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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⁻¹ respectively), with total biomass production peaking in the second year
22 (mean: 11.9 t DM.ha⁻¹). 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⁻¹), but remained constant in spring (mean: 1–2 t DM.ha⁻¹). 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 m² quadrats on each of the four subplots (1 m² 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⁻¹). 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 ⁻¹)		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 *clt* 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

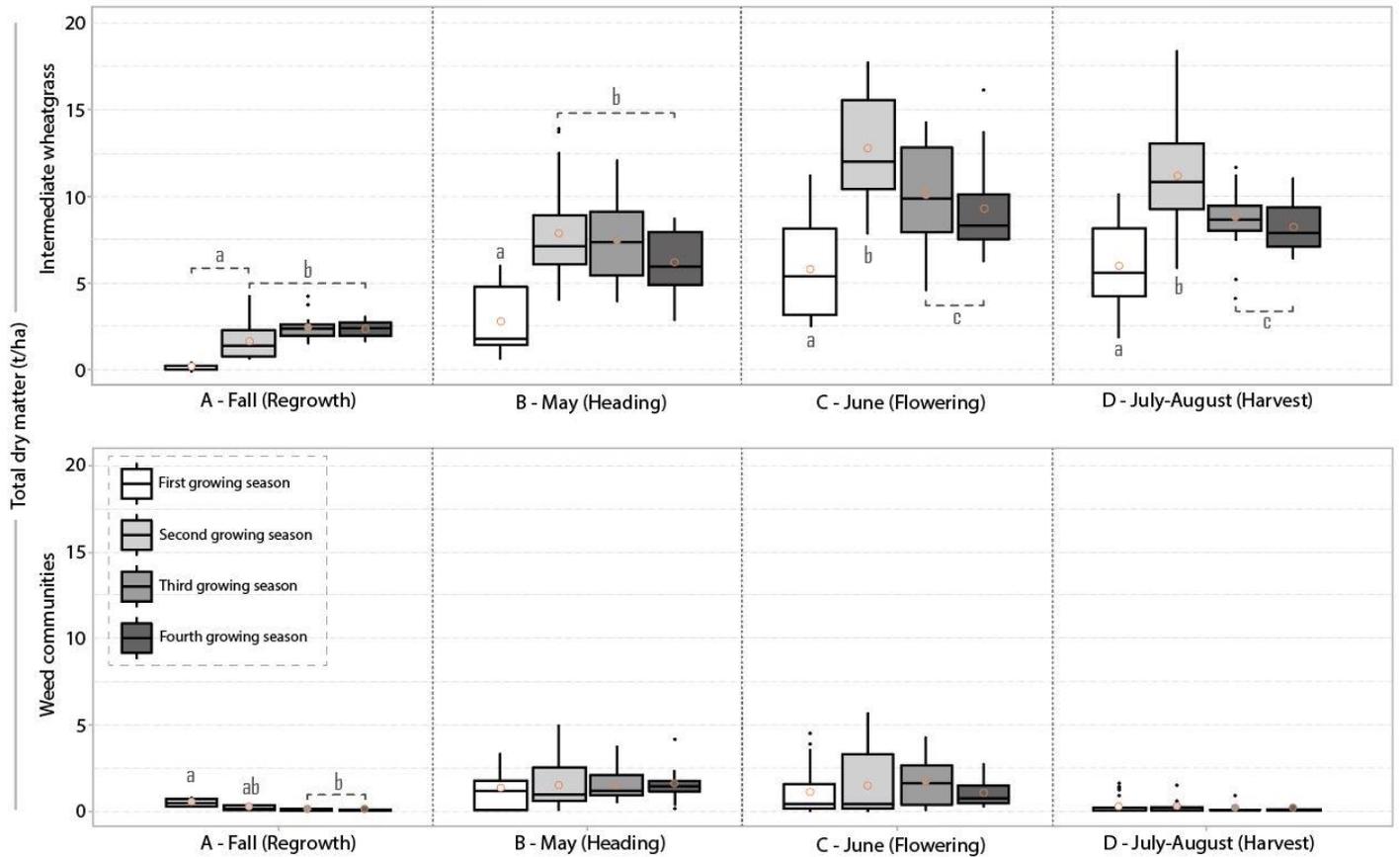
227 the correspondence analysis of the L table. Finally, a fourth-corner statistic was computed
228 (Dray and Legendre, 2008), in which the link between traits and environment variables were
229 measured by Pearson correlation, Chi2, or Pseudo-F index (ratio of between-cluster variance),
230 depending 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***

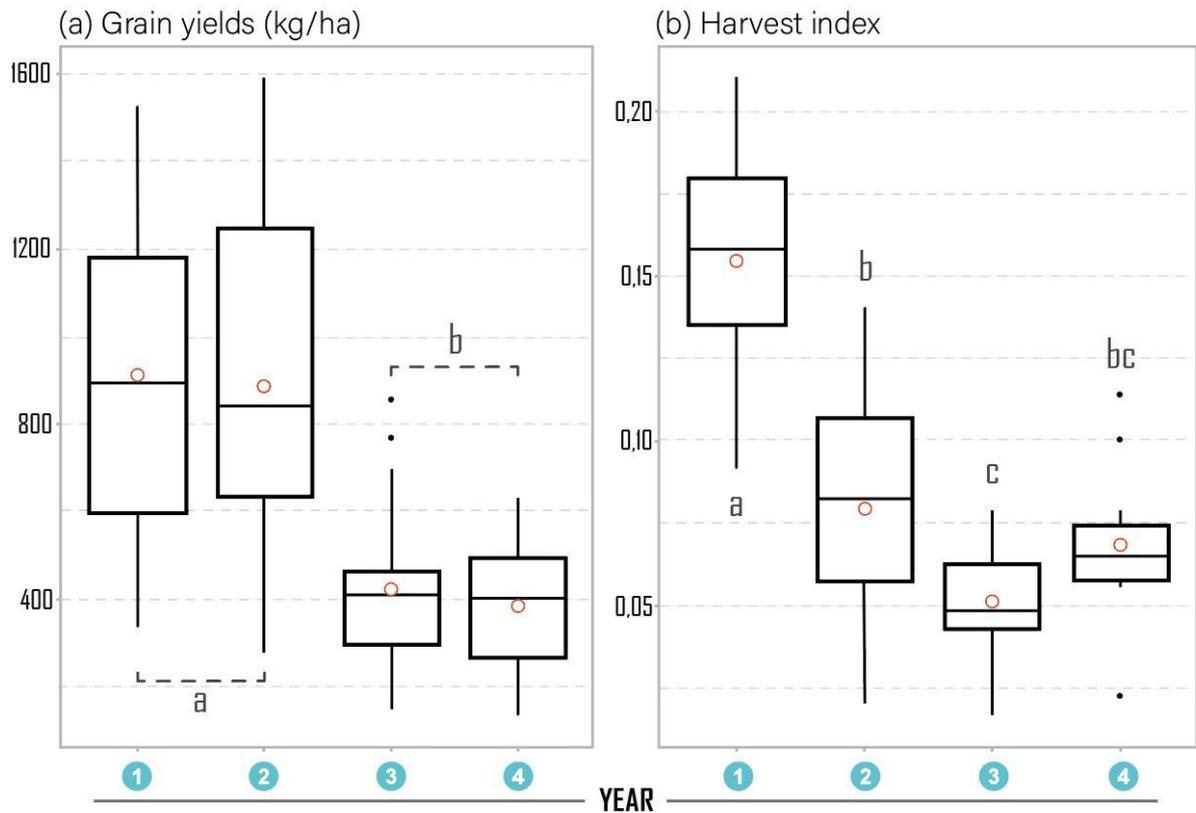
236 The mixed model showed that sampling period, stand age, and their interaction had highly
237 significant effects on IWG biomass (p -value < 0.001). Weed biomass had no significant effect
238 (p -value = 0.15).



