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Impact of 40 Years Use of Different Herbicide Strategies and Crop Rotations on Weed Communities in Two Sites of the Czech Republic

Markéta Mayerová [1,*], Jan Mikulka [1], Michaela Kolářová [2] and Josef Soukup [2]

[1] Division of Crop Management System, Crop Research Institute, Drnovská 507, 161 06 Prague, Czech Republic
[2] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague, Czech Republic
* Correspondence: mayerova@vurv.cz

Abstract: Understanding the impact of farming practices on the composition of weed communities has important implications for the sustainability of weed management strategies. This study analyses data from a 40-year experiment established at two sites in the Czech Republic in 1972. The impact of herbicide strategies on the weed communities was evaluated in multi-crop rotation (MCR) with 50% cereals, and simple crop rotations (SCR) with 75% cereals. The following two herbicide strategies were compared: (1) simple treatment with synthetic auxins only in the simple crop rotation, and (2) targeted herbicide combinations with particular use of sulfonylureas, triazines, ureas and synthetic auxins. Untreated plots were included for reference purposes. Results showed that crop was the major factor determining weed community composition and explained 18% of the total variation in the case of untreated controls. However, herbicide treatment reduced the crop effect to 11%. The highest average increase of Shannon's diversity index was by 25.4% after 44 years at the untreated plots in SCR, and it decreased by 15.1% at the treated plots in MCR. Weed species evenness € increased at all plots by an average of 23%. Simpson's dominance index decreased at untreated plots in both crop rotations and plots treated with auxins, with the highest 44.8% decrease at the untreated plots in SCR. On the contrary, Simpson's index increased by 33,3% on the treated plots in MCR. Herbicide selection depended on the occurrence and density of target weed species, and led to greater decrease in weed diversity and population densities in the diversified crop rotations.

Keywords: herbicide treatment; long-term field trial; species diversity; weed management

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1. Introduction

The evolution of weed communities depends on complex interactions between soil and climate environmental factors and crop management (crop type, soil cultivation and weed control methods) [1,2]. Moreover, the development of these plant communities results from changes in agricultural practices, and many of the observed shifts in weed community composition are caused by intensification. The following changes are especially noted:

(1) Simplified crop rotations, especially the shift from spring to autumn sowing, has reduced the regenerative niche for obligate-germinating species and favours winter annual species such as *Apera spica-venti* (L.) P. Beauv, *Tripleurospermum inodorum* (L.) Sch.Bip., *Veronica persica* Poiret and *Viola arvensis* Murray.
(2) The decline in rye and flax growing then led to suppression of crop specialists such as *Cuscuta epilinum* L., *Silene linicola* C.C.Gmelin, *Lolium remotum* Schrank and *Bromus secalinus* L. [3].
(3) Excessive fertiliser and herbicide use in Europe supported the spread of nitrophilous species including *Chenopodium album* L. and *Stelleria media* (L.) Vill. and also the herbicide resistant or naturally tolerant species such as *Alopecurus myosuroides* Huds., *Amaranthus* spp. L. and *Poa annua* L. [4,5]. In addition, the study of Storkey et al. [6]

from 29 European countries showed that the *Caryophyllaceae*, *Asteraceae* and *Brassicaceae* families make up most of the rare and threatened arable plants, but the families often comprise ecologically different species.

However, the impact of environmental and human management factors on weed population changes has different explanatory weights on different scales. Many factors and their interactions affect weed populations at the landscape scale, and it is therefore difficult to differentiate factors causing specific changes. Although shifts in species composition can be attributed to a particular change in cultural practices at the smaller in-field scale [7], the effects of the farming system at field scale are more evident because of weed species' different sensitivity to herbicide and nitrogen input [8]. Therefore, investigation of the impact of agronomic practices on weed flora changes is most appropriate at the field scale.

It is also difficult to identify the individual drivers of weed composition shifts because of the multiple interactions between management procedures and biotic and abiotic factors. Some researchers suggest crop type is the major factor determining species composition, where the composition is related to different farming practices used for the various crop types [9,10]. However, these management factors can also include the use of specific herbicides associated with the cultivation of individual crops [11]. Widespread use of herbicides has affected weed communities since the second half of the last century [12], and available herbicides have changed considerably over time. For example, synthetic auxins were introduced in the 1940s to control broad-leaved weeds in cereals, but repeated use has increased the distribution of both grass weeds and tolerant broad-leaved weeds [7,13]. In contrast, the abundance of sensitive species, such as *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Capsella bursa pastoris* (L.) Med. and *Thlaspi arvense* L. has temporarily decreased, but despite regular application of synthetic auxins, these susceptible species have maintained their abundance in Europe due to seeds produced by surviving seedlings [14].

Repeated use of a single mode of action has generally resulted in a weed community shift to more herbicide-tolerant species [7]. However, in practice, the long-term repeated application of herbicides with the same active ingredient at the same locality is unlikely, and weed communities are concurrently influenced by other factors. It is therefore difficult to prove that herbicides are the main driver of weed community changes and to predict future trends [9]. Nevertheless, Grundy et al. [15] indicated that the application and timing of a specific herbicide supports certain species by removing susceptible competitor species.

Bàrberi et al. [16] and Hawes et al. [17] revealed that changes in chemical weed control contributed to reduced species diversity in the weed community, and other authors added that these changes caused selection pressure against some species and promoted more adaptable species [5,15]. In contrast, studies have reported inconclusive herbicide treatment effects on weed species diversity. These include: (1) Derksen et al. [18] who recorded no decrease in weed community diversity expressed by Shannon's diversity index and dominance-diversity curves; (2) Ulber et al. [19] who found no significant effect of herbicide treatment on species richness in their short-term field trial; (3) 35-year study of Hume (1987) [14] shows that continued 2,4-D application did not cause significant change in the species present; and finally (4) Mayor and Dessaint [20] compared the impact of chemical, mechanical and integrated weed control on soil seedbank diversity, and although the densities of germinated seeds steadily and significantly increased in plots with mechanical control and decreased in plots with chemical control, there were no differences in Shannon's diversity index values in the strategies used or in different years.

