

1 **Crop rotation effects on weed communities of soybean (*Glycine max* L. Merr.) agricultural fields**
2 **of the Flat Inland Pampa**

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26

26s Abstract

28 Extensive grain crop production systems in the flat inland Pampas mainly include soybean, double
29 crop wheat-soybean and maize in rotation. Due to difficult-to-control weed problems farmers are
30 tending to intensify the rotations in their fields by increasing the number of double crops or by
31 including cover crops before the main crop. Land use intensification may be characterized using
32 the intensification sequence index (ISI), which is the number of crops *per year* considering all
33 crops sown in a particular period; *i.e.* the average number of crops sown in a time unit. To
34 determine how agricultural intensification and crop sequences may modify weed communities, 31
35 soybean fields of commercial farms located in the Flat Pampa of Argentina were surveyed.
36 Frequency of individual weeds within the fields was determined and various statistical methods
37 were used to evaluate changes in weed community composition or function due to intensification
38 (ISI level). A total of fifty-three species, mostly therophytes (28 species), were recorded in
39 soybean crop fields at harvest. Three weed communities were identified, which were related to
40 the ISI level of the fields and to the number of years continuously sown with grain crops (*i.e.*
41 number of years since the last pasture). Weed community under intensified fields was
42 characterized by low species richness ($p<0.05$); *i.e.* the number of weed species was reduced
43 when more crops were sown per year. However, total weed frequency (weed abundance) and
44 weed functional groups were not significantly reduced by field intensification. Since weed
45 problems in grain crops of the Pampas are increasing, mainly due to herbicide resistant weeds,
46 the use of ISI as part of an integrated weed management strategy is discussed.

47

48 Keywords

49 Crop rotations, intensified crop sequences; soybean; weed shift; integrated weed management

50

51

52 1. Introduction

53 The Flat Pampa is one of the five highly productive Pampa ecological zones (Hall et al,
54 1992). It has experienced over the past 50 years huge agricultural changes mainly driven by the
55 expansion of soybean (*Glycine max* L. Merr.) crops. Soybean was introduced as grain crop in the
56 1970s and became the most important summer crop since the 1990s (Satorre, 2001; 2005; 2011).
57 At present, transgenic soybean is sown in almost 60% of flat Pampas with a narrow package of
58 agricultural practices such as no-till cropping, glyphosate resistant varieties, and sowing dates as
59 single-crop or double-crop soybean. The soybean centered crop system has influenced arable
60 weed species shifting. In fact, Pampa's weed community composition was modified and weed
61 abundance was reduced during the early stages of the expansion process of soybean (Vitta et al.,
62 2004; Puricelli and Tuesca, 2005; de la Fuente et al., 2006; Mas et al., 2010). However, recently
63 herbicide resistant weeds have progressed from only one reference in the early 90's to more than
64 27 different biotypes comprising 14 species mostly appearing during the past 10 years (REM
65 AAPRESID, <http://www.aapresid.org.ar/rem/>). Herbicide tolerant and herbicide resistant weeds
66 became the main agriculture problem (Satorre, 2016) and the simplification and repetitive
67 disturbances of the crop production system has been pointed as the main cause (Martínez-Ghersa
68 et al., 2000; Scursoni and Satorre, 2010; de la Fuente and Satorre, 2016). In response to this, the
69 number of herbicide applications and their rate was increased while moment and type of
70 herbicide applications (i.e from systemic foliar absorbed herbicides to residual soil applied
71 herbicides) were simultaneously changed. These have raised concerns, mainly from the
72 associated risks on production (due to cost and phytotoxicity increases), environment and human
73 health. To reduce the negative influence of such weed management changes, land use intensified
74 crops such as double-crops and cover crops have been proposed. Land use intensification is here
75 referred as the number of crops *per* year considering all crops sown in a particular rotation period
76 which is usually quantified by the intensification sequence index (ISI).

77

78 Weed communities may be greatly affected by crop rotation since various, sometimes
79 different, herbicides are applied and some crops may strongly compete for resources (Leroux et
80 al., 1996; Bàrberi et al., 1997; Doucet et al., 1999; Gulden et al., 2010). Moreover, intensified crop
81 rotations (i.e. a rotation with high ISI) may not only reduce growth and fecundity but also weed
82 establishment. The reduction of the fallow period or its absence may reduce weed establishment
83 by regulating dormancy release factors (Satorre and Ghera, 1987; Benech Arnold et al., 2000;
84 Poggio, 2005; Poggio and Ghera, 2011; Andrade et al, 2017). However, the extent to what can be
85 expected as community changes from intensifying crop rotations, have not been documented in
86 real on farm crops.

