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Functional diversity of predators and parasitoids does not explain aphid biocontrol efficiency

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Abstract Many studies demonstrate an important role of natural enemy biodiversity in the regulation of agricultural pests, but the role of different aspects of biodiversity in influencing this crucial ecosystem service remain controversial. We hypothesised that the functional diversity generated by combining divergent consumer groups (roaming coccinellid predators and parasitoid wasps) fosters complementarity, enhancing aphid biocontrol. We tested this using experimental mesocosms containing plants, aphids and natural enemies located in a greenhouse. We compared the aphid control efficiency (final aphid

abundance) of low functional diversity treatments (two parasitoid species, or two predator species) with high functional diversity treatments (all four possible predator-parasitoid combinations). We also included all four enemies as single species treatments to allow calculation of the non-additive effects of combining natural enemies. Results showed that biocontrol (final aphid abundance) was driven by the species identity of natural enemies and positive non-additive effects in two treatments in which the most efficient predator species was combined with a parasitoid species and the other predator species, respectively. Functional diversity did not consistently influence biocontrol or non-additive effects. In conclusion, functional diversity, as defined by differences between roaming predator and parasitoid functional groups, failed to consistently explain biocontrol efficiency in our study. This calls for consideration of finer-scale functional traits and how they govern natural enemy interactions and cascading effects across ecosystems.

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Introduction

A longstanding issue in pest control has been whether to introduce one natural enemy species (NE) or more

than one to maximise suppression of pest populations. This has remained controversial because trials using multiple natural enemy species have had contrasting outcomes on pest control (i.e. negative, positive and neutral) (Denoth et al. 2002; Letourneau et al. 2009). These variable results are likely to be triggered by different ecological mechanisms. For instance, complementarity or facilitation between combined NE species may lead to a positive relationship between NE diversity and the suppression of pest populations (Finke and Snyder 2008; Losey and Denno 1998; Northfield et al. 2010). On the other hand, natural enemies may also engage in intraguild predation (IGP) and/or interspecific competition, e.g., interference (Schoener 1983), which may cancel out positive effects or even result in negative effects of NE diversity on pest suppression (Finke and Denno 2004, 2005; Vance-Chalcraft et al. 2007). Thus, a key challenge in NE biodiversity research is to understand which combinations of natural enemies maximise prey suppression.

The functional diversity (FD) of natural enemies is one aspect of biodiversity that may help to explain variation in the size and direction of NE effects on pest suppression. FD is defined by variation among the traits of individual organisms within communities or trophic groups (McGill et al. 2006; Petchey and Gaston 2002; Tilman 2001). Differences in traits of natural enemies, such as their mouthparts (e.g., chewing, sucking) or mobility (e.g., flying, walking), may underpin differences in their exploitation of pests, such as the size/stage of prey consumed (Wilby et al. 2005), or their spatial foraging domain (e.g., leaf versus ground) (Losey and Denno 1998, 1999). In turn, these niche differences may lead to relaxation of interspecific competition (Northfield et al. 2010) and the generation of positive biodiversity effects, i.e., mixtures of species performing better than expected based only on their component species (Petchey 2003). However, large trait differences between natural enemies may foster negative interactions, leading to competitive dominance, interference and/or IGP. For example, large differences in the body size of predators are associated with IGP (Krenek and Rudolf 2014). Although functional diversity is an increasingly popular lens through which to view ecological communities (Laureto et al. 2015) and studies are emerging linking functional diversity to ecological processes in food webs (Duffy et al. 2016;

Gagic et al. 2015; Schmitz 2009), there remain few explicit tests of the role of natural enemy functional diversity in the context of biocontrol.

Contrasting traits of generalist predators and parasitoids provide a major element of functional diversity within natural enemy assemblages (Snyder and Ives 2003). This functional diversity emerges from a fundamental difference in the nature of prey use and degree of specialisation. Free-living, generalist predators (e.g., coccinellid beetles) attack and consume many individuals and potentially many prey species throughout their lifetime (Evans et al. 1999; Symondson et al. 2002). In contrast, parasitoids develop with a single host individual, and, for just a limited period of their lifespan (a few days after the adult emergence) (Bonet 2009), female parasitoids attack and oviposit on a restricted number of species. There is some evidence of complementarity between generalist predators and specialist parasitoids in the biological control of aphids (Alhadidi et al. 2018; Gontijo et al. 2015; Snyder et al. 2004). However, many studies that have included both predator and parasitoid guilds have reported IGP upon mummified aphids (Bilu and Coll 2007; Snyder and Ives 2003; Wheeler 1977), which has been confirmed by molecular diagnostics on predator guts (Traugott et al. 2012). IGP can reduce complementarity of these NE groups, even on the timescale of a single parasitoid generation, because predation on mummified aphids is redundant (the aphid has already been attacked by a parasitoid) and may ultimately divert predators from attacking healthy aphids (see Casula et al. (2006) for a theoretical treatment). Using a systematic literature search (see Supplementary information 1 for details), we found mixed outcomes of combining generalist predators and specialist parasitoids on aphid biocontrol. The majority of previous experiments (18 of 30) report that combinations of generalist predators and specialist parasitoids enhanced aphid biocontrol. However, over third (11 of 30) report that combinations of generalist predators and specialist parasitoids reduced aphid biocontrol. Although these studies suggest that positive effects are more common, drawing general conclusions is challenging because of differences in experimental design, duration and/or location (see Supplementary information 1), as well as the specific identities of predator and parasitoid species, among studies. There is a particular lack of studies that include multiple unique predator and parasitoid

combinations to assess the generality of effects across species combinations within an individual experimental setting.

