



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

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### ABSTRACT

The development of a perennial grain offers opportunities to diversify annual crop rotations, with potential benefits in terms of soil protection. Perennials could also reduce weed development over time through year-round soil cover and longer growing seasons. However, whether weeds would actually decrease remains mainly theoretical, with field data on perennial grains remaining sparse. Qualitative changes might also have an effect, because disturbance and modifications to resource regimes drive shifts in weed communities. Here, we analyzed weed abundance, composition, and traits in three arable fields containing perennial grain over a 4-year period. Specifically, intermediate wheatgrass (IWG) perennial grain (Kernza) was cultivated. IWG grain production was maximal during the first and second growing seasons (899 and 854 kg.ha<sup>-1</sup> respectively), with total biomass production peaking in the second year (mean: 11.9 t DM.ha<sup>-1</sup>). However, reproductive effort noticeably dropped in the third and fourth years. Weed biomass also gradually reduced during the fall of all years (mean: ~0.4–~0.03 t DM.ha<sup>-1</sup>), but remained constant in spring (mean: 1–2 t DM.ha<sup>-1</sup>). During spring, the community composition of weeds shifted from broadleaves to grass species and, to a lesser extent, from annuals to perennials, with weed species having an earlier phenology than IWG. Thus, relative fitness appeared to be the strongest driver of weed community composition in aging IWG stands. Weed species richness systematically declined over the years. Specific leaf area, height, light, and nitrogen requirement index of weed communities provided poor descriptors of community shifts; however, all weed species with high light requirements were excluded from aging IWG stands. IWG capacity to compete with weeds might have been undermined by its late growing cycle, absence of forage harvest during the growing season, and substantial initial weed development during establishment (first year of growth). Thus, before IWG establishment, initial site conditions and land use history should be considered to implement the best management strategies for each field. Important weed development in perennial grain fields could lead to high weeding requirement, either mechanical or chemical, in conflict with the initial principle of increasing sustainability and reducing inputs.

### 1. Introduction

The perennial grain Kernza™ is the result of ongoing efforts by The Land Institute (Kansas, USA) to domesticate intermediate wheatgrass (IWG, *Thinopyrum intermedium*, (Host) Barkworth & D.R. Dewey). This grain became a figurehead of new perennial grain crops that could revolutionize cropping systems (Crews et al., 2016; DeHaan et al., 2020; Duchene et al., 2019; Ryan et al., 2018). Besides provisioning farmers with food and feed biomass in the form of grain and forage over several years, the deep root system and year round soil cover of this perennial

crop (Sainju et al., 2017; Sakiroglu et al., 2020) could provide important additional benefits, including nutrients leaching mitigation (Culman et al., 2013; Jungers et al., 2019), soil erosion control, and carbon storage, (Audu et al., 2022; Kim et al., 2022; Sprunger et al., 2019).

IWG could also potentially outcompete weeds by increasing the timespan of light and soil resource capture in each year (Lanker et al., 2019; Ryan et al., 2018). This expectation is supported by published studies, in which grasslands were identified as better competitors of weeds compared to annual crops (Domincsek et al., 2021; Schuster et al., 2020). The weed suppression effect from integrating perennial

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

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

Due to the novelty of IWG crops, associated field data about weed development remain scarce, and sometimes contradictory; yet, weed management has been identified by farmers as one of the main challenges in Kernza field trials (Duchene, 2020; Lanker et al., 2019; Law et al., 2021a). Zimbric et al. (2020) and Dick et al. (2018) reported that weed biomass in summer declined during IWG regrowth (second and third year of growth). In contrast, Law et al. (2021a) recorded consistent weed biomass in the summers of successive years, with the development of perennial grass communities (*Poa trivialis*, *Phleum pratense*). During IWG establishment, following sowing in fall, conditions for weed growth are analogous to annual grain stands. Land preparation steps, that vary depending on farming systems, are designed to favor the seed germination and seedling emergence of crops. During the subsequent weeks and months, crop seedlings establish their first roots, leaves, and tillers; consequently, their ability to compete with weeds is initially limited, depending on the rate at which it can occupy space and use resources (i.e., regulated by relative growth rate and sowing density). However, after the first year, the growth and management of IWG widely differs to that of annual grain systems, because regrowth in fall is enabled by perennating organs, such as the root and plant crown; consequently, yearly tillage and soil preparation operation are obsolete. This regrowth ability enhances the efficiency of resource capture and use over time (Culman et al., 2013; De Oliveira et al., 2018; Vico and Brunzell, 2017). However, when IWG is harvested for grain, it cannot be cut at regular intervals during the cropping season, as implemented when harvesting grassland as forage. Only fall or early spring harvest operations are possible, before stem elongation. Thus, a noticeable change in land use is required when cultivating IWG as a perennial grain crop, particularly regarding the disturbance regime (no annual tillage and no regular cutting events in spring) and resource availability over multiple years (because resource capture and use efficiency change over time). Such changes drive shifts in the structure of the weed community (Dominschek et al., 2021; Fried et al., 2022), as demonstrated by Law et al. (2021a) in fields containing IWG grain crops. Therefore, it is important to assess the structure of weed communities over successive growing seasons to determine the importance of IWG cultivation in filtering weed species initially present through habitat change under field conditions.

This study explored how weed communities change under IWG cultivation in the temperate arable fields of western Europe (France),

from crop establishment to 4-years of growth. Biomass, composition, and traits were analyzed. We hypothesized that both weed biomass and community composition in fields would alter in response to changes in the disturbance regime and resource gradients following IWG establishment. Potentially problematic weed species were also identified that might require specific attention and management practices in future research or production fields.