87

88 In the Flat Pampas soybean still is the main crop; although in the last campaign the area
89 under soybean was slightly reduced and that under maize was increased. Few other summer crop
90 species such as sunflower and sorghum are sporadically sown (Ministerio de Agroindustria, 2019;
91 <https://datos.agroindustria.gob.ar/dataset/estimaciones-agricolas>). Despite the prevalence of a
92 reduced number of winter (wheat and barley) and summer crop species (soybean and maize),
93 individual fields experienced various crop sequences and levels of intensification including almost
94 single soybean monocultures to more intensified complex rotations. In this region long time
95 changes in the structure and functioning of weed communities following the expansion of
96 agriculture from mixed beef cattle-grain crop to permanent grain crop production systems have
97 been reported (Ghera and León, 1999; de la Fuente et al., 1999, 2006, 2010; Mas et.al., 2010;
98 Poggio et al., 2010, 2013;). However, little attention has been paid to short term forces of change.
99 Increases in intensification levels (i.e. the set of rotations with greater ISI levels) could rapidly
100 affect the weed communities in the region. A recent experimental approach with two different
101 land intensification levels in the Pampa showed that the frequency of highly common weeds was

102 negatively associated with the number of days with high crop cover (Andrade et al., 2017b). In
103 this paper, the hypothesis that floristic composition and richness of weed communities is changed
104 and that the frequency of predominant weed species is reduced by agricultural intensification
105 levels on farm fields under various crop sequences were tested. For this purpose, commercial
106 fields under a wide range of ISI management levels were evaluated to detect short term weed
107 community shifts on species composition, richness and on species frequency in soybean fields of
108 the Flat Pampas of Argentina.

109

110 **2. Materials and methods**

111 Weed surveys were performed in commercial fields within selected farms located in the
112 main grain crop producing area of the Flat Pampas in the northwest of Buenos Aires province,
113 Argentina (34° 59' to 35° 06' South, 60° 23' to 60° 37' West, approximately; Fig 1 inset). Surveys
114 were performed in soybean fields during the summer of 2012, 2013 and 2014. In the region,
115 climate is temperate, mild and humid with hot summers, historic annual precipitation average is
116 993 mm and historic average temperature is 16 °C. Total summer rainfall was 545.3 mm in 2012,
117 391.03 mm in 2013 and 496 mm in 2014. Fields were chosen after analyzing a historical data base
118 of two groups of farmers (Bragado and Alberdi, Buenos Aires, Argentina) that belong to AACREA
119 organization (<https://www.crea.org.ar/>) Fields represented the variability of actual rotations and
120 crop management of nearly 5,000 km². Average field size was 70 ha to ensure that almost all
121 spontaneous species of the Flat Pampa crop system may be included in the surveys (Mueller-
122 Dombois & Ellenberg, 1974). Surveyed soybean crops were grown under direct drilled rainfed
123 conditions on typic and entic Hapludoll soils which were similarly managed. Weed control was the
124 usual in the region and glyphosate tolerant varieties were used in all fields. During the fallow and
125 after crop emergence, weed control included grass and broadleaf herbicides.

126

127 Sampled areas within the fields fulfilled the following requirements (Mueller-Dombois and
128 Ellenberg, 1974): (i) they were large enough to contain all species belonging to the plant
129 community), (ii) the habitat was uniform within the field area, and (iii) plant cover was
130 homogeneous. Field margins and low areas were avoided, because they may represent different
131 habitats (e.g. different management and soil conditions). A total of thirty one fields were surveyed
132 to assess weed species frequencies and weed community composition at soybean seed-maturing
133 growth stage R7 (Table 1; Fehr et al., 1971). Surveys were made between 15 March and 15 April.
134 This time interval was chosen based on two criteria: (i) spring-summer and autumn-winter weed
135 communities were present; and (ii) the last weed chemical control during the soybean crop cycle
136 had already been applied, in average 70 days before.

