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Integrated effects of crop rotation and different herbicide rates in maize (*Zea mays* L.) production in central Serbia

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ABSTRACT

Successful maize (*Zea mays* L.) cultivation is largely reliable by weed interference. Among weeds, annual species are usually dominant, whereas less prevalent perennials can be challenging to control, too. Driven by profitability, maize is often cultivated continuously using the same management practices over time, resulting in increased weed infestations, particularly perennials. However, crop rotation might reduce the abundance of weed species, lower herbicide impact on the environment, delaying herbicide resistance occurrence in weeds and thus contribute to sustainable maize production,. The aim of this study was to explore the impact of continuous maize cropping (Maize-CC) and a three-crop rotation, maize–winter wheat–soybean (Maize-WW-S), in combination with three weed management treatments: 1) application of a pre-emergence herbicide mixture of acetochlor/S-metolachlor + isoxaflutole at the full label rate, 2) at ½ of full label rate, and 3) an the untreated control, over a 12-year period. The trial was initiated in 2009, and maize was grown in both cropping systems, Maize-CC and Maize-WW-S, in 2012, 2015, 2018, and in 2021. Total weed density, fresh biomass of all annual and perennial weed species and total dry biomass of all weed species was measured four weeks after herbicide application. Maize leaf area index (LAI) was measured at the anthesis, whereas grain yield was measured at the end of the growing cycle. Weed species diversity, number of individuals, weed fresh and dry biomass, were significantly lower with the combination of Maize-WW-S and the herbicide treatments. Grain yield was significantly and negatively correlated with the fresh weight of annual weeds in Maize-CC and was higher in both herbicide treatments, especially in Maize-WW-S. There was no significant difference between pre-emergence herbicide full labelled rate and ½ of the labelled rate in reducing the total fresh weed biomass in Maize-CC (66.3% and 65.9%, respectively) and Maize-WW-S (92.1% and 85.8%, respectively). Thus, the importance of the combined employment of rotation and chemical measures in maize production was confirmed and could be adopted for long-term weed management without compromising yields.

1. Introduction

Maize (*Zea mays* L.) is an important staple crop produced worldwide (FAO, 2023), while its grain yield is mainly dependent on meteorological conditions and applied cropping practices (Dragičević et al., 2015; Gobin et al., 2017; Maitah et al., 2021). In the context of cultivation, well-designed crop rotation involves multiple aspects of ecosystem services and crop management, including different weed management options (Simić et al., 2018; Bowles et al., 2020; Brankov et al., 2021).

In Central Europe, two-crop rotations of maize with winter cereals, or continuous maize cropping are the dominant cropping systems driven by market policies. In Serbia, maize is usually grown in a two-crop rotation with winter wheat (*Triticum aestivum* L., 60%) or soybean (*Glycine max* [L.] Merr., 15%), as well as continuous maize (15%), or in a three-crop rotation (maize–winter wheat–soybean, ~5%) (Videnović et al., 2013).

There are numerous environmental and economic benefits of introduction of legumes into crop rotations (Reckling et al., 2016a), despite that legumes are grown in Europe on less than 2 % of the arable land.

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Abbreviations

Maize-CC	maize continuous cropping
Maize-WW-S	maize-winter wheat-soybean rotation
FLR	full label rate
½; FLR	half of full label rate
Con	control
AI	number of annual weed individuals per species
PI	number of perennial weed individuals per species
FBMA	fresh biomass of annual weeds
FBMP	fresh biomass of perennial weeds
FBMT	fresh total weed biomass
DBMT	dry total weed biomass
LAI	maize leaf area index
GY	maize grain yield

This benefits result in reduction of N pollution by 18–33%, on average, (Reckling et al., 2016b), as well as for N savings. Furthermore, soybean is able to reduce fertiliser inputs by up to 50% (Videnović et al., 2013; Watson et al., 2017). This makes it as an ideal crop to precede maize cultivation. Kollas et al. (2015) indicated that crop rotations can increase crop diversification, and thus the resilience of agricultural systems as a strategy to mitigate climate change.

Maize productivity is closely related to weed interference (Oerke, 2005; Ferrero et al., 2017; Ramesh et al., 2017a; Simić et al., 2022), while yield losses can range 61–72 %, due to delayed weed control, dependently on meteorological conditions, too (Landau et al., 2021). The most commonly used method, for weed control in maize is herbicide application (Moss, 2019). As a rapid and inexpensive method it is widely adopted by farmers. However, intensive and improper use of herbicides can negatively affect agro-ecosystems and human health, and contribute to the development of resistant weed biotypes to herbicides (Owen, 2016). To address farm sustainability, a shift towards less herbicide-dependent cropping systems should be adopted (Tataridas et al., 2022).

Reducing the use of agrochemicals is a key objective of the EU agricultural policy. Thus, a strategy to halt biodiversity loss by 2030 was established, setting specific targets for the EU food system to reduce pesticide use by 50% (European Commission, 2020). On the other hand, using lower herbicide rates than recommended is controversial, due to the possibility of metabolic resistance evolution in weeds (Gressel, 2011). Furthermore, exposure of weeds to sub-lethal herbicide doses may lead directly to non-target site resistance (Vieira et al., 2019).

The Integrated Weed Management (IWM) system combines chemical and non-chemical measures to arrive at the best answer for weed control (Swanton et al., 2008). Those measures are part of any IWM system, which can provide workable weed management. Combining herbicides, as chemical weed control method, with crop rotation, as a cultural method, could be one of the solutions for sustainable weed management. Using the pre-emergence herbicides in a mixture and even in reduced rates, showed the promising results in weed control (Hassan et al., 2010). According to their findings, a half of the recommended dose of s-metolachlor integrated with a maize cultivar may be used to harvest economic yield of maize while keeping the environment intact. Kudsk (2014) found that lower rates could be used to maintain effective weed control only in combination with other non-chemical methods. Furthermore, Nazarko et al. (2005) reported similar approach, where applying of reduced rates can have a positive impact on weed control when it is used as a supplement to other weed control measures.

Rotation gives crops an advantage over weeds, disturbing their lifecycles and does not allow them to multiply or adapt to the agro-ecological conditions (Liebman and Staver, 2001). Also, rotations enable the application of herbicides with different modes of action (MOAs), avoiding or postponing herbicide resistance phenomena. In a crop rotation system, herbicide application rates, especially pre-emergence, can be reduced during rotation (Vasileiadis et al., 2011; Hunt et al., 2017). Brankov et al. (2021) reported that a maize–winter wheat rotation with reduced herbicide application rate could significantly reduce weed densities in maize. Zeller et al. (2021) also showed that in a rotation system of summer crops vs. winter cereal crops, applying herbicides with the same MOA once every five years decreased the density of black-grass (*Alopecurus myosuroides* Huds.) by 23–99%. At this point, it should be noted that the most common practice for weed control in maize fields in the Mediterranean and Balkan Peninsula countries is the application of acetolactate synthase (ALS) inhibitors, as is the case worldwide (Heap, 2014). Herbicides with this MOA have a broad spectrum of weed control, while controlling both grasses and broadleaves (Zhou et al., 2007). However, repeated use of ALS inhibitors over years has led to 102 individual cases of herbicide resistance in maize fields worldwide, 17 of which have been reported in Europe (Heap 2024). Therefore, pre-emergence herbicides offer farmers the opportunity to diversify their herbicide programs with alternative MOAs to prevent further spread of HR weeds in European maize cropping systems. This was the main reason for selecting the tank mixture of isoxaflutole + acetochlor/S-metolachlor for weed control in our study. To date, there are far fewer reports of resistance to pre-emergence herbicides in this crop in Europe (Heap, 2024).

