

1 **Floral presence and flower identity alter cereal aphid endosymbiont communities on adjacent crops**

2 Sharon E. Zytynska^{1,2*}, Sarah Sturm², Cathy Hawes³, Wolfgang W Weisser², Alison Karley³

3 ¹ *Department of Evolution, Ecology, and Behaviour. Institute of Infection, Veterinary and Ecological Sciences.*

4 *University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK*

5 ² *Technical University of Munich, Terrestrial Ecology Research Group, Department of Life Science Systems,*

6 *School of Life Sciences, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany*

7 ³ *Ecological Sciences Department, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK*

8

9 * corresponding author email: sharon.zytynska@liverpool.ac.uk

10

11 **Abstract**

- 12 1. Floral plantings adjacent to crops fields can recruit populations of natural enemies by providing flower
13 nectar and non-crop prey to increase natural pest regulation. Observed variation in success rates
14 might be due to changes in the unseen community of protective endosymbionts hosted by many
15 herbivorous insects, which can confer resistance to various specialist natural enemies, e.g. parasitoid
16 wasps. Reduced insect control may occur if highly protective symbiont combinations increase in
17 frequency via selection effects, and this is expected to be stronger in lower diversity systems.
- 18 2. We used a large-scale field trial to analyse the bacterial endosymbiont communities hosted by cereal
19 aphids (*Sitobion avenae*) collected along transects into strip plots of barley plants managed by either
20 conventional or integrated (including floral field margins and reduced inputs) methods. In addition, we
21 conducted an outdoor pot experiment to analyse endosymbionts in *S. avenae* aphids collected on
22 barley plants that were either grown alone or alongside one of three flowering plants, across three time
23 points.
- 24 3. In the field, aphids hosted up to four symbionts. The abundance of aphids and parasitoid wasps was
25 reduced towards the middle of all fields while aphid symbiont species richness and diversity decreased
26 into the field in conventional, but not integrated, field-strips. The proportion of aphids hosting different
27 symbiont combinations varied across cropping systems, with distances into the fields, and were
28 correlated with parasitoid wasp abundances.
- 29 4. In the pot experiment, aphids hosted up to six symbionts. Flower presence increased natural enemy
30 abundance and diversity, and decreased aphid abundance. The proportion of aphids hosting different
31 symbiont combinations varied across the flower treatment and time, and were correlated with varying
32 abundances of the different specialist parasitoid wasp species recruited by different flowers.
- 33 5. *Synthesis and applications.* Floral plantings and flower identity can have community-wide impacts on
34 the combinations of bacterial endosymbionts hosted by herbivorous insects. Our work highlights the
35 potential of within-season selection for symbiont-mediated pest resistance to natural enemies with
36 biological control impacts. This could be mitigated through increased recruitment of diverse natural
37 enemies by incorporating functional diversity of floral resources into the environment.

38 **Keywords:** insect pests, pest control, pest management, wildflower, sustainable agriculture, insect–plant
39 interactions, *Hamiltonella*, *Regiella*, *Fukatsuia*

40 Introduction

41 Floral plantings are a common measure to recruit and establish natural enemies for natural pest control in
42 adjacent crops under the framework of conservation biological control. However, inconsistent outcomes of
43 floral plantings have hindered more widespread use (Albrecht *et al.* 2020). While we know a substantial
44 amount about the effects of plant identity and diversity on insect herbivores and natural enemies of herbivores,
45 we still lack understanding of how these interactions mediate, or are mediated by, other trophic groups such as
46 insect-associated microbes, e.g. symbiotic bacteria (Hrcek, McLean & Godfray 2016). This has been
47 particularly studied in plant-sucking insects such as aphids, which are major pests of crop worldwide
48 (Zytynska, Tighiouart & Frago 2021). Since many aphid bacterial endosymbionts can also confer resistance to
49 specialist natural enemies it follows that floral plantings that recruit a low diversity of natural enemies may also
50 select for the optimal protective combination of microbes in the aphid host, with reduced biocontrol
51 effectiveness (Zytynska & Meyer 2019).

52 The diversity of the aphid microbiome is surprisingly low (Sugio *et al.* 2015), with one obligate (primary), and
53 nine common facultative (secondary) bacterial symbionts that have been identified in many different aphid
54 species across the world (Zytynska & Weisser 2016). Despite the low diversity, these bacterial symbionts can
55 have strong effects on aphid survival through providing essential nutrients and conferring resistance to natural
56 enemies (parasitoid wasps and entomopathogenic fungi) or resistance to heat-stress, among other potential
57 benefits (Oliver, Smith & Russell 2014; Guo *et al.* 2017). However, aphids experience fecundity and longevity
58 costs of hosting symbionts, with varying costs associated with multiple hosting of different symbiont species
59 (Zytynska, Tighiouart & Frago 2021). Furthermore, effects are highly aphid and symbiont species (and
60 strain/genotype), specific leading to highly variable outcomes. A recent meta-analysis highlighted this variation
61 and the bias towards information on a few common model aphid species (*Acyrtosiphon pisum*, *Sitobion*
62 *avenae* and *Aphis fabae*) (Zytynska, Tighiouart & Frago 2021). Briefly, across multiple aphid species,
63 endosymbionts *Hamiltonella defensa* and *Regiella insecticola* show strong protective effects against parasitism
64 by parasitoid wasps. Other protective effects were supported by too few studies to be assessed in a meta-
65 analysis, but include resistance to entomopathogenic fungi by *R. insecticola*, *Rickettsia*, *Rickettsiella viridis*,
66 *Spiroplasma* and *Fukatsuia symbiotica*; parasitoid resistance by *Serratia symbiotica*, *F. symbiotica*; and heat-
67 shock resistance by *S. symbiotica*, *H. defensa*, *Rickettsia*, and *F. symbiotica* (reviewed in Guo *et al.* (2017).
68 There is also some evidence that infection with *H. defensa* can alter aphid anti-predator behaviour against
69 generalist predators such as ladybirds (Polin, Simon & Outreman 2014; Humphreys, Ruxton & Karley 2022).
70 Generally, field-collected aphids have been found to host 1-4 symbionts per individual (Zytynska & Weisser

71 2016), leading to high chance of aphids co-hosting multiple symbionts with variable costs and benefits.
72 Furthermore, ineffective vertical transmission from mother to offspring (Rock *et al.* 2018) and variable selection
73 pressures over time (e.g. changing natural enemy abundances, Smith *et al.* (2015)), as well as incompatible
74 symbiont combinations in the field (Oliver, Moran & Hunter 2006) result in a polymorphism of infection within
75 populations even in the same host-plant patch (Russell *et al.* 2013; Smith *et al.* 2015; Zytynska *et al.* 2016).
76 Dynamic aphid-symbiont frequencies in a population will feedback to the interacting natural enemy
77 communities with community-wide consequences and potential impacts on biological pest control efforts
78 (Zytynska & Meyer 2019).

79 The impact of protective bacterial symbionts on plant-insect food webs, the regulation of aphid populations,
80 and predator-prey dynamics is only beginning to be considered (McLean *et al.* 2016; Simon, Biere & Sugio
81 2017; Preedy *et al.* 2020; Carpenter *et al.* 2021; Leclair *et al.* 2021). It was previously shown that as plant
82 species richness increased along a gradient (1-4 species, created as mixed plant communities from a pool of
83 20), so did the number and diversity of aphid symbionts identified in three aphid species (each exclusively
84 feeding on a different host plant) in a long-term grassland experiment (Zytynska *et al.* 2016). One hypothesis is
85 that that this is driven by a diversification in the number of selection pressures on the aphids (reviewed in
86 Zytynska & Meyer 2019), through increased natural enemy diversity; a previous study in the long-term
87 grassland experiment showed that as plant species richness increased so did parasitoid wasp diversity
88 (Petermann *et al.* 2010). Therefore, when natural enemy diversity is low (e.g. in crop monocultures) there may
89 be one optimal combination of symbionts that protects against a single main selection pressure (i.e. a
90 dominant natural enemy). If all surviving aphids host the highly protective symbionts, then the diversity of
91 symbionts in the population will be low (all aphids host similar symbiont communities). In a diverse plant
92 community, with higher natural enemy diversity (including various parasitoid wasp species, entomopathogenic
93 fungi and generalist predators), then there may be no single optimal symbiont combination. In this case,
94 variable costs and benefits of co-hosting multiple symbionts could lead to aphids hosting various combinations
95 of the available symbionts (higher diversity) (Hafer-Hahmann & Vorburger 2021). The idea is that with a
96 greater variety of natural enemies each aphid hosts a different symbiont combination but is not protected
97 against all attackers. Co-hosting symbionts can alter associated fitness costs (Zytynska, Tighiouart & Frago
98 2021), which can reduce aphid population growth rates or transmission of symbionts to the next generation
99 (Rock *et al.* 2018). Additionally, the presence of multiple different symbiont combinations in the aphid
100 population could alter competition among natural enemies (McLean & Godfray 2017), and as these selection
101 pressures alter symbiont infection frequencies over time (Carpenter *et al.* 2021; Smith *et al.* 2021) this can

