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Wheat Mixtures as an Effective Option for Weed Management in Organic Farming

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ABSTRACT

In organic cereal cropping systems, crop diversification has emerged as an environmentally friendly strategy for weed management. However, the role of crop mixtures in weed regulation, particularly in Mediterranean conditions, has been largely overlooked. This study evaluates the effects of wheat mixtures on weed suppression and weed species diversity. Over two years, we compared the weed suppression abilities of three wheat monocultures—Florence-Aurora (FA), Forment (FO) and Montcada (MO)—with two wheat mixtures: Florence-Aurora with Forment (FAFO) and Florence-Aurora with Montcada (FAMO) in five commercial fields. We analysed weed cover, weed biomass and weed species community composition and richness. Additionally, we analysed some agronomic and functional wheat traits—including establishment, cover, height, biomass and final tiller number—and correlated them with final weed biomass to better understand the crops' ability to suppress weeds. Results indicated that wheat establishment, cover and height were strongly negatively correlated with weed biomass. Wheat crops differed in both trait values and weed suppression abilities. Crop treatments that exhibited greater establishment, height, and wheat cover, FAFO mixture and FO sole crop, significantly decreased weed cover and biomass. Nevertheless, FAMO did not outperform monocultures, probably due to trait redundancy between the combined plants. Weed species composition was similar across treatments, although FO significantly reduced weed species richness. In conclusion, this study supports the use of wheat mixtures as an effective weed management strategy that also preserves weed species diversity. It further emphasises the importance of including wheat plants with strong functional traits to maximise the mixture weed suppression performance.

1 | Introduction

Sustainable weed management remains one of the biggest challenges in organic cereal systems (Costanzo and Bàrberi 2014). Currently, tillage is still the primary method for weed control, despite growing evidence of its negative environmental

impacts, including biodiversity loss, greenhouse gas emissions and soil degradation (Brooker et al. 2021; MacLaren et al. 2020). Nevertheless, preserving weed biodiversity is not only important for its conservation value, but also provides a range of agroecosystem services, including pest control and pollination (Gurr et al. 2017).

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To address this issue, diversified cropping systems have been proposed as an environmentally friendly option for weed management, as they minimise negative impacts on soil health and field biodiversity often associated with more intensive approaches (Costanzo and Bàrberi 2014; Isbell et al. 2017). Diversified cropping systems, through crop rotation, intercropping or cover crops, can efficiently use resources by improving patterns of light, water and nutrient uptake. This benefit can lead to higher yields, grain quality and weed control, among other services (Brooker et al. 2021; Isbell et al. 2017).

Previous research has demonstrated the strong potential of wheat mixtures to deliver agroecosystem services under field conditions. For example, wheat mixtures can provide resistance to pathogens (Gibson and Nguyen 2021), enhance aphid control (Tooker and Frank 2012; Tous-Fandos, Bragg, et al. 2025), increase grain production (Reiss and Drinkwater 2018) and reduce weed populations (Lazzaro et al. 2018).

Weed regulation in crop mixtures is partly due to weed suppression, defined as the crop's ability to interfere with weed emergence and development (Costanzo and Bàrberi 2014; Isbell et al. 2017). Wheat cultivars and species, hereafter referred to as wheat plants, vary in their weed suppression capacity even under similar crop densities and management practices (Lazzaro et al. 2018; Tous-Fandos, Bragg, et al. 2025; Wu et al. 2021), depending on their set of traits (Barot et al. 2017). On the one hand, combining wheat plants with high variance in traits enhances weed regulation in mixtures, especially when the combination of traits is functionally complementary (Barot et al. 2017; Isbell et al. 2017). On the other hand, the selection effect suggests that increasing crop biodiversity raises the probability of including a genotype with a higher-than-average set of traits that can benefit the crop performance (Loreau and Hector 2001). Key functional aboveground traits linked to weed suppression include crop plant height, period of early vigour, tillering capacity and canopy structure (Andrew et al. 2015).

Although the effects of wheat mixtures on pest management and on yield have been widely studied (Reiss and Drinkwater 2018; Tooker and Frank 2012), their potential for weed control has received less attention in field experiments. To date, only the study conducted by Lazzaro et al. (2018) has directly addressed this aspect under Mediterranean conditions. Furthermore, analysing functional traits in both monocultures and mixtures is necessary to fully understand the mechanisms underlying weed suppression (Barot et al. 2017). Despite this, few studies have employed a trait-based approach to investigate weed suppression in cereal mixtures (Lazzaro et al. 2018).

The primary objective of this study is to assess the effects of wheat mixtures on weed suppression and on weed biodiversity, as indicators of agronomic performance and environmental sustainability, respectively. To achieve these objectives, we conducted a two-year experiment in five commercial organic fields measuring weed cover, final weed biomass, species composition and species richness in two wheat mixtures, and comparing their performance with the corresponding monocultures.

We also assessed how crop parameters (establishment, cover and biomass) and wheat functional traits (height and final tiller number) contribute to weed suppression. We hypothesise that (a) weed suppression would be stronger in wheat mixtures that include a species with a high expression of key traits (b) wheat mixtures would provide weed regulation without altering weed diversity (c) greater wheat establishment, aerial cover, height, wheat biomass and final tiller number would be negatively associated with weed biomass.