Stoate et al. [21] also consider that short-term assessment and comparisons are difficult because herbicide use can change from year to year in response to climate and other factors, and while there have been many observational surveys studying weed communities in Europe [9,11,22,23], long-term field trials similar to ours, focusing on weed management influence on weed communities are unique. Further, although many studies have assessed the effects of herbicides on weed communities [15,18], weed shifts have rarely been studied in the long-term because of problems in determining the impact of changing management practices. Moreover, chemical weed control was not usually included as an explanatory factor because of the wide spectrum of herbicide active ingredients which have changed considerably over time [2,9,11].

A sustainable agriculture approach is to reduce the weed density of harmful competitive species but maintain weed density and species diversity at an acceptable level. Therefore, it is important to know how the long-term use of herbicides affects the weed community, and especially undesirable changes.

The field experiment was established at a time when there was not enough information about the long-term use of herbicides in Czechoslovakia. The initial aim of the experiment was to find out whether repeated use of herbicides with the same mechanism of action and repeated cereals cultivation causes the spread of resistant weed species, and whether this spread can be prevented by crop rotation and changing herbicides.

The objective of this study is to determine and explain changes in weed-community composition in a long-term field experiment with different crop rotations and herbicide treatments. We tested the hypotheses that (1) weed species diversity decreases in plots with long-term herbicide treatment and increases in untreated controls, (2) targeted herbicide treatment suppresses undesirable, highly competitive species and decreases weed density compared to treatment with only auxinic herbicides, and (3) simplification of crop rotation and herbicide use leads to weed-shift.

2. Materials and Methods

2.1. Experimental Sites

The field trial was established in 1972 at two experimental stations of the Crop Research Institute in the Czech Republic: Hněvčeves at 50.31° N, 15.72° E (sugar beet growing region) and Pernolec at 49.77° N, 12.68° E (potato growing region). Their soil and climate characteristics are presented in Table 1. The total area of the experiment was 60 m in length and 45 m in width.

Table 1. Experimental site characteristics with available nutrients (average values over the experimental period).

Experimental Site	Altitude	Average Annual Temperature	Average Annual Rainfall	Soil Classification	pH (KCl)	pH (H_2O)	$Ca_{avail.}$	$P_{avail.}$	$K_{avail.}$	$Mg_{avail.}$
	m	°C	mm				mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Hněvčeves	265	8.2	573	Haplic Luvisol on loess, clay-loam	6.06	6.81	2339	99.9	272.3	241.3
Pernolec	530	7.1	559	Cambisol on orthogneiss, sandy loam	5.09	6.14	1300	60.0	91.6	79.5

The experimental design was split-plot with crop rotation as the main plot and chemical weed control as the sub-plot. The main plot included two different crop rotation systems (CR) with specific cereal percentages: (1) multi-crop CR (MCR) with 50% cereals and 50% broad-leaved crops: winter wheat (*Triticum aestivum* L.)-oilseed rape (*Brassica napus* subsp. *napus* L.)-winter wheat-potato or sugar beet (*Solanum tuberosum* L. or *Beta vulgaris* var. *altissima* L.)-spring barley (*Hordeum vulgare* L.)-pea (*Pisum sativum* L.) and (2) simple CR (SCR) with 75% cereals and 25% legumes: winter wheat-spring barley-spring barley-pea.

Three treatments were used: (1) the untreated control; (2) synthetic auxins in simple crop rotation (MCPA; 2,4-D in cereals, bentazone and PSII inhibitor in pea) and (3) targeted treatment with herbicides and their combinations according to the density of the most important weed species. Photosystem II inhibitors, particularly triazines, were mostly used in the first years of the experiment and during the 1980's. ALS inhibitors, especially sulfonylureas, then prevailed in cereals, and microtubule inhibitors or ACCase inhibitors were used in pea. Photosystem II inhibitors were mostly used in potato and were used together with ACCase inhibitors in sugar beet. The herbicides were applied by small plot sprayer at the rates recommended by manufacturers for common use and no other weed management methods were applied. There were four randomised replications for each 100 m^2 subplot (10 by 10 m). These were established 10 m from field boundaries and separated 1 to 2 m on all sides to eliminate interaction between treatments.

Uniform agronomic practice followed provincial standards and comprised conventional tillage up to 20–25 cm depth, mineral fertilisation, cultivar selection, seeding rates and dates. Fungicides and insecticides were used in case pest pressure.

2.2. Data Collection

Species composition and density were assessed at the beginning of the trial in spring 1972, during the trial, and in 2013–2016 for the purpose of this study. The weed community was sampled when weeds fully emerged, before herbicide application and in an untreated window using four randomly positioned 0.5 by 0.5 m quadrates per subplot, with headlands and plot edges excluded from sampling. Weed species were identified and the number of plants for each species was counted in each quadrate. The densities in four quadrates in each subplot were aggregated to provide the value for 1 m^2. The weeds were identified at species level where possible, and some species such as *Vicia* spp. were identified at the genus level. Botanical nomenclature followed Kubát et al. [24].

2.3. Data Analyses

Weed community species diversity was calculated for each subplot repetition by Simpson's dominance ($D = \sum_i {p_i}^2$), Shannon's diversity ($H' = -\sum_i p_i \ln (p_i)$) and evenness ($E = H/\ln S$) indices; where p_i is the proportion of individuals of 'i'th species in the total number of individuals (S) in the sample quadrate [25]. Analysis of variance was conducted using STATISTICA 13.3 software (TIBCO Software Inc., California, PA, USA), with treatment, locality, and crop as fixed factors and diversity indices as the dependent variables. Scheffe's multiple comparison test at $\alpha = 0.05$ was then employed to determine homogenous groups.

Data on weed species density was log-transformed prior to analysis, and multivariate data analysis in CANOCO 5 software provided data exploration [26]. Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA) were used due to the gradient length on the first canonical axis in compositional turnover in Detrended Correspondence Analysis (DCA). Analysis was performed for each treatment and separately for crop rotation to detect shifts in weed species composition over time. Two explanatory variables were then compiled; the time duration in years identified the long-term shifts in weed composition regardless of the differences in individual years; and crop type comprised winter cereals, spring cereals, legumes, and root crops.