137

138 **Table 1**

139

Year	Farm name	Farm geographic coordinates	Number of fields
2012	San José	34° 52' 26'' S / 60° 39' 28'' W	2
	Los Montes	34° 27' 33'' S / 61° 47' 33'' W	1
	La Suerte	34° 28' 04'' S / 61° 42' 51'' W	3
	La Larga	34° 59' 25'' S / 60° 37' 32'' W	4
	La Manchada	35° 05' 55'' S / 60° 24' 00'' W	4
2013	La Manchada	35° 05' 55'' S / 60° 24' 00'' W	1
	San Felipe	34° 27' 36'' S / 61° 47' 34'' W	4
	La Colorada	35° 04' 13'' S / 60° 23' 56'' W	1
2014	La Colorada	35° 04' 13'' S / 60° 23' 56'' W	3
	San Felipe	34° 27' 36'' S / 61° 47' 34'' W	2
	La Larga	34° 59' 25'' S / 60° 37' 32'' W	3
	La Manchada	35° 05' 55'' S / 60° 24' 00'' W	3

140

141 Table 1 caption: Farm name, geographic coordinates and number of fields surveyed at each one
142 of the three years study.

143

144 Within each field a group of trained persons surveyed weed species presence or absence
145 in 1 m² sample quadrats. Surveys were performed along four radial transects each 30 m long
146 oriented at North, East, South and West. Along each transect quadrats were placed every 2 m and
147 all weeds present were recorded. Therefore, in each field, 60 quadrats were sampled to
148 determine the frequency of each species (proportion of quadrats per field containing a given
149 species) and the species richness (number of species per field) and to estimate weed constancy
150 (percentage of fields containing a given species).

151

152 Arrangement of weed species in functional groups was used to give a better
153 understanding of how weed communities were assembled (Ghersa and León, 1999; Booth and
154 Swanton, 2002). Functional attributes were determined for each weed species considering (i)
155 Raunkiaer life forms into therophytes, geophytes, hemicryptophytes, chamaephytes,
156 phanerophytes; (ii) origin, into native and exotic and; and (iii) growth habit (annual or perennial).
157 Few taxa were only identified at the level of genus and in this case their functional groups were
158 not considered.

159

160 At survey time, fields had various preceding crops, agriculture history length and land use
161 intensification levels (ISI) during the last seven-year cropping period. The number of years under
162 continuous agriculture (YCA) since the last pasture and number of crops after the last maize sown
163 (CALM) were used to characterize the field history and crop sequence. Three categories of ISI
164 were considered for the analysis; *i.e.* ISI-A (from 0.571 to 1.143, 6 fields), ISI-B (from 1.250 to
165 1.286, 16 fields), and ISI-C (from 1.429 to 1.714, 9 fields). Statistical differences among categories
166 were tested and resulted significantly different ($p<0.001$) with a Tukey-Kramer least square means

167 multiple comparison test performed with the GLM procedure of SAS (SAS® University Edition; SAS
168 Institute Inc. 2015, Cary, N.C., USA).

169

170 Weed species composition in the different ISI categories was tested by using the Multi-
171 Response Permutation Procedure (MRPP; Mielke, 1984) using PC-ORD Multivariate Analysis of
172 Ecological Data Version 5.0 with squared Euclidean distances (McCune and Mefford, 1999). Pair-
173 wise comparisons between categories were also performed (Zimmerman et al., 1985). Linear
174 associations among weed assemblages and ISI and YCA as explanatory variables were obtained by
175 mean of canonical correspondence analysis (CCA; ter Braak, 1987). Axis scores were centered and
176 standardized to unit variance, and were scaled for representation of species and surveys. A biplot
177 from CCA was obtained by overlaying a vector diagram based on coefficients from the canonical
178 functions describing each axis.

179

180 The GENMOD procedure of SAS was used to perform generalized linear models and least-
181 square means comparisons. Number of species *per* quadrat was analyzed using a generalized
182 linear model with Poisson distribution. Year, ISI and CALM were considered as sources of variation
183 in the model. The CALM (number of crops after the last maize crop) was categorized for this
184 analysis into two levels, (i) two crops or less (12 fields) and (ii) more than two crops after the last
185 maize grown (19 fields); while the three categories previously defined were considered for ISI.

186

187 The analyses of the proportion of quadrats with presence of weeds (frequency) were
188 performed using a generalized linear model with a binomial distribution and logit link function
189 considering the same sources of variation as previously described. Due to over-dispersion, all
190 statistical parameters were adjusted by the deviance divided by their degrees of freedom. Least-

191 squares means were computed and used to compare mean values of each source of variation
 192 using probability values from the chi-square distribution.

