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Additive effects of predator diversity on pest control caused by few interactions among predator species

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Abstract. 1. Studies of the impact of predator diversity on biological pest control have shown idiosyncratic results. This is often assumed to be as a result of differences among systems in the importance of predator–predator interactions such as facilitation and intraguild predation. The frequency of such interactions may be altered by prey availability and structural complexity. A direct assessment of interactions among predators is needed for a better understanding of the mechanisms affecting prey abundance by complex predator communities.

2. In a field cage experiment, the effect of increased predator diversity (single species *vs.* three-species assemblage) and the presence of weeds (providing structural complexity) on the biological control of cereal aphids were tested and the mechanisms involved were investigated using molecular gut content analysis.

3. The impact of the three-predator species assemblages of aphid populations was found to be similar to those of the single-predator species treatments, and the presence or absence of weeds did not alter the patterns observed. This suggests that both predator facilitation and intraguild predation were absent or weak in this system, or that these interactions had counteracting effects on prey suppression. Molecular gut content analysis of predators provided little evidence for the latter hypothesis: predator facilitation was not detected and intraguild predation occurred at a low frequency.

4. The present study suggests additive effects of predators and, therefore, that predator diversity *per se* neither strengthens nor weakens the biological control of aphids in this system.

Key words. Biocontrol, *Chrysoperla carnea*, *Coccinella septempunctata*, field cage experiment, molecular gut content analysis, multiplex PCR, *Pterostichus melanarius*, trophic interactions.

Introduction

The relationship between predator diversity and the service of biological control of agricultural pests has received much interest in recent years (e.g. Losey & Denno, 1998; Wilby *et al.*, 2005; Straub & Snyder, 2008). In their meta-analysis, Letourneau *et al.* (2009) showed that a high diversity of natural enemies mostly enhances overall prey suppression. The positive effect of predator diversity was, however, more strongly supported by experiments using additive designs (Letourneau *et al.*, 2009), where high predator diversity is positively correlated with total predator abundance. Furthermore, the relationship between predator diversity and biological control may vary among systems and species composition, owing to predator and prey specific traits (Lang, 2003; Tylianakis & Romo, 2010), or as a result of interactions within the predator community (Straub & Snyder, 2006). Positive effects of predator diversity on biological control can arise from predator complementarity, a result of resource partitioning by predator species; and/or facilitation, occurring when one predator species increases prey capture by another predator species (Losey & Denno, 1998). For

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example, resource partitioning occurs among coccinellids and parasitoids which attack aphids on different parts of their host plant (Straub & Snyder, 2008; Gable *et al.*, 2012), or among larvae of two foliage-dwelling predator species [*Chrysoperla plorabunda* (Fitch) and *Coccinella septempunctata* Linnaeus] that occupy different feeding niches on fava bean plants (Chang, 1996). Facilitation has been found to occur when coccinellids foraging in the plant canopy facilitate prey capture by ground beetles by causing aphid prey to fall to the ground (Losey & Denno, 1998; Prasad & Snyder, 2010). Negative effects of predator diversity can arise from predator interference such as intraguild predation. For instance, coccinellids and lacewing larva can be intraguild predators of each other (Michaud & Grant, 2003), and both can be intraguild prey of the large ground-dwelling carabid beetle *Pterostichus melanarius* (Illiger) (Michaud & Grant, 2003).

Habitat complexity can influence biological control in a variety of ways. Greater habitat complexity can reduce the foraging efficiency of predators on their prey (Grabowski *et al.*, 2008). In this way, agricultural weeds could weaken biological control by interfering with predator searching behaviour, or by distracting predators with alternative herbivorous prey or floral resources (Wilby *et al.*, 2013). Additional plant resources could also strengthen biological control by reducing intraguild predation (Langellotto & Denno, 2004; Janssen *et al.*, 2007; Diehl *et al.*, 2011). Clearly, the role of habitat complexity in modifying trophic interaction is complex and difficult to predict *a priori*.

When predator diversity strengthens prey suppression, it can be inferred that positive predator–predator interactions are more important than negative interactions and *vice versa*. Numerous studies have found, however, that increasing predator diversity has no impact on the strength of prey suppression (reviewed by Letourneau *et al.*, 2009). This neutral effect of predator diversity could be caused by an absence of predator–predator interactions (no effect hypothesis) or the counteracting effects of positive and negative predator interactions (counteracting effect hypothesis).

Trophic interactions among arthropods are, in general, difficult to observe and quantify directly (Symondson, 2012). The study of predator diversity effects are therefore often based on indirect indicators, deduced from population count data (Finke & Denno, 2004; Wilby *et al.*, 2005; Griffiths *et al.*, 2008; Straub & Snyder, 2008), although a few studies have used behavioral observations (Straub & Snyder, 2008; Wilby *et al.*, 2013). Thus, direct detection of interactions among predators is needed fully to understand predator–prey food webs and mechanisms behind the biological control of agricultural pests. DNA-based techniques allow detection of ingested prey in predator guts and can be used as a tool to assess links in complex food webs (Symondson, 2012; Traugott *et al.*, 2013). Numerous studies have used molecular techniques to reveal feeding links in complex arthropod food webs (e.g. Gariepy *et al.*, 2007; Campos-Herrera *et al.*, 2011; Eitzinger & Traugott, 2011; Davey *et al.*, 2013; Raso *et al.*, 2014). However, so far no studies have applied molecular techniques to experiments varying predator diversity levels to disentangle the mechanisms behind the effects of predator diversity on prey suppression.

