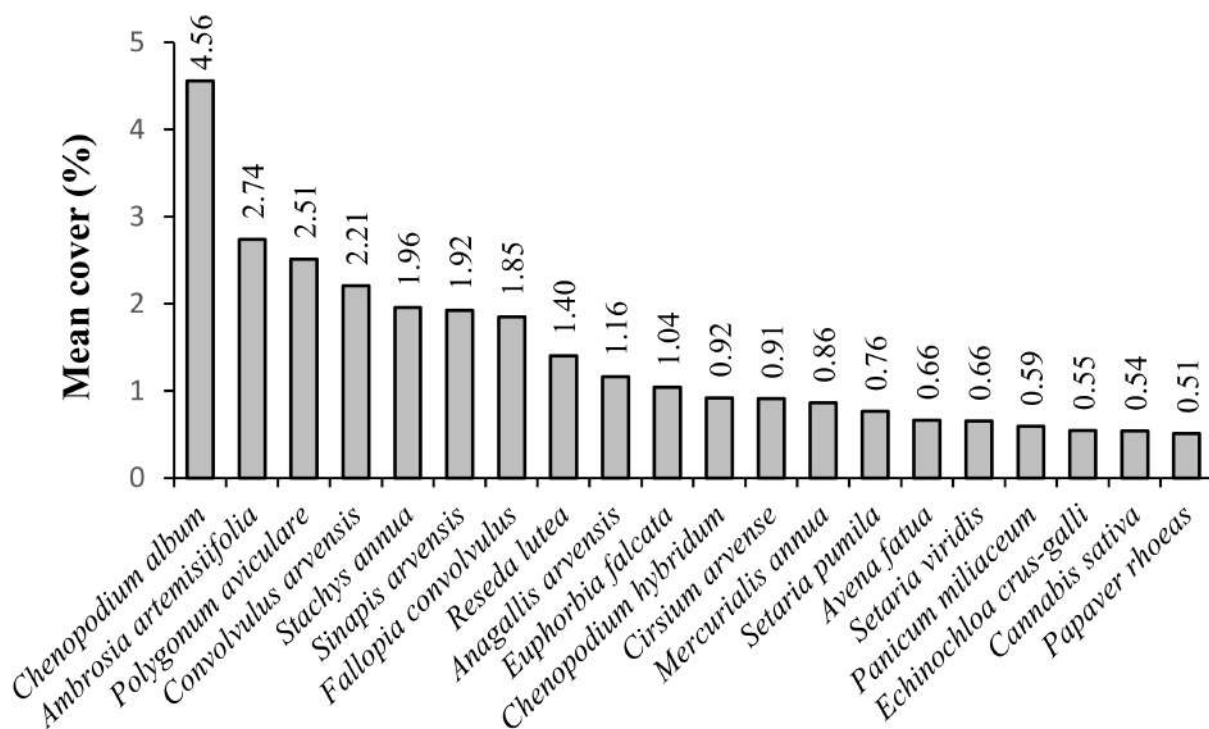


Figure 2. Cont.