102 further affect outcomes of hosting different symbiont combinations. Some symbionts may also be present due
103 to non-natural enemy factors (host-plant or temperature mediated selection), leading to a strong context-
104 dependency effect on interaction outcomes (Lemoine, Engl & Kaltenpoth 2020). Here, we aim to determine if
105 different agricultural management practices can alter these selective pressures with potential consequences
106 for natural pest control in crop systems.

107 Herbivore regulation by natural enemies is one natural ecosystem function that is often disrupted in managed
108 landscapes, such as agroecosystems (Matson *et al.* 1997). The extent to which this is also exacerbated by
109 insect protective symbionts is unknown. Many studies have shown the benefits of increasing plant diversity in
110 agroecosystems (via banker plants or wildflower strips; Gurr *et al.* 2016; Tschumi *et al.* 2016; Balzan 2017), for
111 pollinator and natural enemy populations, but often the effect of plant identity on specific plant-insect
112 interactions is overlooked. Flowering plants offer variable sources of nectar, which is useful for adult insects
113 whose offspring are the control agents (e.g. parasitoid wasps, lacewings, syrphids); parasitoid wasps can
114 survive two weeks longer when a nectar source is offered, increasing search and attack rates (Russell 2015).
115 These additional plants can also host populations of non-pest aphids on which to establish a diverse natural
116 enemy population before pest aphids arrive on the crop plants. In a recent paper, we showed that aphid
117 suppression was a result of numerous weaker interactions between different flower, pest, and natural enemy
118 species, rather than a few dominant interactions (Zytynska *et al.* 2021), and here we used the same outdoor
119 pot experiment to analyse the aphid symbiont communities alongside an additional large-scale field
120 experiment.

121 We analysed bacterial symbiont communities hosted by cereal aphids (*Sitobion avenae* L.) and associations
122 among these symbionts within aphids in two barley crop experiments: The first was a large-scale agricultural
123 field experiment (Scotland), which compares long-term impacts of an integrated cropping system relative to
124 standard commercial practice (Hawes *et al.* 2019) and the second an outdoor pot experiment (South Germany)
125 where previous effects of flower identity on aphid control were demonstrated (Zytynska *et al.* 2021). Both
126 experiments manipulated the presence of flowering plants, defined as the flower strip combined with the
127 integrated approach for the field experiment or by the presence of three different flowering plant species for the
128 pot experiment, across multiple barley varieties. The field experiment additionally examined the effect on aphid
129 symbionts along a transect into the field, expecting decreased insect abundances into the fields (Thies &
130 Tschamntke 1999) while the pot experiment examined the effects across time (Smith *et al.* 2015).

131 Our main hypotheses are (a) the presence of floral resources increases natural enemy abundance/diversity,
132 which reduces aphid abundance, and (b) changes in natural enemy abundances and diversity alters the
133 number, diversity and combination of symbionts hosted by aphids through changing selection pressures. We
134 expect differences to be attributed to specific symbiont combinations as individual symbionts are not
135 independent from one-another inside an aphid host (Mathé-Hubert *et al.* 2019); therefore, we examined effects
136 across common symbiont combinations shared across multiple aphids. For the field experiment, only the total
137 number of parasitoid wasps (via counts of aphid mummies, which host developing wasps) or general predators
138 were collected (via insect traps) and thus we test the hypothesis only for parasitoid abundance rather than
139 diversity. For the pot experiment, more comprehensive data was collected allowing for analysis of each type of
140 parasitoid wasp and predators on aphids and their symbionts.

141

142 **Methods**

143 **Large-scale agricultural field experiment**

144 *Study system and experimental design*

145 The CSC platform at Balruddery Farm near Dundee, Scotland (described in Hawes *et al.* (2019)) is a 42-ha
146 contiguous block of six arable fields, based on a six-year rotation of the commonly grown crops in the region.
147 At the start of the first rotation in 2010, each of the six fields were divided in half. Conventional and integrated
148 management treatments were randomly allocated to each half, and (for the barley fields) in each field-half
149 three different barley varieties were planted as plot strips (i.e. 3 barley variety strips for conventional and 3 for
150 integrated). The conventional treatment is the standard commercial management practice typical for the crop
151 in terms of soil cultivation, fertiliser inputs and herbicide applications, while the integrated system aims to
152 maintain yields, enhance biodiversity and soil biophysical quality, reduce non-renewable inputs and minimise
153 losses from the system relative to conventional practice (Hawes *et al.* 2019). The integrated system includes
154 organic amendment, conservation tillage, soil nutrient supply calculations to minimise use of mineral fertiliser,
155 and reduced reliance on crop protection chemicals (IPM strategies, targeted weed control and species rich
156 wildflower margins), Field margins around the integrated treatments were sown with a wildflower mix in 2015
157 (Balruddery Species Rich Meadow Margin, Scotia Seeds, UK containing seeds of 25 flower species).

158 *Sitobion avenae* aphids were collected on 6th July 2016 and 3rd July 2017, at four distances (5, 15, 30, 50 m)
159 from the edge of the field into each barley variety strip (2016: *Cassata*, *Retriever*, *Saffron* and 2017: *Bazooka*,
160 *Infinity*, *Retriever*). Aphids were stored in 70% ethanol and shipped to Technical University of Munich for
161 symbiont analysis. At each sampling location (distance into the field strip), a maximum five aphids (one per

162 colony) were collected from three adjacent infested tillers in every barley strip (variety); aphids reproduce
163 asexually, and females deposit a group of offspring in one patch before moving away, this approach minimises
164 the chance of collecting aphids from the same clonal mother. Total aphid number was also counted for these
165 three tillers. In total, there were four sampling distances for three barley varieties, repeated across the two
166 management systems, and across two years. Additional data were collected on % cover of the crop and weed
167 plants in the sampled areas using a 1 m² quadrat to estimate the projected area of ground covered by each
168 vegetation type in mid-July. Data on parasitoid and generalist natural enemy abundance were obtained from
169 two yellow sticky traps, one placed at the top and one at the bottom of the plant canopy, at each sampling
170 location, in mid-to-late July.

171 *Aphid endosymbiont analysis*

172 DNA was extracted from individual aphids using the salting out method (Sunnucks & Hales 1996) and
173 examined for nine common bacterial symbionts (*Hamiltonella defensa*, *Regiella insecticola*, *Serratia*
174 *symbiotica*, *Rickettsia sp*, *Spiroplasma*, *Fukatsuia symbiotica*, *Rickettsiella viridis*, *Wolbachia*, and
175 *Arsenophonus*) plus the primary symbiont (*Buchnera aphidicola*) as a positive control using PCR-based
176 assays (Table S1) via gel electrophoresis (Zytynska *et al.* 2016). All samples were run alongside negative
177 controls, with a subset repeated to ensure accuracy.

178 *Data analysis*

179 Data were analysed in R (v. 3.6.3) using RStudio (v. 1.2.5033). We analysed the data at two levels: the
180 individual aphid (i.e. how many and which symbionts are hosted by individual aphids) and the local population
181 level (i.e. how many and which symbionts are hosted across all aphids at each sampling location) where up to
182 five aphids were sampled from three tillers per location.