Here we take a step towards testing the generality of predator–parasitoid interactions in a biocontrol context, by conducting a greenhouse experiment testing the efficiency of aphid biocontrol performed by all six possible combinations of two generalist predatory beetles and two species of parasitoid. Specifically, we used the two-spotted ladybird *Adalia bipunctata* (Linn.) (Coleoptera: Coccinellidae) and the mealybug ladybird *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), and two species of parasitoid, *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae). Although these four natural enemies overlap in traits such as body size and foraging mode, they fall into two distinct functional groups (generalist predators and parasitoids) based on the way they attack the aphids.

We hypothesized that (H1) natural enemies in general suppress aphid density, and (H2) combining natural enemies from two distinct functional groups (predator and parasitoid) will strengthen aphid control, relative to combining natural enemies of the same functional group. Additionally, we also examined the effect of functional diversity after accounting for species identity effects, which may swamp more subtle non-additive effects of functional diversity. We hypothesised that (H3) functional diversity would lead to a relaxation of competition and the emergence of positive non-additive effects despite the occurrence of IGP upon the mummified aphids. Finally, based on previous work (Alhadidi et al. 2018; Griffiths et al. 2008; Wyss et al. 1999), we predicted that (H4) the coccinellid *A. bipunctata* will be the most efficient single NE species and (H5) its combination with the parasitoid *A. ervi* would prove highly complementary and outperform other combinations.

Materials and methods

Greenhouse experiment

To test our hypotheses, the main experiment was conducted using experimental mesocosms containing plants, aphids and natural enemies located in a glass

greenhouse at Swansea University, UK between the 14th of August and 18th of October 2016.

Initial plant growth and creation of mesocosms

Dwarf broad bean plants *Vicia faba* (variety: the Sutton) were planted on 14th of August in 10 cm diameter pots, filled with compost (organic and peat free multi-purpose compost). Plants were watered every four days before introducing the aphids. Aphids were introduced 39 days after planting, and pots were covered with clear cellophane bags (40 µm polypropylene; dimensions (l × w × h): 17.8 × 9.7 × 45.6 cm; Transpack Ltd, Southampton, UK). Cellophane bags were closed around the plant pots using rubber bands to prevent experimental insects from escaping. Throughout the experiment plants were placed individually in a clear plastic container (dimensions 17 × 12 × 3.5 cm; from ZUVO, London, UK) and watered by filling the plastic container with water to the top when dried.

Sourcing and culturing of animals

We established a colony of pea aphids, *Acyrtosiphon pisum*, approximately one year before starting the experiment, in BugDorm-4 insect rearing cages (dimensions 47.5 × 47.5 × 47.5 cm), a constant temperature room (CT) at 20 ± 2 °C, 47 ± 8% RH and a 16:8 L:D photoperiod on dwarf broad bean plants. Natural enemies were supplied by Agralan Ltd (Swindon, UK). Natural enemies were kept in a fridge (4 ± 2 °C) for five days pending estimation of consumption rates of predators (see Supplementary information 2) and to allow for parasitoid mummies to hatch. We used the adults of two generalist predator species in the experiment, the two-spotted ladybird *A. bipunctata* and the mealybug ladybird *C. montrouzieri* and adults of two species of specialist parasitoid the *A. ervi* and *A. abdominalis*. We chose these two parasitoid species among other parasitoids that attack the pea aphids *A. pisum* because they differ in their mummy colour (*A. ervi* mummies are light bronze and *A. abdominalis* mummies are black) making it easier to distinguish between their mummies in the two-parasitoid treatment.

Experimental design and experimental conditions

The design consisted of one NE-free (aphid only) control treatment, all four single-species NE treatments, and all six possible combinations of two species. We used a substitutive approach maintaining respective constant total numbers of predators (6) and parasitoids (24) across treatments. In single species treatments there were six individuals of a predator or 24 individuals of a parasitoid; and in each combination treatment there were three individuals of a predator species and 12 individuals of a parasitoid species. Parasitoids were included at four times greater abundance than predators to compensate for their lower per capita consumption rates and roughly equalize the daily expected rate of predation across treatments. The ratio we used was based on comparing the daily per capita aphid consumption rates of both ladybird species in our CT room trial (see Supplementary information 2) (*A. bipunctata* = 49.3 ± 3.88 SE, *C. montrouzieri* = 46.1 ± 3.27 SE) with representative daily attack rates for the parasitoid taxa used (assuming a sex ratio of 1:1) (He 2008; Couty et al. 2001).