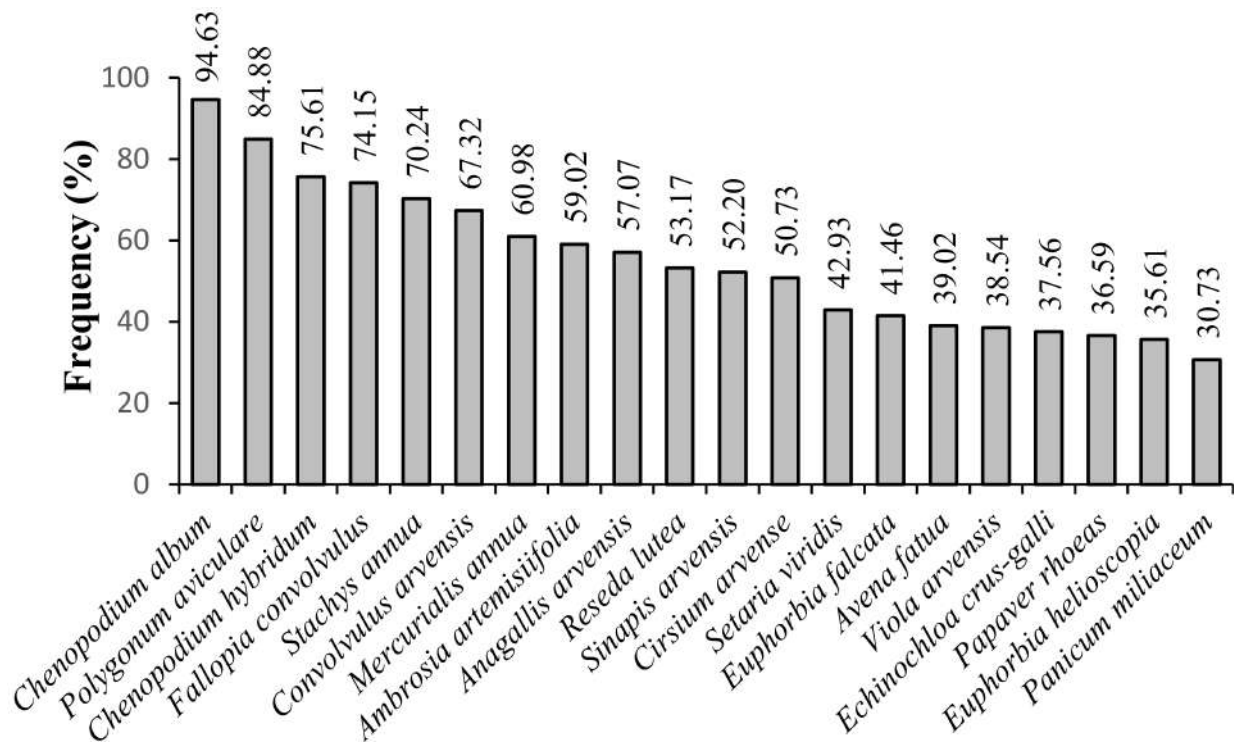


Figure 2. The mean cover values (% of the surface covered) and the frequency of occurrence (% of the fields surveyed) of the twenty most dominant/frequent weed species in the broad-scale survey (BS).

Table 2. Gross and net effects of the explanatory variables on the weed species composition identified using (p)RDA analyses with single explanatory variables in the broad-scale survey (BS).

Factors	d.f.	Gross Effect		Net Effect		F	p-Value
		Explained Variation (%)	R^2_{adj}	Explained Variation (%)	R^2_{adj}		
Soil pH	1	3.083	0.0261	2.164	0.0187	5.172	***
Soil clay content	1	2.598	0.0212	1.959	0.0165	4.683	***
Crop cover	1	2.679	0.0220	1.927	0.0162	4.606	***
Precipitation	1	2.507	0.0203	1.819	0.0150	4.348	***
Linuron	1	2.188	0.0171	1.716	0.0139	4.101	***
Temperature	1	1.531	0.0105	1.323	0.0097	3.163	***
Preceding crop	5	4.413	0.0201	2.965	0.0092	1.418	**
Clpyralid	1	2.273	0.0179	1.175	0.0081	2.809	***
Irrigation	1	1.118	0.0063	1.013	0.0064	2.421	***
Tillage system	1	1.019	0.0053	0.923	0.0054	2.205	**
Soil K content	1	1.150	0.0066	0.840	0.0045	2.008	**

** $p < 0.01$ and *** $p < 0.001$.

The responses of the 10 most associated weed species (the ones with the highest pRDA fit) for each predictor variable are shown in Table 3 for all predictors with just one constrained axis. In the case of the preceding crop, only the first constrained axis was significant (Figure 3). Fields with the previous crops phacelia and cereal had their characteristic species, such as *S. annua* and *P. aviculare*, separated from those previously planted with maize, sunflower and rape along the first axis. The second axis distinguished fields with the spring-sown preceding crops (phacelia, maize and sunflower), which are often abundant in *Amaranthus retroflexus* L. and *Panicum miliaceum* L., from those with the autumn-sown preceding crops (cereal and rape), with the typical species *Ajuga chamaeptyis* (L.) Schreb. and *Capsella bursa-pastoris* (L.) Medik. (Figure 3).

Table 3. Names, fit and score values of species giving the highest fit along the first constrained axis in the partial RDA models of the significant variables specified in Table 2.

