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ARTICLE

Agroecosystems

Associating cultivars or species with complementary traits is key for enhancing aphid control through bottom-up effects

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Abstract

Organic farming promotes diversification strategies to enhance ecological functions. However, early field studies suggested that not all cereal polycultures confer benefits in terms of pest control. Our research involved a trait-based field study to evaluate the advantages of different wheat polycultures on aphid control and yield. We also explored the bottom-up and top-down effects underlying aphid control. We established 10 treatments replicated in five organic fields: three wheat monocultures (Florence-Aurora [FA], Montcada [MO], and Forment [FO]), a mixture with similar-traits cultivars (FAMO), and a mixture with different-traits cultivars (FAFO), each duplicated with and without a burclover undersowing. We analyzed aphid abundance, number of aphids per tiller, parasitism rate, predatory arthropods' abundance, and crop yield. FAFO and burclover undersowing significantly lowered aphid abundance and the number of aphids per tiller on FA. However, the treatments did not affect the abundance of predators or parasitism rates. Finally, wheat yield was similar across treatments, except in 2021 season when FA yielded significantly less. Our findings suggest that polycultures' benefits on aphid control are cultivar specific. Mixing wheat cultivars with complementary functional traits (height and odor profile) and the association of wheat monoculture with a burclover undersowing enhances aphid control by bottom-up effects without compromising crop yield. Nevertheless, stacking the cultivar mixtures with burclover undersowing did not outperform the results of a single diversity practices, probably because of functional redundancy of resistant cultivars and burclover cover.

KEYWORDS

aphid control, bottom-up control, cultivar mixture, functional diversity, legume undersowing, traditional wheat crop

INTRODUCTION

In recent decades, cereal agricultural systems have experienced a notable trend toward simplification with the adoption of monoculture systems and the gradual replacement of traditional cereal cultivars with a limited selection of modern cultivars, which currently account for the 97% of sown cereals (Aragon et al., 2009; Tilman et al., 2011). These modern cultivars are highly reliant on agrochemicals and machinery, and their performance can be negatively affected by drought, high temperatures, and pest infestation, which are common in the Mediterranean basin area (Bonnet et al., 2021; Ficiciyan et al., 2018). Furthermore, the implementation of modern cultivar monocultures led to a significant decrease in diversity, including both the genotypic diversity of crops and the diversity of beneficial arthropods (Dainese et al., 2019; Ficiciyan et al., 2018). This simplification of the system can cause reduced pest resistance and decreasing yield, among other issues (Hatt & Döring, 2023; Reiss & Drinkwater, 2018). To counter this trend, organic farming advocates for diversity-based strategies as a method for increasing in-field complexity and enhancing system functionality (Barot et al., 2017; Gaba et al., 2015; Hatt & Döring, 2023; Wezel et al., 2014).

At the field scale, agrobiodiversity can be classified into two components: planned diversity, which refers to the diversity of cash, forage, or cover crops that are intentionally incorporated into the field, and associated diversity, that includes all the microorganisms, arthropods, and weeds that interact in the system (Altieri & Rogé, 2009). Planned diversity can be either intraspecific or genotypic (intracropping) or interspecific (intercropping). Genotypic diversity is concerned with the association of cultivars, whereas interspecific diversity involves the combination of different species, such as the association of a cash crop with a cover crop, also referred to as undersowing (Andow, 1991).

Polycultures increase field complexity and ecological interactions, which may enhance the provision of ecological functions such as aphid population control and yield stability (Borg et al., 2018; Costanzo & Bàrberi, 2014; Gurr et al., 2017; Malézieux et al., 2009). Aphids are phytophagous insects considered potential pests in cereal crops. In wheat cultivation, aphids can reduce production both directly, by extracting nutrients from the host plant, and indirectly, by serving as vectors for transmitting viruses like the ones causing dwarfism and stunting (such as Barley Yellow Dwarf Virus [BYDV]), among others (Dedryver et al., 2010). Moreover, they are an interesting model organisms for the study of insect-plant interactions (Rodriguez-Saona & Stelinski, 2009).

The potential of cereal polycultures for aphid control relies primarily on two nonexclusive sets of ecological processes: bottom-up and top-down effects. In bottom-up control, aphid population regulation is driven by associational resistance, which is the favorable association between cultivars and nonhost cultivars or crops (Barbosa et al., 2009). Associational resistance supports the disruptive crop hypothesis (Root, 1973), which suggests that potential pests are less likely to find their target host when it is masked by less preferred cultivars or nonhost species. For instance, Shoffner and Tooker (2013) conducted a laboratory experiment that revealed associational resistance when mixing six-line wheat cultivars with varying levels of aphid resistance.

Regarding top-down control, it is impelled by associated diversity, such as predatory arthropods and parasitoid wasps (Gurr et al., 2017). Top-down effects are based on the natural enemies' hypothesis, which states that planned diversification creates conditions that promote the abundance and richness of beneficial arthropods (Altieri & Rogé, 2009; Gurr et al., 2017; Letourneau et al., 2011). For example, early research showed a positive effect of barley mixture on the abundance of ladybirds (Ninkovic et al., 2011). Some other studies have shown that legume undersowing can provide shelter, varied microclimate conditions, and additional resources, for instance, a broader range and greater abundance of alternative prey, that increases the population of aphid predators such as ladybirds or spiders (Dassou & Tixier, 2016; Letourneau et al., 2011).

However, the majority of studies on the functionality of polycultures were conducted in controlled environments, overlooking the complexity found in actual field conditions. Furthermore, prior field research has reported inconsistent evidence regarding the advantages of cereal polycultures for aphid control (Dahlin et al., 2018; Mansion-Vaquié et al., 2019; Ninkovic et al., 2002). This discrepancy can be attributed to the fact that many studies did not take into consideration the functional traits when combining cereal varieties or intercropping, potentially leading to functional redundancies or negative interactions, such as competition. Consequently, increasing the complexity of the cropping system did not necessarily translate into an enhanced system's functionality (Barot et al., 2017; Brooker et al., 2021; Gaba et al., 2015; Ninkovic et al., 2016).