All treatments were replicated seven times (total = 77). During the experiment, the mean daily minimum and maximum temperatures in the greenhouse were 28.6 ± 3.8 °C (SD) and 11.7 ± 4.4 °C, respectively. Mean minimum and maximum RH were $37.2\% \pm 8.9$ and $88.1\% \pm 5.8$, respectively, with natural light.

Establishing the experiment and data collection

We introduced five individual aphids to each plant on the 22nd of September 2016. After allowing almost two weeks for colony growth, on the 4th and 5th of October 2016, we counted the aphid density for each plant, randomly assigned natural enemy treatments to mesocosms, and introduced the enemies to appropriate treatment replicates. The experiment finished on 17th and 18th of October 2016, when aphids and all surviving enemies (Supplementary information 4) were collected directly from the plants. Final data values were collected in a random sequence across mesocosms.

Data analysis

To investigate our main hypotheses, we used a negative binomial generalised linear model with a log link function, including all 11 treatments, combined with planned treatment contrasts. Since variable rates of population growth led to differences in aphid density among mesocosms at the start of the experiment (Supplementary information 3), we used ‘initial aphid density’, i.e., the aphid density when NEs were introduced, as an additive covariate in our model. To investigate H1 we applied a planned linear contrast between control treatment (NE-free) and all natural enemies’ treatments combined, to confirm that natural enemies generally suppressed prey density within our experiment. To investigate whether treatments with higher functional diversity (treatments with both predator and parasitoids) reduce aphid density more than treatments with lower functional diversity (treatments with two predators or two parasitoids) (H2), we contrasted these combinations, excluding the single species treatments from this comparison.

To investigate H3 (i.e., the emergence of positive non-additive effects) we calculated the net diversity effect (the deviation from the expected final aphid density based on the performance of single species treatments; also known as a non-additive multiple predator effect) on the final aphid density (McCoy et al. 2012; Sih et al. 1998). We first calculated the expected final aphid density (N_E) for each combination treatment as:

$$N_E = \sqrt{P_1 P_2} \quad (1)$$

where P_1 and P_2 are the mean proportions of aphids surviving of the treatment containing species 1 (P_1) or species 2 (P_2) at the end of the experiment. We then calculated the net diversity effect by subtracting the observed final aphid density of each combination from the expected final aphid density and divided them by the expected final aphid density. Finally, to test whether the net diversity effect was different in low FD versus high FD treatments, we used Welch’s *t* test for unequal variances and/or unequal sample sizes, which can result in non-integer values for degrees of freedom, following the Welch-Satterthwaite correction (Derrick et al. 2016), and we used one-sample

t test for each combination to find out whether its observed performance differed from the expected value (Eq. 1).

To investigate H4 and H5, we again worked with our main model. To assess which NE species have the greatest effects on aphid suppression (H4), we applied planned contrasts to compare all treatments including each respective species against all other treatments that excluded this species (e.g., all treatments including *Adalia bipunctata* versus all treatments excluding *A. bipunctata*). To assess whether the combination of the ladybird *A. bipunctata* and the parasitic wasps *Aphidius ervi* (Ab + Ae) was the best performing combination in reducing aphid density (H5), we compared aphid densities under each focal combination with those under all other combinations (e.g., (Ab + Ae) versus other combinations). Finally, we used analysis of variance (ANOVA) to test differences on the final density of parasitoid mummies per introduced parasitoid across treatments that included parasitoids. The data were \log_e (final density of parasitoid mummies per number of parasitoids introduced + 1) transformed before analysis to meet linear model assumptions (normality of residuals).

One replicate of the two parasitoid combination treatment was removed before analysis due to existence of spider in the mesocosm, leaving six replicates in this treatment group. All data analysis and figures were performed with the statistical program R version 3.3.3 (R Core Team 2017), using the ‘MASS’ (Venerables and Ripley 2002) and ‘multcomp’ (Hothorn et al. 2008) packages.

Results

As expected, the natural enemy treatments (single species and combinations) reduced final aphid density compared to the enemy-free treatment, a result which was also influenced additively by the initial aphid density (Tables 1 and 2, Fig. 1). We considered the possibility that treatment and initial aphid density had interactive effects, but this was not supported ($P = 0.079$) in comparison with the model where it was included as an additive term (additive $df = 13$, AIC = 823.92; interactive $df = 23$, AIC = 828.67).

Our hypothesis that higher functional diversity will strengthen aphid control was not supported (H2, Table 2). Furthermore, the net diversity effect did

not increase with functional diversity (Fig. 2, H3: $t = -0.468$, $df = 20.109$, $P = 0.645$). One-sample t tests showed that treatments of *A. bipunctata* + *C. montrouzieri* ($t = -6.374$, $df = 6$, $P < 0.001$) and *A. bipunctata* + *A. ervi* ($t = -6.199$, $df = 6$, $P < 0.001$) performed better than expected based on their performance in monoculture, while the performance of other treatments did not differ from the expected results (Fig. 2).

The ladybird *A. bipunctata* was consistently the most efficient species in aphid suppression, while the parasitoid *A. abdominalis* was the least efficient species in this study (H4, Table 2). The best performing combination treatment among our experimental system was that of *A. bipunctata* and *A. ervi* which reflected the efficiency of *A. bipunctata* and the net diversity effect of this combination (H5, Table 2, Figs. 1 and 2).