193

194 3. Results

195 Fifty-three species were recorded from all 31 soybean fields. There were more
 196 therophytes (28 species) than any of the other functional groups. There were also 10
 197 hemicryptophytes species, 8 geophytes, 4 chamaephytes and only one phanerophyte species
 198 (Table 2). Weeds were mainly annuals (28 species). However, 18 species were perennials and one
 199 was annual–biennial and one biennial–perennial. According to species origin almost the same
 200 quantities were natives and exotics (26 and 21, respectively; Table 2).

201

202 **Table 2**

203

Floristic group	Species name	Species code	Plant life form	Status	Growth habit	Species constancy (%)			
						ISI-A	ISI-B	ISI-C	Total
I	<i>Digitaria sanguinalis</i> (L.) Scop.	digsa	Therophytes	Exotic	Annual	100	53	75	68
	<i>Stellaria media</i> (L.) Cirillo	steme	Therophytes	Exotic	Annual	83	53	63	61
	<i>Lamium amplexicaule</i> L.	lamam	Therophytes	Exotic	Annual	50	53	63	55
	<i>Cyperus</i> sp.	cypsp	Geophytes		Perennial	67	41	50	48
	<i>Chenopodium album</i> L.	cheal	Therophytes	Exotic	Annual	50	53	25	45
	<i>Conyza bonariensis</i> (L.) Cronquist.	conbo	Therophytes	Native	Annual	50	47	38	45
	<i>Portulaca oleracea</i> L.	porol	Therophytes	Exotic	Annual	50	41	50	45
	<i>Triticum aestivum</i> L.	triae	Therophytes	Exotic	Annual	50	35	63	45
	<i>Anoda cristata</i> (L.) Schltld.	anocr	Chamaephytes	Native	Perennial	50	47	25	42
	<i>Oxalis conorrhiza</i> Jacq.	oxaco	Hemicryptophytes	Native	Perennial	67	35	38	42
	<i>Amaranthus hybridus</i> L.	amahy	Therophytes	Exotic	Annual	83	24	13	32
	<i>Euphorbia lasiocarpa</i> Klotzsch	eupla	Hemicryptophytes	Native	Perennial	17	29	38	29
	<i>Euphorbia dentata</i> Michx.	eupde	Therophytes	Native	Annual	17	18	38	23
	<i>Cirsium vulgare</i> (Savi) Ten	cirvu	Therophytes	Exotic	Annual	17	18	25	19
	<i>Tagetes minuta</i> L.	tagmi	Therophytes	Native	Annual	17	24	13	19
	<i>Commelina erecta</i> L.	comer	Geophytes	Native	Perennial	17	12	13	13
	<i>Sida rhombifolia</i> L.	sidrh	Chamaephytes	Native	Perennial	33	6	13	13
	<i>Datura ferox</i> L.	datfe	Therophytes	Native	Annual	17	6	13	10

