

Weed communities respond to changes in the diversity of crop sequence composition and double cropping

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Received 17 October 2016

Revised version accepted 10 March 2017

Subject Editor: Guillaume Fried, Anses, France

Summary

Agricultural intensification, besides increasing land productivity, also affects weed communities. We studied weed shifts in cropping sequences differing in the identity and number of crops grown. We also evaluated whether dissimilar weed communities in different cropping systems converge towards more similar communities, when the same sequence is cropped during 2 years. In three locations in the Rolling Pampa, Argentina, field experiments were conducted including five cropping systems in the first year (winter cereal/soyabean, field pea/soyabean, and field pea/maize double crops, and maize and soyabean as single crops), while the same sequence was grown in the following 2 years (wheat/soyabean double crop and maize). Changes in weed community composition and structure were analysed through multivariate analyses and

frequency–species ranking plots. Weed communities differed first among sites, while weed shifts within each site were mainly associated with growing season and crop type. Differences among crop sequences were higher in the first year, mostly related to specific crop grown, rather than to the number of crops in the sequences. Differences were reduced when the same sequence was grown during two consecutive seasons. Frequency of highly common weeds was negatively associated with the number of days with high crop cover. Our findings contribute to understand weed shifts in consecutive growing seasons, which may help readapting crop sequences to reduce the occurrence of abundant weed species.

Keywords: crop cover, crop sequence, crop rotation, cropping system, double cropping, resource use, weed shifts.

ANDRADE JF, SATORRE EH, ERMÁCORA CM & POGGIO SL. (2017). Weed communities respond to changes in the diversity of crop sequence composition and double cropping. *Weed Research*. <https://doi.org/10.1111/wre.12251>.

Introduction

Cropping system design, namely the selection of a crop sequence, is influenced by many off-farm factors, which include the adoption of novel technologies, international grain prices, market policies and the environment. Variability in such factors determines

farmers' decision-making on land use. In Argentina, soyabean (*Glycine max* L. Merr.), introduced as grain crop in the 1970s, became the prevalent summer crop in the 1990s, rapidly displacing maize (*Zea mays* L.). This change in soyabean production in Argentina was related to a high international demand and price of soyabean grain. Moreover, no-till sowing management,

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combined with glyphosate-resistant varieties since 1996, encouraged growing double crops, in which soyabean is sown immediately after the harvest of wheat (*Triticum aestivum* L.), later than its optimum sowing date. These practices consequently increased the number of crops grown each year and the amount of mulch on the soil surface, as well as the soyabean-sown area (Andrade & Satorre, 2015). In the last 5 years, wheat hectareage in Argentina has declined due to domestic market policies, which have accordingly increased the area cultivated with soyabean as single crop and with winter crops alternative to wheat, like barley (*Hordeum vulgare* L.), rapeseed (*Brassica napus* L.) and field pea (*Pisum sativum* L.) (Andrade *et al.*, 2015; <http://www.siaa.gov.ar>).

Land use changes, including modifications in crop sequences and farming practices, unavoidably alter the biotic communities co-existing with crops, such as weeds (de la Fuente *et al.*, 1999; Poggio *et al.*, 2004; Radosevich *et al.*, 2007). Weeds are unwanted by farmers, particularly because of crop yield losses. Integrated weed management strategies can be implemented by designing crop systems to better manage weed problems (Cardina *et al.*, 1999; Radosevich *et al.*, 2007). Crop rotation varies sowing date, growing stage, crop/weed competition dynamics and herbicides, thus reducing weed establishment, growth and fecundity (Covarelli & Tei, 1988; Schreiber, 1992). Moreover, farming practices aimed at increasing crop resource capture can suppress weed growth by competition and reduce establishment by regulating dormancy release factors (Benech Arnold *et al.*, 2000). Weeds can be suppressed by reducing fallow periods or increasing resource capture by crops (Satorre & Ghera, 1987; Poggio, 2005; Poggio & Ghera, 2011). However, both factors have not been tested at the cropping system level by simultaneously reducing fallow periods and increasing both crop resource capture and crop diversity through growing double crops in the crop sequences.

Although double cropping effects on weeds have not yet been experimentally investigated, some studies have evaluated the effects of growing cover crops before summer crops on weed communities (Teasdale, 1993; Teasdale & Mohler, 2000; Brennan & Smith, 2009). Liebman and Davis (2000) indicated that cover crops can suppress weed establishment and growth by changing the environmental factors regulating germination and by competing for resources. Consequently, the number of weed seeds infesting the following crop is reduced. Double cropping in the Argentine Pampas is similar to such systems including cover crops, mostly consisting in growing a summer crop after the harvest of the precedent winter crop. Thus, crop rotation

effects could be added to the increased competition mentioned for double cropping (Liebman & Dyck, 1993). All changes in cropping techniques described also modify species composition in weed communities, known as weed shifts (Swanton *et al.*, 1993; Ghera & León, 1999; Martinez-Ghera *et al.*, 2000).