Here, we report a field cage study where we tested the effects of predator diversity and the presence of weeds on cereal aphid density. We directly investigated aphid, intraguild, and non-pest predation by molecular gut content analysis (MGCA) of the predators to explore the mechanisms behind the observed effects of predator diversity on aphid biological control.

Material and methods

In a manipulative field cage experiment in spring barley (*Hordeum vulgare* Linnaeus) in south-central Sweden, we studied the effect of predator richness and the presence/absence of weeds on cereal aphid [*Rhopalosiphum padi* (Linnaeus) and *Sitobion avenae* (Fabricius)] suppression at stem elongation [crop stage >30 on the Zadoks scale (Zadoks *et al.*, 1974)]. At this crop stage, populations of *R. padi* in Sweden are usually at an exponential growth phase, approaching peak densities in the crop, whereas *S. avenae* have recently colonised the crop and normally occur at lower densities (Caballero-López *et al.*, 2012). We used a substitutive, crossed design with a subset of three taxa of predators: larvae of two foliage-dwelling aphid predator species, *C. septempunctata* and *Chrysoperla carnea* (Stephens) (both species obtained from Katz Biotech AG, Welzheim, Germany), and adults of the autumn breeding ground-dwelling carabid beetle, *P. melanarius*, obtained from the field (Table 1). These predators are commonly found in cereal fields during this crop stage and have been shown to provide significant aphid biological control (Sunderland, 1975; Freier *et al.*, 2007). Moreover, inter-specific interactions potentially affecting biological control, both positively and negatively, have been observed within this predator community (Chang, 1996; Losey & Denno, 1998; Michaud & Grant, 2003; Prasad & Snyder, 2010).

Experimental design

The experiment was conducted at the organic research station 'Ekhaga', belonging to Swedish University of Agricultural Sciences (SLU), close to Uppsala, in south-central Sweden during summer 2012. It was run in $2 \times 2 \times 2\ m^3$ wooden cages with $0.40 \times 0.45\ mm^2$ mesh (LS ECONET ®, Sweden) screening and a Velcro® strip sewn on one side to allow entry. Cages were erected in a newly sown organic barley field (cultivar: Tipple) on 3 May, before cereal aphid colonisation, and were randomly assigned to different treatments. Cages were 2 m apart, and the cage bottom edges were buried under ~10 cm of sand to prevent arthropod movement in and out of the cages. All cages were located at least 10 m from the nearest field edge. Arthropods were removed using pitfall trapping and by hand searching weekly, from 2 weeks prior to aphid introduction until predator introduction. Tiller density within cages was estimated by counting the number of tillers in two representative cages (with and without weeds) at the end of the experiment; they were 1284 and 1384, respectively.

Rhopalosiphum padi (obtained from a laboratory colony maintained on barley) were added to the cages in equal numbers (150 third and fourth instar nymphs on cut barley leaves were placed on growing tillers) on 12 June and population density was monitored weekly until predator introduction. Populations of *R. padi* had reached high infestation levels (347.1 ± 21.9; mean ± SE

Table 1. Predators used in the experiment, foraging behaviour, life stage, and number introduced according to treatments.

Species	Foraging behavior	Life stage at introduction	Daily aphid consumption rate in laboratory	Number of predator introduced per cage (4 m^2)	
				Low spp richness $n = 3*(3+3)$†	High spp richness $n = 6+6$ †
Pterostichus melanarius	Ground-dwelling	Adult	125‡	60	20
Coccinella septempunctata	Foliage-dwelling	First larval instar	25§	300	100
Chrysoperla carnea	Foliage-dwelling	First larval instar	25¶	300	100

†Half of the treatments were assigned to either weeds or no weeds.
‡Chiverton (1988). Assay conducted with *Pterostichus cupreus*.
§Wetzel *et al.* (1982).
¶Atlihan *et al.* (2004).

per 20 tillers) when predators were introduced on July 12 (t_0). *Sitobion avenae* (obtained from Koppert, Berkel en Rodenrijs, Netherlands) were introduced according to the same method (150 third and fourth instar nymphs) on 10 July to mimic natural colonisation, in lower abundance and later in the season than *R. padi*. Numbers of *S. avenae* reached 11.5 ± 1.7 per 20 tillers at t_0.

Predator communities were assembled according to three treatments: no predator-control, low predator diversity (one predator taxon added), and high predator diversity (three predator taxa added). In the low predator diversity treatments, each single species variant was replicated three times. Predator treatments were crossed with the presence/absence of weeds, and the low predator diversity treatment had nine replicates with weeds and nine without weeds. The high predator diversity treatment was replicated six times with weeds, and six times without; and the no-predator control included three replicates with and without weeds.