## 2. Materials & methods

### 2.1. Experimental sites description

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

### 2.2. Data collection

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

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

For all sampling events at the 3 sites, canopy biomass was sampled after weed species had 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), by cutting the aboveground biomass 5 cm above the soil surface. The entire set of weed species identified at each site is provided in Tables 1 and 2. The soil cover of weed species was estimated by visual observation, and was rated on a 10% grade scale. For each sampled quadrat, IWG biomass was separated from weeds, and weed species were separated from each other. IWG and weed biomass were oven-dried at 65 °C for 48 h before weighing. The relative biomass of each weed species was presented as the proportion of total weed biomass per quadrat. This approach allowed us to construct a relative abundance table based on biomass units instead of individual plants (Wilhm, 1968). This table was used for the statistical analyses (diversity indexes, RLQ analysis). Biomass is thereafter expressed in tons of dry matter per hectare (t DM.ha<sup>-1</sup>). In summer, grain yields were

**Table 1**

Information about the on-farm trial sites, including location, main soil type, weather characteristics (OM = Organic Matter, GDD = Growing 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 (0–30 cm)	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, ~15 kg/ha (882 °C)	18/09/2018, ~15 kg/ha (904 °C)	05/09/2017, ~15 kg/ha (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 < 25 cm (10/09/2017) + power harrow (20/09/2017) + tine seeder (20 cm inter-row)	CT: plowing to a depth < 25 cm (14/09/2018) + disc harrow (16/09/2018) + tine seeder (22 cm inter-row)	NT: direct sowing with discer seeder (25 cm 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

obtained by oven-drying and weighing threshed grains in a similar way to the rest of IWG biomass. Yields were calculated and expressed on a 15% humidity basis. The harvest index were calculated as the ratio between grain yields and total aboveground biomass.

### 2.2.2. Traits of weed species

Eight functional traits of weeds were selected as potentially responding to IWG growth under field conditions (Table 2). These traits corresponded to the main ecological features related to resource use. These traits were life history (annual, perennial), phenology, plant division (monocotyledons, broadleaves), plant earliness (month corresponding to the start of the flowering period), average height, specific leaf area (SLA, as a proxy of photosynthetic capacity and growth rate),

nitrogen, moisture and light requirements. The last three were described using Ellenberg's indicator values (Julve, 1998); namely, L - light (from 1- deep shade to 9-full light), F - moisture (from 1-extreme dryness to 12-submerged plant), and N - nitrogen (from 1-extremely infertile to 9-extremely rich). Values for all weed species identified in this study were collected from online databases and the published literature (Table 2).

## 2.3. Data analysis

### 2.3.1. IWG and weed biomass

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

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

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

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

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

**Table 2**

Traits selected to evaluate weeds with their descriptive values (symbol † provided with each trait indicates source reference and 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

†Hill et al. (1999)

††Julve (1998)

†††Kleyer et al. (2008).

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 the correspondence analysis of the L table. Finally, a fourth-corner statistic was computed (Dray and Legendre, 2008), in which the link between traits and environment variables were measured by Pearson correlation, Chi2, or Pseudo-F index (ratio of between-cluster variance), depending on the type of variable (i.e., quantitative or qualitative). Significance was tested by a permutation procedure, resulting in 5000 Monte Carlo permutations of the rows of matrices R and Q (Dolédéc et al., 1996).

### 3. Results

#### 3.1. Biomass of IWG and weeds

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

The mixed model showed that sampling period, stand age, and their interaction had highly significant effects on IWG biomass (p-value <

0.001). Weed biomass had no significant effect (p-value = 0.15).

During the first year of growth (establishment year), IWG biomass was consistently lower compared to the subsequent years, in both fall and spring (Fig. 1, Table S1). IWG biomass was very low (0.087 t DM.ha<sup>-1</sup> on average) during the first fall (Fig. 1a). This low biomass corresponded to small seedlings with four to five leaves maximum. During the first spring biomass was 2.53 and 6.10 t DM.ha<sup>-1</sup> (heading and flowering stages respectively), and rose to and 6.09 t DM.ha<sup>-1</sup> in summer (harvest) (Fig. 1).

On average, IWG biomass during fall increased across the four years (Fig. 1). At flowering and harvest, biomass was highest during the second year of growth (12 and 13 t DM.ha<sup>-1</sup>, respectively). Biomass was similar in the third and fourth growing seasons (9.36 and 8.76 t DM.ha<sup>-1</sup> at flowering, respectively).

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

##### 3.1.2. Weed biomass

Sampling period and its interactions with IWG stand age and biomass significantly explained weed biomass (p-value < 0.01, < 0.05, < 0.01,

