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1 **Weed community shifts during the aging of perennial intermediate wheatgrass**  
2 **crops harvested for grain in arable fields**

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9 **KEYWORDS**

10 Perennial grain, intermediate wheatgrass, weed traits, weed communities, on-farm trials.

11 **ABSTRACT**

12 The development of a perennial grain offers opportunities to diversify annual crop rotations,  
13 with potential benefits in terms of soil protection. Perennials could also reduce weed  
14 development over time through year-round soil cover and longer growing seasons. However,  
15 whether weeds would actually decrease remains mainly theoretical, with field data on perennial  
16 grains remaining sparse. Qualitative changes might also have an effect, because disturbance  
17 and modifications to resource regimes drive shifts in weed communities. Here, we analyzed  
18 weed abundance, composition, and traits in three arable fields containing perennial grain over  
19 a 4-year period. Specifically, intermediate wheatgrass (IWG) perennial grain (Kernza) was  
20 cultivated. IWG grain production was maximal during the first and second growing seasons  
21 (899 and 854 kg.ha<sup>-1</sup> respectively), with total biomass production peaking in the second year  
22 (mean: 11.9 t DM.ha<sup>-1</sup>). However, reproductive effort noticeably dropped in the third and fourth  
23 years. Weed biomass also gradually reduced during the fall of all years (mean: ~0.4--0.03 t  
24 DM.ha<sup>-1</sup>), but remained constant in spring (mean: 1–2 t DM.ha<sup>-1</sup>). During spring, the community

25 composition of weeds shifted from broadleaves to grass species and, to a lesser extent, from  
26 annuals to perennials, with weed species having an earlier phenology than IWG. Thus, relative  
27 fitness appeared to be the strongest driver of weed community composition in aging IWG  
28 stands. Weed species richness systematically declined over the years. Specific leaf area,  
29 height, light, and nitrogen requirement index of weed communities provided poor descriptors  
30 of community shifts; however, all weed species with high light requirements were excluded  
31 from aging IWG stands. IWG capacity to compete with weeds might have been undermined  
32 by its late growing cycle, absence of forage harvest during the growing season, and substantial  
33 initial weed development during establishment (first year of growth). Thus, before IWG  
34 establishment, initial site conditions and land use history should be considered to implement  
35 the best management strategies for each field. Important weed development in perennial grain  
36 fields could lead to high weeding requirement, either mechanical or chemical, in conflict with  
37 the initial principle of increasing sustainability and reducing inputs.

## 38 **1. INTRODUCTION**

39 The perennial grain Kernza™ is the result of ongoing efforts by The Land Institute (Kansas,  
40 USA) to domesticate intermediate wheatgrass (IWG, *Thinopyrum intermedium*, (Host)  
41 Barkworth & D.R. Dewey). This grain became a figurehead of new perennial grain crops that  
42 could revolutionize cropping systems (Crews et al., 2016; DeHaan et al., 2020; Duchene et al.,  
43 2019; Ryan et al., 2018). Besides provisioning farmers with food and feed biomass in the form  
44 of grain and forage over several years, the deep root system and year round soil cover of this  
45 perennial crop (Sainju et al., 2017; Sakiroglu et al., 2020) could provide important additional  
46 benefits, including nutrients leaching mitigation (Culman et al., 2013; Jungers et al., 2019), soil  
47 erosion control, and carbon storage, (Audu et al., 2022; Kim et al., 2022; Sprunger et al., 2019).  
48 IWG could also potentially outcompete weeds by increasing the timespan of light and soil  
49 resource capture in each year (Lanker et al., 2019; Ryan et al., 2018). This expectation is  
50 supported by published studies, in which grasslands were identified as better competitors of

51 weeds compared to annual crops (Dominschek et al., 2021; Schuster et al., 2020). The weed  
52 suppression effect from integrating perennial forage and temporary grasslands in cropping  
53 systems often corresponds to a shift in weed communities, driven by changes in the  
54 disturbance regime and resource gradients (Fried et al., 2022; Gaba et al., 2014). Community  
55 ecology and trait-based approaches can be used to describe the general patterns of these  
56 changes on a functional basis (Fried et al., 2012, 2008; Grime, 2006). For instance, tillage  
57 increases the development of 'ruderal' species (i.e., annuals with fast growing strategies, early  
58 flowering, and higher Specific Leaf Area (SLA; Dominschek et al., 2021; Fried et al., 2022). In  
59 comparison, systems with reduced or no tillage (e.g., grasslands) tend to favor grass and  
60 perennial weed species (Adeux et al., 2022; Meiss et al., 2010a,b). Other contributing factors  
61 include the intensity of grazing and cutting events, which affect how much light penetrates the  
62 canopy, filtering weed species depending on their morphology and ability to regrow (Meiss et  
63 al., 2010a; Meiss et al., 2008; Renne and Tracy, 2007; Schuster et al., 2016; Smith et al.,  
64 2018). Trophic soil conditions represent an orthogonal driver of the structure of weed  
65 communities with, for example, species with greater seed mass and plant height favored in  
66 richer soils compared to poor soils (Fried et al., 2022).

67 Cropping systems that have minimal yield losses due to weed competition tend to correspond  
68 to systems with diversified weed communities, with higher weed evenness and lower weed  
69 biomass, compared to more problematic situations with dominant and competitive weed  
70 species (Adeux et al., 2019). Thus, a shift in the structure of weed communities following  
71 changes to cropping systems could generate problems, depending on the dominance and  
72 fitness of species under novel growing conditions.

73 Due to the novelty of IWG crops, associated field data about weed development remain scarce,  
74 and sometimes contradictory; yet, weed management has been identified by farmers as one  
75 of the main challenges in Kernza field trials (Duchene, 2020; Lanker et al., 2019; Law et al.,  
76 2021a). Zimbric et al. (2020) and Dick et al. (2018) reported that weed biomass in summer  
77 declined during IWG regrowth (second and third year of growth). In contrast, Law et al. (2021a)

78 recorded consistent weed biomass in the summers of successive years, with the development  
79 of perennial grass communities (*Poa trivialis*, *Phleum pratense*). During IWG establishment,  
80 following sowing in fall, conditions for weed growth are analogous to annual grain stands. Land  
81 preparation steps, that vary depending on farming systems, are designed to favor the seed  
82 germination and seedling emergence of crops. During the subsequent weeks and months,  
83 crop seedlings establish their first roots, leaves, and tillers; consequently, their ability to  
84 compete with weeds is initially limited, depending on the rate at which it can occupy space and  
85 use resources (i.e., regulated by relative growth rate and sowing density). However, after the  
86 first year, the growth and management of IWG widely differs to that of annual grain systems,  
87 because regrowth in fall is enabled by perennating organs, such as the root and plant crown;  
88 consequently, yearly tillage and soil preparation operation are obsolete. This regrowth ability  
89 enhances the efficiency of resource capture and use over time (Culman et al., 2013; De  
90 Oliveira et al., 2018; Vico and Brunsell, 2017). However, when IWG is harvested for grain, it  
91 cannot be cut at regular intervals during the cropping season, as implemented when harvesting  
92 grassland as forage. Only fall or early spring harvest operations are possible, before stem  
93 elongation. Thus, a noticeable change in land use is required when cultivating IWG as a  
94 perennial grain crop, particularly regarding the disturbance regime (no annual tillage and no  
95 regular cutting events in spring) and resource availability over multiple years (because  
96 resource capture and use efficiency change over time). Such changes drive shifts in the  
97 structure of the weed community (Dominschek et al., 2021; Fried et al., 2022), as demonstrated  
98 by Law et al. (2021a) in fields containing IWG grain crops. Therefore, it is important to assess  
99 the structure of weed communities over successive growing seasons to determine the  
100 importance of IWG cultivation in filtering weed species initially present through habitat change  
101 under field conditions.

102 This study explored how weed communities change under IWG cultivation in the temperate  
103 arable fields of western Europe (France), from crop establishment to 4-years of growth.  
104 Biomass, composition, and traits were analyzed. We hypothesized that both weed biomass

105 and community composition in fields would alter in response to changes in the disturbance  
106 regime and resource gradients following IWG establishment. Potentially problematic weed  
107 species were also identified that might require specific attention and management practices in  
108 future research or production fields.