Although crop rotations contribute to enhancing weed management, crop productivity, and increase the efficiency of weed control in maize cropping systems, there is lack of information on their integrated effects, causing to the poor farmer's adoption of this practice. We propose that long term experiments studying the combined effects of crop rotations and weed control treatments, as reported in this study, would be essential to help unravel the effects of these treatments on abundance and floristic composition of weeds and maize productivity in long-term. Thus, the objective of this study was to determine the effect of crop rotation, particularly in combination with a ½ and full label rate, influencing variability in weed infestations and maize productivity, as effectiveness of both pre-emergence herbicide rates was previously reported by Brankov et al. (2021). Despite the IWM endorses applying herbicides at the recommended rates, in this study we tested the influence of reduced rates in order to signify the influence of crop rotation as cultural method for weed control. Therefore, we hypothesised that the integrated application of a three-crop rotation, such as maize–winter wheat–soybean, with different pre-emergence herbicide rates could (1) significantly decrease weed infestations in maize, and (2) improve crop productivity in comparison to continuous maize cultivation. We expect that achieved results, as a part of IWM will contribute to its greater adoption by farmers.

2. Materials and methods

2.1. Experimental site and design

The trial was initiated as a split-plot experiment in 2009 in an experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia (44°52′08″ N 20°20′04" E, 81 m above sea level) to examine the integrated effects of crop rotation and pre-emergence herbicides. In fixed experiment, two cropping systems were investigated: continuous maize cropping (Maize-CC) and a three-crop rotation of maize–winter wheat–soybean (Maize-WW-S). Weed control measures are presented in Table 1.

The soil type at the experimental site was a slightly calcareous chernozem-molcal silt loam containing 32% clay, 15% silt, and 53% sand. The soil analysis (Laboratory for agro-chemistry at the Maize Research Institute) are shown in Table 2.

In the twelve-year experiment, maize was grown in both cropping systems (one cycle of Maize-CC and Maize-WW-S) in 2009, 2012, 2015, 2018, and 2021 (2009 was excluded from the analysis as it was the

Table 1
Summary of the weed control measures.

Active ingredients	Product name	Producer	FRL	½ FLR	Control
isoxaflutole	Merlin 750-WG	Bayer Crop Science, Germany	105 g a.i. ha^{-1}	52.5 g a.i. ha^{-1}	–
acetochlor/S-metolachlor	Trophy 786/Dual Gold 960	Nufarm/Syngenta, Switzerland	786/1344 g a.i. ha^{-1}	393 672 g a.i. ha^{-1}	

½ FLR – half of full label rate; FLR – full label rate.

Table 2
Soil characterisation on the experimental field in Zemun Polje (at the beginning of the experiment, (2009)).

Parameter	Soil layer [m]	Estimated values	Method
Organic C [%]	0–0.3	1.9	Walkley and Black (1934)
Total N [%]	0–0.3	0.21	EPA method 351.2 (1993)
pH in H_2O	0–0.3	7.8	
P mg 100 g^{-1} $soil^{-1}$]	0–0.3	14	Watanabe and Olsen (1965)
K mg 100 g^{-1} $soil^{-1}$]	0–0.3	31	Carson (1980)
Total $CaCO_3$ [%]	0–0.3	9.7	Horváth et al. (2005)

initial year when the cropping systems under study had just been established). The split-plot treatments were fixed in the same plots throughout the study (Fig. S1). Measurements were analysed only for maize, whereas winter wheat and soybeans were used as factors influencing maize production in rotation and were not the focus crops.

In the Maize-CC plots, deep ploughing (0.3 m soil depth) was performed after maize harvest, while shallow tillage (0.15 m soil depth) was performed in the spring before sowing. In the Maize-WW-S plot, shallow ploughing (0.1 m soil depth) was performed about 30 days after the wheat harvest, and deep ploughing (in the second half of October) and shallow tillage were performed before maize and soybean sowing in spring. Disc harrowing was performed before sowing the winter wheat.

In autumn, at the same time as deep ploughing, 150 kg ha^{-1} of mono-ammonium phosphate 12:52 (MAP, Granaria Net, Serbia; 18 kg N ha^{-1} and 78 kg P_2O_5 ha^{-1}) were applied. Based on the N-min method (Wehrmann and Scharpf, 1979), additional N (128.9 kg N ha^{-1} in 2012, 100.2 kg N ha^{-1} in 2015, 106.1 kg N ha^{-1} in 2018 and 119.9 kg N ha^{-1} in 2021) in the form of ammonium-nitrate was applied in spring before sowing.

The important dates for cultivation practices and sampling during the maize-growing period in both Maize-CC and Maize-WW-S are presented in Table 3. The Stay Green hybrid ZP 606 was sown for all cycles with a pneumatic drill machine (Majevica, Serbia) in all plots at a density of 62.100 plants ha^{-1} at the optimal time (Table 3).

As a second factor, three weed management treatments were included in maize, representing subplots (Table 1). Until 2013,

Table 3
Timing of maize planting, leaf area measurements, and harvest.

Operation/measurement	Dates			
	2012	2015	2018	2021
Planting and herbicide application	April 27	April 14	April 26	April 29
LAI measurement	July 26	July 29	July 25	July 27
Harvest	September 12	October 7	October 15	October 20

LAI – Leaf area index.

acetochlor was used, and after its ban, S-metolachlor was introduced. The final regulatory action of the EU Commission bans the use of acetochlor as a pesticide. S-metolachlor has the same MOA as acetochlor has; therefore we expected low/no influence on the results. In winter wheat and soybeans, weeds were controlled with herbicides recommended for application. In maize, herbicides were applied immediately after sowing with a CO_2 backpack sprayer with a four-nozzle boom using extended range nozzles (XR11002-SS, TeeJet Spraying Systems, Wheaton, IL, USA) calibrated to deliver a spray volume of 140 L ha^{-1} of solution at 275.8 kPa. Each plot consisted of eight rows of maize, 5 m long with a distance of 0.70 m between rows, for a total of 28 m^2. Each treatment was replicated four times.

2.2. Meteorological conditions

The monthly meteorological data (total precipitation and average temperatures) for the maize-growing period (April–September) are presented in Table 4.

The average air temperature at Zemun Polje varied slightly during the survey years, whereas the total precipitation was significantly higher in 2021 (697.7 mm), 2015 (587.7 mm), and 2018 (545.9 mm) than in 2012 (396.3 mm). In 2018, rainfall totalled 295.0 mm from May to August, but in 2015 it was only 192.1 mm. Adequate rainfall is particularly important in June–July, coinciding with intensive maize stem development and tasselling, favouring 2018 and 2021 as the optimal years (Table 4). However, in 2021, low rainfall and heat waves occurred during grain filling in August and September, negatively affecting crop production.

2.3. Data collection

Total weed density (No m^{-2}) and fresh biomass of all annual (FBMA, g m^{-2}) and perennial weed species (FBMP, g m^{-2}) and of each species and total fresh and dry biomass of all weed species (FBMT and DBMT, g m^{-2}) were measured at four weeks after herbicide application. The number of annual, perennial and total weed species was determined over four replicates and herbicide treatments each year. Weeds were identified after the manual uprooting of two randomly selected points from the centre within each plot using a 0.25 m^2 square. For dry biomass assessment, weed samples were dried at 60 °C in a ventilation dryer (UN 30, Memert, Germany) to a constant weight.