We used a substitutive experimental design to separate the effects of species' richness from total predator abundance (Straub & Snyder, 2006). Thus, the density of each predator in the high diversity treatment was a third of its density in the single predator (low diversity) treatments (Table 1). Predators were released on 12 July (t_0) and the experiment ran for 12 days until 24 July (t_f). The predator densities used were chosen in order to standardise potential aphid consumption rates from published studies, i.e. total potential population consumption rates, and thus expected predation on aphids, would be similar in all predator treatments (Table 1) in the absence of emergent effects of predator interactions, weeds, and any alternative prey available. As data for *P. melanarius* were not available, we used the feeding rate of a related species *Pterostichus cupreus* (Linnaeus) [now *Poecilus cupreus* (L.)] (Chiverton, 1988). We used aphid consumption rates of first instar larvae for *C. septempunctata* (Wetzel *et al.*, 1982) and *C. carnea* (Atlihan *et al.*, 2004). As a result of the high density of aphids present in the cages and to mimic predators' aggregation in areas of high prey density (Sunderland, 2002; Koss *et al.*, 2005), we introduced high densities of predators (Table 1). The densities of each predator species in the high diversity treatment are similar to high predator density situations recorded in agricultural fields (Lovei & Sunderland, 1996; Bosque-Pérez *et al.*, 2002). However, because we employed a substitutive experimental design which isolates the effect of predator diversity by controlling for total predator density, the densities of single species in the low diversity treatment were higher than is typically encountered in the field. To ensure no prey ingested before the experiment would be detected in predator's guts, *P. melanarius* were starved for 3 days before introduction, and *C. septempunctata* and *C. carnea* were raised on pea aphids *Acyrthosiphon pisum* (Harris).

In the weed treatments, a natural community of weeds [10 individuals each of *Galeopsis speciosa* Miller, *Stellaria media* (Linnaeus), and *Matricaria perforata* Mérat] was transplanted from neighbouring fields prior to aphid introduction. All cages were watered four times after weeds were transplanted. Cages assigned to the no-weed treatment were manually weeded on a weekly basis. Weed coverage was recorded at the end of the experiment, and reached $16 \pm 1\%$ (mean $\pm$ SE) of ground cover in the weedy treatment. To assess whether weeds contributed with alternative prey, two individuals of each plant species were collected in each cage at the end of the experiment and examined for the presence of herbivores. None were observed.

Arthropod sampling

Aphid numbers were recorded four times: at predator introduction (t_0), at two time points during the experiment ($t_1 = t_0 + 4$ days and $t_2 = t_0 + 8$ days) and at t_f (12 days after introduction). The cage was divided into quarters, one tiller per quarter was randomly selected, and then four adjacent tillers within the same row of barley were successively assessed for the number of aphids (i.e.,a total of 20 tillers were examined per cage at each time point, corresponding to <1.5% of the total number of tillers). To ensure accurate aphid counts, tillers were cut at the base, and aphids meticulously counted, and determined to species level and growth stage. The number of alates (on the mesh of the cage) were recorded and removed after each aphid count, allowing the number of migrating alates to be estimated every 4 days.

One dry pitfall trap (9 cm diameter × 11 cm depth) was placed at each corner of each cage to recapture *P. melanarius*. Traps were open for 24 h at t_1, t_2 and t_f. Each collected carabid was replaced by a starved one except at t_f when the experiment was terminated. To minimise disturbance, *C. septempunctata* and *C. carnea* were not collected before the final date, when all

Table 2. Targeted taxa and gene, primer names (A and S numbers denote sense and antisense primers, respectively) and sequences, expected product sizes in base pairs [Size (bp)], concentrations in PCR [Conc. (μM)] for primers used in multiplex PCR, and reference.

Target taxa	Target gene	Primer name	Primer sequence (5′–3′)	Size (bp)	Conc. (μM)	Reference
Coccinella septempunctata	COI	Coc-sep-S425	YCC TCC TTT ATC CTC TAA CTT AGC	167	0.07	Present publication
		Coc-sep-A428	CAA AAA GAG GTG TCT TAT CAA GGT			Present publication
Chrysoperla carnea	COI	Chry-car-S426	YCC TTC ATT AAC TTT ATT ACT TGC TT	232	0.2	Present publication
		Chry-car-A429	TAA TGG TAT ACG ATC TAA TGT TAT ATA ACT T			Present publication
Sitobion avenae	COI	Sit-ave-S157	TCA GTY GAT TTA ACT ATT TTT TCA T	257	0.5	von Berg *et al.* (2012)
		Sit-ave-S A103	TCT CCT CCT CCT GCT GGA			von Berg *et al.* (2012)
Rhopalosiphum padi	COI	Rhop-pad-S155	GGA ACA GGA ACA GGA TGA ACA	111	0.1	von Berg *et al.* (2012)
		Rhop-pad-A153	TGA TGA GAT TCC TGC TAA ATG TAG A			von Berg *et al.* (2012)
Earthworms	12 S	12SNF	AAA CTT AAA GAT TTT GGC GGT GTC T	200	0.8	Harper *et al.* (2006)
		12SNR	GCT GCA CTT TGA CCT GAC GTA T			Harper *et al.* (2006)
Collembola	18 S	Coll-S411	GCT CGT AGT TGG ATY TCG GTT T	289	0.2	Present publication
		Coll-A415	**GAA** TTT CAC CTC TAA CGT CGC AG			Sint *et al.* (2012)
Trechus secalis	COI	Tre-sec-S428	CTG GAA TTG CCC ATA GAG G	141	0.1	Present publication
		Tre-sec-A433	TTC GAT CAA AGG TTA TAC CTA TTG G			Present publication
Trechus quadristriatus	COI	Tre-qua-S429	CAG GGA TTG CCC ATA GAG G	143	0.1	Present publication
		Tre-qua-A432	TAC GAT CAA AAG TTA TTC CTA TAG G			Present publication

Bold letters in sequence indicate modifications in published sequence.

tillers were cut and examined for foliage-dwelling predators. All predators were individually collected into 1.5-ml reaction tubes and immediately frozen on dry ice, then stored at −80 °C until MGCA. We recaptured *P. melanarius* at a rate of 9% across the three dates, and *C. septempunctata* at a rate of 15%. Owing to a very low recapture rate of *C. carnea* (0.38%), we decided not to process them molecularly.