	Ax 1 Score	Fit		Ax 1 Score	Fit
Soil pH (+ low, – high)			Soil clay (+ low, – high)		
<i>Alopecurus myosuroides</i>	0.140	0.116	<i>Anagallis arvensis</i>	–0.182	0.127
<i>Reseda lutea</i>	–0.212	0.113	<i>Reseda lutea</i>	–0.161	0.066
<i>Tripleurospermum inodorum</i>	0.080	0.102	<i>Kickxia elatine</i>	–0.033	0.061
<i>Chenopodium polyspermum</i>	0.101	0.073	<i>Anagallis foemina</i>	–0.070	0.061
<i>Stachys annua</i>	–0.191	0.070	<i>Persicaria lapathifolia</i>	–0.100	0.051
<i>Euphorbia falcata</i>	–0.150	0.069	<i>Chenopodium polyspermum</i>	–0.083	0.050
<i>Persicaria lapathifolia</i>	0.103	0.055	<i>Chenopodium album</i>	0.207	0.047
<i>Elymus repens</i>	0.062	0.036	<i>Euphorbia exigua</i>	–0.051	0.043
<i>Mercurialis annua</i>	–0.105	0.035	<i>Euphorbia falcata</i>	–0.102	0.032
<i>Echinochloa crus-galli</i>	0.085	0.034	<i>Artemisia vulgaris</i>	0.015	0.029
Crop cover (+ high, – low)			Precipitation (+ high, – low)		
<i>Ambrosia artemisiifolia</i>	0.258	0.108	<i>Fallopia convolvulus</i>	0.226	0.097
<i>Kickxia elatine</i>	0.041	0.092	<i>Stachys annua</i>	0.157	0.047
<i>Anagallis foemina</i>	0.084	0.088	<i>Setaria viridis</i>	0.127	0.047
<i>Microrrhinum minus</i>	0.046	0.065	<i>Hyoscyamus niger</i>	0.037	0.044
<i>Anagallis arvensis</i>	0.118	0.054	<i>Ambrosia artemisiifolia</i>	–0.160	0.042
<i>Chenopodium hybridum</i>	–0.130	0.053	<i>Mercurialis annua</i>	0.114	0.041
<i>Chenopodium album</i>	–0.208	0.047	<i>Amaranthus blitoides</i>	0.035	0.040
<i>Ajuga chamaepitys</i>	0.052	0.045	<i>Euphorbia exigua</i>	0.048	0.037
<i>Consolida regalis</i>	0.017	0.044	<i>Hibiscus trionum</i>	–0.051	0.029
<i>Lathyrus tuberosus</i>	0.045	0.039	<i>Sinapis arvensis</i>	0.105	0.021
Linuron (+ high, – low)			Temperature (+ low, – high)		
<i>Anagallis arvensis</i>	–0.121	0.056	<i>Setaria viridis</i>	–0.172	0.085
<i>Polygonum aviculare</i>	0.173	0.049	<i>Ajuga chamaepitys</i>	–0.059	0.059
<i>Chenopodium album</i>	–0.191	0.040	<i>Euphorbia falcata</i>	–0.132	0.054
<i>Chenopodium hybridum</i>	–0.112	0.039	<i>Hordeum vulgare</i>	–0.039	0.044
<i>Reseda lutea</i>	0.121	0.037	<i>Euphorbia exigua</i>	0.051	0.043
<i>Papaver rhoeas</i>	–0.070	0.029	<i>Stachys annua</i>	–0.150	0.043
<i>Convolvulus arvensis</i>	0.129	0.029	<i>Hibiscus trionum</i>	–0.059	0.039
<i>Brassica napus</i>	0.042	0.022	<i>Capsella bursa-pastoris</i>	0.048	0.034
<i>Alopecurus myosuroides</i>	0.055	0.018	<i>Fallopia convolvulus</i>	–0.121	0.028
<i>Fallopia convolvulus</i>	0.095	0.017	<i>Thlaspi arvense</i>	–0.036	0.027
Clopyralid (+ high, – low)			Irrigation (+ low, – high)		
<i>Kickxia elatine</i>	0.042	0.096	<i>Solanum nigrum</i>	–0.064	0.049
<i>Convolvulus arvensis</i>	0.220	0.084	<i>Datura stramonium</i>	–0.070	0.048
<i>Euphorbia falcata</i>	0.123	0.047	<i>Chenopodium hybridum</i>	–0.106	0.035
<i>Reseda lutea</i>	0.106	0.028	<i>Sinapis arvensis</i>	–0.126	0.031
<i>Helianthus annuus</i>	–0.038	0.019	<i>Lathyrus tuberosus</i>	0.037	0.027
<i>Anthemis austriaca</i>	–0.039	0.019	<i>Mercurialis annua</i>	–0.087	0.024
<i>Euphorbia exigua</i>	0.032	0.017	<i>Hordeum vulgare</i>	0.025	0.019
<i>Galium aparine</i>	0.027	0.014	<i>Anagallis arvensis</i>	0.065	0.016
<i>Medicago lupulina</i>	–0.023	0.014	<i>Ambrosia artemisiifolia</i>	0.098	0.016
<i>Ajuga chamaepitys</i>	0.028	0.013	<i>Papaver rhoeas</i>	–0.051	0.016
Tillage (+ plough, – no tillage)			Soil K (+ high, – low)		
<i>Mercurialis annua</i>	0.148	0.069	<i>Euphorbia falcata</i>	–0.115	0.041
<i>Anthemis austriaca</i>	–0.051	0.032	<i>Anagallis arvensis</i>	–0.094	0.034
<i>Hordeum vulgare</i>	–0.033	0.031	<i>Anagallis foemina</i>	–0.050	0.032
<i>Avena fatua</i>	–0.086	0.027	<i>Amaranthus blitoides</i>	–0.031	0.031
<i>Datura stramonium</i>	0.048	0.023	<i>Lamium amplexicaule</i>	–0.033	0.029
<i>Silene noctiflora</i>	–0.043	0.021	<i>Panicum miliaceum</i>	–0.078	0.029
<i>Panicum miliaceum</i>	0.067	0.021	<i>Reseda lutea</i>	–0.100	0.025
<i>Cirsium arvense</i>	–0.070	0.020	<i>Artemisia vulgaris</i>	–0.013	0.024
<i>Lathyrus tuberosus</i>	–0.032	0.020	<i>Ajuga chamaepitys</i>	–0.037	0.023
<i>Euphorbia helioscopia</i>	0.042	0.018	<i>Sinapis arvensis</i>	0.106	0.022