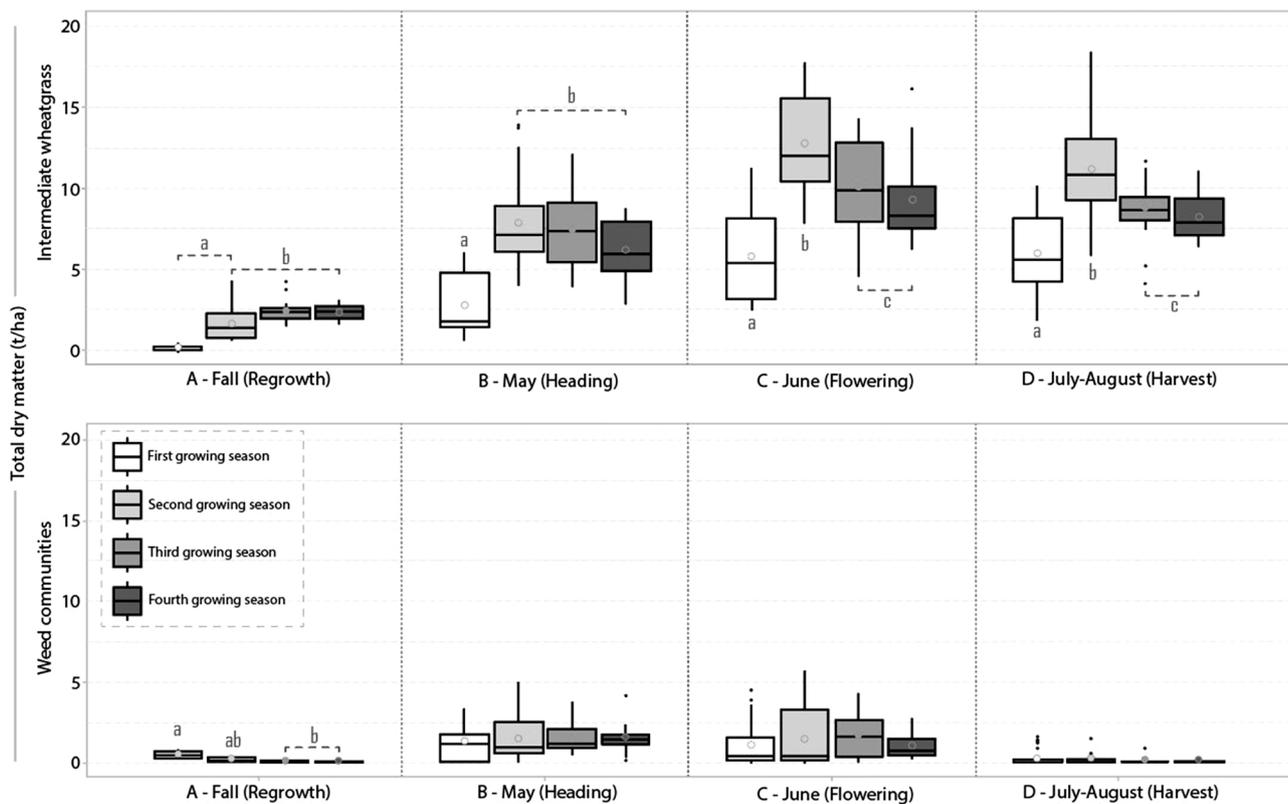


Fig. 1. Total biomass of IWG and weeds (tons of dry matter per hectare) during fall (A), crop heading (B), crop flowering (C), and harvest (D), in the first, second, third, and fourth growing seasons. [Colors useful].

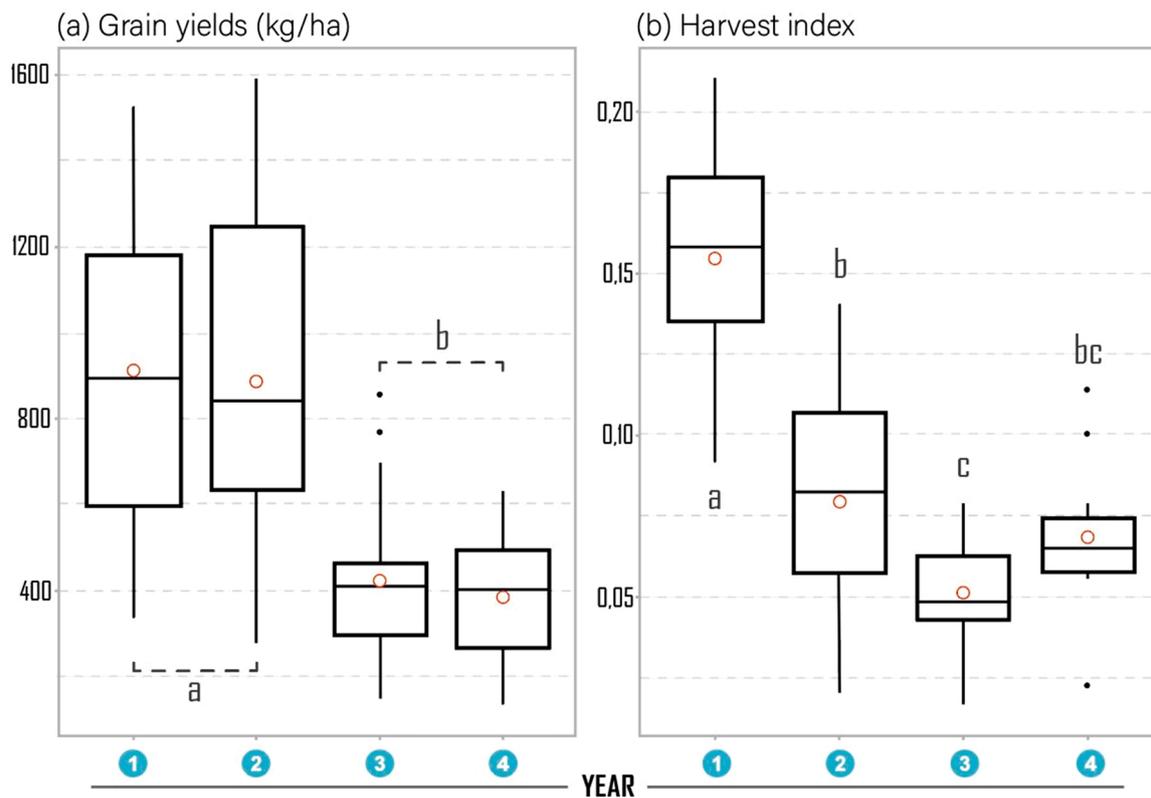


Fig. 2. Grain yield (a) and harvest index (b) of IWG at harvest time during the first, second, third, and fourth growing seasons. Circles in boxplots indicate mean values. Letters indicate statistical differences between years ( $\alpha = 0.05$ ). [Colors not useful].

respectively). In fall, weed biomass was 0.02–0.57 t DM.ha<sup>-1</sup>. Biomass was significantly higher during the establishment year compared to the third and fourth year of growth, in which weed biomass was very low (Fig. 1). A significant negative correlation was observed between the reduction in weed biomass and increase in IWG biomass during fall over the first three years of growth (Figs. 1 and 3), corresponding to a 92.3% reduction in mean weed biomass.