	<i>Senecio pampeanus</i> Cabrera	senpa	Chamaephytes	Native	Perennial	17	6	13	10
	<i>Xanthium strumarium</i> L.	xanst	Therophytes	Native	Annual	17	6	13	10
II	<i>Eleusine indica</i> (L.) Gaertn.	elein	Therophytes	Exotic	Annual	67	35		32
	<i>Lepidium didymum</i> L.	lepdi	Hemicryptophytes	Native	Annual- Biannual	33	12		13
	<i>Bidens subalternans</i> DC.	bidsu	Therophytes	Native	Annual	33	6		10
	<i>Bowlesia incana</i> Ruiz et. Pav.	bowin	Therophytes	Native	Annual	17	12		10
	<i>Dichondra microcalyx</i> (Hallier f.) Fabris	dicmi	Geophytes	Native	Perennial	33	6		10
	<i>Gleditsia triacanthos</i> L.	gletr	Phanerophytes	Exotic	Perennial	17	12		10
	<i>Urtica urens</i> L.	urtur	Therophytes	Exotic	Annual	17	6		6
	<i>Sorghum halepense</i> (L.) Pers.	sorha	Geophytes	Exotic	Perennial	17	6		6
	<i>Senecio vulgaris</i> L.	senvu	Therophytes	Exotic	Annual	17	6		6
	<i>Setaria parviflora</i> (Poir.) Kerguélen	setpa	Geophytes	Native	Perennial	17	6		6
	<i>Gamochaeta</i> sp.	gamsp	Hemicryptophytes			17	6		6
III	<i>Solanum</i> sp.	solsp				17		13	6
	<i>Jaborosa integrifolia</i> Lam.	jabin	Geophytes	Native	Perennial	17		13	6
	<i>Juncus</i> sp.	junsp				17		13	6
IV	<i>Zea mays</i> L.	zeama	Therophytes	Native	Annual	17			3
	<i>Panicum bergii</i> Arechav.	panbe	Hemicryptophytes	Native	Perennial	17			3
	<i>Physalis viscosa</i> L.	phyvi	Geophytes	Native	Perennial	33			6
	<i>Poa annua</i> L.	poaan	Therophytes	Exotic	Annual	17			3
	<i>Eragrostis</i> sp.	erasp	Hemicryptophytes			17			3
V	<i>Amaranthus viridis</i> L.	amavi	Hemicryptophytes	Native	Perennial		12		6
	<i>Chaptalia nutans</i> (L.) Po.	chanu	Hemicryptophytes	Native	Perennial		6		3
	<i>Chloris</i> sp.	chlsp	Hemicryptophytes				12		6
	<i>Sonchus oleraceus</i> L.	sonol	Therophytes	Exotic	Annual		12		6
	<i>Lolium multiflorum</i> Lam.	lolmu	Therophytes	Exotic	Annual		6		3
	<i>Echinochloa colona</i> (L.) Link	echco	Therophytes	Exotic	Annual		6		3
	<i>Convolvulus arvensis</i> L.	conar	Geophytes	Exotic	Perennial		6		3
	<i>Bromus catharticus</i> Vahl	broca	Hemicryptophytes	Native	Biannual- Perennial		6		3
	<i>Carduus acanthoides</i> L.	carac	Therophytes	Exotic	Annual		6		3
	<i>Cenchrus spinifex</i> Cav.	censp	Therophytes	Native	Annual		6		3
VI	<i>Hordeum vulgare</i> L.	horvu	Therophytes	Exotic	Annual			13	3
	<i>Iresine diffusa</i> Humb. & Bonpl. ex Willd.	iredi	Chamaephytes	Native	Perennial			13	3
	<i>Bidens pilosa</i> L.	bidpi	Therophytes	Native	Annual			25	6
	<i>Anagallis arvensis</i> L.	anaar	Therophytes	Exotic	Annual		12	13	10
Total number of species						39	42	27	53

205

206 Table 2 caption: Floristic groups, species list, code (*), function (plant life form, status, growth
207 habit) and constancy according to levels of ISI (ISI-A, ISI-B and ISI-C) registered in the fields
208 surveyed.

