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Flowering areas enhance insect pollinators and biological control agents in wheat intensive agro-ecosystems

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1 **Flowering areas enhance insect pollinators and biological control agents in wheat intensive**  
2 **agro-ecosystems**

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14 **ABSTRACT**

15 1. This four-year study (2018-2021) aimed at investigating the effects of flowering areas inserted  
16 at the margin of soft wheat fields (*Triticum aestivum* L.) in comparison with spontaneous field  
17 margins. The action formed part of a multidisciplinary initiative promoted by the Barilla food  
18 company, aimed at disseminating the sustainable production of soft wheat.

19 2. Specific aims were to study the effects of flowering areas on: **i)** the abundance and species  
20 richness of insect pollinators, including wild bees, syrphids, and butterflies; **ii)** the levels of  
21 wheat aphid and bug infestations and **iii)** the assemblages of biological control agents of  
22 aphids (coccinellids and aphid mummies as a result of parasitization).

23 3. Insect surveys were carried out on 61 wheat-growing farms located in Northern Italy. Insect  
24 pollinators were sampled on all farms, whereas wheat aphids, wheat bugs, and aphid  
25 biological control agents were investigated in a sub-sample of 20 farms.

- 26 4. Results generally indicated a positive impact of flowering areas on multiple taxa in  
27 comparison with spontaneous field margins. The abundance of pollinators and coccinellids  
28 was significantly enhanced by the flowering areas. Aphid mummies showed an interaction  
29 between strategies and distances with more mummies at 5 m in fields close to flowering areas  
30 and at 50 m in the plots close to spontaneous field margin (control).
- 31 5. Flowering areas were validated as an important tool for promoting beneficial taxa in agro-  
32 ecosystems. However, the successful establishment of this ecological infrastructure was  
33 suggested to rely on several factors, which included both the selection of suitable flowering  
34 plant species and the implementation of appropriate management practices.

35 **Keywords:** Coccinellidae, Apoidea, Lepidoptera, ecological infrastructures, agroecological  
36 interventions, Integrated Pest Management, long-term implementation, sustainable food production,  
37 value chain.

## 38 INTRODUCTION

39 The intensification of agriculture has led to a significant loss of natural habitats with negative  
40 consequences for biodiversity (Dudley & Alexander, 2017). The use of resource-intensive  
41 agricultural practices has been shown to influence soil, water, and air quality with serious impacts on  
42 human health (Sabarwal et al., 2018). This trend will likely continue in the future unless specific  
43 actions are taken to implement valuable management strategies to support biodiversity in agro-  
44 ecosystems (Norris, 2008). Within this context, the European Commission (EU) has promoted a plan  
45 to support biodiversity and sustainable food production, based on pesticide and fertilizer reduction  
46 (European Commission, 2020). In the wake of this change, private companies are planning  
47 transformations in their value chains to improve sustainability from field to market. A change of  
48 perspective is necessary, as production processes will have to increasingly consider the social and  
49 environmental value they add (Myers, 2005).

50 Among habitat management practices, agri-environmental schemes are well-known methods for  
51 promoting farmland biodiversity. In addition to a broader focus on preserving agrobiodiversity,

52 several studies have also investigated a targeted 'ecosystem service' approach, specifically  
53 emphasizing biological control or the enhancement of pollination (Crowther et al., 2023; Hatt et al.,  
54 2020; Holland et al., 2016; Uyttenbroeck et al., 2016). The addition of flowering areas (FA) often  
55 constitutes a first step in agroecological management in simplified rural landscapes because of  
56 operational feasibility.

57 The effect of FA on beneficial arthropods has been shown to be dependent on several variables,  
58 including ecological factors and agronomic practices, such as the intensity of pesticide use.  
59 Concerning the ecological factors, it is well known that landscape complexity can affect the response  
60 of arthropods to habitat manipulation (Karp et al., 2018; Saqib et al., 2020), including the  
61 implementation of FA (Batáry et al., 2011; Grass et al., 2016; Tschardt et al., 2005; Tschardt et  
62 al., 2012). Indeed, the effects of habitat management are usually greater in simplified rural landscapes  
63 than in more complex landscapes (Tschardt et al., 2012), although specific responses depend on  
64 the considered taxa. For example, the sowing of flowering strips was shown to be more effective at  
65 increasing the richness and abundance of syrphid species (Diptera, Syrphidae) in simplified farmlands  
66 with a higher proportion of arable crops than in complex landscapes (Haenke et al., 2009; Scheper et  
67 al., 2013).

68 The adoption of habitat management techniques to enhance biodiversity cannot be disentangled from  
69 an evaluation of the associated ecosystem services (i.e., biological control) as well as the possible  
70 disservices. However, the use of arguments based on ecosystem services to justify biodiversity  
71 conservation is not without controversies. Several studies suggested that biodiversity enhances the  
72 magnitude and/or stability of ecosystem functioning, with ecosystem services representing the subset  
73 of services that directly benefit people. In this context, considering the costs of maintaining or  
74 promoting biodiversity is crucial, as financial constraints typically limit the implementation of  
75 practical conservation policies in the real world (Kleijn et al., 2015).