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 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]

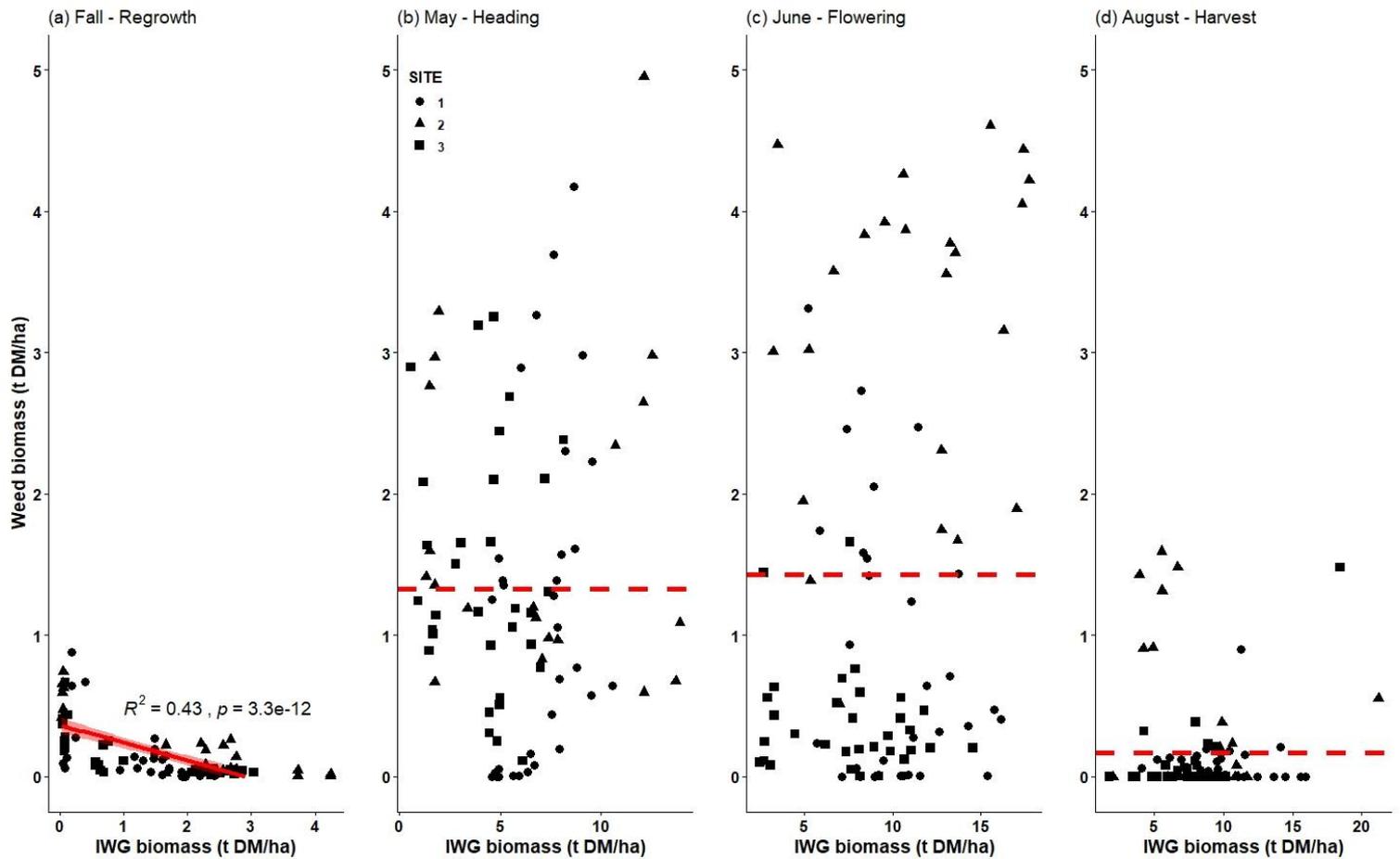
256 The highest IWG grain yield was obtained during the first and second years of growth, and
 257 then dropped in the third and fourth years (Figure 2a). Summer and spring weed biomass did
 258 not significantly affect grain yield. Grain yield was associated with mean harvest indices of 0.05
 259 to 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⁻¹. 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⁻¹ 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⁻¹, 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.