For this study, we established various wheat polycultures to evaluate their functionality under real farming conditions. Functionality was estimated based on the capacity to provide aphid control. To achieve this, we combined the dominant wheat cultivar in the study area, Florence-Aurora, with one cultivar that shared similar functional traits, and with another cultivar that had distinct functional traits. The traits considered to be relevant for aphid control were height, odor profile, and nitrogen content (Barot et al., 2017; Nowak & Komor, 2010; Webster, 2012). Wheat intercropping was established by associating wheat crops with legume undersowing. This decision was driven by the increasing adoption of cereal and legume intercropping in organic farming due to the well-documented benefits of legume plants in enriching soil with nitrogen, and thus increasing cereal yields (Wezel et al., 2014). For the legume undersowing we sowed burclover (*Medicago polymorpha* L.) because it is a common herbaceous plant in Mediterranean arable lands. Finally, we examined whether stacking (combining genotypic and interspecific diversity practices) may lead to a potential synergy boosting aphid population control by providing complementary strategies to the cropping system (Hatt & Döring, 2023).

Therefore, the aim of this study is to assess the effect of contrasted genotypic diversity, interspecific diversity, and their stacking on aphids' populations and their control agents in Mediterranean organic winter wheat crops. We explored the ecological processes underlying aphid control, specifically the associational resistance and the influence of polycultures on parasitism rate and predator's abundance. Finally, we analyzed wheat yield to assess the viability of the wheat polycultures to be used by farmers. We hypothesized that (1) cultivar mixtures with complementary aphid-resistance traits reduce aphid abundance by associational resistance, (2) legume cover crop increases parasitism rate, ground- and foliage-dwelling arthropod predators' abundance, and (3) stacking genotypic and interspecific diversity outperforms monocultures, intracropping and monocultures associated with legume undersowing, and (4) polyculture provides increased yield compared with monocultures.

METHODOLOGY

Study sites

This study was performed during two cropping seasons (2019–2020 and 2020–2021) in the rural area of Gallecs, which is 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 area has a Mediterranean climate with dry and hot summers and mild winters, with a mean annual temperature of 14.6°C and 629.2 mm mean annual rainfall. 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 beginning of the experiment, we analyzed the soil properties of the five experimental fields. The average soil organic matter was 1.7 ± 0.07%, and nitrogen content was 0.17 ± 0.02%. The soil was a slightly alkaline (pH 8.6) loamy clay.

Winter wheat cultivars and legume undersowing

The winter wheat cultivars used in the experiment were selected through collaborative discussions with local farmers and flour makers. We considered three common cultivars with similar agronomic needs and commercial purposes to assure the viability of the cultivar mixtures in a real farming context.

The three wheat cultivars chosen were Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*), which flour is considered excellent for making bread, and two traditional cultivars: Montcada (*T. aestivum* L. subsp. *aestivum*), which is another good variety for making bread, and Forment (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), which confers valuable aromatic properties. The three cultivars have a similar harvest time, and their flour is already mixed for bread manufacturing in mills.

Florence-Aurora and Montcada present similar functional traits related to aphid control but distinct from Forment's. Florence-Aurora and Montcada reach 70 cm at harvest time, whereas Forment reaches nearly 150 cm (Serra-Gironella & Àlvaro, 2017). Aerial architecture can affect aphids' mobility (Barot et al., 2017). Moreover, Florence-Aurora and Montcada have similar odor profiles, which differ from Forment's (Tous-Fandos, Gallinge, et al., 2023). Wheat cultivar odor profile is the composition of volatiles compounds (VOCs) released by the plants. It plays an important role in aphid host localization (Webster, 2012). Finally, we analyzed the tissue nitrogen content by Dumas's combustion of 10 1-month-old individuals grown in pure stands under greenhouse conditions (18–22°C with a light regime of L16:D8 h), as nitrogen content is a host quality trait that conditions aphid host selection (Nowak & Komor, 2010). Florence-Aurora's total nitrogen content was 52.8 ± 0.7 g N kg^{-1}, Montcada was 52.2 ± 0.5 g N kg^{-1} and Forment was 50.9 ± 0.5 g N kg^{-1}.

We selected burclover (*M. polymorpha* L.), a fast-growing sprawling winter annual with weak stems reaching a length of 10–50 cm, as an undersowing crop because it is an N-fixing legume common in the Mediterranean region.

Field experimental design

The experimental design consisted of 10 treatments laid out randomly on 10 experimental plots of 40 m by 18 m established in a commercial field. There were five wheat plot types: three monocultures of Florence-Aurora (FA), Montcada (MO), and Forment (FO), and two cultivar mixtures of Florence-Aurora and Montcada (FAMO) and

Florence-Aurora and Forment (FAFO). Each one of these five wheat plot types was cultivated with the presence (M +) or absence (M−) of a burclover legume undersowing, totaling 10 treatments. The experimental design was replicated in five commercial fields, that were organically managed since 2006, during two consecutive years. To reduce variation between fields, we selected five fields that were similar in size, soil, and surrounding landscape composition, as well as previous agricultural management practices (Chamorro et al., 2017) (Figure 1).

In 2019 and 2020, we applied 30 t ha^{-1} of farmyard composted manure and employed chisel tillage followed by a rotary harrow for seedbed preparation before sowing. Winter wheat cultivars and burclover were sown with an interval of less than 5 days between them (sowing dates: 19–21 November 2019 and 1–3 December 2020) at seed densities of 180 and 2.6 kg ha^{-1} respectively. Cultivar mixtures comprised 50% Florence-Aurora and 50% Montcada or Forment, with prior mixing of the seeds to ensure homogeneity.

Field sampling

Aphids and predators

Cereal aphid and predators' samplings were carried out every 3 weeks from early March to late May during the two cropping seasons. We counted aphids and mummies (parasitized aphids) on 28 wheat tillers evenly distributed along two parallel 20 m transects (14 tillers per transect) situated 5 m apart from the border in each plot. In cultivar mixtures plots, we distinguished wheat cultivars and aphids were counted separately on each cultivar (14 tillers per each cultivar, totaling 28 tillers per plot). Aphid species were identified in the field using magnifiers and visual identification keys for the most common species. We collected mummies containing parasitoids that had not yet emerged and kept them separately in individual vials in the lab at room temperature (20–24°C, with no control over humidity) until the parasitoids emerged. Both the parasitoid adult and the aphid mummies were then preserved in 70% ethanol. We identified hatched primary and secondary parasitoids at the genus level.