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

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

### 3.2. Structure and traits of the weed community in spring

NMDS (stress = 0.163; Fig. 4) showed that perennial IWG stands affected the structure of weed communities in the spring of all four cropping years. Spring sampling time had no effect (crop heading or flowering). The initial situation (first year) differed across the three sites. However, these differences strongly declined over the study period, with year three and four largely overlapping, while being clearly distinct from the first year. The permutational test showed that IWG stand age significantly affected the structure of the weed community (p-value < 0.001). Weed species richness declined from the establishment year to the last growing season at all three sites (from 14 to 5, 12–4, and

18–6, respectively, for sites 1, 2, and 3). For sites 1 and 2, Shannon diversity index declined (from 1.26 to 0.75, and 1.54–0.40, respectively); however, species evenness remained constant at site 1 (0.48–0.46) but dropped at site 2 (from 0.62 to 0.29). This trend at site 2 was attributed to the increasing dominance of *Lolium perenne*, followed by *Poa trivialis*, whereas the other species were highly variable. Site 3 had a stable species diversity index (from 0.96 to 0.90), due to a compensatory effect between lower species richness and higher evenness over the four years (0.33–0.50). The initial dominance of *Galium aparine* and *Veronica persica* was progressively balanced by an increase in other species, such as *Epilobium hirsutum*, *Bromus sterilis*, *Arrhenatherum elatius*, and *Poa trivialis*.

RLQ showed how the weed community changed over time and between sites (Fig. 5). In all three sites, most variation occurred along the first axis (57.17%), which contained (canonical absolute weight of variables > 0.5) plant life history (annual – perennial), division (monocotyledons – broadleaves), and flowering earliness as the main driving traits. The transition from year 1 to year 2, 3, and 4 was characterized by fewer broadleaf plants, shifting towards grass-dominated communities (e.g., *Lolium perenne*, *Alopecurus myosuroides*, *Poa trivialis*, *Bromus sterilis*, *Arrhenatherum elatius*, *Agrostis capillaris*; Fig. 6), and more early (e.g., *Taraxacum* sp., *Alopecurus myosuroides*, *Poa trivialis*) and perennial (e.g., *Arrhenatherum elatius*, *Agrostis capillaris*, *Epilobium hirsutum*, *Lolium perenne*, *Poa trivialis*; species Fig. 6). Fourth corner analysis only identified plant division (i.e., monocotyledons vs broadleaf) as being significantly influenced by the longevity of the IWG stand (p-value < 0.01). Minor drivers identified by RLQ included SLA and weed height, showing that as the community shifted from annuals and broadleaved species towards perennials and grasses, taller species with lower SLA became, to a certain extent, more prominent. The shift from year three to four was marginal compared to shifts in years one and two.