76 Some studies have demonstrated that sowing FA within wheat fields can increase aphid control, in  
77 comparison to wheat grown in monoculture (Hatt et al., 2020; Pollier et al., 2018). Wheat aphids,

78 including *Sitobion avenae* (F.), *Rhopalosiphum padi* (L.) and *Metopolophium dirhodum* (Walker)  
79 (Hemiptera, Aphidae), and wheat bugs, including *Aelia rostrata* Boheman, *Aelia acuminata* (L.)  
80 (Hemiptera, Pentatomidae), *Eurygaster maura* (L.) and *Eurygaster austriaca* (Schrank) (Hemiptera,  
81 Scutelleridae) are Italian key pests that contribute to losses in both wheat yield and grain quality.  
82 Wheat aphids cause direct damage by feeding on stems and leaves, as well as indirect damage by  
83 excreting honeydew or vectoring viruses (Rabbinge et al., 1981). Bugs are mainly responsible for the  
84 depletion in wheat quality, attributable to the injection (via saliva) of proteolytic enzymes into the  
85 kernels (Vaccino et al., 2006). When 3-5% of the kernels are damaged, a reduction in the baking  
86 quality is evident, which then dramatically increases when damaged kernel percentages exceed 10%  
87 (Hariri et al., 2000).

88 The present four-year study (2018-2021) was aimed at investigating the impact of FA implementation  
89 in wheat fields, by a multidisciplinary initiative, promoted by Barilla G. & R. Fratelli S.p.A (Parma,  
90 Italy) and incorporated within the document “Carta del Mulino” (CM) (Supporting Information S1).  
91 The general objective was to explore the contribution of FA in promoting the conservation of  
92 beneficial insects, using a multi taxa approach. Specific aims were to study the effects of FA on: **i)**  
93 the abundance and species richness of insect pollinators, including butterflies (Lepidoptera,  
94 Papilionoidea and Hesperidae), wild bees (Hymenoptera, Apidae) and syrphids (Diptera,  
95 Syrphidae); **ii)** wheat aphid and wheat bug infestations and **iii)** the assemblages of biological control  
96 agents of aphids, including coccinellids (Coleoptera, Coccinellidae) and aphid mummies as result of  
97 parasitization by hymenopteran parasitoids (Hymenoptera, Braconidae).

98

## 99 **MATERIALS AND METHODS**

### 100 **Study sites and experimental design**

101 This four-year study (2018-2021) was carried out in 61 farms located in three regions of Northern  
102 Italy (Emilia-Romagna, Lombardy and Piedmont), where soft wheat (*Triticum aestivum* L.) was  
103 grown as a primary crop. The number of farms increased from the first (n=4) to the last (n=30) year

104 of the project. During the 2020 lockdown due to COVID-19 pandemic, given the difficulties to  
105 cross regional borders, the number of sampled farms was lower (n=7), in comparison to the  
106 previous years (n=20). Farms were selected based on both their adherence to the CM document and  
107 on the availability of farmers to facilitate the sampling activities.

108 FA were sown in either autumn or spring on 3% of the Utilized Agricultural Area cultivated with  
109 soft wheat in each farm, according to the CM document. Farmers could choose among a list of nine  
110 multiflora mixtures of legumes (i.e., *Vicia faba* L., *Medicago sativa* L. and numerous species of  
111 *Trifolium* L.) and herbaceous flowering plants (i.e., *Onobrychis viciifolia* Scop., *Phacelia*  
112 *tanacetifolia* Benth. and *Centaurea cyanus* L.), based on careful consideration of the specific  
113 conditions of each location (Supporting Information S2).

114 During the study, the use of insecticides was banned in all experimental plots, while other  
115 interventions were carried out following the guidelines outlined in the Integrated Pest Management  
116 (IPM) regulations (Pedigo et al., 1986) of the Emilia-Romagna region (Northern Italy) and the CM  
117 document (Supporting Information S1).

118 The experimental plan followed a pair block design in which a single wheat field (> 2 ha) per farm  
119 was considered as a block with two strategies (Fig. 1):

- 120 i) FA, with flowering plants sown adjacently to a margin of the wheat field;
- 121 ii) control, in the opposite side of the wheat field, which was characterized by wild plants growing  
122 spontaneously in the field margin.

123 Insect pollinators (butterflies, wild bees, and syrphids) were monitored in the FA and in the  
124 spontaneous field margins of all 61 farms. Wheat aphids, wheat bugs, aphid mummies and  
125 coccinellids were sampled only in a subset of 20 farms (n=4 in 2018; n=5 in 2019; n=6 in 2020 and  
126 n=6 in 2021) in two plots inside the wheat field (namely, FA plots adjacent to the areas sown with  
127 flowering plants and control plots in the opposite field side adjacent to the spontaneous field  
128 margin).

129 Even if some farms were sampled in multiple years, because of crop rotations, wheat was never  
130 grown in the same fields. Therefore, samples taken in different years from the same farm were  
131 considered as different blocks.

132

### 133 **Insect and vegetational samplings**

#### 134 - **Pollinator insects and evaluation of percentage of flowering species**

135 Insect pollinators (butterflies, wild bees, and syrphids) were visually checked and collected using  
136 an entomological net along a transect of 100 m both in the FA and in the spontaneous field  
137 margins, following the standardized method by Pollard (1977).

138 Most butterflies were identified directly in the field and then immediately released, as they were  
139 often easy to identify visually. On the other hand, most of wild bees were collected and returned to  
140 the laboratory for identification. Butterflies and wild bees were identified either to the genus or  
141 species-morphospecies level. Instead for syrphids, only the total abundance and the relative  
142 abundances of the dominant species were considered.

143 Surveys of insect pollinators were carried out once per year during the flowering period of the  
144 plants in the mixtures (from approximately the beginning of May to the end of June) concurrently  
145 with the assessment of plant species and abundance in both FA and spontaneous field margins  
146 (control).