At the tassel stage, maize leaf area was measured (leaves of five plants per plot) using an LI-COR 3100 area metre (LICOR Biosciences, Lincoln, NE, USA). The leaf area index (LAI) was calculated as follows (Equation (1)):

$$LAI = \frac{Area\ of\ leaf\ coverage\ per\ plant}{Area\ of\ soil\ covered\ per\ plant} \tag{1}$$

At harvest, maize grain yield (GY, t ha^{-1}) was measured from the two central rows in each plot and calculated at 14.0% moisture content.

2.4. Data analyses

The data obtained were processed using STATISTICA 8.0 for Windows (TIBCO software Inc., Palo Alto, CA 94304). Differences between treatments were determined using a three-factorial (year, crop rotation, and herbicide rate) analysis of variance (ANOVA), after assumptions for normality and homogeneity of sample variances were established using the Kolmogorov–Smirnov normality test (Drezner et al., 2010). Means of four replicates were separated using Fisher protected least significant difference test (LSD; Fisher, 1936) when the F-Test showed significant treatment effects ($p = 0.01$).

The correlation between weed biomass and maize GY was presented as a linear regression, while interdependence among weeds, GY and LAI was analysed using Principal Component Analysis (PCA) as a dimensionality-reduction method. Statistical analyses were performed

Table 4
Monthly meteorological conditions at Zemun Polje during the investigation period.

Months Years	IV	V	VI	VII	VIII	IX	X	Average/Sum
Average monthly air temperatures (°C)								
2012	14.4	17.9	24.6	27.1	26.2	22.1	15.4	21.1
2015	12.9	19.1	22.1	26.4	25.7	20.2	12.4	19.8
2018	18.0	21.7	22.7	23.6	25.7	19.8	15.9	21.1
2021	10.7	17.9	23.8	26.7	24.3	21.9	12.6	19.7
Multiyear average	13.8	18.3	22.0	23.5	23.5	18.6	13.6	19.05
Total monthly precipitation sum (mm)								
2012	56.2	58.5	14.8	19.8	4.8	20.7	41.3	216.1
2015	19.7	97.8	31.1	7.2	56.0	73.6	65.1	350.5
2018	24.6	39.0	150.1	61.9	44.0	16.9	20.8	357.3
2021	45.9	73.0	19.5	105.5	38.0	16.5	68.8	367.2
Multiyear average	44.9	61.1	81.1	55.7	48.8	50.0	51.9	388.0

using the SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA). The probability of the distribution of total fresh and dry biomass of weeds in cropping systems was tested using Weibull distribution (MS Excel). This distribution could provide information on reliability of weed abundance and maize productivity based on huge range of data.

3. Results

3.1. Weed species abundance

The most abundant weed species in the maize fields were the annual broadleaf jimsonweed (*Datura stramonium* L.), common lambsquarters (*Chenopodium album* L.), redroot and smooth pigweed (*Amaranthus retroflexus* L. and *A. hybridus* L.), maple-leaved goosefoot (*Chenopodium hybridum* L.) and black nightshade (*Solanum nigrum* L.) (Table 5). Johnsongrass (*Sorghum halepense* [L.] Pers.), Canada thistle (*Cirsium arvense* [L.] Scop.), field-bindweed (*Convolvulus arvensis* L.) and Bermuda grass (*Cynodon dactylon* [L.] Pers.) were the only perennials.

The number of annual weed species declined over time in both cropping systems, Maize-CC and Maize-WW-S, under both herbicide treatments, with the lowest densities in 2018 and 2021. The lowest number of annuals was observed in the three-crop rotation system, Maize-WW-S, and FLR (full label rate), with an average of only two species (Table 5, Fig. S2).

The application of the FLR of the herbicide mixture reduced the number of annual weed species from 6.0 to 2.7 per square meter in Maize-CC. The number of perennial species mostly remained stable, although it decreased in the Maize-WW-S plots with herbicide application at both rates, which was not the case in Maize-CC. Thus, the total number of weed species decreased after pre-emergence herbicide application at both rates, particularly in Maize-WW-S, and was the same for ½FLR and FLR (3.2 species) on average.

Table 5
Total number of weed species over the years and treatments in two maize cropping systems, Maize-CC and Maize-WW-S.

	Maize-CC			Maize-WW-S		
	Control	½FLR	FLR	Control	½FLR	FLR
Annual weed species						
2012	11	10	5	10	4	3
2015	9	5	2	10	1	2
2018	8	3	1	9	4	2
2021	6	6	3	11	0	1
Average	7.75 ± 2.08	6.00 ± 2.94	2.75 ± 1.71	10.00 ± 0.82	2.25 ± 2.06	2.00 ± 0.82
Perennial weed species						
2012	3	3	3	2	2	2
2015	3	3	3	2	0	1
2018	4	3	3	3	2	2
2021	3	3	3	2	0	0
Average	3.25 ± 0.50	3.00 ± 0.00	3 ± 0.00	2.25 ± 0.50	1.00 ± 1.15	1.25 ± 0.96
All weed species						
2012	14	13	8	12	6	5
2015	12	8	5	12	1	3
2018	12	6	4	12	6	4
2021	9	9	6	13	0	1
Average	11.75 ± 2.06	9.50 ± 2.94	5.75 ± 1.71	12.25 ± 0.50	3.25 ± 3.20	3.25 ± 1.71

Average values are presented as mean ± SD (standard deviation); ½FLR – half of full label rate; FLR – full label rate; Maize-CC – maize continuous cropping; Maize-WW-S – maize–winter wheat–soybean rotation.

3.2. Weed density and biomass

Weed density i.e. the number of annual and perennial weed individuals per square meter, as well as their fresh and dry biomasses, decreased significantly with crop rotation and herbicide application (Fig. 1, Table 6, Table 7). Irrespectively that meteorological conditions of the year did not show significant influence on variations of annual and perennial weed density (Table 7), greater fluctuations in weed fresh biomass of annual and perennial weeds were observed in Maize-CC compared to the Maize-WW-S rotation and control treatments than FLR and ½ FLR (Fig. 1). Under the interaction of the cropping system and herbicide rate, the number of annual individuals decreased from 122.2 to 5.2 m^{-2} in Maize-CC and from 61.7 to 6.5 m^{-2} in Maize-WW-S after the FLR treatment. FBMA fluctuated greatly over time with the greatest value in 2015 in Maize-CC (813.1 g m^{-2}) and 2021 in Maize-WW-S (974.9 g m^{-2}) in the untreated control. At the same time, the density of perennial weeds remained stable over time and was not efficiently suppressed, decreasing from 33.7 to 17.2 m^{-2} in Maize-CC and from 14.0 to 3.5 m^{-2} in Maize-WW-S. The number of perennial individuals was relatively low, although they produced a significant quantity of fresh biomass, especially in Maize-CC, which had the highest value in 2018, particularly in the Maize-CC control.

The Weibull distribution, as reliability analyses of greater datasets offers the possibility to compare of various treatments and also to predict trend of different treatments, particularly in long-term. Fig. 2 revealed an increase in both FBMT and DBMT in Maize-CC and Maize-WW-S. However, greater FBMT than DBMT values were present in Maize-CC (particularly at the point of 70% reliability, i.e., 673 g m^{-2} of FBMT and 108 g m^{-2} of DBMT), with a gradual increase in DBMT toward a point of 90% reliability. In contrast, the increase in FBMT and DBMT in Maize-WW-S remained less steep, with the greatest difference achieved at 75% reliability (426 g m^{-2} of FBMT and 137 g m^{-2} of DBMT).