2 | Materials and Methods

2.1 | Study Side

This study was conducted over two cropping seasons (2019–2020 and 2020–2021) in the rural area of Gallecs, a periurban agricultural area of 755 ha located 15 km north of Barcelona (41°33'31.9" N; 2°11'59.5" E, Catalonia, Northeast Spain). The region has a Mediterranean climate, characterised by hot, dry summers and mild winters, with a mean annual temperature of 14.6°C and an average annual rainfall of 629.2 mm. During the two cropping seasons, from December to June, the mean temperature and accumulated precipitation were 13.9°C and 532.3 mm in 2019–2020 and 13.2°C and 83.8 mm in 2020–2021, respectively. At the start of the experiment, soil properties were analysed across the experimental fields. The average soil organic matter was $1.7\% \pm 0.07\%$ and nitrogen content was $0.17\% \pm 0.02\%$. The soil was slightly alkaline (pH 8.6) loamy clay.

2.2 | Crop Wheat Species and Varieties

The crops used in the experiment were winter wheat species and varieties selected in collaboration with local farmers and flour makers. To ensure the viability of wheat mixtures in a real farming context, we chose three widely cultivated wheat crops with similar agronomic requirements and commercial purposes. The selected crops were Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum* cv. Florence-Aurora), a wheat plant commonly used in organic fields for its excellent bread-making flour, Montcada (*Triticum aestivum* L. subsp. *aestivum* cv. Montcada), which also has good baking qualities, and Forment (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn), a durum wheat valued for its distinctive aroma. All three have similar harvest times (June), and their flour is already blended for bread production in the studied region. Considering the goal of commercial applicability, we designed two mixtures: Florence-Aurora with Forment and Florence-Aurora with Montcada, as their flours are already blended in mills. However, the Forment–Montcada mixture did not meet this requirement.

2.3 | Field Experimental Design

The experimental design consisted of five crop treatments: three monocultures (Florence-Aurora (FA), Forment (FO) and Montcada (MO)) and two wheat mixtures (Florence-Aurora with Forment (FAFO) and Florence-Aurora with Montcada (FAMO)). Each treatment was established on an experimental

plot (40×18 m) within a commercial field. The design was replicated across five fields, all organically managed since 2006. To minimise variability, we selected fields of similar size, soil type, surrounding landscape and previous agricultural practices. For the second sampling season, the experimental plots were placed in the exact same positions as in the previous season.

Before sowing, 30 t ha⁻¹ of composted farmyard manure were applied, followed by chisel ploughing and rotary harrowing to prepare the seedbed. Sowing took place on November 19–21, 2019, and December 1–3, 2020, at a seed density of 180 kg ha⁻¹. Wheat mixtures consisted of 50% Florence-Aurora and 50% either Forment or Montcada, with seeds pre-mixed to ensure uniform distribution.

2.4 | Field Sampling

In both years, wheat establishment was assessed after tillering in mid-February by counting the number of wheat plants along a 1 m section sample aligned with the sowing rows. In 2020, eight sampling replicates were placed per plot, whereas in 2021 this number was reduced to four. All samples were placed randomly within each plot, at least 2 m from the plot borders to avoid edge effects.

From March to June, every three weeks, we visually estimated the cover percentage of crop and weeds in four 1 m² quadrats randomly placed in each plot, positioned at least 2 m from the plot edges. During the final sampling, we identified weed species and estimated their specific cover percentages to assess weed species richness and weed composition, at the same 1 m² quadrats. We also recorded the mean height of three wheat tillers per quadrat. In mixtures, we measured wheat height for each species separately. Wheat height was measured from the base to the top of the sheath of the highest leaf.

During the dough development stage in late June of both years, we estimated wheat and weed final abundance by measuring their aboveground dry biomass. Biomass was hand-harvested from four randomly distributed 0.25 m² quadrats per plot, maintaining a 2 m buffer from the edges. The samples collected were then oven-dried at 60°C for 48 h. At the same time, we counted the number of tillers along a 50 cm section aligned with the sowing rows at the same four locations where wheat and weed biomass were sampled.

2.5 | Statistical Analysis

We analysed the response variables using linear or generalised linear mixed-effects model (GLMM) using *glmmTMB* function in R, version 4.3 (Brooks et al. 2017; R Core Team 2024). Gaussian distribution of errors was used to model wheat establishment (square root transformation), final wheat height, weed richness, weed biomass (logarithmic transformation) and wheat biomass, whereas beta distribution was used for weed and wheat cover. Final tiller number was modelled using a Gamma distribution.

To assess the response variables, we fitted five models followed by the model selection approach based on the Akaike Information Criterion corrected for small sample sizes (AICc). We had two fixed explanatory variables: crop treatment (W) (categorical, five levels: FA, FO, MO, FAFO, FAMO) and year (Y) (categorical, two levels: 2020, 2021). Year was included as a fixed factor to account for the substantial rainfall differences across sampling seasons. The field factor was included as a random effect. Weed cover was analysed independently for each year, with sampling (S) month (March, April, May, June) treated as a fixed factor (Table 1).

The significance of fixed effects and their interactions was tested using an *F*-test with the Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT) for GLMMs. Pairwise comparisons were conducted using Tukey-adjusted estimated marginal means from the *emmeans* package (Lenth et al. 2019). Model assumptions were assessed using the *simulateResiduals* function from the *DHARMA* package (Hartig et al. 2024).

