



## Research article

## Intensification of crop rotation affecting weed communities and the use of herbicides in the rolling Pampa



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## ABSTRACT

Weeds are one of the main problems in the cropping systems of the Rolling Pampa (Argentina), where glyphosate resistant varieties of soybean sown with no-tillage system became the most important crop in the rotation. The challenge to solve this problem is to apply alternative approaches that both reduce weediness and the use of chemicals. Thus, the objectives of this work were i) to study the impact of crop rotation intensification on the species composition and richness of weed communities and to identify the relationship with some environmental (soil mineral organic matter) and agronomic variables (intensification, cereal crops in the rotation, biomass production and herbicide applications) and ii) to quantify the use and environmental risk of herbicides related to the intensification of crop rotations. From 2012 to 2019, four rotations were performed on three farms combining crops (soybean, maize, wheat and field pea), cover crops (oats and hairy vetch) and mixed pastures. During spring 2018 and autumn 2019 field and seedbank experiments were performed. PCA using presence-absence of species as response variable and intensification index of rotation (IIR), proportion of cereal crops in the rotation (C), biomass production (B), mineral organic matter (OM) and number of herbicide applications (HA) during the six years of the rotation as explanatory variables. The use and environmental risk of herbicides was also assessed. Surveys and seedbank analysis showed that intensification of crop rotations resulted in differences in the floristic composition of weed communities mainly related to IIR and C. Although the use of herbicides decreased as intensification grew, species richness and abundance did not change. Despite of all the variations considered in this study such as different approaches (emerged weeds and seedbank), locations, crops, pastures and sowing dates, intensification consistently filtered species conforming different weed assemblies and reducing the use of herbicides. Thus, promoting sustainable intensification by increasing cover crops, winter crops, cereal crops and pastures in the rotations would be a useful tool to manage weeds since the use of herbicides can be replaced by increasing the IIR without variations in weed abundance.

## 1. Introduction

Grain crop production systems in the Rolling Pampas mainly include soybean, double crop wheat-soybean and maize in rotation. The Rolling Pampa is a very productive agroecological zone that has experienced important agricultural changes over the past 50 years, mainly driven by the expansion of soybean (*Glycine max* L. Merr.) crops since the 1990s. At present, transgenic glyphosate resistant soybean is sown with no-tillage

cropping, glyphosate resistant varieties as a single-crop or a double-crop wheat-soybean (Satorre, 2011). Despite the efforts made to reduce weediness, weeds constitute one of the main problems in crop production because of the increase of woody species (Ghersa et al., 2002), herbicide tolerant species (Hylvönen and Salonen, 2002), biotypes resistant to herbicides (Heap, 2020) and also because of the negative impact on biodiversity (Satorre et al., 2020) and environmental pollution (Hunt et al., 2017). The challenge to solve these problems is to apply alternative

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approaches to manage weediness and, at the same time, to reduce the use of chemicals, thus avoiding side effects on the agro-ecosystem functioning and pollution.

One possibility is sustainable intensification of the rotations by increasing the number of crops per year or by including different cover crops before the main crop. Sustainable intensification can be defined as the process that uses environmental resources more intensely, maintaining or increasing crop yield by unit area and using chemical inputs in a rational way (Caviglia and Andrade, 2010).

In this context, it is useful to evaluate the impact of sustainable intensification of crop sequences on weed communities and environmental risks related to herbicides. The structure of weed communities is the result of a process that follows several "assembly rules" acting on the set of species as hierarchically organized filters. In this process, the environmental factors affected by rotations restrict the species of the regional group that makes up the local communities. Biotic and abiotic restrictions or filters act on multiple scales by eliminating species that lack specific traits (Ryan et al., 2010). Environmental risks may be reduced by including crops and cover crops in the rotation. These interfere with weeds, generating inhospitable and fatal conditions throughout their life cycle, thus, reducing the need for chemical controls (Davis et al., 2012).

Technological components associated with crop management can also act as filters regulating weed change patterns. In agricultural systems, herbicides constitute one of the main filters against the susceptible species, but there are other filters acting, probably less selectively than herbicides (Ryan et al., 2010). For example, intensification and its relationship with variations in crop sequences and structure, tillage, planting and harvesting system, weed and fertilizer management, are some of the filters that can affect the structure of the community through their effect on weed populations dynamics processes (weed establishment, competition, dispersion, herbicide resistance) (Cordeau et al., 2017).

Effective sequences to manage weeds should include crops that grow in different seasons, maximizing the capture of resources throughout the year, differing in planting and harvesting dates, growth periods, competitive abilities, characteristics of their residues (Melander et al., 2017) and the proportion of cereal crops (Zarina et al., 2015).

Cover crops may also play an important role in sustainable farming systems. They not only reduce soil erosion and nitrate leaching as well as increase soil organic matter, but also suppress weeds without resorting to synthetic herbicides. Instead, cover crops may provide greater efficiency of weed management due to a combination of different inhibitory mechanisms on seed dormancy and germination (soil temperature, light and moisture) and plant growth (competition and allelopathy) (Smith et al., 2015; Melander et al., 2017).

In this context, the hypotheses of this work were that environmental and management factors affected by sustainable intensification i) restrict or filter weed species and functions, thus weed community composition will be different and species abundance and richness will be reduced through intensification of crop rotation and ii) generate fatal conditions for weeds thus, the use herbicides and environmental risks will be reduced with intensification of crop rotation. The objectives of this work were i) to study the impact of intensification of crop rotations on the composition and richness of weed communities and to identify the relationship with some environmental (soil mineral organic matter) and agronomic variables (intensification, cereal crops in the rotation, biomass production and herbicide applications) and ii) to quantify the use and environmental risk of herbicides related to the intensification of crop rotations.

## 2. Materials and methods

The Rolling Pampa is a sub-region of the Río de la Plata grasslands in Argentina (between 34 and 36°S and 58 and 62°W) with a uniform topography, geomorphology, soil types and original vegetation. The landscape is a gently rolling plain (Soriano et al., 1992). The climate is

temperate and humid, with average annual rainfall of 940 mm, concentrated in the spring - summer seasons and a mean annual temperature of 17 °C. Main crops are soybean and, to a lower extent, maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Satorre, 2011).

From 2012 to 2019, crop rotations were carried out on three farms randomly selected from a pool of farms belonging to the Argentine Association of No Tillage Producers (Asociación Argentina de Productores en Siembra Directa, AAPRESID). They were Las Matreras (LMs) located at Irineo Portela, San Nicolás (SN) at Uranga and La Matilde (LMe) at Inés Indart located in the center of the Rolling Pampa (Table 1, Figure 1). A representative field was selected on each farm, counting with similar soils (Argiudol), soil series (INTA, 1990) and topographic position. Each field was divided into plots of similar size (8–20 ha, depending on the field size), where the rotations were located.

The rotations/treatments performed at each farm/block were combinations of crops (soybean, maize, wheat and field pea, *Pisum sativum* L.), cover crops (oats, *Avena strigosa* Schreb. and hairy vetch, *Vicia villosa* Roth) and mixed pastures. The experiment was a completely randomized block design in incomplete factorial arrangement of different levels of two parameters: the intensification index of rotation (IIR) with three levels, high (*h*), mid (*m*) and low (*w*) and the proportion of cereal crops in the rotation (C) with two levels, high (*h*) and low (*w*). This resulted in four rotations: i) pasture, double crop wheat/soybean, double crop field pea/maize, sequence of cover crop oats/soybean (high IIR and low C, rotation *hw*); ii) double crop wheat/maize, sequence of cover crop hairy vetch/maize (mid IIR and high C, rotation *mh*); iii) double crop wheat/soybean, sequence of cover crop hairy vetch/maize, sequence of cover crop oats/soybean (mid IIR and low C, rotation *mw*) and iv) double crop wheat/soybean, winter fallow/maize, winter fallow/soybean (low IIR and low C, rotation *ww*) (Table 2). The latter rotation is the most widespread in this region.

IIR was estimated as the relationship between the time (days) occupied with crops (from crop emergence to physiological maturity) and the total duration of rotation. C was estimated as the ratio between cereal crops and the total crops included in the rotation. For rotations including pastures, the IIR was 1 for the first four years during the pasture cycle and the time of occupation of the crops after drying the pasture. This lowered the IIR to less than 1 (between 0.88 and 0.90).

Crops were grown under no-tillage rain fed system. Inter row distances were 0.18–0.21m for cover crops and winter crops, 0.52 m for maize and 0.38–0.42 m for soybean. Sowing dates were June for winter crops and cover crops, September for maize, November for soybean and December for late maize and soybean. Weed control was the usual in the region, including grass and broadleaf herbicides during the fallow and after crop emergence (Table 3). The pastures were planted in April 2012 on SN and LM and in August 2012 on LMs. All pastures were kept without animals and 3–5 mechanical cuts were made per year to make pasture rolls. In autumn 2016 the pastures were dried with herbicides to start the agricultural cycle.