Crop rotation and herbicide rate, as two interacting cropping practices, expressed the significant influence on fresh biomass of annual and perennial weed species (Tables 6 and 7). On average, for all years, the FBMA was higher in Maize-WW-S (1199.5 g m^{-2}) than in Maize-CC (974.4 g m^{-2}) in the control plot (Table 6). After herbicide application, FBMA decreased at ½ FLR by 65.6% (335.3 g m^{-2}) and 74.5% (306.2 g m^{-2}) in Maize-CC and Maize-WW-S, respectively, indicating

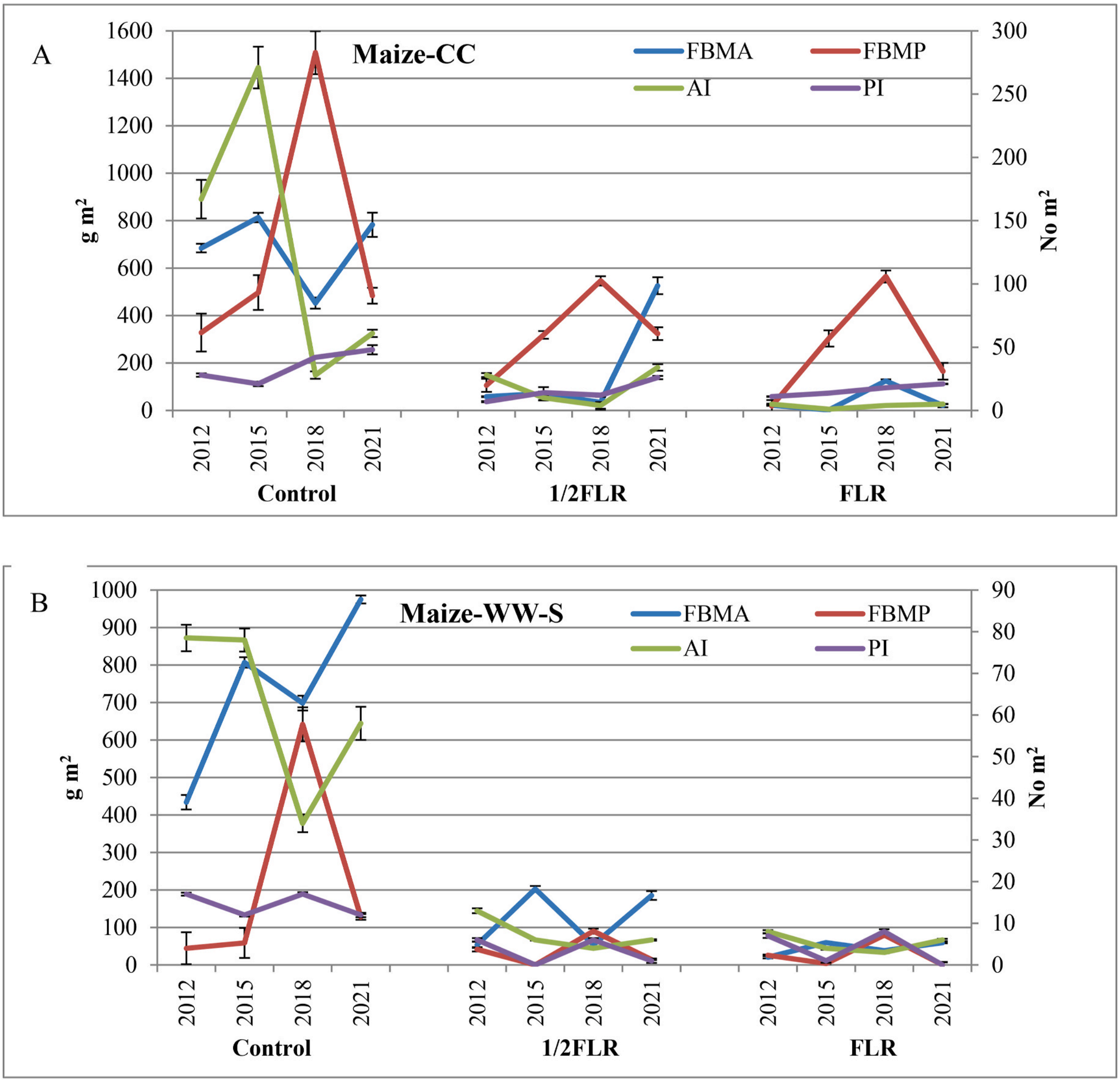

Fig. 1. Dynamics of fresh biomass and number of annual weed species individuals (FBMA and AI), and fresh biomass and number of perennial weed species individuals (FBMP and PI) in maize continuous (Maize-CC) and rotation (Maize-WW-S) (Mean ± SD).

that Maize-WW-S was more effective in controlling annual weeds by 8.9%. Herbicide application, either in the FLR or ½ FLR, reduced the fresh biomass of perennial weeds, particularly in Maize-WW-S. Upon application of the FLR of the herbicide mixture, FBMA was similarly reduced in both cropping systems by 90.0% in Maize-CC (97.3 g m^{-2}) and by 85.4% in Maize-WW-S (175.5 g m^{-2}). The most present and abundant were *Ch. album* and *Ch. hybridum*, which were not totally controlled, particularly in the Maize-CC cropping system. *Anagalis arvensis* L. was not present in the untreated control but developed some biomass in ½ FLR (8.0 g m^{-2}) and FLR (1.2 and 1.4 g m^{-2}). Interestingly, *Reseda lutea* L. was present only in the Maize-WW-S cropping system, while *Lamium purpureum* L. was rarely present in the maize crops in certain years.

Perennial weeds were more difficult to control, and their number was stable in Maize-CC (four species) and slightly reduced in Maize-WW-S to three and two species, respectively (Table 6). The results showed that FBMP in the control plots was significantly higher in Maize-CC (823.2 g m^{-2}) than in Maize-WW-S (175.7 g m^{-2}). In the treated plots, in Maize-CC, there was no difference in FBMP between ½ FLR (358.3 g m^{-2}) and FLR (336.9 g m^{-2}), while in the Maize-WW-S rotation, FBMP in FLR (80.0 g m^{-2}) was half that in ½ FLR (164.3 g m^{-2}).

Comparing the biomass of annuals and perennials, FBMA in the Maize-CC control (974.4 g m^{-2}) was to some extent higher than FBMP (823.2 g m^{-2}), while in the ½ FLR treatment, the difference was insignificant, at 335.3 and 358.3 g m^{-2} (Table 6). Herbicide application at the FLR reduced the biomass of both categories to 97.3 and 336.9 g m^{-2} for FBMA and FBMP, respectively. In contrast, in Maize-WW-S, FBMA was much higher than FBMP in the control (1199.5 g m^{-2}) and herbicide-treated plots (306.2 vs. 164.3 g m^{-2} and 175.5 vs. 80.0 g m^{-2}). This means that FBMT decreased by 61.4% for ½ FLR and 75.8% for FLR in Maize-CC, and 65.8% for ½ FLR and 81.4% for FLR in Maize-WW-S, on average, with herbicide application. This further implied that the reduction in FBMT was 4.3% (½FLR) and 5.6% (FLR) higher in Maize-WW-S than in Maize-CC.