Our aim here was to study the changes in weed communities in different cropping sequences, which differ in the crops starting the sequence. We tested whether the initial crop and associated management make weed communities' composition diverge or converge after applying the same management in the following cropping seasons. We expect weed communities to differ due to the effects of different opening crops. Moreover, we evaluated how the resource use patterns of crops in the sequences are associated with the changes in weed communities' structure, determined by the frequency of each weed species in the community. We expected that the temporal patterns of resource use, particularly the interception of solar radiation by crops, will explain those changes. To test these predictions, weeds were surveyed during the warm seasons of three consecutive growing seasons in three field experiments located in different sites in the Rolling Pampa, Argentina. Crop productivity and resource use patterns were simultaneously characterised in the same field experiments (Andrade *et al.*, 2015, 2017). Our research contributes to understand how changes in weed communities could be predicted and, therefore, may help to readapt crop sequences with the aim of restraining the occurrence of troublesome weed species in subsequent growing seasons.

Materials and methods

Study sites and experimental conditions

Three experiments were conducted under rainfed conditions in the Rolling Pampa, Argentina. Experimental areas were managed as land use units in previous growing seasons (i.e. cropped fields). Although seven crop sequences were included in the experiments (Andrade *et al.*, 2015, 2017), weeds were only surveyed in six treatments. During the first season, the opening crop systems evaluated were wheat/soyabean, barley/soyabean, field pea/soyabean, and field pea/maize as double crops, and maize and soyabean as single crops. Given the high similarity of weed communities in wheat and barley crops, we decided to present them as a single treatment, hereafter referred as 'cereal/soyabean'. In the second and third years of the study, all plots were treated uniformly by growing wheat/soyabean as double crop in year two, and a single maize crop in year three.

One experiment was situated close to Junín (34° 23' S; 60° 48' W), another at Pergamino (33° 55' S; 60° 23' W) and the other next to San Pedro (33° 47' S; 60° 00' W), in Buenos Aires province. Soils in these locations are deep Argiudolls with approximately 3.3% organic matter in the topsoil. Crop genotypes, sowing date, density and row spacing were based on technical recommendations for the region to obtain the highest attainable yield [see Table S1 for details on sowing dates of season 1, and Andrade *et al.* (2015, 2017) for details regarding crop management and site conditions]. Herbicide products applied are presented in Table S2. Each treatment was assigned to plots of 4400 m² (200 m length by 22 m width), with two repetitions per site. Experiments were conducted under the no-till system with the typical machinery used by farmers, with the aim of generating similar conditions to the grain cropping systems of the region.

Soil nutrient status was analysed 20 days before sowing. To complement soil nutrition and supply future crop demands, fertilisers were then applied at sowing according to technical recommendations for the Rolling Pampas (Andrade *et al.*, 2015, 2017). Soyabean and field pea seeds were inoculated before sowing with *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* var. *pisi*, respectively.

Weed sampling

Weed communities were surveyed in February 2011, February 2012 and November 2012. The first assessment was made in maize and soyabean crops, growing either as single crop or as the second crop in double crops, to characterise weed communities standing at the end of the first season. The second and third surveys were carried out before the application of the last herbicide in double-cropped soyabean (2011/2012) and maize crops (2012/2013). Surveys were performed by two trained persons, who walked in zigzags throughout the central area of plots and avoiding their edges. At each plot, the presence of weed species was recorded in 80 quadrat samples of 0.1 m² along the zigzag, registering the number of quadrats where each species occurred, no matter its plant size or phenology (Mas *et al.*, 2010). After that, the frequency of occurrence was calculated for every species.

Temporal patterns of radiation use by crops

To estimate the restriction imposed by crops, the space and time occupancy of crops during winter–spring–summer of the first season was calculated. The fraction of incoming solar radiation reaching the soil was measured with a photosynthetic photon flux sensor bar

(Cavadevices Argentina; <http://www.cavadevices.com>) every 10–15 days, at noon, from emergence to maturity. Values in between measures were extrapolated. Then, the number of days on which each treatment crops intercepted a high proportion of solar radiation (>75%) was quantified. That number was used as a measure of the period in which crops dominated the above-ground space, thus presenting high capacity to suppress the establishment and growth of companion weeds.