The gross effects were tested using separate CCAs or RDAs with single explanatory variables and the net effects were tested using partial CCAs or RDAs with a single explanatory variable and other variables as covariates. The net effects of explanatory variables on weed species composition were tested by Monte-Carlo permutation tests for 999 permutations at $p = 0.05$ significance [22]. The ratio of particular canonical eigenvalues to the sum of all eigenvalues measured the proportion of explained variation. While complete analysis was carried out for Pernolec, there was insufficient data at Hněvčeves for statistical assessment. Therefore, the Hněvčeves species composition in the last monitored years was compared with its composition at trial beginning.

3. Results

3.1. Weed Diversity Indices

Three diversity indices were used to evaluate weed diversity for species richness and evenness, and Table 2 shows the changes in winter wheat diversity after more than 40 years. The average Shannon's diversity index (H′) at the beginning of the trial was higher in multiple crop rotation plots (MCR) at 1.75 at untreated plots and 1.79 at treated plots and lowest at 1.48 at plots with targeted treatment in simple crop rotation (SCR). The highest average H′ increase was by 0.54 after 40 years at the untreated plots in SCR and it decreased by 0.27 at treated plots in MCR. In addition, the weed species evenness (E) increased at all plots by an average of 0.15, and Simpson's dominance index was higher at plots with targeted treatment in SCR, at 0.32 at trial beginning and 0.29 at trial end. Finally, Simpson's index decreased at untreated plots in both crop rotations and plots treated with auxins, with the highest 0.13 decrease at the untreated plots in SCR.

Table 2. Species diversity of weed community expressed by diversity indices—the situations in winter wheat at the trial beginning (1972) and present (2013–2016). The average values of indices (Avg.) ± standard error (SE) include both localities and four replications for each treatment. The averages marked by the same letter in individual columns did not significantly differ at $\alpha = 0.05$ (Scheffe's test).

Treatment	**Sampling Time**	**Shannon's Index**		**Shannon's Evenness**		**Simpson's Index**	
		Avg.	**SE**	**Avg.**	**SE**	**Avg.**	**SE**
untreated MCR	beginning	1.75^{abc}	0.10	0.69^{abc}	0.06	0.24^{ab}	0.06
	present	1.95^{bc}	0.08	0.82^{de}	0.02	0.18^{a}	0.02
targeted MCR	beginning	1.79^{abc}	0.07	0.70^{abc}	0.05	0.21^{ab}	0.04
	present	1.52^{a}	0.15	0.85^{e}	0.01	0.28^{b}	0.03
untreated SCR	beginning	1.59^{ab}	0.12	0.64^{ab}	0.08	0.29^{b}	0.08
	present	2.13^{c}	0.05	0.80^{cde}	0.01	0.16^{a}	0.01
auxin SCR	beginning	1.60^{ab}	0.10	0.64^{ab}	0.08	0.26^{ab}	0.06
	present	2.12^{c}	0.06	0.80^{cde}	0.01	0.16^{a}	0.01
targeted SCR	beginning	1.48^{a}	0.06	0.60^{a}	0.08	0.32^{bc}	0.07
	present	1.51^{a}	0.13	0.75^{bcd}	0.01	0.29^{b}	0.03

Comparison of the differences in weed species diversity between treatments in recent years identified the lowest H′ in plots with targeted treatment, with 1.52 in MCR and 1.51 in SCR, and the highest was at untreated plots at 1.95 in MCR and 2.13 in SCR. However, no differences between treatments were found in E and the values of all diversity indices at plots treated with auxins were comparable to those at untreated plots.

The statistically significant impact of locality on weed species diversity is documented in Table 3. Both Shannon's indices were higher in Pernolec than Hněvčeves, with 1.93 average H′ and 0.77 E, but Simpson's dominance index was higher in Hněvčeves at 0.29 compared to 0.22 at Pernolec. Weed diversity, however, was affected by different crops, and Table 4 highlights that both Shannon's indices were lowest in spring cereals: 1.68 for average H′ and 0.69 for E. The highest average H′ was 1.97 in potato and the highest E was 0.82 in oilseed rape.

Table 3. Species diversity indices in the localities. Average values (avg.) ± standard error (SE) was assessed over years, treatments and crops. The averages marked by the same letter in individual columns did not significantly differ at $\alpha = 0.05$ (Scheffe's test).

Locality	**Shannon's Index**		**Shannon's Evenness**		**Simpson's Index**	
	Avg.	**SE**	**Avg.**	**SE**	**Avg.**	**SE**
Hněvčeves	1.58^{a}	0.03	0.69^{a}	0.01	0.29^{b}	0.01
Pernolec	1.93^{b}	0.03	0.77^{b}	0.01	0.22^{a}	0.01

Table 4. Species diversity indices crops. Average values (avg.) ± standard error (SE) was assessed over years, treatments and localities. The averages marked by the same letter in individual columns did not significantly differ at $\alpha = 0.05$ (Scheffe's test).

Crop	**Shannon's Index**		**Shannon Evenness**		**Simpson Index**	
	Avg.	**SE**	**Avg.**	**SE**	**Avg.**	**SE**
potato	1.97 ^{a}	0.13	0.74 ^{ab}	0.03	0.23 ^{ab}	0.04
pea	1.85 ^{a}	0.07	0.73 ^{ab}	0.01	0.24 ^{ab}	0.02
oilseed rape	1.82 ^{a}	0.04	0.82 ^{b}	0.02	0.21 ^{a}	0.01
winter cereals	1.79 ^{a}	0.04	0.76 ^{b}	0.01	0.23 ^{ab}	0.01
spring cereals	1.68 ^{a}	0.05	0.69 ^{a}	0.02	0.29 ^{b}	0.02

3.2. Impact of Factors Influencing Weed Diversity

The data were separately evaluated for the Hněvčeves and Pernolec localities.