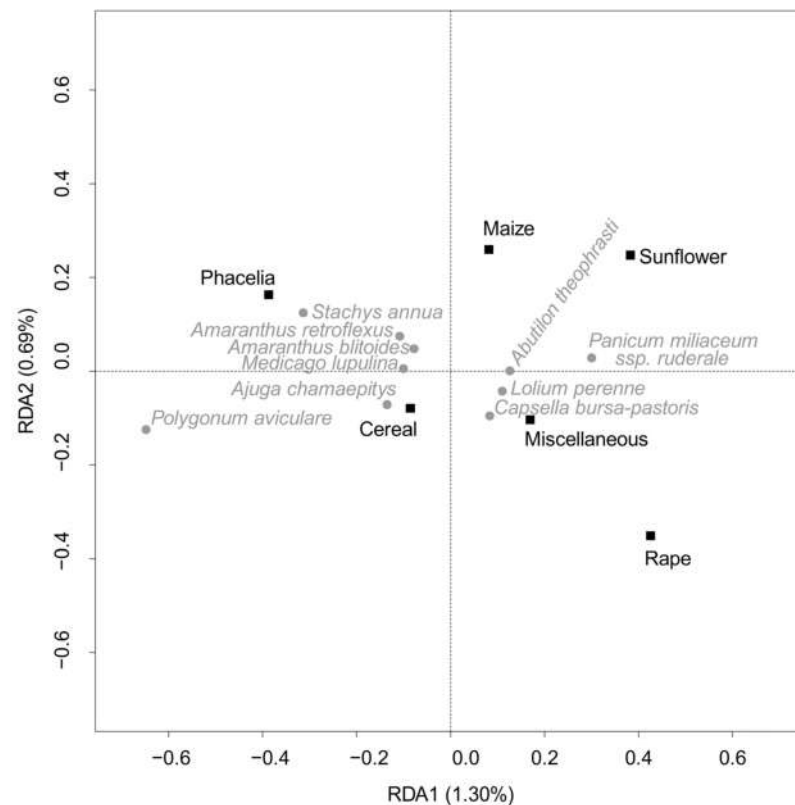


Figure 3. Ordination diagram of the partial RDA model containing the explanatory variable preceding crop in the broad-scale survey (BS). The 10 species with the highest weight on the first two RDA axes are presented. Note that only the first axis is significant at the 2% level.

In the reduced RDA ordination (Figure 4), the first axis can be most related to the explanatory variables soil clay and potassium content, as well as crop cover and clopyralid herbicide, while the second axis is correlated with pH, precipitation and irrigation, as well as linuron application. Samples from the heavier soils containing little potassium, with low crop cover and having been sprayed with clopyralid, which were also typically characterized by the presence of *Setaria pumila* (Poir.) Schult. and *Chenopodium polyspermum* L., generally exhibited positive values on the first RDA axis. In contrast, sites in the less heavy soils, which were also rich in potassium, usually with high crop cover, and which were not sprayed with herbicides and felt the frequent presence of *C. album* and *C. hybridum*, were characterized with low axis 1 values.

The variation partitioning of the RDA model revealed that environmental variables altogether accounted for 1.2 times the variance of non-chemical management variables and were 2.9 times that of herbicides, while non-chemical management practices had 2.4 times more variance than herbicides (Figure 5A). The relative impact of cultural variables was more than 24 times larger than that of mechanical treatments; the relevance of chemical weed control is 9.5 times larger than that of mechanical treatments; and cultural variables altogether had 2.5 times more variance than the chemical weed control variables (Figure 5B).

During the FS, 37 weed species were found. *C. album*, *Setaria viridis* (L.) P.Beauv., *C. hybridum*, *Mercurialis annua* L., *F. convolvulus* and *S. annua* were the top six species in terms of having the largest number of individuals. Besides these six species, *P. aviculare* occurred also in more than 15 fields. Due to the wide confidence intervals, there was no significant difference in weed biomass and abundances between fields treated by clopyralid and left without weed control (Figure 6A). However, there is a tendency toward a lower abundance of *C. album*, *S. viridis*, *C. hybridum* and *P. aviculare*, and total weed abundance and biomass were also slightly lower in fields treated with clopyralid. On the other hand, *M. annua*, *F. convolvulus* and *S. annua* are slightly more abundant in these fields. Applying tine harrow

significantly decreased the total weed biomass and abundance, as well as the abundance of *M. annua*, *F. convolvulus* and *S. annua*, while there were non-significant changes in *C. album*, *C. hybridum* and *P. aviculare* (decrease), as well as *S. viridis* (increase, Figure 6B). The abundance of *M. annua*, *F. convolvulus* and *S. annua* was significantly lower in fields where tine harrow was applied than where clopyralid was (Figure 6C). Total weed biomass and abundance, as well as the abundance of *C. hybridum* and *P. aviculare*, were also lower if tine harrow was applied, but this difference was not significant. *S. viridis* was the only species whose abundance was considerably, but not significantly, higher if tine harrow was applied than in fields with chemical weed control.