## 109 **2. MATERIALS & METHODS**

### 110 **2.1. *Experimental sites description***

111 This study was conducted using three on-farm trial sites (1, 2, and 3) of 0.3–0.4 ha each in the  
112 south-east of France. IWG was established in the fall of 2017 (site 1 and 3) and fall of 2018  
113 (site 2), and was grown until the summer of 2021. Data on IWG yields and weed development  
114 were collected in each successive year to analyze the nature and intensity in the shifts of the  
115 weed community. Table 1 provides information on field locations, management operations,  
116 and main soil and climate characteristics. At each site, samples were taken from four subplots  
117 (10\*20 m) to account for any effect of field heterogeneity. The three sites were cropped for at  
118 least the last 10 years with a three-year rotation of annual crops, which are common in the  
119 region (rapeseed or maize - winter wheat - winter barley or winter rye). Winter wheat preceded  
120 the sowing of IWG at all sites, and maize was grown before wheat. Site 3 was managed with  
121 direct sowing practices, whereas sites 1 and 2 were managed with standard tillage operations,  
122 including mold-board ploughing and harrowing. All sites were managed with herbicides to  
123 control weeds in annual crops and before IWG planting; however, weeds were not treated in  
124 any form (mechanical or chemical) during IWG growth. Each year at grain maturity (after the  
125 last sampling event in summer), direct combine harvesting was used to harvest both grain and  
126 straw.

## 127        2.2.        *Data collection*

### 128                2.2.1. *Canopy biomass, composition, and grain yields*

129        Data collection started in the fall of 2017 (sites 1 and 3) and 2018 (site 2) following crop  
130        establishment, and ended in the summer of 2021, corresponding to the fourth (sites 1 and 3)  
131        or third (site 2) year of IWG growth. During this period, canopy (IWG and weed) biomass and  
132        composition were measured: i) each fall (at the end of November), corresponding to the end  
133        of vegetative growth before winter; ii) each spring at the crop heading period (mid-May) and  
134        flowering stage (mid-June); and iii) in summer at grain maturity (late-July to mid-August). The  
135        two spring sampling periods were chosen to obtain a comprehensive overview of weed  
136        biomass development, because the peak growth period significantly differs among species,  
137        depending on their respective growth dynamics and phenology. In summer, IWG spikes were  
138        collected separately from straw. The spikes were then threshed with a manual thresher  
139        (NEWEEK), and weighed to estimate grain yields.

140        For all sampling events at the 3 sites, canopy biomass was sampled after weed species had  
141        been identified in two 0.5 m<sup>2</sup> quadrats on each of the four subplots (1 m<sup>2</sup> sample per subplot),  
142        by cutting the aboveground biomass 5 cm above the soil surface. The entire set of weed  
143        species identified at each site is provided in Table 1 and 2. The soil cover of weed species was  
144        estimated by visual observation, and was rated on a 10% grade scale. For each sampled  
145        quadrat, IWG biomass was separated from weeds, and weed species were separated from  
146        each other. IWG and weed biomass were oven-dried at 65 °C for 48 h before weighing. The  
147        relative biomass of each weed species was presented as the proportion of total weed biomass  
148        per quadrat. This approach allowed us to construct a relative abundance table based on  
149        biomass units instead of individual plants (Wilhm, 1968). This table was used for the statistical  
150        analyses (diversity indexes, RLQ analysis). Biomass is thereafter expressed in tons of dry  
151        matter per hectare (t DM.ha<sup>-1</sup>). In summer, grain yields were obtained by oven-drying and  
152        weighing threshed grains in a similar way to the rest of IWG biomass. Yields were calculated

153 and expressed on a 15% humidity basis. The harvest index were calculated as the ratio  
154 between grain yields and total aboveground biomass.

### 155 *2.2.2. Traits of weed species*

156 Eight functional traits of weeds were selected as potentially responding to IWG growth under  
157 field conditions (Table 2). These traits corresponded to the main ecological features related to  
158 resource use. These traits were life history (annual, perennial), phenology, plant division  
159 (monocotyledons, broadleaves), plant earliness (month corresponding to the start of the  
160 flowering period), average height, specific leaf area (SLA, as a proxy of photosynthetic capacity  
161 and growth rate), nitrogen, moisture and light requirements. The last three were described  
162 using Ellenberg's indicator values (Julve, 1998); namely, L - light (from 1- deep shade to 9-full  
163 light), F - moisture (from 1-extreme dryness to 12-submerged plant), and N - nitrogen (from 1-  
164 extremely infertile to 9-extremely rich). Values for all weed species identified in this study were  
165 collected from online databases and the published literature (Table 2).

166 **Table 1:** Information about the on-farm trial sites, including location, main soil type, weather characteristics (OM = Organic Matter, GDD = Growing  
 167 Degree Day in base 0 °C), management timing and operations, and identified weed communities.

Site		1	2	3
Surface (ha)		0.31	0.38	0.33
GPS coordinates (longitude; latitude)		5.1251; 45.4250	5.0920; 45.2746	5.1433; 45.3323
Soil characteristics (0-30cm)	Texture	Loam	Sandy-loam	Sandy loam
	pH	7.6	6.7	6.8
	OM (%)	2.1	1.9	2.4
IWG sowing date and rate (accumulated GDD until first frost)		20/09/2017, ~15kg/ha (882°C)	18/09/2018, ~15kg/ha (904°C)	05/09/2017, ~15kg/ha (1 112°C)
Mean temperature during the whole growing season (°C)	2017-2018	12.2	-	10.9
	2018-2019	12.3	11.1	11.3
	2019-2020	13.2	11.9	12.4
	2020-2021	12.6	11.5	11.6
Accumulated GDD during the whole growing season	2017-2018	3775.4	-	3339.2
	2018-2019	3808.5	3512.6	3509
	2019-2020	4080.4	3802.5	3841.5
	2020-2021	3854.1	3606.4	3743
Accumulated rainfall during the whole growing season (mm)	2017-2018	676.8	-	649.6
	2018-2019	535.3	667.2	630.2
	2019-2020	629.5	783.4	731.4
	2020-2021	651.2	832.6	801.4
Tillage practices (CT = conventional tillage; NT = No tillage)		CT: plowing to a depth < 25cm (10/09/2017) + power harrow (20/09/2017) + tine seeder (20cm inter-row)	CT: plowing to a depth < 25cm (14/09/2018) + disc harrow (16/09/2018) + tine seeder (22cm inter-row)	NT: direct sowing with discer seeder (25cm inter-row)
Nitrogen application date (ammonium-nitrate 50 kg N.ha <sup>-1</sup> )		15/03/2018; 24/03/2019; 01/04/2020; 26/03/2021	02/04/2019; 05/04/2020; 27/03/2021	21/03/2018; 05/04/2019; 15/04/2020; 28/03/2021
Harvest date		25/07/2018; 01/08/2019; 27/07/2020; 04/08/2021	11/08/2019; 07/08/2020; 16/08/2021	27/07/2018; 05/08/2019; 08/08/2020; 25/07/2021
Weed species identified on field (code in Table 2)		ACH. CHE. BRO. CAP. PAP. FUM. SON. LAM. POA. VIO. RUM. SEN. CER. STE. VER. ALO. ARR. TAR	BRO. LAM. POA. VIO. SEN. CER. STE. VER. ARR. LOL. CAR. ANA. OXA. AGR	BRO. CHE. PAP. SON. POA. CER. STE. VER. ARR. TAR. POT. LOL. CAR. EPI. GAL. GER. ANA. AGR

168 **Table 2:** Traits selected to evaluate weeds with their descriptive values (symbol † provided with each trait indicates source reference and  
 169 database).

Species	Code	Ellenberg index (†)			Life History (††,†††)	Division (††)	Earliness of flowering (month of the year) (††,†††)	Average canopy height (m) (†††)	Average SLA (†††)
		Light	Moisture	Nitrogen					
<i>Capsella bursa-pastoris</i>	CAP	7	5	7	Annual	Broadleaves	3	0.22	30.02
<i>Papaver rhoeas</i>	PAP	7	5	6	Annual	Broadleaves	4	0.40	33.07
<i>Fumaria officinalis</i>	FUM	6	5	6	Annual	Broadleaves	4	0.20	28.54
<i>Lamium purpureum</i>	LAM	6	5	7	Annual	Broadleaves	3	0.28	38.45
<i>Viola arvensis</i>	VIO	8	4	6	Annual	Broadleaves	3	0.15	24.32
<i>Senecio vulgaris</i>	SEN	7	5	7	Annual	Broadleaves	1	0.20	29.82
<i>Cerastium glomeratum</i>	CER	7	5	5	Annual	Broadleaves	4	0.25	21.75
<i>Stellaria media</i>	STE	7	5	7	Annual	Broadleaves	3	0.23	53.68
<i>Veronica persica</i>	VER	6	5	7	Annual	Broadleaves	3	0.25	39.60
<i>Cardamine hirsuta</i>	CAR	8	5	6	Annual	Broadleaves	3	0.19	27.78
<i>Galium aparine</i>	GAL	6	6	8	Annual	Broadleaves	6	0.68	34.68
<i>Geranium dissectum</i>	GER	5	6	6	Annual	Broadleaves	5	0.35	23.20
<i>Anagallis arvensis</i>	ANA	7	4	5	Annual	Broadleaves	5	0.17	29.22
<i>Achillea millefolium</i>	ACH	8	5	4	Perennial	Broadleaves	6	0.32	19.81
<i>Sonchus arvensis</i>	SON	8	6	6	Perennial	Broadleaves	7	0.88	21.70
<i>Rumex obtusifolius</i>	RUM	7	5	9	Perennial	Broadleaves	6	0.68	29.71