### 2.1. Community composition and richness related to intensification of crop rotations and management variables

During spring 2018 and autumn 2019 field and seedbank experiments were done considering the rotations as treatments and farms as blocks. This time interval was chosen based on two criteria: (a) spring-summer and autumn-winter communities were present and (b) chemical controls had already been applied. During spring 2018, surveys and soil sampling were made in oats for *hw* and *mw* rotations, hairy vetch for *mh* rotations and fallow for *ww* rotations. During autumn 2019, surveys and soil sampling were made in soybean for *hw* and *mw* rotations, soybean/maize for *ww* rotations and maize for *mh* rotations.

Weed surveys (Exp. 1) consisted of a complete list of species present in the entire central area of each plot and they were carried out by two or more trained people walking across the field during 20 min recording all species observed until no more new species were found. Sampled areas

**Table 1.** Farm name, code, location and geographic coordinates and experimental field size and initial soil physico-chemical properties.

Farm name	Code	Location	Geographic coordinates	Field size (ha)	Initial soil physico-chemical properties			
					pH	OM (%) 0–20cm	N total (%)	P (ppm)
Las Matreras	LMS	Irineo Portela	34°19'09"S, 60°28'40"W	59	5.92	3.20	1.78	12.6
San Nicolás	SN	Uranga	33°18'26"S, 60°41'20"W	106	5.78	2.71	1.34	28.4
La Matilde	LMe	Inés Indart	34°00'05"S, 59°39'24"W	39	6.14	3.44	1.65	13.1

**Figure 1.** Geographical location of the study site.

within the plots fulfilled the following requirements (Mueller-Dombois and Ellenberg, 1974): (a) they were large enough to contain all species belonging to the plant community, (b) the habitat was uniform within the plot area, and (iii) plant cover was homogeneous. Plot margins and low

areas were avoided because they may represent different habitats (e.g. different management and soil conditions). The percentage of cover-abundance of individual species was visually estimated, using an adapted Braun–Blanquet scale (Mueller-Dombois and Ellenberg, 1974),

**Table 2.** Rotations/treatments, intensity index (II), the proportion of cereal crops (C), biomass (B), organic matter (OM) and number of herbicide applications (HA) for each treatment including 6 years of rotations (2012/13 to 2017/18). Codes are combinations of first letter indicating level IIR: high (*h*), mid (*m*) and low (*w*) and second letter level of C: high (*h*) and low (*w*).

Farm name	Rotation	Code	IIR	C (%)	B (kg/ha)	OM (%)	HA
LMS	pasture, double crop wheat/soybean, double crop field pea/maize, sequence cover crop oats/soybean	<i>hw</i>	0.88	50	35120	6.1	14
	double crop wheat/maize, sequence cover crop hairy vetch/late maize	<i>mh</i>	0.54	90	45278	4.9	36
	double crop wheat/soybean, sequence cover crop hairy vetch/late maize, sequence cover crop oats/soybean	<i>mw</i>	0.57	36	32842	4.7	48
	double crop wheat/soybean, winter fallow/maize, winter fallow/soybean	<i>ww</i>	0.46	50	40806	5.1	24
SN	pasture, double crop wheat/soybean, double crop field pea/maize, sequence cover crop oats/soybean	<i>hw</i>	0.89	50	30144	5.4	22
	double crop wheat/maize, sequence cover crop hairy vetch/late maize	<i>mh</i>	0.64	92	59035	5.8	26
	double crop wheat/soybean, sequence cover crop hairy vetch/late maize, sequence cover crop oats/soybean	<i>mw</i>	0.65	50	52663	4.1	34
	double crop wheat/soybean, winter fallow/maize, winter fallow/soybean	<i>ww</i>	0.46	50	42260	4.0	43
LMe	pasture, double crop wheat/soybean, double crop field pea/maize, sequence cover crop oats/soybean	<i>hw</i>	0.90	58			
	double crop wheat/maize, sequence cover crop hairy vetch/late maize	<i>mh</i>	0.67	75	83670	5.4	24
	double crop wheat/soybean, sequence cover crop hairy vetch/late maize, sequence cover crop oats/soybean	<i>mw</i>	0.65	45			
	double crop wheat/soybean, winter fallow/maize, winter fallow/soybean	<i>ww</i>	0.53	56	58398	5.0	38

**Table 3.** Mean dose of commercial product applied and mean number of total applications of herbicides used in the different rotations from 2012/13 to 2017/18. Codes are combinations of first letter indicating level IIR: high (*h*), mid (*m*) and low (*w*) and second letter level of C: high (*h*) and low (*w*).

Rotation	Herbicide	Mean dose	Total applications
<i>hw</i>	2,4D 96.3%	655 mL ha <sup>-1</sup>	3.0
	Atrazine 900 g/kg	1 L ha <sup>-1</sup>	1.0
	Chlorsulfuron ethyl 62.5% + Metsulfuron methyl 12.5%	14 g ha <sup>-1</sup>	0.3
	Dicamba 75g	200 mL ha <sup>-1</sup>	1.0
	Flumetsulam 12g	750 mL ha <sup>-1</sup>	0.3
	Glyphosate 54%	2390 mL ha <sup>-1</sup>	1.3
	Glyphosate 60.8%	1584 mL ha <sup>-1</sup>	4.3
	Metolachlor 96 g/L	1200 mL ha <sup>-1</sup>	1.0
<i>mh</i>	Pinoxaden 5% + Cloquintocet-mexyl 1.25%	800 mL ha <sup>-1</sup>	0.3
	2,4D 96.3%	620 mL ha <sup>-1</sup>	3.7
	Atrazine 900 g/kg	1 L ha <sup>-1</sup>	0.7
	Clethodim 12.5 mL/L	950 mL ha <sup>-1</sup>	0.7
	Dicamba 48g	385 mL ha <sup>-1</sup>	0.7
	Glyphosate 54%	2745 mL ha <sup>-1</sup>	4.0
	Glyphosate 60.8%	1700 mL ha <sup>-1</sup>	3.3
<i>mw</i>	Metolachlor 96 g/L	1200 mL ha <sup>-1</sup>	0.7
	2,4D 96.3%	600 mL ha <sup>-1</sup>	4.7
	Atrazine 900 g/kg	1 L ha <sup>-1</sup>	0.7
	Chlorsulfuron ethyl 62.5% + Metsulfuron methyl 12.5%	15 g ha <sup>-1</sup>	0.3
	Diclosulam 84g + Sulfentrazone 50g	27.5 g ha <sup>-1</sup>	0.7
	Glyphosate 506 g/L	2250 g ha <sup>-1</sup>	0.7
	Glyphosate 54%	2136 mL ha <sup>-1</sup>	5.7
	Glyphosate 60.8%	1666 mL ha <sup>-1</sup>	4.0
	Haloxypol methyl 52g/100mL	120 g ha <sup>-1</sup>	0.3
	Haloxypol methyl 3g/100mL	1091 g ha <sup>-1</sup>	0.3
	Imazethapyr 10 g/L	400 mL ha <sup>-1</sup>	0.7
	Metolachlor 96 g/L	1200 mL ha <sup>-1</sup>	0.7
	Paraquat 27.6 g/L	1944 mL ha <sup>-1</sup>	1.0
<i>ww</i>	Penoxsulam 240 g/L	300 mL ha <sup>-1</sup>	0.3
	Sulfometuron methyl 15% + Clorimuronethyl 20%	103 g ha <sup>-1</sup>	0.3
	2,4D 96.3%	487 mL ha <sup>-1</sup>	5.0
	Atrazine 900 g/kg	1 L ha <sup>-1</sup>	1.3
	Chlorsulfuron ethyl 62.5% + Metsulfuron methyl 12.5%	15 g ha <sup>-1</sup>	0.3
	Clethodim 12.5 mL/L	1235 mL ha <sup>-1</sup>	0.7
	Diclosulam 84g + Sulfentrazone 50g	30 g ha <sup>-1</sup>	0.3
	Glyphosate 506 g/L	1700 g ha <sup>-1</sup>	0.7
	Glyphosate 54%	2218 mL ha <sup>-1</sup>	4.7
	Glyphosate 60.8%	1700 mL ha <sup>-1</sup>	4.0
	Haloxypol methyl 52g/100mL	120 g ha <sup>-1</sup>	0.3
	Haloxypol methyl 3g/100mL	1440 g ha <sup>-1</sup>	0.3
	Imazethapyr 10 g/L	490 mL ha <sup>-1</sup>	1.0
Metolachlor 96 g/L	1250 mL ha <sup>-1</sup>	0.7	
Paraquat 27.6 g/L	2000 mL ha <sup>-1</sup>	0.7	
Penoxsulam 240 g/L	250 mL ha <sup>-1</sup>	0.7	
Sulfometuron methyl 15% + Clorimuronethyl 20%	103 g ha <sup>-1</sup>	0.3	

namely with the following intervals: 0–1%, 1–5%, 5–10%, 10–25%, 25–50%, 50–75%, 75–100% (de la Fuente et al., 2010). Mean weed cover-abundance per treatment was estimated as  $\Sigma$  cover-abundance per species per treatment/number of farms.