FBMT and DBMT were the highest in dry 2012 (1393.7 and 379.9 g m^{-2}, respectively) and the lowest in 2015 (523.9 and 108.8 g m^{-2}) and 2021 (613.0 and 109.0 g m^{-2}). Differences in FBMT and DBMT were not significant between cropping systems, although weed biomass was lower in Maize-WW-S, 700.1 and 157.4 g m^{-2}, respectively (Table 7). The herbicide rate caused greater differences in weed biomass, and FBMT and DBMT were significantly higher in the control than in the treatments, whereas the differences between ½ FLR and FLR were not

Table 6

Average weed fresh biomass (g m^{-2}) in maize continuous cropping (Maize-CC) and maize–winter wheat–soybean rotation (Maize-WW-S) for all exanimated years (2012-2015-2018-2021).

	Maize-CC			Maize-WW-S		
	Control	½FLR	FLR	Control	½FLR	FLR
Annual weed species [fresh biomass g m^{-2}]						
Datura stramonium L.	207.18 ± 26.26	48.03 ± 8.13		155.90 ± 16.61	8.15 ± 0.57	
Chenopodium album L.	133.60 ± 17.71	1.95 ± 0.07	3.98 ± 0.32	202.88 ± 25.79	45.08 ± 6.02	
Amaranthus retroflexus L.	125.50 ± 19.45	13.93 ± 0.75		35.38 ± 0.53		
Chenopodium hybridum L.	122.00 ± 24.05	114.28 ± 18.11	35.10 ± 2.56	187.50 ± 23.25		
Solanum nigrum L.	121.33 ± 1.95			101.93 ± 7.36		
Bilderdykia convolvulus (L.) Dumort.	115.83 ± 14.83	87.45 ± 0.69	56.28 ± 5.26	310.25 ± 35.49	236.45 ± 24.01	168.55 ± 5.42
Amaranthus hybridus L.	75.65 ± 11.58	25.90 ± 1.87		22.80 ± 5.29		
Abutilon theophrasti L.	33.48 ± 9.01	4.58 ± 1.09	0.35 ± 0.02	7.83 ± 1.13	1.29 ± 0.09	
Amaranthus albus L.	28.05 ± 3.99	16.75 ± 1.30		53.52 ± 7.55	3.87 ± 0.54	
Hibiscus trionum L.	4.63 ± 0.35	14.4 ± 0.63	0.45 ± 0.17	3.93 ± 0.17		
Stachys annua L.	4.7 ± 0.97			79.03 ± 7.11		2.33 ± 0.48
Setaria viridis [L.] P.Beauv.	1.93 ± 0.17			2.95 ± 0.94		
Ambrosia artermisiifolia L.	0.30 ± 0.03					
Heliotropium europaeum L.	0.23 ± 0.02			0.28 ± 0.02		
Anagalis arvensis L.		8.05 ± 1.24	1.18 ± 0.08			1.45 ± 0.04
Reseda lutea L.				35.23 ± 0.84	11.38 ± 0.81	
Lamium purpureum L.						3.17 ± 0.77
FBMA	974.41	335.32	97.34	1199.51	306.22	175.50
	100%	34.41%	9.99%	100%	25.53%	14.63%
Perennial weed species [fresh biomass g m^{-2}]						
Sorghum halepense [L.] Pers.	621.08 ± 25.86	212.01 ± 15.68	162.40 ± 2.17	153.95 ± 2.12	159.97 ± 4.52	76.08 ± 7.1
Cirsium arvense [L.] Scop.	79.37 ± 14.16	39.41 ± 5.28	24.45 ± 4.30			0.93 ± 0.05
Convolvulus arvensis (L.)	70.02 ± 4.06	54.11 ± 2.96	64.90 ± 6.10	15.60 ± 3.07	4.34 ± 0.48	3.03 ± 0.36
Cynodon dactylon [L.] Pers.	52.72 ± 9.35	52.76 ± 6.99	85.16 ± 2.11	5.65 ± 0.54		
FBMP	823.19	358.29	336.91	174.70	164.31	80.04
	100%	43.52%	40.92%	100%	94.05%	45.81%
All weed species						
FBMT	1797.60	693.71	434.25	1374.21	470.53	255.54
	100%	38.59%	24.16%	100%	34.24%	18.59%

Values are presented as the mean ± SD (standard deviation); FBMA – fresh biomass of annual weeds; FBMP – fresh biomass of perennial weeds; FBMT – fresh biomass of total weeds; ½FLR – half of full label rate; FLR – full label rate; Maize-CC – maize continuous cropping; Maize-WW-S – maize-winter wheat-soybean rotation.

significant.

3.3. Weed interference with maize productivity

Analysing the effects of each factor separately, the results showed that weed and maize productivity parameters were significantly dependent on seasonal variations, i.e., meteorological conditions of the year and herbicide application, whereas crop rotation by itself only influenced maize grain yield significantly (Table 7). Similarly, the LAI and GY were significantly ($P < 0.01$) affected by the meteorological conditions of the year and herbicide application. The highest LAI was achieved in 2021 (3.37) compared to other years and in the treated plots (2.99 (23.4%) and 3.22 (28.9%)) compared to the control (2.29). LAI was not significantly affected by crop rotation and was lower in the Maize-CC treatment (2.69) than in the Maize-WW-S treatment (2.98 (9.7%)). The GY was higher in 2018 (7.77 t ha^{-1}) and 2015 (6.12 t ha^{-1}), and in the treatments (5.74 t ha^{-1} (17.6%) and 6.12 t ha^{-1} (28.6%) in ½ FLR and FLR, respectively) than in the control (4.73 t ha^{-1}). Crop rotation significantly increased the GY by 2.04 t ha^{-1} (31.14%). Herbicide rates did not cause significant differences in LAI or GY.

The weed density, as well as the maize parameters LAI and GY, were significantly influenced by all interactions (Y × CR, Y × HR, CR × HR, and Y × CR × HR), which underlines the joined/combined effects of the meteorological conditions of the year, crop rotation, and herbicide rate on weed control and maize productivity (Table 7). The highest variation was observed under the influence of the herbicide rate for weed parameters, whereas for LAI, the highest variability was under the influence of year, and for GY under the influence of crop rotation. The interaction Y × CR × HR significantly affected weed biomass and maize production parameters (Table 7).

More detailed analysis showed that interaction of crop rotation and herbicide rate caused the variations in weed parameters which were followed with differences in maize GY (Fig. 3). It is clear that greater weed biomass and lower maize yield were present in the Maize-CC. In both systems, a similar trend was observed under the influence of both herbicide rates; the decrease in GY was followed by a significant increase in the biomass of annual weeds in Maize-CC ($R^2 = 0.655$). Nevertheless, a significant positive correlation between GY and FBMP was observed in both cropping systems, and was greater in Maize-CC ($R^2 = 0.735$) than in Maize-WW-S ($R^2 = 0.509$).

According to the PC analysis, the 1st axis contributed 85.83% of the total variability, and the number of annual individuals, FBMT, DBMT, and FBMA were significantly and positively correlated with it, while LAI was negatively correlated. The 2nd axis contributed 12.5% to the total variability, and the number of perennial individuals and FBMP were significantly and positively correlated with it, while GY was negatively correlated. Fig. 4 shows the high variability of GY and LAI in crop rotation (Maize-WW-S), especially at FLR and ½ FLR. Nevertheless, the highest variations of the number of annual and perennial individuals, FBMT, DBMT, FBMA, and FBMP, were in Maize-CC and the control. Small variations in the number of perennial individuals, LAI and FBMP were observed in Maize-CC at both herbicide rates (FLR and ½ FLR). Additionally, small variations in FBMA were observed in Maize-WW-S-Con.