<i>Taraxacum officinale</i>	TAR	7	5	6	Perennial	Broadleaves	4	0.20	34.77
<i>Potentilla reptans</i>	POT	7	5	5	Perennial	Broadleaves	6	0.13	25.09
<i>Epilobium hirsutum</i>	EPI	7	8	7	Perennial	Broadleaves	5	1.15	26.92
<i>Oxalis corniculata</i>	OXA	7	4	5	Perennial	Broadleaves	5	0.15	41.51
<i>Bromus sterilis</i>	BRO	7	4	7	Annual	Monocotyledons	5	0.38	32.20
<i>Alopecurus myosuroides</i>	ALO	6	5	6	Annual	Monocotyledons	4	0.35	27.27
<i>Lolium perenne</i>	LOL	7	5	7	Perenne	Monocotyledons	5	0.35	30.06
<i>Poa trivialis</i>	POA	7	6	6	Perennial	Monocotyledons	4	0.36	30.98
<i>Arrhenatherum elatius</i>	ARR	7	5	7	Perennial	Monocotyledons	5	0.60	28.25
<i>Agrostis capillaris</i>	AGR	6	5	4	Perennial	Monocotyledons	6	0.25	34.43

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171 †Hill et al., 1999

172 ††Julve, 1998

173 †††Kleyer et al., 2008.

## 174 2.3. Data analysis

### 175 2.3.1. IWG and weed biomass

176 Statistical analyses were conducted in R (R Core Team, 2018). Sites corresponded to  
177 replicates, while subplots corresponded to pseudo-replicates. IWG and weed biomass  
178 production were analyzed as response variables with mixed models (lmerTest package)  
179 (Kuznetsova et al., 2019), including three fixed effects. These effects were IWG stand age as  
180 a categorical variable (1- first growing season, 2- second, 3- third, and 4- fourth), sampling  
181 period (fall, mid-spring, late spring, summer), and covariate biomass (weeds or IWG,  
182 depending on which was analyzed as the response or explanatory variable). As our data  
183 collection included a repeated-measures structure (eight measurements taken per year in  
184 twelve subplots), the site effect and nested effect of each subplot per site were treated as  
185 random intercept effects, representing an uncontrolled (not chosen) effect from local conditions  
186 on IWG and weed growth (i.e., weed seed bank, soil, and climate conditions). The model also  
187 fits a random slope to account for the random interactions of sites with sampling period or  
188 stand age factors. Maximum likelihood of model and the covariance between random slopes  
189 and intercepts were checked. The correlations between the random intercept and slope were  
190 low ( $< 0.25$ ). IWG grain yields and harvest index were analyzed using similar models (without  
191 sampling period effect). Variables were log-transformed as necessary to improve normality.  
192 Least-squares means were computed and used for pairwise comparisons (post hoc analysis,  
193  $\alpha = 0.05$ , *lsmeans* and *clm* functions) (Piepho, 2004) to determine the significance among mean  
194 values following significant ( $p$ -value  $< 0.05$ ) factor effects and interactions. When identified as  
195 relevant by the model, correlations between crop and weed biomass were tested with Pearson  
196 coefficients, and their associated significance (t test).

### 197 2.3.2. Structure, diversity, and traits of the weed community

198 Analysis of weed communities during spring was performed using both spring sampling events  
199 (heading and flowering time). Weed diversity was calculated using Shannon's diversity index,  
200 based on biomass units (rather than individuals), according to the following equation:

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$$H' = - \sum_{i=1}^s p_i \ln p_i$$

where  $p$  is the proportion of total weed biomass of a given weed species, and  $s$  is the number of species present. Mean values were calculated for each site for each growing season, and the species evenness index was obtained by dividing  $H'$  by  $\ln(S)$ , which corresponds to  $H_{max}$ . Multivariate analyses were used to examine differences in the structure of the weed community. Nonmetric multidimensional scaling (NMDS) ordination (Oksanen, 2005) was used to visualize differences in community structure. For NMDS, Bray-Curtis dissimilarity coefficients were calculated using transformed (Wisconsin double standardization) species relative abundance values. A minimum stress to halt iterations was set at 0.01 with 100 restarts. The relationship between weed community structure and IWG stand age (duration of crop presence) was tested through permutational multivariate analysis of variance using distance matrices (Adonis function; Oksanen et al., 2020). Considering that the null hypothesis (random dispersion of weed species) was unlikely due to the effect of repeated measures within sites and subplots over the four years, blocking (sites) and nesting (subplots in sites) arguments were added to produce a permutational test to compare the effect of stand age within different groups (here the subplots nested in sites). Then, a three-table ordination method (RLQ analysis) (Dray et al., 2014; Dray and Legendre, 2008) was used to investigate the relationship between weed community traits and IWG growth using the library `ade4` (Chessel et al., 2004). RLQ analysis enabled the joint structure of three matrices to be assessed; namely, R (environmental characteristics of samples), L (species distribution across samples) and Q (species traits) (Dolédéc et al., 1996; Dray et al., 2014). The matrix R contained three environmental variables: site code (1, 2, 3), age of IWG stands, and sampling year for each sample. The matrix L contained the standardized relative abundances of weeds. The matrix Q contained the eight trait values for each of the 26 weed species (Table 2). Correspondence analysis was carried out on the L-matrix. For the R and Q tables, mixed principal component analysis were performed (Hill and Smith, 1976), conserving row weight of

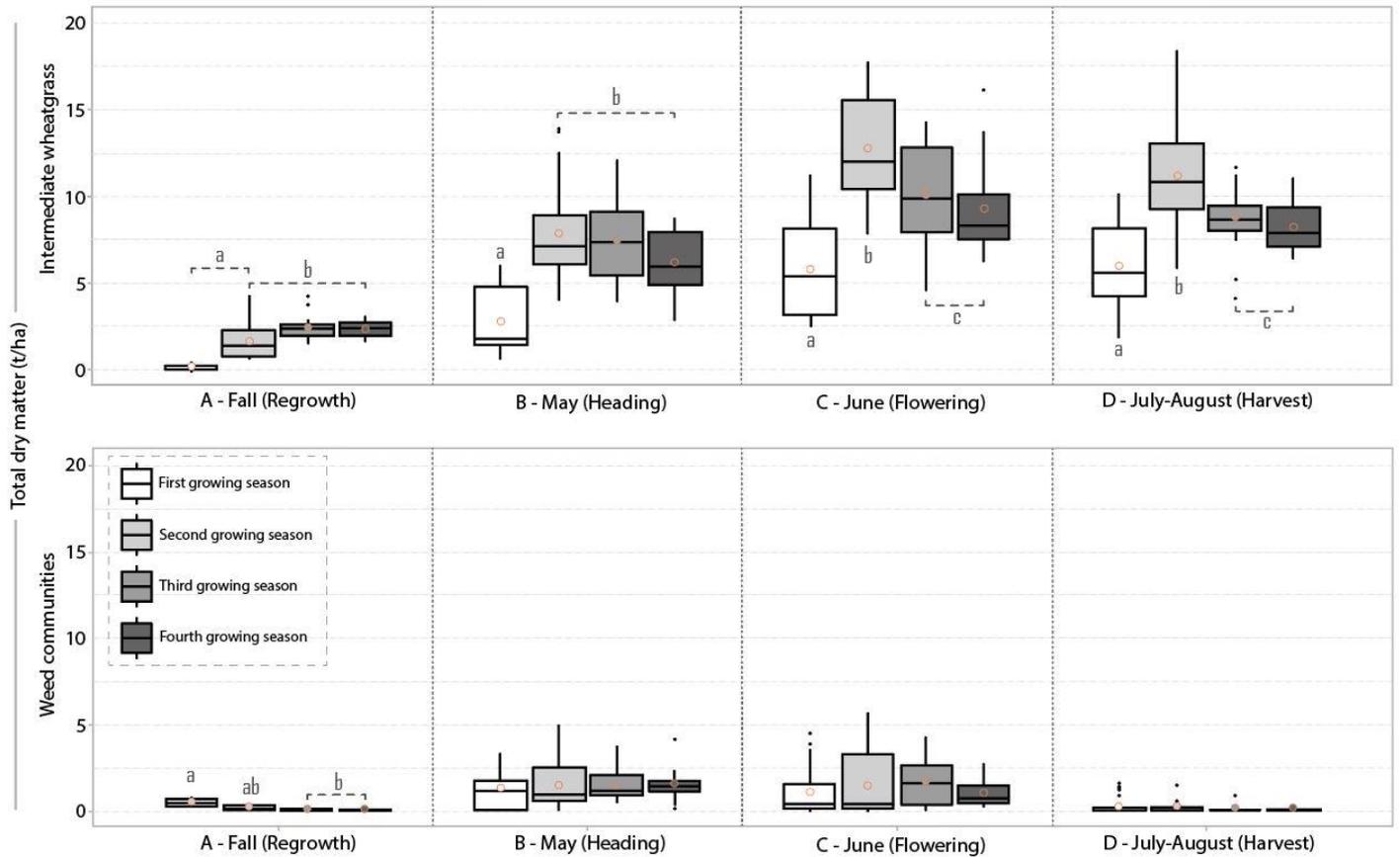
227 the correspondence analysis of the L table. Finally, a fourth-corner statistic was computed  
228 (Dray and Legendre, 2008), in which the link between traits and environment variables were  
229 measured by Pearson correlation, Chi2, or Pseudo-F index (ratio of between-cluster variance),  
230 depending on the type of variable (i.e., quantitative or qualitative). Significance was tested by  
231 a permutation procedure, resulting in 5,000 Monte Carlo permutations of the rows of matrices  
232 R and Q (Dolédec et al., 1996).