Alternative prey species were monitored using sticky traps as described in Kuusk and Ekbom (2010) (per cage: four traps of $5 \times 10\,cm^2$, left for 24 h), soil sampling (per cage: one sampling of $20 \times 20 \times 20\,cm^3$) 20 cm beside each cage, and pitfall trapping (as described above) at t_1, t_2 and t_f. Alternative prey found (mean ± SE) were mainly: Collembola (*Arthropleona* spp. and *Symphypleona* spp.: 44.9 ± 1.13 per sticky trap), earthworms (4.8 ± 0.4 per soil sample), and the small carabid beetle *Trechus* spp. (0.3 ± 0.5 per four pitfall traps per cage). Both earthworms and Collembola are soil-living and could thus not be excluded from the cages. *Trechus* spp. [*Trechus secalis* Paykull and *Trechus quadristriatus* (Schrank)] are mainly autumn breeding predators (Wallin, 1989) and they likely emerged from pupae present in the soil during the field experiment.

Molecular gut content analysis

We evaluated aphid, intraguild, and non-pest prey (i.e. alternative prey that was not a predator) consumption by *P. melanarius* and *C. septempunctata* collected from the low and high predator diversity treatments. A total of 183 *P. melanarius* (104 from the low predator diversity treatment and 79 from the high predator diversity treatment) and 215 *C. septempunctata* (80 from the low predator diversity treatment and 135 from the high predator diversity treatment) were processed.

Multiplex PCR assay

A multiplex PCR assay targeting the cereal aphids *R. padi* and *S. avenae*, the predators *C. septempunctata* and *C. carnea*, the two carabid species within the genus *Trechus* (*T. quadristriatus*, *T. secalis*), as well as the non-pest prey Collembola and earthworms was developed using newly developed primers and ones available in the literature (Table 2). Primer sensitivity to 1000 double-stranded copies per μl of the targeted DNA was balanced for all primers used in the multiplex according to the

procedure described in Sint *et al.* (2012). We tested all non-target taxa encountered in the cages (Table S1) for cross-amplifications with the multiplex-PCR. *Pterostichus melanarius*, the largest predator of this system, was not included in the multiplex PCR assay as it was very unlikely to be preyed upon by any other predator in our experiment. *Coccinella septempunctata* primers were removed from the multiplex PCRs used for testing the cage-collected *C. septempunctata*.

PCRs were performed in 10-µl reactions containing 1.5 µl of extracted DNA, 1 µl of 10× primer mix (in 1× TE buffer, see Table 2 for concentrations), 1 µl 5× Q solution (Qiagen, Hilden, Germany), 0.5 µg bovine serum albumin (BSA), 0.5 µl 1 M tetramethyl ammonium chloride (TMAC), 5 µl 2× Multiplex reaction mix (Multiplex PCR Kit, Qiagen), and 0.5 µl molecular-grade water. Amplifications were carried out in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) under the following thermocycling conditions: 15 min at 95 °C, 35 cycles of 30 s at 94 °C, 90 s at 58 °C, 90 s at 72 °C, and 10 min at 72 °C. We tested for DNA cross-contamination and false negative/positive amplifications by including molecular-grade water and a mix of DNA from all targeted taxa within each PCR. No manipulation errors were revealed.

We describe the multiplex PCR assay (sensitivity/specificity tests and predator screening protocol, i.e. DNA-extraction and electrophoresis) in Document S1.

Statistical analysis

Generalised least squares (GLS) models were used to test the effect of predator treatments, the presence of weeds, and their interaction on aphid density. Time zero counts were subtracted from the aphid counts to account for differences in initial densities. We used the predator treatment, the presence/absence of weeds, and the interaction between the two variables as explanatory variables. We used the compound symmetry correlation structure to account for temporal correlation and repeated measurements in the same cage. Before further analysis the interaction term was removed from the model as there was no interaction effects of weeds and predator treatments (Table 3a) (Crawley, 2007), and to achieve a better fit of the model using the Akaike information criterion. The model selection and optimisation was done according to Zuur *et al.* (2009). On the simplified model, we used a post-hoc test to test different contrasts of the following levels of the predator treatment:

- all predator treatments versus no-predator control: *Contrast 1*
- ground versus foliage-dwelling predator treatments: *Contrast 2*
- *C. septempunctata* versus *C. carnea*: *Contrast 3*

The 'gls' function of the 'nlme' package (Pinheiro *et al.*, 2012) and the 'glht' function of the 'multicomp' package (Hothorn *et al.*, 2008) of R version 2.14.2 (R Development Core Team, 2013) was used for these analyses.

We investigated the effects of predator diversity by comparing an observed reduction of aphid population growth from high predator diversity treatments to an expected reduction according to a null model assuming that the three predators have independent additive effects. In the manner of Griffen (2006), we derived predictions for the expected aphid number in the high diversity treatments based on extrapolation from the low diversity treatments (Eqn. 4).