No consistent trend was observed across the three sites over the four

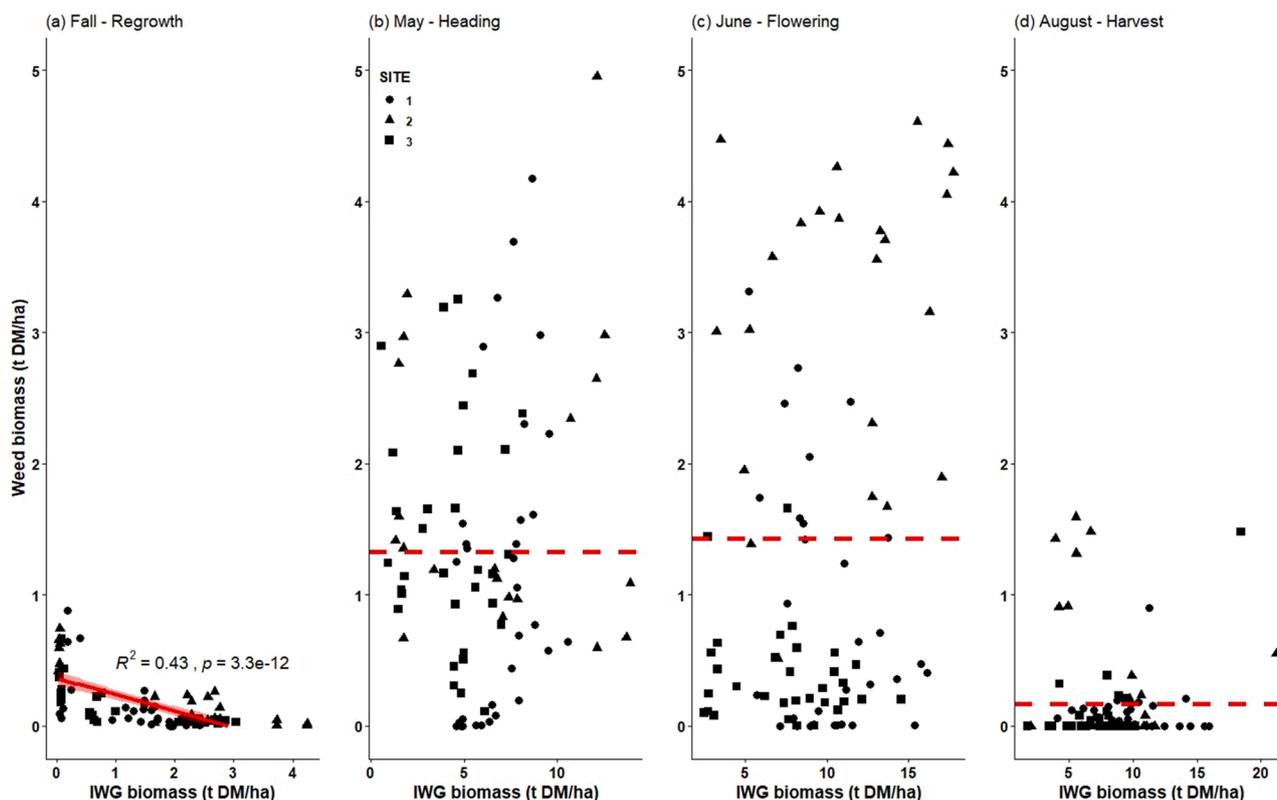
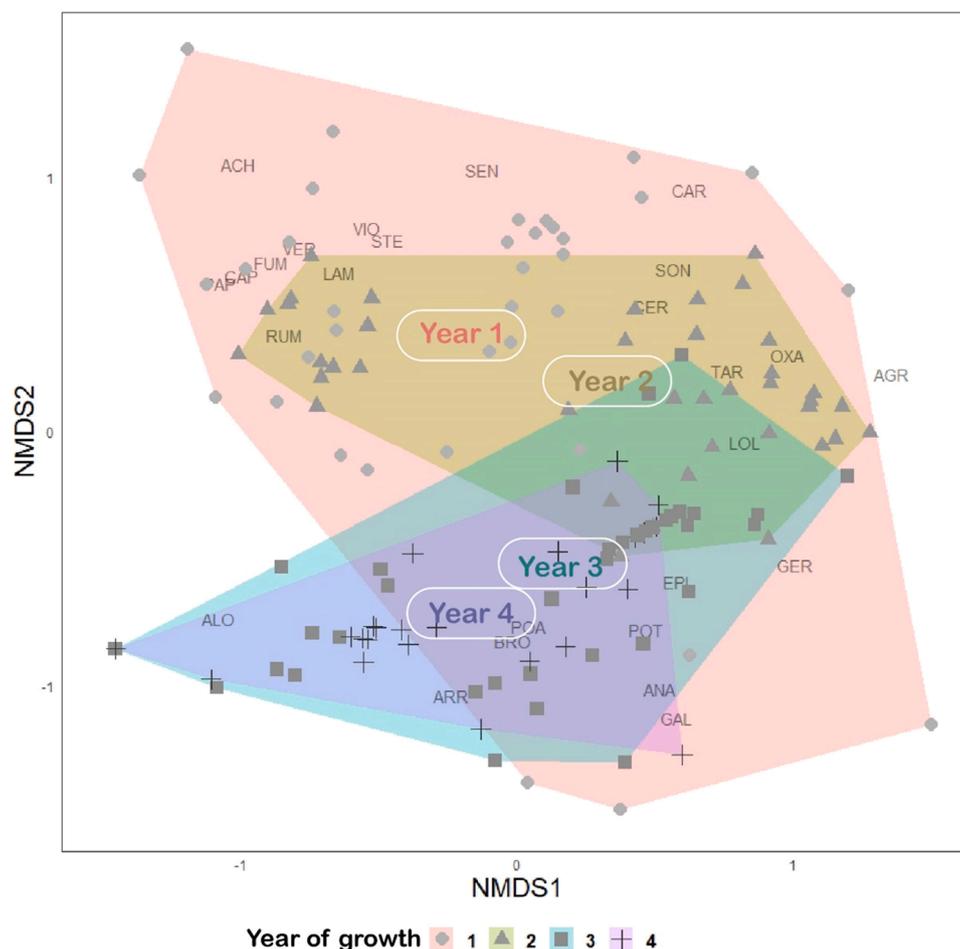


Fig. 3. Weed biomass as a function of intermediate wheatgrass (IWG) biomass in fall (a), crop heading (b), crop flowering (c), and harvest (d). Both parameters are expressed in tons of total dry matter per hectare. Only significant relationships are reported on panels. In the absence of significant correlations,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



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

years on the second axis. This axis was mostly characterized by minor drivers, including the light requirement of weed communities (canonical absolute weight of variables  $> 0.5$ ), along with nitrogen requirement, earliness, and plant division. Overall, site position on the second axis showed the variability of the initial weed communities and its importance of understanding later community assemblages at each site. On the second axis, sites 1 and 2 maintained approximately the same position across years. In contrast, site 3 exhibited a noticeable shift from species with high light requirement (e.g., *Sonchus arvensis* and *Cardamina hirsute*) to less light demanding species (e.g., *Galium aparine*, *Agrostis capillaris*, *Arrhenatherum elatius*, *Bromus sterilis*, *Poa trivialis*, *Lolium perenne*).

## 4. Discussion

### 4.1. Biomass and grain yields of IWG

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

Low grain yields of IWG have been explored by many previous studies (Altendorf et al., 2021; Cassman and Connor, 2022; Cattani, 2017; Tautges et al., 2018), with dedicated breeding programs existing to improve them. Variability in yield might have been exacerbated in our study by the relatively low rate of nitrogen fertilization applied each

year ( $50 \text{ kg N} \cdot \text{ha}^{-1}$ ) compared to the theoretical optimal range identified by previous studies on the role of nitrogen in building grain yields ( $61\text{--}96 \text{ kg N} \cdot \text{ha}^{-1}$ ; Fernandez et al., 2020; Jungers et al., 2017). As nitrogen content was not measured in our study, a definitive conclusion cannot be obtained about the balance between nitrogen input and nitrogen use by plants. Also, the plant allocation strategy in case of nitrogen deficiency is not known (translocation to roots, stems, leaves or grains). At 10 tons of aboveground biomass per hectare at flowering, nitrogen content in the aboveground tissue of IWG is about 1% (Fagnant et al., , under revisions), representing 100 kg nitrogen in plant tissue per hectare. Between flowering and harvest, grain filling would likely cause overall nitrogen demand to slightly increase. Thus, more than 50 kg of the nitrogen required is not provided by fertilizers, and depends nitrogen availability in the soil pool, which tends to be underestimated because nitrogen in roots is not included in this calculation. Besides, heterogeneity of plant emergence at establishment (not measured) and planting dates likely induced heterogeneous seedling vigor initially. However, all sites were planted early enough to allow sufficient GDD accumulation ( $\sim 900$  GDD) and tillering before winter and vernalization, based on Olugbenle et al. (2021). Variation in temperature and moisture conditions between growing seasons also caused heterogeneity in our study. For example, the 2019–2020 growing season was warmer, whereas the 2020–2021 growing season was wetter (Table 1).