### 233 **3. RESULTS**

#### 234 **3.1. *Biomass of IWG and weeds***

##### 235 **3.1.1. *Biomass and yields of IWG***

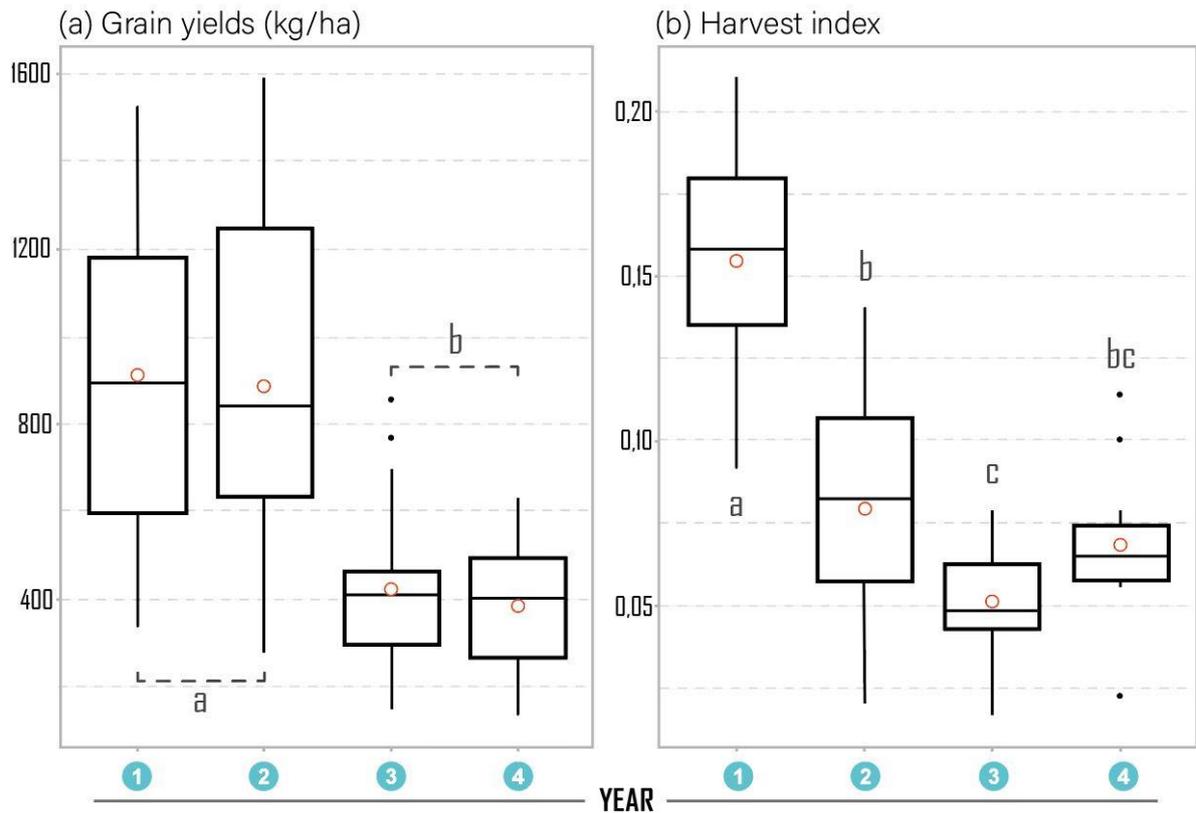
236 The mixed model showed that sampling period, stand age, and their interaction had highly  
237 significant effects on IWG biomass ( $p$ -value < 0.001). Weed biomass had no significant effect  
238 ( $p$ -value = 0.15).



239 **Figure 1:** Total biomass of IWG and weeds (tons of dry matter per hectare) during fall (A),  
 240 crop heading (B), crop flowering (C), and harvest (D), in the first, second, third, and fourth  
 241 growing seasons. [Colors useful]

242 During the first year of growth (establishment year), IWG biomass was consistently lower  
 243 compared to the subsequent years, in both fall and spring (Figure 1, Table S1). IWG biomass  
 244 was very low ( $0.087 \text{ t DM}\cdot\text{ha}^{-1}$  on average) during the first fall (Figure 1a). This low biomass  
 245 corresponded to small seedlings with four to five leaves maximum. During the first spring  
 246 biomass was  $2.53$  and  $6.10 \text{ t DM}\cdot\text{ha}^{-1}$  (heading and flowering stages respectively), and rose to  
 247 and  $6.09 \text{ t DM}\cdot\text{ha}^{-1}$  in summer (harvest) (Figure 1).

248 On average, IWG biomass during fall increased across the four years (Figure 1). At flowering  
 249 and harvest, biomass was highest during the second year of growth ( $12$  and  $13 \text{ t DM}\cdot\text{ha}^{-1}$ ,  
 250 respectively). Biomass was similar in the third and fourth growing seasons ( $9.36$  and  $8.76 \text{ t}$   
 251  $\text{DM}\cdot\text{ha}^{-1}$  at flowering, respectively).



252  
 253 **Figure 2:** Grain yield (a) and harvest index (b) of IWG at harvest time during the first, second,  
 254 third, and fourth growing seasons. Circles in boxplots indicate mean values. Letters indicate  
 255 statistical differences between years ( $\alpha = 0.05$ ). [Colors not useful]