(1) Hněvčeves; time and treatment explained 45.6% and 68.5% of total variation in weed species composition in winter wheat in simple (SCR) and multi-crop rotation (MCR), respectively. Partial Redundancy Analysis detected that the variation in species data explained by the net effect of time duration was 42% in SCR and 63.5% in MCR, and the net effect of treatment was insignificant. Abundance increase was observed in many species; where *Lamium purpureum* L., *Veronica* spp. L., *Viola arvensis*, *Tripleurospermum inodorum* and *Sinapis alba* L. had an overall increase in weed density, and although *Matricaria chamomilla* L. decreased in both crop rotations, the *Apera spica-venti*, *Thlaspi arvense*, *Papaver* spp. L., *Galium aparine and Stellaria media* L. species altered sporadically over time (Figure 1).

(2) Pernolec; combined time and crop type significantly explained over 20% of total variation in weed species composition in all treatments, and the variation explained by net effects was the highest for crop type (Tables 5 and 6). Weed species composition was influenced by monitored variables more at the untreated plots (36.3% and 31.8% in multi- and simple crop rotation, respectively) than at targeted treated plots (30.7% and 20.5% in multi- and simple crop rotation, respectively) and with auxins treated plots (26.1%). Species composition was strongly associated with crop type, and this explained the 18.1% total variation in weed species data at untreated plots in simple crop rotation compared to 11.5% at the auxins treated plots.

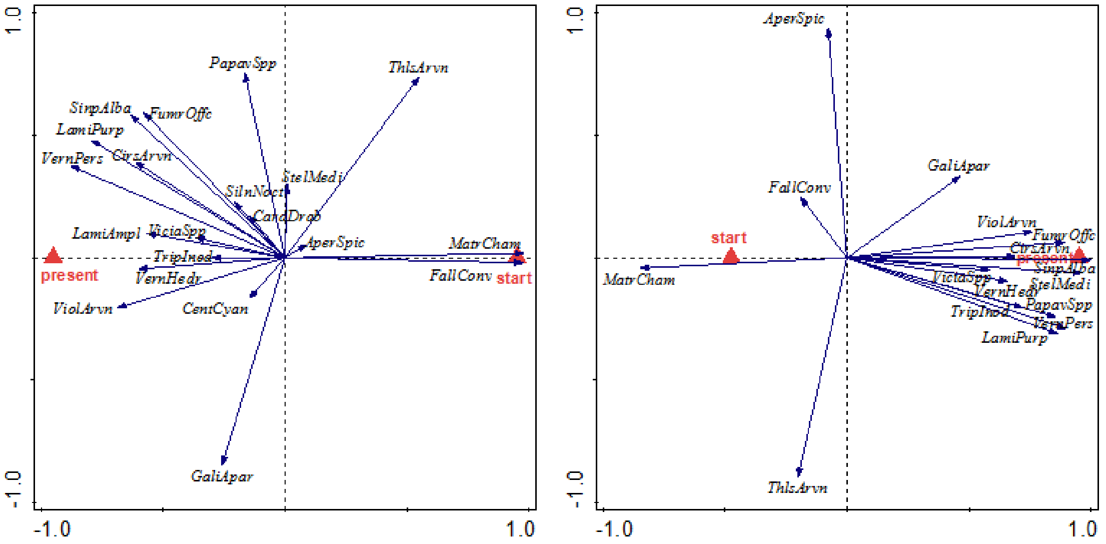

Figure 1. Temporal trends in weed species composition in winter wheat in Hněvčeves, comparison between trial beginning and the present (2016); Redundancy Analysis (RDA); simple crop rotation (**left**), multi-crop rotation (**right**).

Table 5. Net effects of explanatory variables on the weed species composition in Pernolec for multi-crop rotation. Partial RDAs were used when gradient length < 3.0. Eigenvalue = sum of all canonical eigenvalues; % = percentage of explained variance; *F* = ratio for the test of significance of all canonical axes and *p*-value = the corresponding probability from the Monte-Carlo permutation test.

Explanatory Variables	**Untreated**				**Targeted Control**			
	Eigenvalue	**%**	***F***	***P***	**Eigenvalue**	**%**	***F***	***P***
type + year	0.36	36.3	6.7	0.001	0.31	30.7	5.2	0.001
crop type	0.18	17.6	4.3	0.001	0.17	17.1	3.9	0.001
time	0.15	15.4	11.4	0.001	0.1	10.0	6.8	0.001

Table 6. Net effects of explanatory variables on weed species composition in Pernolec for simple rotation. Partial CCAs were used when gradient length > 3.0. Eigenvalue = sum of all canonical eigenvalues (total inertia = 2.07); % = percentage of explained variance; *F* = ratio for the test of significance of all canonical axes and *p*-value = corresponding probability from the Monte-Carlo permutation test.

Explanatory Variables	**Untreated**				**Targeted Control**				**Synthetic Auxins**			
	Eigenvalue	**%**	***F***	***P***	**Eigenvalue**	**%**	***F***	***P***	**Eigenvalue**	**%**	***F***	***P***
type + year	0.66	31.8	8.1	0.001	0.62	20.5	4.5	0.001	0.66	26.1	6.1	0.001
crop type	0.37	18.1	6.9	0.001	0.35	11.6	3.8	0.001	0.29	11.5	8.1	0.001
time	0.28	13.7	10.5	0.001	0.27	8.8	5.8	0.001	0.37	14.8	5.2	0.001

Figures 2–4 depict Pernolec temporal trends in species composition and highlight that the following species receded in both crop rotations regardless of treatment and crop type: *Raphanus raphanistrum*, *Myosotis arvensis* (L.) Hill, *Scleranthus annuus* L. and *Erophila verna* (L.) DC. and simple crop rotation produced similar trends for all herbicide treatments. The density of many weed species in multi-crop rotations was affected more by time and crop type on the untreated plots than on treated plots. For example, the density of *Lamium* spp. L., *Aphanes arvensis* L., *Anagallis arvensis* L. increased more at untreated plots in SCR, and *Tripleurospermum inodorum, Centaurea cyanus* L. *and Thlaspi arvense* increased more in MCR. In addition, *Lycopsis arvensis* L. and *Centaurea cyanus*, which had temporarily receded from the Czech fields, increased at the untreated plots. The density of susceptible species, such as *Raphanus raphanistrum* and *Erophila verna* decreased more rapidly at plots treated with herbicides than at untreated plots, and the density of the more competitive species, including *Centaurea cyanus, Thlaspi arvenese* and *Tripleurospermum inodorum* increased more at untreated plots. In contrast, the density of many of the susceptible species decreased at treated plots; and these included *Arabidopsis thaliana* (L.) Heynh, *Fallopia concolvulus* (L.) Á.Löve, *Anagallis arvensis*, *Galeopsis tetrahit* L. and *Polygonum aviculare* L.