More specifically, because aphid densities at predator introduction differed among replicates (owing to random variability) we first needed to obtain the expected aphid counts for each replicate k of the high predator diversity treatments (Eqn. 3). This was achieved by first estimating r, the aphid (*R. padi*) growth rate in the absence of predators (assuming exponential growth rate of aphids in the no-predator control):

$$r = \frac{\ln\left(N_C\left(t_f\right)\right) - \ln\left(N_C\left(t_0\right)\right)}{t_f - t_0} \quad (1)$$

N_C is the observed number of aphids in the no-predator control treatments at either the introduction of predator (t_0) or at the end of experiment (t_f). A single average growth rate estimate was calculated independently of weed treatments as differences in aphid growth rate was neither expected nor observed in the no-predator control treatment in the presence/absence of weeds.

We then calculated η_{LD_j}, the predicted aphid number at t_f in the absence of predators for all replicates of the low predator diversity treatments j (with $j = 1,2$ and 3 corresponding to *P. melanarius, C. septempunctata, and C. carnea*) as follows:

$$\eta_{\mathrm{LD}_j}\left(t_f\right) = N_{\mathrm{LD}_j}\left(t_0\right) \times \mathrm{e}^{r\left(t_f - t_0\right)} \quad (2)$$

$N_{\mathrm{LD}_j}\left(t_0\right)$ is the observed mean number of aphids at predator introduction in the low predator diversity treatment j.

Similarly, we calculated $\eta_{\mathrm{HD}_k}\left(t_f\right)$, the predicted aphid number at t_f in the absence of predators, for each of the 12 replicates k of the high predator diversity treatment HD_k, from $N_{\mathrm{HD}_k}\left(t_0\right)$, the observed mean number of aphids at the introduction of predators, in the high predator diversity replicate k, i.e.:

$$\eta_{\mathrm{HD}_k}\left(t_f\right) = N_{\mathrm{HD}_k}\left(t_0\right) \times \mathrm{e}^{r\left(t_f - t_0\right)} \quad (3)$$

Finally, $\mathrm{E}\left(N_{\mathrm{HD}_k}\left(t_f\right)\right)$, the expected aphid counts at the end of the experiment for each of the 12 replicates k of the high predator diversity treatment, assuming the three predators have additive effects only, were calculated using the formula (see Document S2 for derivation):

$$\mathrm{E}\left(N_{\mathrm{HD}_k}\left(t_f\right)\right) = \eta_{\mathrm{HD}_k}\left(t_f\right) \times \left(\frac{\mathrm{N}_1\left(t_f\right)}{\eta_{\mathrm{LD}_1}\left(t_f\right)} \times \frac{\mathrm{N}_2\left(t_f\right)}{\eta_{\mathrm{LD}_2}\left(t_f\right)} \times \frac{\mathrm{N}_3\left(t_f\right)}{\eta_{\mathrm{LD}_3}\left(t_f\right)}\right)^{1/3} \quad (4)$$

Where $N_j\left(t_f\right)$ is the mean of the observed aphid counts at the end of the experiment in the low predator diversity treatment $j \in (1, 2, 3)$.

We compared expected and observed values using t-tests to detect whether the three predators of the high diversity treatment had independent effects.

We validated the underlying statistical assumptions of linear models (i.e. the linear relationship between dependent and

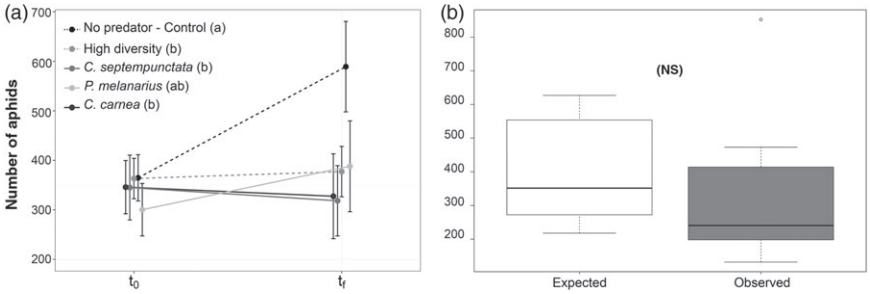

Fig. 1. (a) Numbers of *Rhopalosiphum padi* (mean numbers per 20 tillers ± SE) at predator introduction (t_0) and at the end of the experiment (t_f) according to predator treatments (no-predator control, high diversity, *Coccinella septempunctata*, *Pterostichus melanarius*, and *Chrysoperla carnea*). Different letters after the legend indicate a significant difference in the aphid number between treatments at $\alpha = 0.05$. (b) Comparison of the observed number of *R. padi* at t_f in the high diversity treatment to expected numbers generated by a null-model assuming predators have independent additive effects. NS: $P > 0.05$.

independent variables, the independence and equal variance of errors, and the normal distribution of residuals) graphically.

Finally, we used generalised linear models (GLMs) with binomial error structure to analyse MGCA data from *P. melanarius* and *C. septempunctata*. The quasibinomial error structure was used to correct for underdispersion. The 'glm' function of the 'MASS' package (Venables, 2002) of R was used for these analyses. We tested if the detection frequencies of aphid and alternative prey DNA in predator guts were different in the *P. melanarius*- and *C. septempunctata*-only treatments versus the high predator diversity treatment. We then tested if the detection frequency of intraguild predation in the high predator diversity treatment was different in the presence/absence of weeds. We used presence and absence of DNA as the response variable and predator diversity and presence/absence of weeds as explanatory variables. We finally tested whether the proportions of different prey types detected in the gut were a function of their availability using the abundance of the respective prey taxa recorded in the cage as the explanatory variable.