Data analysis

Weed communities surveyed were described in terms of floristic composition. Multivariate and exponential regression analyses were performed to investigate whether species composition and frequency in the weed communities were affected by any of the crop sequences proposed. Treatments within each site were grouped according to the summer crop grown during the first season to perform a non-metric multidimensional scaling (NMDS) analysis using distance matrices, which is based on Bray–Curtis dissimilarity (Clarke, 1993). NMDS was performed (9999 permutations) with the metaMDS function in the vegan package for R (R Development Core Team, 2014). Function metaMDS uses isoMDS to perform NMDS, but tries to find a stable solution using several random starts (function initMDS) (Anderson, 2001). Unlike multivariate methods that attempt to maximise the variance or correspondence between objects in an ordination, NMDS attempts to represent, as closely as possible, the pairwise dissimilarity between objects in a low dimensional space (two in this case). Then, differences in floristic composition among sites, as well as between groups of treatments along the three sampling seasons at each site, were evaluated with permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001), which was implemented with the adonis function (vegan package for R). The pseudo *F*-ratio statistic was used to compare the total sum of squared dissimilarities among plots belonging to different groups to that of plots belonging to the same group. The pseudo *F*-ratio statistic was calculated as follows:

$$F = \frac{[SS_A/(a-1)]}{[SS_W/(N-a)]} \quad (1)$$

where SS_W is the sum of squared dissimilarities within groups, SS_A is the sum of squared dissimilarities among groups, a is the number of groups, and N is the total number of plots. The terms $(a-1)$ and $(N-a)$ are the degrees of freedom associated with the explanatory factor (the grouping variable) and the residuals respectively (Anderson, 2001).

Changes in weed community structure were analysed through the frequency–ranking relationship to determine the variation of space occupancy of the weed species. Negative exponential regressions (least squares method) based on frequencies of each species were adjusted after ordering from the most to the least frequent species. Then, the intercept and k of the function for each treatment at each survey were determined. The intercept determines how common the most frequent weed species are in each crop treatment, while k represents the evenness of the community and is negatively associated with the dominance of the most common weed species in the community. Parameters of function-adjusted regression (k and intercept), among treatments, were compared by analysis of variance, and then correlated with the number of days with high crop cover in season one. Also, land use intensification was analysed comparing sequences that started with double crops against those with single crops.

Results

Weed community composition

A total of 22 weed species were recorded from the three experiments (15.84 hectares), with 15, 13 and 19 in San Pedro, Pergamino and Junín respectively (Table 1). Most frequent weed species, as the average from the three experiments, were *Echinochloa colona* (L.) Link, *Digitaria sanguinalis* (L.) Scopoli, *Stellaria media* (L.)

Vill., *Conyza bonariensis* (L.) Cronquist var. *bonariensis*, and *Anoda cristata* (L.) Schltld. *Oxalis conorrhiza* Jacq. and *Datura ferox* L. were conspicuous weed species in Junín, while *Commelina erecta* L. was more important in San Pedro [see Table S3 for detailed survey data].

Species composition of weed communities differed between sites (PERMANOVA; $F_2=77.18$; $P < 0.001$). Sites were analysed separately due to the significant interaction between sites and treatments (crop sequence; $F_8 = 2.76$; $P < 0.001$). Although treatment effects were significant ($F_4 = 4.20$; $P < 0.001$), variations in the floristic composition were mainly associated with the particular crop type growing at the sampling time, whether of soyabean or maize. For the same reason, weed communities changed in subsequent years ($F_2 = 31.06$; $P < 0.001$), thus following patterns associated with the crop sequence selected in all plots. Floristic differences between groups of plots with maize and soyabean in the first year were characterised by different contours, which had little or no overlapping areas along the three seasons (Fig. 1, NMDS; PERMANOVA: $F_7=4.33$; 6.16; 13.94 for San Pedro, Pergamino and Junín, respectively; $P < 0.01$). However, the overlapping of those groups of plots increased in two experiments after undergoing the same sequence of crops in the second and third years (Fig. 1).

Weed frequency changes

The structure of weed communities was analysed through the k and intercept values of the regression

Table 1 Scientific name, EPPO code (Bayer code) and mean frequency (%) of weeds along the three surveys at each site. Weeds are ordered from the most to the less common species as an average of the three sites. See Table S3 for full data

	Scientific name	Bayer code	San Pedro	Pergamino	Junín	Average
1	<i>Echinochloa colona</i> (L.) Link.	ECHCO	14.0	31.3	1.58	15.63
2	<i>Digitaria sanguinalis</i> (L.) Scopoli.	DIGSA	0.56	0.27	34.2	11.68
3	<i>Anoda cristata</i> (L.) Schltld.	ANVCR	3.33	0.40	11.4	5.04
4	<i>Stellaria media</i> (L.) Vill.	STEME	0.08	14.4	0.17	4.88
5	<i>Conyza bonariensis</i> (L.) Cronquist var. <i>bonariensis</i>	ERIBO	4.69	1.15	6.63	4.16
6	<i>Commelina erecta</i> L. var. <i>erecta</i>	COMER	10.8	–	0.52	3.77
7	<i>Datura ferox</i> L.	DATFE	0.04	0.04	4.02	1.37
8	<i>Coronopus didymus</i> SM.	COPDI	3.49	0.13	0.67	1.43
9	<i>Oxalis conorrhiza</i> Jacq.	OXACH	0.08	0.04	3.48	1.20
10	<i>Tagetes minuta</i> L.	TAGMI	–	–	2.48	0.83
11	<i>Sonchus oleraceus</i> L.	SONOL	1.65	0.13	0.67	0.82
12	<i>Xanthium spinosum</i> L.	XANSI	–	–	1.96	0.65
13	<i>Euphorbia serpens</i> Kunth.	EPHSN	–	0.58	1.35	0.64
14	<i>Chenopodium album</i> L.	CHEAL	0.06	–	1.33	0.46
15	<i>Lamium amplexicaule</i> L.	LAMAM	–	1.35	–	0.45
16	<i>Xanthium cavanillesii</i> Schouw	XANSP	1.00	–	–	0.33
17	<i>Portulaca oleracea</i> L.	POROL	0.10	0.29	0.44	0.28
18	<i>Amarantus quitensis</i> Kunth	AMAQU	–	–	0.31	0.10
19	<i>Dichondra microcalyx</i> (Hallier f.) Fabris	DIORM	0.10	–	0.08	0.06
20	<i>Cirsium vulgare</i> (Savi) Ten.	CIRVU	–	0.04	0.13	0.06
21	<i>Solanum sisymbriifolium</i> Lam.	SOLSI	0.17	–	–	0.06
22	<i>Taraxacum officinale</i> G. Weber ex F.H. Wigg.	TAROF	–	–	0.03	0.01