To compare weed species community composition across crop treatments, a Bray–Curtis dissimilarity matrix was calculated using the *vegdist* function from the *vegan* package (Oksanen et al. 2022). Permutational multivariate analysis of variance (PERMANOVA) of the dissimilarity matrix was calculated using the *adonis2* function (N permutations = 10,000). The dissimilarities between weed communities across crop treatments were visualised with a non-metric multidimensional scaling (NMDS) plot generated with the *metaMDS* function. Weed species community was analysed separately by year due to strong rainfall differences between sampling seasons.

Kendall's correlation coefficient was used to assess the relationships between above-ground weed biomass and crop establishment, biomass, cover, height and final tiller number. Analyses were conducted separately for each year. In addition, partial correlations between weed biomass and these variables were examined using the *ppcor* function from the *ppcor* package (Kim 2015). Partial correlation measures the relationship between two variables while statistically controlling for the effects of others.

3 | Results

3.1 | Weed Abundance

Weed cover increased over time in all crop treatments, peaking at harvest (June) in 2020 and in May 2021. FO and FAFO treatments presented reduced weed cover compared to FA monoculture. FAMO treatment only lowered weed cover compared to FA in the second year (Table 1) and (Figure 1).

Weed biomass was higher in the second year. Similar to weed cover, weed biomass differed among monocultures, with FO being significantly lower than FA and MO in both years. In 2020, FAMO, FA and MO had comparable weed biomass, but in 2021, FAMO significantly reduced weed biomass compared to FA. Conversely, the FAFO mixture consistently showed lower weed biomass than FA in both years (Tables 1 and 2).

TABLE 1 | Results of the linear (LMM) and generalised linear (GLMM) mixed-effects model selection, examining two fixed covariates wheat (W) and year (Y) and their interactions.

Response variable	Wheat (W)	Year (Y)	Sampling (S)	W×Y	W×S
Weed cover 2020	$\chi_{(4,226)} = 42.33$ $p < 0.001$	—	$\chi_{(3,226)} = 46.56$ $p < 0.001$	—	n.s
Weed cover 2021	$\chi_{(4,270)} = 115.23$ $p < 0.001$	—	$\chi_{(3,270)} = 76.58$ $p < 0.001$	—	n.s
Weed biomass	$F_{(4,83)} = 45.98$ $p < 0.001$	$F_{(1,86)} = 4.05$ $p < 0.05$	—	n.s	—
Weed species richness	$F_{(4,83)} = 17.19$ $p < 0.01$	$F_{(1,86)} = 3.34$ $p = 0.07$	—	n.s	—
Wheat establishment	$F_{(4,83)} = 156.43$ $p < 0.001$	$F_{(1,86)} = 190.92$ $p < 0.001$	—	$F_{(4,83)} = 83.98$ $p < 0.001$	—
Wheat cover	$\chi_{(4,87)} = 144.78$ $p < 0.001$	$\chi_{(1,87)} = 32.80$ $p < 0.001$	—	$\chi_{(4,87)} = 12.00$ $p < 0.05$	—
Wheat height	$F_{(4,83)} = 411.45$ $p < 0.001$	$F_{(1,83)} = 14.12$ $p < 0.001$	—	$F_{(4,83)} = 85.13$ $p < 0.001$	—
Wheat biomass	$F_{(4,83)} = 39.19$ $p < 0.001$	$F_{(1,86)} = 0.05$ $p = 0.81$	—	n.s	—
Final tiller number	$F_{(4,83)} = 290.21$ $p < 0.001$	$F_{(1,86)} = 0.09$ $p = 0.769$	—	$F_{(4,83)} = 8.26$ $p = 0.08$	—

Note: Sampling (S) was added as a fixed covariate to the weed cover model. Only the best model is presented for each response variable. Variables not included in the candidate model set are indicated with '—', whereas variables tested but not retained in the final model are marked as 'n.s.' (not significant).

3.2 | Weed Species Richness and Community Composition

The analysis of weed species community composition revealed no statistically significant differences among crop treatments (PERMANOVA, $df=4$, $R^2=87.72$, $p=0.06$) (Figure S1). Weed species richness averaged 13.97 ± 0.54 species per plot, with a total of 81 weed species observed across two years. The dominant species were *Kickxia spuria* (L.) Dumort. (roundleaf cancerwort), *Fallopia convolvulus* (L.) Á. Löve (black-bindweed) and *Lysimachia arvensis* (L.) U. Manns and Anderb. (scarlet pimpernel), representing 10.4%, 10.0% and 8.9% of the presences, respectively (Table 3). Weed species richness was similar across years. Species richness only declined in the FO treatment during the 2020 season (Tables 1 and 2).

3.3 | Crop Parameters and Wheat Traits

Wheat establishment differed across crop treatments, with FA and FO showing better establishment in 2020. However, FA establishment was 66.3% lower in 2021. Mixtures maintained consistent establishment across seasons.

Overall, wheat cover at harvest time was greater in FO and lower in MO. FAFO presented higher wheat cover than FA. In 2021, FA

cover was 58.6% lower, making it significantly lower than MO but comparable to FAMO. In both years, FO and FAFO were the tallest crops, whereas MO and FAMO were the shortest. Wheat biomass also varied among monocultures, being lowest in MO and highest in FO. Wheat biomass was similar between monocultures and mixtures. Final tiller number was significantly higher in FO compared to all other treatments. Although mixtures had fewer tillers than monocultures in the first season, FAFO and FAMO exceeded FA in tiller production in 2021 (Tables 1 and 4).