Finally, many weed species responded to crop type (Figure 5). While *Apera spica-venti*, *Arabidopsis thaliana*, *Papaver* spp. and *Tripleurospermum inodorum* winter annuals were associated with winter cereals; the *Fallopia convolvulus*, *Chenopodium album*, *Galeopsis tetrahit* and *Anagallis arvensis* summer annuals were associated with spring cereals and the *Plantago major* L., *Taraxacum* sect. *Ruderalia* Kirschner, H.Øllgaard et Štěpánek and *Equisetum arvense* L. perennial weeds were often present in root crops.

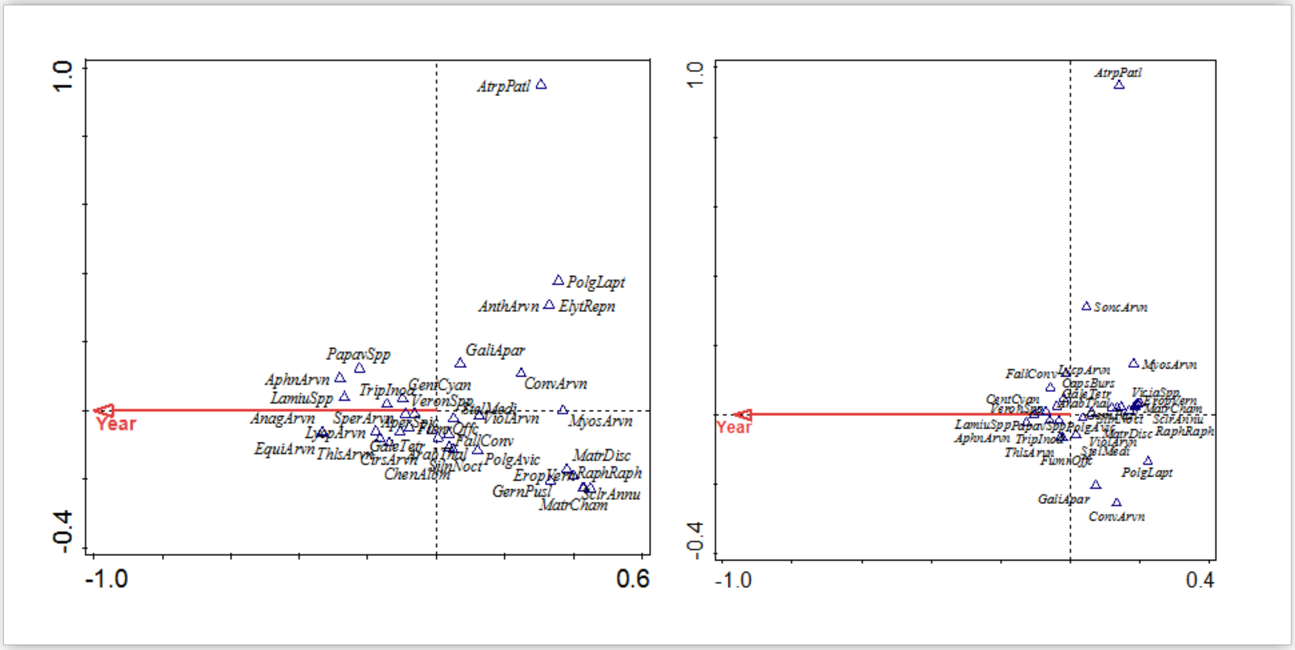

Figure 2. Temporal trends in species composition in Pernolec in simple crop rotation. Ordination diagrams of partial Canonical Correspondence Analysis (CCA) with untreated plots (**right**) and targeted treatment with herbicide combinations (**left**). Year is the explanatory variable and crop type is the covariate; species with low weight are not shown.