Weed seedbank experiments (Exp. 2) consisted of a completely randomized block design with rotation as treatment and farms as blocks. Three seedbank samples of 30 cm long, 20 cm wide and 8 cm depth were randomly taken per plot using a shovel and moved to the greenhouse at the Faculty of Agronomy of Buenos Aires University. The greenhouse had natural temperature and radiation, and only rainfall was prevented. Samples were homogenized there, and four subsamples were placed into plastic trays of 25 cm long, 20 cm wide and 6 cm depth with 5 small holes at the base to favor drainage and then covered with a net to prevent seed

predation, herbivory and contamination. Trays were watered periodically to maintain field capacity. The emerging seedlings were identified, counted and removed once or twice per week depending on emergence rate, until no further emergence was observed. Abundance of individual species was estimated as total number of individuals per species counted by tray. Mean abundance per treatment was estimated as  $\Sigma$  number of individuals per species per treatment/number of trays.

In both experiments weed species constancy was estimated as the percentage of plots containing a given species along a year. Richness was estimated as  $\Sigma$  number species per treatment (Magurran, 1988). Functional groups, defined as clusters of species having resources, habitat, ecophysiological processes or life-history, are the principal determinants of communities' composition (Mooney and Bärberi, 2008). Therefore,



and because functional groups are sensitive to within-field changes (de la Fuente et al., 2010), weed species were classified into functional groups according to family, life cycle (annual, biennial or perennial), morphology (dicotyledons or monocotyledons) and origin (adventitious, naturalized, introduced or native).

Richness in both experiments, cover-abundance per plot in Exp. 1 and abundance per tray in Exp. 2 were transformed to meet the assumptions of analysis of variance model through square root. In Exp. 2 the average subsamples were analyzed. Then, analysis of variance (ANOVA) was performed using Infostat software (2018 version, Di Rienzo et al., 2018). Both homogeneity of variance and normal distribution were tested. When differences among treatments were significant, means were compared using Tukey's significant difference test ( $p < 0.05$ ).

Principal component ordination analysis was done using PC-ORD Multivariate Analysis of Ecological Data Version 5.0 (McCune and Meford, 1999). Ordination helps to identify relationships among species composition at a site and the underlying environmental factors (Digby and Kempton, 1991). It also constructs those linear combinations (axes) of explanatory variables along which the species distributions are maximally separated (terBraak, 1987). This offers potential for examining the response of weed communities to various environmental and agronomic variables (Kenkel et al., 2002). The response variable used was the presence-absence (1–0) of species and the explanatory variables for both experiments were IIR, C, biomass production (B), mineral organic matter (OM) and number of herbicide applications (HA) during the six years of the rotation. B was obtained estimating crop, cover crop and pasture biomass. Crop biomass was estimated considering final yield and harvest index of each crop included in the rotation. Cover crop and pasture biomass was weighed at the end of each cover crop and pasture. HA was estimated by the total number of herbicide applications made for each rotation. To determine associations between the data and the main explanatory variables, a biplot from the PCA was obtained by overlaying a vector diagram on the ordination graph.

## 2.2. Environmental risk of herbicides related to the intensification of crop rotations

The environmental risk assessment was carried out evaluating the use of herbicides in the different rotations of each agricultural year (2012/13 to 2017/18), through the RIPEST 3.0 platform (Ferraro et al., 2020; RIPEST, 2013). RIPEST is a simple fuzzy-based model to estimate the ecotoxicity of pesticides in agricultural systems, based on the link among the toxicity of the different herbicides with the dose used to estimate an environmental potential harmful value. RIPEST is built from ecotoxicological information of formulations registered in the Argentinean National Service for Sanitary and Quality of Agriculture and Food (SENASA), for extensive grain crops. Each active ingredient is characterized by means of two different toxicity values: (1) mammal toxicity and (2) insect toxicity. In order to assess the magnitude of the impact of each application, the values of mammal and insect toxicity are measured using the concept of Toxic Units (TU) in two groups of organisms: mammals and insects.

$$TU_{mi} = Di / LD50_{i \text{ rat}} \quad TU_{ii} = Di / LD50_{i \text{ bee}}$$

Where,  $TU_{mi}$  and  $TU_{ii}$  are the toxic units for mammals and insects, respectively,  $Di$  is the applied dose (g of formulated product  $ha^{-1}$ ) of the pesticide  $i$ ,  $LD50_{i \text{ rat}}$  is the oral acute lethal dose of pesticide  $i$  to kill 50 % of rats ( $mg \text{ kg}^{-1}$ ), and  $LD50_{i \text{ bee}}$  is the pesticide  $i$  acute lethal dose of contact for bees ( $g \text{ bee}^{-1}$ ). After calculating  $LD50$  of a single active ingredient in formulations and mixtures, RIPEST uses the sum of the toxic units (TU) of all the pesticides applied in each field order to calculate the overall toxicity value. Since this work is focusing on weed performance regarding land intensified use with different rotations, only the impact of herbicide use was analyzed independently of other pesticides.

Linear relationships were established between the sum of toxicological units ( $TU_i$  and  $TU_{tm}$ ) at each rotation on each establishment and the intensification rate (IIR), and between HA and IIR, using the Infostat software (2018 version, Di Rienzo et al., 2018).

## 3. Results

### 3.1. Community structure and richness related to intensification of crop rotations and management variables

#### 3.1.1. Weed surveys - Exp. 1

According to the PCA ("principal component analysis"), main three axes explained 61% of the variation during spring 2018 and 58.2% in autumn 2019. In both ordinations a contrast between  $hw$  and  $mh$  rotations was observed in axis 1 ( $hw$  to the left and  $mh$  to the right of the diagram) and a contrast between  $ww$  and the rest of rotations was observed in axis 2 (Figures 2 and 3). In 2018, the main explanatory variables related to the structure of the community were B (62%) and IIR (-55%) in axis 1 and C (-48%) in axis 2. In 2019, the main variables were OM (-52%), IIR (-44%) and HA (43%) in axis 1 and C (-67%) on axis 2 (Figures 2 and 3).

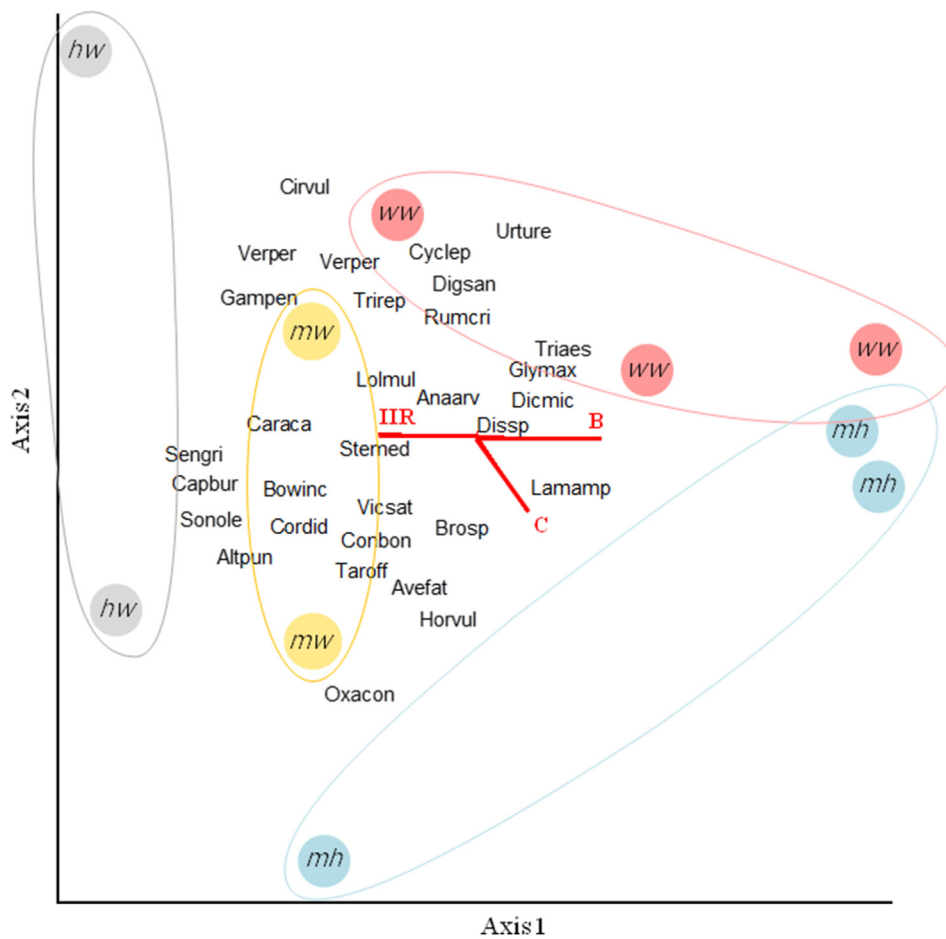
In 2018 spring surveys, high constancy in all rotations was observed for the group consisting of *Stellaria media* (L.) Vill., *Lolium multiflorum* Lam., *Lanium amplexicaule* L., *Coronopus didymus* (L.) Sm., *Conyza bonariensis* (L.) Cronquist, *Urtica urens* L., *Capsella bursa-pastoris* (L.) Medik, *Senecio grisebachii* Baker, *Alternanthera pungens* Kunth and *Sonchus oleraceus* L. The rest of the groups showed differences between rotations. For example, the group *Trifolium repens* L. and *Vicia sativa* L. was present only in rotation  $hl$ , while the group *Cirsium vulgare* (Savi) Ten. and *Gamochaeta pensylvanica* (Willd.) Cabrera, was absent only in rotation  $ih$  (Table 4). In 2019 autumn surveys, *Digitaria sanguinalis* (L.) Scop. and *Commelina erecta* L. presented high constancy in all rotations. The rest of the groups showed differences among rotations. For instance, the group *Dichondra microcalyx* (Hallier f.) Fabris, *L. amplexicaule* was present only in rotation  $hl$ , while the group *Brachiaria platyphylla* (Munro ex C. Wright) Nash, *Echinochloa crus-galli* (L.) P. Beauv. and *Taraxacum officinale* F.H. Wigg. was absent only in this rotation (Table 5).