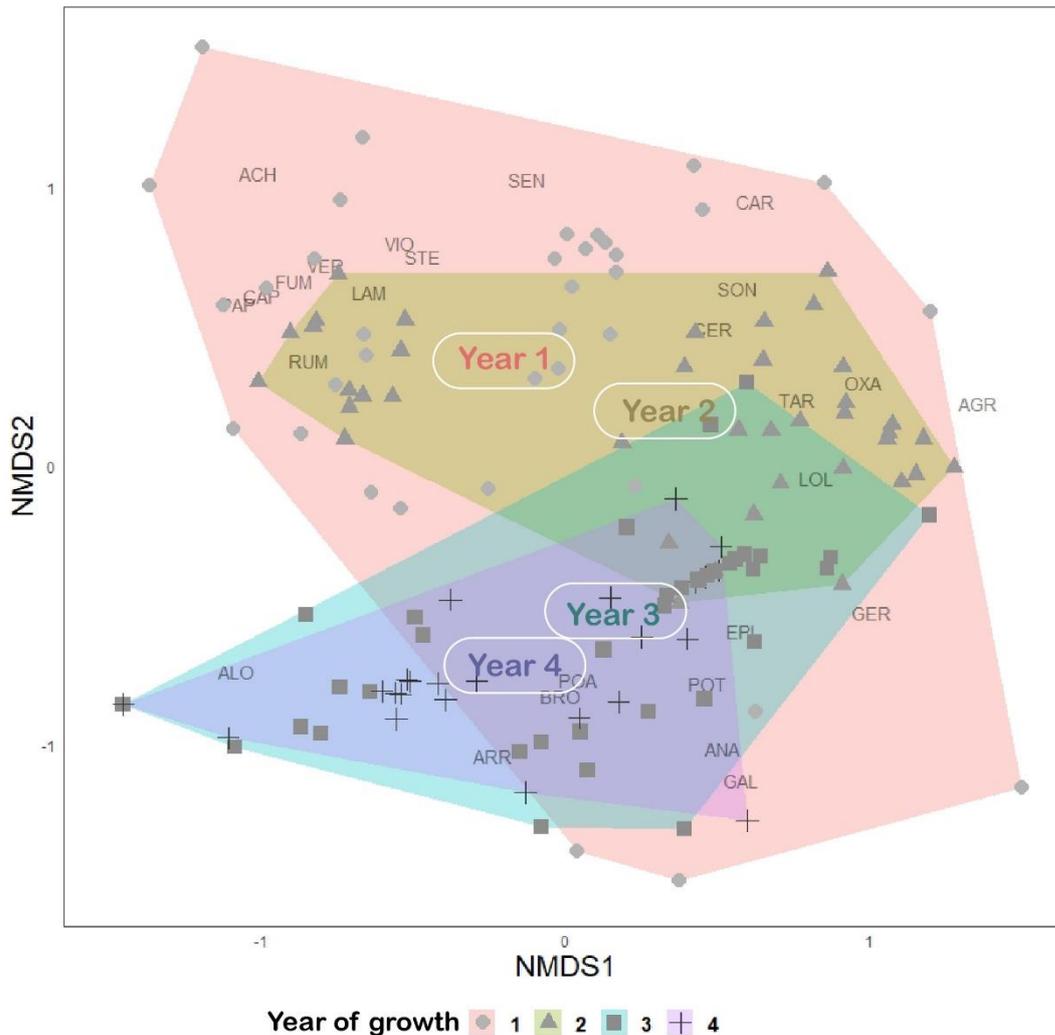
283

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*.