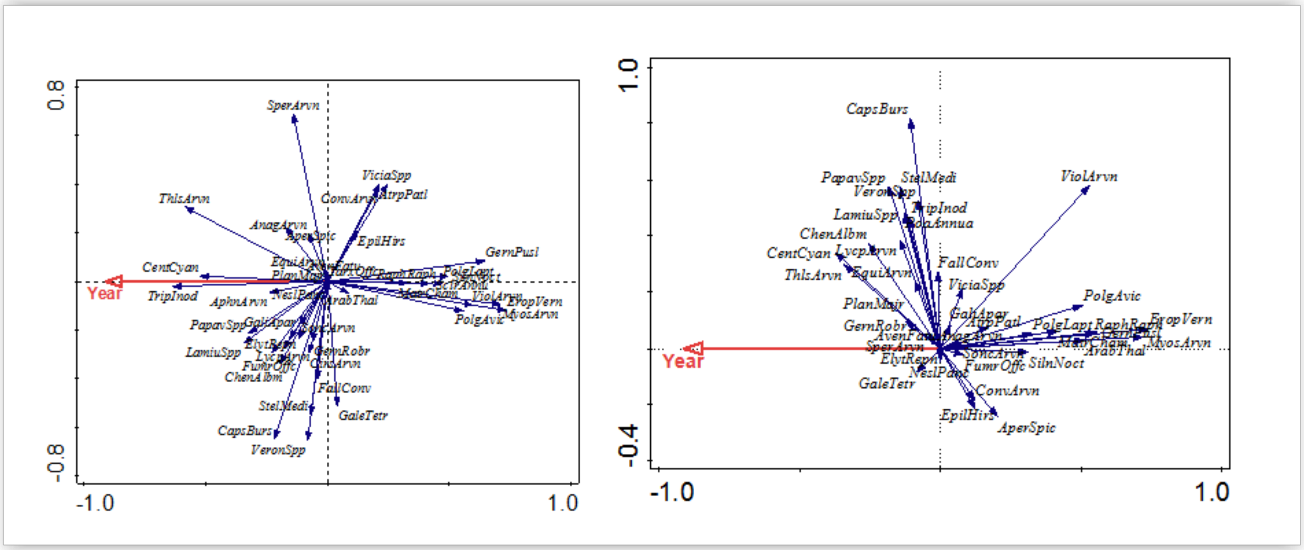

Figure 3. Temporal trends in species composition in Pernolec in multi-crop rotation. Ordination diagrams of partial Redundancy Analysis (RDA) with untreated plots (**right**) and targeted treatment with herbicide combination (**left**). Year is the explanatory variable and crop type is the covariate; species with low weight are not shown.

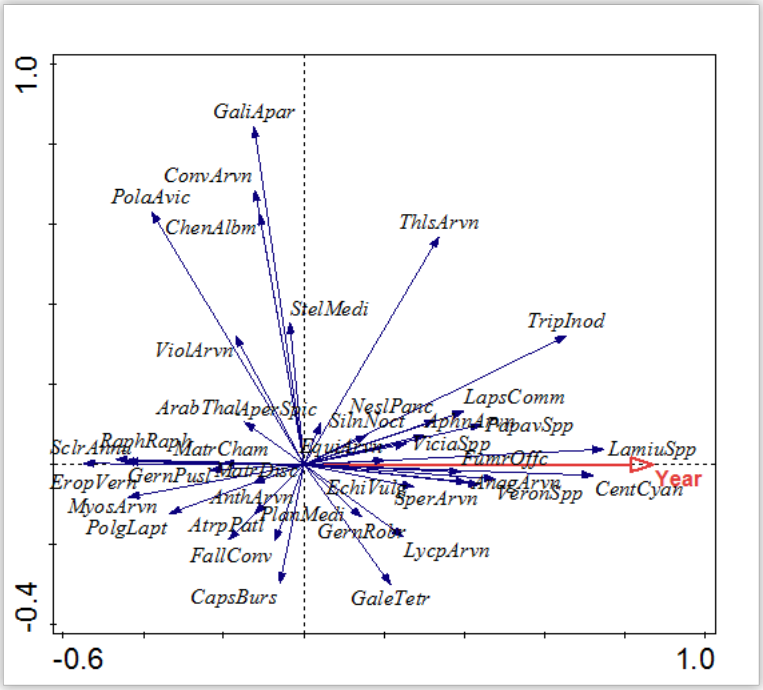

Figure 4. Temporal trends in species composition in Pernolec at plots treated with synthetic auxins. Ordination diagrams of partial Redundancy Analysis (RDA). Year is the explanatory variable and crop type is the covariate; species with low weight are not shown.

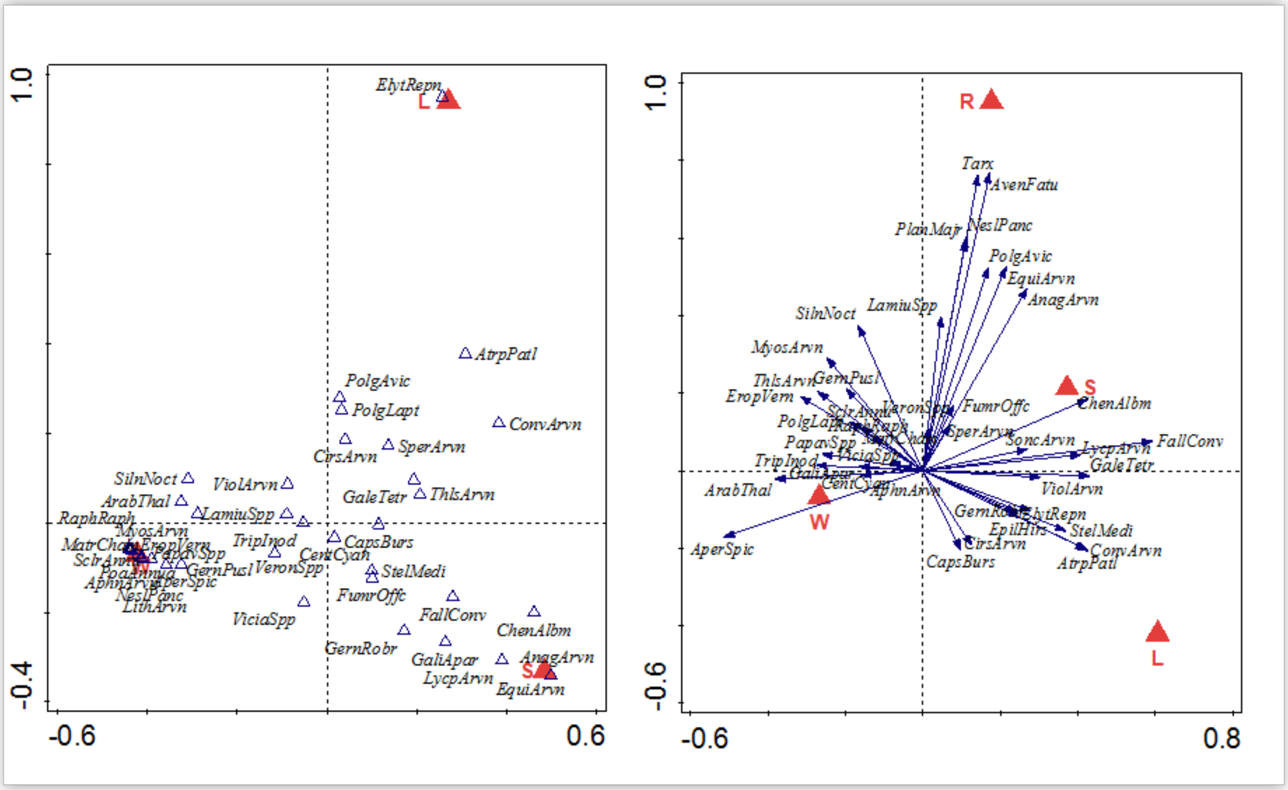

Figure 5. Influence of crop type on weed species composition at untreated Pernolec plots in simple crop rotation (**left**) and multi-crop rotation (**right**). Crop type is the explanatory variable and year is the covariate; species with low weight are not shown. W-winter cereals, S-spring cereals, L-legumes, R-root crops.