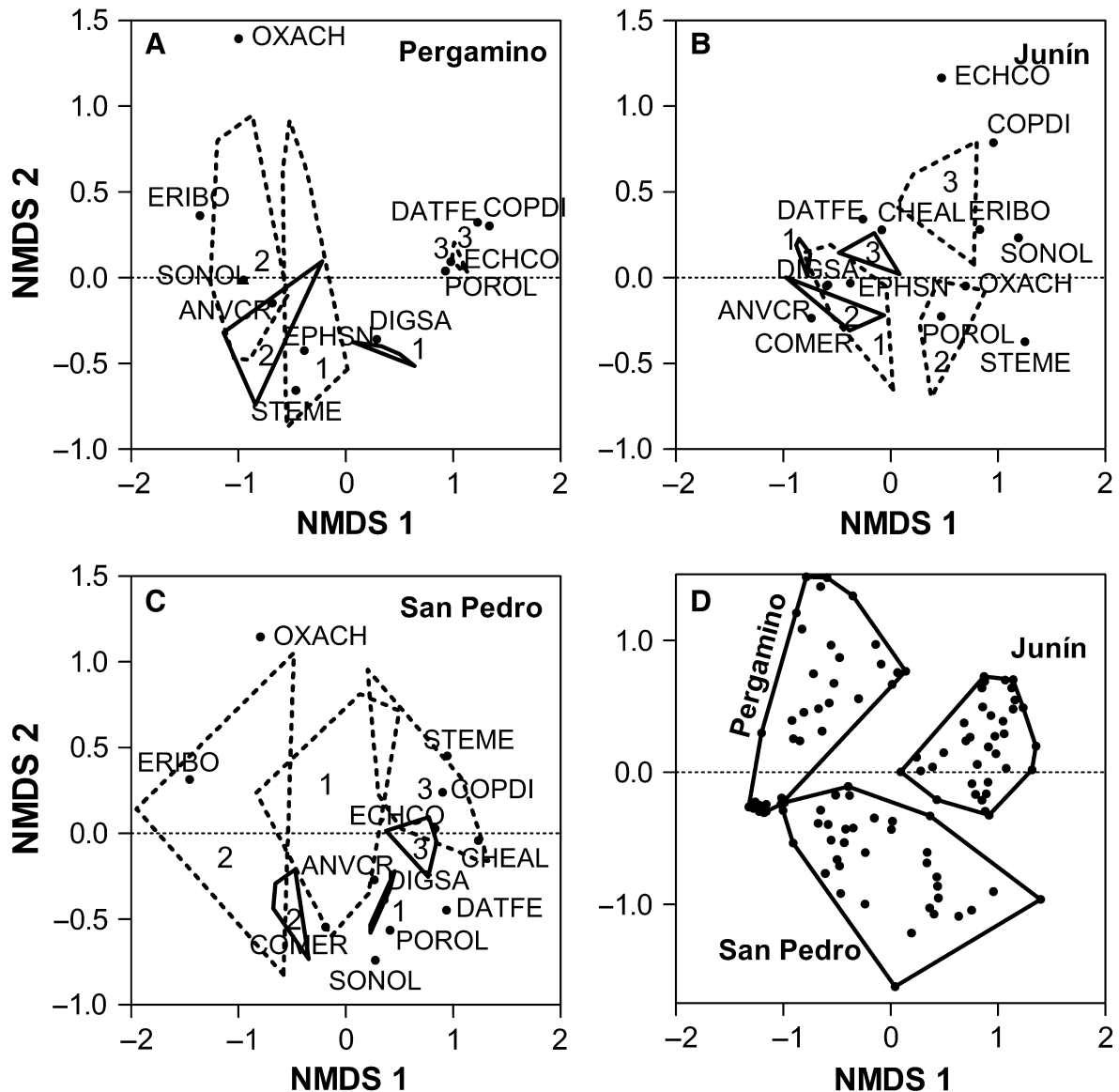


Fig. 1 Non-metric multidimensional scaling analysis (NMDS) based on similitude analysis among sites for the three seasons (D), and among treatments with different initial summer crop on each site (A, B and C). Surveys carried out in the first, second and third seasons are indicated with a contour labelled with the number 1, 2 and 3 respectively. Treatments were grouped according to the summer crop grown during the first season. Discontinuous lines correspond to sequences initiated with soyabean as summer crop (cereal/soyabean, field pea/soyabean and soyabean single crop), whereas continuous lines correspond to sequences initiated with maize as summer crop (field pea/maize and maize single crop). Weed species are indicated with their respective EPPO code (Bayer code).

adjusted in Fig. 2. The evenness (k) of weed communities did not differ among treatments ($P > 0.1$). However, the frequency of the most common weeds (intercept) presented significant differences among treatments and surveys ($P < 0.01$), without interactions between them.

Weed communities differed considerably based on initial crop. Treatments which opened the sequence with single maize crops presented the highest intercept value ($P < 0.05$), while the treatment that opened with cereal/soyabean tended to present the lowest intercept values among the three seasons. The effects of these two

cropping systems were also evident after the second and third seasons. The intercept was depleted in all treatments after cultivating wheat/soyabean in season two ($P < 0.05$). In contrast, all treatments had higher intercepts in the maize crops grown in the last season (Fig. 3).

Double cropping tended to reduce the frequency of most common weeds (intercept value). The intercept was higher with the single maize crop compared with field/pea maize double crop ($P < 0.05$), and a similar trend occurred in soyabean crops, with weed frequencies higher when grown alone than in cereal/soyabean double crop. Differences among treatments were still