3.4 | Correlation Between Weed Biomass and Crop Parameters and Wheat Traits

All variables, except final tiller number, showed significant negative simple correlations with weed biomass. Wheat cover had the strongest correlation, followed by moderate correlations with wheat establishment and biomass. In the first season, wheat height showed a moderate negative correlation, which weakened in the second year. Final tiller number had only a slight correlation with weed biomass (Table 5).

When considering the effect of confounding variables, the partial correlation in 2020 between weed biomass and wheat traits was weak, and no significant relationships were detected. The highest correlation values were observed for wheat cover

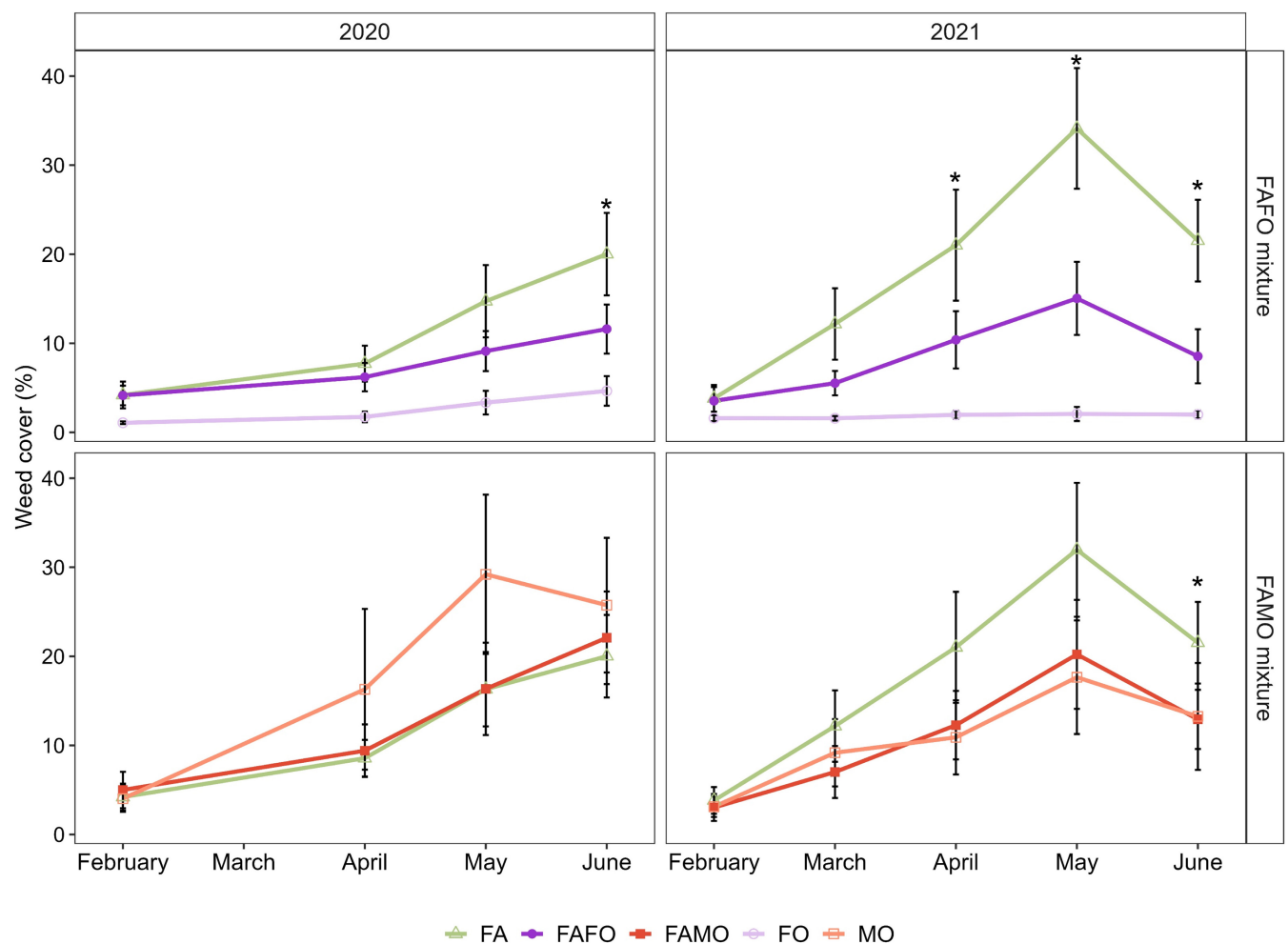


FIGURE 1 | Weed cover (mean $\pm$ SE), expressed as a percentage, across five crop treatments: Florence-Aurora (FA), Forment (FO), and Montcada (MO) monocultures, and Florence-Aurora with Forment (FAFO) and Florence-Aurora with Montcada (FAMO) mixtures. Weed cover was measured monthly. Asterisks indicate significant differences between wheat mixtures and the FA treatment based on Tukey-adjusted pairwise comparisons. Data were analysed separately for each year and sampling.

TABLE 2 | Weed above-ground dry weight biomass (kg/m²) and weed species richness (mean $\pm$ SE) across five crop treatments.