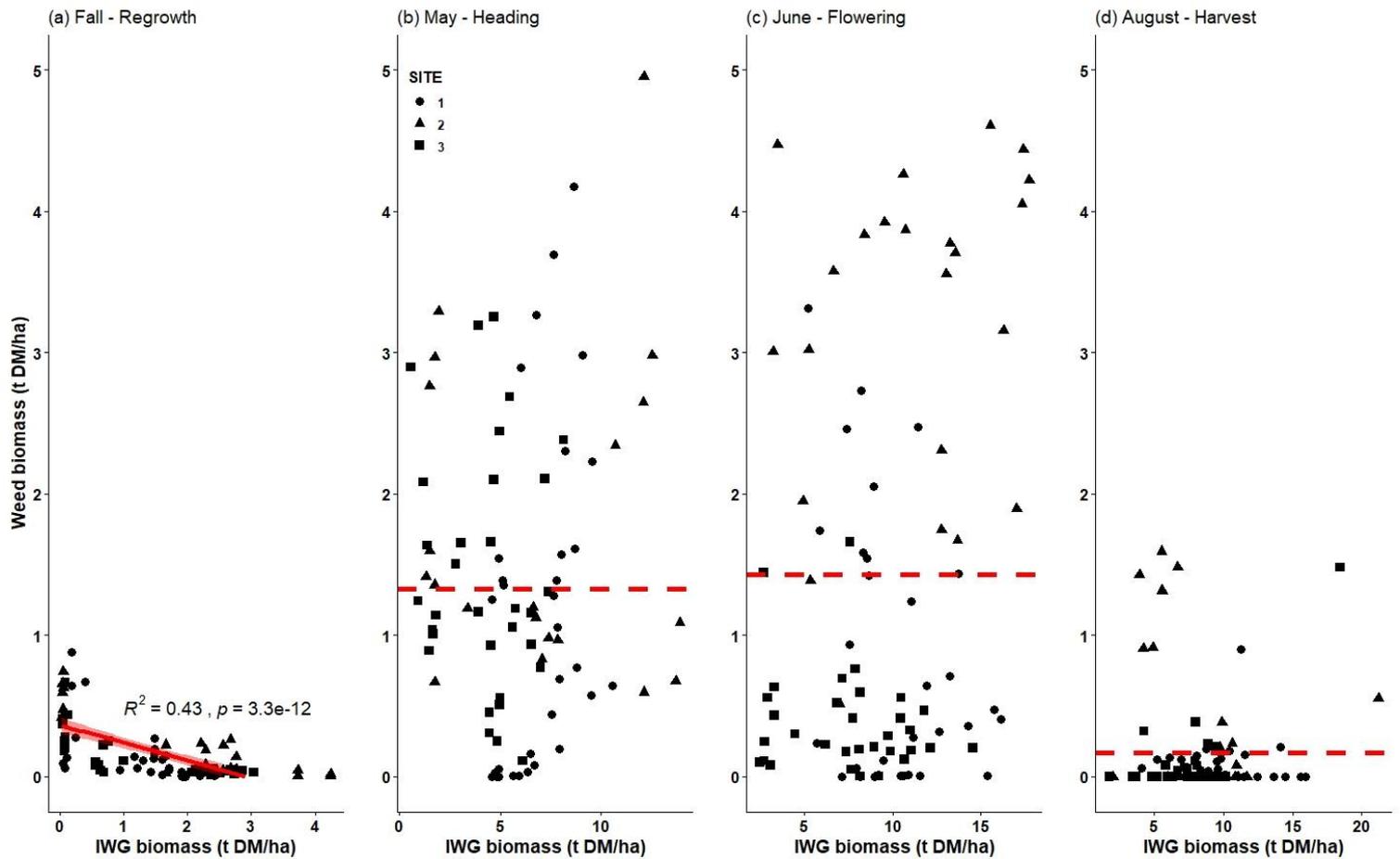
256 The highest IWG grain yield was obtained during the first and second years of growth, and  
 257 then dropped in the third and fourth years (Figure 2a). Summer and spring weed biomass did  
 258 not significantly affect grain yield. Grain yield was associated with mean harvest indices of 0.05  
 259 to 0.16 (Figure 2b). The harvest index was highest in the first year of growth, and then  
 260 decreased in the second year, due to higher IWG biomass not leading to higher grain yields.  
 261 The drop in grain yield during the third growing season caused the harvest index to decline  
 262 further. Biomass production at harvest was a good predictor of grain yield for the first growing  
 263 season ( $R^2 = 0.74$ ,  $p$ -value  $< 0.001$ ); however, the correlation was not significant in the second  
 264 year ( $R^2 = 0.06$ ), and was much weaker in the third and fourth years (0.31 and 0.29,  
 265 respectively;  $p$ -value  $< 0.01$ ).

266           3.1.2. *Weed biomass*

267    Sampling period and its interactions with IWG stand age and biomass significantly explained  
268    weed biomass (p-value < 0.01, < 0.05, < 0.01, respectively). In fall, weed biomass was 0.02 to  
269    0.57 t DM.ha<sup>-1</sup>. Biomass was significantly higher during the establishment year compared to  
270    the third and fourth year of growth, in which weed biomass was very low (Figure 1). A significant  
271    negative correlation was observed between the reduction in weed biomass and increase in  
272    IWG biomass during fall over the first three years of growth (Figures 1 and 3), corresponding  
273    to a 92.3% reduction in mean weed biomass.

274    At crop heading and flowering, weed biomass reached a mean 1.29 and 1.72 t DM.ha<sup>-1</sup> during  
275    the first year, and remained similar in the following three years (Figure 1). At site 3, where  
276    weed biomass was initially the lowest, a significant increase occurred the third year, reaching  
277    levels similar to sites 1 and 2 (Table S1). Overall, in spring, no significant relationship was  
278    found between IWG and weed biomass (Figure 3).

279    At harvest, weed biomass declined in all years (mean: 0 to 0.3 t DM.ha<sup>-1</sup>, Figure 1), and was  
280    not influenced by the biomass or yield of IWG. This phenomenon reflected the delay between  
281    IWG and weed growth cycles, as almost all the weeds were senescent or had decayed when  
282    IWG was harvested.



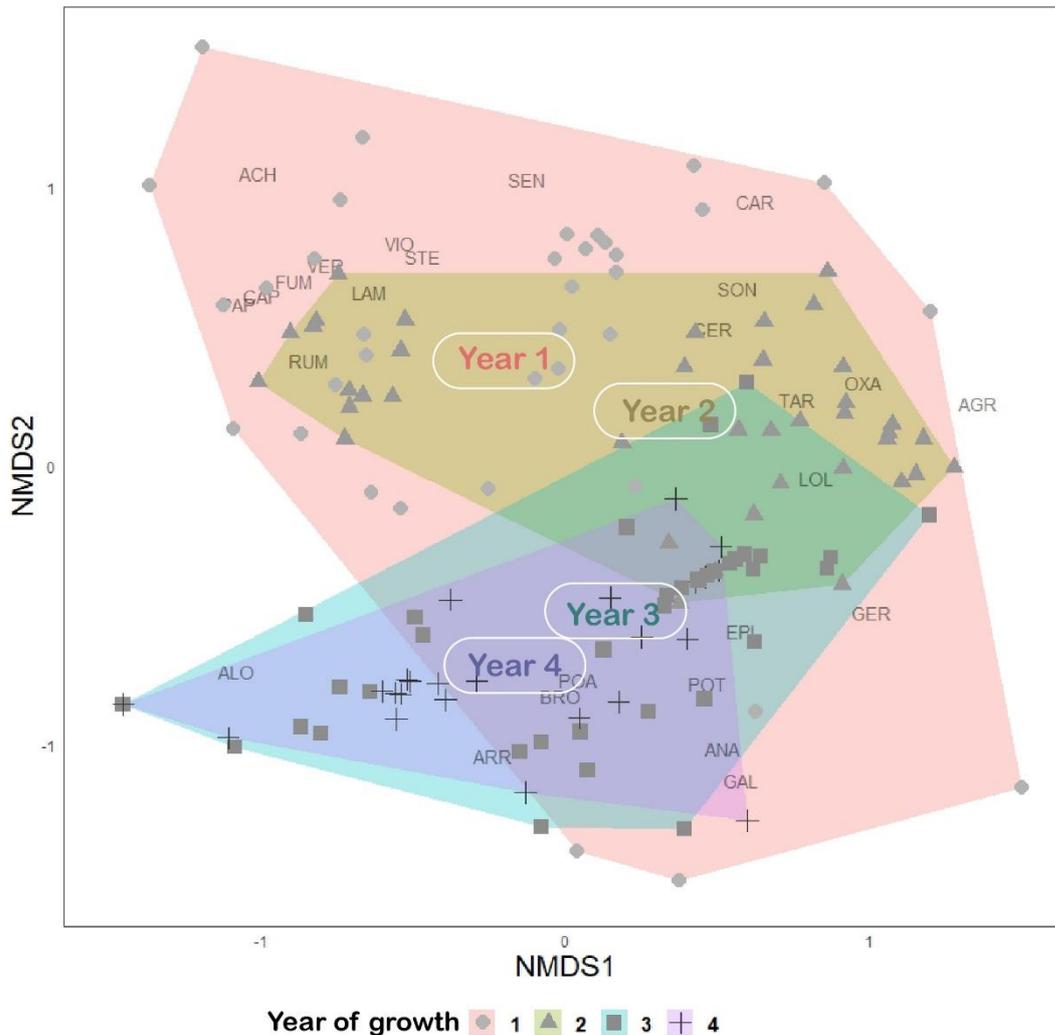
283

284 **Figure 3:** Weed biomass as a function of intermediate wheatgrass (IWG) biomass in fall (a),  
 285 crop heading (b), crop flowering (c), and harvest (d). Both parameters are expressed in tons  
 286 of total dry matter per hectare. Only significant relationships are reported on panels. In the  
 287 absence of significant correlations, horizontal dashed lines indicate the mean value of weed  
 288 biomass. [Colors should be used]

289 **3.2. Structure and traits of the weed community in spring**

290 NMDS (stress = 0.163; Figure 4) showed that perennial IWG stands affected the structure of  
 291 weed communities in the spring of all four cropping years. Spring sampling time had no effect  
 292 (crop heading or flowering). The initial situation (first year) differed across the three sites.  
 293 However, these differences strongly declined over the study period, with year three and four  
 294 largely overlapping, while being clearly distinct from the first year. The permutational test  
 295 showed that IWG stand age significantly affected the structure of the weed community (p-value

296 < 0.001). Weed species richness declined from the establishment year to the last growing  
297 season at all three sites (from 14 to 5, 12 to 4, and 18 to 6, respectively, for sites 1, 2, and 3).  
298 For sites 1 and 2, Shannon diversity index declined (from 1.26 to 0.75, and 1.54 to 0.40,  
299 respectively); however, species evenness remained constant at site 1 (0.48 to 0.46) but  
300 dropped at site 2 (from 0.62 to 0.29). This trend at site 2 was attributed to the increasing  
301 dominance of *Lolium perenne*, followed by *Poa trivialis*, whereas the other species were highly  
302 variable. Site 3 had a stable species diversity index (from 0.96 to 0.90), due to a compensatory  
303 effect between lower species richness and higher evenness over the four years (0.33 to 0.50).  
304 The initial dominance of *Galium aparine* and *Veronica persica* was progressively balanced by  
305 an increase in other species, such as *Epilobium hirsutum*, *Bromus Sterilis*, *Arrhenatherum*  
306 *elatius*, and *Poa trivialis*.