183 We used generalised linear models to analyse the effect of year (2016, 2017), management system
184 (conventional or integrated), distance in the field (5, 15, 30, 50 m), barley variety (Bazooka, Cassata, Infinity,
185 Retriever, Saffron), weed cover and crop cover on overall aphid, parasitoid, and generalist predator abundance
186 (quasipoisson error distribution for count data). Next, we used chi-square analyses to determine significant
187 associations between symbiont species. A linear mixed effects model with year and barley variety as random
188 effects was used to analyse the effect of management system, distance into field, crop cover, aphid
189 abundance and interactions on the number of symbionts hosted by aphids (individual aphid and local
190 population levels) and Shannon diversity of the aphid symbionts (local population level). Models were
191 simplified by removing non-significant terms and minimum adequate models are presented. Further linear

192 models were used to determine influences of management system and distance into the field on crop and
193 weed cover (lmer, using asin transformed data for % cover producing a F-value statistic), and on parasitoid
194 and predator abundances (glmer, using poisson error distribution for count data producing a Chi-square
195 statistic). We analysed the effect of the management system, distance into the field and the abundance of
196 parasitoid wasps (and interactions) on the proportion of aphids hosting different symbiont species (averaged
197 across combinations) or the different symbiont combinations using generalised mixed effects models with
198 binomial error distribution including year as a random effect. Lastly, we used Structural Equation Modelling
199 (SEM) in the R package 'piecewise' (Lefcheck 2016), where responses included the proportion of aphids
200 hosting each different symbiont combination, as well as aphid and parasitoid abundances, and relevant crop
201 traits; predictors included experimental variables of management system and distance into the field. Model fit
202 was evaluated using Fisher's C statistic with the presented model reproducing the data well ($P > 0.05$).

203

204 **Outside pot experiment**

205 *Study system and experimental design*

206 The experiment was set up in Freising, Bavaria, South Germany on a paved surface next to a natural meadow,
207 May-July 2018. We used four spring barley varieties that varied in aphid susceptibility in laboratory trials
208 (Barke, Chevallier, Grace, and Scarlett) (Zytynska *et al.* 2020; Zytynska *et al.* 2021), and three flowering plant
209 species commonly used as companion/intercropping plants with barley [Buckwheat (*Fagopyrum esculentum*),
210 and Red clover (*Trifolium pratense*) from Rühlemann's Kräuter & Duftpflanzen, Germany; and, Faba bean
211 (*Vicia faba cv. Perla*), from Kiepenkerl seeds].

212 We used a factorial randomised block experimental design that was focused on the effect of flower
213 presence/absence and flower identity on aphid and natural enemy population dynamics on the barley (see
214 Zytynska *et al.* 2021). The plants were placed in a grid system (4 x 5 plants per block), with 50 cm between
215 each barley-flower combination, and 1.5 m between blocks. Here, we focus on *Sitobion avenae* aphids
216 collected from barley plants that were grown either alone, or next to *Fagopyrum*, *Trifolium* or *Vicia*. The
217 experiment also had a treatment with all three flower species (mixed) but aphid control was so effective here
218 (Zytynska *et al.* 2021) that insufficient aphids could be collected for symbiont analysis. Seeds were germinated
219 and grown in individual pots (2 litre pots filled with Floragard B Pot Medium-Coarse potting substrate, pH 5.6,
220 NPK 1-0.6-1.2); one plant per pot for barley, *Fagopyrum* and *Vicia* was used while *Trifolium* plants were
221 established from 15-20 sown seeds.

222 All plants were initially grown under an outdoor covered shelter for three weeks and then transferred fully
223 outdoors on 29th May 2018 and placed into individual 1 litre capacity pot trays. For the no flower treatment
224 there was a single barley pot, for the treatments with one flower there was one barley pot and one flower pot.
225 To avoid confounding factors related to 'barrier effects' of the plants, the flowers were placed behind the barley
226 plants, away from the natural meadow (i.e. not between the barley and the meadow). Once per week, a full
227 invertebrate survey was conducted by carefully examining every plant (both barley and flower) and recording
228 the number of all generalist predators, specialist parasitoid wasp adults (if observed), and parasitoid mummies
229 (hardened shell after an aphid has been parasitized, using form and colour to differentiate between mummies
230 formed by *Aphidius sp.*, *Aphelinus sp.* or *Praon sp.*). Aphids were identified to species, and winged/unwinged
231 aphids were counted separately. Aphid and parasitoid DNA was amplified from a fragment of the insect CO1
232 gene using universal primers LCO1490 and HCO2198, to confirm species identification (Table S1). The
233 dominant parasitoid species were identified as *Praon volucre* (pink mummies, with cocoon under the body) and
234 *Aphidius rhopalosiphii* (copper-coloured mummies). No *Aphelinus sp.* parasitoid DNA was detected in any
235 collected aphid samples, but black mummies (representative of this genus) were observed during the
236 experiment.

237 *Aphid endosymbiont analysis*

238 Aphids were collected for endosymbiont analysis on 23rd June (late June), 11th July (early July) and 25th July
239 (late July). One aphid per colony on every plant was collected and stored in 70% ethanol. Only *Sitobion*
240 *avenae* aphids were present in sufficient numbers for symbiont analysis. DNA extraction from whole aphids,
241 and subsequent endosymbiont analyses using PCR-based assays (Table S1) were performed as for the large-
242 scale experiment.

243 *Data analysis*

244 Data were analysed in R (v. 3.4.3) using RStudio (v. 1.0.143). We first analysed the effect of the presence and
245 identity of the flowers, date of collection, as well as barley variety on overall *Sitobion avenae* aphid, parasitoid
246 and generalist predator abundance using generalised linear models with quasipoisson error distributions. We
247 also calculated natural enemy diversity using Shannon diversity and analysed the same factors as for
248 abundance data but using a linear model with normal error distribution. Similarly, for the field experiment, we
249 used Chi-square analyses to identify non-random associations among the symbiont species, important to
250 interpret effects of symbiont combination as opposed to single individual symbiont species. Linear mixed
251 effects models with experimental block and row as random factors were used to analyse the effect of date of
252 collection, aphid abundance, barley variety, and flower treatment (and interactions) on the number of

253 symbionts hosted by aphids (symbiont richness at the individual aphid and plant level, i.e. collected from the
254 same plant) and on the Shannon diversity of symbionts at the plant level. We analysed the effect of symbiont
255 species/combinations, flower treatment, and the natural enemy Shannon diversity and abundances of the
256 different species (and interactions) on the proportion of aphids hosting different symbiont species or
257 combinations (hosting or not hosting the symbiont) using generalised linear models with binomial error
258 distribution. We also used Structural Equation Modelling (SEM) in the R package 'piecewise' (Lefcheck 2016),
259 using linear mixed effect models with block and row as random effects. Responses included the proportion of
260 aphids hosting each different symbiont combination, as well as abundance of aphids (winged and unwinged
261 separated), predator (all species grouped together), and parasitoid (separated by genus); the number of non-
262 pest aphids on the flowers was initially included but removed as it was non-significant in all models. Each
263 aphid-symbiont combination was analysed using the number of aphids and natural enemies observed at the
264 time point of collection, to account for differences in time of collection. Predictors included each flower
265 resource as an individual variable to determine flower identity effects. Model fit was evaluated using Fisher's C
266 statistic with the presented model reproducing the data well ($P > 0.05$).

267

268 **Results**

269 **Effects of integrated management practices on aphid endosymbionts (CSC field experiment)**

270 *Sitobion avenae* aphids (N=219) were collected from 41 sampling areas across the fields (termed local
271 populations, 1-10 aphids per population, mean = 5.34 ± 0.29 SE, median/mode = 6). This included 144 aphids
272 (71 from conventional, 73 from integrated) from 24 local populations in 2016, and 75 aphids (11 from
273 conventional, 64 from integrated) from 17 local populations in 2017 from field strips that were managed
274 according to either a standard conventional cropping practice in one field half or an integrated management
275 system in the other field half (Hawes *et al.* 2019). We observed no significant effect of field management on
276 total aphid abundance ($F_{1,36}=1.46$, $P=0.235$; Fig. 1a) despite collecting fewer aphids from conventional fields in
277 2017 (but not 2016) – suggesting there were fewer but larger colonies in this year compared to integrated
278 fields with more but smaller colonies (allowing for a greater number of aphids to be collected). We also
279 observed no effect of management system on parasitoid wasp abundance ($F_{1,36}=0.71$, $P=0.403$; Fig. 1b), but
280 insect abundances decreased into the field-halves (aphids: $F_{1,36}=6.17$, $P=0.018$, Fig. 1a; parasitoids:
281 $F_{1,36}=8.08$, $P=0.007$; Fig. 1b).