The total number of aphids counted in each plot throughout the entire sampling season served as a proxy for measuring aphid abundance. It expressed overall aphid pressure. At aphid peak time, in late April 2020 and early May 2021, we measured the number of aphids per tiller. The number of aphids per tiller was calculated as the mean number of aphids per single cultivar tiller per plot. Every cultivar was analyzed separately. Hence, it enabled us to compare the aphid infestation on each cultivar when grown in different conditions: monoculture, cultivar mixture, and with or without burclover undersowing. We used the number of aphids per tiller as an indicator for the disruptive crop hypothesis from

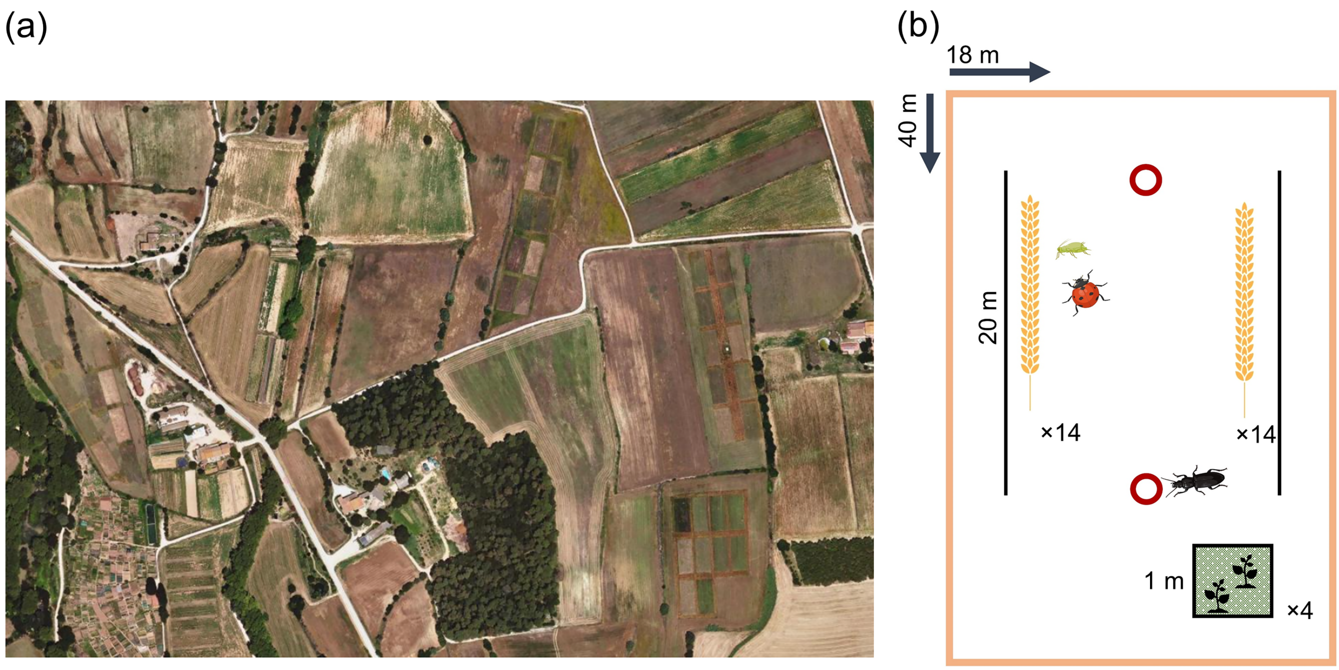

FIGURE 1 Aerial photograph of the study area and sampling design: (a) Orthophoto of three experimental fields in Gallecs obtained from Vissir v3.35 (2020) from the Institut Cartogràfic i Geològic de Catalunya (ICGC); (b) schematic of the experimental plot design, showing the two transects for sampling aphids, parasitoids, and foliage-dwelling predators (black lines), the two pitfall traps for sampling ground-dwelling predators (brown circles), and burclover cover samplings (square).

bottom-up aphid control. In contrast, to test the natural enemies' hypothesis, we used parasitism rate, and predators' abundance distinguishing between foliage-dwelling and ground-dwelling predators. The parasitism rate was calculated by dividing the total number of mummies by the sum of non-mummified and mummified aphids per plot.

We visually recorded the abundance of foliage-dwelling predators along the 20 m transects in 5 min. The walking speed was 2 m min^{-1}, and we counted all predators within 1 m on either side of each transect. To improve our observations, we carefully examined the spikes and tillers of the wheat, and we adjusted our view angle to ensure that every area was visible. The target groups were ladybirds (Coleoptera: Coccinellidae) in the adult and larval stages, hoverflies (Diptera: Syrphidae) in the larval stage, adult soldier beetles (Coleoptera: Cantharidae) and adult spiders (Araneae). Foliage-dwelling predators recording was conducted from 9:00 a.m. to 5:00 p.m. in fair weather conditions.

We installed two pitfall traps along the central line of each plot, 9 m from the lateral edges and 10 m from either end, to assess ground-dwelling predators. Each trap comprised a plastic cup (diameter = 65 mm, 100 mm deep) filled with propylene glycol (diluted at 30% in distilled water) as a preservative solution. We covered the pitfall traps with a floor tile to limit the bycatch of small vertebrates or the interference with birds or rain throughout the open period. The traps were installed for periods of 48 h each, three times in 2020 and four times in 2021. We stored the collected samples in 70% ethanol. The identification of ground-dwelling predators was at the order level (i.e., Dermaptera, Opiliones), at the family level in rove beetles (Coleoptera: Staphylinidae), and at the genera level in ground beetles (Coleoptera: Carabidae) and spiders (Araneae). Ground beetle and spider richness were analyzed.

Burclover ground cover

At aphid peak time, we analyzed the relation between the ground cover of burclover and aphid abundance in plots with burclover undersowing to deepen the effect of nonhost cover on aphid control. The sampling was performed every 3 weeks from March to June in the 2020 season. Well-trained samplers visually estimated the percentage of burclover cover in four random 1 m^2 quadrats $plot^{-1}$.

Crop yield

The total dry grain mass per plot (in kilograms per hectare) served as an estimate of crop yield. The plots were harvested at the end of June (20–23 June both years) by a commercial harvester, and the grain was weighed on-site with a crane scale. Then, 800–1000 g grain aliquots were saved, separated from impurities in the laboratory, and dried (48 h oven-dried at 60°C) to extrapolate grain dry mass without impurities.

Our sampling had two limitations: The 2020 season started 1 month later than 2021 due to COVID-19 pandemic restrictions. Consequently, only three out of four samplings per year were evaluated. Second, burclover did not grow well in 2021. The poor establishment of burclover prevented the inclusion of this factor in the statistical analysis of the data collected in 2021.