Irrespective of site or climatic year, biomass production peaked during the second growing season, and likely corresponded to a peak in reproductive growth (i.e., many fertile tillers that induced stems elongation; Altendorf et al., 2021; Fernandez et al., 2020; Hunter et al., 2020a). However, the harvest index dropped after the first year

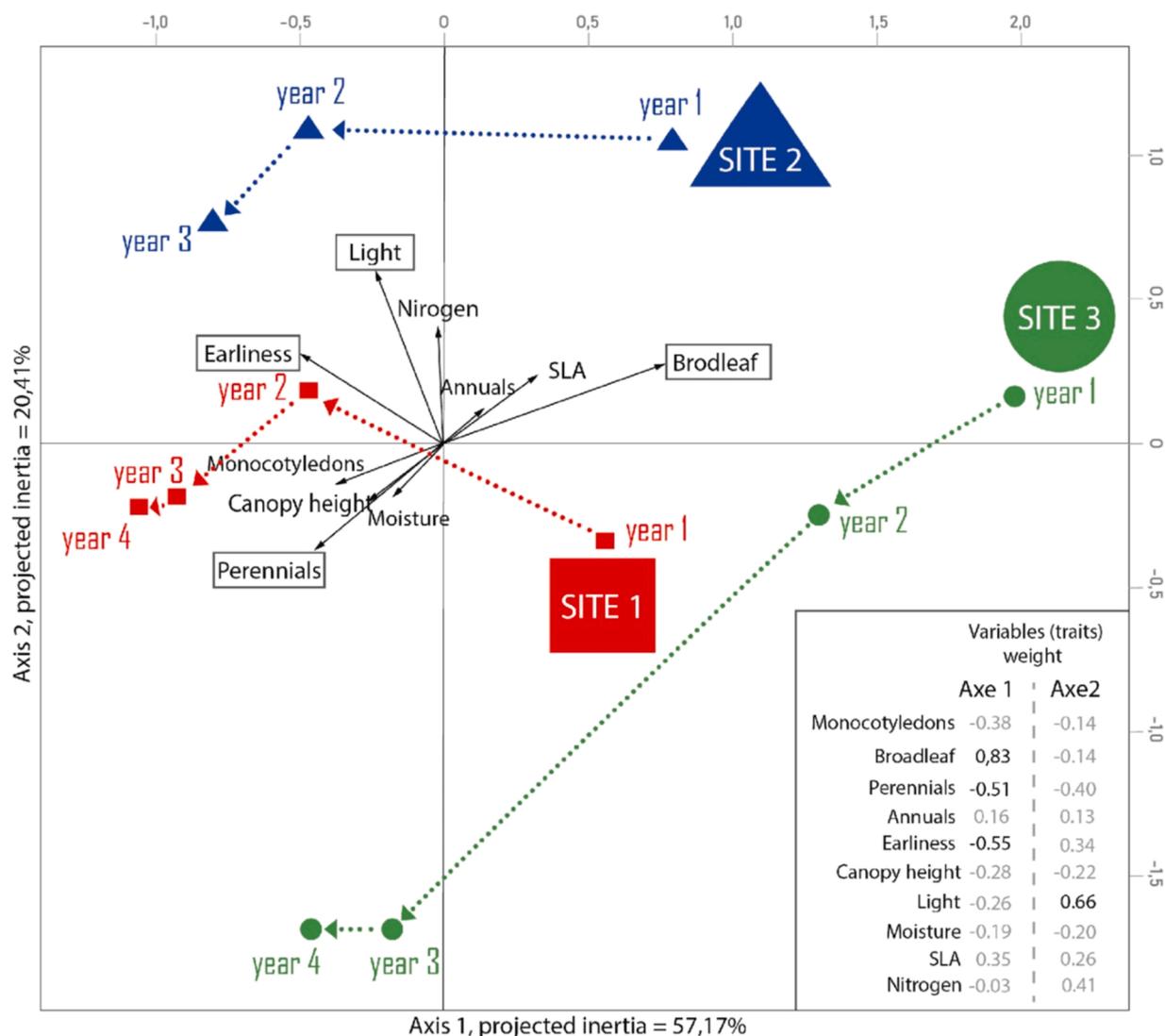


Fig. 5. RLQ analysis of the multiple relationships between species traits (life history, division, earliness of flowering, average canopy height, average SLA, light-moisture-nitrogen; Ellenberg index; Table 2), experimental site, and IWG growth (longevity). First and second axes summarized 57.17% and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

(Fig. 2b), indicating a potential trade-off between tiller density and tiller fertility. Previous studies also reported a decline in grain yield over time, driven by a decrease in the fertility of tillers and spikes (Altendorf et al., 2021; Fernandez et al., 2020; Hunter et al., 2020). Regardless of the underlying yield components involved, this reduced fertility led to very low grain yields in the third and fourth years of growth (Fig. 2a). Tillering is a complex process in grasses, as it is under multifactorial control (e.g., light quality, nutrient and water availability, defoliation regime, temperature; Assuero and Tognetti, 2010). This process is especially complex in perennial species, like IWG, for which the regulation of tiller emergence and differentiation in relation to environmental cues remain poorly understood (Lafarge and Durand, 2011; Rouet et al., 2021). Thus, more research is required to clarify tillering dynamics, which would allow appropriate management practices to be designed (e.g., planting design, mowing, grazing, fertilization, residue management) that maintain the production of fertile tillers over multiple growing seasons.