282 We observed no effect of management system or distance in the field for generalist predators (system:
283 $F_{1,36}=0.12$, $P=0.730$; distance: $F_{1,36}=0.02$, $P=0.895$; Fig. 1c). Hence, our overall hypothesis that floral resources

284 increase natural enemies and reduce aphids was not supported in this field system. However, across all field
285 locations when there were more parasitoid wasps we observed fewer aphids ($X^2_1=10.59$, $P=0.001$), but the
286 opposite with generalist predators where more predators were found in areas with higher aphid numbers
287 ($X^2_1=6.34$, $df=1$, $P=0.012$). Lastly, integrated field-halves had reduced crop cover ($F_{1,36}=4.95$, $P=0.032$) with
288 correlated increases in weed cover ($r= -0.65$, $n=41$, $P<0.001$). Generalist predator abundance was positively
289 associated with higher crop ($X^2_1=13.81$, $P<0.001$) and weed cover ($X^2_1=3.84$, $P=0.05$), with no significant
290 effects on the abundance of specialist parasitoid wasps.

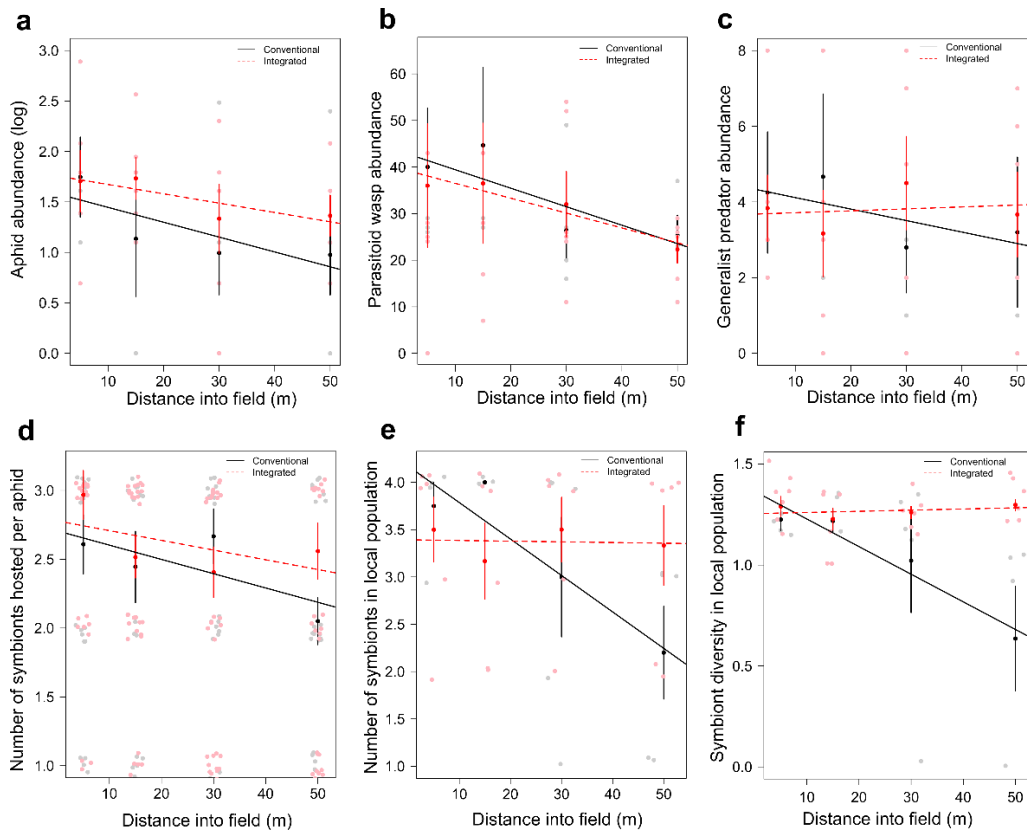
291 We identified four bacterial endosymbionts with high prevalence within the sampled aphids: *Hamiltonella*
292 *defensa* (Hd) hosted by 63.9% of aphids; *Regiella insecticola* (Ri) hosted by 67.0% of aphids; *Serratia*
293 *symbiotica* (Ss) hosted by 59.8% of aphids; and *Fukatsuia symbiotica* (Fs) hosted by 63.5% of aphids. Fifteen
294 different combinations of these symbionts were identified in the aphids, with 20.1% of aphids (44/219) hosting
295 all four. The most frequent symbiont combination included the three symbionts without *Serratia* (HdRiFs:
296 55/219, 25.1% of aphids), followed by aphids hosting all four symbionts and then those aphids singly hosting
297 *Serratia* (Ss: 34/219, 15.5% of aphids). The average number of symbionts per aphid was 2.5 ± 0.1 , while the
298 average number of symbionts per sampling location (local population) was 3.3 ± 0.2 . Fewer aphids (than
299 expected at random) co-hosted any symbiont with *Serratia*, while more aphids co-hosted combinations of
300 *Hamiltonella-Regiella* ($X^2_1=36.0$, $df=1$, $P<0.001$), *Hamiltonella-Fukatsuia* ($X^2_1=34.6$, $df=1$, $P<0.001$) and
301 *Regiella-Fukatsuia* ($X^2_1=50.1$, $df=1$, $P<0.001$).

302 Aphids collected in integrated managed field strips co-hosted a slightly, but significantly higher number of
303 symbionts (2.60 ± 0.09) than those in the conventional field-halves (2.45 ± 0.11), and symbiont richness per
304 aphid decreased with distance into both field types by 18.6 % (Distance into field: Table 1, Fig. 1d). At the local
305 population level, symbiont richness and diversity among aphids were also higher in the integrated fields ($3.38 \pm$
306 0.18) than conventional (3.12 ± 0.28 ; Table 1). Symbiont species richness and diversity decreased into the
307 field but only under conventional management, while remaining relatively constant into the field under
308 integrated management (management system x distance into field, Table 1, Fig. 1e,f). The number of
309 symbionts hosted by individual aphids strongly increased with increasing crop cover (Table 1), despite no
310 effects of crop cover on aphid abundance ($F_{1,28}=0.01$, $P=0.927$). With a crop cover of 20-60%, individual
311 aphids hosted 1.85 ± 0.20 symbionts compared to 3.13 ± 0.12 symbionts with high crop cover of 65-95%.
312 There was no effect of crop cover on the richness and diversity of symbionts at the local population level
313 (Table 1).

314 **Table 1. Effects of field management and distance into field on aphid symbiont communities**

	Symbiont richness						Symbiont diversity		
	Individual aphid level			Local population level			Local population level		
	df	F	P	df	F	P	df	F	P
Crop cover	1,212	14.22	<0.001	1,34	0.20	0.730	1,34	3.69	0.066
Aphid abundance	1,212	0.16	0.686	1,34	1.63	0.210	1,34	0.29	0.593
Parasitoid abundance	1,212	0.29	0.590	1,34	0.04	0.852	1,34	1.19	0.282
Management system	1,212	8.94	0.003	1,34	0.74	0.396	1,34	0.01	0.986
Distance into field	1,212	9.33	0.003	1,34	5.19	0.029	1,34	7.83	0.009
System x Distance	1,212	2.42	0.121	1,34	7.59	0.009	1,34	9.70	0.004

315 *Linear mixed effects models (lmer) with barley variety and year as random effects. Distance into the field is*
 316 *fitted as a continuous variable.*
 317