The average density of *A. ervi* mummies (per adult *A. ervi* introduced) did not vary significantly across treatments that included them ($F_{3,23} = 2.17$, $P = 0.12$; Fig. 3a). However, the average density of *A. abdominalis* mummies (per adult *A. abdominalis* introduced) differed significantly among treatments where they were included ($F_{3,23} = 8.98$, $P < 0.001$; Fig. 3b). Thus the combination treatment of *C. montrouzieri* and *A. abdominalis* had the highest density of *A. abdominalis* mummies (Fig. 3b). This suggests that complementarity increased survivorship of *A. abdominalis*. Notably, however, this increased survivorship did not increase aphid suppression in this combination. Thus, these results suggested the aphids experienced no consequence of IGP upon either parasitoid species in our study (H3).

Discussion

Contrary to our main hypothesis, combining natural enemies from two distinct functional groups (predator and parasitoid) did not strengthen aphid control compared to combining natural enemies of the same functional group. Rather, aphid control was determined by a species identity effect of *A. bipunctata* and its complementary effects with the parasitoid *A. ervi* and the other predator *C. montrouzieri* (Table 2, Fig. 1 and 2).

By including four unique combinations of predators and parasitoids in a single experiment, we were able to

Table 1 Analysis of deviance table for a negative binomial GLM testing the effects of initial aphid density (at the time of first enemy introduction) and the different experimentaltreatments ($n = 7$ replicates each, except combination of *Aphidius ervi* and *Aphelinus abdominalis* $n = 6$ replicates) on the final aphid density

Final aphid density	<i>df</i>	Deviance residual	Residual <i>df</i>	Residual deviance	P value
NULL	–	–	75	196.042	–
Initial aphid density	1	11.23	74	184.82	< 0.001
Treatments	10	97.99	64	86.83	< 0.001

Table 2 Planned contrasts testing the null hypotheses corresponding to our original hypotheses H1, H2, H4 and H5 (i.e., H₀1: Pres = Abs; H₀2: HFD = LFD; H₀4: each individualsingle species = all other species combined; and H₀5: each two species combination = all other species combined)

Hypothesis No.	Corresponding null hypothesis	Estimate	SE	<i>z</i> value	P value
H1	Pres – Abs = 0	– 1.001	0.3528	– 2.836	0.0432
H2	HFD – LFD = 0	0.320	0.768	0.416	0.999
H4	Ab – others = 0	– 5.293	1.118	– 4.735	< 0.001
H4	Cm – others = 0	1.602	1.081	1.483	0.683
H4	Ae – others = 0	– 1.647	1.251	– 1.317	0.793
H4	Aa – others = 0	4.326	1.143	3.785	< 0.001
H5	(Ab + Ae) – combinations = 0	– 1.646	0.509	– 3.233	0.012
H5	(Ab + Aa) – combinations = 0	0.781	0.569	1.373	0.757
H5	(Ab + Cm) – combinations = 0	– 0.909	0.396	– 2.298	0.189
H5	(Ae + Aa) – combinations = 0	0.654	0.526	1.242	0.836
H5	(Cm + Aa) – combinations = 0	0.844	0.453	1.863	0.410
H5	(Cm + Ae) – combinations = 0	– 0.909	0.396	– 2.298	0.177

Labels indicate: natural enemies present (Pres), natural enemies absent (Abs), high functional diversity (HFD), low functional diversity (LFD), *Adalia bipunctata* (Ab), *Cryptolaemus montrouzieri* (Cm), *Aphidius ervi* (Ae), *Aphelinus abdominalis* (Aa), combination treatments (combinations) and all treatments except the natural enemy-free treatment (others). Estimates and SE for linear hypotheses represent differences between \log_{10} [mean (number of aphids)] in contrasted treatments, e.g., H1; \log_{10} [mean (number of aphids in the NEs-present)] – \log_{10} [mean (number of aphid in NEs-absent)]. The *z* values are based on a Wald χ^2 test

explore the generality of predator–parasitoid interactions, revealing variability across species compositions. In our study, notably, the frequency of positive effects of combining predators and parasitoids (1 in 4) was no greater than that of combining multiple predators (1 in 2). This finding indicates that interactions between predators and parasitoids, and the resulting level of biocontrol, is not determined by the combination of predators and parasitoids (functional diversity) per se. Ecologists and biocontrol practitioners should not, therefore, assume that combining natural enemies with different foraging traits (predator versus parasitoid) enhances biocontrol.