Results

Predator effect on aphid density

Aphid density was significantly reduced in the predator treatments compared with the predator-free control (Fig. 1a; Table 3b/*Contrast 1*). Although predators were not able to reduce aphid numbers below initial densities, in many replicates they prevented a further increase in aphid abundance. *Pterostichus melanarius* was the least efficient predator treatment in controlling aphid density, although differences compared with other predator treatments were not significant (Fig. 1a).

The density of *R. padi* stayed high in all treatments [at t_f: per 20 tillers per cage: 396.2 ± 34.6 (mean ± SE); per sticky trap: 32.0 ± 3.6]. *Sitobion avenae* were rare (at t_f: per 20 tillers per cage: 25.4 ± 3.8; per sticky trap: 0.2 ± 0.1) and *S. avenae* density was too low to be analysed separately. When pooling the two aphid species, treatment effects on aphid density gave similar results to when analysing *R. padi* only, so for simplicity only data involving *R. padi* are presented here.

The number of alates produced and the initial population of aphids were significantly correlated (Pearson's product-moment correlation, $r = 0.52$, $P < 0.001$), and as adding number of alates as a covariate did not change the fit and output of the model, it is not included in the model results shown below.

Table 3. Analysis of the predator and weed treatment effects. (a) Output of the ANOVA of the generalised least squares model where the response variable is the difference between the number of *Rhopalosiphum padi* per 20 tillers at each sampling date and the initial number at start of experiment. (b) Result of the post-hoc tests examining the pre-defined predator treatment contrast matrix.

(a) Source of variation	numDF	denDF	*F*-value	*P*-value
(Intercept)	1	98	0.402	0.528
Treatment	4	98	2.982	0.023
Weeds	1	98	1.368	0.245
Treatment : weeds	4	98	0.299	0.878

(b) Contrast	Estimate	SE	z-value	Pr(>|z|)
Contrast 1: Predator treatments versus no-predator control	1.544	0.637	2.424	0.0357
Contrast 2: Ground-dwelling versus foliage-dwelling predator treatments	0.062	0.374	0.164	0.6213
Contrast 3: *C. septempunctata* versus *C. carnea* treatments	0.118	0.229	0.514	0.6213

We used predator treatment (no-predator control, high predator diversity, *Coccinella septempunctata*, *Chrysoperla carnea*, and *Pterostichus melanarius*) and the presence of weeds as explanatory variables. Cage (1–36) nested in sampling date (t_1, t_2, t_f) was used in the correlation structure to account for temporal autocorrelation and repeated measures. *P*-values are corrected for multiple comparison using Hochberg (1988) corrections.

Aphid density was neither significantly affected by habitat domain of predators (ground-dwelling vs. foliage-dwelling) (Fig. 1a, Table 3b/*Contrast 2*) nor by predator identity (*C. septempunctata* vs. *C. carnea*) in the foliage-dwelling predator treatments (Fig. 1a; Table 3b/*Contrast 3*). The presence of weeds did not influence aphid density or alter any of the predator treatment effects (Table 3a).

Observed aphid density in the high diversity treatment was not statistically different from expected values using eqn 4, i.e. assuming independent additive effects of the three predator

Table 4. DNA detection rates of aphids (*Rhopalosiphum padi* and *Sitobion avenae*), intraguild prey (*Coccinella septempunctata* and *Chrysoperla carnea*) and alternative prey in the gut of *Pterostichus melanarius* and *Coccinella septempunctata*.

Predator	Aphids	Intraguild prey†	Alternative prey
P. melanarius ($n = 183$)	*R. padi*: 97.3%	*C. septempunctata*: 5.1%	Collembola: 24.6%
	S. avenae: 0%	*C. carnea*: 0%	*Trechus* spp.: 2.2%
			Earthworms: 2.7%
C. septempunctata ($n = 215$)	*R. padi*: 97.7%	*C. carnea*: 1.5%	Collembola: 0.9%
	S. avenae: 3.3%		*Trechus* spp.: 0%
			Earthworms: 0%

†Intraguild prey was detected in the high diversity treatment only. *P. melanarius*: $n = 79$; *C. septempunctata*: $n = 135$.

species (Fig. 1b; ANOVA, $F_{1,8} = 0.02$, $P = 0.90$). We included weed treatment as an explanatory variable, but the result did not change.

Predation on aphids, intraguild, and non-pest prey

Aphids were extensively detected in the predator guts (Table 4, Fig. 2): 97.3% of *P. melanarius* and 97.7% of *C. septempunctata* were positive for *R. padi*. *Sitobion avenae* was not detected at all in *P. melanarius* and was only detected in 3.3% of *C. septempunctata*. For both predators, *R. padi* detection rates did not differ depending on predator diversity (low vs. high predator diversity treatments) (GLM, family = quasibinomial, *P. melanarius*: Estimate = 0.70, SE = 0.95, $P = 0.46$; *C. septempunctata*: Estimate = −0.75, SE = 0.89, $P = 0.41$). The aphid DNA detection rates were independent of the weed treatment (GLM, family = quasibinomial, *P. melanarius*: Estimate: 0.83, SE = 1.23, $P = 0.50$; *C. septempunctata*: Estimate: 0.61, SE = 0.88, $P = 0.50$), aphid density (GLM, family = quasibinomial, *P. melanarius*: Estimate = −0.36, SE = 0.46, $P = 0.44$; *C. septempunctata*: Estimate = −0.43, SE = 0.47, P = 0.38), and the density of Collembola in the cages (GLM, family = quasibinomial, *P. melanarius*: Estimate = −0.37, SE = 0.47, $P = 0.44$; *C. septempunctata*: Estimate = −0.43, SE = 0.47, $P = 0.38$).