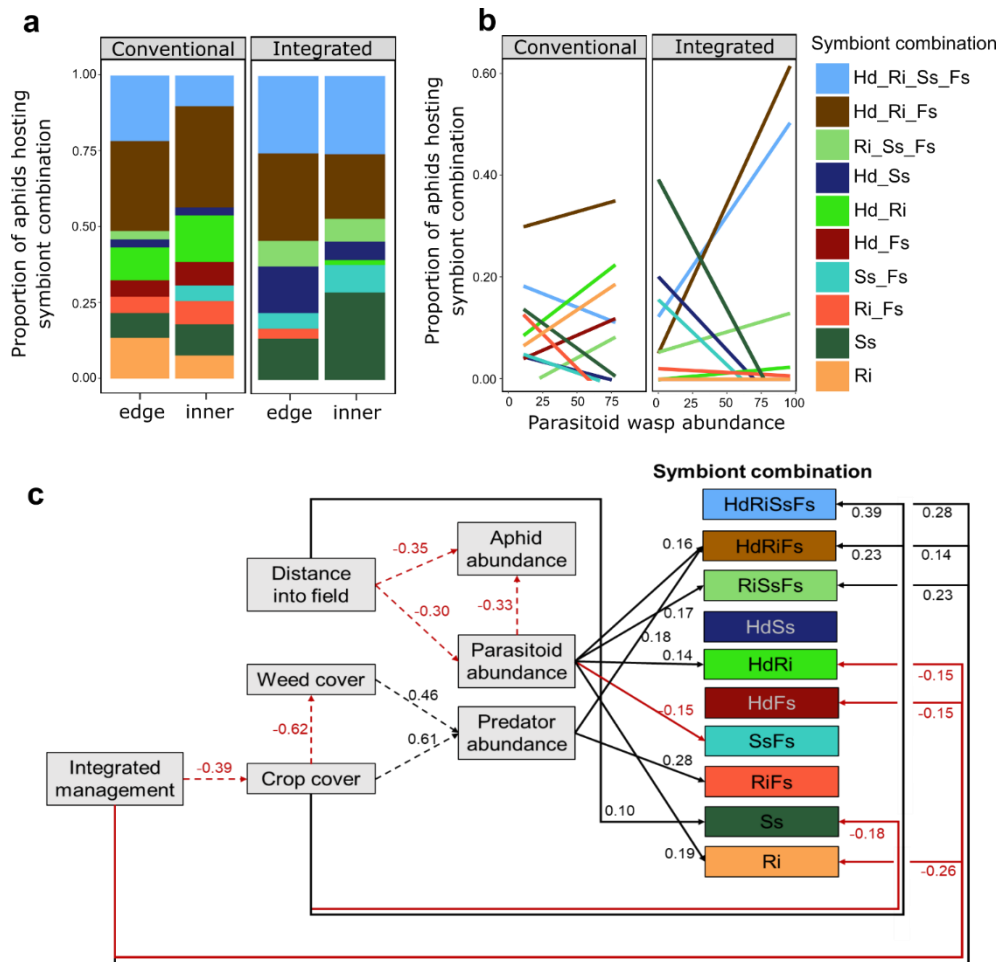
318 **Figure 1. Effects of field management and distance into field on (a) aphid abundance (log transformed),**
 319 **(b) parasitoid abundance, (c) generalist predator abundance, (d) the number of symbionts hosted by individual**
 320 **aphids, (e) the number of symbionts within the local population (sampling area), and (f) the Shannon diversity of**
 321 **symbionts within the local population. Error bars represent ± 1 SE.**
 322
 323
 324
 325

326 For further in-depth analysis of the endosymbiont combinations in aphids, we focus on the ten combinations
 327 that are hosted by a minimum of 5% of aphids within one management system (N=201 out of 219 aphids). The
 328 proportion of aphids hosting the different symbiont species varied across the management type ($X^2_1=43.03$,
 329 $P<0.001$, Fig. S1a) but not with the distance into the field ($X^2_3=2.19$, $P=0.533$, Fig. S1a). Aphids collected from
 330 conventional field-halves were more likely to host *Regiella*, while those from integrated field-halves were more
 331 likely to host *Serratia*. This is reflected in the common combinations of symbionts hosted by aphids
 332 (combination x management system: $X^2_9=26.23$, $P=0.002$; combination x distance: $X^2_9=8.41$, $P=0.493$) (Fig.
 2a). Here, it can also be seen that aphids collected from conventional field-halves host (on average)

333 combinations with fewer symbionts than aphids from integrated ones, with more aphids hosting 1-2 symbionts
 334 in the inner part of conventional fields (Fig. 2a).

335 The abundance of parasitoid wasps in the local sampling area influenced the number of aphids hosting the
 336 different symbiont species ($X^2_3=50.87$, $P<0.001$; Fig. S1b) or combinations ($X^2_9=48.84$, $P<0.001$) (Fig. 2b).

337 This was also dependent on the management system ($X^2_2=20.77$, $P<0.001$; Fig. 2b) but not the distance into
 338 the field, perhaps due to the mobility of the parasitoids. Using structural equation modelling (Fig 2c), we show
 339 that there are direct positive and negative effects of integrated management as well as parasitoid and predator
 340 abundances on the number of aphids hosting specific symbiont combinations. Indirect effects via the distance
 341 into the field, and effects of management on crop and weed cover further mediate effects of natural enemies
 342 on the number of aphids hosting the different symbiont combinations. The level of protection provided by these
 343 different symbiont combinations against natural enemies remains to be tested.

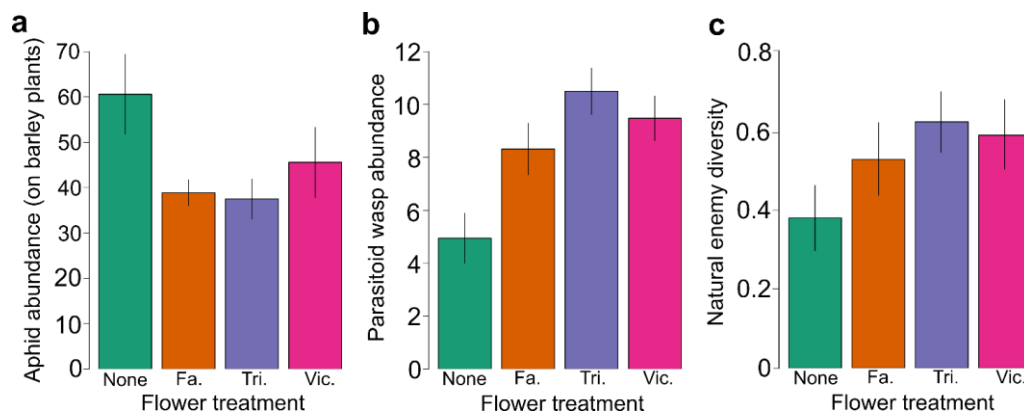


344
 345 **Figure 2. Field experiment: effects of field variables on the aphid symbiont community.** The proportion
 346 of aphids hosting the ten most common most common combinations of symbionts within integrated and
 347 conventionally managed fields across (a) distance into the field (edge, 5-15m or inner, 30-50m), and (b)
 348 parasitoid wasp abundance, highlighting variation due to symbiont combinations as opposed to overall
 349 symbiont richness or diversity (Table 1). Further explored in (c) via structural equation modelling showing
 350 significant links between variables and symbiont combinations. Dashed lines show analysis at the level of
 351 sampling location, with solid lines showing results at the individual aphid level. *Serratia symbiotica* (Ss),
 352 *Rickettsia* (Rk), *Wolbachia* (W), *Regiella insecticola* (Ri), *Hamiltonella defensa* (Hd) and *Spiroplasma* (Sp).

353 **Flower identity effects on aphid symbionts (outdoor pot experiment)**

354 **Outside pot experiment**

355 Aphid abundance was reduced on barley plants by 30 % when grown next to a flower ($F_{1,118}=10.06$, $P=0.002$),
356 with fewest aphids on barley grown next to *Fagopyrum* or *Trifolium* ($F_{2,93}=3.92$, $P=0.024$; Fig 3a). Parasitoid
357 wasp abundance varied strongly with flower treatment and identity ($F_{3,116}=5.76$, $P=0.001$) and was lowest on
358 barley next to no flower while highest on barley next to *Trifolium* (120% higher, Fig. 3b), with effects on overall
359 natural enemy diversity incorporating parasitoid wasp species as well as generalist predators such as ladybird
360 and lacewing larvae primarily driven by the presence of flowers more than the identity ($F_{1,94}=3.78$, $P=0.055$,
361 Fig. 3c). The abundance of *S. avenae* aphids was higher on barley varieties Grace and Scarlett than on Barke
362 or Chevallier ($F_{3,110}=3.33$, $P=0.022$), while there were no differences across barley varieties in parasitoid
363 abundances ($F_{3,91}=0.85$, $P=0.467$) or natural enemy diversity ($F_{3,91}=1.43$, $P=0.239$).