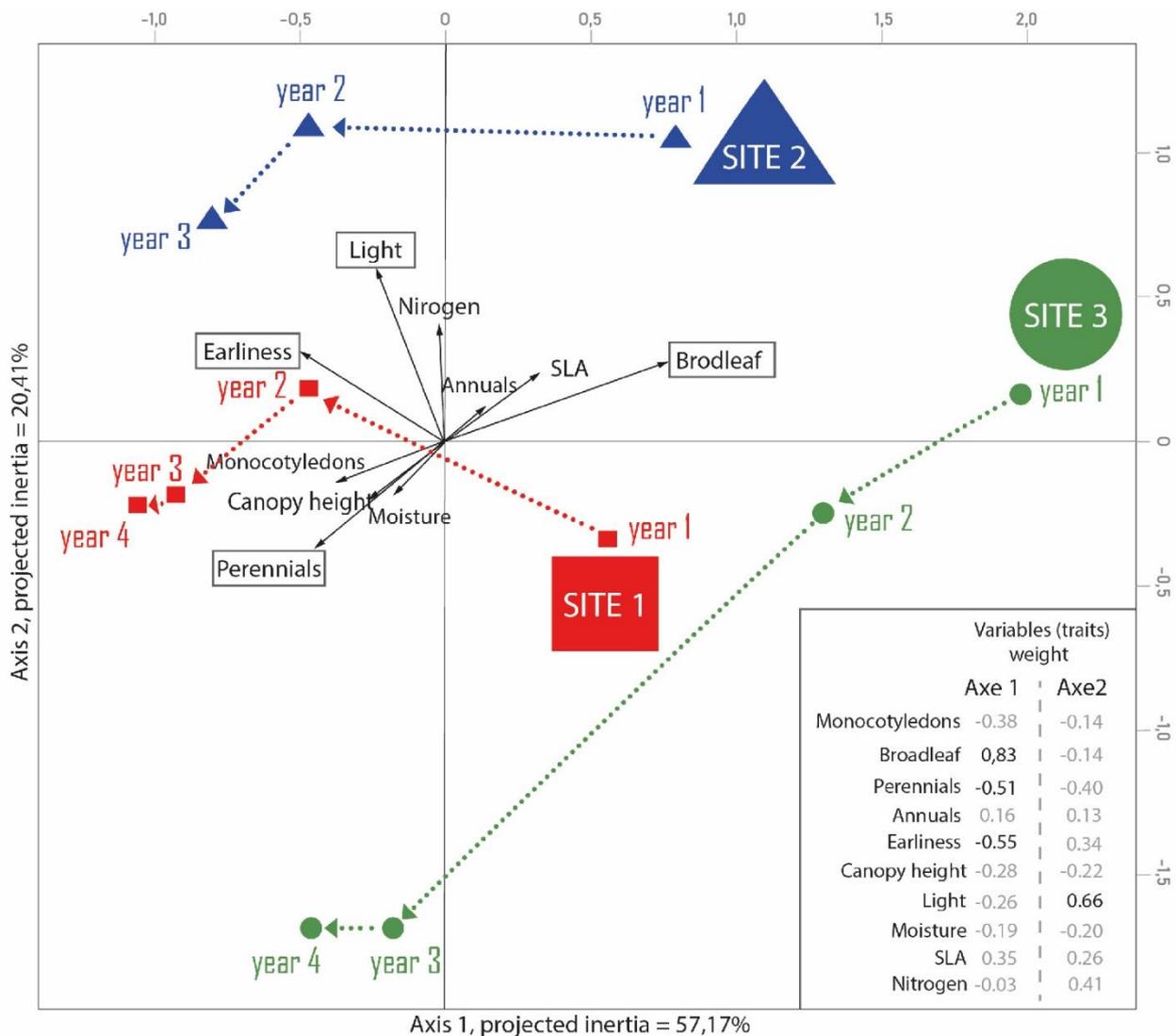


307 **Figure 4:** Non-Metric Multidimensional Scaling (NMDS) analysis of the relative abundance of  
 308 weed species in IWG crops during spring in the first, second, third, and fourth years of growth.  
 309 The centroid of each species and year is given by labeled positions. [Colors useful]

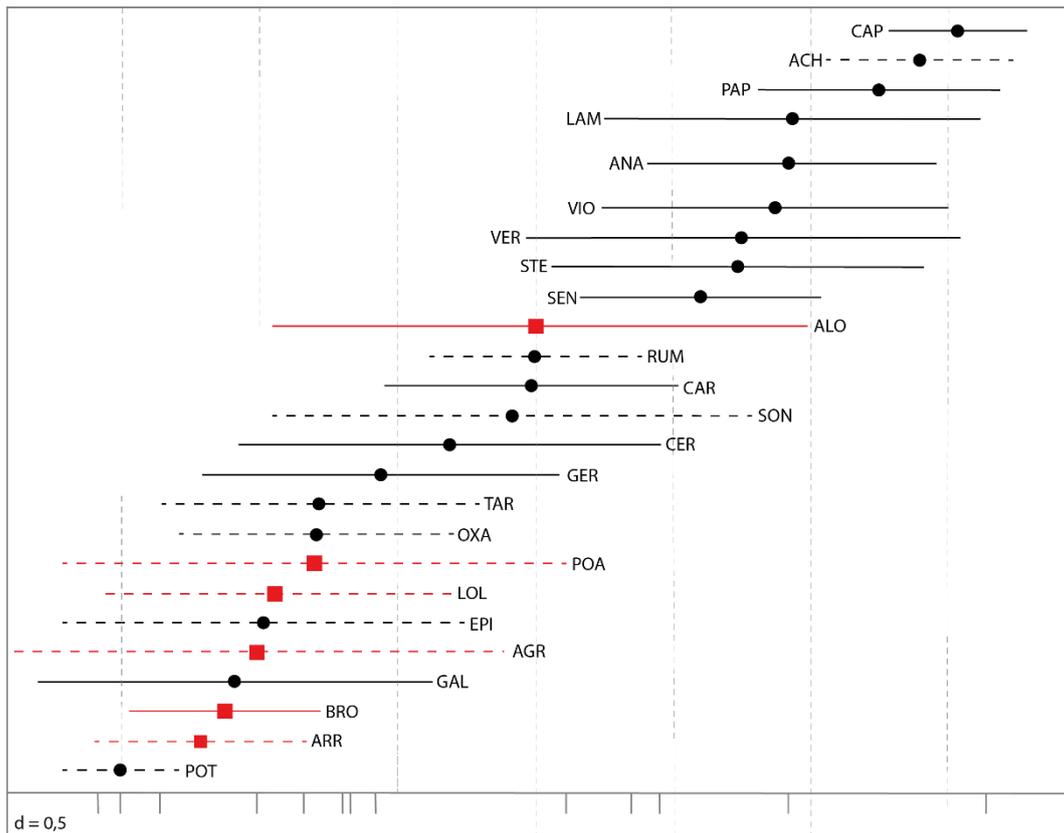
310 RLQ showed how the weed community changed over time and between sites (Figure 5). In all  
 311 three sites, most variation occurred along the first axis (57.17%), which contained (canonical  
 312 absolute weight of variables > 0.5) plant life history (annual – perennial), division  
 313 (monocotyledons – broadleaves), and flowering earliness as the main driving traits. The  
 314 transition from year 1 to year 2, 3, and 4 was characterized by fewer broadleaf plants, shifting  
 315 towards grass-dominated communities (e.g., *Lolium perenne*, *Alopecurus myosuroides*, *Poa*  
 316 *trivialis*, *Bromus sterilis*, *Arrhenatherum elatius*, *Agrostis capillaris*; Figure 6), and more early  
 317 (e.g., *Taraxacum sp.*, *Alopecurus myosuroides*, *Poa trivialis*) and perennial (e.g.,

318 *Arrhenatherum elatius*, *Agrostis capillaris*, *Epilobium hirsutum*, *Lolium perenne*, *Poa trivialis*;  
319 species (Figure 6). Fourth corner analysis only identified plant division (i.e., monocotyledons  
320 vs broadleaf) as being significantly influenced by the longevity of the IWG stand (p-value <  
321 0.01). Minor drivers identified by RLQ included SLA and weed height, showing that as the  
322 community shifted from annuals and broadleaved species towards perennials and grasses,  
323 taller species with lower SLA became, to a certain extent, more prominent. The shift from year  
324 three to four was marginal compared to shifts in years one and two.