Total species richness was 31 in spring 2018 and 32 in autumn 2019. Annual dicotyledons (many of them from *Asteraceae* and *Fabaceae* families) predominate over monocotyledons (mainly annual *Poaceae*) and there were no marked differences between native and exotic species (Tables 4 and 5). In both years no significant differences were found in mean richness (2018  $p = 0.22$  and 2019  $p = 0.39$ ) and mean cover abundance (2018  $p = 0.23$  and 2019  $p = 0.34$ ) among rotations (Table 6).

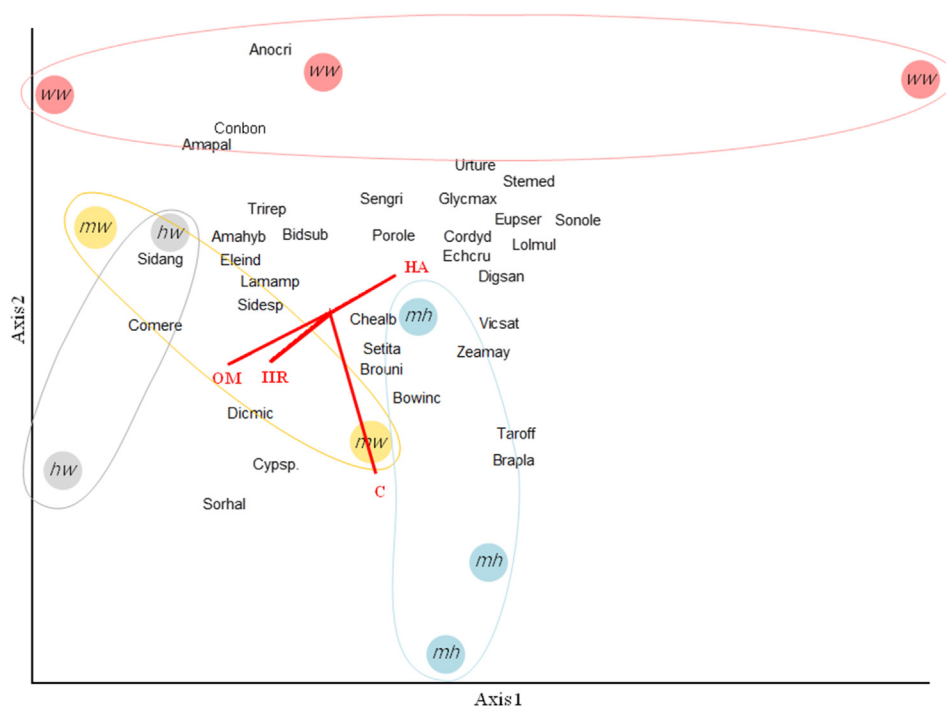
#### 3.1.2. Weed seedbank experiments - Exp. 2

According to the PCA main three axes explained 50.9% of the variation in 2018 and 44% in 2019. A  $mw$  and  $mh$  contrast ( $mw$  to the top left and  $mh$  to the bottom right of the Figure 4) was found in 2018 and a contrast between  $hw$  and the rest of rotations ( $hw$  to the left of the Figure 5) was found in 2019 in axes 1 and 2. The main variables that explained the structure of the communities were HA (-35 %) and C (22 %) related to axis 1 and OM (-52%), C (-40 %) and HA (31%) related to axis 2 in 2018, and IIR (-76%) and B (48%) related to axis 1 and B (41 %) related to axis 2 in 2019 (Figures 4 and 5).

In 2018, the group formed by *C. bonariensis*, *Amaranthus hybridus* L. and *Portulaca oleracea* L. showed high constancy in the seedbank of all rotations. The rest of the groups showed differences among rotations. For example, the group *Melilotus* sp., *T. repens*, *Polygonum* sp., *D. microcalyx* and *Sida rhombifolia* was present only in rotation  $hw$ , the group *E. crus-galli* and *D. sanguinalis* was absent only in rotation  $mw$  and the group *C. didymus*, *T. officinale*, *E. indica* and *Cyperus* sp. was absent only in rotation  $mh$  (Table 7). In 2019, the group formed by *C. bonariensis*, *L. amplexicaule*, *E. indica*, *S. media*, *P. oleracea* and *G. pensylvanica* showed high constancy in the seedbank of all rotations. The rest of the groups



**Figure 2.** PCA ordination of the data surveyed (Exp1) during spring 2018. Rotations code (ww, mw, mh and hw) and weeds code: first 3 letters of the genus and first 3 letters of the species. Vectors represent the main explanatory variables: intensification index of rotation (IIR), biomass production (B) and proportion of cereal crops in the rotation (C). Species codes: *Stellaria media* (Stemed); *Lolium multiflorum* (Lolmul); *Lamium amplexicaule* (Lamamp); *Coronopus didymus* (Cordid); *Conyza bonariensis* (Conbon); *Urtica urens* (Urture); *Capsella bursa-pastoris* (Capbur); *Senecio grisebachii* (Sengri); *Alternanthera pungens* (Altpun); *Sonchus oleraceus* (Sonole); *Cirsium vulgare* (Cirvu); *Gamochaeta pensylvanica* (Gampen); *Trifolium repens* (Trirep); *Vicia sativa* (Vicsat); *Cyclosporum leptophyllum* (Cyclep); *Veronica persica* (Verper); *Digitaria sanguinalis* (Digsan); *Carduus acanthoides* (Caraca); *Taraxacum officinale* (Taroff); *Rumex crispus* (Rumcric); *Veronica peregrina* (Verper); *Bowlesia incana* (Bowinc); *Oxalis conorrhiza* (Oxacon); *Avena fatua* (Avefat); *Hordeum vulgare* (Horvul); *Bromus sp.* (Bros); *Anagallis arvensis* (Anaarv); *Glycine max* (Glymax); *Triticum aestivum* (Triaes); *Dichondra microcalyx* (Dicmic); *Distichlis sp.* (Dissp).



**Figure 3.** PCA ordination of the data surveyed (Exp1) during autumn 2019. Rotations code (ww, mw, mh and hw) and weeds code: first 3 letters of the genus and first 3 letters of the species. Vectors represent the main explanatory variables: proportion of cereal crops in the rotation (C), herbicide applications (HA), mineral organic matter (OM) and intensification index of rotation (IIR). Species codes: *Digitaria sanguinalis* (Digsan); *Commelina erecta* (Comere); *Anoda cristata* (Anocri); *Conyza bonariensis* (Conbon); *Eleusine indica* (Eleind); *Sida angustifolia* (Sidang); *Dichondra microcalyx* (Dicmic); *Lamium amplexicaule* (Lamamp); *Stellaria media* (Stemed); *Cyperus sp.* (Cypsp); *Sorghum halepense* (Sorhal); *Brachiaria platyphylla* (Brapla); *Echinochloa crus-galli* (Echcru); *Taraxacum officinale* (Taroff); *Vicia sativa* (Vicsat); *Zea mays* (Zeamay); *Setaria itálica* (Setita); *Euphorbia serpens* (Eupser); *Lolium multiflorum* (Lolmul); *Coronopus didymus* (Cordid); *Senecio grisebachii* (Sengri); *Glycine max* (Glymax); *Sonchus oleraceus* (Sonole); *Bidens subalternans* (Bidsub); *Trifolium repens* (Trirep); *Urtica urens* (Urture); *Portulaca oleracea* (Porole); *Amaranthus hybridus* (Amahyb); *Chenopodium album* (Chealb); *Bowlesia incana* (Bowinc); *Amaranthus palmeri* (Amapal); *Bromus unioloides* (Brouni).

**Table 4.** Weed communities related to each rotation surveyed (Exp. 1) during spring 2018. Floristic groups, species, functions, constancy and total richness. D: dicot, M: monocot, A: annual, P: perennial, AD: adventitial, NAT: naturalized, I: introduced, N: native. Codes are combinations of first letter indicating level IIR: high (*h*), intermediate (*m*) and low (*w*) and second letter indicating level of C: high (*h*) and low (*w*).