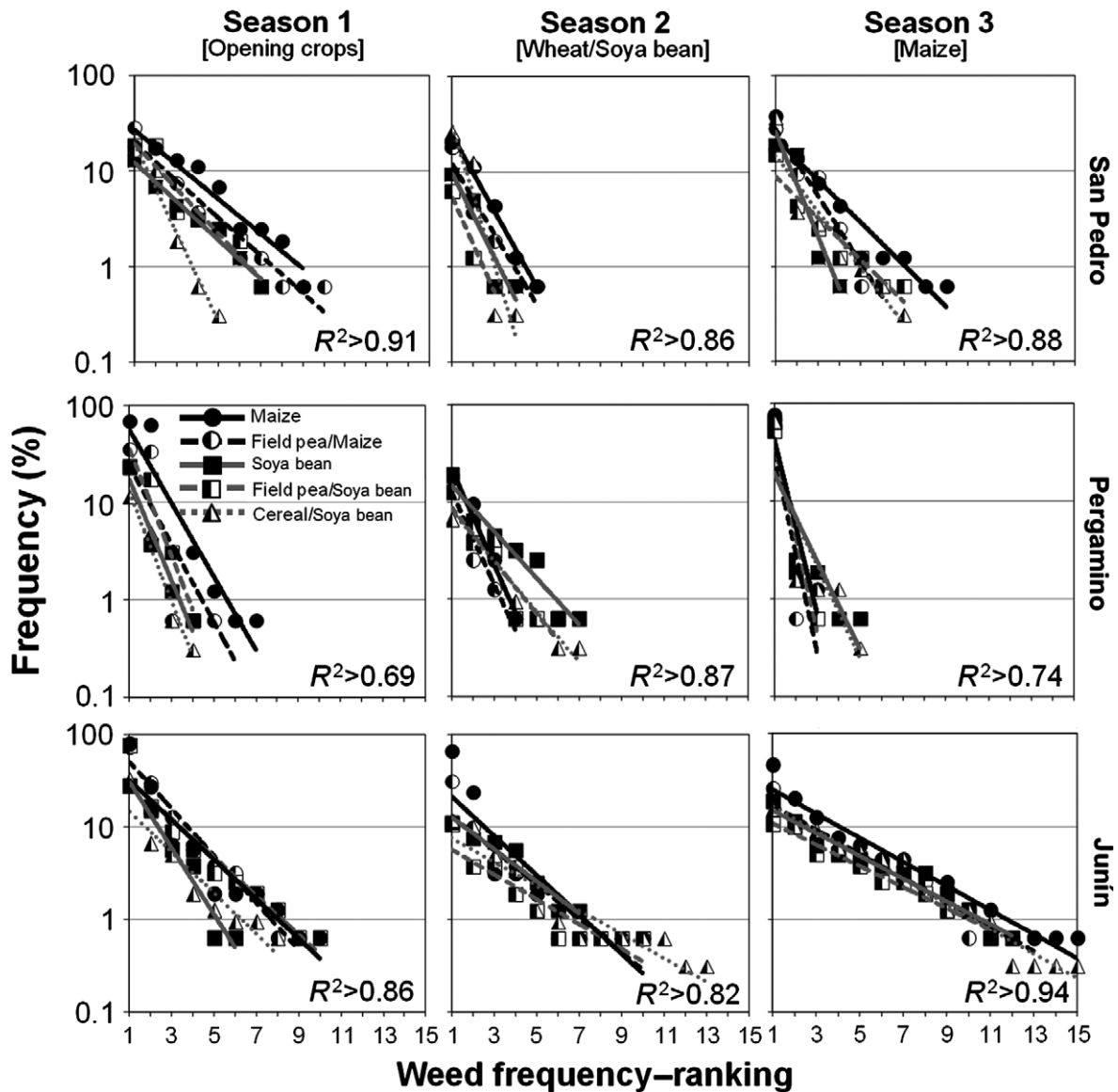


Fig. 2 Weed species frequency as a function of the frequency–ranking in the community for the three seasons at each site. Negative exponential regressions were adjusted after ordering from the most to the least frequent species.

detected in the second and third seasons, when the same crops were established in all plots (Fig. 3).

Growing conditions for weeds

Cropping systems greatly differed in the interception patterns of solar radiation during the first year. Consequently, the temporal dynamics of radiation interception through that first season and global annual non-intercepted solar radiation available for weeds greatly differed among treatments (Fig. 4). Radiation intercepted by single crops was obviously null during autumn and winter and low during spring. When field pea was grown before summer crops, a higher proportion of solar radiation during spring was captured compared with single summer crops and, to

some extent, delayed the crop cycle of the second summer crop (Table S1). Growing winter cereals increased the interception of solar radiation in the cool season, even during winter. The highest differences among treatments were found in San Pedro, given the clayey soil type that intensified some drought effect during summer (Fig. 4). Differences among plots were smaller in the second and third seasons, given that the same crop sequence was grown in all treatments.

Association between weed community structure and crop type

At the end of the first season, there was a negative correlation between the intercept of the adjusted functions in Fig. 2 and the number of days with high cover of

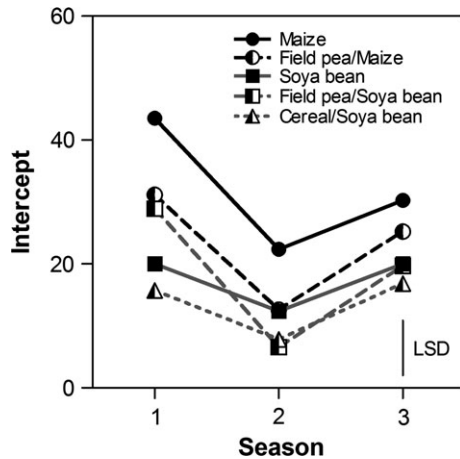


Fig. 3 Average intercept values of the adjusted regressions in Figure 2 for each treatment along the three seasons. Each intercept value is the average of the three experimental sites. LSD: Least significant difference ($P < 0.05$) for comparison of means for the three seasons.

crops ($P < 0.01$; $R^2 = 0.48$; Fig. 5). The number of days with high crop cover in the first season was also negatively associated with the intercept of the fitted functions in the second season ($P < 0.01$; $R^2 = 0.52$; Fig. 5), thus showing some residual effect in the structure of weed communities. In the last season, those two variables were not associated ($P > 0.1$; Fig. 5).