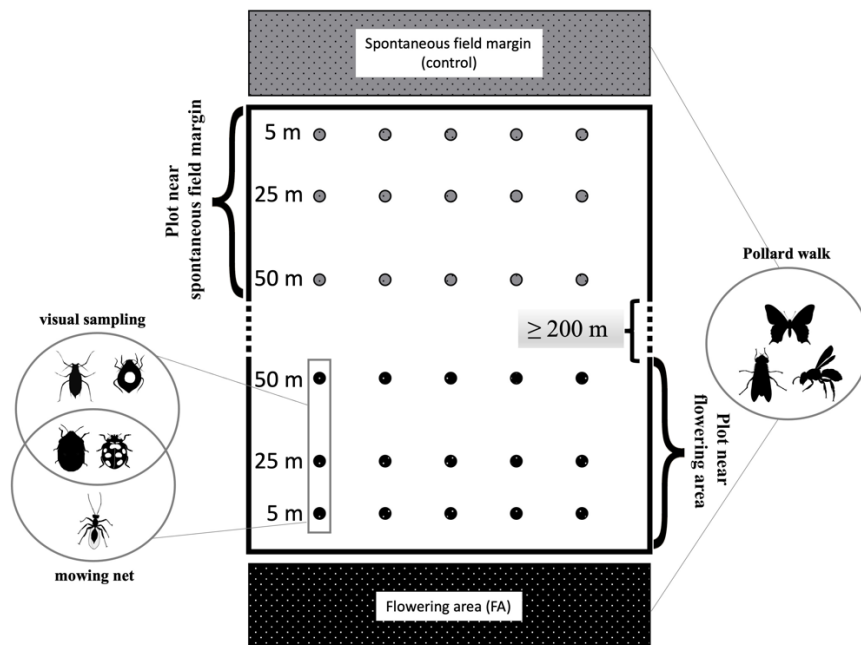
147 The plant abundance was estimated following the Braun-Blanquet scale (Braun-Blanquet, 1932),  
148 modified according to Pacini et al., 2003. Further, the percentage of flowering species was  
149 calculated by visually estimating the total soil coverage of different flowering species (defined as >  
150 90% plants for each species at the reproductive stage) within each survey spot delimited in FA and  
151 spontaneous field margins (totaling three spots of 50 m<sup>2</sup> per strategy in each farm) (Ashworth et al.,  
152 2022).

153

154

155 - **Wheat pests and aphid biological control agents**

156 Surveys of wheat aphids, wheat bugs, and aphid biological control agents (adults, pupae, and larvae  
157 of aphidophagous coccinellids and aphid mummies as result of parasitization) were conducted  
158 twice per year during the FA flowering period by sampling insects at three progressive distances (5  
159 m, 25 m, and 50 m) inside the wheat field both for the FA plots and for the plots near to the  
160 spontaneous field margin. At each distance five sampling points, 10 m apart from each other, were  
161 replicated (Fig. 1). Wheat aphids and aphid mummies, including both aphids still parasitized and  
162 aphids with the hole of wasp emergence, were evaluated by visually checking 1 m<sup>2</sup> of soft wheat  
163 plants per sampling point. Wheat bugs and coccinellids were collected with a sweeping net by  
164 performing ten sweeps back and forth at each sampling point.  
165 All target insects were counted, except for the wheat aphids, for which only the percentages of  
166 infested tillers were recorded. Only aphidophagous coccinellids were considered.  
167



**Figure 1** Experimental design established in each farm. Insect pollinators were sampled once in all the farms (n =61), whereas pests and biological control agents were sampled twice in each of the farms included in the sub-sample (n =20).

168 **Statistical analysis**

169 The number of individuals of butterflies, wild bees and syrphids was compared between FA and the  
170 spontaneous field margin, fitting three generalized linear mixed models (GLMM) with Poisson  
171 error distribution and logarithmic link function. Strategy (FA vs spontaneous field margins) was  
172 used as a fixed factor and farm was included as a random block effect. Kenward–Roger method  
173 was used to estimate the degrees of freedom.

174 Flowering plant percentages were compared between FA and spontaneous field margins by  
175 Wilcoxon Signed Ranks Test for paired samples. FA and spontaneous field margin in the same farm  
176 were considered as paired. Because of the crop rotations across years, same farms sampled in  
177 different years were included as different blocks.

178 Multiple linear regressions were used for modelling the effects of wheat field margin (FA vs  
179 spontaneous field margins) and the percentage of flowering plants in the margins on the number of  
180 individuals of each pollinator group counted by Pollard walks. The kind of field margin was coded  
181 as a dummy variable set at 1 for FA and 0 for the spontaneous field margin. No outliers were  
182 detected analysing Cook’s distances, nor the numbers of large residuals exceed what could be  
183 expected given the sample size. Standard Errors (SE), Confidence Intervals at 95% (CI 95%), and  
184 p-values of the coefficients were calculated by bias corrected and accelerated bootstrap based on  
185 1000 resamplings.

186 The number of wheat plants infested by aphids out of the total number of tillers inspected was  
187 analyzed using a GLMM with binomial error distribution, probit link function, and Kenward–Roger  
188 method. Strategy (FA plot vs spontaneous field margin plot) and the distances from the border  
189 inwards into the wheat fields (5, 25, and 50 m, respectively) were included as fixed and interacting  
190 factors. Farms were modeled as a random block factor.

191 Finally, to test the effects of FA on aphid mummies, wheat bugs, and coccinellids, we run GLMMs  
192 with a similar structure as above, with the only exception of using the Poisson error distribution to

193 model count data. Bonferroni sequential method was used when needed to accommodate for  
194 multiple comparisons among the three levels of the distance factor.  
195 Data on the wheat bugs, which were collected by sweeping nets, were converted to square meter  
196 density using the formula reported in Tonkyn, 1980 to compare our data with the economic  
197 thresholds reported for the Piedmont region, Italy.  
198 The software package IBM SPSS Statistics ver. 23 (IBM Corp., Armonk, NY) (IBM 2019) was  
199 used for all the statistical analyses.