Floristic group	Species	Families	Morpho type	Growth cycle	Origin	Species constancy (%) in each rotation			
						<i>hw</i>	<i>ww</i>	<i>mw</i>	<i>mh</i>
I	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	D	A	AD	100	100	100	66.7
	<i>Lolium multiflorum</i> Lam.	Poáceas	M	A-B	I	50	33.3	50	33.3
	<i>Lamium amplexicaule</i> L.	Lamiáceas	D	A	AD	100	100	50	100
	<i>Coronopus didymus</i> (L.) Sm.	Brassicaceae	D	A	N	100	33.3	100	66.7
	<i>Conyza bonariensis</i> (L.) Cronquist	Asteraceae	D	A	N	50	100	100	33.3
	<i>Urtica urens</i> L.	Urticaceae	D	A	NAT	100	100	50	33.3
	<i>Capsella bursa-pastoris</i> (L.) Medik.	Brassicaceae	D	A-B	AD	100	33.3	100	33.3
	<i>Senecio grisebachii</i> Baker	Asteraceae	D	P	N	100	33.3	100	33.3
	<i>Alternanthera pungens</i> Kunth	Amaranthaceae	D	P	N	100	33.3	100	33.3
	<i>Sonchus oleraceus</i> L.	Asteraceae	D	A	AD	100	33.3	50	33.3
II	<i>Cirsium vulgare</i> (Savi) Ten.	Asteraceae	D	A-B	NAT	100	33.3	100	
	<i>Gamochaeta pensylvanica</i> (Willd.) Cabrera	Asteraceae	D	B-P	N	100	66.7	100	
III	<i>Trifolium repens</i> L.	Fabaceae	D	P	AD	50			
	<i>Vicia sativa</i> L.	Fabaceae	D	A	AD	50			
IV	<i>Cyclosporum leptophyllum</i> (Pers.) Sprague & Britton & P. Wilson	Apiaceae	D	A	N	50	66.7		
	<i>Veronica persica</i> Poir.	Plantaginaceae	D	A	AD	100	33.3		
	<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	M	A	AD	50	33.3		
V	<i>Carduus acanthoides</i> L.	Asteraceae	D	A	N	100		50	
	<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	D	P	AD	50		50	
	<i>Rumex crispus</i> L.	Polygonaceae	D	P	AD	50		50	
	<i>Veronica peregrina</i> L.	Plantaginaceae	D	A	AD	50		50	
VI	<i>Bowlesia incana</i> Ruiz & Pav.	Umbelíferas	D	A	N	100			33.3
	<i>Oxalis conorrhiza</i> Jacq.	Fabaceae	D	P	N	50			33.3
VII	<i>Avena fatua</i> L.	Poaceae	M	A	I				33.3
	<i>Hordeum vulgare</i> L.	Poaceae	M	A	AD				33.3
	<i>Bromus</i> sp.	Poaceae	M					50	33.3
	<i>Anagallis arvensis</i> L.	Primulaceae	D	A	AD			50	
VIII	<i>Glycine max</i> (L.) Merr.	Fabaceae	D	A	I		33.3		
	<i>Triticum aestivum</i> L.	Gramineae	M	A	I		33.3		
	<i>Dichondra microcalyx</i> (Hallier f.) Fabris	Convolvulaceae	D	P	N		33.3		
	<i>Distichlis</i> sp.	Poaceae	M				33.3		
Total richness						23	19	18	15

showed differences among rotations. For example, the group *C. didymus*, *C. album* and *T. repens* was absent only in rotation *ww* (Table 8).

The total richness was 20 in 2018 and 24 in 2019, mainly summer species that had not emerged when the field surveys were made (Tables 7 and 8). In 2018 no significant differences were found in mean richness ( $p = 0.45$ ) and abundance ( $p = 0.81$ ) among rotations. While in 2019, differences were found among rotations in mean richness ( $p = 0.05$ ), being higher in *hw* (8.9 species) than in *ww* (3 species) and in abundance ( $p = 0.04$ ), being higher in *hw* (98.63 species) than in *ww* (14.41 species) (Table 6). Annual dicotyledons (many of them from *Fabaceae* family) predominate over monocotyledons (mainly annual *Poaceae*) and there were no marked differences between natives, adventitial and introduced (Tables 7 and 8).

### 3.2. Use and environmental risk of herbicides related to the intensification of crop rotations

The total number of herbicide applications made for each rotation (HA) was significantly reduced ( $r^2 = 0.46$ ,  $p = 0.03$ ) as IIR increased. Main differences in the use of herbicides occurred between high and low IIR (Figure 6, Table 3).

Environmental risk assessed through TUM ( $r^2 = 0.32$ ,  $p = 0.08$ ) and TUI ( $r^2 = 0.37$ ,  $p = 0.06$ ) was not related to intensification of the rotation. However, TUM and TUI were significantly different between high

and low IIR (Figure 6). These differences are explained mainly by the use of Paraquat with high TUM and TUI in *mw* and *ww* rotations and the high number of applications of 2,4-D with high TUM in *ww* rotations (Table 3).

## 4. Discussion

Results of the present research, considering emerged weeds but also seedbank, supported the hypothesis that intensification of crop rotations act as ecological filters structuring weed communities and reducing the use of herbicides. The filtering effect agree with previous research (Moonen and Barberi, 2004; Satorre et al., 2020) based on observational studies in crop fields, while in the present study an original experimental approach, including an IIR gradient under field and controlled conditions was used. Data on the weed seedbank contain useful information on past field management and are an important tool for the evaluation of the effectiveness of alternative crop and weed management systems (Moonen and Barberi, 2004).

Moreover, this approach covered not only traditional crops of this region (soybean, maize, wheat), but also alternative crops (field pea), cover crops (hairy vetch and oats) and pastures, and not only optimum sowing dates for single crops and first crop in double crops, but also delayed sowing date of the second crop (soybean and maize) in double crops and cover crops sequence. Despite of all these variations in the

**Table 5.** Weed communities related to each rotation surveyed (Exp. 1) during autumn 2019. Floristic groups, species, functions, constancy and total richness. D: dicot, M: monocot, A: annual, P: perennial, AD: adventitial, NAT: naturalized, I: introduced, N: native. Codes are combinations of first letter indicating level IIR: high (h), intermediate (m) and low (w) and second letter indicating level of C: high (h) and low (w).

Floristic group	Species	Families	Morpho type	Growth cycle	Origin	Species constancy (%) in each rotation			
						hw	ww	mw	mh
I	<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	M	A	AD	50	100	50	100
	<i>Commelina erecta</i> L.	Commelinaceae	D	P	N	100	33.3	50	33.3
II	<i>Anoda cristata</i> (L.) Schldt.	Malvaceae	D	A	N	50	100	50	
	<i>Conyza bonariensis</i> (L.) Cronquist	Asteraceae	D	A		100	100	100	
	<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	M	A	AD	50	33.3	50	
	<i>Sida angustifolia</i> Lam.	Malvaceae				100	33.3	50	
III	<i>Dichondra microcalyx</i> (Hallier f.) Fabris	Convolvulaceae	D	P	N	50			
	<i>Lamium amplexicaule</i> L.	Lamiaceae	D	A	A	50			
IV	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	D	A	AD	100	66.7		33.3
	<i>Cyperus</i> sp.	Cyperaceae	M	P		50			33.3
	<i>Sorghum halepense</i> (L.) Pers.	Poaceae	M	P		100		50	66.7
	<i>Brachiaria platyphylla</i> (Munro ex C. Wright) Nash	Poaceae	M	A	N		33.3	50	66.7
	<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Poaceae	M	A	I		33.3	50	66.7
	<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	D	P	AD		33.3	50	66.7
V	<i>Vicia sativa</i> L.	Fabaceae	D	A	AD		33.3		66.7
	<i>Zea mays</i> L.	Poaceae	M	A	N		33.3		33.3
	<i>Setaria itálica</i> (L.) P. Beauv.	Poaceae	M	A			33.3		66.7
	<i>Euphorbia serpens</i> Kunth	Euphorbiaceae	D	A			33.3		33.3
VI	<i>Lolium multiflorum</i> Lam.	Poaceae	M	A-B	I		33.3		
	<i>Coronopus didymus</i> (L.) Sm.	Brassicaceae	D	A	N		33.3		
	<i>Senecio grisebachii</i> Baker	Asteraceae	D	P	N		33.3		
	<i>Glycine max</i> (L.) Merr.	Fabaceae	D	A	I		33.3		
	<i>Sonchus oleraceus</i> L.	Asteraceae	D	A	AD		33.3		
	<i>Bidens subalternans</i> DC.	Asteraceae	D	A	N		33.3		
	<i>Trifolium repens</i> L.	Fabaceae	D	P	A		33.3		
	<i>Urtica urens</i> L.	Urticaceae	D	A	NAT		66.7		
	<i>Portulaca oleracea</i> L.	Portulacacaceae	D	A	AD		33.3		
	<i>Amaranthus hybridus</i> L.	Amaranthaceae	D	A	AD		33.3		
VII	<i>Chenopodium album</i> L.	Chenopodiaceae	D	A	AD				33.3
	<i>Bowlesia incana</i> Ruiz & Pav.	Umbelliferae	D	A	N				33.3
VIII	<i>Amaranthus palmeri</i> S. Watson	Amaranthaceae	D	A	I		66.7	50	
	<i>Bromus unioloides</i> Kunth	Poaceae	M	A-B	N			50	
Total richness						11	25	12	14

**Table 6.** Mean cover abundance (%) and richness (number of species plot<sup>-1</sup>) in Exp. 1 and mean abundance (plants tray<sup>-1</sup>) and richness (number of species tray<sup>-1</sup>) in Exp. 2 of weed communities related to each rotation during 2018 and 2019 and probability (p) from ANOVA. Codes are combinations of first letter indicating level IIR: high (h), intermediate (m) and low (w) and second letter indicating level of C: high (h) and low (w).