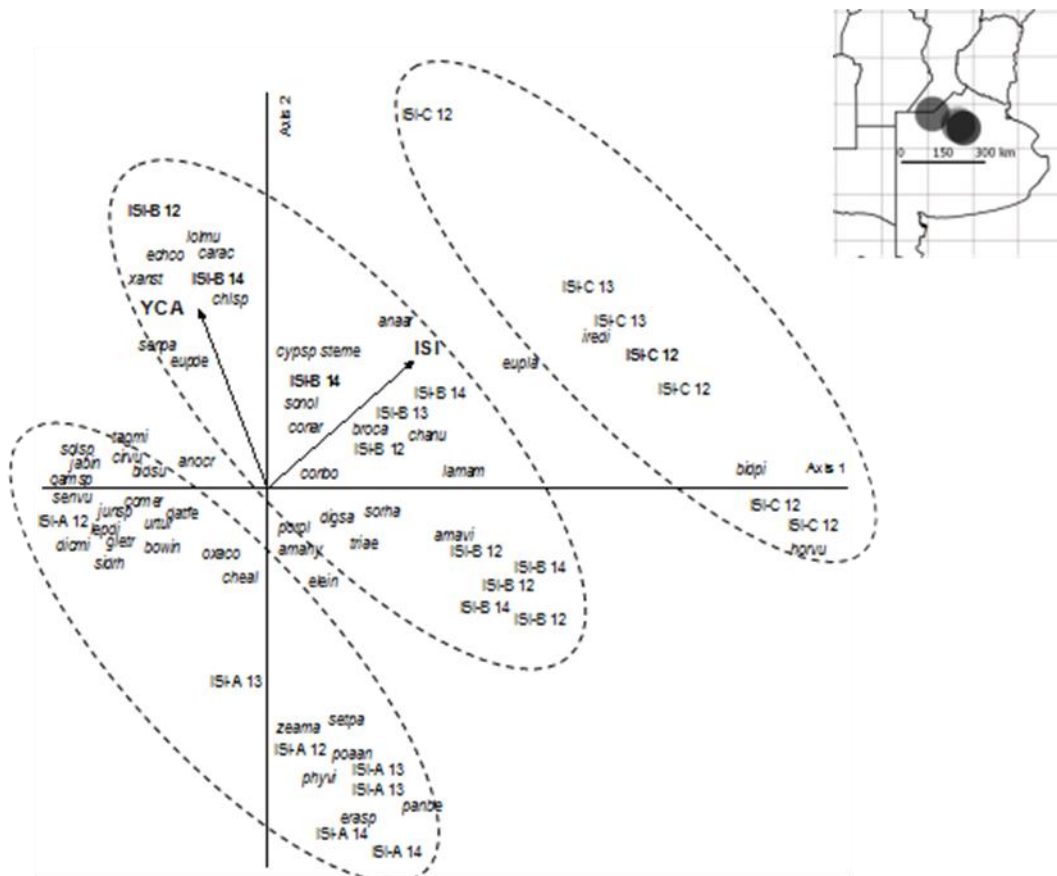
209

210 Level of intensification (ISI) determined three different weed communities, one for each
211 ISI category (see material and methods), which varied in the arrangement of floristic groups. Six
212 floristic groups were recognized according to the species composition and constancy. Floristic
213 group I was represented by species present with high constancy values in all the ISI ranges.
214 Floristic group II was present in ISI-A and B while floristic group III was present in ISI-A and C.
215 Floristic groups IV, V and VI were only present in ISI-A, ISI-B and ISI-C, respectively (Table 2). The
216 richness of ISI A, B and C weed communities was 39, 41 and 26 respectively ($p < 0.05$); i.e. species
217 richness decreases as intensification increases. Floristic composition differed statistically
218 according to MRPP only between weed communities related to extreme ISI (ISI-A and ISI-C,
219 $p < 0.05$) but functional composition was similar among weed communities ($p > 0.10$, Table 2).

220

221 The CCA arranged weed species in both axis regarding to the years under continuous
222 agriculture (YCA) and the intensification sequence index (ISI). Axis 1 explained 30 % of the total
223 variance and was mostly related to the ISI values of fields; therefore it strongly separate floristic
224 group VI from floristic groups II and III (Fig. 1). Floristic group V was between the previously
225 mentioned floristic groups and it was associated to ISI-B fields. Within each ISI group the distance
226 in axis 2 explained 20 % of the total variance, and it was mostly related to the number of years
227 under continuous agriculture of fields (YCA; Fig. 1). All ISI categories and floristic groups were
228 represented by fields surveyed in different years and with long and short agricultural history.

229



231
 232 Caption Fig. 1: Ordination diagram of weeds (code: three-first letters, genus; two last letters,
 233 specific epithet, see Table 2) in the two principal axes of the CCA. The relative lengths of the
 234 arrows indicate the importance of a variable. The distribution of data points in the analysis is
 235 represented by the ISI level (ISI-A, B, and C) followed by the last two numbers of the year of the
 236 survey (12, 13, and 14). Inset indicates the position and area where the surveys were performed.

237
 238 The probability of finding together different species in the same quadrat was overall low
 239 (1.03 species m⁻² on average from all fields, Table 3). However, there were significant differences

240 among ISI categories (0.75 for ISI-C and 1.12 for ISI-A, Table 3). The frequency of some species
 241 from floristic group I was analyzed separately (Table 3). Some species were negatively ($p < 0.05$)
 242 affected by crop sequence intensification of the fields while others were promoted or remained
 243 unaffected. The frequency of *Digitaria sanguinalis* and *Oxalis conorrhiza* diminished as the ISI
 244 increased but in the case of *Euphorbia dentata* the frequency increased as the ISI also increased.
 245 All the other species explored (*Amaranthus hybridus*, *Conyza bonariensis*, *Cyperus* sp. and
 246 *Portulaca oleracea*) remained statistically unaffected by ISI (Table 3). Weed frequency, as a
 247 measure of the weed infested area of the fields, was relatively high (62 %; Table 3) and it was not
 248 significantly affected by ISI, *i.e.* weed infested areas were similar in fields with different ISI levels.