Discussion

Our research showed that weed community composition differs when accompanying different crops during the same growing season, while it may converge after applying the same management in the following seasons. However, the impact of starting with particular crop types on the frequency of the most common weeds may remain in the following seasons. The highest differences in weed communities were found among sites and, in second place, due to the crop species growing at survey time. These results agree with previous research (Anderson & Milberg, 1998; Smith & Gross, 2007; Meiss *et al.*, 2010; Pinke *et al.*, 2016), but our findings have been obtained by applying an original experimental approach in field conditions.

Most studies on the effect of crop sequences on weed communities are based on observational approaches in crop fields (de la Fuente *et al.*, 1999; Suárez *et al.*, 2001; Lososová *et al.*, 2004; Poggio *et al.*, 2004; Fried *et al.*, 2015), whereas experimental approaches, usually applied in plots relatively smaller than a crop field, have been used for testing the effects of agricultural practices, such as fertilisation, ploughing or herbicide use (Pyšek & Lepš, 1991; Bärberi *et al.*, 1998; Doucet *et al.*, 1999). Our study covers an

unexplored scale of analysis between observational and experimental approaches on weed communities, and at the same time, contributes to link them both.

In our study, herbicides were applied with the purpose of maintaining weed density below the thresholds of significant damage according to technical recommendations for the region. Herbicide applied therefore varied among treatments in the first year. Thus, the experimental design does not allow disaggregating the effects of herbicide management from those of sequences. In future long-term field experiments at large scales, several factors should be considered to elucidate the effects of chemical control in different crop rotations, such as the timing of application and the use of residual herbicides.