364 **Figure 3. Outdoor pot experiment: effects of flower treatment** on (a) overall aphid abundance, (b)
365 parasitoid wasp abundance, (c) natural enemy diversity. None: no flower, Fa: *Fagopyrum esculentum*, Tri:
366 *Trifolium pratense*, Vic: *Vicia faba*. Error bars represent ± 1 SE.

367

368 For symbiont analysis, 127 *Sitobion avenae* aphids were collected from barley plants grown alone (N=34), next
369 to *Fagopyrum* (N=33), *Trifolium* (N=23) or *Vicia* (N=37) plants, with 29-36 aphids collected from each barley
370 variety across flower treatments. Of these aphids, we collected 63 in late June, 43 in early July and 21 in late
371 July. We identified six bacterial symbionts within the sampled aphids: *Serratia symbiotica* (Ss) was hosted by
372 all sampled aphids (100%), *Rickettsia* (Rk) and *Wolbachia* (W) each were hosted by 77/127 (60.6%) aphids,
373 *Regiella insecticola* (Ri) and *Hamiltonella defensa* (Hd) were hosted by 72/127 (56.7%) aphids while
374 *Spiroplasma* (Sp) was hosted by 54/127 (42.5%) aphids. Thus, all aphids hosted at least one symbiont and 8
375 (6.3 %) aphids hosted all 6 symbionts. There were 29 different combinations of symbionts observed across all
376 aphids, however only eight combinations were each hosted by at least 5% of the population and these are the
377 focus for the analyses (N=84 aphids of 127 total aphids with symbionts). The remaining 21 less abundant
378 combinations had an average infection frequency of 2.0 ± 0.29 %. The most frequent symbiont combinations

379 were the 4-symbiont combination of SsRkHdW (10.2%) and RiSsRkW (8.7%). There was a positive
 380 association between frequencies of symbionts *Wolbachia* and *Regiella* ($X^2_1=5.41$, $P=0.020$), and between
 381 *Wolbachia* and *Rickettsia* ($X^2_1=24.50$, $P<0.001$), while we detected a negative association between *Regiella*
 382 and *Hamiltonella* ($X^2_1=21.46$, $P<0.001$).

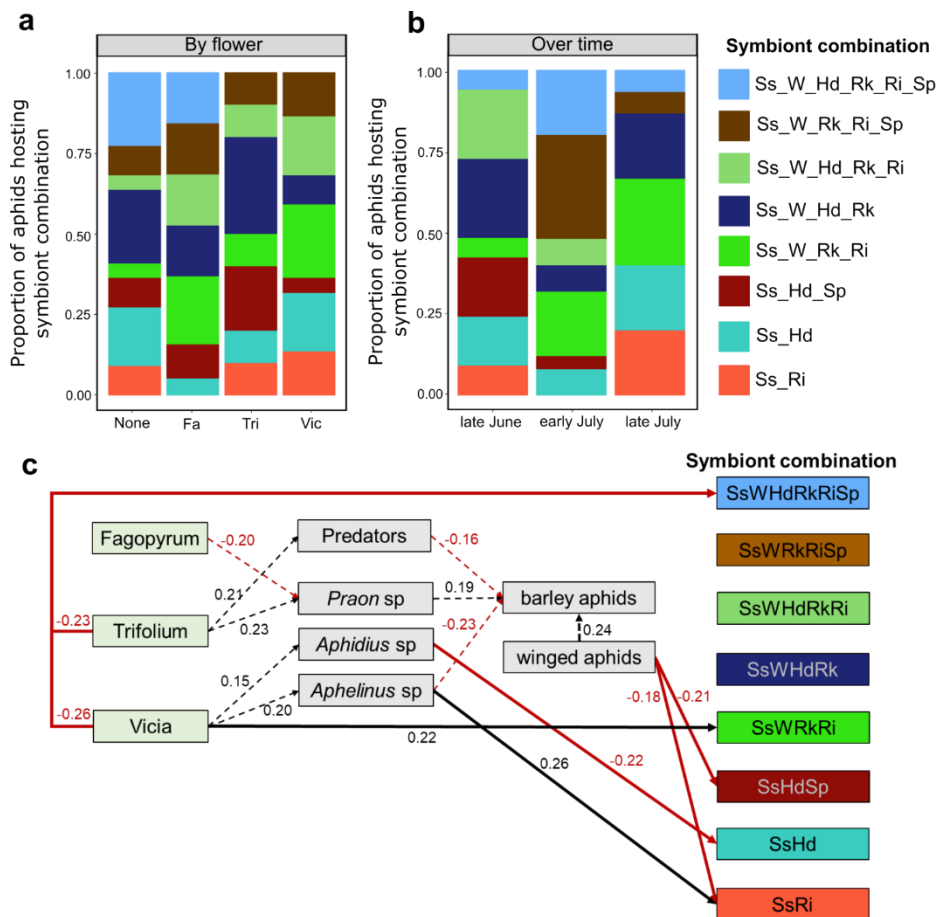
383 The number of symbionts co-hosted by individual aphids, and the number and diversity across all aphids
 384 collected from the same plant (local population level) was strongly influenced by the date of collection and the
 385 overall abundance of aphids on each plant (Table 2). The number of symbionts hosted by aphids increased
 386 from late June (3.56 ± 0.14) to early July (4.26 ± 0.17), and then dropped again towards the end of July
 387 (3.43 ± 0.17). Flower identity influenced the number of symbionts hosted by individual aphids and at the
 388 population level (Table 2), with aphids hosting fewer symbionts when collected from barley grown next to
 389 Trifolium or Vicia. At the population level, barley variety was not a significant main effect for symbiont species
 390 richness or diversity. Nevertheless, the effect of the flower treatment was dependent on the barley variety
 391 driven by a strong decrease in the number of symbionts hosted by aphids on *Grace* barley next to Trifolium.

392 **Table 2. Effects of flower presence and identity on aphid symbiont communities**

	Symbiont richness						Symbiont diversity		
	Individual aphid level			Plant level			Plant level		
	df	F	P	df	F	P	df	F	P
Date of collection	2,107	7.27	<0.001	2,56	8.91	<0.001	2,56	6.79	0.002
Aphid abundance	1,107	4.04	0.047	1,56	5.12	0.028	1,56	4.79	0.038
Nat. enemy diversity	1,107	0.13	0.717	1,56	2.57	0.114	1,56	4.24	0.044
Barley variety	3,107	1.30	0.278	3,56	1.83	0.152	3,65	2.44	0.074
Flower PA	1,107	0.72	0.397	1,56	0.11	0.741	1,56	0.03	0.873
Flower identity	3,107	3.35	0.039	3,56	3.71	0.031	1,56	2.68	0.078
Barley x Flower id	9,107	0.87	0.551	9,56	2.25	0.032	3,56	2.25	0.031

393 *Linear mixed effects models (block and row as random), aphid number is by date of collection. No significant*
 394 *interactions with date of collection were observed.*