Although we found no general effect of combining generalist predators with specialist parasitoids, the best performing combination in our study was indeed a combination of predator and parasitoid (*A. bipunctata* and *A. ervi*) (Table 2). Because this positive effect did not extend to other predator–parasitoid combinations, we can conclude that combining predators and parasitoids is not sufficient to generate positive effects on aphid biocontrol. Other species-specific traits (e.g., behavioural or physiological) of *A. bipunctata* and *A. ervi*, beyond those considered here, must also have been involved. We were unable to ascertain what the enemies were fed before we used them in our

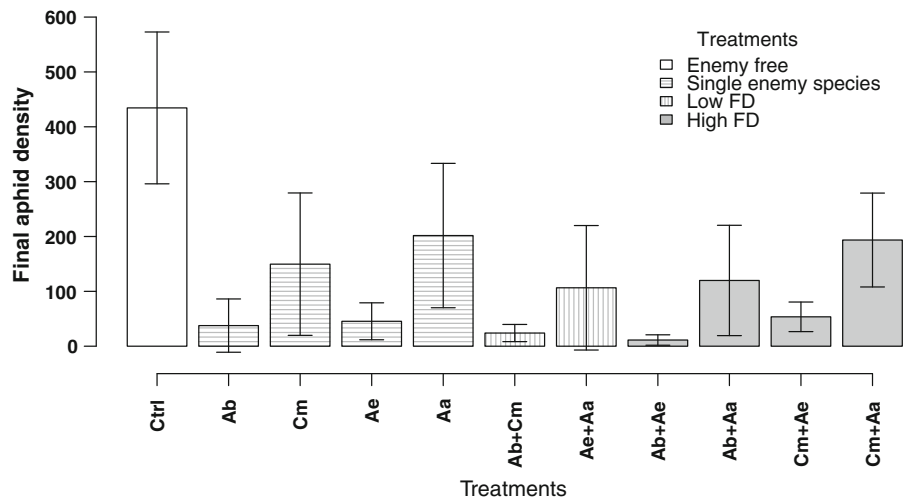


Fig. 1 Final aphid density response to different treatments of natural enemies. Treatments are labelled as enemy-free (Ctrl); single enemy treatments [*Adalia bipunctata* (Ab), *Cryptolaemus montrouzieri* (Cm), *Aphidius ervi* (Ae), *Aphelinus abdominalis* (Aa)]; low functional diversity treatments (*A. bipunctata* + *C.*

montrouzieri, *A. ervi* + *A. abdominalis*); high functional diversity treatments (*A. bipunctata* + *A. abdominalis*, *C. montrouzieri* + *A. ervi*, *C. montrouzieri* + *A. abdominalis*, *A. bipunctata* + *A. ervi*). Error bars show \pm 95% CIs. See Table 1 for results of planned comparisons among groups

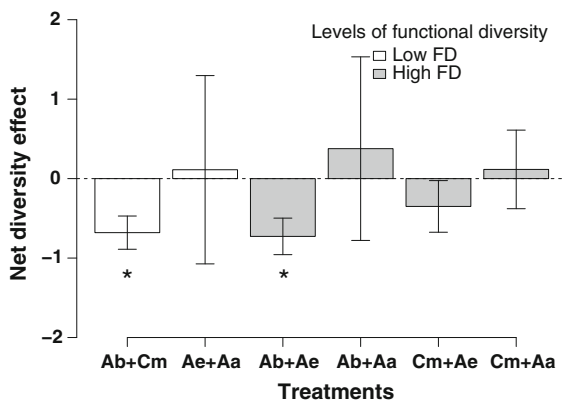
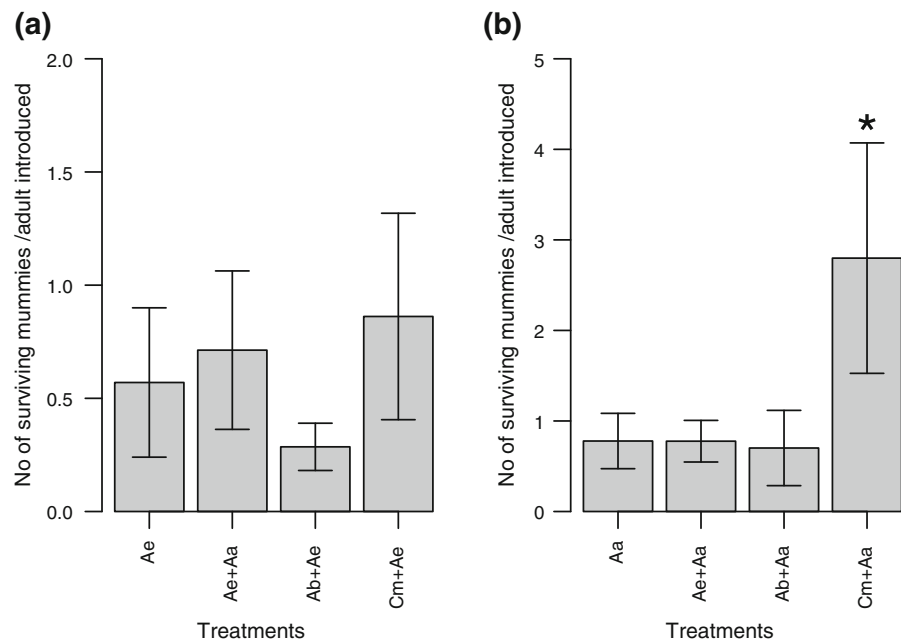