4. Discussion

4.1. Impact of Treatment on Weed Diversity Indices

Our results confirmed an increased Shannon's diversity index (H') to some extent at plots without herbicide treatment compared to treated plots after more than 40 years of research at Hněvčeves and Pernolec. This is supported by the following authors; Edesi et al. [27] proved decreasing H' tendency from herbicide use in conventional farming compared to organic farming in a five-year trial; similar results were presented in Mahn's report [28] that herbicides reduced weed community structure by reduced H' and Hyvönen and Salonen's six year experiment [29] found insignificantly higher H' in a low-input cropping system without herbicides.

Weed community diversity is also influenced by the intensity of chemical control. For example, Légère et al. [1] observed higher H' in minimum than in moderate or intensive chemical weed management, but lower evenness (E) in moderate than in minimum or intensive weed management. Moreover, our trial revealed that H' increased in plots with less intensive treatment with synthetic auxins, while H' decreased in targeted treatment plots in multiple crop rotation (MCR) but remained at the same level in simple crop rotation (SCR). This partly agrees with Hume [14] who reported that the long-term effect of 2,4-D altered species proportions without significant effect on species richness, and Zengín [30] recorded that the abundance of many species decreased after three or four years repeated 2,4-D applications in spring wheat. In contrast, authors such as Derksen et al. [18] and Ulber et al. [19] recorded no significant effect of herbicide treatment on species richness in short-term trials.

Although weed species evenness (E) increased at all plots in our experiment, Simpson's dominance index decreased at untreated plots and increased on plots treated by targeted herbicide combinations. This may indicate density increase in dominant species at the treated plots. Derksen et al. [18] also showed E increase or stagnation after the application of herbicides, and the increase in community evenness at treated plots can be explained by niche exploitation of species with earlier low relative density due to the decline in dominant species suppressed by herbicide application [31]. Moreover, while changes in H' can occur relatively rapidly, changes in E take effect over longer periods; some authors, therefore, did not notice significant differences in E between treatments [17]. For example, Armengot et al. [32] indicated significant decrease in weed species richness from herbicide treatment in their one-year study.

4.2. Impact of Locality and Crop on Weed Diversity Indices

We identified differences in weed species diversity between experimental localities; where the Pernolec district had higher average values in all diversity indices over treatments than in Hněvčeves which is situated in a more intensive production area. Although 53 weed species were recorded in Pernolec and only 29 in Hněvčeves (Supplementary Materials), a higher proportion of competitive species was observed at all Hněvčeves trial plots. These locality differences could be explained by higher Hněvčeves soil fertility, higher crop competitiveness and greater land management intensity close to the trial area.

We also confirmed statistically significant impact of crop type on weed species diversit,; where this was higher in dicotyledonous crops than in winter cereals and the lowest was observed in spring cereals. This agrees with Gulden et al. [33] and Bellinder et al. [34] who found that weed diversity was affected by different crops. In addition, Pyšek et al. [35] recorded the highest species richness in root crops but, in contrast to our research, they reported higher species richness in spring cereals than winter cereals.

Although Lososová et al.'s report [22] of higher species richness in cereals than in root crops contrasted with our research, their study into crop classification of weed vegetation combined the perennial and annual crops with different sowing times in the same categories; for example, "Cereals" included also rape and fodder crops. This could have resulted in higher species richness in the "Cereals" category. However, Barroso et al. [36] support our results of relatively small differences in weed diversity in different weed management and in different crops. Moreover, although weed communities change as a result of different crops and management practices, the total number and relative abundance of weed species does not necessarily change over the long-term, and treatments on crops with similar diversity levels can also have different weed community composition [1].

4.3. Impact of Factors Influencing the Weed Diversity

Evaluated variables of crop type, time and treatment explained only part of the total variation in weed species composition, and the unexplained variation was associated with other factors, particularly environmental conditions. For example, Grundy et al. [15] reported that changes in species composition are more strongly associated with season than with individual herbicide treatment, but De Mol et al. [2] recorded that annual changes made only a small contribution to variation in species composition. Results of Lososová and Cimalová [10] and Fried et al. [9] confirmed our strong impact of crop type on species composition.

Our weed species composition was more influenced by monitored variables at the untreated plots than in treated plots, where the targeted treatment contributes to the variation and reduced time impacts in both crop rotations and crop type impacts in simple crop rotation. In contrast, however, Bàrberi et al. [16] reported that the effects of rotation and the preceding crop on the weed flora composition in winter wheat were evident only with reduced herbicide use. We also recorded temporal trends in weed community composition in both localities and found that the *Apera spica-venti*, *Arabidopsis thaliana*, *Stellaria media* and *Fallopia convolvulus* species occurred more or less evenly from the beginning of the experiment to the present. Although the density of many susceptible species such as *Arabidopsis thaliana* (L.) Heynh, *Anagallis arvensis*, *Galeopsis tetrahit* L. and *Polygonum aviculare* L. decreased at treated plots, they were not eliminated. The long-term use of herbicides in our experiment did not eliminate species from the species pool because the seed bank partly compensated for damaging herbicidal effects. Moreover, the targeted treatments concentrated on crop and weed density, and while herbicides with the same active ingredient were not applied long enough to cause significant shifts in weed species composition, they could still prevent increase in weeds adapted to a particular crop's management strategy.