Table 7
Analyses of variance for the effects of year, cropping system, and herbicide rate and their interaction with each other the number of annul weeds, number of perennial weeds, fresh biomass of annul weeds, fresh biomass of perennial weeds; fresh biomass of total weeds, dry biomass of total weeds, leaf area index, and grain yield.

	AI No m^2	PI No m^2	FBMA g m^{-2}	FBMP g m^{-2}	FBMT g m^{-2}	DBMT g m^{-2}	LAI m^2 m^{-2}	GY t ha^{-1}
Year								
2012	42.17ns	14.33ns	981.5a	412.2ns	1393.7a	379.9a	2.76b	4.61b
2015	61.67ns	10.67ns	327.3b	196.6ns	523.9b	108.8b	2.55b	6.12 ab
2018	16.17ns	15.17ns	323.3b	496.6ns	819.9 ab	175.5 ab	2.66b	7.55a
2021	30.17ns	18.17ns	426.7b	186.2ns	613.0b	109.0b	3.37a	3.83b
CV%	*2.47*	*1.45*	*3.21*	*2.93*	*6.92*	*3.23*	*1.41*	*0.28*
Cropping system								
Maize-CC	49.83ns	22.08a	469.0ns	506.2a	975.2ns	229.2ns	2.69ns	4.51b
Maize-WW-S	25.25ns	7.05b	560.4ns	139.7b	700.1ns	157.4ns	2.98ns	6.55a
CV%	*0.52*	*0.68*	*1.68*	*1.95*	*7.30*	*3.94*	*0.45*	*0.39*
Herbicide rate								
Control	92.00a	23.88a	1086.9a	499.0a	1585.9b	371.7b	2.29b	4.73b
1/2FLR	14.75b	9.50b	320.8b	261.3 ab	582.1a	127.2a	2.99a	5.74a
FLR	5.88b	10.38b	136.4b	208.5b	344.9a	81.1a	3.22a	6.12a
CV%	*1.22*	*0.98*	*5.44*	*4.95*	*9.42*	*2.31*	*0.53*	*0.36*
ANOVA, Probability (F)								
Year	2.37[a]	1.07[a]	8.00[b]	2.93[a]	6.39[b]	10.02[b]	7.02[b]	20.59[b]
Crop rotation	3.80[a]	33.81[b]	0.55	18.05[b]	2.76ns	2.48ns	3.64ns	24.32[b]
Herbicide rate	29.07[b]	11.89[b]	41.44[b]	3.85[a]	35.88[b]	22.35[b]	19.0[b]	3.40[a]
Year × Crop rotation	2.30[a]	8.14[b]	3.54[b]	5.91[b]	3.44[b]	4.84[b]	6.46[b]	21.0[b]
Year × Herbicide rate	9.55[b]	1.58[a]	17.10[b]	1.76[a]	9.84[b]	9.90[b]	9.59[b]	7.20[b]
Crop rotation × Herbicide r.	46.99	10.84	444.5[a]	398.4[a]	15.85[b]	10.80[b]	10.01[b]	7.40[b]
Year × Crop rot. × Herbicide r.	34.7[b]	13,26[b]	373.1[b]	439.8[b]	5.12[b]	5.65[b]	9.39[b]	9.14[b]

LSD test.
[a] Significant at 0.05 level.
[b] Significant at 0.01 level; ns – non-significant; the values signed with the same letter are not significantly different at the 0.05 level of significance; AI – number of annul weeds individuals; PI– number of perennial weeds individuals; FBMA – fresh biomass of annul weeds; FBMP– fresh biomass of perennial weeds; FBMT – fresh biomass of total weeds; DBMT – dry biomass of total weeds; 1/2FLR – half of full label rate; FLR – full label rate; Maize-CC – maize continuous cropping; Maize-WW-S – maize–winter wheat–soybean rotation; LAI – leaf area index; GY – grain yield; CV%- coefficient of variation; LSD – least significant difference.

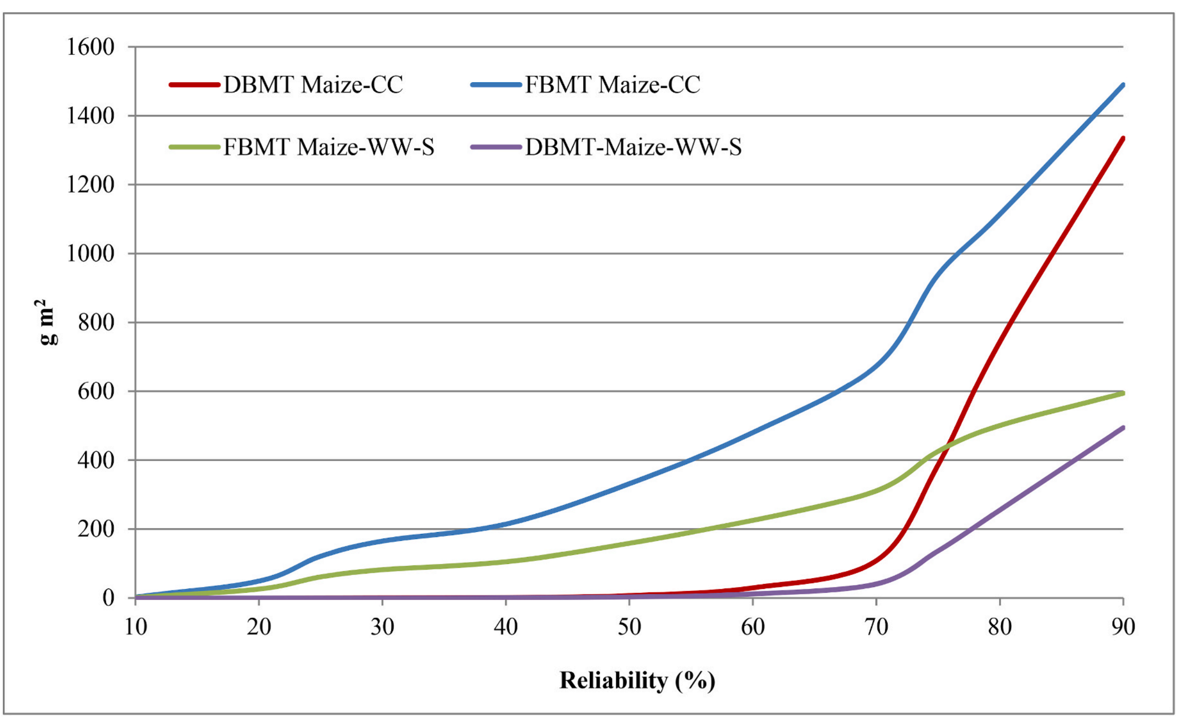

Fig. 2. Probability and trend of total fresh biomass distribution (FBMT) and total dry biomass distribution (DBMT) of weeds in Maize-CC and Maize-WW-S (using Weibull distribution).