Fig. 2 The net diversity effect under all two-species combinations of natural enemies. Net diversity represents the deviation from the expected mean aphid density based on final aphid density of single species treatments (the dashed horizontal line) (H3 [net diversity effect \neq 0]: $t = -0.468$, $df = 20.109$, $P = 0.645$). Treatments are categorised as low functional diversity treatments (*Adalia bipunctata* + *Cryptolaemus montrouzieri* (Ab + Cm), *Aphidius ervi* + *Aphelinus abdominalis* (Ae + Aa)); or high functional diversity treatments (*A. bipunctata* + *A. ervi* (Ab + Ae), *A. bipunctata* + *A. abdominalis* (Ab + Aa), *C. montrouzieri* + *A. ervi* (Cm + Ae), *C. montrouzieri* + *A. abdominalis* (Cm + Aa)). Error bars show \pm 95% CIs. * Denotes combinations with net diversity effect significantly different from zero (expected mean aphid density based on final aphid density of single species treatments) at $\alpha = 0.05$

experiments. Other research has demonstrated that feeding history can affect natural enemies attacking choice (Finke and Snyder 2008; Turlings et al. 1993). For instance, the parasitoid wasp *Venturia canescens* (Gravenhorst) prefers to deposit eggs in hosts of the same species from which they themselves emerged, when exposed to more than one host species (Thorpe and Jones 1937). However, this tendency (attraction to host-related cues) was found to be moderated by adult parasitoid learning in many other parasitoid species. Thus there was more contribution from adult parasitoid learning to the foraging success of insects than pre-mature learning (Turlings et al. 1993). Although positive predator-parasitoid interactions like this one are restricted to particular species combinations, they are common (see Supplementary information 1, Greenop et al. 2018). More focused assessments (e.g., including behavioural observations) of such positive interactions may point to the particular mechanisms involved.

We also found that the combination of two generalist predators enhanced biocontrol. Although the trait-basis of this is not clear, it could have emerged from fine-scale differences in traits (e.g., timing of foraging) resulting in stronger intraspecific versus interspecific interference (Griffin et al. 2008; Griffiths et al. 2008). This result provides further evidence that species that are assumed to be ecologically redundant

Fig. 3 Average density of parasitoid mummies at the end of the experiment. **a** Number of *Aphidius ervi* mummies per adult introduced, **b** number of *Aphelinus abdominalis* mummies per adult introduced. Species labelled as *Adalia bipunctata* (Ab), *Cryptolaemus montrouzieri* (Cm), *A. ervi*, (Ae), *A. abdominalis* (Aa). Error bars show \pm 95% Cls. *Denotes significance differences between treatment groups at $\alpha = 0.05$



(two ladybirds) may actually show complementarity and enhance ecological processes such as biocontrol (Griffin and Silliman 2011). The trait basis of positive multiple predator effects does not, therefore, lie exclusively in gross differences between functional groups (e.g., predators and parasitoids), or between taxonomically distant relatives (Griffin et al. 2013; Northfield et al. 2014), but can also emerge from finer-scale differences that may occur between close relatives. Further work is needed to elucidate which traits predict interactions in food webs and in biocontrol contexts.

Species identity also had an important effect in our study, consistent with numerous previous studies of aphid biocontrol (Denoth et al. 2002; Long and Finke 2014; Schmitz and Suttle 2001; Sokol-Hessner and Schmitz 2002; Straub and Snyder 2006). The traits of species (i.e., functional identity) ultimately underpins differences in the performance of natural enemies. *A. bipunctata* achieved the highest level of aphid suppression in our experiment, suggesting that its traits are particularly well suited to efficient aphid biocontrol, consistent with previous studies (Alhadidi et al. 2018; Griffiths et al. 2008; Wyss et al. 1999). Other traits, such as mobility, metabolic rate or degree of dietary specialisation, might help to explain species differences (especially the strong effect of *A. bipunctata*) and better define the functional role of species in

biocontrol systems. Once these traits are known, weighted averages of these traits could be used instead of (or in combination with) functional diversity to effectively explain biocontrol (Gagic et al. 2015).

IGP of coccinellid predators upon parasitoids, through consumption of parasitized aphids, has been reported previously (Bilu and Coll 2007; Snyder and Ives 2003; Wheeler 1977). We did not find evidence to support the presence of IGP. On the contrary, the number of surviving *A. abdominalis* mummies (per adult introduced), was in fact significantly higher when it was combined with the predator *C. montrouzieri* (Fig. 3b). In light of the substitutive design we used, which effectively compares interspecific to intraspecific interactions, it seems that intraspecific competition among *A. abdominalis* exceeded any negative interspecific interactions (competition, IGP) between *A. abdominalis* and *C. montrouzieri*. Notwithstanding, this apparent relaxation of negative effects on mummy survival did not translate into a positive effect of this combination of predator and parasitoid on aphid biocontrol, suggesting that, at least in our experiment, it is not an important mechanism.