200

## 201 **RESULTS**

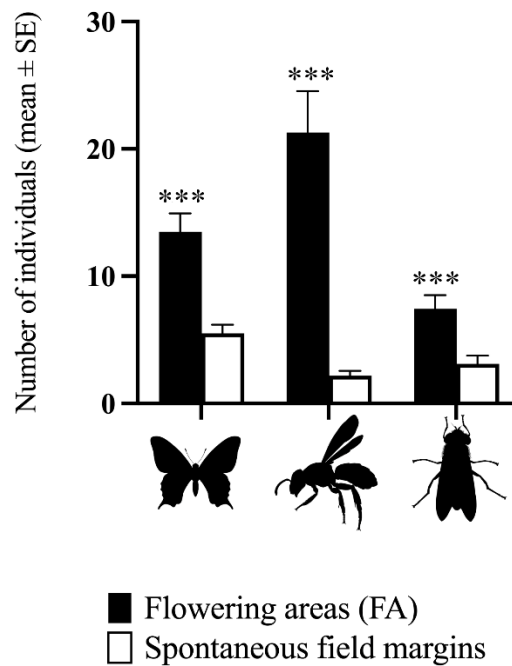
### 202 **Insect pollinators and percentage of flowering species**

203 During the entire study, 1160 butterflies belonging to 33 species were sampled along the transects.  
204 Of these, 823 were found in the FA and 337 in the spontaneous field margins. This was equivalent  
205 to a mean of  $13.49 \pm 1.44$  and  $5.52 \pm 0.66$  (SE) per 100 m transect in FA and the spontaneous field  
206 margin, respectively. *Pieris* (Lepidoptera, Pieridae) was the dominant genus, accounting for 31% of  
207 all the butterflies sampled across the transects (Supporting Information S3). The total number of  
208 butterfly species ranges from 29 in FA to 25 in spontaneous field margin.

209 Overall, 1432 wild bee individuals were collected, of which 1063 were identified to genus or  
210 species-morphospecies level (Supporting Information S4). The large majority (1001) of individuals  
211 were found in the FA, whereas in the spontaneous field margins only 62 wild bees were counted.  
212 This was equivalent to a mean of  $21.29 \pm 3.24$  and  $2.20 \pm 0.36$  per FA and spontaneous field margin  
213 transects, respectively. The total number of species/morphospecies was 81 (69 in FA and 28 in  
214 spontaneous field margin), and *Bombus* was the most abundant genus (67.3%).

215 Syrphids were less abundant than other pollinator groups, with a total of 644 individuals, of which  
216 454 were counted in the FA and 190 in the spontaneous field margin. The mean number per transect  
217 was  $7.44 \pm 1.07$  and  $3.11 \pm 0.65$  for the FA and the control, respectively. The most abundant

218 species (pooling both FA and spontaneous field margin transects) was *Sphaerophoria scripta* (L.)  
 219 accounting for 70.41% of the individuals sampled.  
 220 GLMM showed a significantly higher abundance of butterflies ( $F_{(1, 120)}=190.61$ ;  $P<0.001$ ), wild  
 221 bees ( $F_{(1, 120)}=626.75$ ;  $P<0.001$ ), and syrphids ( $F_{(1, 120)}=101.05$ ;  $P<0.001$ ) in FA in comparison to  
 222 spontaneous field margins (Fig. 2).



**Figure 2** Abundances of butterflies, wild bees, and syrphids sampled in the flowering areas (FA) and spontaneous field margins (control). Asterisks indicate significant differences between strategies (GLMMs,  $***P<0.001$ ).

223  
 224 The percentage of flowering plants was significantly higher in FA (median = 54.50%; 25<sup>th</sup>- 75<sup>th</sup>  
 225 percentiles = 42.38 – 72.25%) than in the spontaneous field margins (median = 11.70%; 25<sup>th</sup>- 75<sup>th</sup>  
 226 percentiles = 4.47 – 24.06%) ( $Z = -6.37$ ;  $P<0.001$ ). In particular, *Phacelia tanacetifolia* Benth.,  
 227 *Papaver rhoeas* L., *Trifolium pratense* L., *Trifolium repens* L., and *Onobrychis viciifolia* Scop were  
 228 the most abundant species in the FA. On the other hand, in spontaneous field margins the dominant  
 229 species were *Ammi majus* L., *Convolvulus arvensis* L., *Matricaria chamomilla* L., *Papaver rhoeas*

230 *L.* and *Picris* spp. The flowering periods of several plant species showed a partial overlap in the  
 231 surveys.

232 The multiple linear models, run to test the effect of wheat field margin and the percentage of  
 233 flowering plants on the number of individuals of butterflies ( $R^2 = 0.28$ ;  $F_{(2, 117)} = 23.17$ ;  $P < 0.001$ ),  
 234 bees ( $R^2 = 0.24$ ;  $F_{(2, 117)} = 18.90$ ;  $P < 0.001$ ) and syrphids ( $R^2 = 0.12$ ;  $F_{(2, 117)} = 8.10$ ;  $P < 0.001$ ),  
 235 were all statistically supported. For butterflies and syrphids, the significant factor was the  
 236 percentage of flowering plants in the margin where the Pollard walk transect was carried out (Tab.  
 237 1). On the other hand, for bees a significant effect was detected only for the presence of the FA.  
 238

**Table 1.** Effects of wheat field margin (FA vs spontaneous field margins) and the percentage of flowering plants on the abundance of the pollinators group as assessed by Pollard walks. Linear regression models with confidence intervals, standard errors (SE) and p-values estimated by 1000 bootstrap resamplings.