This is partly supported by Hyvönen et al. [37] who found that sulfonylurea application caused no changes in spring cereal weed community structure in the 1980–1990 decade in Finland. Even long-term application of synthetic auxins did not cause the expected change in species composition, and this finding is supported by Zengín's report [30] of their decreased efficiency on the *Centaurea cyanus*, *Veronica spp.*, *Lamium spp.* and *Fumaria officinalis L.* broad-leaved weeds and subsequent increase in the density of these species.

Some authors reported increased density in perennial species such as *Elytrigia repens* (L.) Nevski and *Cirsium arvenese* (L.) Scop. due to long-term herbicide application against the annual broad-leaved species in cereals [29], but we did not detect this effect because we employed deep ploughing and crop rotations. Our trial, however, can be partly compared with Pallut and Moll's results [38] which found that the permanent application of reduced herbicide doses in a 12-year long-term trial caused increased density in *Centaurea cyanus*, *Matricaria* spp., *Apera spica-venti* and *Viola arvensis* species.

The results also indicate that long-term herbicide application tended to reduce the differences in weed species density, especially in simple crop rotations, and Hume [14] observed similar effects after long-term annual 2,4-D application in wheat rotation, where reduced density of the susceptible *Chenopodium album* and *Thlaspi arvenese* species, decreased the competitive effects of the tolerant *Setaria viridis* (L.), *Fallopia convolvulus* and *Solanum triflorum* Nutt. species and increased their density.

Our work herein also confirmed the well-known response of weed species on the cultivated crop [2,39,40] where crop type effect was slightly higher in the multi-crop rotations. This finding is supported by Hyvönen and Salonen [29] who found crop type more important in affecting weed community composition than weed management methods with or without herbicide application. In addition, Gulden et al. [33] explained the total variation in the seedbank density of the three major weeds primarily by crop and in-crop herbicide omission (>50%), but most variability in minor species (>80%) was unexplained. These results may reflect the regular glyphosate application before crop sowing in this trial.

Although the weed community composition generally changed over time in our long-term trial, these changes are not clearly attributable to herbicide effects, and this contrasts with Rotchés-Ribalta et al.'s. report [5] that long-term herbicide application led to selection pressure against susceptible weed species and the promotion of naturally tolerant and resistant species such as *Alopecurus myosuroides*, *Chenopodium polyspermum* L., and *Capsella bursa-pastoris* L. In addition, Salonen et al. [41] also showed that herbicide use explained most variation in weed community species composition in Finland; with the most dominant *Chenopodium album*, *Poa annua*, *Stellaria media* and *Galium spurium* L. species associated with the use of phenoxy acids and ploughing.

Modern weed management concepts consider approaches which reduce weed density but still maintain species diversity at an acceptable level, and despite long-term herbicide application, we found no decrease in species evenness or elimination of susceptible species from the species pool. This is supported by Ulber et al. [19] and Jones and Smith [42] who reported that treatment with selective herbicides provides considerable weed control but retains species diversity.

5. Conclusions

Our study highlights that the most significant proportion of weed diversity is explained by factors other than herbicide use. Herein, we established that crop type and rotation are the major factors determining species composition, and we therefore suggest the use of more diversified crop rotations which will support both weed species diversity and acceptable weed density. Finally, the selection of effective herbicides according to the occurrence and density of target weed species and omitting repeated application of herbicides with the same active ingredient will most certainly contribute to decreased population densities of potentially dominant and highly competitive species, and thus encourage minor species with lower competitiveness. These findings are in line with the new approach to biodiversity-friendly weed management which requires a balance between sufficient crop production and maintaining satisfactory species diversity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13010102/s1.

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Abbreviations

Abbreviations for Figures 1–5: AnthArvn (*Anthemis arvensis*), AnagArvn (*Anagallis arvensis*), AperSpic (*Apera spica–venti*), AphnArvn (*Aphanes arvensis*), ArabThal (*Arabidopsis thaliana*), AtrpPatl (*Atriplex patula*), AvenFatu (*Avena fatua*), CapsBurs (*Capsella bursa-pastoris*), CardDrab (*Cardaria draba*), CentCyan (*Centaurea cyanus*), ChenAlbm (*Chenopodium album*), CisrArvn (*Cirsium arvense*), ConvArvn

(*Convolvulus arvensis*), ElytRepn (*Elytrigia repens*), EpilHirs (*Epilobium hirsutum*), EquiArvn (*Equisetum arvense*), EropVern (*Erophila verna*), FallConv (*Fallopia convolvulus*), FumOffc (*Fumaria officinalis*), GaleTetr (*Galeopsis tetrahit*), GaliApar (*Galium aparine*), GernPusl (*Geranium pusillum*), GernRobr (*Geranium robertianum*), LamiAmpl (*Lamium amplexicaule*), LamiPurp (*Lamium purpureum*) LamiuSpp (*Lamium* spp.), LapsComm (*Lapsana communis*), LithArvn (*Lithospermum arvense*), LycpArvn (*Lycopsis arvensis*), MatrDisc (*Matricaria discoidea*), MatrCham (*Matricaria chamomilla*), MyosArvn (*Myosotis arvensis*), NeslPan (*Neslia paniculata*), PapaSpp (*Papaver* spp.), PlanMajr (*Plantago major*), PlanMedi (*Plantago media*), PoaAnnua (*Poa annua*), PolgAvic (*Polygonum aviculare*), PolgLapt (*Polygonum lapathifolium*), RaphRaph (*Raphanus raphanistrum*), SclrAnnu (*Scleranthus annuus*), SilnNoct (*Silene noctiflorum*), SinpAlba (Sinapis alba), SoncArvn (*Sonchus arvensis*), SperArvn (*Spergula arvensis*), StelMedi (*Stellaria media*), Tarx (*Taraxacum* sect. *Ruderalia*), ThlsArvn (*Thlaspi arvense*), TripInod (*Tripleurospermum inodorum*), VernHedr (*Veronica hederifolia*), VernPers (*Veronica persica*), VeronSpp (*Veronica* spp.), ViciaSpp (*Vicia* spp.), ViolArvn (*Viola arvensis*).

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