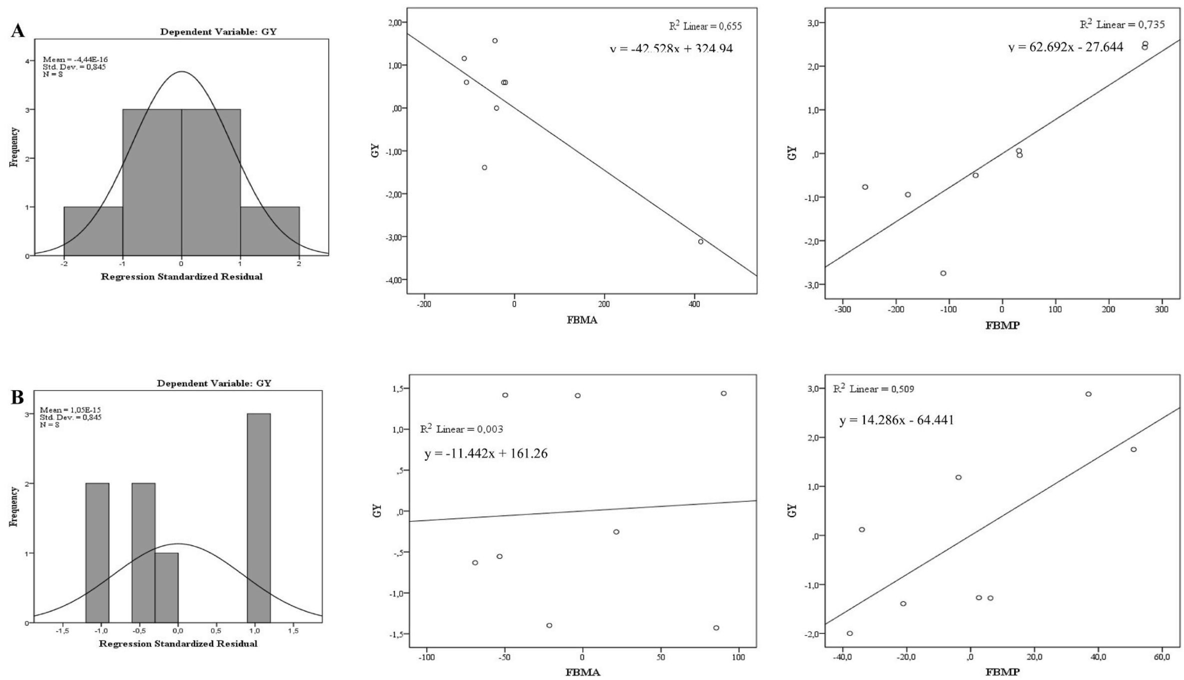

Fig. 3. Interdependence of maize yield (GY) and fresh biomass of annual weed species (FBMA) and perennial weed species (FBMP) in FLR and 1/2FLR in maize continuous cropping (A) and maize rotation (B). Regression analyses; $p = 0.05$.

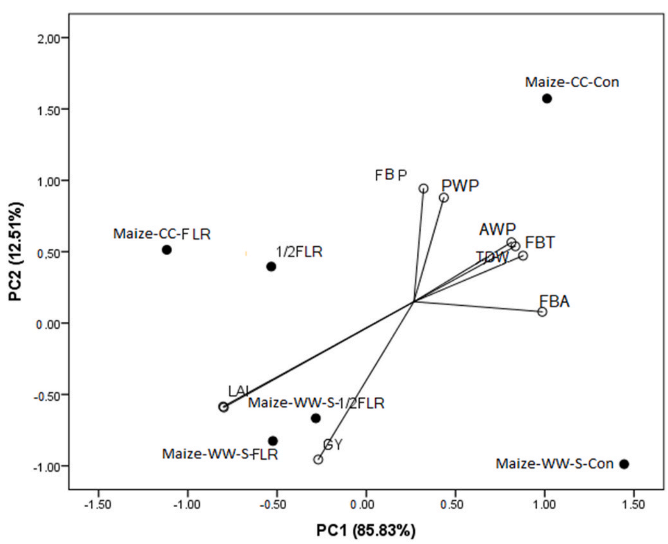

Fig. 4. Principal component analysis of the number of weed individuals per species (annual – AI, perennial – PI), fresh weed biomass (annual – FBMA, perennial – FBMP, total – FBMT), total dry biomass of weeds (DBMT), maize leaf area index (LAI) and maize grain yield (GY) in maize continuous cropping (Maize-CC) and maize–winter wheat–soybean rotation (Maize-WW-S) with herbicide application in the full labelled rate (FLR), half of the full labelled rate (1/2FLR) and untreated control (Con).

4. Discussion

4.1. Weed species abundance and density

The most abundant annual weed species (*D. stramonium*, *Ch. album*, *A. retroflexus*, *A. hybridus*, *Ch. hybridum* and *S. nigrum*), as well as perennial weeds species (*S. halepense*, *C. arvense* and *C. arvensis*), represent the core of the weed community in summer arable crops throughout Central Serbia in long-term (Stefanović et al., 2011) and were also dominant in both maize cropping systems, either Maize-CC or Maize-WW-S. Also, some perennial species with greater adaptability to arable areas and warm climate, such as *S. halepense*, *C. dactylon* and *C. arvense* were present. They have the potential to cause significant losses in yield and grain quality in maize (Simić et al., 2021). However, well-adapted spring annual species in maize have enormous spreading potential, due to the production of a large number of seeds (Nguyen and Liebman, 2022).

It is well known that variations in weed biodiversity are mainly dependent on environmental conditions and applied cultural practices (Fried et al., 2008; Peters et al., 2014). Over the last 60 years, the most popular measure for weed control in maize has been herbicide application, which can result in the development of herbicide resistance, and some issues such as herbicide drift, environmental pollution, and health issues (Moss, 2019). Crop diversification in rotation has the potential to sustainably control weeds (Sharma et al., 2021). With rotation, the complete crop growing technology is changing, including soil tillage and herbicides with different MOAs: in soybean, herbicides target grass weeds, whereas in wheat, as a dense crop, herbicides target broad-leaf weeds. This practice disturbs weed composition and the participation of annual and perennial species. In this study in Maize-CC, the number of annual weed species decreased over time, particularly in the FLR treatment, whereas the number of perennial species remained constant (four species), which was supported by their biology and the MOA of pre-emergence herbicides targeting annual weeds. The dominant annual species are well adapted to the maize growth cycle and crop arrangement pattern (Oljača et al., 2007; Nguyen and Liebman, 2022), usually producing greater biomass than perennials. However, the existence and survival of perennial weeds are facilitated by spreading through seed dispersal and especially by vegetative sprouting (e.g. roots and rhizomes). Thus, in Maize-CC, perennials are usually favoured and efficiently compete with annuals (Simić et al., 2016; Butkevičiene et al., 2021), so FBMA showed a significantly increasing trend (1199.5 g m^{-2}) in the Maize-WW-S compared to the Maize-CC (974.4 g m^{-2}), while for perennial weeds, the trend was completely opposite (174.7 and 823.2 g m^{-2}, respectively). Due to the diversification of crop species in rotation, perennials could be successfully managed in more sustainable way than being controlled with herbicides alone (Butkevičiene et al., 2021).

Crop rotation reduces weed abundance, density, and biomass (Brankov et al., 2021), disturbs their niche and adaptability to certain agro-ecosystem services, breaks their lifecycles and prevents the establishment of crop patterns (Satorre et al., 2020). What is more, the combined application of Maize-WW-S and herbicides, even at ½ FLR, significantly reduced the biomass of weeds (Simić et al., 2016; Shahzad et al., 2021). Thus, perennial weed species abundance decreased significantly in Maize-WW-S over time, while decrease was insignificant after herbicide application at both rates. Irrespective that elementary plots were relatively small, experiment was fixed and allowed to follow changes in weed flora, indicating that crop rotation is one of the most important part of cropping technology, with the optional use of herbicides to reduce perennial weed species abundance.