Coccinella septempunctata were detected at a low frequency (5.1%) in the gut of *P. melanarius* in the high predator diversity treatment, whereas *C. carnea* was not detected at all (Table 4, Fig. 2). Two of the 135 *C. septempunctata* collected in the high predator diversity treatment screened positive for *C. carnea* (1.5%). The detection of intraguild predation by *P. melanarius* on *C. septempunctata* was independent of the aphid density (GLM, Estimate = 0.06, SE = 0.05, $P = 0.22$) and the date (GLM, Estimate = −0.60, SE = 0.54, $P = 0.26$).

Except for Collembola, the detection rate of alternative prey was low: 2.2% of *P. melanarius* were tested positive for *Trechus* spp. and 2.7% for earthworms. Collembola were detected in 24.6% of the screened *P. melanarius* and were the only alternative prey detected in *C. septempunctata* at a rate of 0.9% (Table 4, Fig. 2). The detection rate of Collembola in *P. melanarius* was not dependent on their density (GLM, Estimate = −0.002; SE = 0.001, $P = 0.12$).

Discussion

Effect of predator diversity

The impact of predator diversity on aphid biological control in this experiment was found to be additive. The aphid density observed in the high predator diversity treatment at the end of the experiment did not differ from the expected aphid density, assuming that the three predator species have only additive effects. Thus, based on the results from MGCA, we can, with fairly strong confidence, reject the counteracting effect hypothesis where strong negative and positive effects of predator diversity cancel each other. We found no evidence of facilitation among predators, and only a few negative inter-specific interactions were detected in the form of intraguild predation by *P. melanarius* and *C. septempunctata.*

MGCA provided no evidence of functional synergy between foliage-dwelling predators (*C. septempunctata* and *C. carnea)* and the ground-dwelling predator *P. melanarius*, because the proportion of aphid DNA detected in the guts of *P. melanarius* and *C. septempunctata* were identical in the low and high predator diversity treatments. However, we acknowledge that the *per capita* predation rate on aphids cannot be quantified using MGCA, and the detection frequency of predation events is used here as a proxy for trophic interaction strength.

Furthermore, MGCA revealed a low frequency of intraguild predation events among the predators (Fig. 2). We found only low levels of predation by *P. melanarius* on *C. septempunctata* (5.1%), and no predation by *P. melanarius* on *C. carnea*. *Chrysoperla carnea* was detected at low rates (1.5%) in the guts of *C. septempunctata. Coccinella septempunctata* were occasionally found at ground level (on sticky traps, nine individuals over four sampling dates) whereas *C. carnea* larvae were not encountered on the ground (i.e. not found on sticky traps). Thus, the few intraguild predation events that were detected probably occurred among predators found in the same niche. Other studies have, however, shown that intraguild predation among these predators could happen when confined to Petri dishes (Dinter, 1998; Hindayana *et al.*, 2001; Noppe *et al.*, 2012).

We still cannot rule out the possibility that intraguild predation among *C. septempunctata* and *C. carnea* had occurred earlier in the experiment as we recaptured and analysed both predators at the end of the experiment only and the detection of predation events with MGCA is unlikely after 24–36 h. Moreover, we could not investigate *C. carnea's* gut content because of its very low recapture rate, possibly owing to larvae entering the pupal stage (although no pupae were observed).

The low occurrence of intraguild predation might result from the high prey density present in our cages. Other studies found that a high prey density decreases the encounter and attack rates between predators (Polis *et al.*, 1989; Lucas *et al.*, 1998; Griffen, 2006; Werling *et al.*, 2012), and that predator diversity effects on prey suppression are less likely with high prey–predator ratios (Tylianakis & Romo, 2010; Wilby & Orwin, 2013). For example, Werling *et al.* (2012) found similar additive effects of