Statistical analysis

All statistical analyses were conducted using R, version 4.1.2 (R Core Team, 2021). The measured variables of aphid abundance, the number of aphids per tiller, total parasitism rate, total abundance of ground-dwelling predators and total abundance of foliage-dwelling predators were analyzed with generalized linear mixed effects models (GLMM). The crop yield model was assessed with a linear mixed effects model (LMM). The total number of ground- and foliage-dwelling predatory individuals was insufficient to conduct a rigorous GLMM analysis for each taxonomic group. For modeling, we used the *glmmTMB* function from the *glmmTMB* package (Brooks et al., 2017).

Five models were fitted with three fixed explanatory variables: wheat treatment (W) (categorical with five levels: FA, MO, FO, FAMO, FAFO), burclover undersowing (M) (categorical with two levels: M+, M−), year (Y) (categorical with two levels: 2020, 2021), and their interactions. The field factor was included as a random effect variable. Model 1 contemplated three-way interaction ($W \times M \times Y$), Model 2 did not consider any interactions, Model 3 accounted for the two-way interaction between wheat and burclover undersowing ($W \times M$), Model 4 contemplated the two-way interaction between wheat and year ($W \times Y$), and Model 5 considered the two-way interaction between burclover undersowing and year ($M \times Y$). The significance of fixed effect factors and their interactions was determined with an *F* test with Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT) for GLMMs. Pairwise comparisons were carried out using Tukey-adjusted estimated marginal means from the *emmeans* package (Lenth et al., 2019). The best model for each response variable was considered according to the corrected Akaike information criterion for small sample sizes (AIC_c). The simulateResiduals function from the *DHARMa* package was utilized to examine normality, homoscedasticity and residual distribution (Hartig, 2022).

Aphid abundance was analyzed with a negative binomial function. For the statistical evaluation of the number of aphids per tiller, each cultivar was analyzed independently. Hence, we categorized the wheat treatment factor into two distinct levels: monoculture (FA, MO, or FO) and crop mixture (FAMO or FAFO). To equalize the sowing ratios between monocultures and mixtures, the total number of aphids per single wheat tiller cultivar in mixture plots was doubled. Then, the mean number of aphids per tiller was fitted to a negative binomial function. The total parasitism rate was analyzed with a binomial model. The ground- and foliage-dwelling predator abundance were fitted to a negative binomial function.

We employed a linear regression to examine the relationship between aphid abundance and burclover cover.

RESULTS

Aphid abundance

We recorded a total of 27,417 aphids in five fields during the two sample seasons belonging to 7 species, the most abundant being *Sitobion avenae* (Fabricius, 1775), which represented the 78% of the total aphid abundance (see Appendix S1: Table S1 for a complete list of all aphid species found). Given the predominance of *S. avenae* throughout all treatments, we refrained from conducting species-level analyses for aphid variables.

The analysis of aphid abundance revealed significant effects of year, wheat and burclover factors, as well as W × Y interaction (Table 1). Aphids were more abundant in 2021 ($p = 0.007$). Over both sampling seasons, FA plots supported higher aphid abundance compared with FO and MO ($p < 0.001$). Cultivar mixtures displayed varied outcomes depending on identity and year. On the one hand, FAFO exhibited similar aphid abundance to FO, and significantly lower than FA ($p = 0.005$), consistently across years. On the other hand, FAMO, FA and MO presented comparable aphid abundances in 2020. However, in 2021, MO had a significantly lower abundance, FAMO was intermediate, and FA had a significantly higher one ($p = 0.03$). Aphid abundance was higher in FAMO than in FAFO plots, but this difference was significant only in 2021 ($p = 0.04$). Aphid abundance was lower in plots with burclover undersowing, except in FAFO plots. Burclover effect was statistically significant in FA and FO plots ($p = 0.03$) (Figure 2).

Number of aphids per tiller

The analysis of the number of aphids per tiller of FA cultivar presented a significant effect of wheat treatment and W × M interaction (Table 1). Both years, the number of aphids per tiller tended to be reduced in FA grown in mixtures, but only those in FAFO presented a significantly lower value ($p = 0.02$). Additionally, the presence of burclover undersowing had divergent effects. It decreased the number of aphids per tiller in FA plots ($p = 0.03$) but increased it in FAFO ($p = 0.04$) (Figure 3a). The number of aphids per tiller of MO and FO cultivars was not influenced by any studied treatment (Figure 3b,c).

Burclover ground cover and aphid abundance relationship

When examining the overall relationship between burclover cover and aphid abundance, we found a significantly negative association between estimated burclover ground cover percentage and aphid abundance ($p < 0.001$, $r^2 = 0.36$) (Figure 4a). This pattern was consistent when individual wheat treatments were analyzed separately, with statistical significance observed in FA and FO treatments ($p = 0.02$, $r^2 = 0.41$; $p < 0.001$, $r^2 = 0.73$, respectively) (Figure 4b). Nevertheless, there was no correlation between aphid abundance and burclover cover in FAFO plots (Figure 4c).

Parasitoid and aphid predator abundance

We collected 2276 mummies (open and closed) during the two sample seasons. We identified nine wasp genera, with *Aphidius* dominating as the main primary parasitoid genus (60%) and *Asaphes* as secondary parasitoids (10%) (further details Appendix S1: Table S1). The overall parasitism rate was 11.5% in 2020 and decreased to 5.1% in 2021 ($p < 0.001$).

Wheat treatment significantly affected parasitism rates both years (Table 1). Nonetheless, the results were discordant. In 2020, FO plots exhibited a lower parasitism rate (7.2% ± 1.3%; mean ± SE) when compared with FA (11.4% ± 2.9) and FAMO plots (12.1% ± 2.7). In 2021, however, FAMO had the highest parasitism rate (5.4 ± 0.7), and FA showed the lowest rate (3.9% ± 0.7). Burclover undersowing did not influence the parasitism rate (Table 1).

We recorded a total of 2050 foliage-dwelling predatory arthropods from the surveys. The most abundant group was ladybirds, represented by 57.0%. The total abundance

TABLE 1 Results of the linear mixed effects model (LMM) and generalized linear mixed effects models (GLMM) selection relating three fixed covariables: Wheat (W), burclover undersowing (M), year (Y) and their interactions.