4.2. Association between weeds and maize productivity

Maize and weeds share the same environment and use the same resources, competing with each other, the outcome of which differs from year to year. Ferrero et al. (2017) indicated that maize is less competitive in cold years, allowing higher weed infestations and, consequently, reduced crop yields. Although crops are designed to achieve high yields, their resilience to environmental fluctuations is limited, while weeds are highly adaptive and have greater production potential (Adeux et al., 2019; Bourgeois et al., 2019; MacLaren et al., 2020; Simić et al., 2020a). It is well known that maize productivity is mainly driven by meteorological factors. Thus, in 2015, as a relatively cold season with more precipitation than usual in May (97.8 mm), greater LAI and higher GY were achieved, while the lowest yield values were achieved in 2012 and 2021, as seasons with drought presence in June (sum of precipitation $<$20 mm and average air temperature $>$23 °C). It is well known that drought occurrence can thereby affect maize growth and yield potential (Kim and Lee, 2023). Nevertheless, the majority of the weed species present are adapted to higher temperatures (Ramesh et al., 2017b) while the potential to develop significant biomass was reduced. In colder seasons, this could be a consequence of the synergistic effect of the greater coverage and competition achieved by maize and lower temperatures.

Although weed species produce greater biomass under a sufficient water supply, efficacy of pre-emergence (PRE) herbicides is greater under higher soil moisture (Rastgordani et al., 2023). Therefore, weed control is greatly influenced by the complex interactions between soil properties, particularly soil moisture (Sebastian et al., 2017). Meteorological variations also influence efficacy of PRE herbicides and thus, further competition between weeds and crops in terms of their productivity potential (Ramesh et al., 2017a; Simić et al., 2020b). Proper water and nutrient management are important tools for crop-weed interference as part of the IWM (Kaur et al., 2018), so adaptive specialisation in the community context is driven by strategic trade-offs (Agrawal, 2020). From this standpoint, rotation reduces weed adaptability and survival over time, in the community context, giving space to maize to uphold, and increase competitiveness, while in Maize-CC, the adaptability traits and spreading potential of weed communities are greatly supported.

Cropping systems, such as Maize-WW-S rotation aimed at supporting maize productivity strongly affect both annuals and perennials, especially when combined with herbicides (Brankov et al., 2021; Weisberger et al., 2019). Accordingly, the annual and perennial biomasses were lower on average in Maize-WW-S, but significantly only FBMP. A meta-analysis conducted by Zhao et al. (2020) reported a 20% increase in crop yield owing to crop rotation, whereas a meta-analysis by Weisberger et al. (2019) showed a 49% reduction in weed density owing to crop rotation. Nevertheless, in Maize-CC, weed species tend to rapidly adapt to management practices repeated in each season, promoting weed competitiveness over crops in resource-limited environments (Singh et al., 2022).

Herbicide application significantly reduced weed biomass (FBMT and DBMT) in both Maize-CC and Maize-WW-S, compared to the control, resulting in greater LAI and GY values, over time. Present variations signified the importance of meteorological conditions in the expression of herbicide efficacy (Simić et al., 2020b; Landau et al., 2021). It is important to emphasise that when all three factors were considered (Y $\times$ CR $\times$ HR), only GY was significantly affected and was 31.14% higher in Maize-WW-S than in Maize-CC, and by 6.21% and 22.70% in ½FLR and FLR, respectively, compared to the control. The integrated effect of crop rotation and herbicides was profoundly reflected on FBMT and DBMT reduction, particularly in Maize-WW-S, without differences between the ½ FLR and FLR, similar to the results of Brankov et al. (2021). While Nazarko et al. (2005) emphasized that the application of reduced rates of herbicides is possible, Zeller et al. (2021) showed that the use of herbicides with the same MOAs only once every five years in crop rotation contributed to a 23–99% reduction in black-grass. Importantly, perennial species cannot develop and successfully reproduce in rotation systems as successfully as they can in Maize-CC (Simić et al., 2021).

The fluctuation in meteorological conditions over the 12 years of this experiment significantly influenced weed composition and distribution over time, supporting review of Peters et al. (2014). The results indicated that long-term Maize-CC tended to support the growth of perennial weeds, while a rotation system provided favourable conditions for annual weeds, mainly due to their mode of propagation (Fonteyne et al., 2020; Simić et al., 2021). In general, the high density of annuals reduced LAI, whereas perennials, due to their number and biomass (FBMP), mainly reduced GY. Maize productivity in Maize-WW-S is supported by the combined effects of rotation and herbicide use. Applying rotation alone reduces the weed productivity (Bajwa et al., 2019; Siddiqui et al., 2022), which could be the main reason for the two-fold reduction in DBMT and the two-fold increase in GY.

The novelty of this study regarding maize cultivation and potential to increase its competitiveness over weeds presents, a sustainable strategy to uphold reasonable crop yields with minimising open niches for weeds (Lakara et al., 2019). The novelty and strength of study toward the joint effect of Maize-WW-S and different rates of pre-emergence herbicides is present in the positive connection between FBMP and GY, providing evidence of reduced weed pressure on maize (MacLaren et al., 2020) as a result of strategic ecological trade-offs. Thus, maize productivity was supported by the better use of agro-ecosystem services. What is more, there was no significant difference between FLR and ½ FLR treatment in Maize-WW-S, demonstrating that crop rotation is the first-line defence in the control of weed infestation, and that even lower chemical inputs could be highly effective in combination with cultural measures (Vasileiadis et al., 2011; Tataridas et al., 2022). This is of particular importance when climate change at local and global level was considered, supporting agro-ecosystem protection and overall sustainability.

While this study offers a comprehensive analysis of the integrated effect of maize rotation and herbicide effectiveness over a 12-year period, there are several limitations of the study, such as: 1) the lack of multi-year continuity regarding winter wheat and soybean (variation in their productivity and weed infestation over time); 2) a cost-benefit analysis of the examined cropping systems, as the most important factor for farmers, even though reduced pesticides use brings lower production costs. This provides opportunities to further improve research towards solutions that could be recommended to farmers. They might benefit from the integration of crop rotation and pre-emergence herbicides in order to control weed on satisfactory level, and accordingly support maize productivity.

5. Conclusions

Growing maize in a maize–winter wheat–soybean rotation with reduced rates of pre-emergence herbicides could contribute to a significant reduction in weed infestation over time (up to 92.1 %). The application of pre-emergence herbicides at both rates, FLR and ½ FLR, effectively reduced the number and biomass of annual weed species in both Maize-WW-S and Maize-CC, whereas the number and biomass of perennial weeds remained stable (particularly in Maize-CC). In Maize-WW-S, the positive correlation between FBMP and maize grain yield indicated that the interference of perennials was buffered by increased crop diversification. To reduce weed density and weed biomass and subsequently increase maize GY and its stability over time, crop rotation should be included in cropping technology, and the use of herbicides should only be an additional weed control measure. In surplus to sustainability in maize production, a lower herbicide input such as ½ FLR, which showed the same effectiveness as FLR, could be applied.

The results confirm the importance of the combined employment of rotation and chemical measures in maize production as part of the IWM, recruiting agro-ecosystem services to a higher degree. To reduce pesticide application, research should focus on an integrated approach using the diversification of cropping systems, including more factors related to crop–weed–environment interactions. As this technology could offer benefits for crop production, within this technology farmers still might face with some challenges to implement this practice, from organizational and economical point of view.

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CRediT authorship contribution statement

Milena Simić: Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vesna Dragičević:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Alexandros Tataridas:** Writing – review & editing, Writing – original draft, Formal analysis. **Tsvetelina Krachunova:** Writing – review & editing. **Jelena Srdić:** Formal analysis. **Ioannis Gazoulis:** Writing – review & editing, Visualization. **Milan Brankov:** Writing – review & editing, Supervision, Investigation, Formal analysis, 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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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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