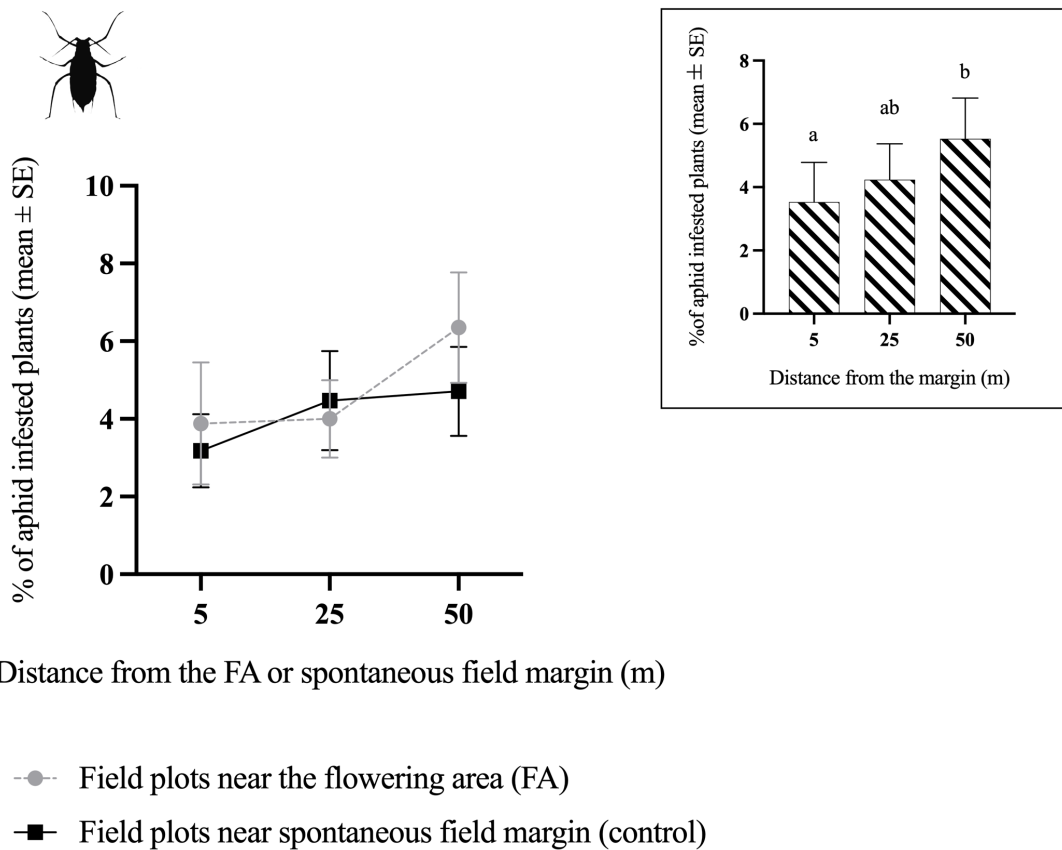
Pollinator Group		B	SE	Confidence Intervals 95%	Standardized $\beta$	P
Butterflies	Constant	3.11	0.78	1.45 – 4.84		<0.001
	FA vs Spontaneous field margins	2.15	1.68	-0.87 – 5.22	0.11	0.19
	Flowering percentage	0.16	0.04	0.08 – 0.24	0.45	<0.001
Bees	Constant	0.07	1.48	-3.43 – 3.15		0.96
	FA vs Spontaneous field margins	14.34	3.58	8.46 – 21.96	0.35	<0.001
	Flowering percentage	0.14	0.09	-0.02 – 0.31	0.18	0.15
Syrphids	Constant	2.12	0.84	0.37 – 3.64		0.01
	FA vs Spontaneous field margins	1.89	1.26	-0.40 – 4.57	0.13	0.13
	Flowering percentage	0.07	0.03	0.02 – 0.12	0.25	0.02

239

#### 240 **Soft wheat pests**

241 The percentage of plants infested by aphids was significantly lower at 5 m in comparison to 50 m in  
 242 both in the wheat plots located on the side with FA and in those near to spontaneous field margins  
 243 (GLMM: “distance”;  $F_{(2, 114)} = 9.26$ ,  $P < 0.001$ ; Fig. 3). The maximum recorded percentage of infested

244 tillers was 20% in the investigated farms. Hence, aphid infestations never exceeded the economic  
 245 threshold reported in the IPM guidelines of Emilia-Romagna region, which is set at 80% of tillers  
 246 with aphid colonies.



**Figure 3** Mean percentage ( $\pm$  SE) of aphid infested plants obtained by visual sampling in FA and control plots at three progressive distances from the flowering area (FA) and the spontaneous field margin. The GLMM detected significant differences ( $P < 0.001$ ) among distances, which are shown with different letters in the chart on the right.