	Florence-Aurora		Forment		Montcada		Florence-Aurora with Forment		Florence-Aurora with Montcada	
2020										
Weed biomass	0.3±0.08	c	0.05±0.02	a	0.4±0.13	c	0.1±0.04	b	0.3±0.10	c
Weed species richness	14.9±1.90	b	9.3±1.20	a	15.1±2.17	b	12.7±1.90	ab	13.9±0.9	b
2021										
Weed biomass	0.6±0.08	c	0.08±0.02	a	0.4±0.13	bc	0.2±0.04	b	0.3±0.12	b
Weed species richness	15.9±2.10	a	12.2±1.30	a	15.0±1.80	a	15.7±1.50	a	15.3±1.20	a

Note: Letters indicate significant differences based on Tukey-adjusted pairwise comparisons. Post hoc analyses were conducted separately for each year.

and establishment variables ($r = -0.18$, $p = 0.11$ and $r = -0.17$, $p = 0.11$, respectively). In contrast, in 2021, a significant moderate negative partial correlation was found between weed

biomass and wheat cover ($r = -0.32$, $p < 0.001$), along with a weak but significant negative correlation between weed biomass and establishment ($r = -0.23$, $p < 0.05$).

TABLE 3 | Weed species observed in every crop treatment during the 2020 and 2021 seasons combined... Only the 10 most frequent species per treatment are included.

sp	Florence-Aurora		Forment		Montcada		Florence-Aurora with Forment		Florence-Aurora with Montcada	
	ni	pi	ni	pi	ni	pi	ni	pi	ni	pi
<i>Agrostemma githago</i> L.	—	—	12	4.4	—	—	10	3.0	—	—
<i>Anacyclus valentinus</i> L.	12	8.6	—	—	13	3.3	—	—	—	—
<i>Chenopodium album</i> L.	18	11.3	19	7.0	28	7.1	18	5.4	26	7.7
<i>Chenopodium vulvaria</i> L.	18	11.3	15	5.5	23	5.9	16	4.8	17	5.1
<i>Convolvulus arvensis</i> L.	11	7.3	13	4.8	20	5.1	12	3.6	21	6.3
<i>Diplotaxis eruroides</i> L. DC.	11	7.3	—	—	17	4.3	—	—	15	4.5
<i>Euphorbia falcata</i> L.	—	—	—	—	—	—	—	—	10	3.0
<i>Fallopia convolvulus</i> L. Á. Löve	22	16.2	36	13.2	47	12.0	27	8.1	32	9.5
<i>Kickxia spuria</i> L. Dumort	27	19.9	33	12.1	42	10.7	31	9.3	37	11.0
<i>Lysimachia arvensis</i> (L.) U. Manns and Anderb.	27	19.9	25	9.2	39	9.9	25	7.5	30	8.9
<i>Medicago lupulina</i> L.	—	—	—	—	—	—	14	4.2	—	—
<i>Papaver rhoeas</i> L.	19	12.3	7	2.6	15	3.8	13	3.9	14	4.2
<i>Polygonum aviculare</i> L.	26	16.9	29	10.7	39	9.9	18	5.4	32	9.5
<i>Vaccaria hispanica</i> Mill.	—	—	11	4.0	—	—	—	—	—	—
	N = 302		N = 272		N = 393		N = 335		N = 336	

Note: ni indicates the total number of observations of each species recorded in both years, N represents the total number of species observations recorded for each treatment, and pi is the percentage of a species' observation relative to the total species observation, calculated as $(ni/N) \times 100$.

TABLE 4 | Wheat establishment, wheat height (cm), wheat cover at harvest (%), wheat biomass (kg/m²) and final tiller number (mean $\pm$ SE) across five crop treatments.

	Florence-Aurora		Forment		Montcada		Florence-Aurora with Forment		Florence-Aurora with Montcada	
2020										
Establishment	23.84 ± 1.1	b	29.35 ± 1.2	c	20.46 ± 1.0	a	24.77 ± 1.1	b	21.66 ± 1.1	a
Wheat cover	29.44 ± 2.7	a	62.75 ± 6.6	c	21.54 ± 7.7	a	39.23 ± 4.4	b	26.53 ± 3.6	ab
Height	74.81 ± 2.0	b	112.83 ± 1.8	d	61.13 ± 1.4	a	91.11 ± 2.4	c	66.59 ± 2.7	a
Wheat biomass	2.90 ± 0.2	b	3.20 ± 0.2	b	2.10 ± 0.2	a	2.90 ± 0.2	b	2.60 ± 0.3	ab
Final tiller number	26.98 ± 1.9	b	41.80 ± 2.1	c	27.08 ± 1.6	b	16.39 ± 1.0	a	15.59 ± 1.0	a
2021										
Establishment	8.03 ± 0.9	a	22.08 ± 1.8	d	21.62 ± 1.7	d	16.38 ± 1.3	c	13.80 ± 1.4	b
Wheat cover	12.19 ± 1.2	a	45.13 ± 5.3	c	21.93 ± 2.1	b	25.64 ± 1.6	b	18.38 ± 1.6	ab
Height	66.59 ± 2.7	c	105.63 ± 2.5	e	51.55 ± 2.0	a	83.47 ± 2.0	d	58.92 ± 2.2	b
Wheat biomass	2.30 ± 0.2	a	3.60 ± 0.3	b	2.20 ± 0.2	a	2.90 ± 0.1	ab	2.50 ± 0.4	a
Final tiller number	9.17 ± 1.7	a	34.80 ± 1.5	c	31.96 ± 3.0	c	16.79 ± 0.7	b	15.81 ± 1.3	b

Note: Letters indicate significant differences based on Tukey-adjusted pairwise comparisons. Each year was analysed independently.