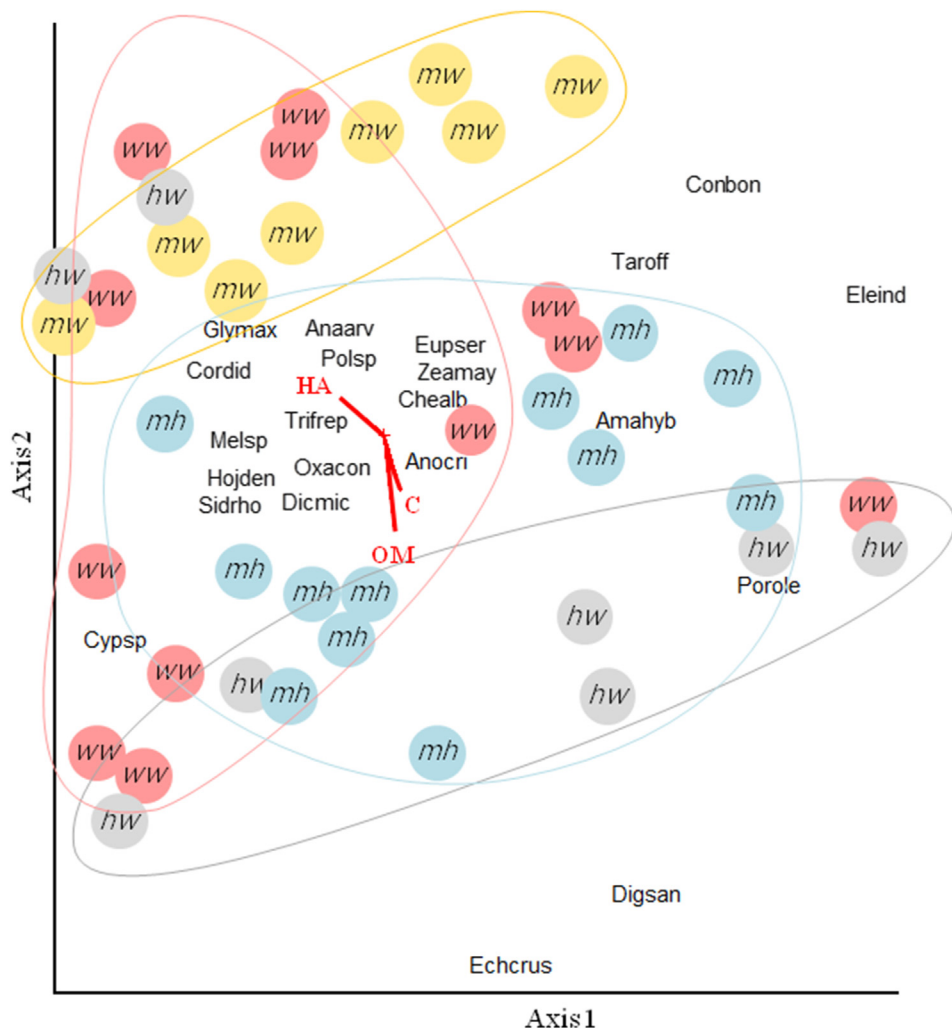
Variable	Exp.	Year	Rotations				p
			hw	ww	mw	mh	
Mean cover abundance (%)	1	2018	25.3	3.5	9.8	15.4	0,23
		2019	5.6	11.6	4.6	7.8	0,34
Mean abundance (plants tray <sup>-1</sup> )	2	2018	48.6	55.8	11.1	62.9	0,81
		2019	98.6	14.4	35	32.2	0,04
Mean richness (number of species plot <sup>-1</sup> )	1	2018	18	9.3	12	6.3	0,22
		2019	7.5	11	6.5	7.3	0,39
Mean richness (number of species tray <sup>-1</sup> )	2	2018	5.4	3.6	3.1	4.9	0,45
		2019	8.9	3	5.9	5	0,05

agricultural environment, intensification consistently filtered species conforming different weed assemblies, since main variables explaining weed community structure in both experiments and years included IIR and C.

The role of IIR as explanatory variable was expected, since the IIR changes the agricultural and microclimatic environment and thus, filters

species of the community through their effect on weed establishment, survival, fecundity and dispersion. However, it was remarkable to obtain the same Results combining different approaches, years, crop sequences, biomass production and quality, crop structures, herbicide modes of action and timing, planting and harvesting timing. These results agree with Satorre et al. (2020) who found that crop sequence intensification





**Figure 4.** PCA ordination of the data surveyed in seed bank (Exp 2) during spring 2018. Rotations code (ww, mw, mh and hw) and weeds code: first 3 letters of the genus and first 3 letters of the species. Vectors represent the main explanatory variables: herbicide applications (HA), mineral organic matter (OM) and proportion of cereal crops in the rotation (C). Species codes *Coryza bonariensis* (Conbon); *Amaranthus hybridus* (Amahyb); *Portulaca oleracea* (Porole); *Coronopus didymus* (Cordid); *Taraxacum officinale* (Taroff); *Eleusine indica* (Eleind); *Cyperus sp.* (Cypsp); *Echinochloa crus-galli* (Echcrus); *Digitaria sanguinalis* (Digsan); *Euphorbia serpens* (Eupser); *Oxalis conorrhiza* (Oxacon); *Chenopodium album* (Chealb); *Melilotus sp.* (Melsp); *Trifolium repens* (Trifrep); *Polygonum sp.* (Polsp); *Dichondra microcalyx* (Dicmic); *Glycine max* (Glymax); *Sida rhombifolia* (Sidrho); *Zea mays* (Zeamay); *Anagallis arvensis* (Anaarv).

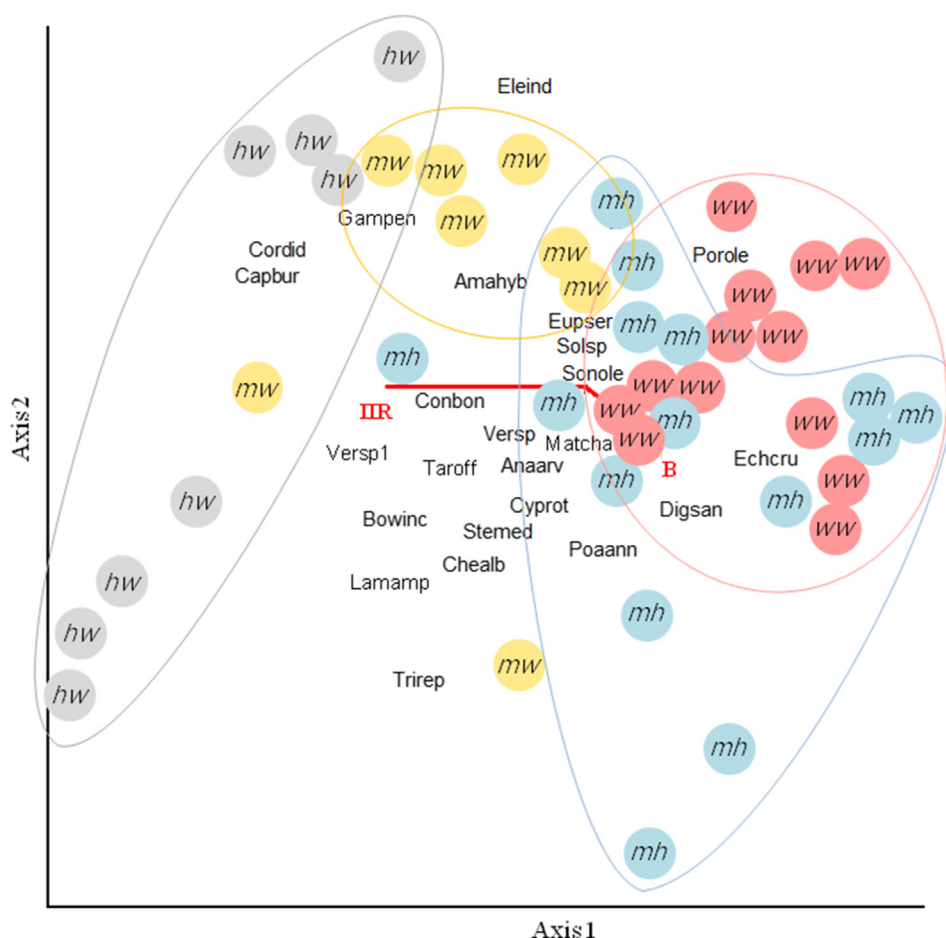
modified weed community within the agricultural fields of the Flat Pampa in the Northwest of Buenos Aires. Intensified crop rotations may not only reduce weed growth and fecundity by weed-crop competition but also weed establishment by crop canopy effect on soil seedbank environment. As IIR increases weed constancy of species needing terminating factors to release dormancy, such as fluctuating temperatures and light may be reduced (Batlla et al., 2019). This could be the case of the absence of *A. hybridus* and *P. oleracea* (Kruk et al., 2006) with high IIR in Exp. 1 during 2019. Moreover, the lack of requirements of terminating factors may explain the high constancy of some species in all rotations, such as *C. bonariensis* (Valencia-Gredilla et al., 2020) and *S. media* (Grundy et al., 2000) (Tables 4, 5, 7, and 8). Sequences with high proportion of cover crops or pastures in the rotations had high IIR. Cover crops could have influenced the weed community, either as living plants or plant residue after the cover crop ends, by modifying the seedbank environmental factors affecting the weed establishment processes and by competing for resources modifying weed growth (Teasdale and Mirsky 2015; Smith et al., 2015).