In our study, mixed models (Section 3.1.1) and regression analysis (Section 3.1.2, Fig. 3) did not indicate that high or low weed biomass depressed IWG yields. This result supported that of Zimbric et al. (2020), who showed that yield did not increase in plots where weeds were

removed (starting from the second growing season) compared to unweeded plots. However, weeds are a major cause of yield loss in grain production (Adeux et al., 2019; Barberi, 2002; Oerke, 2006), largely due to competition for resources (light, nutrients, water; Zimdahl, 2007). This competition is particularly deleterious for wheat yields during stem elongation and flowering (Fischer, 2020 and references therein). Yet in the current study, the highest weed biomass was recorded in late spring, during the elongation phase of IWG, up to anthesis (Figs. 1 and 3). Overall, our results (together with previous findings; Zimbric et al., 2020) raise the question of why IWG biomass and grain production appear to be relatively insensitive to important spring weed biomass compared to annual grains. A recent study investigating how different weed communities impact winter cereals showed that weed biomass alone, especially when sampled late in the season, is a poor predictor of yield loss (Adeux et al., 2019). The authors showed that weed-crop interference is better captured in terms of niche competition. In other words, weed traits are critical for understanding their impact, with weeds that occupy the same niche as crops being the most deleterious. IWG likely displays high ecological complementarity with the weed flora encountered in typical annually disturbed agrosystems, such as those of this study, initially mitigating the impact of weeds on IWG yield.

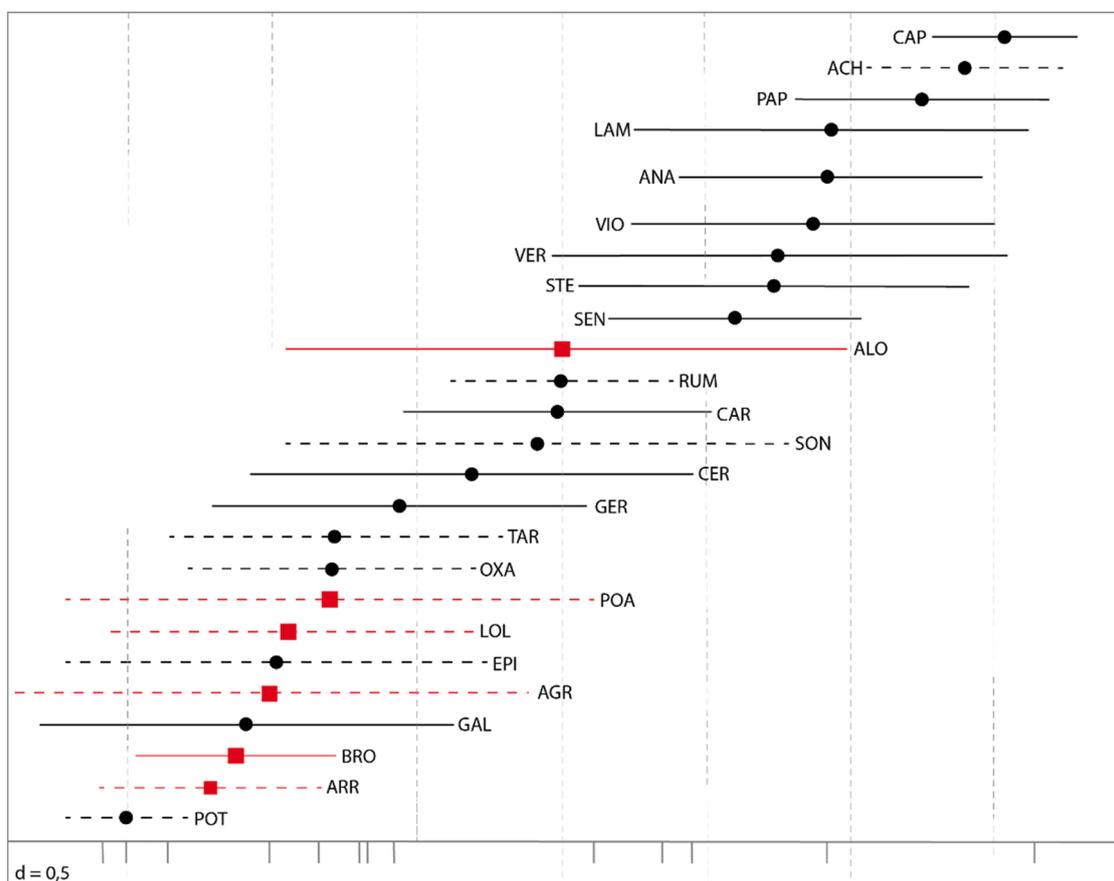


Fig. 6. Average position and standard deviation of each species at crop flowering according to normalized site scores (x axis; RLQ analysis). Dispersion along the x-axis represents the distance between species in the context of growth (i.e., site and year conditions). Grass species are represented with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

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

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

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

In spring, weed biomass was already high in the first year, and remained high throughout the experimental period, despite higher IWG biomass in spring during the regrowth years. These weed biomass levels tended to be higher than that recorded for grain crops, such as wheat and barley, under organic management, for which weed biomass rarely exceeds  $0.5 \text{ t DM}\cdot\text{ha}^{-1}$  (Drews et al., 2009; Lundkvist et al., 2008). Such high weed abundance is not likely to be tolerated by farmers in strict grain cropping systems where weed biomass cannot be valorized as forage, and controlling weed seedlings is an important issue. Law et al. (2021a) reported stable weed biomass in spring over three years in IWG fields, whereas Zimbric et al. (2020) reported a significant decline after

the establishment year. Yet in the latter study, weed biomass was sampled at grain harvest in summer, when early weeds were likely to have already senesced. In fact, the significant decline in weed biomass recorded from the spring to harvest sampling periods in our study (Fig. 1) shows that sampling weeds at IWG harvest might largely underestimate weed development, as most observed species grew in spring, and were already senesced at the IWG harvest stage. In addition, the initial mean weed biomass recorded in the current study was double that recorded by Zimbric et al. (2020); thus, weed pressure was less challenging in this previous study, and the diversity of weed species was lower (notably very few grass species).