Response variable	W	M	Y	W × M	W × Y	M × Y	W × M × Y
Aphid abundance							
χ	125.2	5.1	123.0	...	17.2	...	...
df	4, 7	1, 10			4, 7		
p	0.001	0.04	0.001	...	0.007	...	...
No. aphids/Florence-Aurora tiller							
χ	14.4	0.01	11.5	13.4	15.0	0.1	10.7
df	2, 10	1, 3	1, 11	2, 10	2, 10	1, 11	2, 10
p	0.001	0.89	0.001	0.005	0.001	0.76	0.008
No. aphids/Montcada tiller							
χ	0.8	1.5	0.01	...	...	...	...
df	1, 3	1, 4	1, 3				
p	0.36	0.21	0.92	...	...	...	...
No. aphids/Forment tiller							
χ	0.3	0.61	9.7	...	...	4.6	...
df	1, 4	1, 11	1, 4			1, 4	
p	0.60	0.60	0.009	...	...	0.04	...
Parasitism rate							
χ	17.1	0.60	33.8	...	...	...	...
df	4, 8	1, 6	1, 11				
p	0.004	0.43	0.001	...	...	...	...
Total no. ground-dwelling predators							
χ	2.3	0.6	129.7	...	...	...	...
df	4, 3	1, 7	1, 6				
p	0.68	0.84	0.001	...	...	...	...
Total no. foliage-dwelling predators							
χ	11.8	5.3	37.8	...	...	2.2	...
df	4, 4	1, 7	1, 7			1, 7	
p	0.02	0.04	0.001	...	...	0.14	...
Crop yield							
F	3.9	0.6	0.003	...	22.8	...	...
df	4, 7	1, 10	1, 10		1, 10		
p	0.45	0.82	0.99	...	0.001	...	...

Note: The significance of fixed effect factors and their interactions was determined with an *F* test with Kenward-Roger approximation for LMMs or a likelihood ratio test for GLMM. Field factor was included as a random effect variable. Only the best model is presented for each response variable.

of foliage-dwelling predators presented a significant effect of year, being higher in 2020 than in 2021 ($p < 0.001$). In 2020, both diversification practices influenced the abundance of foliage-dwelling predators. FO plots showed a lower abundance of foliage-dwelling predators, while burclover undersowing significantly decreased their abundance (Tables 1 and 2).

In total, we collected 3113 ground-dwelling predators through pitfall trapping in 2020 and 2021. Spiders represented 58.3% of all individuals followed by rove beetles with 32.3% and ground beetles with 5.3%. We identified 15 families of spiders and the family richness of spiders per trap per sampling was on average 0.48 ± 0.06 in 2020 and 0.99 ± 0.06 in 2021. Moreover, we identified a total of 16 ground beetle genera. The genus richness of ground beetles per trap per sampling was 0.2 ± 0.07 in 2020 and 0.7 ± 0.09 in 2021. The statistical analysis of the total abundance of ground-dwelling predators

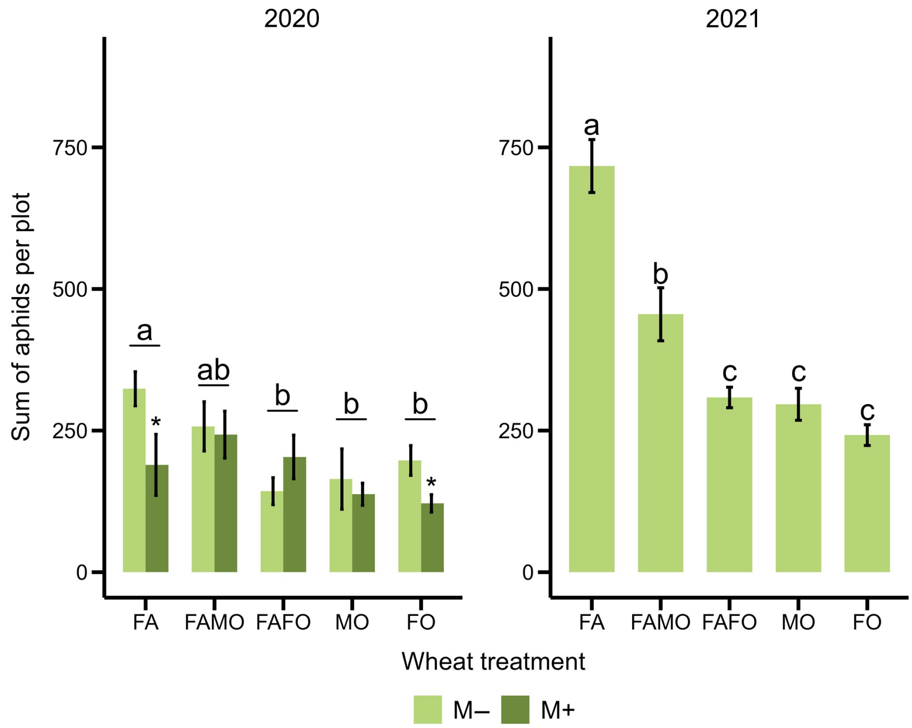

FIGURE 2 Aphid abundance (mean ± SE) over the 2020 and 2021 seasons on 5 wheat treatments: Florence-Aurora monoculture (FA), Florence-Aurora and Montcada mixture (FAMO), Florence-Aurora and Forment mixture (FAFO), Montcada (MO) and Forment (FO) monoculture with (M+) or without (M−) burclover undersowing. Burclover treatment was dismissed in 2021 for its poor establishment. Letters indicate significant differences within wheat treatment and asterisks indicate significant differences within burclover treatment according to Tukey-adjusted pairwise comparisons ($p < 0.05$). Every year was analyzed independently.

showed a strong interannual variation ($p < 0.001$). Wheat and burclover treatments had no significant effect (Tables 1 and 3).

Crop yield

Wheat yield was statistically similar in both harvest seasons, and it was not affected by wheat or burclover treatments. However, W × Y interaction was statistically significant (Table 1). This effect was notably observed in FA treatment. In 2021, FA yield decreased by 43.5% compared with 2020. Moreover, in 2021, FA plots had lower yield compared with other wheat treatments ($p < 0.001$) (Tables 1 and 4).

DISCUSSION

The influence of wheat cultivar mixture on aphid abundance and natural enemies

Our research evaluates the effect of wheat polycultures, specifically two contrasted cultivar mixtures and the incorporation of burclover undersowing, as well as their stacking on aphid control under farming conditions.