C in rotation could have directly influenced weed success, through differences in the modes of action of herbicides used to control weeds, the crop canopy characteristics affecting weed – crop competition and the stubble quantity and quality modulating weed emergence. Indirect influence could have been related to crop-specific management practices resulting in different resource availability (Caviglia and Andrade, 2010). In addition, including cereal crops and grass cover crops in the rotation could change some of the soil properties such as organic matter and the residues they produce modulating the seedling establishment processes

by reducing light transmittance to the soil, soil daily maximum temperature, fluctuating temperatures and moisture (Oreja et al., 2020). This could be the case of species such as of *E. indica*, present in mw and absent in mh (Tables 5 and 7), whose emergence declines as crop residues increases (Chauhan and Johnson, 2008). However, these modifications depend on the quantity and type of stubble, which depends on biomass produced by the precedent crops and the quality of the biomass. Usually, cereals produce higher quantities of residues and with higher contents of carbon or higher C/N ratio (Caviglia and Andrade, 2010) than non cereal crops. So, these residues remain longer and produce greater modifications on seedbank environment and during longer periods than residues from non cereal crops, with stronger effects on the weed community.

B was also an important variable in 2018, while HA and OM were explanatory variables in 2019. B, OM and HA were related to IIR and C, since usually the highest values of B and OM and the lowest of HA were related to the highest of IIR and C. B may be related to the influence of pasture, crop and cover crop growth on the weed establishment by affecting the signals that release dormancy of species that are sensitive to the canopy presence (Kruk et al., 2006), weed survival and fecundity by competition. High cover crop biomass production reduces weed growth and fecundity (Moonen and Barberi, 2004; Melander et al., 2017). HA used on each rotation was an important factor determining the weed community composition, filtering species susceptible to specific herbicides used on the rotations (Fried et al., 2019).

The influence of OM as an indicator of the weed community was also reported by Jiang et al. (2018) and Ahmad et al. (2016). Particularly Jiang et al. (2018) reported that in wheat fields *Coryza canadensis* was



**Figure 5.** PCA ordination of the data surveyed in seed bank (Exp 2) during autumn 2019. Rotations code (ww, mw, mh and hw) and weeds code: first 3 letters of the genus and first 3 letters of the species. Vectors represent the main explanatory variables: herbicide applications (IIR) and biomass production (B). Species codes: *Conyza bonariensis* (Conbon); *Lamium amplexicaule* (Lamamp); *Eleusine indica* (Eleind); *Stellaria media* (Stemed); *Portulaca oleracea* (Porole); *Gomchaeta pensylvanica* (Gampen); *Capsella bursa-pastoris* (Capbur); *Amaranthus hybridus* (Amahyb); *Bowlesia incana* (Bowinc); *Veronica* sp. (Versp); *Coronopus didymus* (Cordid); *Trifolium repens* (Trirep); *Chenopodium album* (Chealb); *Taraxacum officinale* (Taroff); *Cyperus rotundus* (Cyprot); *Verbena* sp. (Versp); *Sonchus oleraceus* (Sonole); *Poa annua* (Poaann); *Solanum* sp. (Solsp); *Matricaria chamomilla* (Matcha); *Digitaria sanguinalis* (Digsan); *Echinochloa crus-galli* (Echcru); *Anagallis arvensis* (Anaarv); *Euphorbia serpens* (Eupser).

**Table 7.** Weed communities related to seed bank in each rotation surveyed (Exp. 2) during spring 2018. Floristic groups, species, functions, constancy and total richness. D: dicot, M: monocot, A: annual, P: perennial, AD: adventitial, NAT: naturalized, I: introduced, N: native. Codes are combinations of first letter indicating level IIR: high (h), intermediate (m) and low (w) and second letter indicating level of C: high (h) and low (w).

Floristic group	Species	Families	Morpho type	Growth cycle	Origin	Species constancy (%) in each rotation			
						hw	ww	mw	mh
I	<i>Conyza bonariensis</i> (L.) Cronquist	Asteraceae	D	A	N	100	66.7	100	100
	<i>Amaranthus hybridus</i> L.	Amaranthaceae	D	A	AD	50	66.7	100	100
	<i>Portulaca oleracea</i> L.	Portulacaceae	D	A	AD	50	33.3	50	100
II	<i>Coronopus didymus</i> (L.) Sm.	Brassicaceae	D	A	N	100	100	100	
	<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	D	P	AD	100	66.7	100	
	<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	M	A	AD	50	33.3	100	
	<i>Cyperus</i> sp.	Cyperaceae	M	P		100	33.3	50	
III	<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Poaceae	M	A	I	100	66.7		100
	<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	M	A	AD	100	66.7		100
IV	<i>Euphorbia serpens</i> Kunth	Euphorbiaceae	D	A			33.3	50	100
	<i>Oxalis conorrhiza</i> J. acq.	Fabaceae	D	A	N		33.3		100
	<i>Glycine max</i> (L.) Merr.	Fabaceae	D	A	I		33.3		
	<i>Chenopodium album</i> L.	Chenopodiaceae	D	A	AD	50	33.3		
V	<i>Melilotus</i> sp.	Fabaceae	D			50			
	<i>Trifolium repens</i> L.	Fabaceae	D	P	AD	50			
	<i>Polygonum</i> sp.	Polygonaceae	D	A		50			
	<i>Dichondra microcalyx</i> (Hallier f.) Fabris	Convolvulaceae	D	P	N	50			
	<i>Sida rhombifolia</i> L.	Malvaceae	D	A	N	50			
VI	<i>Zea mays</i> L.	Poaceae	M	A	N			50	
	<i>Anagallis arvensis</i> L.	Primulaceae	D	A	AD			50	
Total richness						15	13	10	7

**Table 8.** Weed communities related to seed bank (Exp. 2) in each rotation surveyed during autumn 2019. Floristic groups, species, functions, constancy, richness and mean cover abundance. D: dicot, M: monocot, A: annual, P: perennial, AD: adventitious, NAT: naturalized, I: introduced, N: native. Codes are combinations of first letter indicating level IIR: high (*h*), intermediate (*m*) and low (*w*) and second letter indicating level of C: high (*h*) and low (*w*).

Floristic group	Species	Families	Morpho type	Growth cycle	Origin	Species constancy (%) in each rotation			
						<i>hw</i>	<i>ww</i>	<i>mw</i>	<i>mh</i>
I	<i>Coryza bonariensis</i> (L.) Cronquist	Asteraceae	D	A	N	100	100	100	100
	<i>Lamium amplexicaule</i> L.	Lamiaceae	D	A	AD	50	66.7	50	66.7
	<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	M	A	AD	50	33.3	100	33.3
	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	D	A	AD	100	100	100	66.7
	<i>Portulaca oleracea</i> L.	Portulacacaceae	D	A	AD	50	66.7	50	66.7
	<i>Gamochaeta pensylvanica</i> (Willd.) Cabrera	Asteraceae	D	B-P	N	100	66.7	100	100
II	<i>Capsella bursa-pastoris</i> (L.) Medik	Brassicaceae	D	A-B	AD	100	33.3	100	
	<i>Amaranthus hybridus</i> L.	Amaranthaceae	D	A	AD	50			
	<i>Bowlesia incana</i> Ruiz & Pav.	Umbelíferae	D	A	N	50		50	
	<i>Veronica</i> sp.	Plantaginaceae				100		50	
III	<i>Coronopus didymus</i> (L.) Sm.	Brassicaceae	D	A	N	100		100	66.7
	<i>Trifolium repens</i> L.	Fabaceae	D	P	AD	50		50	66.7
	<i>Chenopodium album</i> L.	Chenopodiaceae	D	A	AD	50		50	33.3
IV	<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	D	P	AD	50			33.3
	<i>Cyperus rotundus</i> L.	Cyperaceae		P		50			66.7
	<i>Verbena</i> sp.	Verbenaceae	D			50			33.3
	<i>Sonchus oleraceus</i> L.	Asteraceae	D	A	AD	3			66.7
	<i>Poa annua</i> .	Poaceae	M	A		12			66.7
	<i>Solanum</i> sp	Solanaceae	D				33.3	50	33.3
V	<i>Matricaria chamomilla</i> L.	Asteraceae	D	A	AD		33.3		33.3
	<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	M	A	AD		33.3		66.7
	<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Poaceae	M	A	I		66.7		100
	<i>Anagallis arvensis</i> L.	Primulaceae	D	A	AD				33.3
	<i>Euphorbia serpens</i> Kunth	Euphorbiaceae	D	A			33.3		
	Total richness						18	12	13

negatively associated with OM soil content, which agrees with our Results for *Coryza* sp. On the other hand, Ahmad et al. (2016) reported that one of the species with a high positive association with the OM content was *Cyperus rotundus* L., as observed in the weed surveys in the plots as well as in the seedbank experiment (Exp 1 and Exp 2). This species was only present in the rotations with high or intermediate IIR and C, which are usually the rotations with the highest OM values.