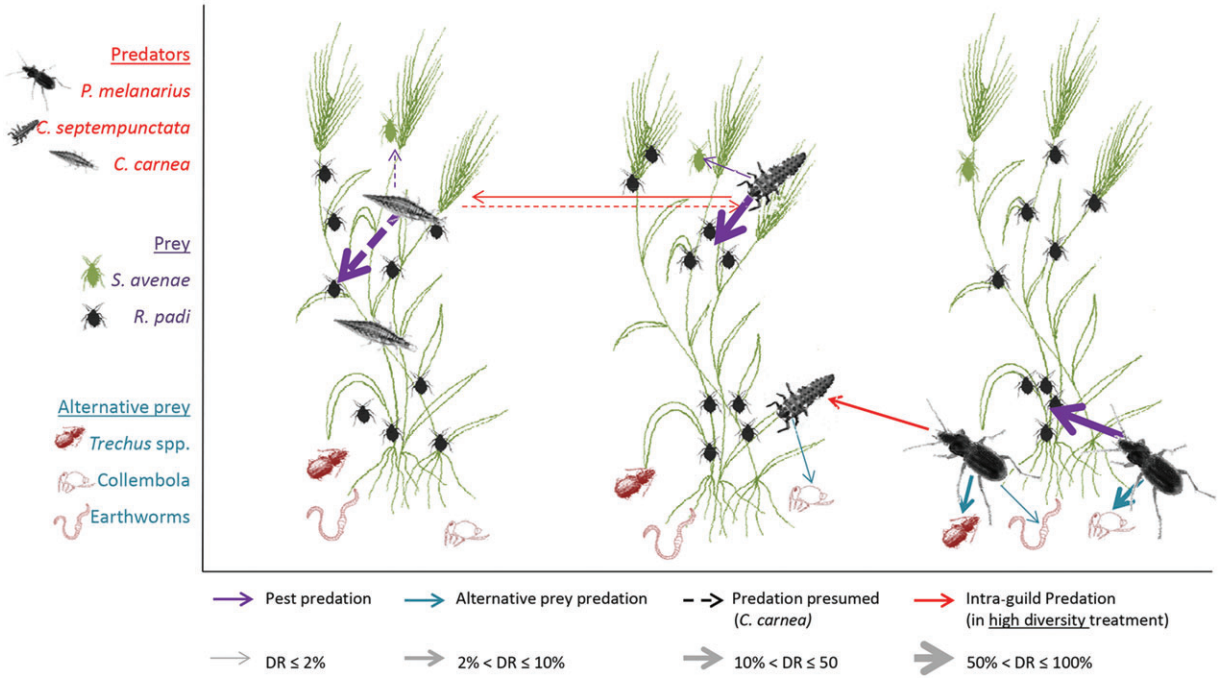

Fig. 2. Food web revealed by molecular gut content analysis (MGCA). The three groups of tillers represent the three low diversity treatments (*Chrysoperla carnea*, *Coccinella septempunctata*, and *Pterostichus melanarius*). The high diversity treatment is represented by the assemblage of the three low diversity treatments presented with intraguild predation links (i.e. the whole picture). The line weight varies according to the frequency of the detection rate (DR) deducted from MGCA. No difference in link weights (i.e. prey DNA detection frequency) has been observed between low and high diversity treatments. Links for *C. carnea* are presumed, assuming a similar DR for the two foliage dwelling predators.

predator diversity [between *Coleomegilla maculata* (De Geer) and *Podisus maculiventris* (Say)] at a high prey density of the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)], but not at a low density, where synergistic effects were found.

Effect of weeds

We did not find an effect of the presence of weeds on aphid biological control. In our system, weeds were primarily a source of structural complexity as they did not supplement additional alternative prey (although some flowers were present). Our result thus suggests that additional structural complexity has neither enhanced nor disrupted the top-down suppression of aphids. MGCA confirms this finding, with a similar detection rate of aphid predation and intraguild predation events in *P. melanarius* and *C. septempunctata* regardless of weed treatments. However, as only a few intraguild predation events were detected, a reduction would have been hard to discern. The high prey density present in our system might have reduced the potential impact of the structural effects of weeds on the reduction of intraguild predation.

Although our experiment was primarily designed to study the structural effects of weeds on trophic interaction in a short term, additional effects may arise in a longer term. For instance, weeds can improve the microclimate for predators, provide alternative resources such as pollen or nectar, increase densities of prey, and/or add alternative prey species, which together might favour predator abundances in a longer perspective (Langellotto & Denno, 2004). This effect of weeds was confirmed by Diehl *et al.* (2011), who found an increased activity density of carabids in weedy plots but not in plots were only an additional structure was provided. They conclude that weeds can benefit predator communities in the long term through the promotion of predator establishment and increased predator fitness. To date, no long-term studies on the effects of weeds on biological pest control are available.

Conclusion

Our study shows that under the conditions studied here, *P. melanarius*, *C. septempunctata*, and *C. carnea* have additive effects on aphid density, implying no effect of predator diversity on the biological control of aphids on barley at the maturing stage.

MGCA indicated that facilitation was absent, and intraguild predation occurred at a low frequency among the foliage and ground-dwelling predators involved in this experiment. This suggests that the additive effect of predator species on biological control was not as a result of strong synergistic or antagonistic interactions, rejecting the counteracting effect hypothesis. We also found no effect of weeds on aphid biological control.

The absence of effects of predator diversity and the presence of weeds on biological control might, however, have resulted from the conditions of high herbivore densities. High prey densities

probably reduced the likelihood of detecting positive effects of functional diversity of foliage and ground-dwelling predators. Our results thus suggest that in the short term, at least when aphid densities are high, it is possible to predict the combined effect of these multiple predator species on the biological control of cereal aphids by extrapolating data from the single predator treatments. Thus, it may be equally effective to enhance populations of individual species as to enhance biodiversity of predators under these conditions. Our results furthermore imply that, under such a scenario, it might be possible to predict the outcome of biological control using a dynamic multispecies trophic model without estimating non-linear predator–predator interaction terms.

Predator diversity is, however, expected to insure against environmental fluctuations and provide more stable control of agricultural pests when a longer time scale is considered (Yachi & Loreau, 1999). Similarly, a longer time scale might strengthen the impact of weeds, by providing alternative food sources at times when pests are scarce. Future studies, therefore, need to explore how predator and plant biodiversity contributes to delivery and stability of biological control over a longer time scale and at lower pest density, e.g. at the aphid crop colonisation phase.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12188

Document S1. Multiplex PCR assay.

Document S2. Derivation of expression for expected aphid abundance in combined species treatment.

Table S1. Non-target taxa tested for cross-amplification with the multiplex PCR. We qualified as non-targets all taxa encountered in our field cages.

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