There are several important limitations of our study, which may also have contributed to variability in the effects of predators and parasitoids, and the lack of any general effect of functional diversity. First, including only two species within each functional

group in our experiment limited the interactions between the competitors and may have allowed one species (*A. bipunctata*) and its superior traits to drive the process of aphid control in this study. Another possible reason for the lack of a general FD effect is the small experimental arena we used, which limited the scope for spatial complementarity between species, and/or spatial shifts due to non-consumptive ('fear') effects (Michaud et al. 2016). Furthermore, the limited duration of the experiment did not allow the parasitoids or the predators to have multiple generations. This would have limited the potential for complementarity between the NE groups to strengthen aphid control via increased NE population densities and/or overlapping their life history stages. The size of experimental arena also determines how many details of any component of an ecosystem can be examined and, consequently, how the findings are interpreted. Thus, it is impossible for any single experiment to manipulate all components of biological diversity and measure all the ecological functions in an individual ecosystem (Symstad et al. 2003), particularly a small one such as our experiment. Moreover, greater prey diversity can be another relevant factor worth considering, that was beyond the scope of our experiment. Greater prey diversity could support an effect of functional diversity in aphid control, thus morphological and behavioural differences among prey species can boost functional complementarity between predators and parasitoids and ultimately increase aphid consumption (Gagic et al. 2015; Wilby et al. 2005). Therefore, given the widespread use of both generalist predators and parasitoids in biocontrol and their coexistence in agricultural landscapes, we encourage future, larger and more complex scale work on their interactions.

In conclusion, the functional diversity of natural enemies failed to explain the performance of predator-parasitoid combinations in our study system. Nevertheless, there was evidence of important non-additive interactions between natural enemies in our study, with one predator and parasitoid combination (*A. bipunctata* and *A. ervi*) showing especially strong aphid control—a combination we therefore recommend to control pea aphids, particularly on broad bean plants. Future studies may wish to consider additional natural enemies' traits and/or different functional trait metrics [e.g., FD, community weighted means

(Petchey and Gaston 2002)] to help explain variation in biocontrol in this and similar systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no potential conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors, and all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent No individuals have participated in this study.

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References

- Alhadidi SN, Griffin JN, Fowler MS (2018) Natural enemy composition rather than richness determines pest suppression. *BioControl* 63:575–584. <https://doi.org/10.1007/s10526-018-9870-z>
- Bilu E, Coll M (2007) The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. *BioControl* 52:753–763
- Bonet A (2009) Parasitoid wasps, natural enemies of insects. In: Claro KD, Oliveira PS, Rico-Gray V (eds) *Tropical biology and conservation management*, vol 7. *Phytopathology and entomology*. Eolss Publishers, Paris, pp 185–207
- Casula P, Wilby A, Thomas MB (2006) Understanding biodiversity effects on prey in multi-enemy systems. *Ecol Lett* 9:995–1004
- Couty A, Clark S, Poppy G (2001) Are fecundity and longevity of female *Aphelinus abdominalis* affected by development in GNA-dosed *Macrosiphum euphorbiae*? *Physiol Entomol* 26:287–293
- Denoth M, Frid L, Myers JH (2002) Multiple agents in biological control: improving the odds? *Biol Control* 24:20–30
- Derrick B, Toher D, White P (2016) Why Welch's test is type I error robust. *Quant Meth Psych* 12:30–38
- Duffy JE, Lefcheck JS, Stuart-Smith RD, Navarrete SA, Edgar GJ (2016) Biodiversity enhances reef fish biomass and resistance to climate change. *Proc Natl Acad Sci USA* 113:6230–6235