Results didn't support the hypothesis that abundance, richness and environmental risks will be reduced through intensification of crop rotation. In both experiments and years, mean species richness and abundance were similar among communities, except for the seedbank experiment in 2019, being higher in *hl* than in *ll*. The lack of differences in richness indicate that, while the weed community change as a result of differential crops and crop management practices, the total number and abundance of weed species does not necessarily change. This is in accordance with previous authors working on different crops and across different management systems (Armengot et al., 2013), but disagree with Satorre et al. (2020) who found that weed species richness was greater under low than high intensifications. The differences could be probably due to the different crops and cover crops included in the rotations, timings of the surveys, approaches (experimental vs observational studies) and tillage systems (no tillage vs conventional tillage). Functional composition was similar among communities, annual dicotyledons species predominate over monocotyledons and there were no marked differences among native, adventitious and introduced species. This result is consistent in weed communities in the Pampas (de la Fuente et al., 2010). Apparent disorder and randomness at one level of integration (species level) can produce order at higher levels (agroecosystem level) (Solbrig, 1993). Functional composition is more stable than floristic composition despite environmental heterogeneity due to rotations.

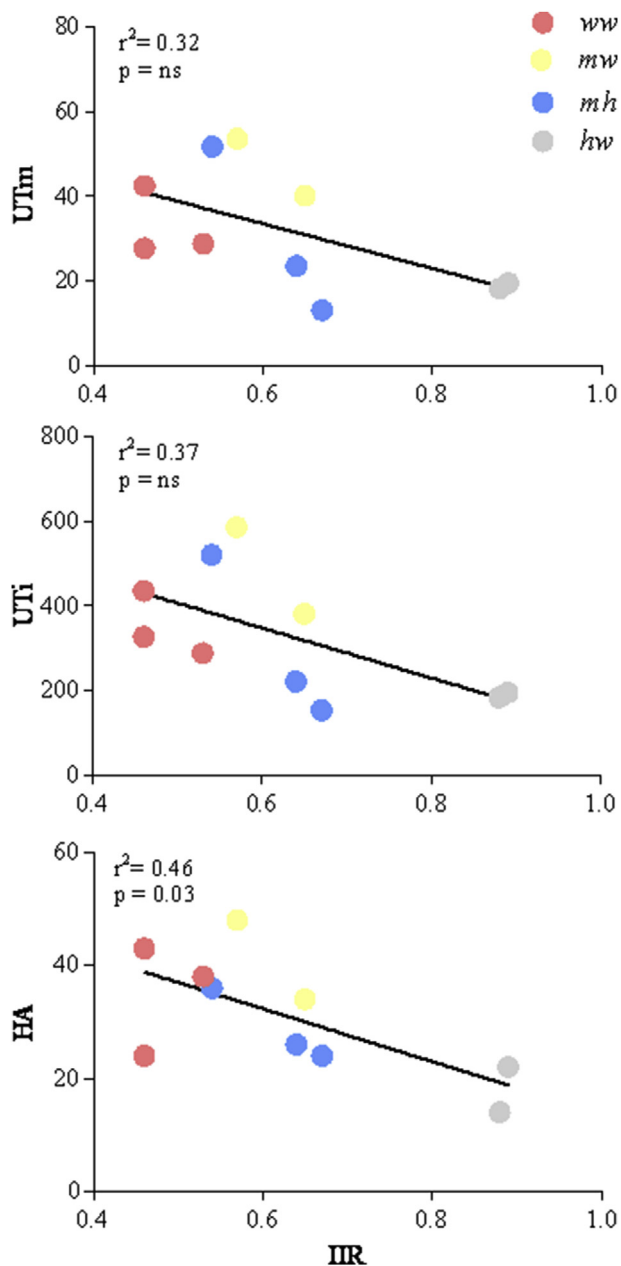
Although the list of species and the floristic groups structuring each community were not the same between experiments and years, the

analysis always identified four different communities related to each rotation (Tables 4, 5, 7, and 8). Considering that there are some environmental differences associated with climate, soil and management among farms, finding differences among rotations indicates that IIR is a very important filter structuring the community.

A group of species with high constancy in all the rotations was found in all experiments and years. This is a common result as, in general, they are species adapted to favorable environmental conditions for crops (de la Fuente et al., 2010). In both years, eight floristic groups were registered in weed surveys and five to six floristic groups were registered in seedbank experiments. Some floristic groups were present in specific rotations and absent in others. Species from groups I and II with a high constancy among rotations, such as *S. media*, *L. multiflorum*, *L. amplexicaule*, *C. didymus*, *S. oleraceus*, *C. bonariensis*, *E. indica*, *A. hybridus* and *P. oleracea*, are reported as highly constant (de la Fuente et al., 2010; Satorre et al., 2020) in other surveys from the region.

Rotations including hairy vetch are expected to have higher levels of nitrogen. This could explain in part the presence of floristic group VII in spring 2018, formed almost exclusively by grasses (*Avena fatua* L., *Bromus* sp. and *Hordeum vulgare* L.) with high response to soil nitrogen, which are absent in rotations without hairy vetch. Pastures filter weed species by competition and modification of the seedbank environment in a similar way to cover crops (Entz et al., 2002). Floristic group III in spring 2018 was the only group of species registered exclusively on this rotation and with forage species probably related to the pasture. The pasture also seemed to have some influence on group V, with typical species of pasture weed communities.

A negative relationship was observed between the number of herbicide applications and the IIR. Since weed abundance was almost invariable among rotations, weed management based on the use of herbicides can be replaced by increasing the IIR to obtain similar Results. In the present work rotations with the high IIR included cover crops, which are



**Figure 6.** Relationship of mammals' toxicological unit TUm (top), insects' toxicological unit TUi (middle) and HA (bottom) with herbicide applications (IIR) in all rotations (*ww*, *mw*, *mh* and *hw*). ns denotes non-significant relationship.

broadly used as a tool to reduce the use of herbicides (Snapp et al., 2005) and the impact of herbicide resistant biotypes (Palhano et al., 2018) and/or tolerant weed species (Cholette et al., 2018). It is accepted that the more diverse the crops in the rotation, the more difficult for problematic weeds to find a niche to establish (Melander et al., 2017). The inclusion of cover crops and winter crops in the rotation reduces the use herbicides like 2,4D, an herbicide widely used to control broadleaf species before summer crops sowing dates and paraquat an herbicide used to control particularly *Conyza* sp in double knock down strategies. Both herbicides presented high TU in agreement with other assessments of environmental risks for 2,4-D (Gaona et al., 2019) and paraquat (Kim and Kim, 2020). Also, cover crops replace the use of some residual herbicides used in the fallow period or before the sowing date of crops (Pittman et al., 2019). Pastures are excellent competitors against weeds and are characterized by the use of low number of herbicides compared with

grain crops; therefore, the inclusion in the rotation is quite positive to reduce the environmental risks of the system. The risk assessment presented here together with other assessments would form an information base from which growers could make decisions based on quantitative differences in risk.

## 5. Conclusions

Surveys and seedbank analysis showed that intensification of crop rotations had a great impact on the floristic structure of weed communities but not in their functional structure or species richness and abundance. Different floristic groups were identified in both experiments and years associated to each rotation and characterized by the presence or absence of different groups. The main explaining variables of the communities were IIR and C. The use of herbicides decreased, and the risk of herbicides tended to decrease as intensification grew. Thus, increasing the intensification and the proportion of cereals of rotations would be a particularly useful tool to manage weeds without affecting species diversity and at the same time reducing the use of herbicides and environmental pollution. The way to increase the IIR could include cover crops, winter crops and pastures in the rotation. Moreover, the sustainable intensification of the rotations may help to protect soil from erosion, increase organic matter content and soil fertility, improve soil structure and water retention capacity, break pest cycles and enhance soil micro-organism activity.

## Declarations

### Author contribution statement

Elba B. de la Fuente; Fernando. H. Oreja; Adriana. E. Lenardis: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marianne Torcat Fuentes: Performed the experiments; Analyzed and interpreted the data.

Belén Agosti: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Antonio Barrio; Alejandra Gil; Martín Marzetti; Eugenia Niccia: Performed the experiments.

Santiago Barberis; Julieta Robredo: Analyzed and interpreted the data.

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### Data availability statement

Data included in article/supplementary material/referenced in article.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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