Crop identity determines weed community composition

Our results confirmed that crop identity determines the composition of associated weed communities (Anderson & Milberg, 1998; Poggio *et al.*, 2004; Smith & Gross, 2007; Fried *et al.*, 2008). Contours of the same season, either if open with maize or soyabean, change positions together in seasons 2 and 3 (Fig. 1). This indicates that crop identity was the most important source of variation at each site. Moreover, the differences generated in the first season were attenuated in the following two seasons, when the crop sequence was the same for all treatments.

When agricultural systems are studied, efforts are focused on the disturbance patterns and the magnitude of the effects generated, although the response largely depends on the initial condition of the system (Booth & Swanton, 2002). The process explained in the previous paragraph occurred in two of three sites. In Junín, weed community among treatments did not converge when the crop sequence was unified given a high frequency of *D. sanguinalis* in the entire experimental area. This was evident in the contour distribution around this weed species in the multivariate analysis (Fig. 1), being closer to those plots that open with maize, either as single or second crop. Despite past crop management was similar among sites, it seems that in Junín previous weed management was ineffective in controlling *D. sanguinalis*, which might explain the high abundance of this weed species.

Land use intensification reduces weed frequency

The frequency of the most common weed species was reduced in treatments combining high temporal land occupancy with high crop cover. Weed suppression by reducing fallow periods or by increasing crop

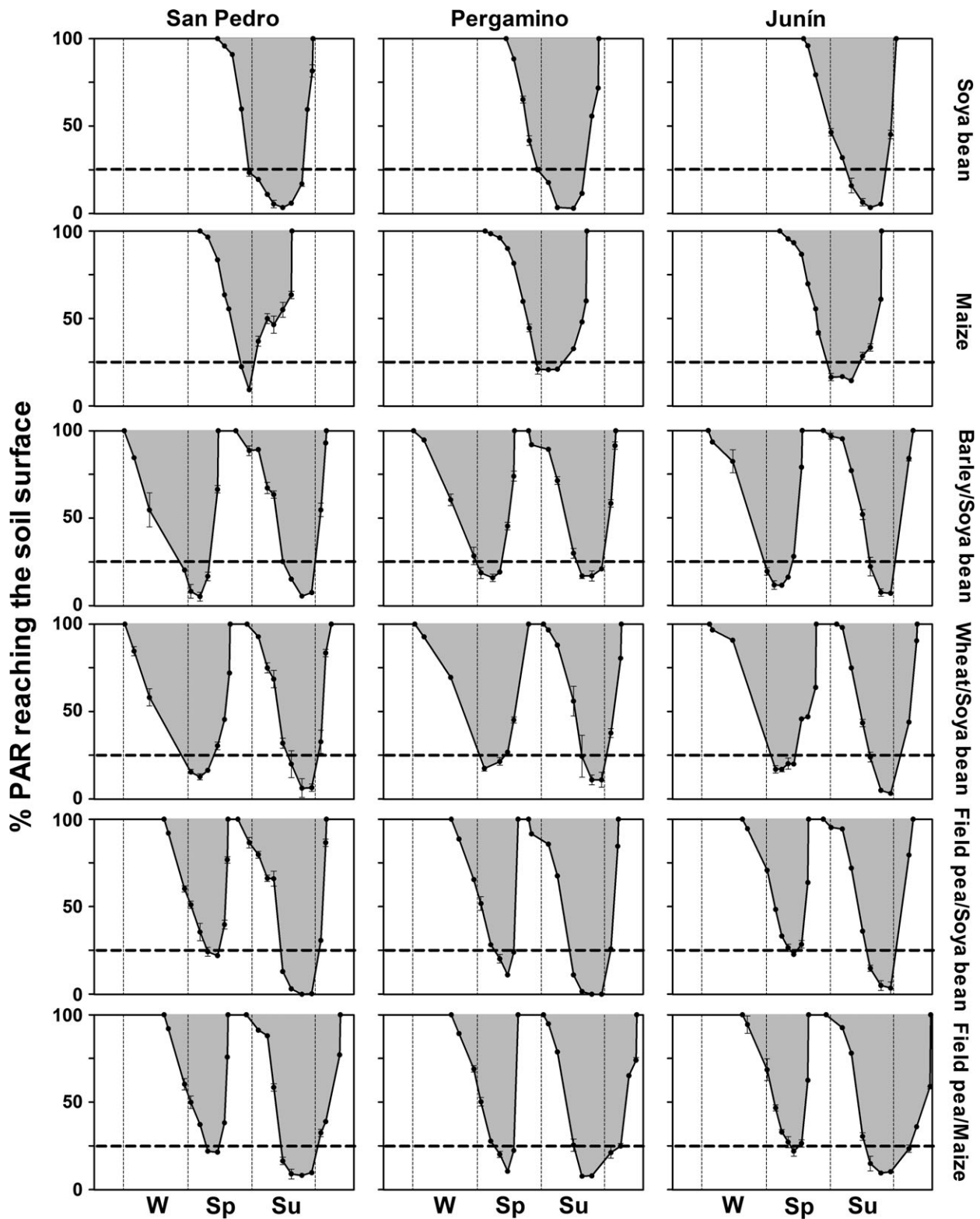


Fig. 4 Dynamics of photosynthetically active radiation (PAR) reaching the soil surface during the first season for all treatments in the three sites. Horizontal dashed lines indicate 75% of PAR intercepted by crops. Vertical lines indicate standard deviation. W: Winter; Sp: spring; Su: summer.