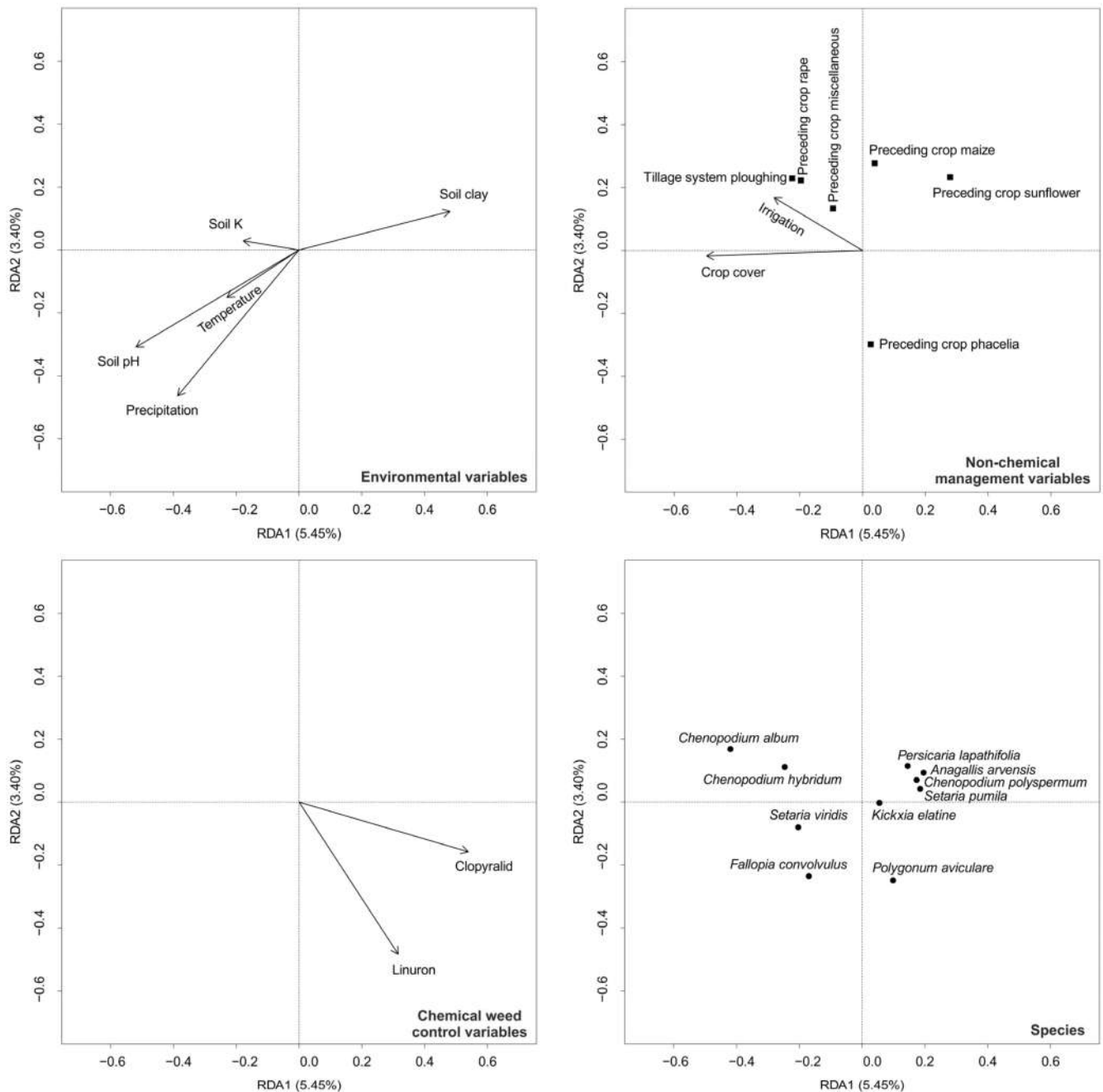


Figure 4. Ordination diagrams of the reduced RDA model containing the 18 significant explanatory variables and the species in the broad-scale survey (BS). Only the species with the highest weights on the first two RDA axes are presented.