The three wheat cultivars used in this study differed in aphid susceptibility. The evaluation of aphid abundance on monoculture plots revealed that Florence-Aurora was more prone to aphid infestation than Montcada and Forment cultivars. As demonstrated in earlier research, one possible reason why Florence-Aurora supports larger aphid populations could be its high tissue nitrogen content (Aqueel & Leather, 2011; Nowak & Komor, 2010).

Concerning genotypic diversity, we assessed two contrasted mixtures. The first mixture, FAMO, had similar aphid-related functional traits such as odor profile, plant height and nitrogen content (Serra-Gironella & Àlvaro, 2017; Tous-Fandos, Gallinge, et al., 2023). This mixture failed to reduce aphid abundance as well as the number of aphids per tiller and, hence, did not offer associational resistance. The second mixture, FAFO combined cultivars with different functional traits, Florence-Aurora and Forment. FAFO plots presented promising aphid control potential in both sampling years because it presented a decrease in aphid abundance. This is the first time that a two-line wheat cultivar mixture

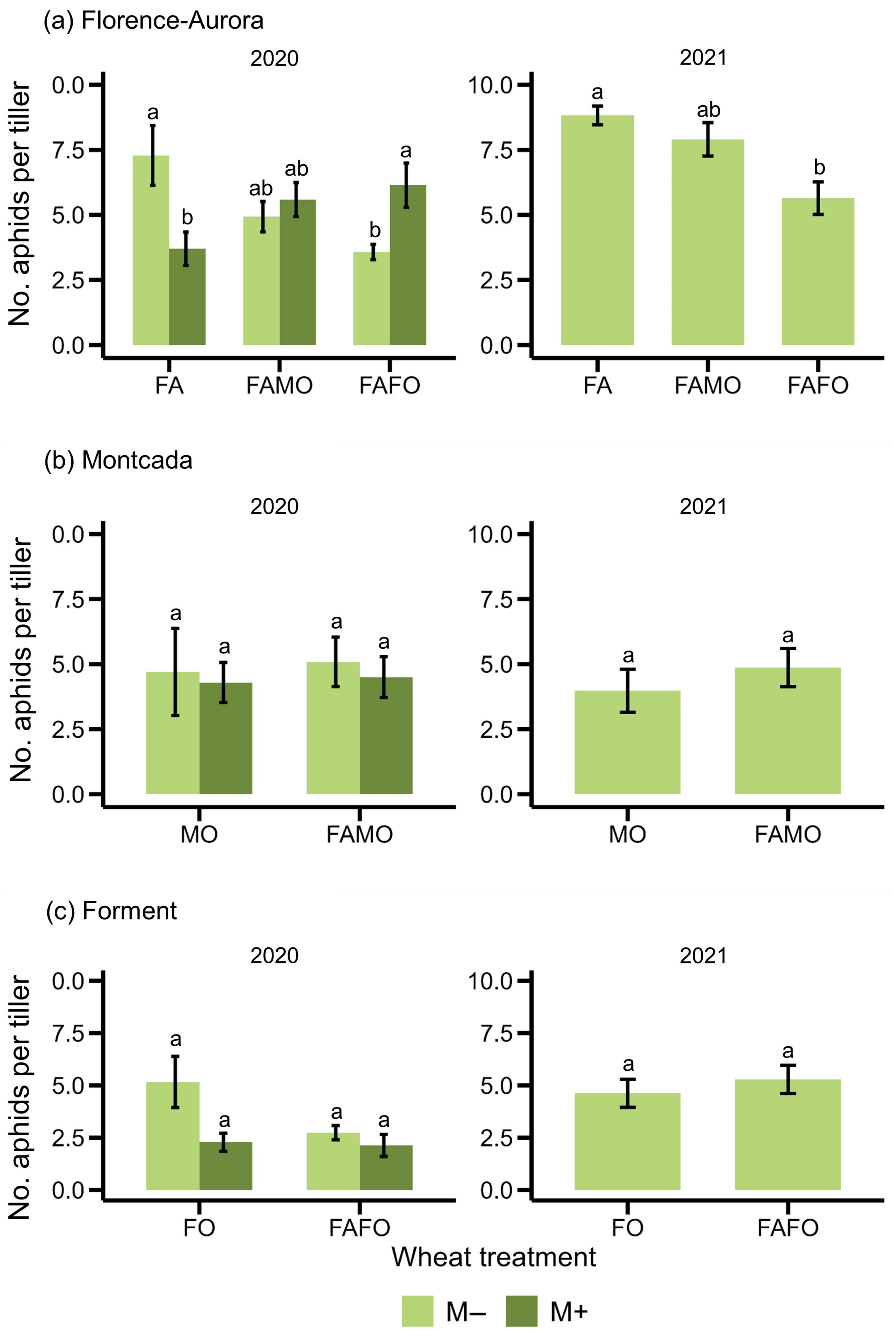

FIGURE 3 Number of aphids per tiller (mean ± SE) at aphid peak time (late April 2020, early May 2021) in each wheat cultivar: (a) Florence-Aurora cultivar grown in monoculture (FA), mixed with Montcada (FAMO), and mixed with Forment (FAFO); (b) Montcada cultivar grown in monoculture (MO) or mixed with Florence-Aurora (FAMO); (c) Forment cultivar grown in monoculture (FO) or mixed with Florence-Aurora (FAFO). All crop types were intercropped with (M+) or without (M−) burclover undersowing. Burclover treatment was dismissed in 2021 for its poor establishment. Letters indicate significant differences within wheat treatment according to Tukey-adjusted pairwise comparisons ($p < 0.05$). Every year was analyzed independently.

provides pest control compared with previous field studies (Mansion-Vaquié et al., 2019).