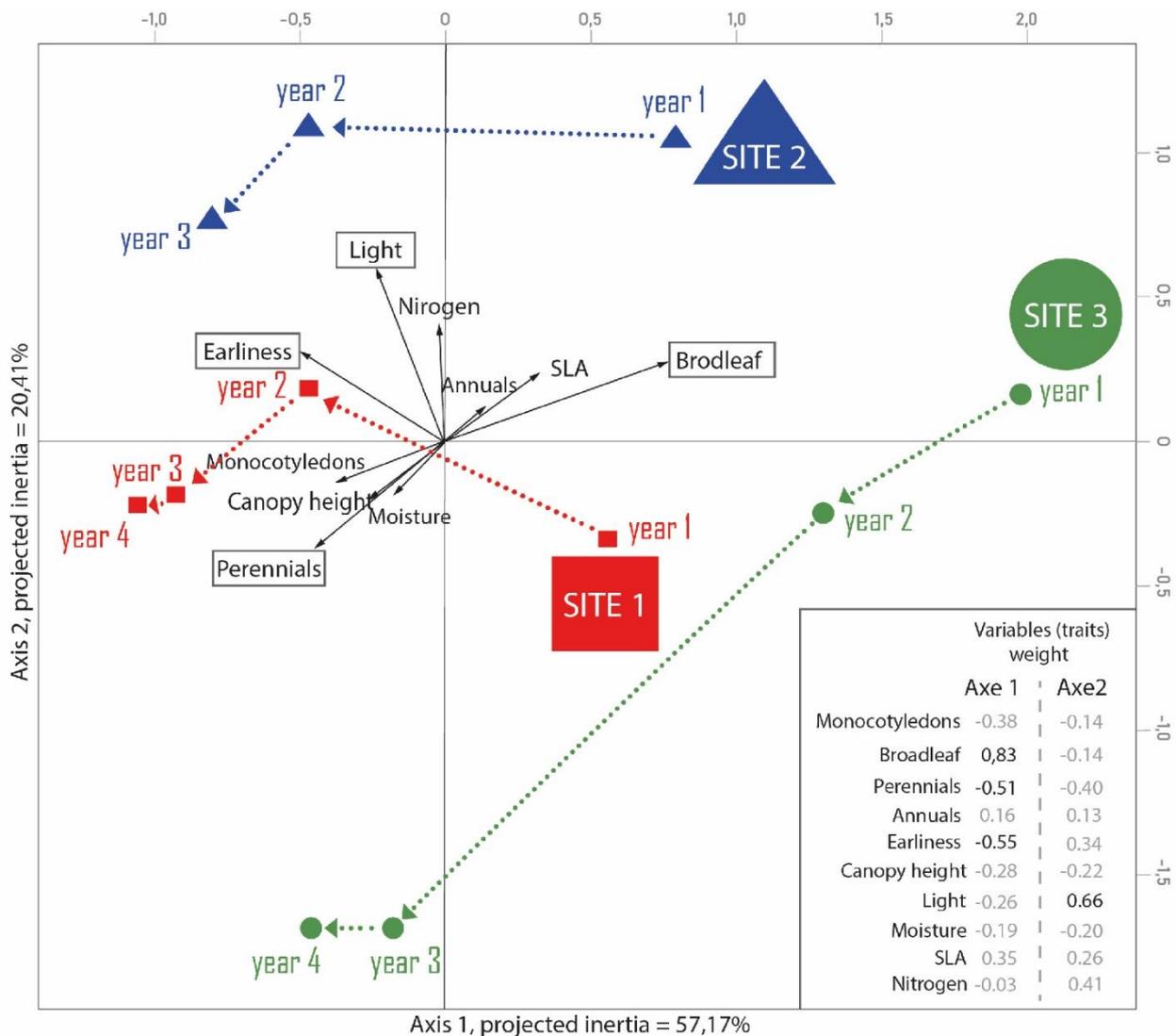


307 **Figure 4:** Non-Metric Multidimensional Scaling (NMDS) analysis of the relative abundance of
 308 weed species in IWG crops during spring in the first, second, third, and fourth years of growth.