TABLE 5 | Kendall correlation analysis of weed biomass in relation to establishment and key wheat traits (aerial cover, height, biomass and final tiller number).

	Establishment		Wheat cover		Height		Wheat biomass		Final tiller number	
2020	−0.44	*	−0.47	*	−0.43	*	−0.37	*	−0.22	ns
2021	−0.40	*	−0.52	*	−0.24	ns	−0.33	*	−0.14	ns

Note: Coefficient Kendall values with asterisks denote statistically significant correlations. Data were analysed separately for each year.

4 | Discussion

4.1 | The Effects of Crop Treatments in Weed Suppression

Wheat plants grown in monoculture presented differences in weed regulation capacities. Weed cover and biomass measurements revealed that Forment monoculture was more efficient in weed suppression than Florence-Aurora and Montcada treatments. The literature shows that different wheat species and cultivars possess unique traits that can benefit the cropping system, for instance, enhancing aphid control or yield stability (Reiss and Drinkwater 2018; Tous-Fandos, Bragg, et al. 2025; Tous-Fandos, Chamorro-Lorenzo, et al. 2025). In this study, Forment stood out as an excellent wheat species for weed suppression due to its favourable functional trait values that will be discussed below.

The combination of Florence-Aurora and Forment consistently enhanced weed regulation, specifically weed cover at the late development stage and weed biomass at harvest time, when compared to Florence-Aurora monoculture. The good performance of the mixture can be attributed to the presence of Forment, which exhibited high values for key wheat traits associated with weed suppression. The increased cover and height provided by Forment likely enhanced light and nutrient uptake, strengthening the mixture's competitive ability against weeds (Andrew et al. 2015). These findings support the interpretation that improved weed regulation in our study was primarily driven by selection effects (Loreau and Hector 2001).

This research presents novel findings, as it is the first to demonstrate that a two-cereal crop mixture can successfully enhance weed suppression. Lazzaro et al. (2018) examined wheat mixtures under Mediterranean conditions for weed regulation and yield improvement and concluded that only mixtures with a high number of components (6–12 wheat species) were effective in delivering those agroecosystem services. Similarly, Kaut et al. (2009) found no evidence of weed suppression by cereal mixtures, with observed effects linked more to weed tolerance (the reduced impact of weed competition on crop performance) than actual suppression.

Our results agree with previous research, showing that increased crop diversity does not always lead to increased agroecosystem services. Instead, achieving the desired benefits in a mixture strongly depends on the trait profile of the combined plants (Barot et al. 2017; Gaba et al. 2015). Florence-Aurora with Montcada mixture did not outperform its individual components in monoculture, probably due to trait redundancy between the combined wheat plants. In addition, Montcada wheat proved

to be a weaker competitor against weeds, so incorporating it into the cropping system is unlikely to enhance weed regulation function in the field.

Nevertheless, it is necessary to highlight that wheat monocultures, in particular Florence-Aurora, in the second sampling season showed a poorer establishment resulting in higher weed abundance. This decline in establishment is likely related to the lower rainfall recorded during that period, which may have triggered seed germination and early development.

In contrast, both mixtures maintained similar establishment values as in the previous year. Under these conditions, the Florence-Aurora with Montcada mixture exhibited significantly lower weed cover and biomass compared to the Florence-Aurora treatment. This finding hints at a buffering effect, where mixtures help stabilise crop performance over time (Yachi and Loreau 1999).

4.2 | Crop Treatments Influence on Weed Species Richness

In this study, we also examined the impact of wheat crops on weed diversity, given their conservation value and contribution to agroecosystem services (Gurr et al. 2017). Wheat mixtures stand as an environmentally friendly weed management option since they enhance weed control without significantly altering weed diversity. In the first place, weed species richness of the two mixtures remained comparable to that of Florence-Aurora and Montcada monocultures. Only Forment treatment showed a decline in weed species richness when compared to other monocultures and mixtures, which coincided with its lower overall weed abundance.

In addition, weed species community composition was not statistically different across crop treatments. Weed communities were dominated by common weeds, although we identified some segetal rare species including *Vaccaria hispanica* (Mill.) Rauschert (cowherb) and *Agrostemma githago* L. (corncockle). The presence of rare weeds in wheat mixtures is particularly significant from a biodiversity conservation perspective (Rotchés-Ribalta et al. 2015).

4.3 | The Role of Crop Parameters and Wheat Traits in Weed Suppression Ability

A trait-based approach is widely recognised as an effective strategy for enhancing agroecosystem services, yet its application to

cereal mixtures remains underexplored (Barot et al. 2017). The present study examined crop parameters, including establishment, wheat cover and biomass, and functional wheat traits, wheat height and final tiller number, in monocultures and mixtures to deepen our understanding of the weed regulation ability of wheat crops. Analysis of these variables indicated heterogeneity among monocultures and differences in crop performance between monocultures and mixtures. The three selected wheat plants, Florence-Aurora, Forment and Montcada, exhibited distinct trait values, reflecting considerable variability in functional attributes. In this context, Forment crops emerged as the most efficient for weed suppression due to its superior establishment rate, aerial cover, height and tillering capacity. In contrast, Montcada crops had the weakest establishment, shortest height, and lowest biomass. These differences likely account for the distinct weed suppression effects observed among the three wheat monocultures.