Additionally, we found a decrease in the number of aphids per tiller on Florence-Aurora plants grown in FAFO, suggesting lower aphid infestation in Florence-Aurora plants due to bottom-up effects (Barbosa et al., 2009). While acknowledging the potential impact of induced changes in the physiology of the more favored plants, as seen in other studies (Barbosa et al., 2009; Dahlin et al., 2018), it is probable that the associational resistance in FAFO is mediated through odor or physical masking as suggested in the disruptive hypothesis (Root, 1973). Mixing Florence-Aurora

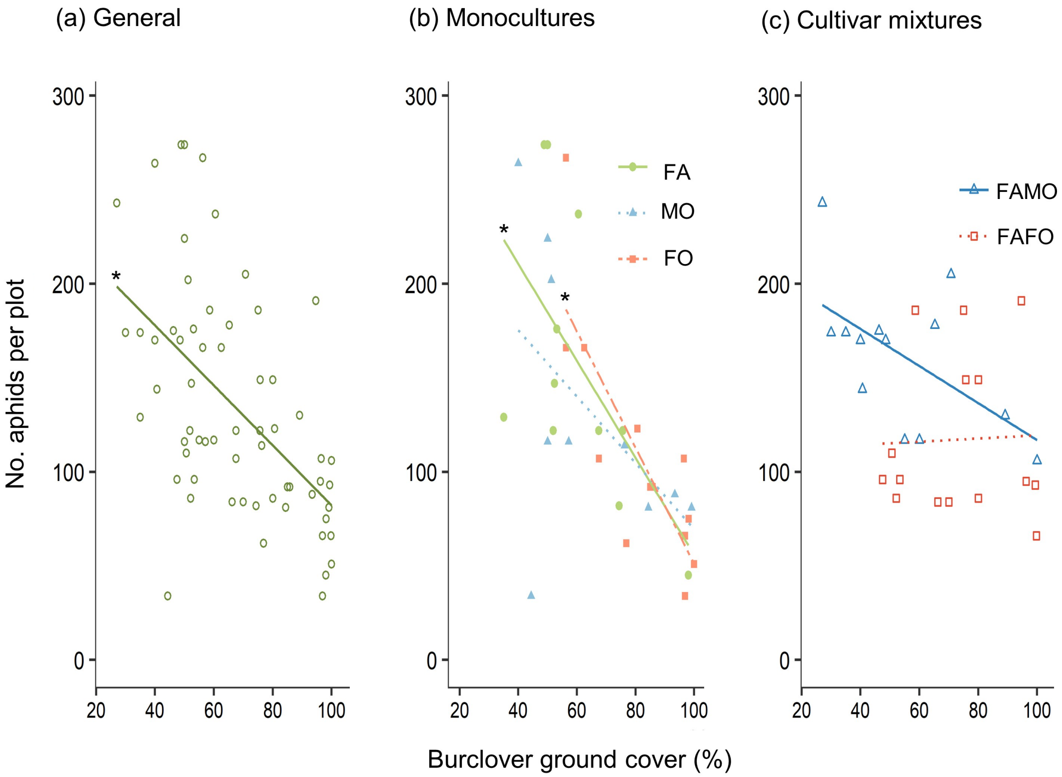

FIGURE 4 Regression analyses relating burclover ground cover and number of aphids at peak time (late April 2020, early May 2021). First-order polynomials best described the relation between burclover ground cover and number of aphids per plot in all the analyses. (a) General analysis including all plots; (b) Monocultures: Florence-Aurora, Montcada (MO) and Forment (FO); (c) Cultivar mixtures: Florence-Aurora and Montcada mixture (FAMO), Florence-Aurora and Forment mixture (FAFO). Asterisks indicate a significant relationship analyzed by linear regression ($p < 0.05$).

plants with the less attractive and taller cultivar Forment decreases the likelihood of aphids locating the preferred host, Florence-Aurora. In this regard, previous research has shown that the odor profile of FAFO is less attractive to *S. avenae* compared with the FA odor profile (Tous-Fandos, Gallinge, et al., 2023).

Our results agree with previous research wherein the benefits of genotypic diversity on aphid control were cultivar-specific and depended on the identity and interactions between the cultivar mixtures. These findings highlight the importance of selecting favorable cultivar with complementary traits for ensuring functional polycultures (Dahlin et al., 2018; Ninkovic et al., 2002). For the evaluation of top-down effects, we analyzed the parasitism rate and the abundance of beneficial arthropods. Cultivar mixtures did not enhance parasitism rate. Moreover, wheat plots with a higher parasitism rate did not present a lower aphid population. Thus, it is uncertain whether parasitism played a substantial role in aphid control (Helms & Hunter, 2005; Mansion-Vaquié et al., 2019). On the other hand, cultivar mixtures did not have any effect on aphid predators' abundance or richness. Nonetheless, early studies did not find a connection between higher predator abundance and predation rate (Grettenberger & Tooker, 2017). Therefore, further research into predation activity is needed to complement the results obtained in this study.

Burclover undersowing effect on aphid abundance and natural enemies

A higher ground cover of burclover undersowing was negatively associated with aphid abundance, particularly in FA and FO plots. Cover crops can provide aphid control via bottom-up effects, wherein the chemical and physical concealment of the primary crop diminishes the probability of aphids locating their preferred host plant (Dassou & Tixier, 2016; Hatt et al., 2018; Lopes et al., 2016; Médiène et al., 2011). In this respect, Mansion-Vaquié et al. (2020) demonstrated in a lab experiment that clover undersowing physically obstructed aphid movement, impeding its spread and establishment in wheat crops. In contrast, burclover undersowing slightly affected aphid abundance or the number of aphids per tiller in MO plots.

TABLE 2 Abundance of foliage-dwelling predator groups (in individuals per square meter; mean ± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Auora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M+) or absence (M−) of burclover undersowing.

Taxonomic group	Burclover undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
2020						
Araneae	M+	0.04 ± 0.06	0.1 ± 0.07	0.03 ± 0.02	0.04 ± 0.01	0.1 ± 0.03
	M−	0.1 ± 0.02	0.3 ± 0.03	0.09 ± 0.01	0.1 ± 0.02	0.1 ± 0.02
Coleoptera						
Coccinellidae	M+	0.5 ± 0.01	0.4 ± 0.04	0.1 ± 0.0	0.6 ± 0.01	0.3 ± 0.06
	M−	0.9 ± 0.2	0.5 ± 0.1	0.2 ± 0.03	0.5 ± 0.01	0.3 ± 0.06
Cantharidae	M+	0.1 ± 0.0	0.2 ± 0.05	0.07 ± 0.01	0.1 ± 0.05	0.08 ± 0.03
	M−	0.08 ± 0.08	0.1 ± 0.07	0.03 ± 0.02	0.1 ± 0.04	0.07 ± 0.01
Total		1.7 ± 0.7	1.6 ± 0.4	**0.5 ± 0.2**	1.4 ± 0.5	0.95 ± 0.5
2021						
Araneae	M−	0.07 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	0.07 ± 0.01
Coleoptera						
Coccinellidae	M−	0.2 ± 0.04	0.4 ± 0.1	0.2 ± 0.05	0.3 ± 0.09	0.2 ± 0.06
Cantharidae	M−	0.05 ± 0.01	0.09 ± 0.02	0.05 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
Total		0.32 ± 0.02	0.59 ± 0.03	0.35 ± 0.03	0.49 ± 0.05	0.32 ± 0.02

Note: Boldface indicates significant relationships in the total amount of foliage-dwelling predators according to Tukey-adjusted pairwise comparisons ($p < 0.05$). Every year was analyzed independently.