247  
 248 Wheat bugs in the genus *Eurygaster* (n=748 outnumbered the individuals belonging to *Aelia* genus  
 249 (n=10). Abundances of these key pests was higher in FA plots than in control plots (GLMM:  
 250 “strategy”:  $F_{(1, 114)} = 24.50$ ,  $P < 0.001$ ; Fig. 4). Nevertheless, the mean number of wheat bugs per  $m^2$ ,  
 251 obtained applying the formula by Tonkyn (1980) was far below the economic threshold reported in

252 IPM guidelines of Piedmont region (=5 bugs / m<sup>2</sup>). A maximum density of 0.35 bugs per m<sup>2</sup> was  
 253 recorded considering all the farms and both the strategies.  
 254

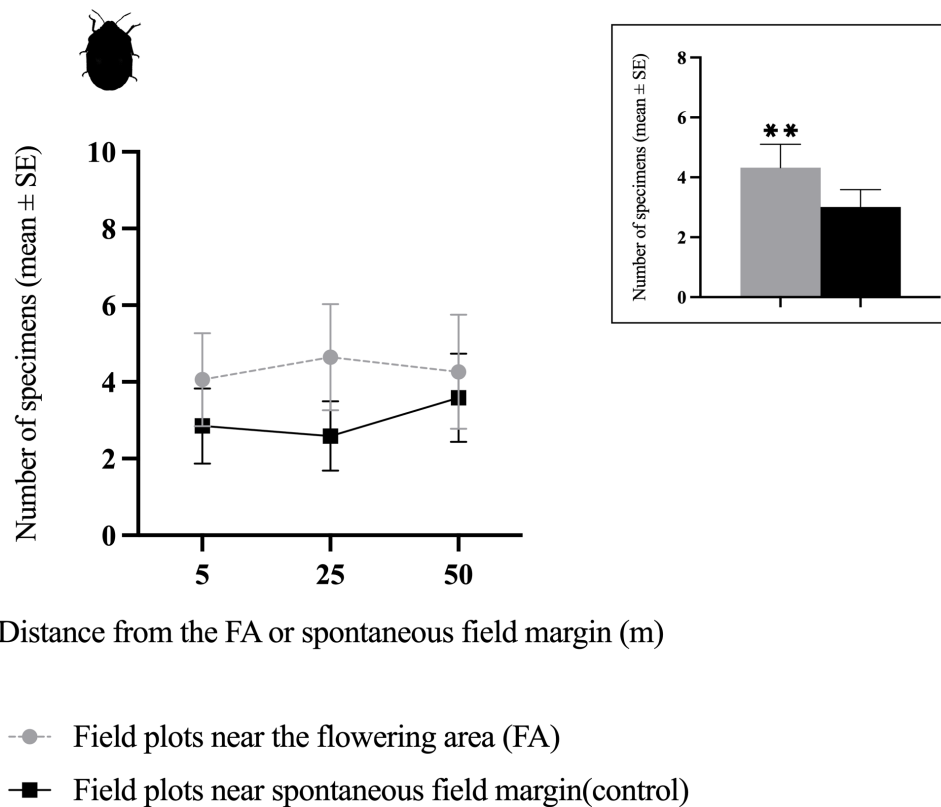
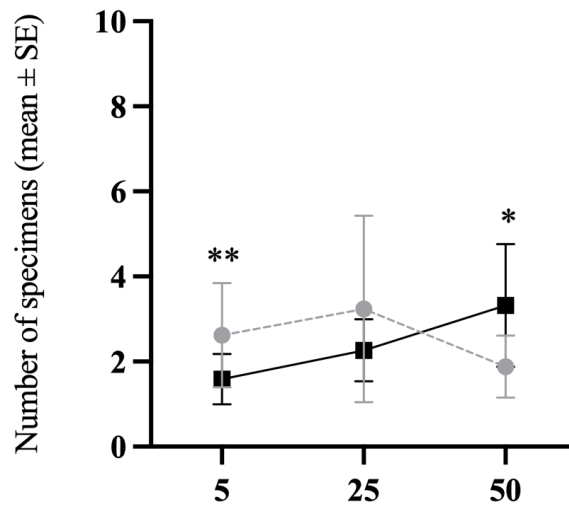


Figure 4 Mean number of wheat bugs sampled by the sweeping net wheat plots at three progressive distances from the flowering area (FA) and the spontaneous field margin. The GLMM detected significant differences ( $P < 0.001$ ) between strategies, which are shown by asterisks in the chart on the right.

255

### 256 **Biological control agents**

257 A significant interaction “distance\*strategy” was detected for the number of aphid mummies on  
 258 wheat plants, ( $F_{(2, 114)} = 27.37, P < 0.001$ ; Fig. 5A). At a 5 m distance from the borders, more  
 259 mummies were found in the wheat plots adjacent to the FA than in the plots close to the  
 260 spontaneous field margins, while at 50 m inwards from the border, more mummies were counted in  
 261 the wheat plots grown close to the spontaneous margins than in the plots near to the FA.



Distance from the FA or spontaneous field margin (m)

- Field plots near the flowering area (FA)
- Field plots near control (spontaneous field margin)

Figure 5 Mean number of aphid mummies sampled by visual sampling. Asterisks indicate significant differences between strategies which were compared within each distance (GLMM; \*\*\* $P < 0.001$ ; \* $P < 0.05$ ).

262 The most abundant species of adult coccinellids was *Hippodamia variegata* Goeze accounting for  
263 the 41.53% of the total individuals, followed by *Coccinella septempunctata* L. (37.70%) and  
264 *Propylea quatuordecimpunctata* L. (14.94%). The remaining 5.83% of coccinellid species included  
265 few individuals of *Oenopia conglobata* L., and *Harmonia axyridis* Pallas.

266 The mean number of the aphidophagous coccinellids resulted significantly higher in plots adjacent  
267 to the FA, than in wheat plots near to spontaneous field margins (“strategy”:  $F_{(1, 44)} = 17.28$ ,  
268  $P < 0.001$ ; Fig.6B). Moreover, a significant effect of the distance was also highlighted, with more  
269 coccinellids at 5 m than at the remaining distances, irrespective of the strategy (“distance”:  $F_{(2, 144)}$   
270  $= 6.82$ ,  $P < 0.01$ ; Fig.6B).