249

250 Among the variables considered to characterize the structure of the crop sequence of the
 251 fields, CALM (the number of crops after the last maize sown) significantly affected some of the
 252 weed populations. The frequency of *A. hybridus* was higher and that of *E. dentata* was lower
 253 when maize crops were grown less than two years before the surveyed soybean crop (CALM < 2
 254 years, $p < 0.05$, Table 3).

255

256 **Table 3**

Variable	Effects		$p > \text{chi-square}$	Lsmeans (*)	Overall mean	deviance/df
	$p > \text{chi-square}$	Lsmeans (*)				
Richness	<0.0001	ISI-A 1.12 a	<0.0001	L- 1.12 a	1.03	1.19
		ISI-B 1.08 a		H- 0.84 b		
		ISI-C 0.75 b				
Total frequency of weed species	0.7324	ISI-A 0.71 a	0.5717	L- 0.68 a	0.62	24.6
		ISI-B 0.64 a		H- 0.60 a		
		ISI-C 0.57 a				
Frequency of: <i>Digitaria sanguinalis</i>	0.0363	ISI-A 0.40 a ISI-B 0.07 b ISI-C 0.15 ab	0.0614	L- 0.31 a H- 0.09 a	0.22	26.1

<i>Cyperus</i> sp.	0.4905	ISI-A 0.01 a ISI-B 0.05 a ISI-C 0.04 a	0.5457	L- 0.02 a H- 0.04 a	0.04	7.3
<i>Conyza bonariensis</i>	0.3273	ISI-A 0.02 a ISI-B 0.03 a ISI-C 0.06 a	0.5916	L- 0.03 a H- 0.04 a	0.04	4.2
<i>Portulaca oleracea</i>	0.5687	ISI-A 0.02 a ISI-B 0.03 a ISI-C 0.01 a	0.1275	L- 0.03 a H- 0.01 a	0.02	3.6
<i>Oxalis conorrhiza</i>	0.0053	ISI-A 0.046 a ISI-B 0.012 b ISI-C 0.005 b	0.1143	L- 0.022 a H- 0.009 a	0.02	2.2
<i>Amaranthus hybridus</i>	0.3144	ISI-A 0.017 a ISI-B 0.035 a ISI-C 0.006 a	0.0007	L- 0.051 a H- 0.005 b	0.03	4.4
<i>Euphorbia dentata</i>	0.0313	ISI-A 0.0011 a ISI-B 0.0044 a ISI-C 0.0154 b	<.0001	L- 0.0006 a H- 0.0284 b	0.03	2.3

257 (*) Least-square means of the same effect with different letters are different at $p < 0.05$.

258

259 Table 3 caption: Effects of ISI and CALM on (i) richness, as average number of weed species per
 260 square meter, (ii) total frequency of weed species, and (iii) the frequency of some abundant
 261 summer-crop weed species. If effects are significant ($p < 0.05$) different letters are presented to
 262 identify different means. Categories of ISI were ISI-A, 0.57 to 1.14, ISI-B, from 1.25 to 1.29, and ISI-
 263 C, from 1.43 to 1.71. Levels of CALM considered were equal or less than two crops (L) and more
 264 than two crops (H). Overall least-square mean values and deviance are also presented for each
 265 effect and level.

266

267 4. Discussion

268 Agricultural intensification reduced floristic richness of weed communities in agricultural
 269 fields of the Flat Pampa (Table 2, Fig. 1). Various authors indicated that intensified agriculture may

270 reduce plant biodiversity (Vandermeer et al., 1998; Yeates and Bongers 1999; Chapin et al., 2000;
271 Norris et al., 2003; Butler et al., 2007). In addition, there are evidences that land use
272 intensification greatly modify the habitat, resource availability or soil properties which may be
273 responsible of changes in plant biodiversity and weed community composition (Lacher et al.,
274 1999; Tschardtke, 2002; Benton et al., 2003; Foley et al., 2005; Andrade et al., 2015, 2017a)
275 independently from the herbicides used as weed control strategies. Large canopy gaps due to
276 fallow periods, which are usually more frequent in single than double crops were signaled to
277 reduce crop competition and to promote greater weed richness in low intensified fields (Poggio et
278 al., 2005). Although weed richness was reduced, it appeared that overall weed infestation was not
279 significantly reduced by ISI (Table 3). As it was expected, some weed populations may persist and
280 grow under intensified rotations while others may not (Kruk, 2015). Weeds differed in their
281 performance when ISI was modified (Fig. 1, Table 2 and 3). Various mechanisms could take part in
282 the persistence and growth of some weed populations and the failure of others under the studied
283 conditions. For example, it may be hypothesized that *Eleusine indica* establishment has been
284 affected by the higher amount of plant residues on top soil under direct drilled more intensified
285 fields since plant residues reduce alternating temperatures which are necessary for germination
286 of *E. indica* (Ismail et al., 2002; Chauhan and Johnson, 2008). Other species, such as *Bidens*
287 *subalternans*, *Sonchus oleraceus*, *Carduus acanthoides* and *Physalis viscosa* are very sensitive to
288 soil degradation (de la Fuente et al., 1999) which may occurred in fields under continuous
289 soybean which was frequent in poorly intensified (ISI-A) conditions. On the contrary, *Iresine*
290 *diffusa*, from floristic group VI present in intensified fields (ISI-C), is a perennial species, less
291 dependent on dormancy terminating factors and tolerant to glyphosate (Puricelli and Faccini,
292 2009). To recognize and consider the various weed responses involved is important if ISI is going
293 to be part of an integrated weed management strategy.