325 No consistent trend was observed across the three sites over the four years on the second  
326 axis. This axis was mostly characterized by minor drivers, including the light requirement of  
327 weed communities (canonical absolute weight of variables > 0.5), along with nitrogen  
328 requirement, earliness, and plant division. Overall, site position on the second axis showed the  
329 variability of the initial weed communities and its importance of understanding later community  
330 assemblages at each site. On the second axis, sites 1 and 2 maintained approximately the  
331 same position across years. In contrast, site 3 exhibited a noticeable shift from species with  
332 high light requirement (e.g., *Sonchus arvensis* and *Cardamina hirsute*) to less light demanding  
333 species (e.g., *Galium aparine*, *Agrostis Capillaris*, *Arrhenatherum elatius*, *Bromus sterilis*, *Poa*  
334 *trivialis*, *Lolium perenne*).



335 **Figure 5:** RLQ analysis of the multiple relationships between species traits (life history,  
 336 division, earliness of flowering, average canopy height, average SLA, light-moisture-nitrogen;  
 337 Ellenberg index; Table 2), experimental site, and IWG growth (longevity). First and second  
 338 axes summarized 57.17 and 20.41% of inertia, respectively. Trait variables with a canonical  
 339 weight > 0.5 (absolute value) on RLQ axes 1 or 2 are shown. Each point in the ordination plot  
 340 represents the site-year position modeled (canonical weight) according to its traits on RLQ  
 341 axes 1 and 2. Squares = site 1, triangles = site 2, circles = site 3. [Colors should be used]



342 **Figure 6:** Average position and standard deviation of each species at crop flowering according  
 343 to normalized site scores (x axis; RLQ analysis). Dispersion along the x-axis represents the  
 344 distance between species in the context of growth (i.e., site and year conditions). Grass  
 345 species are represented with red lines and squares. Perennial species are represented with  
 346 dashed lines. Species codes are listed in Table 2. [Colors should be used]

## 347 **4. DISCUSSION**

### 348 **4.1. Biomass and grain yields of IWG**

349 The biomass and yield of IWG in this study were consistent with those of previous studies  
 350 (Fernandez et al., 2020; Hunter et al., 2020a,b; Law et al., 2021a; Zimbric et al., 2020).  
 351 Importantly, low grain yields, and variability in both grain and biomass production, likely  
 352 represent a significant hurdle in adopting IWG as a novel perennial grain crop by farmers.

353 Low grain yields of IWG have been explored by many previous studies (Altendorf et al., 2021;  
 354 Cassman and Connor, 2022; Cattani, 2017; Tautges et al., 2018), with dedicated breeding  
 355 programs existing to improve them. Variability in yield might have been exacerbated in our

356 study by the relatively low rate of nitrogen fertilization applied each year (50 kg N.ha<sup>-1</sup>)  
357 compared to the theoretical optimal range identified by previous studies on the role of nitrogen  
358 in building grain yields (61 to 96 kg N.ha<sup>-1</sup>; Fernandez et al., 2020; Jungers et al., 2017). As  
359 nitrogen content was not measured in our study, a definitive conclusion cannot be obtained  
360 about the balance between nitrogen input and nitrogen use by plants. Also, the plant allocation  
361 strategy in case of nitrogen deficiency is not known (translocation to roots, stems, leaves or  
362 grains). At 10 tons of aboveground biomass per hectare at flowering, nitrogen content in the  
363 aboveground tissue of IGW is about 1% (Fagnant et al., *under revisions*), representing 100 kg  
364 nitrogen in plant tissue per hectare. Between flowering and harvest, grain filling would likely  
365 cause overall nitrogen demand to slightly increase. Thus, more than 50 kg of the nitrogen  
366 required is not provided by fertilizers, and depends nitrogen availability in the soil pool, which  
367 tends to be underestimated because nitrogen in roots is not included in this calculation.  
368 Besides, heterogeneity of plant emergence at establishment (not measured) and planting  
369 dates likely induced heterogeneous seedling vigor initially. However, all sites were planted  
370 early enough to allow sufficient GDD accumulation (~900 GDD) and tillering before winter and  
371 vernalization, based on Olugbenle et al. (2021). Variation in temperature and moisture  
372 conditions between growing seasons also caused heterogeneity in our study. For example, the  
373 2019–2020 growing season was warmer, whereas the 2020–2021 growing season was wetter  
374 (Table 1).

375 Irrespective of site or climatic year, biomass production peaked during the second growing  
376 season, and likely corresponded to a peak in reproductive growth (i.e., many fertile tillers that  
377 induced stems elongation; Altendorf et al., 2021; Fernandez et al., 2020; Hunter et al., 2020a).  
378 However, the harvest index dropped after the first year (Figure 2b), indicating a potential trade-  
379 off between tiller density and tiller fertility. Previous studies also reported a decline in grain  
380 yield over time, driven by a decrease in the fertility of tillers and spikes (Altendorf et al., 2021;  
381 Fernandez et al., 2020; Hunter et al., 2020). Regardless of the underlying yield components  
382 involved, this reduced fertility led to very low grain yields in the third and fourth years of growth

383 (Figure 2a). Tillering is a complex process in grasses, as it is under multifactorial control (e.g.,  
384 light quality, nutrient and water availability, defoliation regime, temperature; Assuero and  
385 Tognetti, 2010). This process is especially complex in perennial species, like IWG, for which  
386 the regulation of tiller emergence and differentiation in relation to environmental cues remain  
387 poorly understood (Lafarge and Durand, 2011; Rouet et al., 2021). Thus, more research is  
388 required to clarify tillering dynamics, which would allow appropriate management practices to  
389 be designed (e.g., planting design, mowing, grazing, fertilization, residue management) that  
390 maintain the production of fertile tillers over multiple growing seasons.

391 In our study, mixed models (section 3.1.1) and regression analysis (section 3.1.2, Figure 3) did  
392 not indicate that high or low weed biomass depressed IWG yields. This result supported that  
393 of Zimbric et al. (2020), who showed that yield did not increase in plots where weeds were  
394 removed (starting from the second growing season) compared to unweeded plots. However,  
395 weeds are a major cause of yield loss in grain production (Adeux et al., 2019; Barberi, 2002;  
396 Oerke, 2006), largely due to competition for resources (light, nutrients, water; Zimdahl, 2007).  
397 This competition is particularly deleterious for wheat yields during stem elongation and  
398 flowering (Fischer, 2020 and references therein). Yet in the current study, the highest weed  
399 biomass was recorded in late spring, during the elongation phase of IWG, up to anthesis  
400 (Figures 1 and 3). Overall, our results (together with previous findings; Zimbric et al., 2020)  
401 raise the question of why IWG biomass and grain production appear to be relatively insensitive  
402 to important spring weed biomass compared to annual grains. A recent study investigating how  
403 different weed communities impact winter cereals showed that weed biomass alone, especially  
404 when sampled late in the season, is a poor predictor of yield loss (Adeux et al, 2019). The  
405 authors showed that weed-crop interference is better captured in terms of niche competition.  
406 In other words, weed traits are critical for understanding their impact, with weeds that occupy  
407 the same niche as crops being the most deleterious. IWG likely displays high ecological  
408 complementarity with the weed flora encountered in typical annually disturbed agrosystems,  
409 such as those of this study, initially mitigating the impact of weeds on IWG yield. Further studies

410 are required to test this hypothesis, and potentially identify the weed traits that are the most  
411 deleterious to IWG growth and grain yield in the field.

#### 412 4.2. *Does weed biomass change under IWG over time?*

413 Our results showed that weed biomass declined during fall in all four years of IWG growth  
414 (Figure 1 and 3). This decline might have been due to increased competition with the crop in  
415 fall, as IWG biomass was significantly higher during regrowth periods compared to when it was  
416 establishment in the first fall (Figure 1). Alternatively, weed emergence might have declined  
417 because soil preparation and tillage operations were not implemented after sowing the crop.  
418 Also, the composition of the weed community might have shifted towards grasses, which  
419 remain vegetative in fall. In any case, slow IWG growth during establishment in the first fall  
420 clearly made the crop prone to substantial weed development in the first months after sowing,  
421 with potentially enduring effects in terms of crop growth and weed management for farmers.