- Evans EW, Stevenson AT, Richards DR (1999) Essential versus alternative foods of insect predators: benefits of a mixed diet. *Oecologia* 121:107–112
- Finke DL, Denno RF (2004) Predator diversity dampens trophic cascades. *Nature* 429:407–410
- Finke DL, Denno RF (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol Lett* 8:1299–1306
- Finke DL, Snyder WE (2008) Niche partitioning increases resource exploitation by diverse communities. *Science* 321:1488–1490
- Gagic V, Bartomeus I, Jonsson T, Taylor A, Winqvist C, Fischer C, Slade EM, Steffan-Dewenter I, Emmerson M, Potts SG (2015) Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proc R Soc Lond B* 282:20142620
- Gontijo LM, Beers EH, Snyder WE (2015) Complementary suppression of aphids by predators and parasitoids. *Biol Control* 90:83–91
- Greenop A, Woodcock BA, Wilby A, Cook SM, Pywell RF (2018) Functional diversity positively affects prey suppression by invertebrate predators: a meta-analysis. *Ecology* 99:1771–1782. <https://doi.org/10.1002/ecy.2378>
- Griffin JN, Silliman BR (2011) Predator diversity stabilizes and strengthens trophic control of a keystone grazer. *Biol Lett* 7:79–82
- Griffin JN, De La Haye KL, Hawkins SJ, Thompson RC, Jenkins SR (2008) Predator diversity and ecosystem functioning: density modifies the effect of resource partitioning. *Ecology* 89:298–305
- Griffin JN, Byrnes JE, Cardinale BJ (2013) Effects of predator richness on prey suppression: a meta-analysis. *Ecology* 94:2180–2187
- Griffiths GJ, Wilby A, Crawley MJ, Thomas MB (2008) Density-dependent effects of predator species-richness in diversity–function studies. *Ecology* 89:2986–2993
- He XZ (2008) Reproductive behaviour of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae): a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant science (Entomology) at Massey University, Palmerston North, New Zealand
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- Krenek L, Rudolf VH (2014) Allometric scaling of indirect effects: body size ratios predict non-consumptive effects in multi-predator systems. *J Anim Ecol* 83:1461–1468
- Laureto LMO, Cianciaruso MV, Samia DSM (2015) Functional diversity: an overview of its history and applicability. *Nat Conserv* 13:112–116
- Letourneau DK, Jedlicka JA, Bothwell SG, Moreno CR (2009) Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. *Annu Rev Ecol Evol Syst* 40:573–592
- Long EY, Finke DL (2014) Contribution of predator identity to the suppression of herbivores by a diverse predator assemblage. *Environ Entomol* 43:569–576
- Losey JE, Denno RF (1998) Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. *Ecology* 79:2143–2152
- Losey JE, Denno RF (1999) Factors facilitating synergistic predation: the central role of synchrony. *Ecol Appl* 9:378–386
- McCoy MW, Stier AC, Osenberg CW (2012) Emergent effects of multiple predators on prey survival: the importance of depletion and the functional response. *Ecol Lett* 15:1449–1456
- McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. *Trends Ecol Evol* 21:178–185
- Michaud J, Barbosa PR, Bain CL, Torres JB (2016) Extending the “Ecology of Fear” beyond prey: reciprocal nonconsumptive effects among competing aphid predators. *Environ Entomol* 45:1398–1403
- Northfield TD, Snyder GB, Ives AR, Snyder WE (2010) Niche saturation reveals resource partitioning among consumers. *Ecol Lett* 13:338–348
- Northfield TD, Crowder DW, Takizawa T, Snyder WE (2014) Pairwise interactions between functional groups improve biological control. *Biol Control* 78:49–54
- Petchey OL (2003) Integrating methods that investigate how complementarity influences ecosystem functioning. *Oikos* 101:323–330
- Petchey OL, Gaston KJ (2002) Functional diversity (FD), species richness and community composition. *Ecol Lett* 5:402–411
- R Core Team (2017). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.r-project.org/>
- Schmitz OJ (2009) Effects of predator functional diversity on grassland ecosystem function. *Ecology* 90:2339–2345
- Schmitz OJ, Suttle KB (2001) Effects of top predator species on direct and indirect interactions in a food web. *Ecology* 82:2072–2081
- Schoener TW (1983) Field experiments on interspecific competition. *Am Nat* 122:240–285
- Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. *Trends Ecol Evol* 13:350–355
- Snyder WE, Ives AR (2003) Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. *Ecology* 84:91–107
- Snyder WE, Ballard SN, Yang S, Clevenger GM, Miller TD, Ahn JJ, Hatten TD, Berryman AA (2004) Complementary biocontrol of aphids by the ladybird beetle *Harmonia axyridis* and the parasitoid *Aphelinus asychis* on greenhouse roses. *Biol Control* 30:229–235
- Sokol-Hessner L, Schmitz OJ (2002) Aggregate effects of multiple predator species on a shared prey. *Ecology* 83:2367–2372
- Straub CS, Snyder WE (2006) Experimental approaches to understanding the relationship between predator biodiversity and biological control. In: Brodeur J, Boivin G (eds) *Trophic and guild in biological interactions control*. Springer, Dordrecht, pp 221–239
- Symondson W, Sunderland K, Greenstone M (2002) Can generalist predators be effective biocontrol agents? *Annu Rev Entomol* 47:561–594
- Symstad AJ, Chapin FS, Wall DH, Gross KL, Huenneke LF, Mittelbach GG, Peters DP, Tilman D (2003) Long-term and large-scale perspectives on the relationship between

- biodiversity and ecosystem functioning. *AIBS Bull* 53:89–98
- Thorpe WH, Jones FG (1937) Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. *Proc R Soc Lond B* 124:56–81
- Tilman D (2001) Functional diversity. In: Levin SA (ed) *Encyclopedia of biodiversity*. Academic Press, San Diego, pp 109–120
- Traugott M, Bell J, Raso L, Sint D, Symondson W (2012) Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation. *Bull Entomol Res* 102:239–247
- Turlings TC, Wäckers FL, Vet LE, Lewis WJ, Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC (eds) *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York, pp 51–78
- Vance-Chalcraft HD, Rosenheim JA, Vonesh JR, Osenberg CW, Sih A (2007) The influence of intraguild predation on prey suppression and prey release: a meta-analysis. *Ecol* 88:2689–2696
- Venerables W, Ripley B (2002) *Modern applied statistics with S*. Springer, New York
- Wheeler A (1977) Studies on the arthropod fauna of alfalfa: VII. Predaceous insects. *Can Entomol* 109:423–427
- Wilby A, Villareal S, Lan L, Heong K, Thomas MB (2005) Functional benefits of predator species diversity depend on prey identity. *Ecol Entomol* 30:497–501
- Wyss E, Villiger M, Hemptinne JL, Müller-schärer H (1999) Effects of augmentative releases of eggs and larvae of the ladybird beetle, *Adalia bipunctata*, on the abundance of the rosy apple aphid, *Dysaphis plantaginea*, in organic apple orchards. *Entomol Exp Appl* 90:167–173

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