271

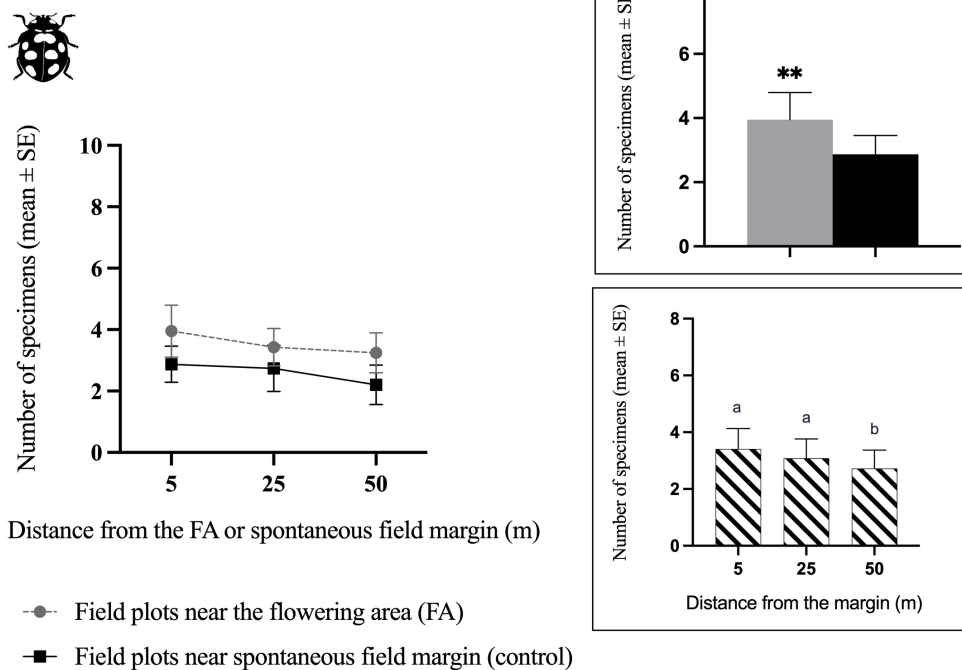


Figure 6 Mean number of coccinellids sampled by the sweeping net method in wheat plots at three progressive distances from either the flowering area (FA) or the spontaneous field margin. The charts on the right indicate significant differences between strategies (GLMM; \*\*\*P<0.001) and between distances (GLMM; different letters: P<0.05).

272

## 273 DISCUSSION

### 274 - Insect pollinators

275 The abundance of butterflies, wild bees, and syrphids showed a significant positive response to the  
 276 presence of the FA with an increase of 486, 939, and 190 individual numbers, respectively. Also,  
 277 the number of pollinator species was positively influenced by FA. This last result should be  
 278 interpreted as a mere attraction of FA towards pollinator insects, rather than as a stable increase in  
 279 species richness for which additional interventions are usually necessary. The availability of semi-  
 280 natural habitats at the landscape level has been identified as an important factor in providing  
 281 alternative floral resources, host plants, nesting sites, and overwintering refuges for pollinator taxa  
 282 (von Königslöw et al., 2021).

283 In detail, there was an increase of 4 butterfly species and 41 wild bee species on FA compared to  
284 spontaneous field margin. However, the number of butterfly species found on the FA in the present  
285 study was only a small fraction of the species that might be expected for similar habitats in the  
286 Italian checklist (Villa et al., 2009). This is partly attributable to the fact that only a single sampling  
287 was carried out during the flowering period of the FA. Additionally, the lack of host plants for the  
288 larvae of most of the Italian species was certainly a major driver for the low number of species  
289 found in the present study sites.

290 The same points were likely also valid for the wild bees. While most bee species are polylectic, and  
291 can collect pollen from a variety of plants, several others exhibit a narrow host range, typically  
292 limited to a single plant family. This aspect, coupled with the lack of nesting sites, may have played  
293 an important role in limiting the long-term persistence of wild bee species in the investigated agro-  
294 ecosystems (Amy et al., 2018; Kennedy et al., 2013).

295 Finally, as multiple linear regressions revealed, abundances of butterflies and syrphids were strictly  
296 related to the percentage of flowering species, regardless from the strategy (FA and spontaneous  
297 field margins). On the other hand, wild bees were found to be significantly influenced by the  
298 presence of flowering areas. These results seem to be coherent with the requirement of wild bees,  
299 because plant species within FA were more suitable for bee foraging; for example, legumes are  
300 particularly appreciated by long-tongued bees such as *Bombus* sp. Butterflies are more generalist in  
301 plant foraging and their abundance is strongly affected by the presence of flowers, which are more  
302 abundant within FA. Moreover, in spite syrphids are considered rather opportunistic as flower  
303 visitors, they show strong preferences for particular flower morphologies (i.e., flowers with open  
304 corolla and exposed nectaries). A more specific flowering mixture for syrphids (like the short-  
305 tongue *S. scripta*, which was the most abundant species in our study) should include plants with  
306 more accessible flowers (Doyle et al., 2020), for example Apiaceae, Brassicaceae, Asteraceae and  
307 species like *Lobularia maritima* (L.) and *Fagopyrum exculentum* Moench. (Irvin et al., 2021). A

308 supplementary source of attraction for syrphids could be represented by stimuli offered by aphid  
309 infested weeds, which are typically present in spontaneous field margins of our study.