cover had been reported in previous investigations (Satorre & Ghersa, 1987; Hald, 1999; Liebman & Davis, 2000; Poggio, 2005; Meiss *et al.*, 2010; Poggio

& Ghersa, 2011). Here, the number of days with high crop cover (>75% of intercepted radiation) was considered to combine both processes. The intercept of

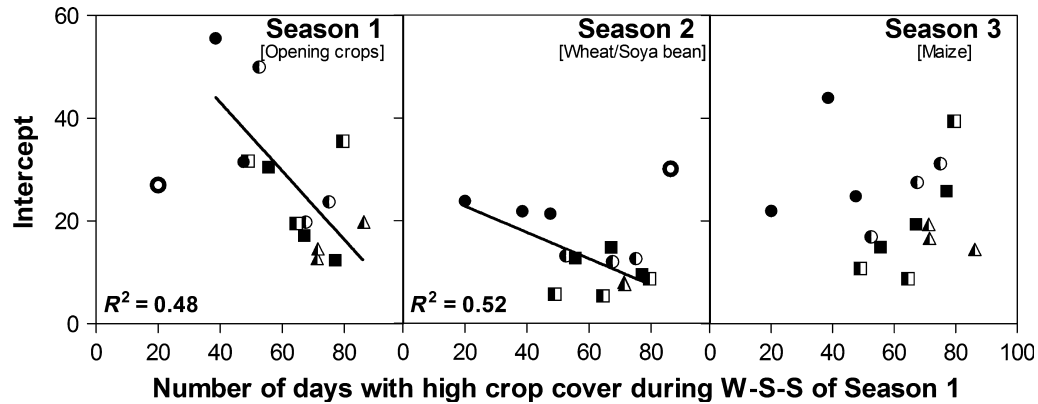


Fig. 5 Intercept values of the regressions fitted in Figure 2 as a function of the number of days with crop cover higher than 75% during winter, spring and summer of season 1, indicated in Figure 4. Black circles correspond to sequences opening with single maize crop, black/white circles with field pea/maize, black squares with soyabean single crop, black/white squares with field pea/soya-bean and black/white triangles with cereal/soyabean. Open circles were considered outliers given that those growing crops underwent high water stress in Season 1 (maize single crop; Andrade *et al.*, 2015) and nutritional stress in Season 2 (wheat; Andrade *et al.*, 2017).

the frequency–rank-adjusted function decreased on treatments with high crop cover during extended periods of time (i.e. double cropping, especially cereal/soyabean double crop), even at some point in the following season (Fig. 5). Moreover, the increased amount of mulch in double cropping (Andrade *et al.*, 2015) could have enhanced weed suppression (Teasdale, 1993; Teasdale & Mohler, 2000). Despite the frequency changes in the most common weeds, evenness in the community (represented by k) was almost unaffected, suggesting that frequency of the less common weeds followed the same direction of changes as the dominant species.

The analysis of intercept variations determined how frequent the most common weed species were in each crop treatment for the three cropping seasons, which also showed some residual effects of the opening crops on the intercept values (Fig. 3). The reduction of intercept values by double cropping was also evident in the lower intercept values after the second season, when wheat/soyabean double crops were grown in all plots. After that, frequency of most common weeds increased (Fig. 3), because maize intercepted a low amount of radiation during the third season (Andrade *et al.*, 2017).

Conclusions

Weed shifts were mainly associated with crop identity. Weed frequency changes were influenced by temporal patterns of resource use by crops, particularly of solar radiation. Cropping systems with high resource exploitation reduced the frequency of the most common weeds. For these reasons, weed shifting dynamics may

serve as an indicator to redesign crop production systems. Our research, albeit carried out during a restricted period, contributes to a better understanding of how changes in weed communities in subsequent growing seasons can be predicted. Our findings may thus help readapt crop sequences with the aim of reducing the occurrence of abundant weed species. The observed residual effects may require conducting long-term experiments to evaluate effects over longer periods.

Acknowledgements

Authors thank the CREA advisors' group of Northern Buenos Aires province, who collaborated in the experimental design, and to Ing. Agrs. Lucas Ratto, Juan Pablo Cobutta and Ezequiel Gandino for their collaboration in weed surveys. JFA and SLP are members of CONICET, the National Research Council of Argentina.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Sowing date for all crops in all treatments evaluated as the average of the three experiments in season 1.

Table S2 Herbicides used in all treatments at the three sites over each season.

Table S3 Weed frequencies (%) for every crop sequence over the three seasons as the average of two replicates at each site.