 309 The centroid of each species and year is given by labeled positions. [Colors useful]

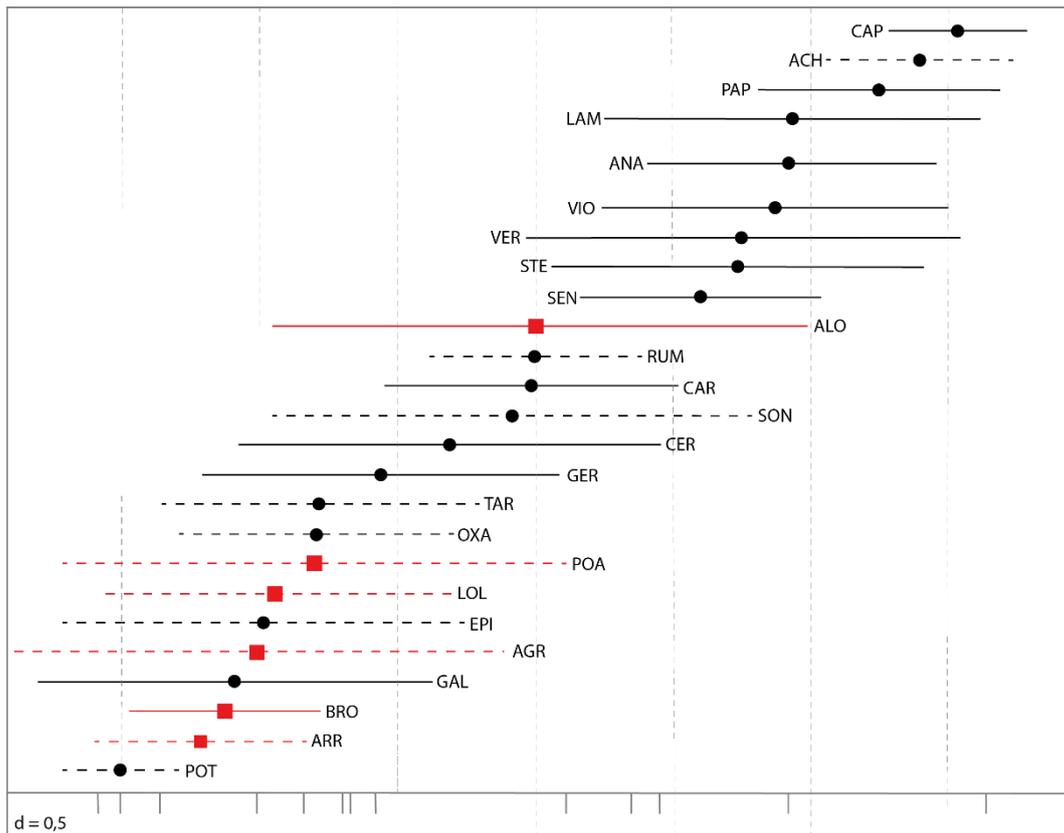
310 RLQ showed how the weed community changed over time and between sites (Figure 5). In all
 311 three sites, most variation occurred along the first axis (57.17%), which contained (canonical
 312 absolute weight of variables > 0.5) plant life history (annual – perennial), division
 313 (monocotyledons – 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⁻¹)
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⁻¹; 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⁻¹
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 1st to flowering; Duchene et
493 al., 2021) compared to earlier temperate grass (<1300 GDD accumulation from February 1st
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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