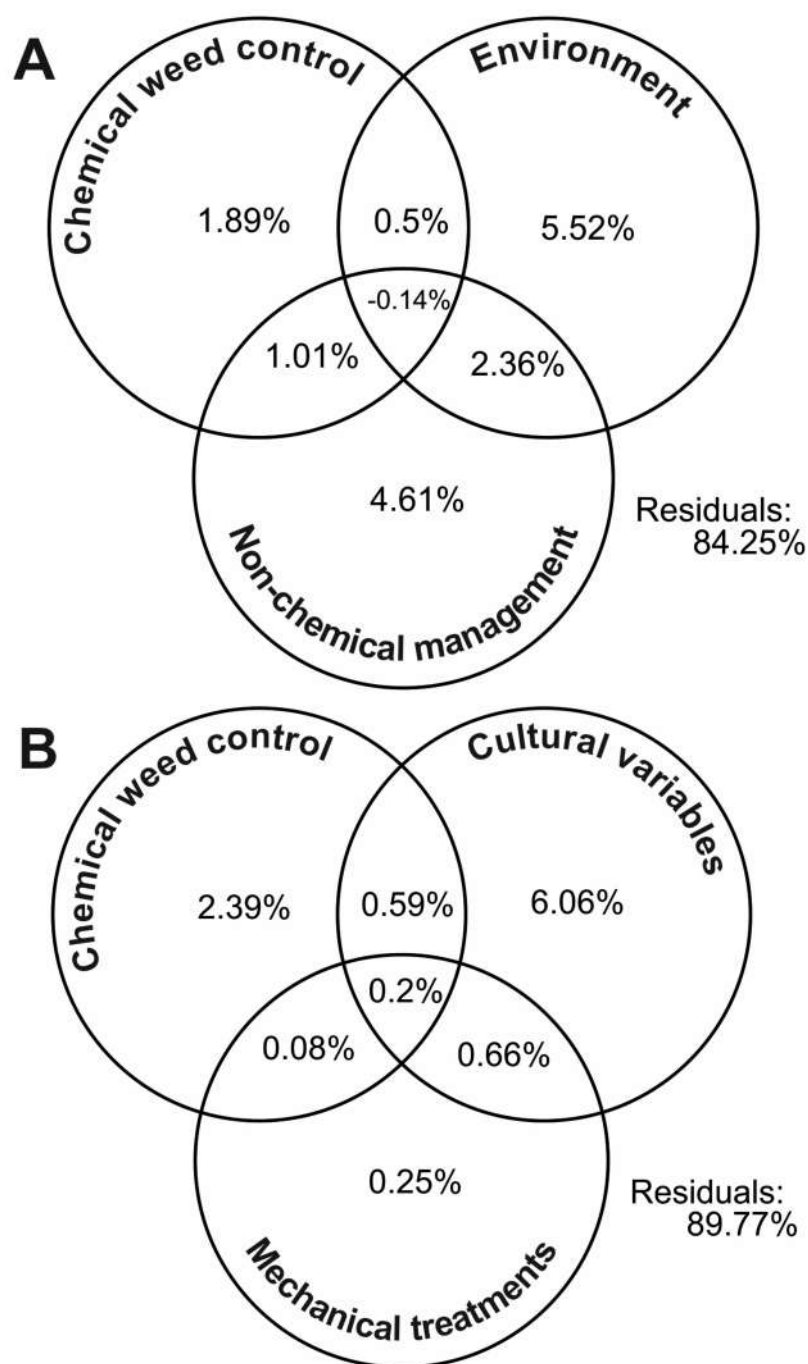


Figure 5. Percentage contributions of groups of significant explanatory variables to the variation in weed species composition, identified via variation partitioning in the broad-scale survey (BS). **(A):** Environmental vs. non-chemical management vs. chemical weed control variables; **(B):** cultural vs. mechanical vs. chemical components of weed management (environment variables are among the residuals here).

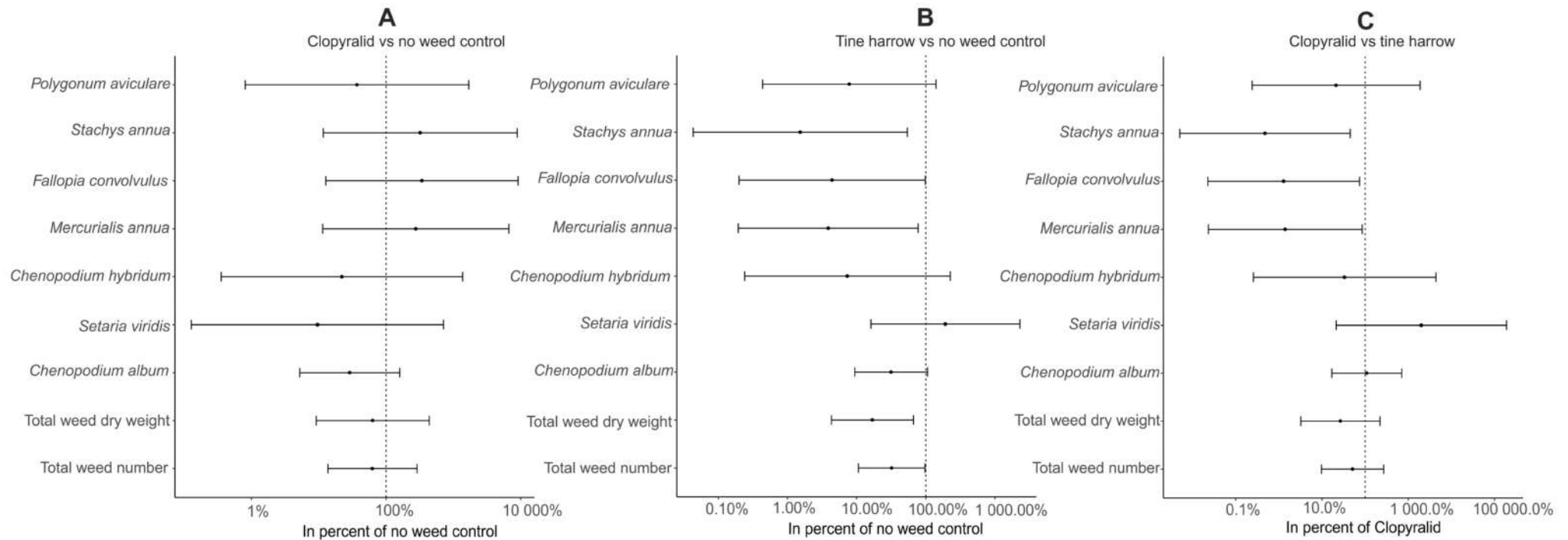


Figure 6. Differences between three weed management treatments (clopyralid, tine harrow and no weed control) in the fine-scale survey (FS): relative differences in (=ratios of) weed abundances and total biomass values for each pair of treatments (A–C). Dots: mean relative difference; whiskers: 95% confidence intervals based on Tukey-type post hoc test of GLMM models. A relative difference is significant at 5% level, if its confidence interval does not cross the vertical dotted line at 100%.

4. Discussion

4.1. Environmental Variables

Our analyses revealed that for the BS two soil properties, pH and clay content were the most important factors affecting the weed flora; moreover, the soil potassium content, though to a lesser extent, was also influential (Table 2). These parameters were also among the most important variables in our earlier studies in summer annual weed vegetation [41] and in soybean fields [32], as well as in other European investigations [55–57]. The great influences of these variables suggest relatively long soil gradients in the area studied [58] and could be also due to the fact that relatively large parts of phacelia fields were managed extensively or even organically (65% of the fields were not treated with herbicide and the proportion of organic fields was 12%). Accordingly, in these fields, the sensitive soil indicator species were not eliminated with chemical managements. Among these, the signaling weeds, for instance, *S. annua*, *Euphorbia falcata* L. and *M. annua*, preferred basic soils, while *Persicaria lapathifolia* (L.) Delarbre and *Chenopodium polyspermum* L. were associated with higher clay content (Table 3).