310 - **Wheat pests and aphid biological control agents**

311 Wheat plots near FA were in general associated with a greater abundance of biological control  
312 agents in comparison to plots close to spontaneous field margins. The present study corroborates the  
313 existing literature, demonstrating that an enhancement of biological control agents can be achieved  
314 through the implementation of FA (Balzan et al., 2016; Blaauw & Isaacs, 2014; Skirvin et al., 2011;  
315 Tschumi et al., 2016). Coccinellids were more abundant in wheat plots close to the FA than in the  
316 plots near to the spontaneous field margins. In both FA and control plots, there was a decreasing  
317 gradient effect from the field margin which is typical of many beneficial insects and cyclic  
318 colonizers (Marshall & Moonen, 2002). The abundance of both aphidophagous coccinellids and  
319 aphid mummies on wheat tillers was higher at 5 m distance from the FA than at the same distance  
320 from the spontaneous field margins. The increased density of aphid biological control agents could  
321 be due to both food sources and buffer effects provided by the FA. The buffer effect could be  
322 particularly significant in agro-ecosystems with a high intensification, similar to that investigated in  
323 the present study. In high intensification systems, the FA may have played a role as a refuge for  
324 biological control agents, protecting them from pesticide inputs in the adjacent fields.

325 While aphid mummies were more abundant at 5 m from the field borders in FA plots, this pattern  
326 was reversed in the spontaneous field margin plots, with mummies being more abundant at 50 m  
327 from the borders. A possible explanation of these trends is that aphid parasitoids were not only  
328 influenced by the presence of diversified floral resources but also by other ecological factors,  
329 including host/prey availability (Toivonen et al., 2018). Another possible explanation could be that  
330 the higher density of mummies, observed in spontaneous field margin plots at 50 m, may have been  
331 fostered by the lower abundance of coccinellids at the same distance because predators may have a  
332 negative influence on parasitoids through their exploitation of aphid colonies (Muller et al., 1999).  
333 On the other hand, the higher density of coccinellids and aphid mummies on wheat plants at 5 m

334 from the FA could be attributable to the strong gradient effect of these ecological infrastructures as  
335 buffer zones, which may have compensated for the competitive interactions between predators and  
336 parasitoids.

337 Coccinellids and parasitoids may have played a role in suppressing wheat aphids as the infestation  
338 levels were always below the economic threshold, set at 80% of infested tillers by the IPM  
339 guidelines of the Emilia-Romagna region. Moreover, the pooled data of the aphid infested plants  
340 showed a lower infestation at 5 m, where coccinellid density was higher, in comparison to 50 m, in  
341 both strategies. Usually, aphidophagous coccinellids show a positive response to aphid density  
342 (Elliott & Kieckhefer, 2000; Evans, 2003). On the contrary, only some species of hymenopteran  
343 parasitoids exhibit a positive correlation of parasitism rates with aphid densities (Walde &  
344 Murdoch, 1988). Nevertheless, both coccinellids and hymenopteran parasitoids may cover long  
345 distances to find prey or hosts (Boivin et al., 2012).

346 Outbreaks of wheat bugs have been reported in Northwestern Italy, raising serious concerns  
347 (Vaccino et al., 2006). An overall lower number of wheat bugs per m<sup>2</sup> was found in this study.  
348 However, statistical analyses showed a higher density of wheat bugs sampled by the sweeping net  
349 method in wheat plots adjacent to the FA in comparison with plots near to the spontaneous field  
350 margins. This indicates a possible disservice attributable to the flowering mixtures. Most wheat  
351 bugs feed predominantly on Gramineae with strong preference for wheat, however, grass species  
352 such as *Poa* spp. can be exploited as well (Edde, 2022). *Poa pratensis* L. was used, although at a  
353 low percentage, in some of the flowering mixtures sown in the present study, thus representing a  
354 possible explanation for these findings. The use of grasses in the mixture should be carefully  
355 reconsidered for flowering strips intended to be sown in agro-ecosystem dominated by cereal crops.

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360 **CONCLUSIONS**

361 In conclusion, FA adoption could represent an important element for pollinators and sustainable  
362 pest management in rural landscapes dominated by arable crops, leading to opportunities for an  
363 agroecological transition of farming systems.

364 General considerations for the successful establishment of these ecological infrastructures for both  
365 natural enemies and pollinator insects should rely on: i) the careful selection of flowering plant  
366 species to avoid or mitigate any potential disadvantages, ii) the appropriate management practices,  
367 and iii) the motivation of farmers in the application of sustainable production strategies.

368 Moreover, to render the FA more resilient and effective, it is crucial to carefully consider and limit  
369 the use of pesticides, avoiding any insecticide sprays against pests that do not exceed the economic  
370 thresholds.

371 Although soft wheat cultivation cannot directly benefit from pollinator insects, the use of FA can be  
372 an important tool for promoting beneficial taxa that provide multiple ecosystem services in such  
373 agro-ecosystems. FA may have a remarkable impact on the local availability of nectar and pollen  
374 with positive consequences on the fitness and abundance of butterflies, wild bees, and syrphids on a  
375 landscape scale. However, FA rarely provides nesting sites for bees as well as host plants for  
376 butterfly larvae. Therefore, while FA can represent a first and relatively easy step for improving  
377 insect abundance, their adoption is not enough to guarantee biodiversity increases in the medium-  
378 long term. For this reason, supplementary strategies, such as perennial (semi) natural habitats and  
379 other ecological interventions, should also be applied to improve landscape configuration and  
380 connectivity.

381

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391 The authors declare the following financial interests/personal relationships which may be  
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396

397 **Data availability**

398 The data that support the findings of this study are available on request from the corresponding  
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