294 Some important soybean weeds were not reduced under high ISI fields. Most of these
295 species were characterized within floristic group I (Table 2) including *D. sanguinalis*, *A. hybridus*
296 and *Chenopodium album*. These species have been indicated as highly competitive (Guglielmini et
297 al., 2017) causing great crop yield losses. Moreover, herbicide resistant biotypes of some of these
298 species have already been recognized under field conditions in the region (REM AAPRESID,
299 <http://www.aapresid.org.ar/rem/>). Our results call the attention that rotation intensification
300 should not be considered individually as an easy solution but a weed management alternative to
301 temporarily reduce herbicide use and to diversify weed control methods. As already occurred
302 with soybean in the simple low ISI predominant cropping system, if farmers tend to rely in easy
303 solutions to weed problems, it seems that few more competitive and adapted weed populations
304 may be selected in intensified fields.

305

306 CALM affected the frequency of some weed species and weed community composition
307 was also slightly modulated by YCA. On one hand, as YCA increases, soil resources are depleted in
308 the grain extensive low input flat Pampa agriculture. On the other hand, as CALM is low (L), fallow
309 periods are long and more soil and light resources are available for some weed populations to
310 grow. Therefore crop rotation length and crop sequence may differently release resources and
311 affect establishment, growth and/or dispersion of weed populations (Soriano et al., 1971; Ghersa
312 and León, 1999). This is particularly true in weed communities mainly composed by therophyte
313 species (Fig. 1, Table 3), whose life form implies that they must rely exclusively on seed
314 production and plant establishment for its persistence (Benvenuti, 2007).

315

316 Overall weed frequency, as estimator of weed abundance (Magurran, 1988), was not
317 significantly affected by intensification in the studied fields (Table 3) but, as previously
318 mentioned, floristic richness was significantly reduced by ISI (Table 2 and 3). It is recognized that

319 weed control is a particularly conflicting environmental issue since weeds can cause yield loss but
320 support farmland biodiversity (Bretagnolle and Gaba, 2015; Petit et al., 2016). This study mitigates
321 the conflict since it showed that intensified rotations may cause the reduction of species richness
322 while maintaining weed frequency and some community functions (i.e. life forms, growth habit).
323 Therefore, intensification may be seen as a useful tool in sustainable agriculture by integrating
324 weed management and functional landscape biodiversity.

325

326 **5. Conclusions**

327 Average intensified sequence index (ISI) is less than 1.1 in the overall Pampa region
328 (Andrade and Satorre, 2015) and at present, weed control is kept at the expense of herbicide
329 inputs and raising costs which are forcing farmers to look to more integrated weed management
330 strategies. In order to deal with specific weed problems which are usually aggressive herbicide
331 tolerant or resistant weeds, intensified rotations are promoted. From our results, crop sequence
332 intensification itself modified weed community, reduced weed species richness and changed the
333 frequency of some weed populations within the agricultural fields of the Flat Pampa in Northwest
334 of Buenos Aires. Thus, crop sequence intensification may be considered a useful tool in weed
335 management, as a part of an integrated solution. However, it also appear that it should not be
336 considered a simple, isolated permanent tool since weed species were differently affected and
337 new harmful weed problems may be promoted. Finally, more research on individual processes of
338 some target weed species need to be explored if we aim to predict weed dynamics and use
339 intensified rotations as effective parts of integrated weed management systems aiming to
340 efficiently neutralize the growth and persistence of weeds.

341

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347

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