These results suggest that the success of undersowing in enhancing aphid control depends on the characteristics of wheat used and how these plants interact with each other and with aphids.

Furthermore, cover crops may provide shelter and secondary food resources which enhances the abundance of beneficial arthropods (Dassou & Tixier, 2016; Gurr et al., 2017). In our research, however, burclover undersowing had no influence on both the total abundance, the richness of aphid predators and the parasitism rate. Possibly because the burclover undersowing did not present attractive floral resources or a complex aerial plant architecture (Hatt et al., 2018; Lopes et al., 2016).

The potential of the stacking genotype and interspecific diversity for aphid control

Some studies have suggested that stacking different levels of crop diversity may lead to higher aphid control by complementary strategies (Hatt & Döring, 2023). Nonetheless, the plots with the highest diversity (wheat cultivar mixtures with burclover undersowing) did not outperform the plots with only one diversity practice. Specifically, in our study we observed an unfavorable association between FAFO and burclover undersowing, perhaps because both diversification practices benefited the cropping system by bottom-up effects, which may lead to functional redundancy. These results tied up with previous field research (Mansion-Vaquié et al., 2019). In this regard, many studies have already highlighted the need for complementary ecological processes to ensure functional polycultures (Barot et al., 2017; Gaba et al., 2015).

Performance of wheat polycultures on yield

Our research addresses the provision of pest control services through diversification practices in real farming conditions, so far overlooked in the scientific literature to date. For that reason, we mixed wheat varieties whose flour is already blended for bread-making production in this region and analyzed the possible effect of diversity strategies on the crop yield. In the 2020 season, yield was comparable among treatments; however, in the 2021 season, cultivar mixtures outperformed modern cultivar Florence-Aurora monoculture plot. We should underscore that in 2021 the region experienced a particularly dry cropping season (~80% reduction in rainfall compared with the mean annual rainfall). Our results indicate the advantages of traditional cultivars and cultivar mixtures over modern cultivar monocultures in maintaining high yields across seasons, regardless of

TABLE 3 Abundance of grown-dwelling predator groups (individuals per trap; mean ± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Auora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M+) or absence (M−) of burclover undersowing.

Taxonomic group	Burclover undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
2020						
Araneae	M+	1.1 ± 0.2	1.1 ± 0.3	1.3 ± 0.3	1.0 ± 0.2	1.5 ± 0.3
	M−	0.7 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	1.6 ± 0.4	1.0 ± 0.1
Coleoptera						
Staphylinidae	M+	0.6 ± 0.2	0.5 ± 0.1	0.8 ± 0.3	0.5 ± 0.2	0.8 ± 0.2
	M−	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.4
Carabidae	M+	0.2 ± 0.1	0.02 ± 0.0	0.04 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
	M−	0.1 ± 0.1	0.1 ± 0.04	0.02 ± 0.02	0.2 ± 0.1	0.02 ± 0.0
Total		1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.5	2.0 ± 0.4	2.2 ± 0.4
2021						
Araneae	M−	2.5 ± 0.5	2.6 ± 0.5	2.8 ± 0.4	2.1 ± 0.3	2.4 ± 0.4
Coleoptera						
Staphylinidae	M−	1.3 ± 0.2	1.2 ± 0.2	2.0 ± 0.6	1.4 ± 0.3	1.4 ± 0.2
Carabidae	M−	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Total		4.2 ± 0.5	4.2 ± 0.6	5.1 ± 0.8	3.8 ± 0.3	4.1 ± 0.4

TABLE 4 Crop yield (grain, in kilograms per hecatre; mean ± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Aurora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M+) or absence (M−) of burclover undersowing.

Burclover undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
2020					
M−	4380 ± 626	4535 ± 993	3792 ± 219	5765 ± 973	4342 ± 685
M+	5813 ± 631	4084 ± 268	3798 ± 271	3654 ± 855	4453 ± 624
2021					
M−	**2881 ± 594**	5000 ± 513	5530 ± 572	4459 ± 1036	4106 ± 618

Note: Boldface indicates significant differences within wheat treatment according to Tukey-adjusted pairwise comparisons ($p < 0.05$). Every year was analyzed independently.

meteorological conditions (Reiss & Drinkwater, 2018). Our findings agree with Borg et al. (2018) meta-analyze which showed a grain yield increase of 4.3%–5.7% in winter wheat mixtures.

When testing the feasibility of a potential weed as a cover crop, it is essential to evaluate its impact on the crop yield to reduce the negative effects of competition. Burclover undersowing, reduced aphid abundance without affecting wheat yield, suggesting a facilitation relationship between the two species (Lopes et al., 2016; Verret et al., 2017). Nevertheless, there are some limitations to the use of burclover in Mediterranean fields, as its establishment and growth are affected by meteorological conditions rendering it unsuitable during dry cropping seasons.

To conclude, this study revealed the potential of genotypic (cultivar mixtures) and interspecific (burclover undersowing) diversity for enhancing bottom-up ecological processes related to aphid population control in organic winter wheat crops. Moreover, it provides significant support for cultivar-specific effects on associational resistance. Cultivar mixture with complementary traits has a great potential on aphid control, while mixing cultivars with similar traits or stacking diversity practices with comparable bottom-up effects do not cause any further reductions in aphid abundance. The advantages of burclover undersowing were found to be closely tied to the identity of the cultivar associated. Thus, trait-approach research is needed to reinforce functional polycultures.

Finally, the diverse strategies employed in this experiment had no detrimental effect on crop yields, demonstrating their suitability for polyculture in Mediterranean organic farming conditions.

AUTHOR CONTRIBUTIONS

F. Xavier Sans, Lourdes Chamorro-Lorenzo, Berta Caballero-López, Alejandro Pérez-Ferrer and José M. Blanco-Moreno conceived the experimental setup. Data collection was performed by all authors. Alba Tous-Fandos analyzed the data. Alice Casiraghi identified the parasitoid wasps' genera. Alba Tous-Fandos wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Tous-Fandos, Chamorro Lorenzo, et al., 2023) are available from CORA: https://doi.org/10.34810/data909.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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