422 In spring, weed biomass was already high in the first year, and remained high throughout the  
423 experimental period, despite higher IWG biomass in spring during the regrowth years. These  
424 weed biomass levels tended to be higher than that recorded for grain crops, such as wheat  
425 and barley, under organic management, for which weed biomass rarely exceeds 0.5 t DM.ha<sup>-1</sup>  
426 (Drews et al., 2009; Lundkvist et al., 2008). Such high weed abundance is not likely to be  
427 tolerated by farmers in strict grain cropping systems where weed biomass cannot be valorized  
428 as forage, and controlling weed seedlings is an important issue. Law et al. (2021a) reported  
429 stable weed biomass in spring over three years in IWG fields, whereas Zimbric et al. (2020)  
430 reported a significant decline after the establishment year. Yet in the latter study, weed  
431 biomass was sampled at grain harvest in summer, when early weeds were likely to have  
432 already senesced. In fact, the significant decline in weed biomass recorded from the spring to  
433 harvest sampling periods in our study (Figure 1) shows that sampling weeds at IWG harvest  
434 might largely underestimate weed development, as most observed species grew in spring, and  
435 were already senesced at the IWG harvest stage. In addition, the initial mean weed biomass  
436 recorded in the current study was double that recorded by Zimbric et al. (2020); thus, weed

437 pressure was less challenging in this previous study, and the diversity of weed species was  
438 lower (notably very few grass species).

439 The fact that weed biomass did not decline in spring over the four years contrasts with previous  
440 studies highlighting the value of temporary grasslands in promoting weed regulation for crop  
441 rotations (Bretagnolle et al., 2011; Dominschek et al., 2021; Lemaire et al., 2015; Meiss et al.,  
442 2010a). However, data on temporary grasslands cannot be easily transposed to IWG fields  
443 because management practices notably differ. For instance, absence of mowing and grazing  
444 are absent in IWG fields, but are common practices during the entire spring period in  
445 grasslands (Schuster et al., 2018, 2016). Since grain production is at stake, IWG tillers cannot  
446 be cut or grazed after the start of stem elongation until grain maturity in late July or early  
447 August. Consequently, many weed communities are able to complete their entire life cycle and  
448 produce new seeds for the following year before IWG is harvested. This phenomenon is  
449 particularly an issue with narrow-row planting (as is the case in this study), where mechanical  
450 weeding is technically challenging.

451 Unlike IWG grown in pure stands, grasslands generally harbor a mixture of different grass  
452 and/or legume species. Through complementarity and stabilizing niche differences (Cardinale  
453 et al., 2007; Duchene et al., 2017; HilleRisLambers et al., 2012), functional diversity in mixtures  
454 generally allow a better use of resources, leaving less vacant space for weed species to  
455 develop. As such, previous studies highlighted the benefit of IWG intercropping with legumes  
456 to reduce weed biomass (Law et al., 2021b), but also to increase forage value (Favre et al.,  
457 2019) and stabilize grain yields over multiple years (Dimitrova Mårtensson et al., 2022; Tautges  
458 et al., 2018).

#### 459 *4.3. Do weed communities change with ageing IWG stands?*

460 In our study, weed communities under ageing stands of IWG had lower diversity compared to  
461 those under young stands. Furthermore, as IWG stands aged, the composition of weed  
462 communities significantly shifted from primarily annual broadleaved weeds towards grass

463 species and, to a lesser extent, perennial and earlier flowering species (Figure 5 and 6). A  
464 larger number of sites is needed to confirm whether the life history and earliness of plants are  
465 robust drivers. Also, weed trait values could be improved by measuring the traits of studied  
466 samples rather than using trait values provided in databases at the species level. While this  
467 approach is sufficiently robust for some traits (e.g., plant division or life history), it does not  
468 account for the plasticity of other traits in a given environment (e.g., SLA, height). At our study  
469 sites, which had a history of annual rotation, a minimum of three years was required to observe  
470 shifts in the weed community under IWG. Similar changes were observed at other studies for  
471 both IWG (Law et al., 2021a; Zimbric et al., 2020) and temporary grasslands (Bretagnolle et  
472 al., 2011; Hiltbrunner et al., 2008; Meiss et al., 2010a), with these studies reporting the  
473 selection of grass weed species in perennial stands over time (e.g., *Poa trivialis*, *Phleum*  
474 *pratense*).

475 At our study sites, the main grass species for which abundance increased included *Poa*  
476 *trivialis*, *Lolium perenne*, *Agrostis capillaris*, *Arrhenatherum elatius*, and *Bromus sterilis*. These  
477 species were mostly perennials that flowered earlier than IWG (Table 2; Figure 6). At one of  
478 the sites, the early annual *Alopecurus myosuroides* was also present; however, it was already  
479 relatively abundant in the first year, and no further increase was recorded. Among the few  
480 broadleaf species that were also able to grow under IWG aging stands, almost all were  
481 perennials (e.g., *Taraxacum officinale*, *Epilobium hirsutum*, and *Potentilla reptans*), except  
482 *Galium aparine*.

483 The selection of species more adapted to co-existing with IWG reflected an interplay between  
484 differences in relative fitness and stabilizing niches (HilleRisLambers et al., 2012). The  
485 important changes that occurred when switching from an annually tilled system to a perennial  
486 system without tillage modify the fitness optimum for all species growing in a given  
487 agroecosystem, acting as a filter of weed species. In the current study, this phenomenon  
488 resulted in the clustering of weed species over time around two main functional traits: grass  
489 species and perennial habit, supporting previous studies (Dominschek et al., 2021; Fried et al.,

490 2022; Mainardis et al., 2020). Furthermore, functional dissimilarity, such as different growing  
491 cycles, also facilitate coexistence by limiting competition between species. The later phenology  
492 of IWG (about 1630 average GDD accumulation from February 1<sup>st</sup> to flowering; Duchene et  
493 al., 2021) compared to earlier temperate grass (<1300 GDD accumulation from February 1<sup>st</sup>  
494 to flowering; e.g. *Poa pratense*, *Arrhenantherum elatius*, *Lolium perenne*, *Dactylis glomerata*,  
495 *Festuca arundinacea*, *Alopecurus myosuroides*; Cruz et al., 2010) might explain why earlier  
496 grasses thrive in IWG fields. Changes to resource gradients (i.e., light, nitrogen, moisture)  
497 minimally affected our trait-based analysis (Figure 6). Still, light demanding species (e.g., *Viola*  
498 *arvensis*, *Cardamine hirsute*, *Achillea millefolium*, *Sonchus arvensis*; Table 2) were  
499 systematically hindered, and there was a clear change in the light requirement of weed  
500 communities at site 3, likely due to species that were only found at this location (e.g.,  
501 *Cardamine hirsute*, *Sonchus arvensis*, *Galium aparine*).

502 Among broadleaf species that continued to grow under IWG aging stands were *Epilobium*  
503 *hirsutum* and *Galium aparine*, which have a climbing morphology, contrasting to broadleaves  
504 with upright stature that are generally found in temporary grasslands (Meiss et al., 2010a). As  
505 mowing and grazing were not implemented at our three sites, the potential role of these two  
506 species in promoting or suppressing other weed species remains unexplored. However, as the  
507 value of IWG perennial grain is linked to its ability to produce both grain and forage (Favre et  
508 al., 2019; Law et al., 2022; Pugliese et al., 2019), the interactions between different defoliation  
509 regimes and the assemblages of weed communities requires further research.

## 510 **5. CONCLUSIONS**

511 This study provided new evidence on the evolution of weed abundance and community  
512 composition under ageing IWG stands (establishment to four years). Biomass and yield  
513 performance of the perennial grain crop supported previous studies, with biomass production  
514 being lower during the first year, while grain yield substantially declined after the second  
515 harvest. While weed biomass tended to decrease in the fall of each year, it remained constant

516 in each spring; however, the composition of the weed communities shifted. The species  
517 richness of weeds was also consistently lower under aging IWG stands, whereas evenness  
518 remained dependent on local site characteristics and initial weed communities. Grasses and  
519 perennials were largely favored over annual broadleaves; thus, strong fitness differences  
520 appear to drive community composition towards species with similar functional traits. In  
521 contrast, dissimilarity (e.g., phenological differences) might help to stabilize niche differences  
522 and favor particular species. The abundance of weed species co-existing with IWG  
523 demonstrates the need to assess the legacy effect of perennial grain in crop rotations. Such  
524 information could help optimize optimal management practices, including planting time and  
525 design, covered seeding, mechanical weeding, intercropping or mowing.

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## 530 **CONTRIBUTIONS**

531 O.D., C.D., and F.C. conceived and planned the experiments. O.D. carried out the  
532 experiments, sampling, formal analysis, and figure design. O.D., C.B. and F.C contributed to  
533 the interpretation of results. O.D. led the writing the manuscript. C.B. participated to the writing,  
534 editing and reviewing. C.D., B.D., and F.C. participated to reviewing and provided critical  
535 feedback.

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