Even though the mixtures were composed of equal proportions of each wheat cultivar, the crop parameters and trait values in mixtures closely resembled those of the best-performing component when grown in monoculture, indicating that the most competitive wheat cultivar or species tends to dominate within mixtures. This pattern found in mixtures overall improves crop performance and may even surpass that of less effective monocultures.

Regarding the correlation between wheat performance and weed biomass, the results pointed out that wheat establishment and cover were the most important variables for weed suppression, followed by wheat biomass. Crop establishment has been proposed as one essential variable for good competition against weeds (Hoad et al. 2012) and it has already been demonstrated that suboptimal wheat establishment leads to high weed abundance (Lazzaro et al. 2017).

Wheat cover and biomass play a key role in light availability under the crop canopy. Crop plants with a canopy structure that increases ground cover—such as those with large, non-rigid leaves and a non-erect growth habit—not only capture light more efficiently but also suppress weed growth by reducing light penetration (Andrew et al. 2015). Wheat height, although showing inconsistent results in this experiment, was also a good predictor of weed suppression. Several studies highlight the weed-suppressing benefits of taller wheat. For example, Wicks et al. (2004) compared thirteen wheat cultivars and found a negative correlation between weed density and mature wheat height.

Although final tiller number is often described as a weed competition trait, particularly in low-density cereal crops (Andrew et al. 2015), our results indicate it has little impact on weed suppression. Previous studies had even reported a slight positive correlation between the tiller index of wheat plants and both weed density and biomass (Lazzaro et al. 2018).

Further exploration on phenology or physiology traits would complement the results provided by this work. For instance, other traits such as root length or specific leaf area may have an effect on the weed suppression capacity of the crop (Andrew et al. 2015).

5 | Conclusions

This study concludes that wheat mixtures offer an effective strategy for improving weed regulation while maintaining weed species diversity in Mediterranean organic cereal systems. To further enhance weed suppression, it is preferable to include wheat plants with strong crop parameters and functional traits, such as high establishment rates, extensive canopy cover, and greater height.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in CORA.Repositori de Dades de Recerca at <https://doi.org/10.34810/data2209>.

References

- Andrew, I. K. S., J. Storkey, and D. L. Sparkes. 2015. "A Review of the Potential for Competitive Cereal Cultivars as a Tool in Integrated Weed Management." *Weed Research* 55: 239–248. <https://doi.org/10.1111/wre.12137>.
- Barot, S., V. Allard, A. Cantarel, et al. 2017. "Designing Mixtures of Varieties for Multifunctional Agriculture With the Help of Ecology. A Review." *Agronomy for Sustainable Development* 37: 1–20. <https://doi.org/10.1007/s13593-017-0418-x>.
- Brooker, R. W., T. S. George, Z. Homulle, et al. 2021. "Facilitation and Biodiversity–Ecosystem Function Relationships in Crop Production Systems and Their Role in Sustainable Farming." *Journal of Ecology* 109: 2054–2067. <https://doi.org/10.1111/1365-2745.13592>.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, et al. 2017. "glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling." *R Journal* 9: 378–400. <https://doi.org/10.32614/rj-2017-066>.
- Costanzo, A., and P. Bàrberi. 2014. "Functional Agrobiodiversity and Agroecosystem Services in Sustainable Wheat Production. A Review." *Agronomy for Sustainable Development* 34: 327–348. <https://doi.org/10.1007/s13593-013-0178-1>.
- Gaba, S., F. Lescouret, S. Boudsocq, et al. 2015. "Multiple Cropping Systems as Drivers for Providing Multiple Ecosystem Services: From Concepts to Design." *Agronomy for Sustainable Development* 35: 607–623. <https://doi.org/10.1007/s13593-014-0272-z>.
- Gibson, A. K., and A. E. Nguyen. 2021. "Does Genetic Diversity Protect Host Populations From Parasites? A Meta-Analysis Across Natural and