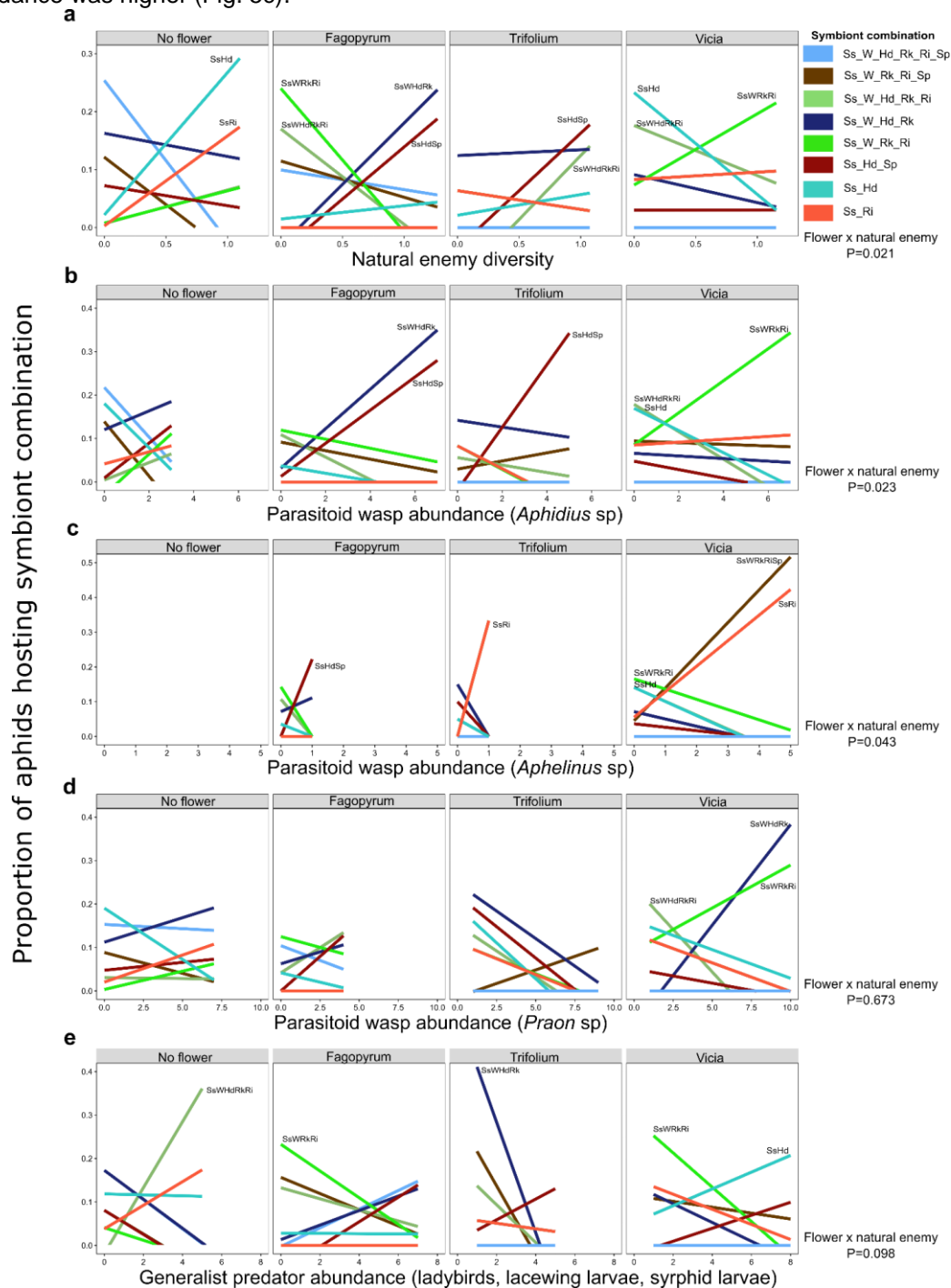
395
 396 Flower identity strongly affected the symbiont combinations hosted by cereal aphids on the adjacent barley
 397 plants. The proportion of aphids hosting each symbiont species ($X^2_5=25.37$, $P=0.045$; Fig. S2) and combination
 398 of symbionts ($X^2_7=42.43$, $P=0.004$; Fig. 4a) differed among the flower treatments. Aphids collected next to
 399 Trifolium and Vicia (the legumes) did not host the 6-symbiont combination of symbionts, and no aphids hosting
 400 the 2-symbiont combination of *Regiella-Serratia* were collected in the Fagopyrum treatment. The date of
 401 collection altered the frequency of aphids hosting the different symbiont combinations ($X^2_2=65.30$, $P<0.001$;
 402 Fig. 4b). Of note, the six-symbiont combination plus the 5-symbiont combination without *Hamiltonella* was
 403 hosted by half of all aphids collected in early July (peak aphid and natural enemy abundances), with a strong
 404 decrease in these abundances by late July.



405 **Figure 4. Outdoor pot experiment: the effect of flower treatment and time on aphid symbiont**
 406 **communities.** The proportion of aphids hosting the eight most common symbiont combinations (a) across
 407 flower treatments, and (b) over time, highlighting variation on specific combinations of symbionts as well as on
 408 overall richness and diversity effects (Table 1). Further explored in (c) via structural equation modelling
 409 showing significant links between variables and symbiont combinations, using data combined across all dates
 410 but incorporating appropriate abundance data relating to aphid collection date (see methods). Dashed lines
 411 show analysis at the level of plant, with solid lines showing results at the individual aphid level. *Serratia*
 412 *symbiotica* (Ss), *Rickettsia* (Rk), *Wolbachia* (W), *Regiella insecticola* (Ri), *Hamiltonella defensa* (Hd) and
 413 *Spiroplasma* (Sp).
 414

415 Lastly, we found that the number of aphids hosting the dominant symbiont combinations varied dependent on
 416 the flower treatment and the natural enemy community recruited by the different flowers (Fig. 4c). Using
 417 structural equation modelling, we were able to highlight direct and indirect effects of flower identity on the
 418 number of aphids hosting the various symbiont combinations. For example, *Vicia* plants led to an increase in
 419 *Aphidius sp.* parasitoid wasps that were negatively associated with aphids hosting *Serratia-Hamiltonella*
 420 symbionts, and an increase in *Aphidius sp.* parasitoids that were positively associated with aphids hosting
 421 *Serratia-Regiella* symbionts (Fig. 4c). We found that natural enemy diversity did not correlate with overall
 422 symbiont diversity, except in the *Trifolium* treatments (Fig S3), but rather via abundances of specific individual
 423 symbiont combinations (Fig. 5). By analysing correlations between natural enemy diversity and the proportion
 424 of aphids hosting the different common symbiont combinations ($X^2_{7}=36.13$, $P=0.021$; Fig. 5a) we identified
 425 those symbiont combinations most associated with changes in natural enemy diversity and abundance of each
 426 natural enemy group (Fig. 5b-e). While the level of protection provided by these different symbiont

427 combinations against natural enemies remains to be tested, we can highlight some interactions worthy of
 428 future focus: a consistent increase in aphids hosting symbiont combination *Serratia-Hamiltonella-Spiroplasma*
 429 (SsHdSp) was seen with increasing *Aphidius* sp. parasitoid wasp abundance while those hosting SsHd
 430 decreased (Fig. 5b); *Aphelinus* sp. parasitoids were not present on barley plants next to no flower, but for
 431 those next to Trifolium or Vicia more aphids hosting *Regiella-Serratia* (RiSs) were collected when parasitoid
 432 abundance was higher (Fig. 5c).



433 **Figure 5. Outdoor pot experiment: summary of associations between natural enemy abundance and**
 434 **aphid symbiont communities across the flower treatments.** The proportion of aphids hosting the eight
 435 most common symbiont combinations across (a) natural enemy Shannon diversity, and abundance of (b)
 436 *Aphidius* sp. (c) *Aphelinus* sp. (d) *Praon* sp. parasitoid wasps and (e) generalist predators. Statistical
 437 significance of interaction shown to the right. *Serratia symbiotica* (Ss), *Rickettsia* (Rk), *Wolbachia* (W),
 438 *Regiella insecticola* (Ri), *Hamiltonella defensa* (Hd) and *Spiroplasma* (Sp).