Despite the relatively small extent of the study area (Figure 1), and its narrow altitudinal range (115–125 m), climatic conditions seem to have been diverse enough to influence the weed species composition: precipitation was the fourth, while temperature was the sixth most important variable. This is in accordance with the findings of other country-wide Hungarian [32,33,41] and similar European studies [44,59,60], where climatic factors were also highly important. *Hibiscus trionum* L., a well-known indicator for thermophile sites in Hungary and Central Europe [32,33,61], was clearly associated with the warmest and driest locations also in our study, even if it was not among the most abundant weeds in the surveyed phacelia fields. On the contrary, *Capsella bursa-pastoris* (L.) Medik. was found to be more abundant in the cooler sites and *F. convolvulus* in the more humid sites (Table 3).

4.2. Non-Chemical Management Variables

4.2.1. Cultural Practices

Among cultural variables, crop cover appeared to be the most important factor shaping the weed vegetation in our BS. This variable was also found to be important in the Hungarian oil pumpkin fields [33], and it can be regarded as an indirect cultural variable, which integrates the effects of several other, more direct cultural practices and variables, such as seeding rate, plant density, cultivar type and fertilizer use. These practices target the development of a dense crop canopy as early as possible, which can then overwhelm the emerging weed populations [39]. The investigation of Andrade et al. [62] also confirmed that the frequency of highly common weeds was negatively associated with the number of days with high crop cover. The fact that neither seeding rate nor crop row spacing, nor fertilizers were significant in our BS underlines the complexities of the development of a dense crop canopy. Similar complexities were documented in experiments in Turkey, where once the narrow [63], then the wide row spacing [64] proved to be the best for the phacelia growing. Nevertheless, the examinations of Szabó and Horváth [65] in central Hungary showed that smaller row spacings and higher seeding rates can provide better weed-suppressing capacity for the phacelia crop.

Interestingly, *A. artemisiifolia* was one of the weeds that were associated with lower crop cover in the BS (Table 3). According to a recent investigation in sunflower, soybean, maize and pumpkin fields in the Austrian–Hungarian borderland, crop cover was also the most important variable correlated with the abundance of this species, showing also higher infestations if crop cover was lower [66]. It is known that despite its relatively high potential stature, this species performs poorly at low light intensities [67]. In contrast, the other species we found to be strongly associated with low phacelia cover, such as *Kickxia elatine* (L.) Dumort., *Anagallis foemina* Mill., *A. arvensis* L., *Microrrhinum minus* (L.) Fourr. and *A. chamaepitys*, are characterized by short stature, which makes them less competitive in dense crop stands with reduced light [68]. Our results also suggest that some taller weeds, such as *C. album*, were quite abundant even in denser phacelia stands (Table 3),

probably because of their taller stature. In the case of *C. album*, tall stature is one of the attributes that is considered to be responsible for the global success of this weed [69].

The effect of preceding crop was also remarkable in our study. With respect to this variable, we could distinguish two groups among the weed species, from two different perspectives (Figure 3). First, the weed flora after cereals and phacelia formed a joint fraction against maize, sunflower and rape. This can be explained by the fact that the weed vegetation of phacelia is reminiscent of that of cereals, because these crops are ordinarily not mechanically disturbed after sowing, and they have a similar stature and the same mid-summer harvesting season. Similarly, many of the most characteristic weeds of phacelia, including *S. annua*, *A. arvensis* and *Euphorbia falcata* L., are typical also in cereal stubbles [70]. In contrast, maize and sunflower are often hoed after sowing, they are much taller with broader row spacings, with peculiar conditions for the light competition, and they also have a long growing season until autumn. Rape has a similar growing season as cereals, but it usually forms very dense stands in the area studied. Secondly, the weed flora after preceding crops sown in spring (phacelia, maize and sunflower) was also separated from autumn-sown preceding crops (cereal and rape). This result is in accordance with other European studies highlighting the relevance of the sowing season as a proxy for timing patterns in soil cultivation [71–73]. Different sowing dates induce the development of distinct weed communities, whose impact can be traced also in the subsequently grown crops. Consequently, alternating crops with distinct life cycles can break the development of crop–weed associations; thus, the proper selection of the preceding crop can be one of the most efficient tools of cultural weed management [39], which can reduce herbicide use [74].

Irrigation was also relevant for the weed species composition in phacelia fields. As Christoffoleti et al. [75] highlighted, irrigation water can transport weed seeds to the fields, and, in addition, weed communities [76] and their diversity [77] can also be altered by watering. The increased water supply is not only beneficial for the crop, but it can also be beneficial for certain weeds, as was indicated by the greater abundance of *Solanum nigrum* L., *Datura stramonium* L., *C. hybridum*, *S. arvensis* and *M. annua* in response to irrigation in our study (Table 3). Shrestha et al. [78] also reported that *S. nigrum* could grow quite large and contributed a substantial amount of biomass in irrigated tomato fields.

The tillage system was also significant during our investigation, indicating larger abundances of some perennials, such as *Cirsium arvense* (L.) Scop. and *Lathyrus tuberosus* L., in fields without ploughing (Table 3). This is in accordance with the findings of some classical [79,80] and newer studies [81,82], showing an increase in perennial weeds with reduced tillage compared to ploughing.