- Agricultural Systems.” *Evolution Letters* 5: 16–32. <https://doi.org/10.1002/evl3.206>.
- Gurr, G. M., S. D. Wratten, D. A. Landis, and M. You. 2017. “Habitat Management to Suppress Pest Populations: Progress and Prospects.” *Annual Review of Entomology* 62: 91–109. <https://doi.org/10.1146/annur-ev-ento-031616-035050>.
- Hartig, F., L. Loshe, and M. de Souza. 2024. “DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.” R Package Version 0.4.6. <https://CRAN.R-project.org/package=DHARMa>.
- Hoad, S. P., N.-Ø. Bertholdsson, D. Neuhoﬀ, and U. Köpke. 2012. “Approaches to Breed for Improved Weed Suppression in Organically Grown Cereals.” In *Organic Crop Breeding*, edited by E. T. Lammerts van Bueren and J. R. Myers, 61–76. Wiley-Blackwell.
- Isbell, F., P. R. Adler, N. Eisenhauer, et al. 2017. “Benefits of Increasing Plant Diversity in Sustainable Agroecosystems.” *Journal of Ecology* 105: 871–879. <https://doi.org/10.1111/1365-2745.12789>.
- Kaut, A. H. E. E., H. E. Mason, A. Navabi, J. T. O’Donovan, and D. Spaner. 2009. “Performance and Stability of Performance of Spring Wheat Variety Mixtures in Organic and Conventional Management Systems in Western Canada.” *Journal of Agricultural Science* 147: 141–153. <https://doi.org/10.1017/S0021859608008319>.
- Kim, S. 2015. “Ppcor: An R Package for a Fast Calculation to Semi-Partial Correlation Coefficients.” *Communications for Statistical Applications and Methods* 22: 665–674. <https://doi.org/10.5351/CSAM.2015.22.6.665>.
- Lazzaro, M., A. Costanzo, and P. Bàrberi. 2018. “Single vs Multiple Agroecosystem Services Provided by Common Wheat Cultivar Mixtures: Weed Suppression, Grain Yield and Quality.” *Field Crops Research* 221: 277–297. <https://doi.org/10.1016/j.fcr.2017.10.006>.
- Lazzaro, M., A. Costanzo, D. H. Farag, and P. Bàrberi. 2017. “Grain Yield and Competitive Ability Against Weeds in Modern and Heritage Common Wheat Cultivars Are Differently Influenced by Sowing Density.” *Italian Journal of Agronomy* 12: 343–349. <https://doi.org/10.4081/ija.2017.901>.
- Lenth, R., H. Singmann, J. Love, et al. 2019. “Emmeans: Estimated Marginal Means, Aka Least-Squares Means (R Package Version 1.1) [Computer Software].” *The R Journal*. <https://doi.org/10.1080/00031305.1980.10483031>.
- Loreau, M., and A. Hector. 2001. “Partitioning Selection and Complementarity in Biodiversity Experiments.” *Nature* 412: 72–76. <https://doi.org/10.1038/35083573>.
- MacLaren, C., J. Storkey, A. Menegat, H. Metcalfe, and K. Dehnen-Schmutz. 2020. “An Ecological Future for Weed Science to Sustain Crop Production and the Environment. A Review.” *Agronomy for Sustainable Development* 40: 1–29. <https://doi.org/10.1007/s13593-020-00631-6>.
- Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2022. “Package Vegan.” <https://github.com/vegandevs/vegan>.
- R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Reiss, E. R., and L. E. Drinkwater. 2018. “Cultivar Mixtures: A Meta-Analysis of the Effect of Intraspecific Diversity on Crop Yield.” *Ecological Applications* 28: 62–77. <https://doi.org/10.1002/eap.1629>.
- Rotchés-Ribalta, R., J. M. Blanco-Moreno, L. Armengot, L. José-María, and F. X. Sans. 2015. “Which Conditions Determine the Presence of Rare Weeds in Arable Fields?” *Agriculture, Ecosystems and Environment* 203: 55–61. <https://doi.org/10.1016/j.agee.2015.01.022>.
- Tooker, J. F., and S. D. Frank. 2012. “Genotypically Diverse Cultivar Mixtures for Insect Pest Management and Increased Crop Yields.” *Journal of Applied Ecology* 49: 974–985. <https://doi.org/10.1111/j.1365-2664.2012.02173.x>.
- Tous-Fandos, A., D. Bragg, J. M. Blanco-Moreno, L. Chamorro-Lorenzo, and F. X. Sans. 2025. “Wheat Cultivar Mixtures Enhance the Delivery of Agroecosystem Services Compared to Monocultures Under Contrasted Tillage Intensities and Fertilization.” *Agronomy for Sustainable Development* 45: 1–13. <https://doi.org/10.1007/s13593-024-00996-y>.
- Tous-Fandos, A., L. Chamorro-Lorenzo, B. Caballero-López, et al. 2025. “Associating Cultivars or Species With Complementary Traits Is Key for Enhancing Aphid Control Through Bottom-Up Effects.” *Ecosphere* 16: 1–15. <https://doi.org/10.1002/ecs2.70076>.
- Wicks, G. A., P. T. Nordquist, P. S. Baenziger, R. N. Klein, R. H. Hammons, and J. E. Watkins. 2004. “Winter Wheat Cultivar Characteristics Affect Annual Weed Suppression.” *Weed Technology* 18: 988–998. <https://doi.org/10.1614/WT-03-158R1>.
- Wu, Y., N. Xi, J. Weiner, and D. Y. Zhang. 2021. “Differences in Weed Suppression Between Two Modern and Two Old Wheat Cultivars at Different Sowing Densities.” *Agronomy* 11: 253. <https://doi.org/10.3390/agronomy11020253>.
- Yachi, S., and M. Loreau. 1999. “Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis.” *Proceedings of the National Academy of Sciences of the United States of America* 96: 1463–1468. <https://doi.org/10.1073/pnas.96.4.1463>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Non-metric multidimensional scaling (NMDS) plot illustrating the Bray-Curtis dissimilarities of the weed species compositions from the two sampling seasons together across wheat crops ($n = 80$). Florence-Aurora (FA), Forment (FO) and Montcada (MO) monocultures and Florence-Aurora with Forment mixture (FAFO) and Florence-Aurora with Montcada (FAMO) mixtures. Ellipses show the 95% confidence region around the centroid of each treatment, illustrating the dispersion of samples and the degree of overlap among communities.