439 Discussion

440 The outdoor pot experiment showed a clear reduction in aphids on barley plants in the presence of flowers,
441 with correlated increases in natural enemy abundance and diversity; the mixed flower treatment suppressed
442 aphids so strongly that insufficient aphids could be collected for endosymbiont analyses (further explored in
443 Zytynska *et al.* (2021)). Flower identity affected the type of natural enemy recruited (species of parasitoid wasp
444 or combined generalist predators) and abundances of these correlated with the proportion of aphids hosting
445 different endosymbiont communities. Variation across time further demonstrated the importance of temporal
446 changes in aphid and natural enemy abundances on endosymbiont communities, also shown in pea aphid
447 systems by Smith *et al.* (2015) and to a limited extent by Leclair *et al.* (2021). The large-scale field experiment
448 comparing aphids collected from field plot strips grown under an integrated or conventional management
449 system (Hawes *et al.* 2019) did not result in reduced aphid or increased natural enemy abundances in
450 integrated-managed fields. However, in accordance with the pot experiment we again identified a range of
451 associations between natural enemy abundances and the proportion of aphids hosting specific endosymbiont
452 combinations. There were further links identified between integrated management and crop cover on aphid
453 endosymbiont communities independent of natural enemy variables, indicating additional effects beyond those
454 studied here. Overall, we observed aphids co-hosting a greater number of endosymbionts in integrated field
455 strips, while in the pot experiment this effect was dependent on the natural enemy abundance and driven by
456 effects with *Trifolium* plants. This highlights the context-dependency aphid-endosymbiont-natural enemy
457 effects, but suggests management changes could have immediate effects on the surrounding ecological
458 community with potential within-season impacts for pest control of cereal aphids.

459 In the field experiment, aphids collected in the integrated field-strips hosted higher numbers of endosymbionts
460 (3- and 4-species combinations). While our study did not empirically test for symbiont-induced costs or the
461 protective benefits of hosting these symbionts, we infer that correlations with parasitoid abundances indicate
462 protective effects: aphids surviving in areas of higher parasitism are more likely to host protective symbionts.
463 The costs of co-hosting multiple symbionts also remain to be tested for these specific aphids and symbionts,
464 but since aphid abundances did not differ between conventional and integrated systems this may indirectly
465 indicate increased hosting costs. Previous work, predominantly on other aphid species, has shown that
466 parasitoid resistance can be conferred by all the symbionts we identified (reviewed by Guo *et al.* 2017). In
467 *Sitobion avenae* aphids experimental work with endosymbionts has shown *R. insecticola* to reduce parasitoid
468 emergence rates (Luo *et al.* 2020) while infection with *H. defensa* had no protective effect (Lukasik *et al.* 2013;
469 Zepeda-Paulo, Villegas & Lavandero 2017; Li *et al.* 2018). Effects of the other endosymbionts has not been

470 empirically tested in *S. avenae* sufficiently to make conclusions, but our results suggest that examining
471 variation between *Serratia symbiotica* and *R. insecticola* would be an informed starting point. Future work
472 should also focus on naturally occurring combinations of symbionts as opposed to single infections since these
473 drive the responses we identified rather than individual effects of one symbiont species.

474 In the pot experiment, aphid abundance was reduced and natural enemy diversity/abundance increased on
475 barley plants grown next to a flower through effects on non-pest aphids on the flowers before aphid
476 colonisation on the adjacent barley (also see Zytynska *et al.* 2021). Thus, our experiment confirms effects of
477 wildflower strips seen in small scale experiments (Tschumi *et al.* 2016; Balzan 2017; Albrecht *et al.* 2020), and
478 indicates a need for more in-field (close-range) floral resources to maximise effects; for example, via
479 intercropping or in-field floral rows. We observed significant variation in the symbionts hosted by aphids due to
480 flower identity as well as presence, and these correlated with abundances of the different natural enemies
481 recruited. These correlations can inform future studies to identify specific costs and benefits, which will allow a
482 greater understanding of how this trade-off differs among symbiont combinations to alter aphid population
483 dynamics. Of particular interest are the correlations of multi-symbiont combinations SsWRkRi, SsHdSp and
484 SsWHdRk with *Aphidius* parasitoids and SsRi with *Aphelinus* parasitoids, again highlighting the potential
485 importance of *R. insecticola* (Ri) in these aphids (Luo *et al.* 2020). In our previous study (Zytynska *et al.* 2021)
486 we highlighted interactions among the different natural enemies within flower treatment and these could have
487 impacts also for the aphid endosymbiont community. Parasitoid wasps avoid attacking aphids that they detect
488 to have been previously attacked, with this effect lasting up to two days (Outreman *et al.* 2001). This will
489 increase the survival chance of aphids resistant to the original parasitoid but perhaps not the second attacker,
490 especially if it is a different species. In contrast, generalist predators readily consume parasitized aphids before
491 mummification (Meisner *et al.* 2011), with some evidence that *R. insecticola* symbionts may also increase
492 predation risk of aphids by ladybirds (Ramírez-Cáceres *et al.* 2019). Predation of parasitised aphids removes
493 the parasitoid from the next generation via intraguild predation, thus the effect on biocontrol will be dependent
494 on the local aphid, symbiont, and natural enemy community composition. The rare but ecologically-important
495 potential of horizontal transmission of symbionts among aphids via natural enemies (Oliver *et al.* 2010; Gehrler
496 & Vorburger 2012; Zytynska & Venturino 2018) may further explain the ability of aphid populations to respond
497 rapidly to changing natural enemy abundances. Overall, this leads to complex community dynamics and
498 diverse selection pressures acting on the symbiont communities, particularly where there is high natural enemy
499 diversity; any effect of aphid or parasitoid genotype (not studied here) can further influence outcomes. We also
500 observed effects of the number of winged aphids on the proportion of unwinged aphids hosting 2- and 3-

501 symbiont combinations. Winged aphids are long-distance dispersal morphs and could be important if they
502 bring in novel symbiont combinations, assuming reproduction within the crop, but few studies have so far
503 assessed symbionts in winged aphids (but see Smith *et al.* (2021)). Identifying source populations in the wider
504 ecological landscape, e.g. from surrounding cereal fields, will help us understand community dynamics and
505 potential effects on local biological control efforts.

506 Our hypothesis was that a low diversity of natural enemies favours selection for a combination of symbionts
507 that provides the highest level of protection (Oliver *et al.* 2008) and subsequent extinction of the natural enemy
508 (Sanders *et al.* 2016; Vorburger 2018). From our data, a dominance of *Aphelinus* parasitoids might select for
509 aphids hosting *Serratia-Regiella*, while a dominance of *Aphidius* parasitoids might select for those hosting
510 *Serratia-Hamiltonella-Spiroplasma*. In a diverse system (e.g. with floral resources increasing natural enemy
511 diversity), there will be a mixture of these natural enemies leading to a mixture of symbiont combinations.
512 Variable effects of different natural enemies and interactions among them is key to disrupting selection for
513 symbiont combinations with high protective effects but low fecundity costs (Zytynska & Meyer 2019). We
514 showed this does not necessarily translate to increased diversity of aphid symbionts in a local population but
515 rather that it is specific to the symbiont combinations that are hosted and the interactions experienced by the
516 population. When every interaction differs for the various natural enemy species this can contribute to natural
517 regulation of pest populations (e.g. Preedy *et al.* 2020), by naturally diversifying biological control strategies for
518 crop protection (Pimentel 1991).

519 In conclusion, we show that floral plantings, and flower identity, can have community-wide effects on the
520 diversity of natural enemies of herbivores, aphid populations, and the bacterial endosymbionts hosted by the
521 aphids. Management of agricultural systems via conservation biological control and regenerative approaches
522 requires ecological knowledge to predict how these might impact other parts of the system to optimise yield
523 outputs. Unravelling what drives herbivore population regulation enables us to understand the important role of
524 complex multi-species interactions and highlights the processes we should aim to promote to enhance natural
525 pest regulation. Designing tailored floral plantings (e.g. Tschumi *et al.* 2016) to contain sufficient functional
526 diversity to recruit and establish an abundant and diverse community of natural enemies (via nectar and non-
527 pest prey) is key to increasing their impact for pest control on neighbouring crops. A greater diversity of natural
528 enemies is likely to reduce in-field selection pressures favouring protective endosymbiont communities of pest
529 insects, which might otherwise undermine natural pest regulation, and suggests a mechanism by which
530 diversity can be utilised for internal system regulation, reducing reliance on agrochemical inputs for pest
531 control.

532 Author contributions

533 SEZ, AK, CH designed the study and collected the data. SS performed the molecular analyses to identify the
534 symbionts. SEZ analysed the data, WWW supported the interpretation of results. SEZ wrote the manuscript
535 with comments from all authors.

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