4.2.2. Mechanical Weed Management

Among the 205 surveyed fields, 31 were treated with tine harrow, three with cultivating tillage and one was hand-hoed. These operations were grouped as a single treatment in the BS analysis, where they did not prove to be significant, possibly due to the low prevalence of this treatment. In the narrower FS study, which had fewer predictors in a more balanced design, tine harrow significantly reduced the number of several weeds (such as *M. annua*, *F. convolvulus* and *S. annua*), as well as the total number and the total dry weight of weeds. Other abundant weeds, such as *C. album*, *C. hybridum* and *P. aviculare*, were also reduced, although just to an insignificant extent (Figure 6B). The different outcomes of the two investigations (BS and FS) can be explained by many reasons. First, the sampling strategy in the FS survey, with weed counting and dry weight measuring within microplots, is more accurate than estimating plant cover values in large plots. Second, the more diverse multidimensional environment can also have masked the effects of mechanical treatments in the BS. This is especially true for the soil conditions, as Mouazen et al. [83] emphasized that soil texture and properties had important effects on the mechanical action of the tine harrow. During soil penetration, the tine movement is mainly influenced by moisture content and soil aggregate size. Johnson and Luo [27] also reported that wet

soils reduced the performance and weed control benefits of the tine harrow in peanut plantations. Consequently, the efficiency of tine harrow could be decreased in heavy clay soils, which sites accounted for about one third of the tine harrowed fields in our BS. In contrast, our FS was conducted in loamy soils alone, possibly allowing more favorable circumstances for an efficient tine harrow application. Interestingly, tine harrow appeared to increase the number of *S. viridis* individuals (Figure 6B). A similar phenomenon was also detected earlier in oil pumpkin fields, where cultivating tillage had an encouraging impact on some weed populations [33]. This can be explained by the fact that in addition to its weeding action, the soil disturbance of mechanical equipment might also stimulate weed seed germination [40]. However, the newly emerged weeds after the passage of tine harrower were quite small in their stature compared to that of phacelia and, according to our subsequent observations, were not able to grow through phacelia canopy, which had become dense in the meantime.

4.3. Herbicides

According to the experiments of Doma et al. [21], phacelia is very susceptible to most of the chemical weedkillers, and can be easily eliminated if it appears as a volunteer crop [84]. Only three herbicides were applied in the phacelia cultures studied by us: clopyralid for some dicotyledonous weeds, quizalofop-P-ethyl against grass weeds [17] and linuron, the herbicide that had been considered to be most efficient by the growers until it was withdrawn in the third year of our study. Nevertheless, we found significant effects on weeds in the case of linuron and clopyralid. *C. album*, which was by far the most prevalent weed in our study (Figure 2), and also *C. hybridum* seem to be particularly sensitive to linuron (Table 3). This is why the withdrawal of this herbicide became such a great challenge for phacelia seed growers, who had, thus, lost their best tool for controlling the most troublesome weed. Linuron had also been a mainstay in pumpkin [33], bean [20] and carrot [19] management, and its withdrawal creates similar challenges in other parts of the world [85]. The substitution is not easy: for example, clopyralid, which was developed to provide selective control of broadleaf weeds (e.g., *C. arvensis*, [86]), has a very low efficiency against *C. album* [87]. In Hungary, clopyralid is used in many cultures against pernicious weeds from the *Asteraceae* family, e.g., *A. artemisiifolia* and *C. arvensis* [17]. According to our results, two plants from this tribe, volunteer sunflower and *Anthemis austriaca* Jacq., were significantly susceptible to this ingredient (Table 3). Anyway, the fact that this herbicide was applied often with patch spraying made tracing its efficiency more difficult in our BS.

Although the fields involved in our FS investigation were not infested with weeds from the *Asteraceae* family, our results suggest that clopyralid can also reduce some other weeds, even *C. album*, but it was not able to significantly decrease the total weed number and biomass (Figure 6A). Although this ingredient can be credited in the integrated management of phacelia crop in the case of the high infestation of troublesome *Asteraceae* weeds, our results suggest that tine harrow itself can produce greater weed control efficiency than clopyralid for the weeds outside of this taxonomical group (Figure 6C).

4.4. Biodiversity Issues

We could register eight red list weed species (*Agrostemma githago* L., *Anchusa arvensis* (L.) M. Bieb., *Anthemis cotula* L., *Chenopodium vulvaria* L., *Galium tricornutum* Dandy, *Melampyrum arvense* L., *Misopates orontium* (L.) Raf. and *Neslia paniculata* (L.) Desv.) during our survey, albeit with low frequency values. These species always occurred in extensively or organically managed fields, which sometimes harbored large populations of further rare insect-pollinated plants with large biodiversity values. For example, *S. annua*, a retreating melliferous weed with great ecological and historical value [88], sometimes thrived in large quantities in such fields. In contrast, the spayed phacelia crops usually hosted only a few common weed species, such as the majority of conventional farmlands in Europe, where agricultural intensification absolutely impoverished the arable weed flora [89]. This

highlights the importance of extensification in phacelia crops for the conservation of rare arable plants in Europe [90].

5. Conclusions

Our research highlighted that environmental and cultural variables together are responsible for most of the variance in the weed species composition of phacelia fields in Hungary. The most prevalent weed, *C. album*, seemed to be highly sensitive to linuron. Anyway, this herbicide had been banned in the third year of our study; the remaining alternatives did not show significant effects on this species (even though tine harrow and clopyralid could reduce it to an insignificant extent). Nevertheless, our results also suggest that tine harrow can efficiently decrease the total number and biomass of weeds, even if its efficiency might be affected by soil properties. Accordingly, tine harrow is a promising tool in the weed management of phacelia, which should be inserted into the integrated management of this challenging crop species.

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