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

Unlike IWG grown in pure stands, grasslands generally harbor a mixture of different grass and/or legume species. Through

complementarity and stabilizing niche differences (Cardinale et al., 2007; Duchene et al., 2017; HilleRisLambers et al., 2012), functional diversity in mixtures generally allow a better use of resources, leaving less vacant space for weed species to develop. As such, previous studies highlighted the benefit of IWG intercropping with legumes to reduce weed biomass (Law et al., 2021b), but also to increase forage value (Favre et al., 2019) and stabilize grain yields over multiple years (Dimitrova Mårtensson et al., 2022; Tautges et al., 2018).

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

In our study, weed communities under ageing stands of IWG had lower diversity compared to those under young stands. Furthermore, as IWG stands aged, the composition of weed communities significantly shifted from primarily annual broadleaved weeds towards grass species and, to a lesser extent, perennial and earlier flowering species (Figs. 5 and 6). A larger number of sites is needed to confirm whether the life history and earliness of plants are robust drivers. Also, weed trait values could be improved by measuring the traits of studied samples rather than using trait values provided in databases at the species level. While this approach is sufficiently robust for some traits (e.g., plant division or life history), it does not account for the plasticity of other traits in a given environment (e.g., SLA, height). At our study sites, which had a history of annual rotation, a minimum of three years was required to observe shifts in the weed community under IWG. Similar changes were observed at other studies for both IWG (Law et al., 2021a; Zimbric et al., 2020) and temporary grasslands (Bretagnolle et al., 2011; Hiltbrunner et al., 2008; Meiss et al., 2010a, 2010b), with these studies reporting the selection of grass weed species in perennial stands over time (e.g., *Poa trivialis*, *Phleum pratense*).

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

The selection of species more adapted to co-existing with IWG reflected an interplay between differences in relative fitness and stabilizing niches (HilleRisLambers et al., 2012). The important changes that occurred when switching from an annually tilled system to a perennial system without tillage modify the fitness optimum for all species growing in a given agroecosystem, acting as a filter of weed species. In the current study, this phenomenon resulted in the clustering of weed species over time around two main functional traits: grass species and perennial habit, supporting previous studies (Dominschek et al., 2021; Fried et al., 2022; Mainardis et al., 2020). Furthermore, functional dissimilarity, such as different growing cycles, also facilitate coexistence by limiting competition between species. The later phenology of IWG (about 1630 average GDD accumulation from February 1st to flowering; Duchene et al., 2021) compared to earlier temperate grass (<1300 GDD accumulation from February 1st to flowering; e.g. *Poa pratense*, *Arrhenatherum elatius*, *Lolium perenne*, *Dactylis glomerata*, *Festuca arundinacea*, *Alopecurus myosuroides*; Cruz et al., 2010) might explain why earlier grasses thrive in IWG fields. Changes to resource gradients (i.e., light, nitrogen, moisture) minimally affected our trait-based analysis (Fig. 6). Still, light demanding species (e.g., *Viola arvensis*, *Cardamine hirsute*, *Achillea millefolium*, *Sonchus arvensis*; Table 2) were systematically hindered, and there was a clear change in the light requirement of weed communities at site 3, likely due to species that were only found at this location (e.g., *Cardamine hirsute*, *Sonchus arvensis*, *Galium aparine*).

Among broadleaf species that continued to grow under IWG aging stands were *Epilobium hirsutum* and *Galium aparine*, which have a

climbing morphology, contrasting to broadleaves with upright stature that are generally found in temporary grasslands (Meiss et al., 2010a, 2010b). As mowing and grazing were not implemented at our three sites, the potential role of these two species in promoting or suppressing other weed species remains unexplored. However, as the value of IWG perennial grain is linked to its ability to produce both grain and forage (Favre et al., 2019; Law et al., 2022; Pugliese et al., 2019), the interactions between different defoliation regimes and the assemblages of weed communities requires further research.

## 5. Conclusions

This study provided new evidence on the evolution of weed abundance and community composition under ageing IWG stands (establishment to four years). Biomass and yield performance of the perennial grain crop supported previous studies, with biomass production being lower during the first year, while grain yield substantially declined after the second harvest. While weed biomass tended to decrease in the fall of each year, it remained constant in each spring; however, the composition of the weed communities shifted. The species richness of weeds was also consistently lower under aging IWG stands, whereas evenness remained dependent on local site characteristics and initial weed communities. Grasses and perennials were largely favored over annual broadleaves; thus, strong fitness differences appear to drive community composition towards species with similar functional traits. In contrast, dissimilarity (e.g., phenological differences) might help to stabilize niche differences and favor particular species. The abundance of weed species co-existing with IWG demonstrates the need to assess the legacy effect of perennial grain in crop rotations. Such information could help optimize optimal management practices, including planting time and design, covered seeding, mechanical weeding, intercropping or mowing.

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## CRedit authorship contribution statement

Olivier Duchene, Christophe David, and Florian Celette conceived and planned the experiments. Olivier Duchene carried out the experiments, sampling, formal analysis, and figure design. Olivier Duchene, Camille Bathellier and Florian Celette contributed to the interpretation of results. Olivier Duchene led the writing the manuscript. Camille Bathellier participated to the writing, editing and reviewing. Christophe David, Benjamin Dumont, and Florian Celette participated to reviewing and provided critical feedback.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126721.

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